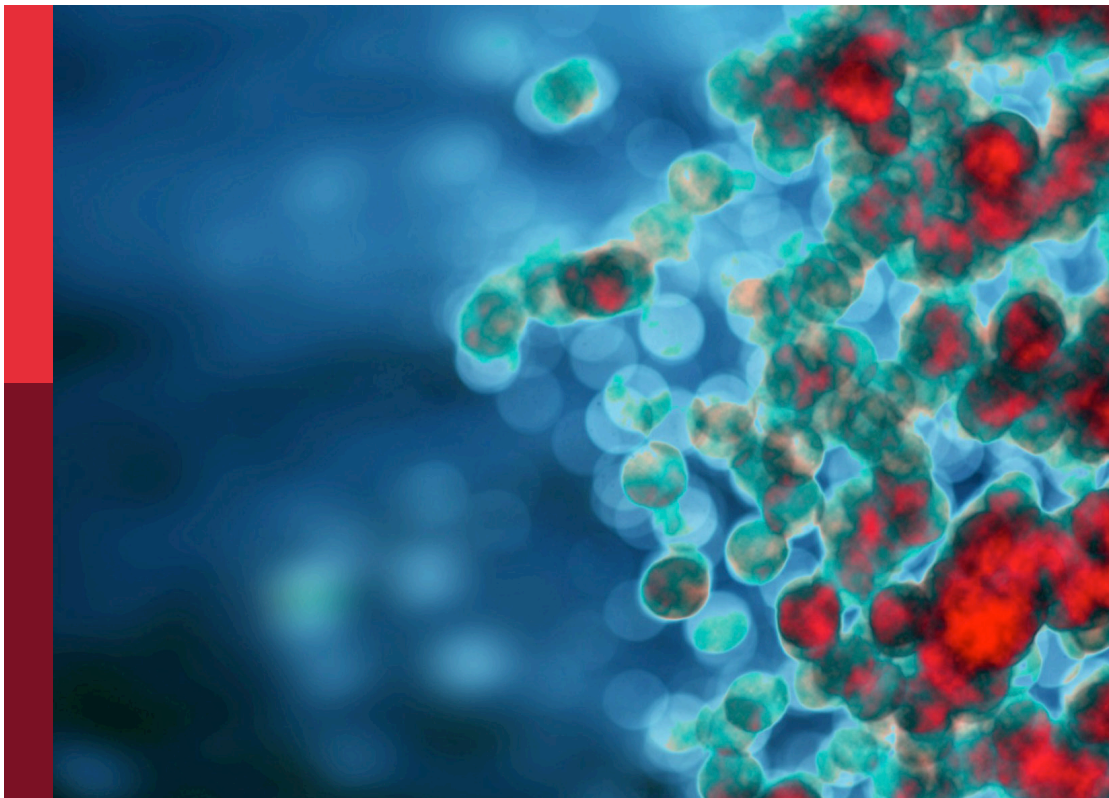
frontiers | Research Topics

Host-microbiota immuno-interactions for personalized microbial therapeutics

Edited by
Nar Singh Chauhan, Sunil Kumar Raghav and Shashank Gupta

Published in
Frontiers in Immunology



FRONTIERS EBOOK COPYRIGHT STATEMENT

The copyright in the text of individual articles in this ebook is the property of their respective authors or their respective institutions or funders. The copyright in graphics and images within each article may be subject to copyright of other parties. In both cases this is subject to a license granted to Frontiers.

The compilation of articles constituting this ebook is the property of Frontiers.

Each article within this ebook, and the ebook itself, are published under the most recent version of the Creative Commons CC-BY licence. The version current at the date of publication of this ebook is CC-BY 4.0. If the CC-BY licence is updated, the licence granted by Frontiers is automatically updated to the new version.

When exercising any right under the CC-BY licence, Frontiers must be attributed as the original publisher of the article or ebook, as applicable.

Authors have the responsibility of ensuring that any graphics or other materials which are the property of others may be included in the CC-BY licence, but this should be checked before relying on the CC-BY licence to reproduce those materials. Any copyright notices relating to those materials must be complied with.

Copyright and source acknowledgement notices may not be removed and must be displayed in any copy, derivative work or partial copy which includes the elements in question.

All copyright, and all rights therein, are protected by national and international copyright laws. The above represents a summary only. For further information please read Frontiers' Conditions for Website Use and Copyright Statement, and the applicable CC-BY licence.

ISSN 1664-8714
ISBN 978-2-8325-7118-7
DOI 10.3389/978-2-8325-7118-7

Generative AI statement
Any alternative text (Alt text) provided alongside figures in the articles in this ebook has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Host-microbiota immuno-interactions for personalized microbial therapeutics

Topic editors

Nar Singh Chauhan — University of Pittsburgh, United States
Sunil Kumar Raghav — Louisiana State University, United States
Shashank Gupta — Louisiana State University, United States

Citation

Chauhan, N. S., Raghav, S. K., Gupta, S., eds. (2025). *Host-microbiota immuno-interactions for personalized microbial therapeutics*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-7118-7

Table of contents

05 Editorial: Host-microbiota immuno-interactions for personalized microbial therapeutics
Shashank Gupta, Sunil Kumar Raghav and Nar Singh Chauhan

07 Commensal bacteria education history calibrates the naivety and activation threshold of adaptive antiviral immune system
Baohua Li, Zhou Sha, Li Mou, Feng Zhang, Lifeng Jia, Yan Zhu, Yan Guo, Guohong Deng, Haibo Wu, Hong Wei, Yuzhang Wu, Lilin Ye, Changjiang Liu, Jian Li and Yanyan Zhang

16 Chaihushugan powder regulates the gut microbiota to alleviate mitochondrial oxidative stress in the gastric tissues of rats with functional dyspepsia
Xuejiao Liu, Keming Yang, Yuebo Jia, Yeliya Yeertai, Chenheng Wu, Xiangxiang Wang, Qingling Jia, Zhijian Gu, Jun Cong and Jianghong Ling

40 Harnessing the human gut microbiota: an emerging frontier in combatting multidrug-resistant bacteria
Wenwen Ding, Yiwen Cheng, Xia Liu, Zhangcheng Zhu, Lingbin Wu, Jie Gao, Wenhui Lei, Yating Li, Xin Zhou, Jian Wu, Yongtao Gao, Zongxin Ling and Ruilai Jiang

66 *Pseudomonas aeruginosa* enhances anti-PD-1 efficacy in colorectal cancer by activating cytotoxic $CD8^+$ T cells
Lu Chen, Guangcong Ruan, Xuefei Zhao, Ailin Yi, Zhifeng Xiao, Yuting Tian, Yi Cheng, Dongfeng Chen and Yanling Wei

79 Therapeutic targeting of the host-microbiota-immune axis: implications for precision health
Asiya Nazir, Fathima Hasnain Nadeem Hussain, Tuahir Hassan Nadeem Hussain, Rania Al Dweik and Afsheen Raza

97 Microbiota as diagnostic biomarkers: advancing early cancer detection and personalized therapeutic approaches through microbiome profiling
Majid Eslami, Ramtin Naderian, Aisa Bahar, Ali Babaeizad, Solaleh Rezanavaz Gheshlagh, Valentyn Oksenych and Hamed Tahmasebi

115 The gut-immune axis in primary immune thrombocytopenia (ITP): a paradigm shifts in treatment approaches
Xuejun Guo, Ke Wang, Qianhui Liu, Natalia Baran and Wenxue Ma

131 Harnessing the microbiome to improve clinical outcomes for cancer, transplant, and immunocompromised patients in the intensive care unit (ICU)
Lizbeth Nieves, Alexandra Roach, Joseph Hunter, Sarah Smeh, Andrew Islas, Ariana Islas, Joseph Blattman and Michelle Di Palma

142 Clinical and biochemical characterization of hepatitis B surface antigen-positive patients with or without *Helicobacter pylori* co-infection at the University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia
Mekuriaw Belayneh, Bantayehu Addis Tegegne, Mulualem Lemma, Zenahebezu Abay and Yeshambel Belyhun

151 Interaction of vaginal microbiota and biomarkers in Premature rupture of membranes: from bench to beside
Yudi Deng, Yang Li, Tiana Liu and Fuju Wu

170 Role of the microbiota–gut–lung axis in the pathogenesis of pulmonary disease in children and novel therapeutic strategies
Zhifang Wang, Jiayao Yu, Yaqi Liu, Jiaqi Gong, Zepeng Hu and Zheng Liu

frontiers | Frontiers in Immunology

TYPE Editorial
PUBLISHED 28 October 2025
DOI 10.3389/fimmu.2025.1716098

Check for updates

OPEN ACCESS

EDITED AND REVIEWED BY
Pietro Ghezzi,
Brighton and Sussex Medical School,
United Kingdom

*CORRESPONDENCE
Nar Singh Chauhan
nschauhan@mdurohtak.ac.in

RECEIVED 30 September 2025
ACCEPTED 20 October 2025
PUBLISHED 28 October 2025

CITATION
Gupta S, Raghav SK and Chauhan NS (2025) Editorial: Host-microbiota immuno-interactions for personalized microbial therapeutics.
Front. Immunol. 16:1716098.
doi: 10.3389/fimmu.2025.1716098

COPYRIGHT
© 2025 Gupta, Raghav and Chauhan. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Editorial: Host-microbiota immuno-interactions for personalized microbial therapeutics

Shashank Gupta[1], Sunil Kumar Raghav[2] and Nar Singh Chauhan[3]*

[1]School of Medical Sciences, Faculty of Medicine and Health, Örebro University, Örebro, Sweden, [2]Laboratory of Immuno-genomics & Systems Biology, Institute of Life Sciences, Bhubaneswar, Orissa, India, [3]Department of Biochemistry, Maharshi Dayanand University, Rohtak, Haryana, India

KEYWORDS

human microbiota, host-microbe interactions, personalized therapy, microbiome therapeutics, microbiota dysbiosis

Editorial on Research Topic
Host-microbiota immuno-interactions for personalized microbial therapeutics

The human gut microbiota plays a crucial role in modulating the immune system by regulating both innate and adaptive immune responses (1). Dysbiosis of the gut microbiota can disrupt these immune-interactions and predispose individuals to immune-mediated physiological and metabolic disorders (2). Dysbiosis of the human gut microbiota often manifests as increased gut permeability, aberrant immune responses, and chronic inflammation (3). Restoring a balanced microbiota through microbial therapeutics has emerged as a promising strategy to mitigate these detrimental effects (4). Personalized microbial therapeutics are developed to exploit host-microbiota interactions in a manner tailored to an individual's microbiome composition and immune profile (5). Recent developments in human microbiome research highlight the need for personalized therapeutic approaches that address the complex interplay between the immune system and the microbiota (6). This Research Topic brings together compelling articles that highlight diverse ways in which host-microbiota immune interactions can be leveraged to improve personalized microbial therapeutics.

Research and review articles

Disease-focused clinical perspectives highlight the role of microbial communities in pathophysiology and patient outcomes. Belayneh et al. delineated the role of *Helicobacter pylori* co-infection in the alteration of clinical features in hepatitis B patients, providing evidence that microbial status can influence viral disease trajectories. Deng et al. connected alterations in the vaginal microbiota to preterm premature rupture of membranes as prognostic biomarkers, offering a pathway for early intervention in pregnancy

complications. Guo et al. highlighted the gut–immune axis in primary immune thrombocytopenia, reframing autoimmune pathology through a microbiome lens and pointing toward novel treatment paradigms. Eslami et al. explored microbiota as diagnostic biomarkers, emphasizing their potential for early cancer detection and personalized treatment planning. Chen et al. demonstrated that *Pseudomonas aeruginosa* can enhance the effectiveness of anti-PD-1 therapy for colorectal cancer by activating $CD8^+$ T cells, underscoring the potential for synergistic effects between microbiota and immunotherapy. Complementing these insights, Nieves et al. discussed how microbiome modulation could improve clinical outcomes in immunocompromised patients. Together, these findings highlight the promise of microbiota-informed diagnostics and therapeutic strategies in diverse clinical settings.

Microbial therapeutic innovations also provide a broader framework for personalized therapeutics. Nazir et al. summarized approaches to therapeutically target the host–microbiota–immune axis, emphasizing the potential of precision microbial medicine. Simultaneously, Ding et al. highlighted the role of the microbiome in combating antimicrobial resistance, projecting microbiota-based interventions as a promising frontier in addressing one of the greatest global challenges. Additionally, Wang et al. summarized microbial and metabolite targets that could be leveraged to develop personalized microbiota-based therapies for pediatric pulmonary diseases. Experimental studies enrich our clinical and translational understanding with insights into host–microbe biology. Li et al. showed the role of commensal bacterial history in calibrating antiviral immune readiness and influencing the threshold for adaptive immune activation. Liu et al. demonstrated the role of Chaihushugan powder in alleviating functional dyspepsia in rats by reshaping the gut microbiota and reducing mitochondrial oxidative stress in gastric tissue. These findings highlight the innovative approaches to harnessing the microbiota for therapeutic purposes.

This Research Topic charts a promising roadmap for translating microbiome science into precision therapeutics. The integration of mechanistic insights, translational studies, and clinical evidence provides a robust framework that advances microbiota-informed personalized medicine as a foundational paradigm in future healthcare (7).

Author contributions

SG: Writing – original draft, Writing – review & editing. SR: Writing – original draft, Writing – review & editing. NC: Writing – original draft, Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Yadav M, Chauhan NS. Overview of the rules of the microbial engagement in the gut microbiome: a step towards microbiome therapeutics. *J Appl Microbiol.* (2021) 130:1425–41. doi: 10.1111/jam.14883

2. Yang Q, Cai Y, Guo S, Wang Z, Wang Y, Yu X, et al. Decoding immune interactions of gut microbiota for understanding the mechanisms of diseases and treatment. *Front Microbiol.* (2023) 14:1238822. doi: 10.3389/fmicb.2023.1238822

3. Yadav M, Chauhan NS. Role of gut-microbiota in disease severity and clinical outcomes, Brief. *Funct Genom.* (2024) 23:24–37. doi: 10.1093/bfgp/elac037

4. Mkilima T. Synthetic biology approaches for restoring gut microbial balance and engineering disease-specific microbiome therapeutics. *Microb Pathog.* (2025) 207:107931. doi: 10.1016/j.micpath.2025.107931

5. Matusheski NV, Caffrey A, Christensen L, Mezgec S, Surendran S, Hjorth MF, et al. Diets, nutrients, genes and the microbiome: recent advances in personalised nutrition. *Br J Nutr.* (2021) 126:1489–97. doi: 10.1017/S0007114521000374

6. Filardo S, Di Pietro M, Sessa R. Current progresses and challenges for microbiome research in human health: a perspective. *Front Cell Infect Microbiol.* (2024) 14:1377012. doi: 10.3389/fcimb.2024.1377012

7. Ratiner K, Ciocan D, Abdeen SK, Elinav E. Utilization of the microbiome in personalized medicine. *Nat Rev Microbiol.* (2024) 22:291–308. doi: 10.1038/s41579-023-00998-9

frontiers | Frontiers in Immunology

TYPE Original Research
PUBLISHED 07 February 2025
DOI 10.3389/fimmu.2025.1519023

Check for updates

OPEN ACCESS

EDITED BY
Nar Singh Chauhan,
Maharshi Dayanand University, India

REVIEWED BY
Manu Bhambi,
Zigzoo Marketing Services Pvt Ltd, India
Asiya Nazir,
Abu Dhabi University, United Arab Emirates

*CORRESPONDENCE
Yanyan Zhang
yzhangtmmu@163.com
Jian Li
seraph-2004@163.com
Changjiang Liu
cj_514@163.com
Lilin Ye
yelilinlcmv@tmmu.edu.cn

†These authors share first authorship

RECEIVED 29 October 2024
ACCEPTED 22 January 2025
PUBLISHED 07 February 2025

CITATION
Li B, Sha Z, Mou L, Zhang F, Jia L, Zhu Y, Guo Y, Deng G, Wu H, Wei H, Wu Y, Ye L, Liu C, Li J and Zhang Y (2025) Commensal bacteria education history calibrates the naivety and activation threshold of adaptive antiviral immune system. *Front. Immunol.* 16:1519023. doi: 10.3389/fimmu.2025.1519023

COPYRIGHT
© 2025 Li, Sha, Mou, Zhang, Jia, Zhu, Guo, Deng, Wu, Wei, Wu, Ye, Liu, Li and Zhang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Commensal bacteria education history calibrates the naivety and activation threshold of adaptive antiviral immune system

Baohua Li[1†], Zhou Sha[2†], Li Mou[3†], Feng Zhang[4], Lifeng Jia[5], Yan Zhu[1], Yan Guo[1], Guohong Deng[1], Haibo Wu[2], Hong Wei[6], Yuzhang Wu[7], Lilin Ye[7*], Changjiang Liu[3*], Jian Li[7*] and Yanyan Zhang[5,8,9*]

[1]Department of Infectious Diseases, Southwest Hospital, Third Military Medical University (Army Medical University), Chongqing, China, [2]School of Life Sciences, Chongqing University, Chongqing, China, [3]National Health Commission (NHC) Key Laboratory of Birth Defects and Reproductive Health, Chongqing Population and Family Planning Science and Technology Research Institute, Chongqing, China, [4]Department of Hepatobiliary Surgery, Tongren Municipal People's Hospital, Tongren, Guizhou, China, [5]Medical Research Center, Chongqing General Hospital, Chongqing University, Chongqing, China, [6]Department of Animal Science and Animal Medicine, Huazhong Agricultural University, Wuhan, China, [7]Institute of Immunology, Third Military Medical University, Chongqing, China, [8]College of Life Sciences, Chongqing College, University of Chinese Academy of Sciences, Chongqing, China, [9]School of Biomedical Engineering, Chongqing Medical University, Chongqing, China

Exhaustion of the immune system's ability to adapt to novelty suggests that the changes it undergoes might be a consequence of an evolutionary unpredictable antigenic exposure over a lifetime. Thus, we raise the question of whether a naive immune system can manage new antigens better than an educated immune system. Here, by employing the naive immune system of germ-free (GF) mice without a history of microbial exposure, we compared their adaptive immune responses with those of the conventional (Conv) mice upon new viral infection. Interestingly, the naive GF immune system showed robust T-cell responses, with more potent memory T cells established for long-term protection, even in the condition of primary lower T-cell levels for naive GF mice. Furthermore, we found that the ABX-treated Conv mice showed impaired T-cell responses, compared with the untreated Conv ones. With the microbiota eliminated, the ABX mice still have a history of microbial exposure and education for their immune system. In summary, commensal bacteria education history calibrates the naivety and the activation threshold of the adaptive antiviral immune system.

KEYWORDS

commensal microbiota, adaptive antiviral immunity, germ-free mice, ABX-treated mice, immune education history, T cell immune effect and memory, immune naivety and activation threshold, LCMV (lymphocytic choriomeningitis virus)

Highlights

- Considering the condition of primary lower T-cell level, the immune system of naive GF mice showed more robust T-cell responses upon LCMV infection.
- With the microbiota eliminated, the ABX-treated mice show impaired T-cell responses, unlike GF mice upon LCMV infection.
- The naive GF immune system shows greater plasticity and sensitivity in launching robust adaptive immune responses against new viral infections.

Introduction

Adults were thought to have a stronger immunity than children, as they have a more mature immune system with established powerful memory T and B cells against reinfection with experienced pathogens (1). Nevertheless, the clinical course of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in children, even in neonates and infants, is milder than that in adults. Children also respond better than adults in the case of other novel viral infections, such as in the 2002 SARS-CoV-1 outbreak, the 2012 MERS-CoV outbreak, and even in the massive 1918 H1N1 influenza pandemic (2–6). Moreover, children also show better effects of vaccination than adults (7, 8). The less-experienced humoral immunity in children, as evidenced by the higher IgM levels, might induce the production of more potent antibodies upon SARS-CoV-2 infection (9). The acute phase of coronavirus disease 2019 (COVID-19) in humans is associated with strong T-cell lymphopenia in severe disease, with a bias toward $CD8^+$ T cells (10). Importantly, lymphocyte levels in infected children are not reduced as much as those in adults (9). This central role for T cells makes them a desirable target for assessing immune responses to novel viral infections. The immune system of children is likely prepared to react to novelty in the early years of life to build the pool of memory T and B cells for preventing reinfection, a function that might be dampened in adults.

Exhaustion of the immune system's ability to adapt to novelty suggests that the changes it undergoes might be a consequence of an evolutionary unpredictable antigenic exposure over a lifetime. From the above, we raise the question of whether a naive immune system is able to manage new antigens better than an educated one. Here, to address the above question, we employed the naive immune system of GF mice without a history of microbial exposure and compared their adaptive immune responses with those of the conventional (Conv) mice (regularly maintained in an SPF condition) at the same age of 6–8 weeks upon new viral infection. For the new viral infection model, we applied the relatively mature LCMV (*lymphocytic choriomeningitis virus*)-P14 CD8 or LCMV-SMARTA CD4 T-cell response system, which can help us to identify the LCMV-specific P14 CD8 (transgenic TCR that is specific for the MHC class I molecule H2-Db-gp33-41 peptide) T-cell responses or the LCMV-specific SMARTA CD4 (transgenic TCR that is specific for the MHC class II molecule H2-I-Ab-gp61-80 peptide) T-cell responses, respectively (11). LCMV is an ideal model to study adaptive immune response, and T- and B-cell responses to LCMV are sensitive and robust, which allows investigators to assess T- and B-cell responses more accurately (11).

Comparing the condition of primary lower T-cell levels of GF mice compared with that of Conv mice, surprisingly, the naive immune system of GF mice showed more robust T-cell responses once they encountered LCMV infection, also with more potent memory $CD8^+$ T cells, especially those of Tcm (central memory) and Trm (resident memory) cells, established for long-term protection. Furthermore, in mice treated with ABX (with antibiotic treatment to abolish the microbiota in Conv mice), we found that they showed impaired T-cell responses, not similar to that of GF mice upon LCMV infection. With the microbiota eliminated, the immune system of ABX mice still showed microbial exposure history and education; in other words, their immune system has been educated and is not that naive. Collectively, the above results suggested that the education history of commensal bacteria calibrates the naivety and activation threshold of the adaptive antiviral immune system. This finding, at least from one standpoint, explains the immune system's naivety-related differences in adapting to novel stimuli.

Results

Mice without a history of microbial education developed robust adaptive immune responses upon encountering new pathogens

To test whether a naive immune system has a weaker or stronger immunity than an educated immune system upon new viral infection, we studied the immune system of GF mice without a history of microbial exposure and compared their T-cell responses to LCMV infection with those of Conv mice. Firstly, we sorted CD90.1 P14 CD8 T cells from CD90.1 P14 mice and transferred the same quantity of cells into CD90.2 Conv mice or CD90.2 GF mice, respectively, following acute viral infection with the new pathogen LCMV Armstrong (Figure 1A). Then, we analyzed the CD8 T-cell responses on the indicated days. Surprisingly, GF mice exhibited a more robust CD8 T-cell immunity than Conv mice, with both the cell proportion and the cell number of the LCMV-specific P14 CD8 T cells elevated dramatically in the spleen on day 8 (Figure 1B, Supplementary Table S1).

As the microbiota is largely associated with intestinal microbial populations, we also analyzed small intestinal lymphocytes. The intestine contains the largest number of immune cells of any organ in the body, and the intestinal mucosal immune system is composed of three major lymphoid areas: the lamina propria (LP, which lies just underneath the basement membrane in the intestinal villi), the intraepithelial compartment (IEL, which contains intraepithelial lymphocytes and is located between the columnar epithelial cells), and Peyer's patches (PPs, which are embedded in the gut wall) (12, 13). Here, we first analyzed CD8 T cells in the LP of the small intestine (SI) and found that both the transferred LCMV-specific P14 CD8 T cells and the polyclonal $CD44^+$ CD8 T cells of recipient mice were robustly enriched in the GF group compared with those in the Conv group

10.3389/fimmu.2025.1519023

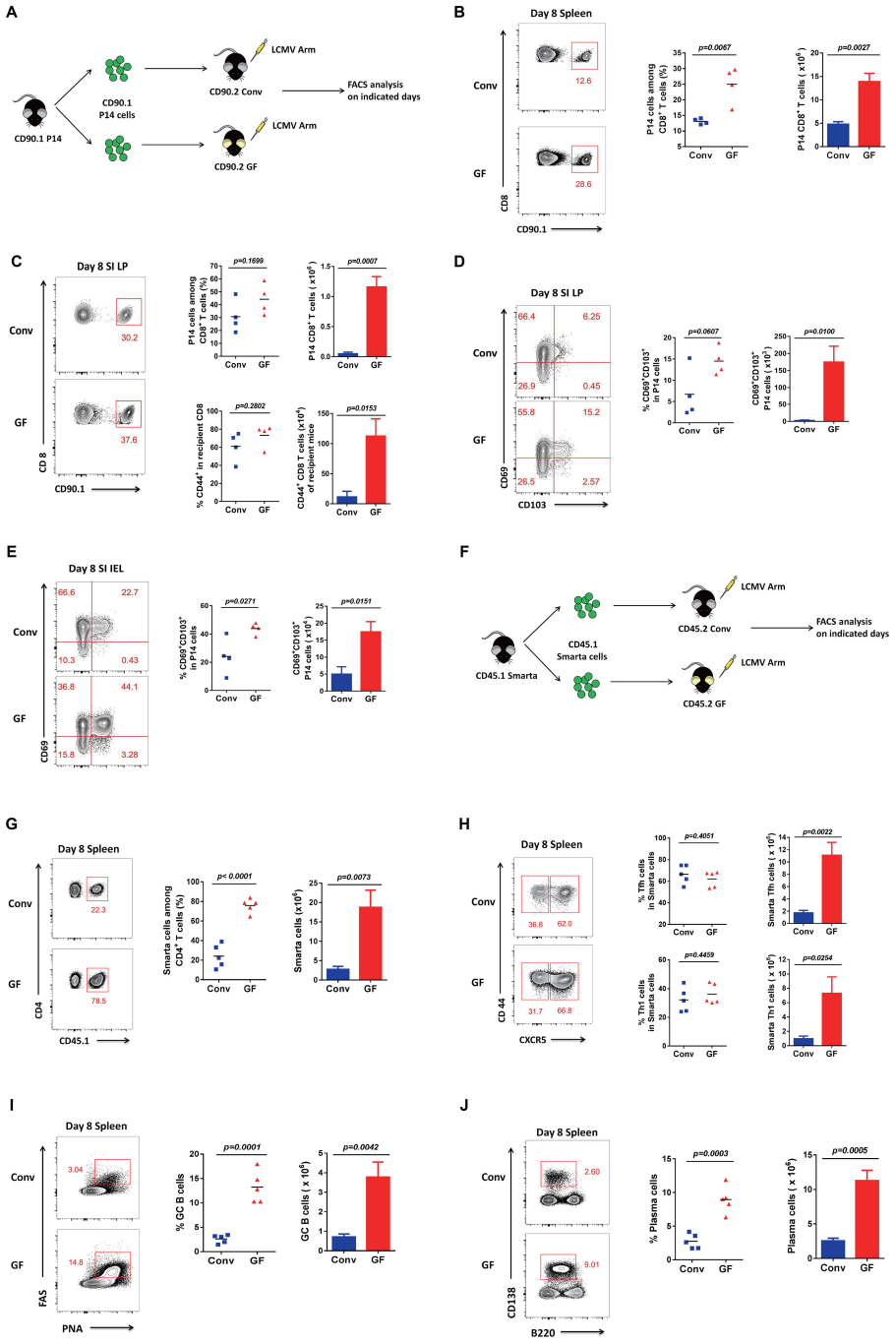


FIGURE 1
Adaptive immune responses in GF and Conv mice after LCMV infection. **(A)** Experimental design. **(B)** FACS analysis of P14 CD8 T cells among total CD8$^+$ T cells of Conv or GF mouse spleens on day 8 post-infection (p.i.). **(C)** FACS analysis of P14 cells among total CD8 T cells or total CD44$^+$CD8 T cells among non-P14 CD8 T cells of recipient mice in the SI LP of Conv or GF mice on day 8 p.i. **(D, E)** FACS analysis of CD69$^+$CD103$^+$ P14 cells (Trm) in the SI IEL or SI LP of Conv or GF mice on day 8 p.i. **(F)** Experimental design. CD45.1 SMARTA cells were adoptively transferred into CD45.2 naive Conv or GF mice following LCMV-Arm infection the next day, and splenocytes were harvested on the indicated days for analysis. **(G)** FACS analysis of CD45.1$^+$ SMARTA cells among total CD4 T cells of Conv or GF mouse spleens on day 8 p.i. **(H)** FACS analysis of CD44$^+$CXCR5$^+$ SMARTA T_{FH} cells or CD44$^+$CXCR5$^-$ SMARTA T_H1 cells in the spleen of Conv or GF mice on day 8 p.i. **(I, J)** FACS analysis of PNA^{hi}FAS^{hi}B220$^+$CD19$^+$ GC B cells **(I)** or CD138^{hi}B220^{lo} plasma cells **(J)** in the spleen of Conv or GF mice on day 8 p.i.

(Figure 1C). We also tested CD69$^+$CD103$^+$ Trm (resident memory T cell) precursors on day 8 in the LP and found that both the cell proportion and the cell number of Trm precursors were overtly increased in GF mice compared with those in Conv mice (Figure 1D). We then detected and confirmed that the Trm precursors on day 8 in the IEL also showed a robust increase in both cell proportion and cell number in GF mice compared with those in Conv mice (Figure 1E, Supplementary Figure S1).

Furthermore, to investigate CD4 T-cell responses, we sorted CD45.1 SMARTA CD4 T cells from CD45.1 SMARTA mice and

transferred the same quantity of cells into CD45.2 Conv mice or CD45.2 GF mice, followed by infection with LCMV (Figure 1F). Then, we analyzed the CD4 T-cell responses on the indicated days. Consistently, both the cell proportion and the cell number of LCMV-specific SMARTA cells were dramatically augmented in GF mice compared with those in Conv mice (Figure 1G), followed by obvious increases in both follicular helper T (Tfh) and T helper 1 (Th1) cell numbers (Figure 1H, Supplementary Figure S3). Because of the helper functions of Tfh cells for germinal center B-cell (GC B cell) maturation for antibody production, we further detected both germinal center B cells and plasma cells. Likewise, both the cell proportion and the cell number of GC B cells and plasma cells showed a robust increase in GF mice compared with those in Conv mice (Figures 1I, J, Supplementary Figure S4). These findings demonstrated that mice without a history of microbial education developed robust adaptive immune responses to novel viral infections.

Mice without a history of microbial education established potent memory CD8 T cells for long-term protection

To investigate whether mice without a history of microbial education can establish long-term protection, we first sorted CD90.1/CD45.1 P14 CD8 T cells from CD90.1/CD45.1 P14 mice and transferred the same quantity of cells into CD90.2/CD45.2 Conv mice or CD90.2/CD45.2 GF mice, followed by acute viral infection with the new pathogen LCMV Armstrong (Figure 2A). Then, we examined LCMV virus-specific P14 CD8 T cells on day 39 in the spleen and found that both the cell proportion and the cell number of P14 CD8 T cells were robustly increased in GF mice compared with those in Conv mice (Figure 2B). We further analyzed $CD127^+KLRG1^-$ memory CD8 T cells, especially $CD127^+CD62L^+$ Tcm (central memory T) cells, in P14 cells on day 75 in the spleen. Consistently, not only memory CD8 T cells but also Tcm cells increased in number in GF mice compared with those in Conv mice (Figure 2C). We then examined the polyclonal $CD127^+KLRG1^-$ memory CD8 and $CD127^+CD62L^+$ Tcm cells among $CD44^+$ CD8 T cells of the recipient mice. The results were consistent with those for the transferred P14 CD8 T cells, with augmentation of both memory CD8 and Tcm cell levels among the polyclonal $CD44^+$ CD8 T cells of the recipient GF mice (Figure 2D). As laboratory evidence from clinical patients showed greater clonal expansion of T cells with more IL-2 and IFN-γ-producing T cells in moderate disease than in severe disease for COVID-19, we further applied ICC analysis on day 57 in the spleen, testing for IL-2 and IFN-γ, for a deeper understanding of memory CD8 T-cell functions and found that GF mice showed higher IL-2 and IFN-γ levels in both cell proportion and mean fluorescence intensity (MFI) in both P14 CD8 T cells and polyclonal $CD44^+$ CD8 T cells of the recipient GF mice compared with those of Conv mice (Figures 2E, F, Supplementary Figure S2, Supplementary Table S1).

We also analyzed small intestinal lymphocytes. First, we found that both the cell proportion and the cell number of LCMV-specific P14 CD8 T cells showed a robust increase on day 39 in the IEL of GF mice compared with those of Conv mice (Figure 2G). Furthermore, we analyzed $CD69^+CD103^+$ Trm cells among P14 CD8 T cells and found that Trm cells also showed an obvious increase in both cell frequency and cell number on day 39 in the IEL in GF mice compared with those in Conv mice (Figure 2H). We then examined both LCMV-specific P14 cells and Trm cells on day 57 in the LP, and the results were consistent with those for the IEL (Figures 2I, J). We further confirmed the robust increase in both the cell frequency and the cell number of Trm cells among polyclonal $CD44^+$ CD8 T cells of the recipient GF mice (Figure 2K). Finally, to verify the phenotype, we also examined P14 cells in another tissue, the lung, with repeatable results showing a robust increase in both the frequency and the number of P14 cells on day 57 in the lungs of GF mice compared with those of conditional mice (Figure 2L). Collectively, the above results showed that mice without a history of microbial exposure established more potent memory CD8 T cells for long-term protection against viral reinfection.

Mice without a history of microbial education showed primary lower T-cell levels

Furthermore, we wanted to determine whether naive GF mice have primary lower or higher T-cell levels than Conv mice without LCMV infection. We thus found that both CD8 and CD4 T cells, including regulatory T cells (Tregs with $CD4^+FOXP3^+$), showed a lower primary cell number in naive GF mice than in Conv mice (Figures 3A–C, Supplementary Table S1).

In short, naive GF mice showed lower levels of primary T cells. This finding was consistent with previous reports (13–15). On the other hand, the above overall results indicated greater plasticity with sensitivity of the naive immune system of GF mice, as shown by the robust adaptive immune responses and immune memory established upon new viral infection, even in the condition of primary lower T cell levels for GF mice compared with that of Conv mice.

The ABX-treated mice with a history of microbial education exhibited impaired T-cell immunity to new viral infection

To investigate whether the microbiota impacts the T-cell responses in Conv mice, we treated mice with antibiotics to eliminate the microbiota (Figure 4A). Thus, we compared LCMV-specific P14 CD8 T cells and polyclonal $CD44^+$ CD8 T cells of recipients on day 8 in the spleen of the ABX-treated mice with those of normal Conv mice as a control, and we found that both P14 cells and the polyclonal CD8 T cells of the recipient ABX-treated mice showed a decreased cell number (Figure 4B). Because of the importance of small intestinal lymphocytes with large intestinal microbiota populations, we also examined P14 cells and polyclonal activated CD8 T cells on day 12 in the IEL and LP, respectively, and found that the results were consistent with those in the spleen; both P14 cells and polyclonal CD8 T cells of the recipient ABX-treated mice exhibited decreased cell numbers in both the IEL and LP (Figures 4C-F). Furthermore, we also detected polyclonal memory CD8 T cells and Trm cells for long-term protection on day 61 in the

10.3389/fimmu.2025.1519023

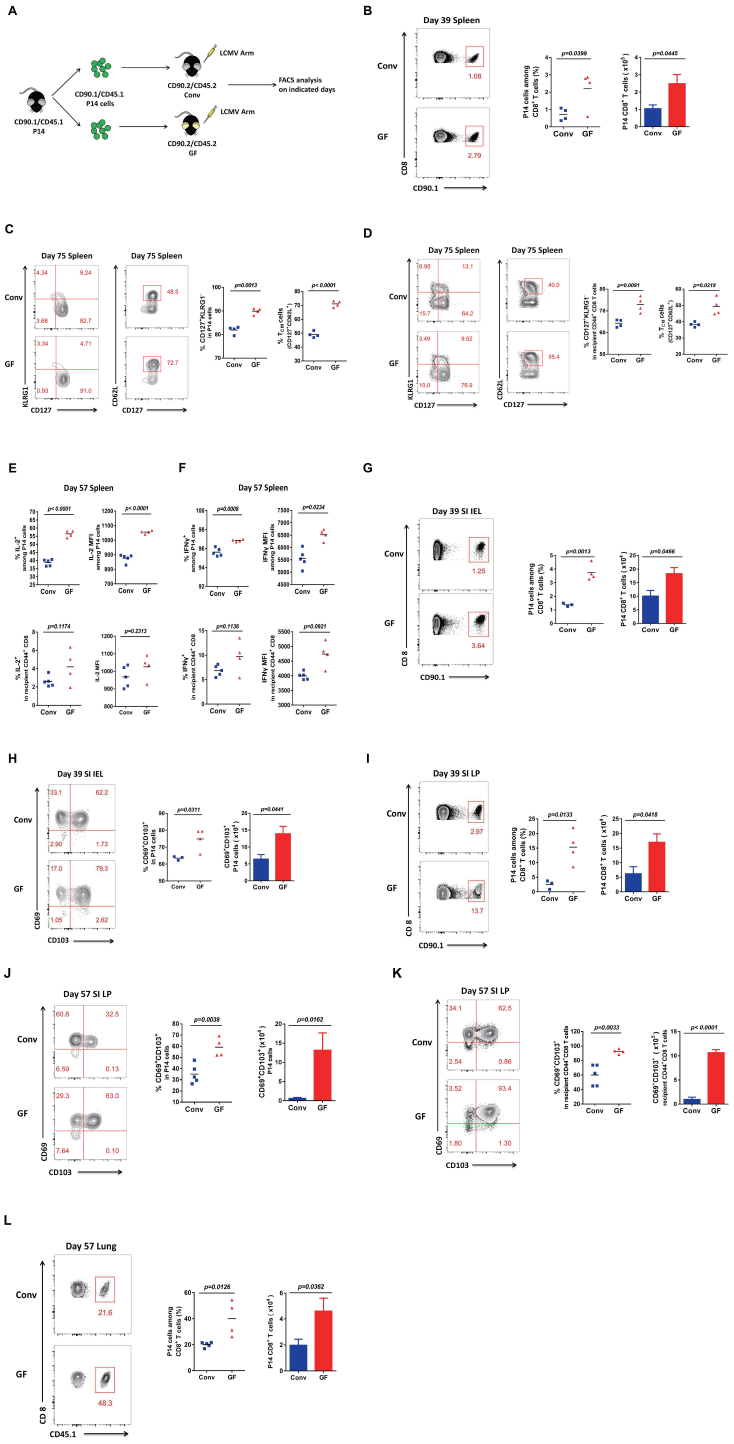


FIGURE 2
Memory CD8 T-cell responses in GF and Conv mice for long-term protection. **(A)** Experimental design. **(B)** FACS analysis of P14 cells among total CD8 T cells of Conv or GF mouse spleens on day 39 p.i. **(C, D)** FACS analysis of CD127^{+}KLRG1^{-} memory or CD127^{+}CD62L^{+} Tcm cells among P14 cells **(C)** or among CD44^{+}CD8 T cells of recipient mice **(D)** in Conv or GF mouse spleens on day 75 p.i. **(E, F)** The proportion and MFI of IL-2^{+} **(E)** or IFN-γ^{+} **(F)** cells among P14 cells or among CD44^{+}CD8 T cells of recipient Conv or GF mouse spleens on day 57 p.i. **(G, H)** FACS analysis of P14 cells **(G)** or CD69^{+}CD103^{+} Trm P14 cells **(H)** in the SI IEL of Conv or GF mice on day 39 p.i. **(I, J)** FACS analysis of P14 cells **(I)** or CD69^{+}CD103^{+} Trm P14 cells **(J)** in the SI LP of Conv or GF mice on day 39 p.i. **(I)** or on day 57 p.i. **(J)**. **(K)** FACS analysis of CD69^{+}CD103^{+} Trm cells among CD44^{+}CD8 T cells of recipient mice in the SI LP on day 57 p.i. **(L)** FACS analysis of P14 cells among total CD8 T cells of the lung on day 57 p.i.

10.3389/fimmu.2025.1519023

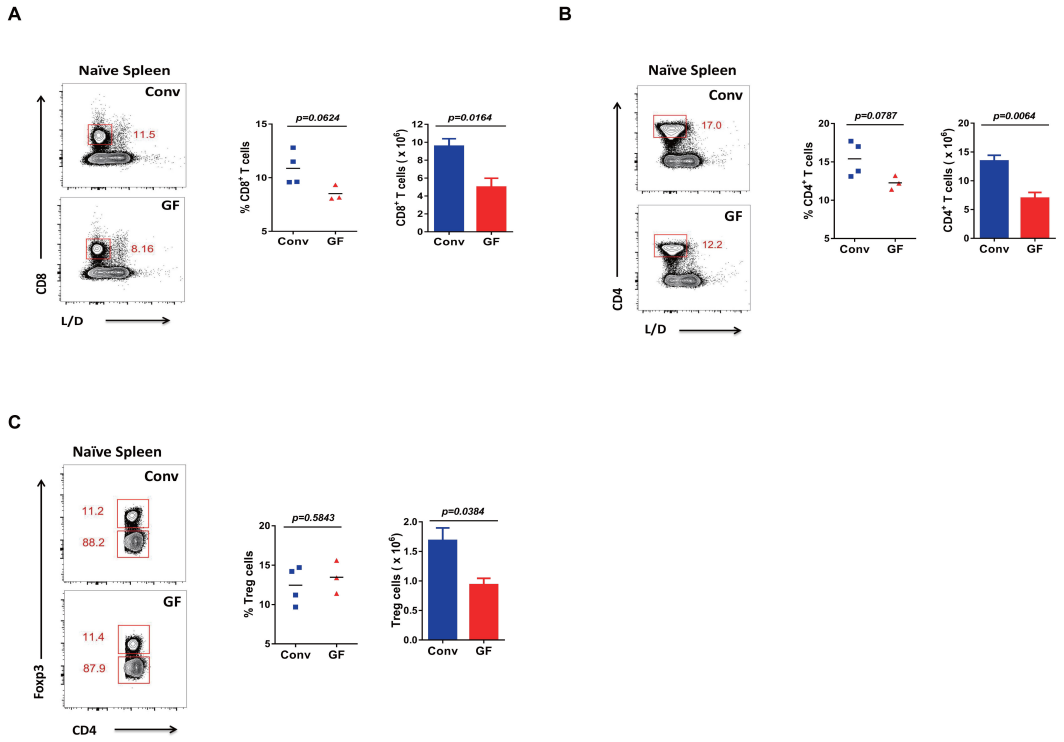


FIGURE 3
Primary T cell levels in GF and Conv mice. **(A–C)** FACS analysis of L/D$^-$CD8$^+$ T cells **(A)**, L/D$^-$CD4$^+$ T cells **(B)**, or L/D$^-$Foxp3$^+$CD4$^+$ Tregs **(C)** in the spleen of naive Conv or GF mice.

IEL and LP of the recipient ABX-treated mice and Conv mice, with all of them showing a consistently lower number in the ABX-treated mice than in control mice (Figures 4G–J, Supplementary Table S1).

In brief, with the microbiota eliminated, the ABX-treated mice did not show the same immune responses as the GF mice upon new viral infection, compared with normal Conv mice, respectively. The major difference in their immune system is that the ABX-treated mice still have a history of microbial education; in other words, their immune system is not that naive. Although naive GF mice have fewer primary T cells than Conv mice, they exhibited more robust T-cell responses after new viral infection, including an elevated cell proportion and cell function of memory CD8 T cells for long-term protection. The naive immune system of GF mice with a much abundant naive T-cell repertoire seems to have greater plasticity with sensitivity for aspects of T cells, including T-cell activation, proliferation, differentiation, and function, which is consistent in different organs.

Discussion

Exhaustion of the immune system's ability to adapt to novelty suggests that the changes it undergoes might be a consequence of an evolutionary unpredictable antigenic exposure over a lifetime. From the above, we raise the question of whether a naive immune system is able to manage new antigens better than an educated one. Here, by employing the naive immune system of GF mice, we compared the adaptive immune responses of GF mice with those of Conv mice upon LCMV infection. Surprisingly, we found that the naive immune system of GF mice shows robust T-cell responses upon new viral infection, with also more potent memory CD8 T cells established for long-term protection, even in the condition of primary lower T-cell levels for naive GF mice than for Conv ones. Furthermore, we found that the ABX-treated mice showed impaired T-cell responses, unlike GF mice, upon new viral infection. However, even with the microbiota eliminated, the ABX-treated mice still have a history of microbial exposure in their immune system; in other words, their immune system has been educated and is not that naive. In short, the commensal bacteria education history calibrates the naivety and the activation threshold of the adaptive antiviral immune system.

One explanation is that the naive immune system of GF mice also includes both DCs and Tregs that play important roles in T-cell outcomes upon infection. As newly evidenced in the COVID-19 pandemic, the interplay between dendritic cells and CD8 T lymphocytes is a crucial component of SARS-CoV-2 infection immunity (16–18). Moreover, along with the expansion of the memory T-cell pool with continual pathogen exposure, the cell proportion of naive T cells decreases. Under these circumstances, there would be much lower efficiency for DCs to present antigens to the fittest naive T cells. From this perspective, the naive GF immune system with a more abundant naive T-cell repertoire shows greater plasticity and sensitivity to launch more potent T-cell responses upon encountering new viral infection, which at least from one standpoint explains the immune system naivety-related differences in adapting to novelty.

10.3389/fimmu.2025.1519023

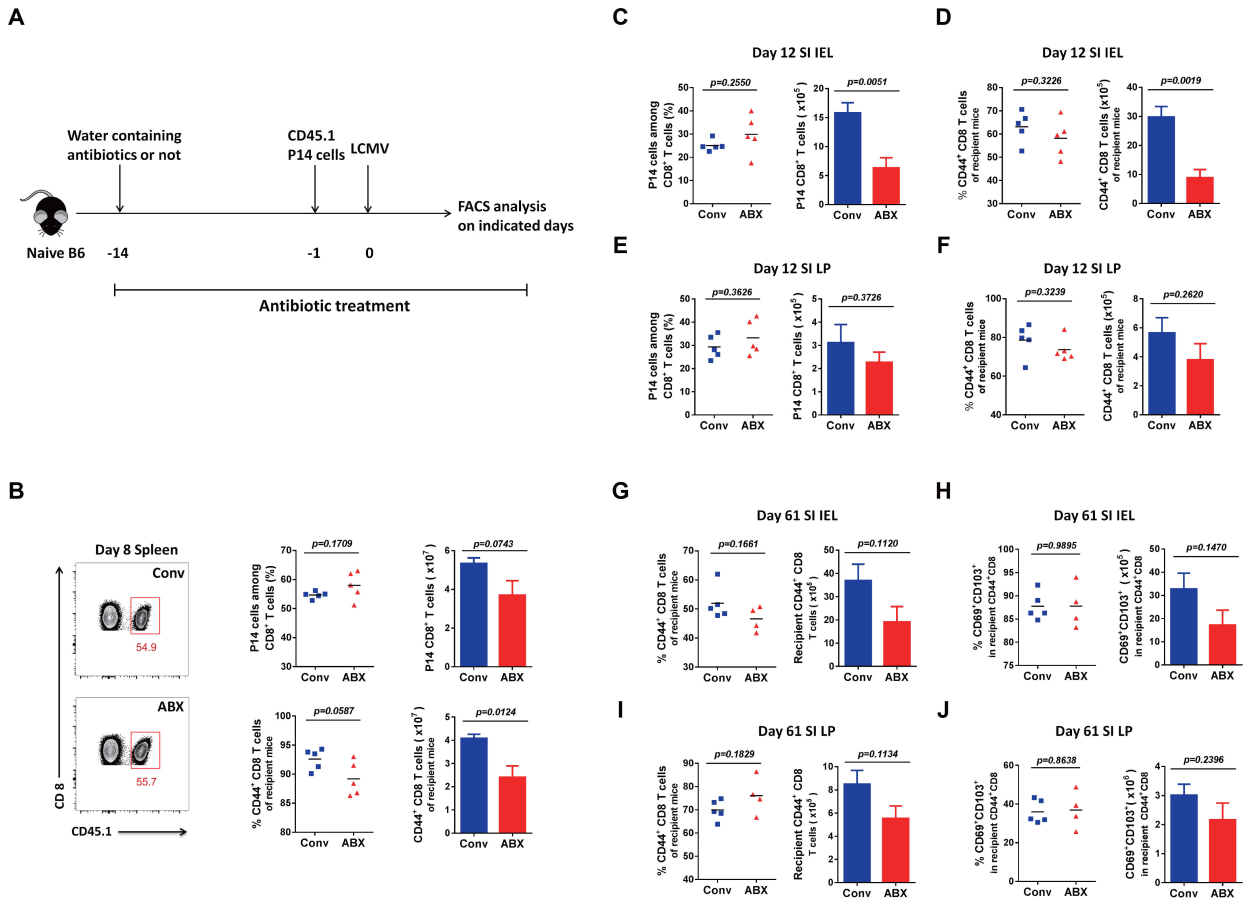


FIGURE 4
T-cell immunity to LCMV infection in ABX-treated mice and Conv mice. **(A)** Diagram of antibiotic treatment. **(B)** FACS analysis of P14 cells among total CD8 T cells or CD44$^+$CD8 T cells among the non-P14 CD8 T cells of recipient mice in the spleen of Conv or ABX-treated mice on day 8 p.i. **(C–F)** FACS analysis of P14 cells among total CD8 T cells or CD44$^+$CD8 T cells among the non-P14 CD8 T cells of recipient mice in the SI IEL **(C, D)** or SI LP **(E, F)** of Conv or ABX-treated mice on day 12 p.i. **(G–J)** FACS analysis of CD44$^+$CD8 total memory T cells or CD69$^+$CD103$^+$ Trm cells among CD44$^+$CD8 T cells of recipient mice in the SI IEL **(G, H)** or SI LP **(I, J)** of Conv or ABX-treated mice on day 61 p.i.

On the one hand, there are two major classes of Tregs in Conv mice: thymus-derived natural Tregs (nTregs) and intestinally induced Tregs (iTregs) (19). Importantly, iTregs have antigen-specific TCRs for microbiota *in-situ* tolerization, which are different from those of nTregs, and the adoptive transfer of iTregs has been found to affect neither the primary antiviral CD8 T-cell responses nor the viral clearance (20). Moreover, LCMV Armstrong acute infection barely induces iTregs (21). As a result, the T-cell responses that are systemically stronger in the intestines, spleens, and lungs of GF mice than in those of Conv mice are supposedly not due to the effects of Tregs.

Based on the above, we have a suggested perspective on the use of the GF mouse system in intestinal microbiota studies. Compared to GF mice, it would be more appropriate to use ABX-treated mice for research on the impact of intestinal microbiota disruption on immune disturbance. GF mice do not have the same immune starting point as Conv mice. Consistent with previous reports, naive GF mice had primary lower T-cell levels in our study (14), and importantly, their immune system did not have the same history of microbial exposure and education as that of the Conv mice. Regarding humans, there are patients with antibiotic-disrupted microbiota, but there are no GF patients. On the other hand, the GF mouse system is assumed to be more suitable for research on the impact of microbiota colonization on intestinal immune system development.

Materials and methods

Mice and virus infection

P14 (CD90.1 or CD45.1) and SMARTA (CD45.1) TCR transgenic mice were obtained from Dr. Rafi Ahmed (Emory University). C57BL/6J (CD45.2) mice were purchased from the Jackson Laboratories. Germ-free mice were provided by Dr. Hong Wei and the Animal Facility of Third Military Medical University, and these GF mice were bred in sterile plastic isolators with autoclaved food and water. All handling procedures for GF mice, including LCMV infection, were carried out under sterile conditions. All mice analyzed were 6–8 weeks of age, and both

male and female mice were included without randomization or blinding. LCMV Armstrong was provided by Dr. Rafi Ahmed, and 2 × 10^5 PFU (i.v.) were used to establish acute infection in mice. Mice infected with LCMV were housed in accordance with Institutional Biosafety Regulations of Third Military Medical University. All experimental protocols were approved by the Institutional Animal Care and Use Committees of the Third Military Medical University. All methods were carried out in accordance with relevant guidelines and regulations that were approved by the Ethics Committees of the Third Military Medical University. This study was carried out in compliance with the ARRIVE guidelines.

Adoptive cell transfer

In each individual experiment, a total of 2 × 10^4 naive CD45.1$^+$ (or CD90.1$^+$) P14 cells or SMARTA cells were adoptively transferred into naive wild-type CD45.2$^+$ (or CD90.2$^+$) mice, and then these recipient mice were infected with 2 × 10^5 PFU LCMV Armstrong (i.v.) the next day.

Lymphocyte isolation from the small intestine and lung

For the isolation of SI IELs, the small intestine and Peyer's patches were removed, and the intestine was cut longitudinally into 0.5 cm pieces. The intestine pieces were incubated with 0.154 mg/mL of dithioerythritol (DTE) in 10% HBSS/HEPES bicarbonate (30 min at 37°C, 250 rpm) to extract the IEL. For lamina propria lymphocyte (LPL) isolation, gut pieces were further treated with 100 U/mL of type I collagenase (Worthington, Lakewood, USA) in RPMI 1640, 5% FBS, 2 mM of $MgCl_2$, and 2 mM of $CaCl_2$ for 2 h at 37°C, stirring at 250 rpm. For the isolation of lung lymphocytes, the lung was removed and cut into small pieces, and the pieces were incubated with 1.3 mM of EDTA in HBSS (30 min at 37°C, 250 rpm), followed by treatment with 100 U/mL of type I collagenase (Worthington) in RPMI 1640, 5% FBS, 2 mM of $MgCl_2$, and 2 mM of $CaCl_2$ (1 h at 37°C, 250 rpm). After enzymatic treatment, lymphocytes were then purified on a 44/67% Percoll gradient (800*g* at 23°C for 20 min).

Flow cytometry and antibodies

Flow cytometry data were acquired by FACSCantoII (BD Biosciences, Franklin Lakes, USA) and analyzed with the FlowJo software (Tree Star, Ashland, USA). The antibodies and reagents used for flow cytometry staining are listed in Supplementary Table S1. Surface staining was performed in PBS containing 2% BSA or FBS (wt/vol). For CXCR5 staining, cells were stained with purified anti-CXCR5 (BD Biosciences) for 1 h at 4°C, followed by biotinylated antirat immunoglobulin G (IgG) (Jackson ImmunoResearch, West Grove, USA) and then fluorescently labeled streptavidin (eBioscience, San Diego, California) for 30 min on ice. Foxp3 staining was performed with the Foxp3/Transcription Factor Staining Buffer Set (eBioscience) after surface staining. For detection of cytokine production, splenocytes were first stimulated with the indicated peptide (0.2 μg/mL), GolgiPlug, GolgiStop, anti-CD107a, and anti-CD107b antibodies (BD Biosciences) at 37°C for 5 h. Following surface staining, intracellular cytokine staining was performed with a Cytofix/Cytoperm Fixation/Permeabilization Kit according to the manufacturer's instructions.

Antibiotic treatment

Mice were provided with autoclaved drinking water supplemented with ampicillin (0.5 mg/mL, Solarbio, Beijing, China), gentamicin (0.5 mg/mL, Solarbio), metronidazole (0.5 mg/mL, Solarbio), neomycin (0.5 mg/mL, Solarbio), and vancomycin (0.25 mg/mL, Solarbio). Antibiotic treatment was started 2 weeks before infection and continued for the duration of the experiments.

Statistical analysis

Statistical analysis was conducted using Prism 6.0 (GraphPad, San Diego, USA). The unpaired two-tailed *t*-test with 95% confidence interval was used for the calculation of *P*-values.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The animal study was approved by the Institutional Biosafety Regulations of the Third Military Medical University. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

YYZ: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. BL: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Validation, Writing – original draft. ZS: Investigation, Validation, Writing – original draft. LM: Software, Writing – original draft. FZ: Software, Validation, Writing – review & editing. LJ: Writing – review & editing. YZ: Writing – review & editing. YG: Writing – review & editing. GD: Writing – review & editing. HBW: Writing – review & editing. HW: Writing – review & editing. YW: Supervision, Visualization, Writing – review & editing. LY: Writing – review & editing. CL: Writing – review & editing. JL: Data curation, Investigation, Methodology, Validation, Writing – original draft.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by grants from the National Natural Science Foundation of China (82471793 to YYZ), Chongqing Talent Program (CQYC2021059730 to YYZ), Chongqing Talent Innovation Program (CX2022081 to YYZ), Senior Medical Talents Program of Chongqing for Young and Middle-aged (YXGD202448 to YYZ), National Natural Science Foundation of China (32300737 to BL), and Natural Science Foundation of Tibet Autonomous Region (XZ202401ZR0059 to BL).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2025.1519023/full#supplementary-material.

References

1. Simon AK, Hollander GA, McMichael A. Evolution of the immune system in humans from infancy to old age. *Proc Royal Soc B: Biological Sci* (2015) 282 (1821):20143085. doi: 10.1098/rspb.2014.3085

2. Hon KL, Leung CW, Cheng WTF, Chan PKS, Chu WCW, Kwan YW, et al. Clinical presentations and outcome of severe acute respiratory syndrome in children. *Lancet*. (2003) 361(9370):1701–3. doi: 10.1016/S0140-6736(03)13364-8

3. Morens DM, Fauci AS. The 1918 influenza pandemic: lessons for 2009 and the future. *Crit Care Med*. (2010) 38(7):1018–28. doi: 10.1097/CCM.0b013e3181ceb25b

4. Li G, Li S, Qi F, Mei G. Mild encephalitis/encephalopathy in children with a reversible. *splenial lesion. Radiol Infect Dis*. (2018) 5:118–22. doi: 10.1016/j.jrid.2018.08.005

5. Shehata MM, Gomaa MR, Ali MA, Kayali G. Middle East respiratory syndrome coronavirus: a comprehensive review. *Front Med*. (2016) 10:120–36. doi: 10.1007/s11684-016-0430-6

6. Alfaraj S. H., Al-Tawfiq J. A., Altuwaijri T. A., Memish Z. A.. Middle East respiratory syndrome coronavirus in pediatrics: a report of seven cases from Saudi Arabia. *Front Med*. (2019) 13(1):126–30. doi: 10.1007/s11684-017-0603-y

7. Han B, Song Y, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy children and adolescents: a double-blind, randomised, controlled, phase 1/2 clinical trial. *Lancet Infect Dis*. (2021) 21:1645–53. doi: 10.1016/S1473-3099(21)00319-4

8. Lefebvre JS, Lorenzo EC, Masters AR, Hopkins JW, Eaton SM, Smiley ST, et al. Vaccine efficacy and T helper cell differentiation change with aging. *Oncotarget*. (2016) 7(23):33581–94. doi: 10.18632/oncotarget.v7i23

9. Fialkowski A, Gernez Y, Arya P, Weinacht KG, Kinane TB, Yonker LM. Insight into the pediatric and adult dichotomy of COVID-19: Age-related differences in the immune response to SARS-CoV-2 infection. *Pediatr Pulmonology*. (2020) 55(10):2556–64. doi: 10.1002/ppul.v55.10

10. Chen Z, John Wherry E. T cell responses in patients with COVID-19. *Nat Rev Immunol*. (2020) 20:529–36. doi: 10.1038/s41577-020-0402-6

11. Fousteri G, Dave Jhatakia A. Viral infections and autoimmune disease: roles of LCMV in delineating mechanisms of immune tolerance. *Viruses*. (2019) 11:885. doi: 10.3390/v11100885

12. Round JL, Mazmanian SK. The gut microbiota shapes intestinal immune responses during health and disease. *Nat Rev Immunol*. (2009) 9:313–23. doi: 10.1038/nri2515

13. Gensollen T, Iyer SS, Kasper DL, Blumberg RS. How colonization by microbiota in early life shapes the immune system. *Sci*. (2016) 352(6285):539–44. doi: 10.1126/science.aad9378

14. Kennedy EA, King KY, Baldridge MT. Mouse microbiota models: comparing germ-free mice and antibiotics treatment as tools for modifying gut bacteria. *Front Physiol*. (2018) 9:1534. doi: 10.3389/fphys.2018.01534

15. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. *Cell*. (2014) 157:121–41. doi: 10.1016/j.cell.2014.03.011

16. Buttenschön J, Mattner J. The interplay between dendritic cells and CD8 T lymphocytes is a crucial component of SARS-CoV-2 immunity. *Cell Mol Immunol*. (2021) 18:247–9. doi: 10.1038/s41423-020-00624-1

17. Zhou R, To KK-W, Wong Y-C, Liu L, Zhou B, Li X, et al. Acute SARS-coV-2 infection impairs dendritic cell and T cell responses. *Immunity*. (2020) 53:864–877.e5. doi: 10.1016/j.immuni.2020.07.026

18. Zhang Y, Li B, Bai Q, Wang P, Wei G, Li Z, et al. The lncRNA Snhg1-Vps13D vesicle trafficking system promotes memory CD8 T cell establishment via regulating the dual effects of IL-7 signaling. *Signal Transduction Targeted Ther*. (2021) 6(1):126. doi: 10.1038/s41392-021-00492-9

19. Brown EM, Kenny DJ, Xavier RJ. Gut microbiota regulation of T cells during inflammation and autoimmunity. *Annu Rev Immunol*. (2019) 37:599–624. doi: 10.1146/annurev-immunol-042718-041841

20. Baca Jones C, Pagni PP, Fousteri G, Sachithanantham S, Dave A, Rodriguez-Calvo T, et al. Regulatory T cells control diabetes without compromising acute anti-viral defense. *Clin Immunol*. (2014) 153(2):298–307. doi: 10.1016/j.clim.2014.05.006

21. Punkosdy GA, Blain M, Glass DD, Lozano MM, O'Mara L, Dudley JP, et al. Regulatory T-cell expansion during chronic viral infection is dependent on endogenous retroviral superantigens. *Proc Natl Acad Sci*. (2011) 108(9):3677–82. doi: 10.1073/pnas.1100213108

frontiers | Frontiers in Immunology

TYPE Original Research
PUBLISHED 18 February 2025
DOI 10.3389/fimmu.2025.1549554

Check for updates

OPEN ACCESS

EDITED BY
Nar Singh Chauhan,
Maharshi Dayanand University, India

REVIEWED BY
Jiezuan Yang,
Zhejiang University, China
Avinash Lomash,
Medanta The Medicity Hospital, India

*CORRESPONDENCE
Zhijian Gu
guzhijian@shutcm.edu.cn
Jun Cong
jencongcong@hotmail.com
Jianghong Ling
ljh18817424778@163.com

†These authors have contributed equally to this work and share first authorship

RECEIVED 21 December 2024
ACCEPTED 29 January 2025
PUBLISHED 18 February 2025

CITATION
Liu X, Yang K, Jia Y, Yeertai Y, Wu C, Wang X, Jia Q, Gu Z, Cong J and Ling J (2025) Chaihushugan powder regulates the gut microbiota to alleviate mitochondrial oxidative stress in the gastric tissues of rats with functional dyspepsia.
Front. Immunol. 16:1549554.
doi: 10.3389/fimmu.2025.1549554

COPYRIGHT
© 2025 Liu, Yang, Jia, Yeertai, Wu, Wang, Jia, Gu, Cong and Ling. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Chaihushugan powder regulates the gut microbiota to alleviate mitochondrial oxidative stress in the gastric tissues of rats with functional dyspepsia

Xuejiao Liu[1†], Keming Yang[2†], Yuebo Jia[1†], Yeliya Yeertai[1], Chenheng Wu[1], Xiangxiang Wang[3], Qingling Jia[1], Zhijian Gu[1*], Jun Cong[1*] and Jianghong Ling[1*]

[1]Department of Gastroenterology, Shuguang Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, China, [2]Department of Gastroenterology, Shenzhen Traditional Chinese Medicine Hospital, Shenzhen, Guangdong, China, [3]School of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, China

Introduction: Functional dyspepsia (FD) is a prevalent functional gastrointestinal disorder associated with oxidative stress (OS) and dysbiosis. Chaihushugan powder (CHSGP) demonstrates efficacy in treating FD; however, the underlying therapeutic mechanism is not yet elucidated. This study aims to investigate the effects of CHSGP on OS and gut microbiota (GM) in FD rats, with a particular emphasis on the role of GM as a potential target for the antioxidant properties of CHSGP.

Methods: The FD rat model was established with a modified tail-clamp stimulation and the administration of the CHSGP decoction at a dosage of 9.6 g/kg via gavage for a duration of 4 weeks. The GM was depleted by the administration of a cocktail of metronidazole (200 mg/kg), ampicillin (200 mg/kg), neomycin sulfate (200 mg/kg), and vancomycin (100 mg/kg). Fecal microbiota transplantation (FMT) was performed with CHSGP-treated fecal supernatant at a dosage of 10 mL/kg. The gastrointestinal motility was measured using the rates of gastric emptying and small intestine propulsion. Hematoxylin and eosin staining was employed to elucidate the pathological changes, while the transmission electron microscope was used to examine the microstructures of the interstitial cells of Cajal (ICC). Chemiluminescence, colorimetric assay, immunofluorescence co-staining, and western blot assay were employed to identify the OS-related markers (ROS, SOD, NOX4, PRDX1, and TRX2). Sequencing of fecal microbiota was performed utilizing 16S rDNA.

Results: The CHSGP decoction promoted gastrointestinal motility, protected the microstructure of ICC, and reduced OS in FD rats. The GM composition was also regulated by CHSGP. However, these effects disappeared after microbiota depletion. Fortunately, the FMT therapy reinstated them.

Conclusion: Chaihushugan powder decoction might regulate the GM to alleviate mitochondrial OS in the gastric tissues of FD rats.

KEYWORDS

functional dyspepsia, Chinese medicine, gastric dynamics, gut microbiota, fecal microbiota transplantation, oxidative stress

1 Introduction

Functional dyspepsia (FD) is the clinical syndrome characterized by post-meal fullness, premature satiety, epigastric discomfort, or burning. No identifiable structural cause has been found to explain these symptoms (1). According to the Rome criteria, the global prevalence of undiagnosed dyspepsia ranges from 6.9% to 62.8% (2). Patients with FD often suffer from psychological co-morbidity, somatization, low quality of life, and increased financial burdens, alongside an increased burden on social healthcare resources (2). Primary treatments for FD include prokinetic drugs, proton pump inhibitors, *Helicobacter pylori* eradication, neuromodulators, psychotherapy, and probiotics (3). However, treatment outcomes have been unsatisfactory, and patients often experience relapses. Increasing amount of clinical and basic research has shown that traditional Chinese medicine (TCM) is effective for FD (4, 5).

The exact pathogenesis of FD is incompletely understood. Researches have shown that oxidative stress (OS) and gut microbiota (GM) dysbiosis significantly contribute to the pathogenic mechanisms of FD (6, 7). OS refers to the disruption in cellular redox signaling pathways caused by increased concentration of reactive oxygen species (ROS) that exceeds the capability of antioxidant defenses (8). These reactive species cause structural modifications in lipids, proteins, and DNA, potentially resulting in cellular damage (8). The resulting cellular or tissue injury, along with the activation of apoptotic signaling pathways, can subsequently trigger inflammation and contribute to the development of gastrointestinal (GI) mucosal diseases (9). Our previous basic studies confirmed that mitochondrial OS in interstitial cells of Cajal (ICC) caused GI motility disorders in FD (10, 11). On the other hand, gut dysbiosis can lead to impaired duodenal barrier function and low-grade inflammation, which are key mechanisms in the pathogenesis of FD (12). Our prior clinical studies have shown that the diversity and richness of the GM are lower in patients with FD compared to healthy individuals (4). It is worth noting that gut dysbiosis has been recognized as a factor that may affect OS (13). Certain bacteria generate formyl peptides that induce epithelial cell inflammation and elevate intracellular ROS levels (14). Meanwhile, research has demonstrated that probiotics have antioxidative properties and help reduce OS damage (15). Consequently, the pathogenic mechanism of FD may be linked to OS resulting from GM dysregulation. Furthermore, research have demonstrated that drugs exert their effects through GM (16). Numerous studies suggested that the effectiveness of herbal treatments arise from their capacity to influence GM and metabolites (17, 18).

FD is known as "Pi Man" in TCM, with liver qi stagnation identified as the principal reason. TCM theory thinks that Qi is a subtle substance continuously circulating within the human body, playing a vital role in enhancing the functional activities of organs. The function of the liver is to convey and disperse qi and promote its circulation. Consequently, the FD treatment rule is to alleviate liver qi stagnation. Chaihushugan powder (CHSGP) originates from the Ming dynasty, as documented in Jingyue's Complete Works. It is distinguished remedy that specifically targets liver qi imbalances. This formula has historical significance and has been widely used to manage digestive disorders. It consists of seven traditional plants: *Cyperus rotundus* L. [Cyperaceae], *Conioselinum anthriscoides 'Chuanxiong'* [Apiaceae], *Bupleurum falcatum* L. [Apiaceae], *Paeonia lactiflora Pall.* [Paeoniaceae], *Citrus* × *aurantium* L. [Rutaceae], *Citrus aurantium f. deliciosa* (Ten.). M. Hiroe [Rutaceae], and *Glycyrrhiza glabra* L. [Fabaceae]. Our preliminary clinical research indicated that CHSGP had considerable effectiveness in the management of FD (4). It effectively improved the dyspepsia symptom scale scores, enhanced the rate of barium strip gastric emptying, and increased the scores of hamilton anxiety scale, hamilton depression scale, and quality of life (4). Additionally, it modulated the composition of GM (4). In fundamental research, CHSGP treatment reduced mitochondrial ROS and serum malondialdehyde (MDA) levels, elevated citrate synthase (CS) and superoxide dismutase (SOD) levels in the gastric tissue, and improved the mitochondrial membrane potential (10, 11). These findings indicated that CHSGP treatment might ameliorate mitochondrial OS and modulate GM in FD rats. However, it remains unclear whether GM is the core target for CHSGP to exert its antioxidant effects. The use of antibiotic cocktails (ABX) significantly reduced the diversity of GM, subsequently impacting cell counts, signaling pathways, and organ functions, with the results resembling those in germ-free animals (19). Consequently, pseudo-sterile animals can be developed utilizing ABX for studies linked to GM depletion (20). Fecal microbiota transplantation (FMT) involves the transfer of healthy microbiota to dysbiotic host to restore beneficial GM and achieve eubiosis (21). FMT can establish the donor microbiota after microbiota depletion using ABX (22). In the majority of TCM research protocols, animals administered herbs or herbal substances for a duration are chosen as FMT donors to explore GM-dependent effects of herbal treatments (18, 23). In this study, we employed ABX and FMT to investigate whether CHSGP treatment

could improve mitochondrial OS in the gastric tissues of FD rats by influencing GM.

2 Methods and materials

2.1 Animals and establishment of the FD rat model

The sample size for the study was established via the KISS approach, as referenced in Festing's research (24). Power study indicated that a sample size of six rats per group achieved 90% power to identify a 20% change, with a 5% significance level and a two-sided test. Seventy-two male Sprague-Dawley rats, each weighing between 180 and 200 g, were obtained from Shanghai SLAC Animal Biotechnology Co. Ltd. (2017-0005, Shanghai, China). The rats were housed in a specific pathogen-free controlled environment with humidity levels regulated at 55% ± 2%, temperature set at 22°C ± 2°C, and 12-h light/dark cycle. They were offered the unlimited supply of food and water. After a week of adaptation, FD was induced by administering the tail-clamp stimulation for 30 min, twice daily, for 4 weeks (11). Clinical studies found that patients with FD predominantly exhibited the decrease in gastric emptying rate (4) and showed no organic causes upon upper endoscopy (25). Consequently, experimental research utilized the reduction in 24-hour GI transit rate and hematoxylin eosin (HE) staining of gastric tissue, which revealed no pathological changes such as erosion or ulcers, as criteria for FD model establishment (10, 11). The Ethics Committee of the Shanghai University of TCM approved this study on March 17, 2023 (Ethics No. PZSHUTCM2303240005). All animal experimental procedures followed the experimental animal management regulations of the Shanghai University of TCM.

2.2 CHSGP preparation and pathway prediction for therapeutic efficacy

CHSGP decoction was composed of the following herbs: 12 g each of *Citrus aurantium f. deliciosa* (Ten.). M. Hiroe [Rutaceae] (20221225, Zhejiang, China) and *Bupleurum falcatum* L. [Apiaceae] (2022121508, Gansu, China); 9 g of *Citrus aurantium* L. [Rutaceae] (20210915, Jiangxi, China), *Ligusticum chuanxiong* [Apiaceae] (2023021907, Sichuan, China), *Paeonia lactiflora Pall.* [Paeoniaceae] (2022121908, Anhui, China), and *Cyperus rotundus* L. [Cyperaceae] (2022102308, Shandong, China); and 3 g of *Glycyrrhiza glabra* L. [Fabaceae] (2023022307, Xinjiang, China) (11). CHSGP decoction was heated in a 37°C water bath and administered to rats via gavage at a dosage of 9.6 g/kg, as previously established (26). We employed ultraperformance liquid chromatography-tandem quadrupole mass spectrometer (UPLC-MS/MS) to analyze and identify CHSGP compounds, subsequently utilizing the CTD and swisstarget databases to determine the action targets of these compounds. Finally, we enriched the cellular components, molecular functions, biological processes, and Kyoto Encyclopedia of Genes and Genomes (KEGG) (KEGG)-related pathways associated with CHSGP using Gene Ontology and the KEGG databases. Relevant results can be found in Supplementary Tables 1-6.

2.3 Probiotics preparation

The probiotics tablets used were *Bifidobacterium* quadruple viable bacteria tablets (KDJ3YSP, Hangzhou Grand Biologic Pharmaceutical Inc., Hangzhou, China) that were composed of *Bifidobacterium*, *Lactobacillus*, *Enterococcus*, and *Bacillus*. Probiotics were administered at a dosage of 0.945 g/kg following the protocols (27).

2.4 Antibiotic cocktail preparation

Consistent with previous studies (28), a combination of four antibiotics, namely metronidazole at 200 mg/kg, ampicillin at 200 mg/kg, vancomycin at 100 mg/kg, and neomycin sulfate at 200 mg/kg, was used to deplete GM. The antibiotics were obtained from Solarbio Science & Technology Co., Ltd. (Beijing, China). The dosage administered was 1 mL per 100 g of body weight.

2.5 Fecal microbiota transplantation

We massaged the abdominal region of rats in the FD group treated with CHSGP (FD + CHSGP) to induce defecation and collected the fecal samples using aseptic techniques. Fecal samples were mixed with PBS at a 1:10 ratio, homogenized for 10 min, and centrifuged at 6000 rpm for 15 min. The supernatant was collected and stored at 4°C until transplantation. The administered dosage was 1 ml per 100 g of body mass.

2.6 Experimental procedures

We conducted three experiments that used the following protocol:

I. To evaluate the influence of CHSGP decoction on the mitochondrial OS and GM in FD rats, after a week of adaptation, 24 rats were grouped using the randomized block design. The rats were first categorized by body weight, followed by the generation of random numbers through SPSS software to assign them randomly into four groups (n=6): the control group (control), the FD model group (FD), the FD + CHSGP group, and the FD group treated with probiotics (FD + probiotics). FD was induced in all groups except for the control group. FD, FD + CHSGP, and FD + probiotics group rats were administered oral saline, CHSGP decoction, and the probiotics solution, respectively. The dosage was 1.0 mL/100 g given twice daily for 4 weeks. The rats were euthanized under anesthesia on day 29, and samples were collected. The detailed procedure is illustrated in Supplementary Figure S1A.

II. To investigate the potential impact of GM on the antioxidant properties of CHSGP, 24 rats were allocated into four groups using the randomized block design (n=6): the control group (control), the group treated with ABX, the group with an ABX-induced FD model [ABX (FD)], and the group with ABX-induced FD treated with

CHSGP [ABX (FD + CHSGP)]. After a week of adaptation, the antibiotic cocktail was administered daily via gavage to the ABX, ABX (FD), and ABX (FD + CHSGP) groups for 7 days to induce the pseudo-germ-free state. Subsequently, the ABX (FD) and ABX (FD + CHSGP) groups received saline or the CHSGP decoction via gavage twice daily for 4 weeks concurrent with the tail-clamp simulation. In contrast, we administered saline to the control and ABX groups. On day 36, the rats were euthanized under anesthesia, and the samples were collected. The detailed experimental procedure is illustrated in Supplementary Figure S1B.

III. FMT study was conducted to assess the antioxidant properties of CHSGP mediated by GM. Utilizing the randomized block design, 24 rats were divided into four groups (n=6): a control group (control), a group with an FD model (FD), a group with an FD model subjected to ABX-disposed FMT treatment (ABX (FD + FMT)), and a group with an FD model treated with FMT (FD + FMT). From days 1 to 7, the ABX (FD+FMT) group received the ABX intragastrically once a day. From days 8 to 35, the FD and ABX (FD + FMT) groups were administered saline and the CHSGP fecal bacterial solution, respectively, via gavage twice daily, following the 4 weeks of tail-clamp modeling. We administered saline to the control group. The rats were euthanized under anesthesia on day 36, and samples were collected. The detailed procedure is illustrated in Supplementary Figure S1C. The timeline for the experimental procedures I-III is illustrated in Supplementary Figure S2.

2.7 Gastric emptying and intestinal propulsion rate measurements

Semi-solid paste with carbon powder was prepared based on the prior study (10). After the final administration, the rats underwent the fasting duration of 12 h. After 30 min, we anesthetized the rats and opened their abdomens. The stomach and small intestine were excised, and the total weight of the stomach was recorded. The stomach contents were extracted, and the weights of the empty stomachs were recorded. The maximum distance traveled by the black semi-solid paste within the small intestine and the overall length of the small intestine were measured. The gastric emptying rate and the small intestinal propulsion rate were calculated using the following formulas (10): Gastric emptying rate (%) = [1 − (total stomach weight − empty stomach weight)/weight of semi-solid paste] × 100%, and small bowel propulsion rate (%) = furthest distance of carbon powder/total length of small bowel × 100%.

2.8 Histopathology

The gastric tissue samples, measuring 3 mm × 3 mm, were preserved in the 4% paraformaldehyde solution. They were dehydrated, coated with paraffin, and sliced into thin sections approximately 4–5 μm thick. After the dewaxing process, the gastric tissue was subjected to hematoxylin and eosin (H&E) staining, dehydrated, penetrated, and encapsulated with neutral resin. The optical microscope (Cx31rtsf, Olympus, China) was employed to examine pathological changes in stomach tissue.

2.9 Transmission electron microscopy

The gastric tissues (1 mm^3) were fixed in the 2.5% glutaraldehyde solution. Ultra-thin sections of 0.5 mm × 0.3 mm were made after rinsing, fixing, rinsing, dehydration, osmosis, and embedding. The morphological features of the ICC were observed after staining using the transmission electron microscope (TEM) (FEI Tecnai G2 Spirit, Czech Republic), emphasizing mitochondrial swelling and vacuoles.

2.10 Chemiluminescence

The gastric mitochondria was extracted using the mitochondrial extraction kit (SM0020, Solarbio, China). The mitochondrial supernatant was incubated with a 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) working solution or PBS solution at 37°C for 30 min according to the instructions of the ROS detection kit (E004-1-1, NJJCBIO, China). The wavelength of the enzyme-labeled instrument (SynergyLX, BioTek, Norcross, GA, USA) was set, and the levels of ROS were measured.

2.11 Colorimetric assay

The mitochondrial extraction kit (SM0020, Solarbio, China) was utilized to isolate the stomach mitochondria. The mitochondrial supernatant protein was measured using the bicinchoninic acid assay (BCA) kit (P0010S, Beyotime, China). We prepared the substrate application solutions and enzyme working solutions following the instructions provided in the SOD assay kit (A001-3-2, NJJCbio, China). The samples were incubated on plates, and the SOD levels were measured using the enzyme-labeled instrument (SynergyLX, BioTek).

2.12 Immunofluorescence co-staining

The histological sections of the gastric tissues were deparaffinized, antigenically repaired using the citrate buffer for 15 min, and blocked with 10% bovine serum albumin (BSA) for 1 h. The sections were incubated overnight at 4°C with primary antibodies NADPH oxidase 4 (NOX4) (1:800, 67681-1-Ig, Proteintech, China), Thioredoxin2 (TRX2) (1:500, 13089-1-AP, Proteintech, China), Peroxiredoxin-1 (PRDX1) (1:800, 66820-1-Ig, Proteintech, China), and Cytochrome oxidase IV (COIV) (1:500, 66110-1-Ig, Proteintech, China). Following the washing steps, the sections were incubated with anti-mouse IgG (A0216, Beyotime, China) and anti-rabbit IgG (A0208, Beyotime, China) at 37°C for 1 h. Following the washing process, the slices were sealed with 4',6-diamidino-2-phenylindole (DAPI) for 5 min. Finally, the laser confocal microscope (Leica SP8, Germany) was used to examine the samples.

2.13 Western blot

Mitochondria from the stomach were obtained using the mitochondrial extraction kit (SM0020, Solarbio, China). The

radioimmunoprecipitation assay (RIPA) lysis buffer (P0013E, Beyotime, China) was employed to extract the mitochondrial proteins. After quantification, the proteins were electrophoresed and transferred onto the polyvinylidene fluoride membrane (IPVH00010, Millipore, Ireland). After treatment with 5% skimmed milk, the membranes were co-incubated overnight at 4°C with the following primary antibodies: anti-NOX4 (1:1000, 67681-1-Ig, Proteintech, China), anti-TRX2 (1:500, 13089-1-AP, Proteintech, China), anti-PRDX1 (1:5000, 66820-1-Ig, Proteintech, China), and anti-GAPDH (1:50000, 60004-1-Ig, Proteintech, China). The samples were incubated at room temperature with anti-mouse IgG (1:1000, A0216, Beyotime, China) and anti-rabbit IgG (1:1000, A0208, Beyotime, China) for 1 h. The protein expression was detected using the automated chemiluminescent fluorescence imaging analysis system (5200 Multi, Tanon, China) and quantified with ImageJ software.

2.14 GM testing

Rat fecal samples were obtained and stored in liquid nitrogen, held at −80°C until analysis. Genomic DNA from fecal material was extracted from 0.1 g of frozen fecal samples using the E.Z.N.A.® soil DNA Kit (Omega Bio-Tek, Norcross, GA, U.S). The DNA concentration and purity were determined using 1.0% agarose gel electrophoresis and a NanoDrop® ND-2000 spectrophotometer (Thermo Scientific Inc., USA) to ensure that the quality of the samples met the criteria for subsequent analysis. The hypervariable region V3-V4 of the bacterial 16S rRNA gene was amplified with primer pairs 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (29) using the T100 Thermal Cycler (BIO-RAD, USA). PCR quantification and purification were conducted. Finally, the Illumina NextSeq 2000 PE300 platform (Illumina, San Diego, CA, USA) was selected for high-throughput sequencing and species annotation according to the standard protocols established by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

2.15 Statistical analysis

Statistical analyses were performed using SPSS version 26.0 and GraphPad Prism version 9.0. The Shapiro-Wilk test employed to assess the normality of the data, and the findings indicated conformity with the normal distribution. The data were presented as means ± standard deviations (x ± SD). Levene's method was used to test the homogeneity of variances in the data, and the results showed that the variances were homogeneous. Consequently, one-way analysis of variance (ANOVA)was used to compare multiple groups, whereas the Fisher's least significant difference (LSD) method was used to compare two groups.

The GM structure analysis was based on the α- and β-diversity. The Ace and Chao indices indicated community richness, while the Shannon and Simpson indices assessed community diversity principal coordinate analysis (PCoA), principal component analysis (PCA) and non-metric multidimensional scaling (NMDS) were applicable for comparing species diversity among communities. Additionally, we used the similarity analysis to assess the importance of the detected changes in community structure. The significance level of $p < 0.05$ denoted the significant difference, while a $R > 0$ indicated that the variation between groups was greater than the variation within groups (30). P values were calculated via one-way ANOVA for multiple group comparisons, and Wilcoxon rank-sum test for two groups comparisons.

3 Results

3.1 CHSGP promoted gastrointestinal motility in FD rats

FD is characterized by reduced GI motility without any stomach mucosal pathology (11). The results of this study revealed that the FD model, induced by clamp-tail stimulation, exhibited decreased gastric emptying rate ($p < 0.01$) and slower small intestinal movement ($p < 0.01$) compared with the control group, as illustrated in Figure 1A. The CHSGP treatment promoted the gastric emptying ($p < 0.01$) and small intestinal propulsion rates ($p < 0.01$). Additionally, H&E staining demonstrated that the gastric tissue glandular structures across all groups were normal and showed no signs of pathological changes such as erosion or ulceration (Figure 1B).

3.2 CHSGP protected the gastric tissue mitochondria and alleviated OS in FD rats

Wang (11) discovered that the damage of ICC could slow the gastric motility. TEM was used to examine the ultrastructures of the gastric tissue ICC. The results revealed that ICC in the control group had preserved morphologies characterized by elongated or oval shapes, intact nuclear membranes, complete organelles, abundant mitochondria, and clear cytoplasmic structures. Conversely, the FD groups showed noticeable swelling, distortion, and vacuolar changes, accompanied by the absence of mitochondrial cristae. Following the CHSGP treatment, the mitochondria appeared clear and intact with minimal swelling and deformation, as depicted in Figure 2.

The ultrastructure of mitochondria can be compromised by OS (31). The gastric mitochondria were analyzed for levels of ROS, SOD, NOX4, PRDX1, and TRX2 related to OS. The findings indicated that the FD group had increased mitochondrial ROS ($p < 0.01$) and reduced SOD ($p < 0.01$) compared with the control group (Figures 3A, B). After CHSGP treatment, the levels of mitochondrial ROS were decreased ($p < 0.01$), whereas SOD levels were increased ($p < 0.05$) (Figures 3A, B). The levels of mitochondrial OS were assessed by the immunofluorescence co-localization method for relative quantification. The mitochondrial marker CoxIV (pink) was co-localized with the OS markers NOX4 (green), PRDX1 (red), and TRX2 (turquoise). The co-localized expression of NOX4 and COXIV was significantly elevated in the FD group compared with the control group ($p < 0.01$), as shown in Figures 3C, D. Conversely, the co-localized expression of PRDX1 ($p < 0.05$) or TRX2 ($p < 0.01$) with COXIV exhibited a significant decrease, as shown in Figures 3E-H. CHSGP intervention reversed the above

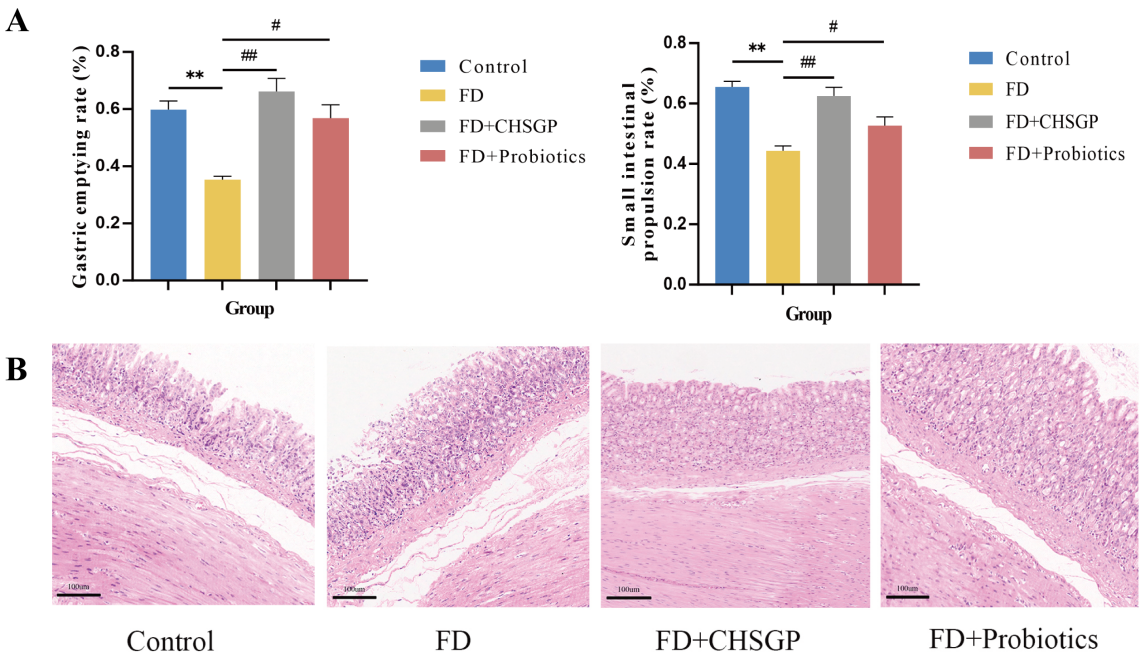


FIGURE 1
CHSGP promoted the gastrointestinal motility and protected the gastric mucosa in FD rats. **(A)** Test of gastric emptying rate and small intestinal propulsion rate. **(B)** Representative H&E-stained image (200 x magnification). Data were expressed as mean ± standard deviation (x ± SD) (n=6). P values were calculated using One-Way ANOVA followed by Fisher's LSD test. vs control group, **p < 0.01; vs FD group, #p < 0.05, ##p < 0.01.

results. Notably, the co-localized expression of NOX4 and COXIV was markedly reduced ($p < 0.01$), while the expression levels of PRDX1 ($p < 0.01$) or TRX2 with COXIV had risen ($p < 0.05$) (Figures 3C-H). Additionally, the western blot (WB) analysis revealed that the FD group had elevated levels of the NOX4 protein ($p < 0.01$) and decreased levels of the PRDX1 ($p < 0.01$) and TRX2 proteins ($p < 0.05$) compared with the control group, as illustrated in Figures 3I-L. Following the administration of CHSGP decoction, NOX4 protein levels decreased ($p < 0.01$), while PRDX1 ($p < 0.01$) and TRX2 ($p < 0.01$) protein levels increased (Figures 3I-L). These findings indicated that CHSGP treatment ameliorated mitochondrial OS damage.

3.3 CHSGP regulated the diversity and composition of GM in FD rats

High-throughput gene sequencing analysis of 16S rDNA extracted from the rat fecal microbiota DNA was performed to evaluate the impact of the CHSGP therapy on the microbial community. The pan/core curve illustrated the common or core species found within the sample. The sequencing curve reached a plateau, suggesting that it was satisfactory for capturing the complete spectrum of species in the analyzed samples (Figures 4A, B). Subsequent analysis that used the existing data was considered appropriate. α- and β-diversity analyses were performed to assess the variability within GM. Several operational taxonomic unit (OTU)-based indices, including the Simpson, Shannon, Chao, and Ace indices, showed that no notable differences among the four groups ($p > 0.05$). This indicated similar microbial abundance and homogeneity in the α-diversity analysis (Figures 4C-F). The β-diversity of the microbiota was illustrated by the PCoA, PCA, and NMDS analyses. The PCA dissimilarities across the groups were assessed using the analysis of similarities, and PCoA was performed using the Bray-Curtis distance metric. The PCA showed no statistical difference among the four groups of GM ($p > 0.05$) (Figure 4G); however, the PCoA indicated statistical significance among the groups ($p < 0.01$) (Figure 4H). Therefore, the FD group exhibited the distinct clustering pattern in comparison to the control group, indicating that the structural composition of the sample was dissimilar (Figure 4H). Furthermore, the bacterial composition of the CHSGP group exhibited greater similarity to that of the control group (Figure 4H). Researchers have used NMDS analyses to transform entities from the multidimensional space into the lower-dimensional space. This contributes to their positioning, analysis, and classification. It also preserves the original relationships between the objects. We tested the NMDS analysis by measuring the stress value. The results showed that GM of the FD group significantly differed from those of the control group ($p < 0.01$) (Figure 4I). Furthermore, the GM composition of the CHSGP group was more similar to that of the control group. Next, we used Venn diagrams to analyze the differences in the number of OUTs for the four sample communities. A total of 578 OTUs were identified as common across all groups. Specifically, the control group exhibited 772 OTUs, while the FD group contained 737 OTUs. The FD + CHSGP and FD + probiotics groups each included 740 OTUs (Figure 4J).

The species compositions of the control, FD, and FD + CHSGP groups were analyzed at the phylum and genus levels. Fourteen bacterial categories were identified at the phylum level, with Firmicutes and Bacteroidetes being the most common, constituting roughly 90% of the total bacterial population (Figure 5A). Compared

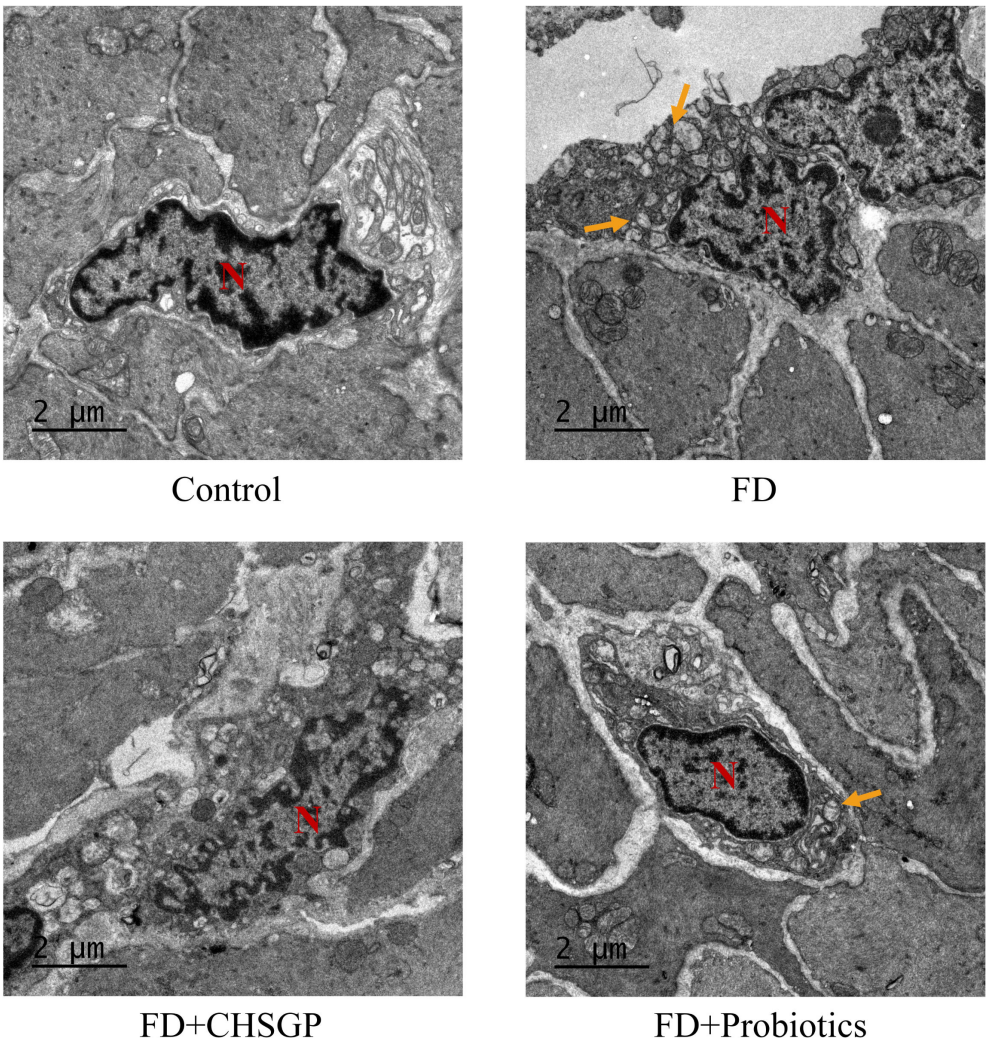


FIGURE 2
CHSGP protected the gastric tissue ICC mitochondria in FD rats. ICC morphology was observed with a TEM (6000 x magnification). Red N represents the nucleus, and yellow arrows represent the damaged mitochondria.

with the control group, the FD group had a rise in Bacteroidetes ($p < 0.05$) and a decline in Firmicutes ($p < 0.05$). This result indicated the disrupted microbiota (Figure 5B). CHSGP treatment restored the microbial composition of the FD. Compared with the FD group, the CHSGP group had reduced Bacteroidetes ($p < 0.05$) and increased Firmicutes ($p < 0.05$) (Figure 5C).

At the genus level, 38 bacteria were identified. They primarily included *norank_f_Muribaculaceae*, *unclassified_o:Clostridia_UCG-014*, *UCG-005*, *Lactobacillus*, *unclassified_f:Lachnospiraceae*, *Romboutsia*, and *Bacteroides* (Figure 5D). The FD group had reduced levels of *Ligilactobacillus* ($p < 0.05$) and *Bifidobacterium* ($p < 0.05$) while higher levels of *Erysipelotrichaceae_UCG-003* ($p < 0.01$) and *Collinsella* ($p < 0.05$) compared to the control group (Figure 5E). After the CHSGP treatment, there was a decrease in the proportions of *Prevotellaceae_UCG-001* ($p < 0.05$), *Colidextribacter* ($p < 0.05$), *Alistipes* ($p < 0.05$), and *Akkermansia* ($p < 0.01$), while the proportions of *UCG-005* ($p < 0.05$), *Oribacterium* ($p < 0.01$), *Negativibacillus* ($p < 0.05$), and *Bifidobacterium* ($p < 0.05$) were raised compared with the FD group (Figure 5F).

3.4 CHSGP regulated the GM to alleviate OS

Pearson's correlation analysis was employed to evaluate the relationship between GM and the indicators of OS (ROS, SOD, NOX4, TRX2, and PRDX1) across the control, FD, and FD + CHSGP groups. At the phylum level, Bacteroidota exhibited negative correlation with TRX2 ($p < 0.05$), while Cyanobacteria had negative correlation with PRDX1 ($p < 0.05$). Conversely, Deferribacterota exhibited positive correlation with ROS ($p < 0.05$) and NOX4 ($p < 0.05$), Firmicutes showed positive correlation with TRX2 ($p < 0.05$), and Campylobacterota shown positive correlation with SOD ($p < 0.05$) (Figure 6A). At the genus

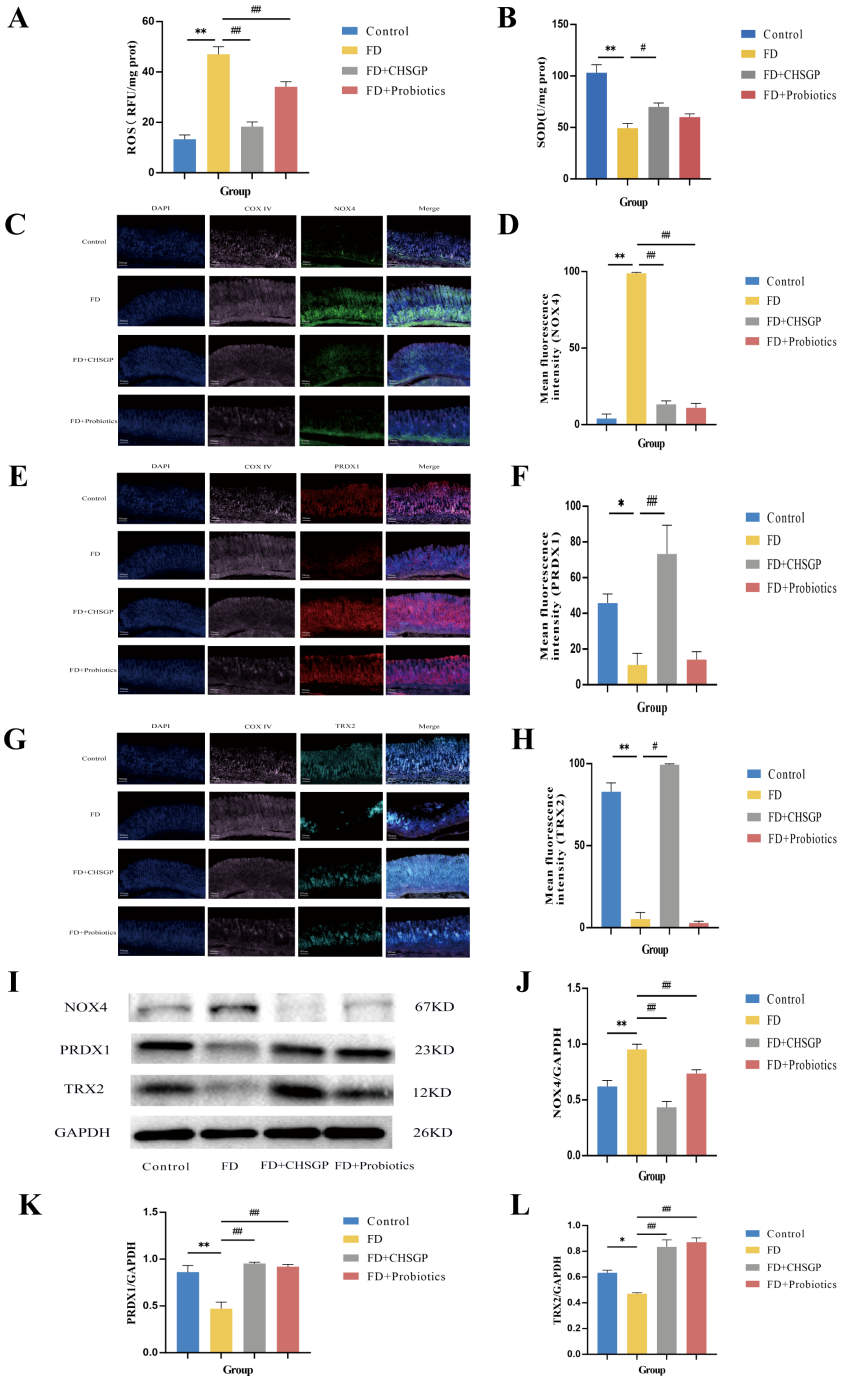


FIGURE 3
CHSGP alleviated the mitochondrial OS in FD rats. **(A)** ROS content analyzed by chemiluminescence. **(B)** SOD content analyzed by colourimetric assay. **(C)** Staining of gastric tissue with the mitochondrial marker COX IV (pink) and the OS marker NOX4 (green). **(D)** Mean fluorescence intensity of NOX4. **(E)** Staining of gastric tissue with the mitochondrial marker COX IV (pink) and the OS marker PRDX1 (red). **(F)** Mean fluorescence intensity of PRDX1. **(G)** Staining of gastric tissue with the mitochondrial marker COX IV (pink) and the OS marker TRX2 (turquoise). **(H)** Mean fluorescence intensity of TRX2. **(I)** The protein expression levels of NOX4, PRDX1 and TRX2 were analyzed by WB. **(J-L)** Density calculations for NOX4, PRDX1 and TRX2. Data were expressed as mean ± standard deviation (x ± SD) (n=6). P values were calculated using One-Way ANOVA followed by Fisher's LSD test. vs control group, $*p < 0.05$, $**p < 0.01$; vs FD group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$.

level, *Prevotellaceae-UCG-001* exhibited positive correlation with ROS ($p < 0.05$), while negative correlation with PRDX1 ($p < 0.05$). *Alloprevotella* showed positive correlations with ROS ($p < 0.05$) and NOX4 ($p < 0.05$), but it had negative correlations with PRDX1 ($p < 0.01$) and TRX2 ($p < 0.05$). *UCG-005* correlated negatively with ROS ($p < 0.05$). *Unclassified-o-Gastranaerophilales* and *Enterohabdus* exhibited negative correlation with PRDX1 (both $p < 0.05$). *Romboutsia* had positive correlation with TRX2 ($p < 0.05$), while *unclassified-f-Muribaculaceae* demonstrated negative correlation with TRX2 ($p < 0.05$) (Figure 6B).

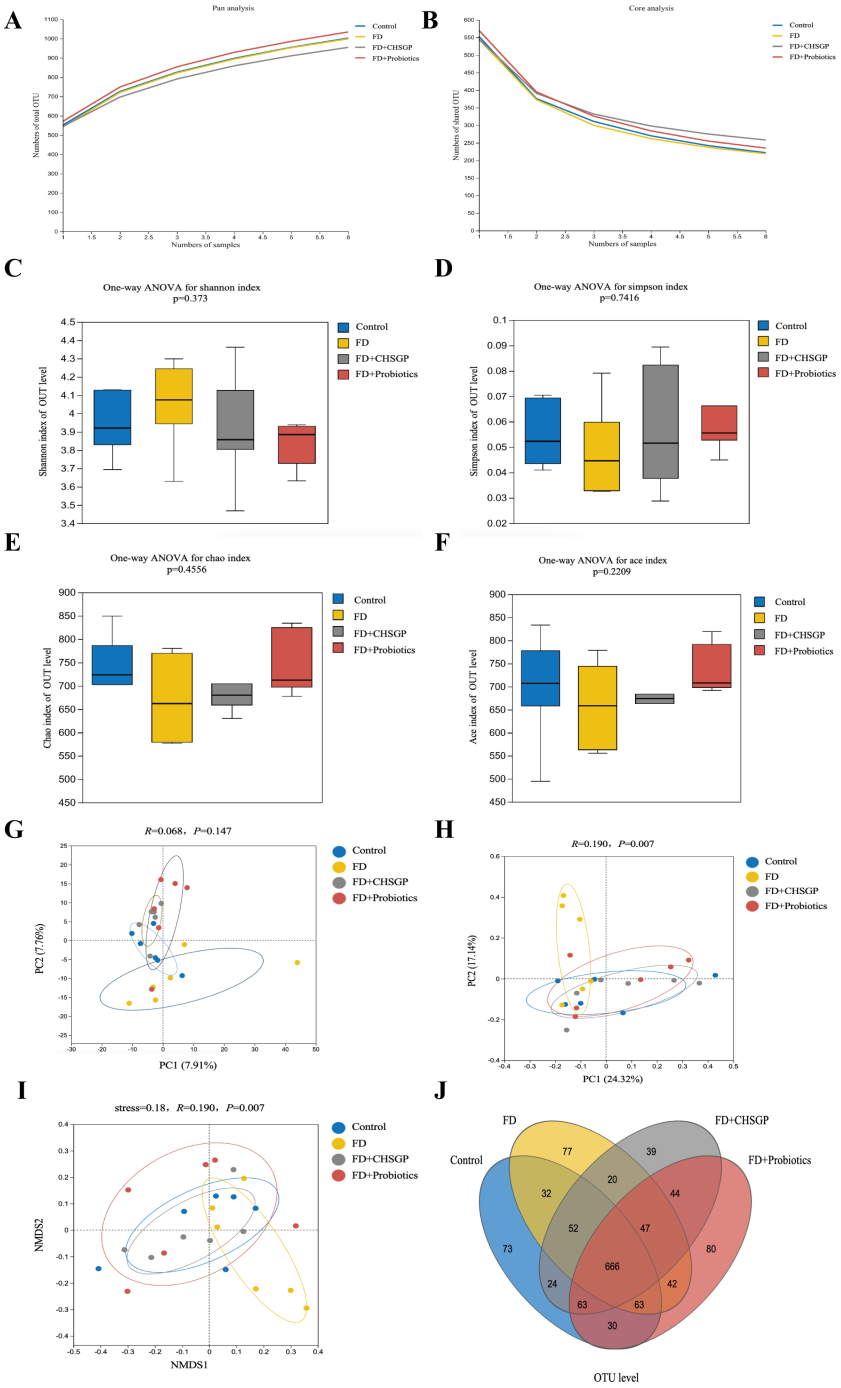


FIGURE 4
CHSGP regulated the diversity of the GM in FD rats. **(A, B)** Pan/Core curve. **(C-F)** Alpha diversity analysis. Data were expressed as mean ± standard deviation (x ± SD) (n = 6). Differences between samples were compared using the ANOSIM test. **(C)** Shannon index analysis. **(D)** Simpson index analysis. **(E)** Chao index analysis. **(F)** Ace index analysis. **(G-I)** Beta diversity analysis. Data were expressed as mean ± standard deviation (x ± SD) (n = 6). Differences between samples were compared using the ANOSIM test. **(G)** PCA analysis. **(H)** PCOA analysis. **(I)** NMDS analysis. **(J)** OTU analysis: Venn diagrams.

3.5 CHSGP relied on the GM to promote gastrointestinal motility in FD rats

3.5.1 Gastrointestinal motility changes in FD rats after ABX treatment

The rats were administered ABX to deplete their GM seven days prior to experiment II. The ABX group exhibited a notably slower gastric emptying rate than the control group ($p < 0.05$). The ABX (FD) and ABX (FD + CHSGP) groups had lower gastric emptying rates ($p < 0.01$ and $p < 0.05$, respectively) and slower small intestinal propulsion rates ($p < 0.01$ and $p < 0.05$, respectively) than the control group (Figure 7A). However, no statistically significant differences were seen between the ABX (FD) and ABX (FD + CHSGP) groups (both $p > 0.05$) (Figure 7A). The H&E staining showed structurally intact gastric tissues

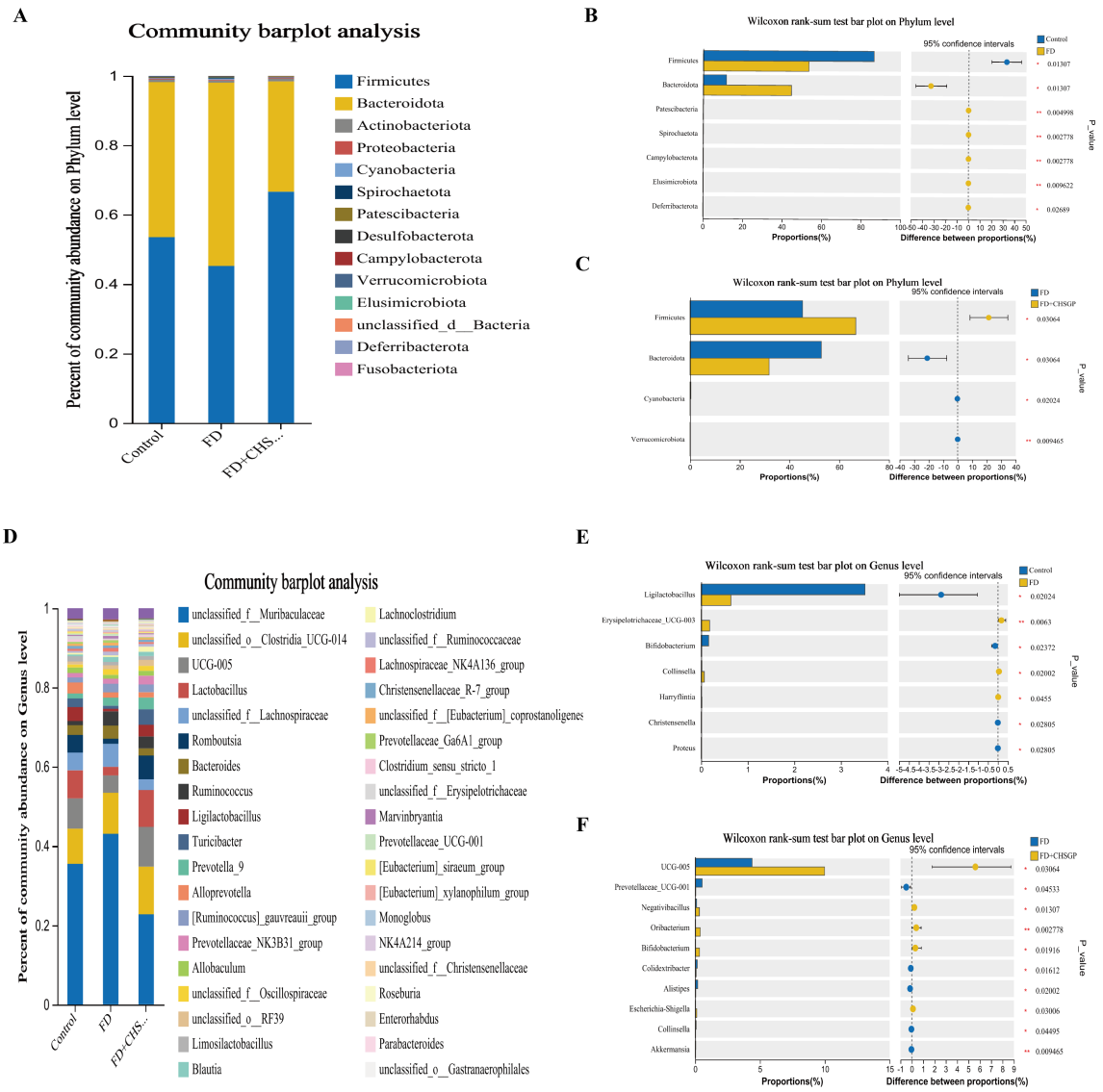


FIGURE 5
CHSGP regulated the species composition of the GM in FD rats. **(A)** Columnar stack diagram of species composition at the phylum level between the control, FD, and FD + CHSGP groups. **(B)** Differential strains at the phylum level between control and FD groups. **(C)** Differential strains at the phylum level between FD and FD + CHSGP groups. **(D)** Columnar stack diagram of species composition at the genus level between the control, FD, and FD + CHSGP groups. **(E)** Differential strains at the genus level between control and FD groups. **(F)** Differential strains at the genus level between FD and FD + CHSGP groups. Data were expressed as mean ± standard deviation (x ± SD) (n=6). P values were calculated using Wilcoxon rank-sum test. *$p < 0.05$, **$p < 0.01$.

in the ABX, ABX (FD), and ABX (FD + CHSGP) groups, without any signs of organic damage such as erosion or ulcers (Figure 7B). However, a slight neutrophil infiltration was observed in the mucosal layer. The aforementioned findings suggested that GM depletion led to the disappearance of GI propulsion mediated by CHSGP.

3.5.2 Gastrointestinal motility changes in FD rats after FMT treatment

The rat fecal samples treated with FD + CHSGP were collected early and stored at −80°C. The fecal bacterial solution was transferred to rats with or without depleted GM. After the FMT, the results showed that the ABX (FD + FMT) and FD + FMT groups exhibited increased gastric emptying rates ($p < 0.01$ and $p < 0.05$, respectively) and small intestinal propulsion rates (both $p < 0.01$) compared with the FD group (Figure 7C). Additionally, the HE sections showed no structural damage or organic lesions in any group (Figure 7D). Therefore, CHSGP relied on the GM to enhance GI motility in FD.

3.6 CHSGP relied on GM to protect ICC's mitochondria in FD rats

3.6.1 Mitochondrial ultrastructure changes in FD rats after ABX treatment

The TEM images revealed significant swelling, distortion, and vacuolization in the mitochondria of ICC following the antibiotic

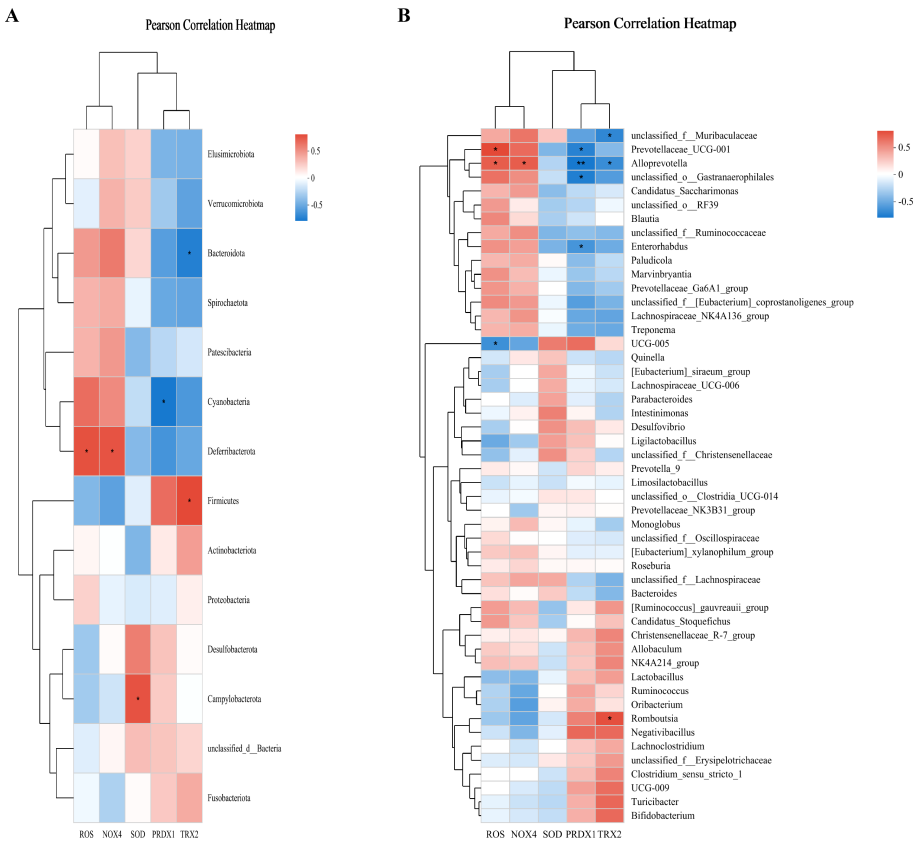


FIGURE 6
CHSGP regulated the GM to alleviate OS. **(A)** Heatmap of the correlation between the differential microbiota at the phylum level among the control group, FD group, and FD + CHSGP group and the indicators of OS. **(B)** Heatmap of the correlation between the differential microbiota at the genus level among the control group, FD group, and FD + CHSGP group and the indicators of OS. We calculated the correlation between variables using the pearson correlation coefficient. *$p < 0.05$, **$p < 0.01$.

treatment. This was accompanied by the disappearance of cristae in the groups treated with ABX, ABX (FD), and ABX (FD + CHSGP) (Figure 8A). This indicated that the protective role of CHSGP in mitochondria was lost after GM depletion. As a result, GM contributed to the protective effect of CHSGP on the mitochondria.

3.6.2 Mitochondrial ultrastructure changes in FD rats after FMT treatment

Mitochondria of the ICC showed swelling and deformities in the FD + FMT group after the FMT treatment, while they remained clear and intact in the ABX (FD + FMT) group (Figure 8B). These results indicated that the CHSGP fecal microbiota offered specific protection against the mitochondrial damage resulting from dysbiosis of microbiota in FD.

3.7 CHSGP relied on GM to alleviate mitochondrial OS in FD rats

3.7.1 Mitochondrial OS changes in FD rats after ABX treatment

Following antibiotic administration, there was the significant increase in ROS levels ($p < 0.01$) and a decrease in SOD levels ($p < 0.05$) in the gastric tissue mitochondria compared with the control group (Figures 9A, B). However, there were no significant differences in ROS and SOD levels between the ABX (FD) and ABX (FD + CHSGP) group (both $p > 0.05$) (Figures 9A, B). The co-localization expression intensities of NOX4, PRDX1, and TRX2 were elevated in the ABX, ABX (FD), and ABX (FD + CHSGP) groups compared with the control group ($p < 0.01$ or $p < 0.05$) (Figures 9C-H). No significant differences were found between the ABX (FD) and ABX (FD + CHSGP) groups (both $p > 0.05$) (Figures 9C-H). Additionally, the ABX, ABX (FD), and ABX (FD + CHSGP) groups showed an increase in NOX4 protein expressions (both $p < 0.01$), a decrease in PRDX1 protein expressions (both $p < 0.05$), and a reduction in TRX2 protein expressions (both $p < 0.01$) compared with the control group (Figures 9I-L). However, no significant differences were seen in the protein levels of NOX4, PRDX1, and TRX2 between the ABX (FD) and ABX (FD + CHSGP) groups (both $p > 0.05$) (Figures 9I-L). This indicated that the beneficial effect of CHSGP on mitochondrial OS was lost after GM depletion. Consequently, the GM contributed to the antioxidant effects of CHSGP on the mitochondria.

3.7.2 Mitochondrial OS changes in FD rats after the FMT treatment

Rats, regardless of GM depletion, received fecal solutions from CHSGP-treated FD rats. Following the FMT treatment in experiment III, there was a significant reduction in mitochondrial ROS levels ($p < 0.01$)and an increase in SOD levels ($p < 0.05$) compared to the FD

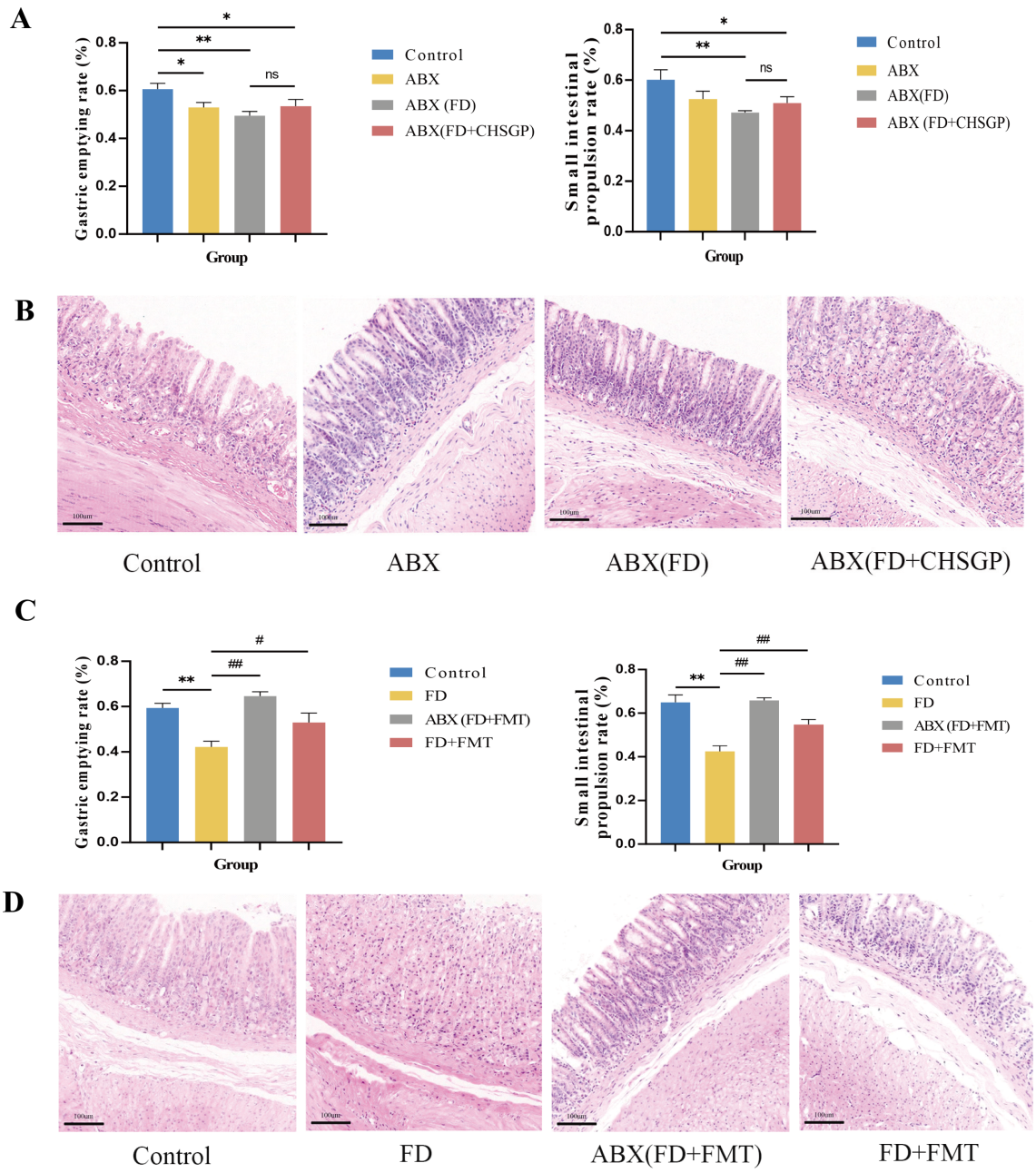


FIGURE 7
CHSGP relied on the GM to promote gastrointestinal motility in FD rats. **(A-B)** Changes of the gastrointestinal motility and gastric mucosa in FD after ABX: **(A)** Test of the gastric emptying rate and small intestinal propulsion rate. **(B)** Representative H&E-stained image (200 × magnification). **(C, D)** Changes of the gastrointestinal motility and gastric mucosa in FD after FMT: **(C)**Test of the gastric emptying rate and small intestinal propulsion rate. **(D)** Representative H&E-stained image (200 x magnification). Data were expressed as mean ± standard deviation (x ± SD) (n=6). P values were calculated using One-Way ANOVA followed by Fisher's LSD test. vs control group, $^{*}p < 0.05$, $^{**}p < 0.01$; ABX (FD) vs ABX (FD + CHSGP) group, ns$p > 0.05$; vs FD group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$.

group (Figures 10A, B).Additionally, the NOX4 protein content was notably reduced in the ABX (FD+FMT) and FD + FMT groups (both $p < 0.01$). In comparison, the PRDX1 proteins exhibited markedly elevated ($p < 0.01$ and $p < 0.05$ respectively) (Figures 10C-E), while the TRX2 proteins displayed no significant differences between the groups (both $p > 0.05$) compared with the FD group (Figure 10F). The ABX (FD + FMT) group showed the stronger antioxidant effect, characterized by reduced NOX4 expression and elevated PRDX1 expression, compared to the FD + FMT group (Figures 10C-E). Moreover, there was the reduced co-localization of NOX4 and Cytochrome oxidase IV (COX IV) ($p < 0.05$) and the increased co-localization of PRDX1 and COX IV ($p < 0.01$) compared with the FD group (Figures 10G-J). However, there were no statistically significant differences in the co-localization expression of TRX2 and Cytochrome oxidase IV (COX IV) between the ABX (FD+FMT) or FD+FMT groups compared to the FD group (both $p > 0.05$) (Figures 10K-L). Therefore, the antioxidant effects of CHSGP on the mitochondria depended on the GM.

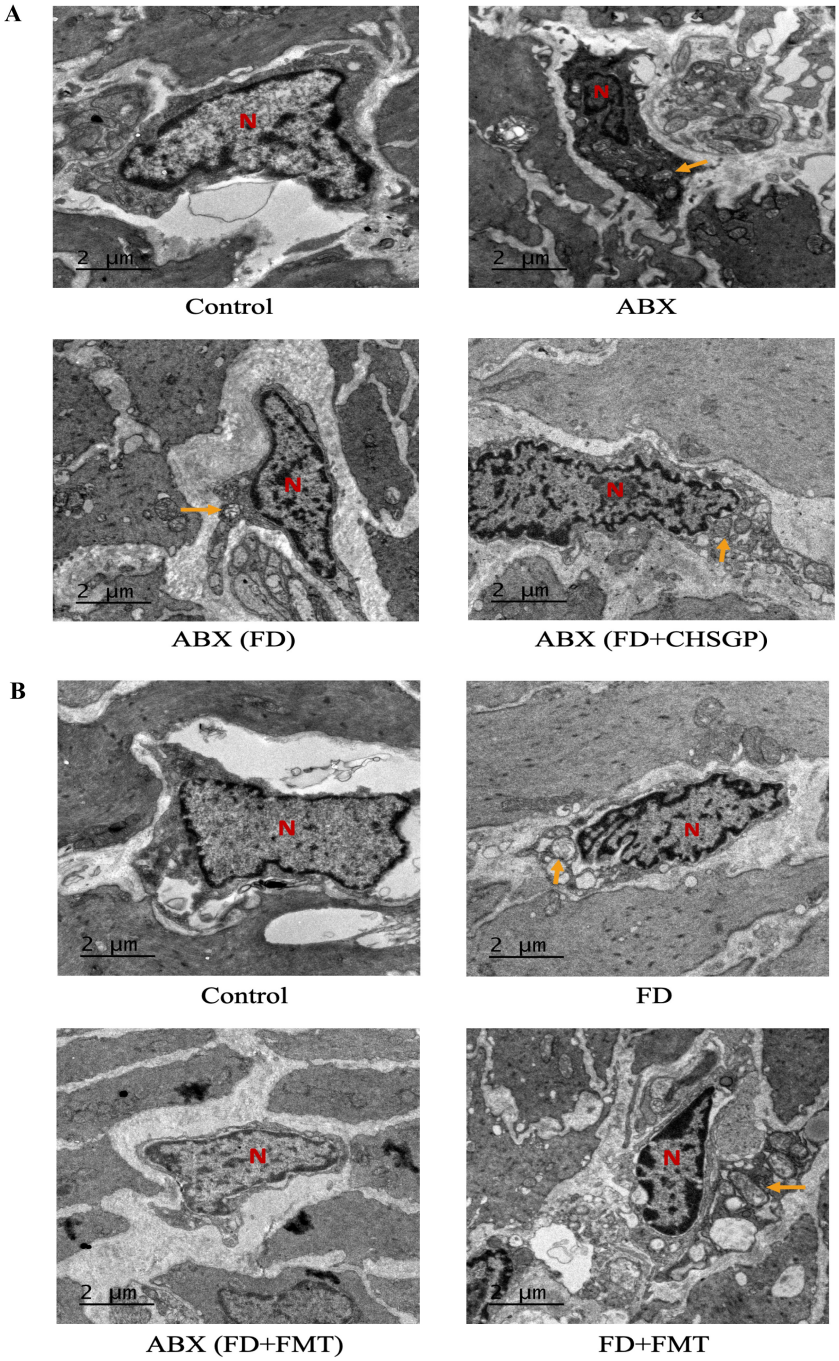


FIGURE 8
CHSGP relied on the GM to protect ICC mitochondria in FD rats. **(A)** ICC morphology was observed with a TEM (6000 x magnification) after ABX. **(B)** ICC morphology was observed with a TEM (6000 x magnification) after FMT. Red N represents the nucleus, and yellow arrows represent the damaged mitochondria.

3.8 CHSGP exerted antioxidant effects by regulating the diversity and composition of the GM in FD rats

3.8.1 Diversity and composition changes of the GM in FD rats after ABX treatment

The pan/core curve analysis suggested that the number of sequenced samples adequately met the required criteria (Figures 11A, B). The α-diversity analysis showed no statistically significant difference in the Simpson and Shannon indices across groups (both $p > 0.05$) (Figures 11C, D). However, the statistically significant difference was observed in the Ace and Chao indices (both $p < 0.01$). This result indicated that ABX could reduce the richness of the microbiota, but ABX (FD) and ABX (FD + CHSGP) could increase the abundance of the microbiota (Figures 11E, F). The PCA, PCoA, and NMDS analyses demonstrated significant changes in GM composition in the antibiotic-depleted groups (ABX, ABX (FD), and ABX (FD +

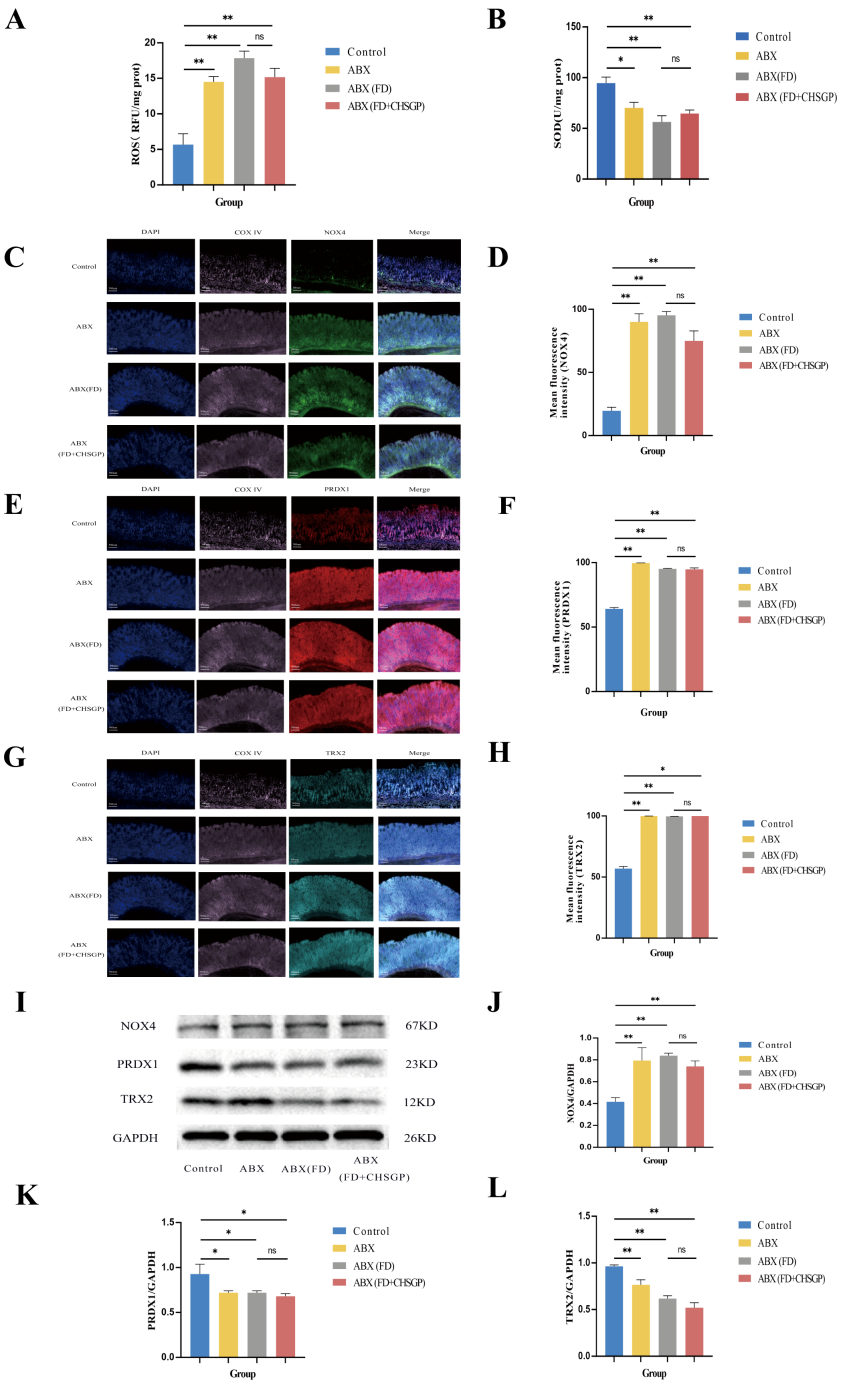


FIGURE 9
Changes of the mitochondrial OS in FD rats after ABX. **(A)** ROS content analyzed by chemiluminescence. **(B)** SOD content analyzed by colourimetric assay. **(C)** Staining of gastric tissue with the mitochondrial marker COX IV (pink) and the OS marker NOX4 (green). **(D)** Mean fluorescence intensity of NOX4. **(E)** Staining of gastric tissue with the mitochondrial marker COX IV (pink) and the OS marker PRDX1 (red). **(F)** Mean fluorescence intensity of PRDX1. **(G)** Staining of gastric tissue with the mitochondrial marker COX IV (pink) and the OS marker TRX2 (turquoise). **(H)** Mean fluorescence intensity of TRX2. **(I)** The protein expression levels of NOX4, PRDX1 and TRX2 were analyzed by WB. **(J-L)** Density calculations for NOX4, PRDX1 and TRX2. Data were expressed as mean ± standard deviation (x ± SD) (n=6). P values were calculated using One-Way ANOVA followed by Fisher's LSD test. vs control group, *$p < 0.05$, **$p < 0.01$; ABX (FD) vs ABX (FD+CHSGP) group, ns$p > 0.05$.

CHSGP)) compared to the control group (both $p < 0.01$) (Figures 11G-I). The comparative analysis indicated no significant differences in the overall microbiota structure among the ABX, ABX (FD), and ABX (FD + CHSGP) groups ($p > 0.05$) (Figures 11G-I). These results indicated that CHSGP administration after the antibiotic treatment did not affect the overall composition of the GM in FD rats. The Venn diagram showed that all four groups collectively shared 514 OTUs, while the control group had 1003 OTUs. In addition, the ABX group contained 838 OTUs, the ABX (FD) group comprised 1369, and

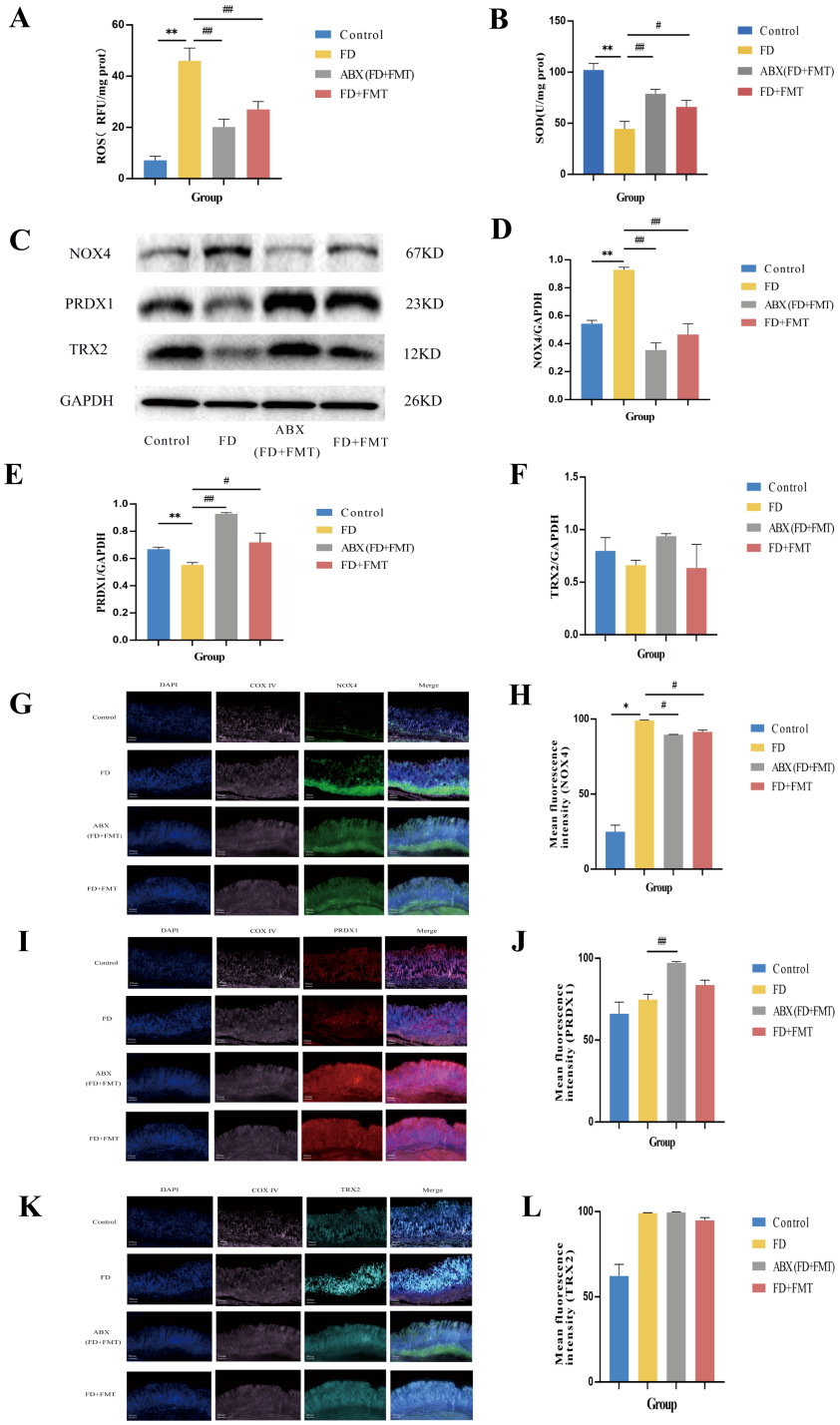


FIGURE 10
Changes of the mitochondrial OS in FD rats after FMT. **(A)** ROS content analyzed by chemiluminescence. **(B)** SOD content analyzed by colourimetric assay. **(C)** The protein expression levels of NOX4, PRDX1 and TRX2 were analyzed by WB. **(D-F)** Density calculations for NOX4, PRDX1 and TRX2. **(G)** Staining of gastric tissue with the mitochondrial marker COX IV (pink) and the OS marker NOX4 (green). **(H)** Mean fluorescence intensity of NOX4. **(I)** Staining of gastric tissue with the mitochondrial marker COX IV (pink) and the OS marker PRDX1 (red). **(J)** Mean fluorescence intensity of PRDX1. **(K)** Staining of gastric tissue with the mitochondrial marker COX IV (pink) and the OS marker TRX2 (turquoise). **(L)** Mean fluorescence intensity of TRX2. Data were expressed as mean ± standard deviation (x ± SD) (n=6). P values were calculated using One-Way ANOVA followed by Fisher's LSD test. vs control group, $*p < 0.05$, $**p < 0.01$; vs FD group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$.

the ABX (FD + CHSGP) group included 1270. (Figure 11J). The comparison with the control group revealed a drop in microbial OTUs in the ABX group, suggesting the effective depletion of the GM in rats due to ABX treatment.

Ten principal bacterial phyla were identified within the groupings, with Firmicutes and Bacteroidetes being the most dominant, constituting almost 90% of the overall bacterial population (Figure 12A). In comparison to the control group, the

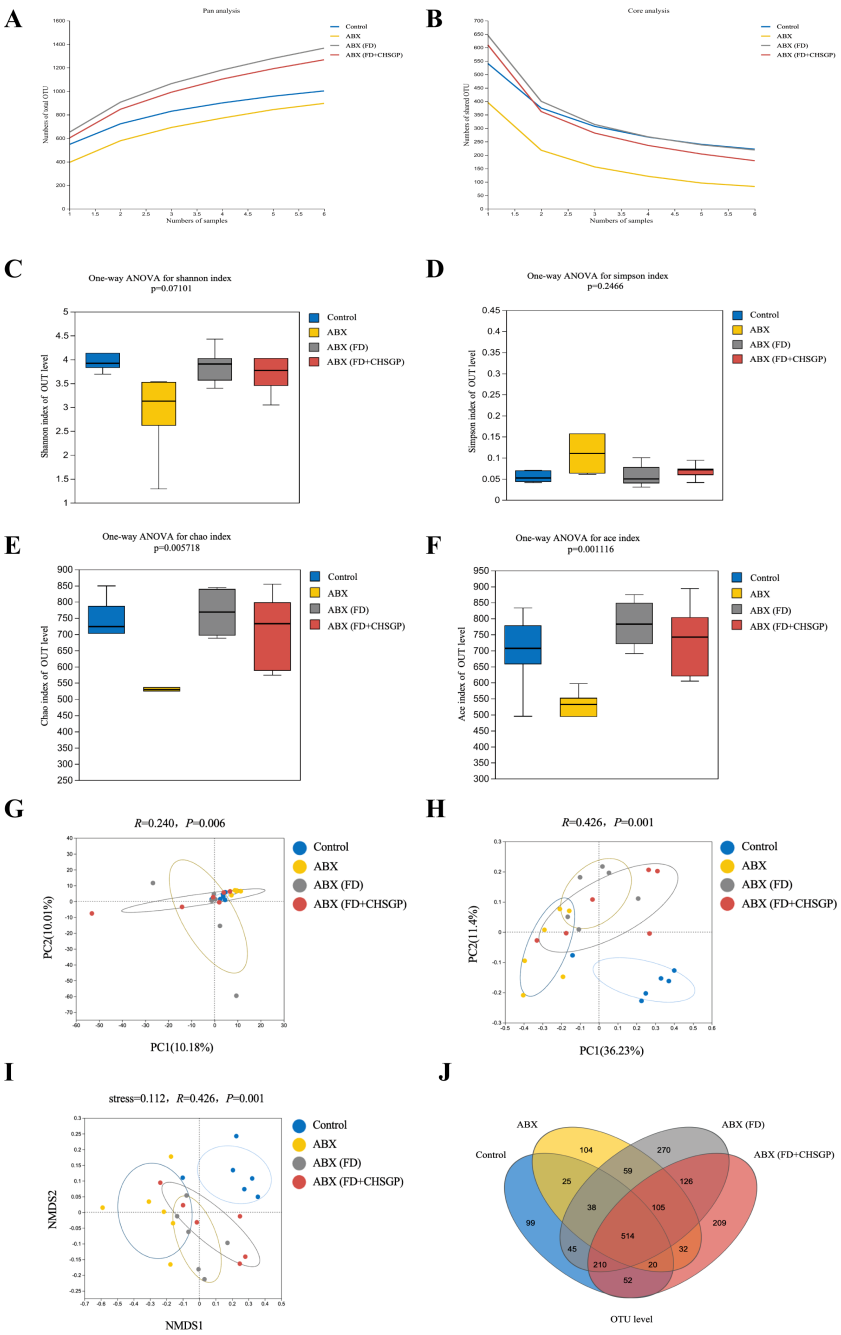


FIGURE 11
Diversity analysis of the GM of FD rats after ABX. **(A, B)** Pan/Core curve. **(C-F)** Alpha diversity analysis. **(C)** Shannon index analysis. **(D)** Simpson index analysis. **(E)** Chao index analysis. **(F)** Ace index analysis. **(G-I)** Beta diversity analysis. **(G)** PCA analysis. **(H)** PCOA analysis. **(I)** NMDS analysis. **(J)** OTU analysis: venn diagrams. Data were expressed as mean ± standard deviation (x ± SD) (n=6). Differences between samples were compared using the ANOSIM test.

richness of Bacteroidetes declined ($p < 0.01$), while the richness of Firmicutes increased ($p < 0.01$) in the ABX, ABX (FD), and ABX (FD + CHSGP) groups (Figure 12B). However, the above strains exhibited no statistically significant difference between the ABX (FD) and ABX (FD + CHSGP) groups ($p > 0.05$) (Figure 12C). These results indicated that ABX could disrupt the GM structure at the phylum level. Meanwhile, CHSGP administration did not affect the GM of FD rats. This indicated that after GM consumption, the regulatory effect of CHSGP on microbial communities at the phylum level was not significant.

Twelve predominant bacterial genera were identified in the groups that accounted for approximately 80% of the overall bacterial population. These included *unclassified_f_Lachnospiraceae*, *norank_f_Muribaculaceae*, *Romboutsia*, *Turicibacter*, *Ligilactobacillus*, *Bifidobacterium*, *Lactobacillus*, and *Alistipes* (Figure 12D). Compared with the control group, the

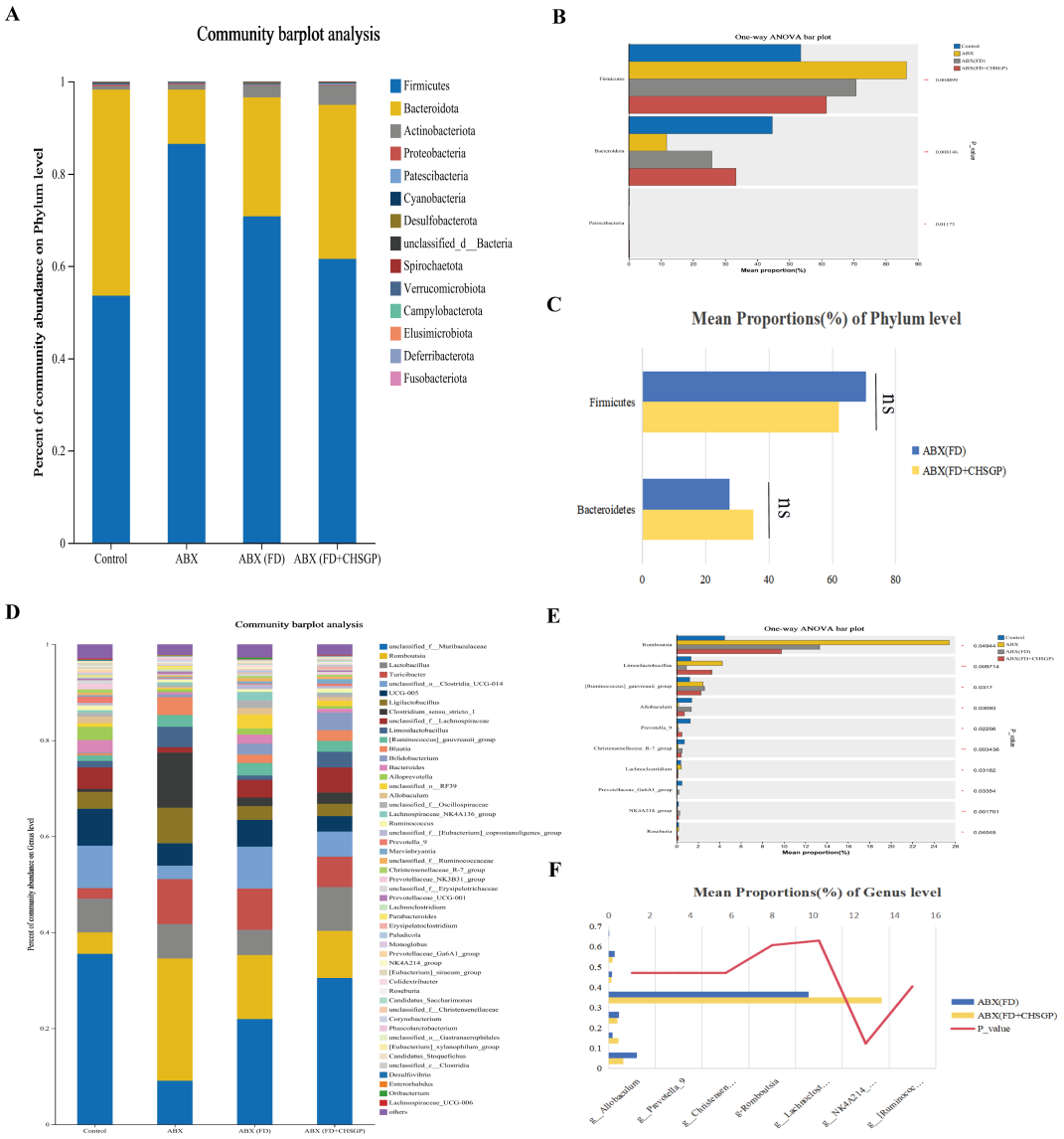


FIGURE 12
Species composition analysis of the GM of FD rats after ABX. **(A)** Columnar stack diagram of species composition at the phylum level between control, ABX, ABX (FD) and ABX (FD + CHSGP) groups. **(B)** Differential strains at the phylum level between control, ABX, ABX (FD) and ABX (FD + CHSGP) groups. **(C)** Differential strains at the phylum level between ABX (FD) and ABX (FD + CHSGP) groups. **(D)** Columnar stack diagram of species composition at the genus level between control, ABX, ABX (FD) and ABX (FD + CHSGP) groups. **(E)** Differential strains at the genus level between control, ABX, ABX (FD) and ABX (FD + CHSGP) groups. **(F)** Differential strains at the genus level between ABX (FD) and ABX (FD + CHSGP). Data were expressed as mean ± standard deviation (x ± SD) (n=6). P values were calculated using one-way ANOVA to compare multiple groups, and Wilcoxon rank-sum test to compare two groups. *$p < 0.05$, **$p < 0.01$, ns, $p > 0.05$.

abundances of *Allobaculum* ($p < 0.05$), *Prevotella_9* ($p < 0.05$), the *Christensenellaceae_R-7_group* ($p < 0.01$), *Lachnoclostridium* ($p < 0.05$), and the *NK4A214_group* ($p < 0.01$) decreased, while the abundances of *Romboutsia* ($p < 0.05$) and the *[Ruminococcus]_gauvreauii_group* increased ($p < 0.05$) in the ABX, ABX (FD), and ABX (FD + CHSGP) groups (Figure 12E). In addition, the abundances of *non-Muribaculaceae*, *Alistipes*, *Romboutsia*, *Turicibacter*, *Bifidobacterium*, *Lactobacillus*, and *Ligilactobacillus* were not statistically significant between the ABX (FD) and ABX (FD + CHSGP) groups (both $p > 0.05$) (Figure 12F). These findings showed that ABX could disturb the structure of the GM at the genus level. Accordingly, CHSGP administration did not affect the GM of FD rats. This indicated that CHSGP could lose its ability to regulate the microbiota at the genus level following GM depletion.

3.8.2 Diversity and composition changes of the GM in FD rats after the FMT treatment

Rats, regardless of GM depletion status, received fecal solutions from CHSGP-treated FD rats. The pan/core curve showed that the sequencing sample number adequately met the established criteria (Figures 13A, B). No statistical differences were observed in the Shannon, Simpson, Chao, and Ace indices across the groups in the α -

diversity analysis (both $p > 0.05$) (Figures 13C-F). This result indicated that the four groups had similar richness and diversity at the α-diversity level. The PCA, PCOA, and NMDS analyses indicated that the GM composition had changed among the control group, FD group, ABX (FD + FMT) group, and FD + FMT group (both $p < 0.01$) (Figures 13G-I). The ABX (FD + FMT) and FD + FMT groups had distinct alterations in the GM compared with the FD group. The Venn diagram showed the intersection of the 620 OTUs in all of the groups. The control group had 1003 OTUs, the FD group had 999, the ABX (FD + FMT) group had 1229, and the FD + FMT group had 1213 (Figure 13J). This result indicated that the FMT intervention could alter the microbial diversity of rats with FD.

Ten bacterial groups were identified at the phylum level, with Firmicutes, Bacteroidetes, and Actinobacteriota being the most predominant in each group (Figure 14A). The GM structures were regulated after the FMT. A decrease in Bacteroidetes (both $p < 0.01$) and an increase in Firmicutes (both $p < 0.01$) and Actinobacteriota ($p < 0.05$ and $p < 0.01$, respectively) were observed in comparison to the FD group. These results indicated that the FMT might influence the composition of GMat the phylum level in FD rats.

Eleven predominant bacterial genera were identified at the genus level, accounting for nearly 80% of the total bacterial population. These genera included *unclassified_f_Lachnospiraceae*, *norank_f_Muribaculaceae*, *Romboutsia*, *Turicibacter*, *Ligilactobacillus*, *Bifidobacterium*, *Lactobacillus*, and *Alistipes*. (Figure 14D). The FMT administration promoted changes in the GM at the genus level in FD rats. Compared to the FD group, the ABX (FD + FMT) group showed a notable reduction in the levels of *Bacteroides* ($p < 0.05$), *Alloprevotella* ($p < 0.05$), and the *Lachnospiraceae_NK4A136_group* ($p < 0.05$), whereas *Romboutsia* ($p < 0.01$), *Bifidobacterium* ($p < 0.01$), *Turicibacter* ($p < 0.01$), *Corynebacterium* ($p < 0.01$), *Clostridium_sensu_stricto_1* ($p < 0.01$), and *Blautia* rose ($p < 0.05$) (Figure 14E). The FD + FMT group had a decrease in *Bacteroides* ($p < 0.01$), while *Romboutsia* ($p < 0.01$), *Bifidobacterium* ($p < 0.01$), *Turicibacter* ($p < 0.01$), *UCG-005* ($p < 0.05$), *Corynebacterium* ($p < 0.01$), and *Clostridium_sensu_stricto_1* increased ($p < 0.01$) (Figure 14F). These results indicated that the FMT might influence the compositions of GM at the genus level in FD rats.

3.9 FMT regulated the GM to alleviate OS

Pearson's correlation analysis was employed to evaluate the association across the GM and OS indicators (ROS, SOD, NOX4, TRX2, and PRDX1) in the control, FD, and FD + FMT groups. At the phylum level, Firmicutes was positively correlated with PRDX1 ($p < 0.05$) and negatively correlated with NOX4 ($p < 0.05$). Patescibacteria was positively correlated with ROS ($p < 0.05$). Bacteroidota, Deferribacterota, and Proteobacteria were positively correlated with NOX4 (both $p < 0.05$) (Figure 15A). At the genus level, *Enterorhabdus* was positively correlated with ROS ($p < 0.05$) and NOX4 ($p < 0.01$), while it was negatively correlated with PRDX1 ($p < 0.01$). *Romboutsia* was positively correlated with PRDX1 ($p < 0.05$), while it was negatively correlated with NOX4 ($p < 0.05$). *Unclassified-o-RF39*, the *Lachnospiraceae-NK4A136-group*, the *[Eubacterium]-xylanophilum-group*, and *Unclassified-f-oscillospiraceae* exhibited positive correlations withNOX4 ($p < 0.01$, $p < 0.05$, $p < 0.05$, and $p < 0.05$, respectively), demonstrating negative correlations with PRDX1 ($p < 0.05$, $p < 0.05$, $p < 0.01$, and $p < 0.05$, respectively). *Alloprevotella* and *Prevotella-9* showed positive correlations with NOX4 (both $p < 0.05$) (Figure 15B).

4 Discussion

Previous studies emphasized that dyskinesia of the GI tract and disorders of GM were key pathological mechanisms of FD (4). TCM significantly enhanced mitochondrial function, GI motility improvements, and GM regulation in FD (5, 10, 11). We found that CHSGP relied on the GM to relieve mitochondrial OS damage in gastric tissue, hence enhancing gastrointestinal motility. This investigation was divided into three portions. We first explored the impact of CHSGP on OS-related markers (ROS, SOD, NOX4, PRDX1, and TRX2) and GM in FD rats. We then implemented GM depletion using a four-antibiotic cocktail to evaluate whether the effect of CHSGP on OS was GM-dependent in FD rats. Finally, we examined the effect of the fecal microbiota from rats treated with FD + CHSGP on the OS and GM under different GM conditions. The schematic representation of the findings and mechanisms is illustrated in Supplementary Figure S3 (By Figdraw).

The emergence of numerous GI diseases is associated with OS. These diseases include neurogastrointestinal disorders, inflammatory bowel disease, peptic ulcers, and GI malignancies (32). OS arises when the generation of ROS exceeds the capacity of cells to eliminate or neutralize them via antioxidants (32). The NOX family, known as NOXs, consists of transmembrane proteins that facilitate electron transfer across biological membranes to catalyze the reduction of oxygen to superoxide (O_{2-}) (33). Pathological stimuli such as inflammation, hypoxia, and ischemia can induce the expression of NOX4, subsequently promoting the production of ROS (33). Antioxidant mechanisms involve enzymes that produce reduced forms of antioxidants and ROS-interacting enzymes, including peroxidases, SOD, and oxidoreductases. These mechanisms are crucial for the regulation of ROS levels *in vivo*. SOD serves as the principal defensive mechanism against diseases or damage caused by ROS. SOD comprises a class of metalloenzymes that uses metal ions in their active sites to catalyze the conversion of ROS into molecular oxygen and H_2O_2 for further use in different biological processes. The PRDX oxidoreductase contains an ionized thiol that converts ONOO– and other peroxides to H_2O_2 by oxidizing the conserved thiolate to -SOH intermediates. These can interact with resolving cysteine thiols (-SH). The Trx system comprises Trx and its associated thioredoxin reductase (TR). TR uses NADPH as an electron donor to facilitate the reduction and reconstruction of the dithiol active site in oxidized Trx. Mishina (34) stated that the Trx system was crucial for establishing an intracellular H_2O_2 gradient creation and for inhibiting the diffusion of H_2O_2 throughout the cytoplasm. Experiment I showed that the mitochondrial structure of the ICC is

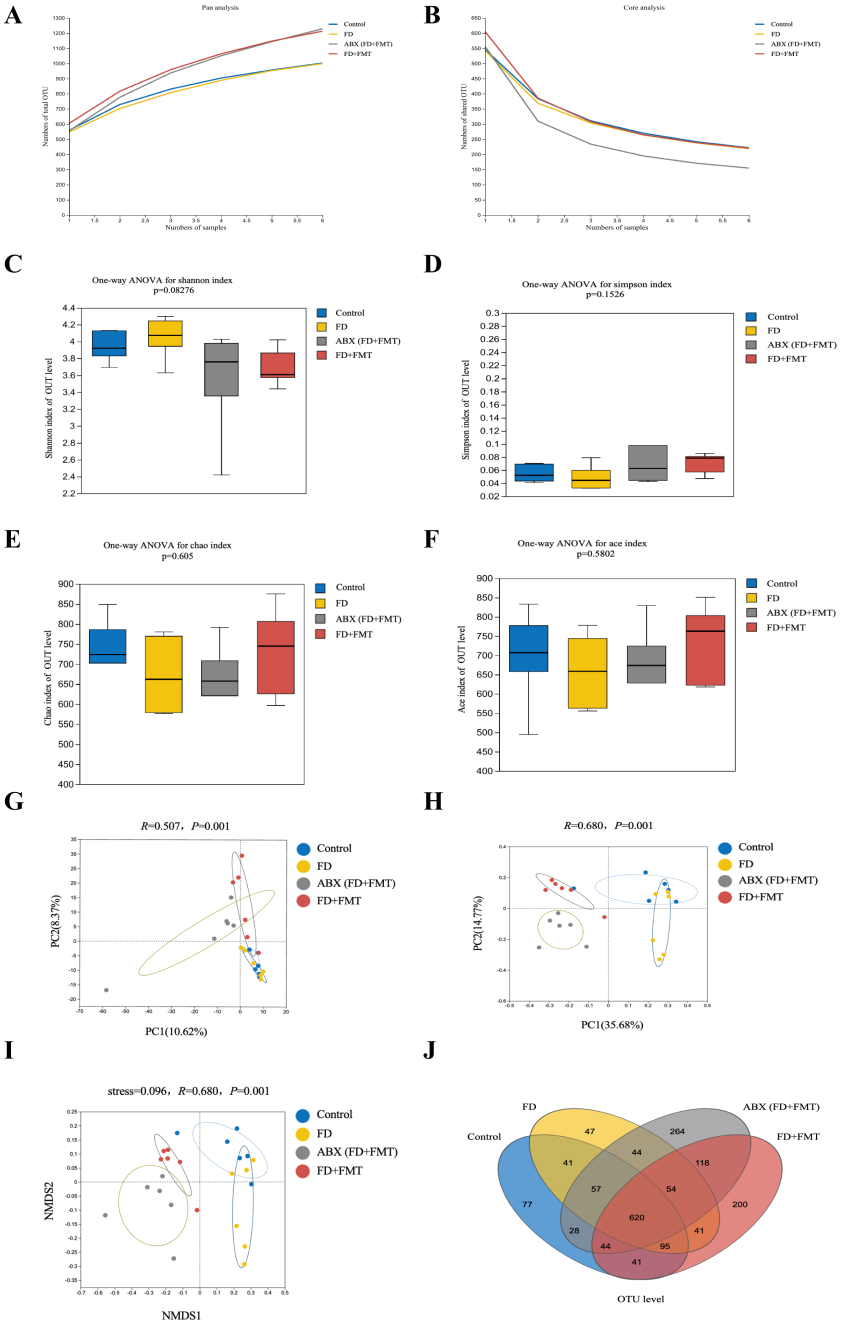


FIGURE 13
Diversity analysis of the GM of FD rats after FMT. **(A, B)** Pan/Core curve. **(C-F)** Alpha diversity analysis. **(C)** Shannon index analysis. **(D)** Simpson index analysis. **(E)** Chao index analysis. **(F)** Ace index analysis. **(G-I)** Beta diversity analysis. **(G)** PCA analysis. **(H)** PCOA analysis. **(I)** NMDS analysis. **(J)** OTU analysis: venn diagrams. Data were expressed as mean ± standard deviation (x ± SD) (n=6). Differences between samples were compared using the ANOSIM test.

compromised, along with alterations in OS indicators in FD group. These changes suggested that dysregulation of gastric motility was associated with OS damage to gastric tissue mitochondria in FD. In addition, research has linked OS development to peptic ulcer disease and non-ulcer dyspepsia (35). After CHSGP treatment, all of the mentioned pathological changes were reversed. This suggested that CHSGP alleviated mitochondrial OS in the stomach tissues of FD rats. Wei Kangning also treated indigestion using antioxidant methods (5). Except for FD, the common functional gastrointestinal disorders (FGIDs) included functional constipation and irritable bowel syndrome (IBS). Some studies have shown that TCM can treat these conditions by alleviating OS. For slow-transmitting constipation, the Zhizhu decoction can alleviate intestinal inflammation and OS by activating the SIRT1/FoxO1 antioxidant signaling pathway in the colon (36). STW5 reduced the 5-hydroxytryptamine (5-HT) signaling pathway in the brain-gut axis and combated stress-induced OS to facilitate the treatment of IBS (37).

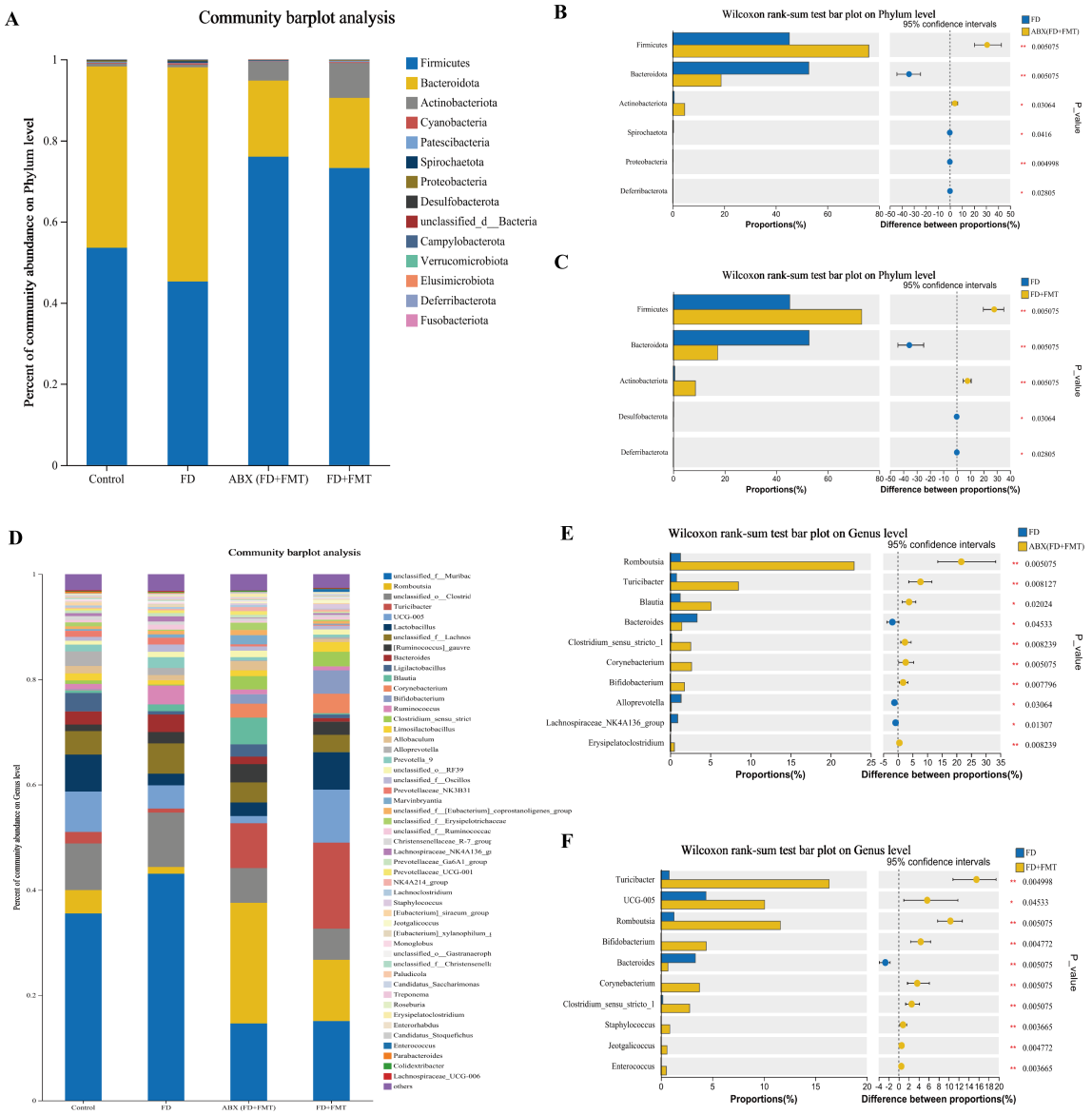


FIGURE 14
Species composition analysis of the GM of FD rats after FMT. **(A)** Columnar stack diagram of species composition at the phylum level between control, FD, ABX (FD + FMT) and FD + FMT groups. **(B)** Differential strains at the phylum level between FD and ABX (FD + FMT) groups. **(C)** Differential strains at the phylum level between FD and FD + FMT groups. **(D)** Columnar stack diagram of species composition at the genus level between control, FD, ABX (FD + FMT) and FD + FMT groups. **(E)** Differential strains at the genus level between FD and ABX (FD + FMT) groups. **(F)** Differential strains at the genus level between FD and FD + FMT groups. Data were expressed as mean ± standard deviation (x ± SD) (n=6). P values were calculated using Wilcoxon rank-sum test. *$p < 0.05$, **$p < 0.01$.

Microbiota dysbiosis refers to a disruption in the microbiota structure and function, affected by environmental and host-related factors (38). This disruption surpasses the resilience and recovery capacity of microbial ecosystems, leading to GI disorders (38). Experiment I revealed that the FD group exhibited microbial dysbiosis. The literatures also confirmed that the microbial disorders that were investigated in this study contributed to FD development. For example, 16S rRNA miseq sequencing showed a link between dyspepsia symptoms and the Firmicutes and Bacteroides levels (7). The *Ligilactobacillus* and *Bifidobacterium* genera are recognized as beneficial bacteria that can stimulate the protective response of the host immune system (39, 40). Studies have shown the connection between the development of functional GI disorders and the absence of these bacteria (41, 42). This study revealed that FD might result in a reduction in Firmicutes, *Ligilactobacillus*, and *Bifidobacterium* while simultaneously elevating levels of Bacteroidetes, *Erysipelotrichaceae_UCG-003*, and *Collinsella*. The CHSGP decoction modulated the GM of FD by decreasing the levels of Bacteroidetes, *Prevotellaceae_UCG-001*, *Colidextribacter*, *Alistipes*, and *Akkermansia*, while increasing the levels of Firmicutes, *UCG-005*, *Oribacterium*, *Negativibacillus*, and *Bifidobacterium*. Moreover, the literature review suggested that the child compound endothelium corneum enhanced Firmicutes and diminished Bacteroidetes in rats, playing a therapeutic role in

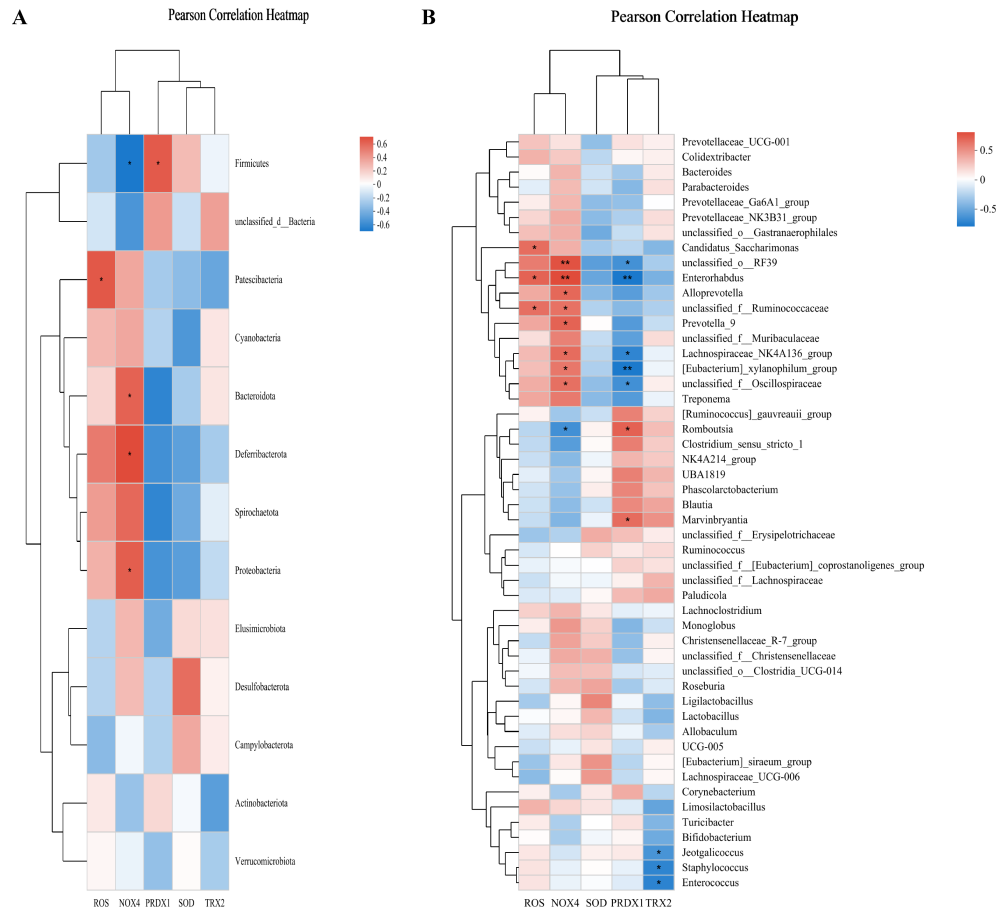


FIGURE 15
FMT regulated the GM to alleviate OS. **(A)** Heatmap of the correlation between the differential microbiota at the phylum level among the control, FD, and FD + FMT groups and the indicators of OS. **(B)** Heatmap of the correlation between the differential microbiota at the genus level among the control, FD, and FD + FMT groups and the indicators of OS. Average was used for both clinical factors and species hierarchical clustering methods. We calculated the correlation between variables using the pearson correlation coefficient. $*p < 0.05$, $**p < 0.01$.

FD (7). Chinese medicine can treat other FGIDs by affecting the GM. The XiaoChengQi decoction alleviated slow-transmitting constipation by increasing the relative abundances of *Lactobacillus*, *Muribaculaceae*, the *Prevotellaceae_NK3B31_group*, and *Roseburia* in feces and decreasing the relative abundances of the *Lachnospiraceae_ NK4A136_group* and *Desulfovibrio* (43). Tong-Xie-Yao-Fang has demonstrated efficacy in alleviating symptoms of irritable bowel syndrome with diarrhea (IBS-D) by enhancing the GM diversity and modifying the relative abundance of *Akkermansia* and *Clostridium sensu stricto 1* within gut microbial communities (44).

Microbial changes can affect the OS. Inflammation generated by *Helicobacter pylori* alters the thiol-disulfide equilibrium in the cellular redox system, a pivotal process in the pathogenesis of non-ulcer dyspepsia (45). Several studies have indicated that the GM could induce OS in various clinical contexts, including neurodegenerative diseases (46), atherosclerosis (47), and type 2 diabetic (48). Therefore, we can use drugs and probiotics to regulate the GM to alleviate OS (15). In Experiment I, we performed the correlation analysis of the differing microbial communities and OS indicators among the control, FD, and FD + CHSGP groups to investigate whether the CHSGP decoction could alleviate OS by modulating the GM. The results revealed some specific microbial species linked to OS at the phylum and genus levels. To evaluate the influence of GM depletion on the antioxidant properties of the CHSGP decoction, the rats were treated with four antibiotics to deplete their GM and observe changes in the OS-related indices (Experiment II). There were significant differences in the GM compositions between the control and ABX groups, indicating the effectiveness of antibiotics in depleting the microbiota. We compared OS-related indicators in the ABX (FD) and ABX (FD + CHSGP) groups in Experiment II. The results indicated that the CHSGP decoction failed to adequately modulate OS after the microbiota depletion. To verify that CHSGP relied on the GM to alleviate OS, we administered a fecal microbiota solution from the FD + CHSGP group to monitor changes in the OS indicators. The findings showed that the FMT treatment alleviated OS and repaired the mitochondrial structure damage. This result confirmed that CHSGP relied on the GM to reduce OS in FD rats. Interestingly, the ABX (FD + FMT) group showed a more significant protective effect against mitochondrial OS than the FD + FMT group. This result was likely attributable to the interference from the natural

microbiota in rats. Microbiota sequencing analysis showed changes in the microbiota composition in the ABX (FD + FMT) and FD + FMT groups compared with the FD group. We conducted the correlation analysis between microbial species differences across various groups and OS markers. The results indicated that the FMT increased the abundance of microbiota that inhibited OS. These bacteria included Firmicutes and *Romboutsia*. The FMT also suppressed microbiota that promoted OS, including Bacteroidota, Deferribacterota, *Enterorhabdus*, *Alloprevotella*, and *Prevotella-9*. Studies have proven that the above bacterial changes affect the OS process. For instance, researchers have demonstrated that Firmicutes affects glutathione production by modulating the activity of the essential enzyme glutamate-cysteine ligase catalytic subunit (Gclc) (49). Additionally, these bacteria suppress ROS accumulation by activating the cAMP response element-binding pathway (49). *Group A Streptococcus* belongs to Firmicutes, and its metabolic byproducts, including SOD, NADH oxidase, TRX, nicotinamide adenine dinucleotide oxidase, alkyl hydroperoxide reductase, and glutathione reductase, exhibit antioxidant properties (50). Furthermore, *Group A Streptococcus* possesses proteins that facilitate DNA repair and the restoration of proteins damaged by ROS. It also contains metal ion transporters that indirectly control metal homeostasis to fight OS (51). *Bacteroides fragilis* is an anaerobic bacterium that is part of the Bacteroides genus found in the human intestine. Research has indicated that purified *Bacterotoxin fragile* triggers a rise in spermine oxidase (SMO) levels in HT29/c1 and T84 colonic epithelial cells. This causes ROS production in an SMO-dependent manner (52). Although there are no particular investigations on the impact of *Romboutsia* on OS, inflammation in the pig colon has demonstrated a reduction in both *Romboutsia* levels and total antioxidant capacity activity (53). This suggested a potential correlation between the two. Deferribacterota are anaerobic bacteria capable of oxidizing many complex organic compounds and organic acids. They are classified as sulfate-reducing bacteria, and researchers have confirmed their phylogenetic position as an incomplete oxidizer based on the dissimilatory sulfate reductase phylogeny (54). An increase in *Enterorhabdus* is associated with OS-related diseases such as colitis (55) and diabetes (48), and thus, this genus is often considered an OS-promoting strain. *Alloprevotella* and *Prevotella-9* are Gram-negative anaerobic bacteria within the Prevotella genus, characterized by low tissue redox potentials (35). Santos (56) used matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF/TOF) tandem mass spectrometry to reveal that the differentially regulated protein sequences in *Prevotella* intermedia are associated with antioxidant and redox regulatory functions. Our findings demonstrated that CHSGP might alleviate mitochondrial OS in the gastric tissue of FD rats by decreasing the abundances of OS-promoting strains (Bacteroidota, Deferribacterota, *Enterorhabdus*, *Alloprevotella*, and *Prevotella-9*) and increasing the abundances of OS-inhibiting strains (Firmicutes and *Romboutsia*).

Consistent with the findings of the current investigation, researchers have found that the Shen-Ling-Bai-Zhu-San therapy modulates microbial structure, leading to the enhancement of energy metabolic pathways and the reduction in OSto treat FD (57). Furthermore, the literature has proven that herbal medicines can treat other FGIDs by modulating the relationship between OS and the GM. Network pharmacology and molecular docking analyses have revealed that the rhubarb peony decoction may provide a comprehensive therapeutic effect on the overlapping syndromes of ulcerative colitis and IBS through a multi-component, multi-target, multi-pathway biological mechanism addressing OS, immune dysfunction, and gut microbial dysbiosis (58). The Simo decoction can increase the abundance of beneficial GM to relieve OS and treat constipation (59).

The mechanism by which the CHSGP decoction restored GI movement may be associated with the alleviation of OS due to GM regulation. Specifically, the CHSGP decoction enhanced the abundances of OS-inhibiting strains (Firmicutes and *Romboutsia*) while decreasing the abundances of OS-promoting strains (Bacteroidota, Deferribacterota, *Enterorhabdus*, *Alloprevotella*, and *Prevotella-9*). Future research can be pursued from two perspectives. Firstly, we will extract and purify the chosen core microbial communities, such as the OS-inhibitory strains Firmicutes and *Romboutsia*, to assess their efficacy and safety in treating FD rats. The data gained will be implemented in the clinical treatment of patients with FD to assess their therapeutic benefits. Secondly, in terms of fundamental research, the GM exerts its effects through metabolic products; therefore, we will subsequently investigate the specific mechanisms by which CHSGP counteracts mitochondrial OS in ICC from the metabolomics perspective. On the other hand, microbial dysbiosis underlies the symptoms associated with FGIDs (60). This study also inspires us to explore the role of the microbiota in the pathogenesis and treatment of FGIDs, based on the symbiotic relationship between the microbiota and the host.

However, this study has some limitations. First, the model reproduced the diminished gastrointestinal motility characteristic with FD. However, the pathological mechanisms of FD in humans encompass not only delayed gastric emptying but also impaired gastric accommodation, hypersensitivity to gastric distension, and abnormalities in the gut-brain axis (25). Therefore, this study does not fully represent human FD. Second, differences in genetic background can affect the therapeutic applicability of findings derived from animal studies. Third, microbiota dysbiosis is a central mechanism underlying the occurrence of FD. Broad-spectrum antibiotics can reduce the GM, which not only affects the OS of ICC but may also influence GI motility through other mechanisms. For instance, the administration of ampicillin in mice resulted in the decrease in the number of enteric neurons within the colonic muscle tissue, thereby impairing GI motility (61). This study specifically evaluated OS, and we cannot rule out the possibility that the GM may contribute to the development of FD through other pathways.

Data availability statement

The data presented in the study are deposited in the Figshare repository, accession number 10.6084/m9.figshare.28389212.

Ethics statement

The animal study was approved by the ethics committee of the Shanghai University of Traditional Chinese Medicine. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

XL: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. KY: Conceptualization, Data curation, Methodology, Visualization, Writing – original draft, Writing – review & editing. YJ: Conceptualization, Methodology, Writing – review & editing, Writing – original draft. YY: Data curation, Formal analysis, Visualization, Writing – review & editing. CW: Data curation, Formal analysis, Investigation, Writing – review & editing. XW: Data curation, Methodology, Validation, Visualization, Writing – review & editing. QJ: Conceptualization, Methodology, Project administration, Writing – review & editing. ZG: Data curation, Resources, Writing – review & editing. JC: Funding acquisition, Project administration, Resources, Writing – review & editing, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization. JL: Conceptualization, Funding acquisition, Project administration, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This work was supported by the National Natural Science Foundation of China (NO.82174309) and China Medical Association of Minorities (CMAM) (No.2022Z1048-460302).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2025.1549554/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Experimental operation diagram.

SUPPLEMENTARY FIGURE 2
Experimental procedure timeline diagram.

SUPPLEMENTARY FIGURE 3
Mechanistic diagram.

References

1. Drossman DA, Hasler WL. Rome IV-functional GI disorders: disorders of gut-brain interaction. *Gastroenterology*. (2016) 150:1257–61. doi: 10.1053/j.gastro.2016.03.035

2. Barberio B, Mahadeva S, Black CJ, Savarino EV, Ford AC. Systematic review with meta-analysis: global prevalence of uninvestigated dyspepsia according to the Rome criteria. *Aliment Pharmacol Ther*. (2020) 52:762–73. doi: 10.1111/apt.16006

3. Masuy I, Van Oudenhove L, Tack J. Review article: treatment options for functional dyspepsia. *Aliment Pharmacol Ther*. (2019) 49:1134–72. doi: 10.1111/apt.15191

4. Wang Y, Jia Y, Liu X, Yang K, Lin Y, Shao Q, et al. Effect of Chaihu-Shugan-San on functional dyspepsia and gut microbiota: A randomized, double-blind, placebo-controlled trial. *J Ethnopharmacol*. (2024) 322:117659. doi: 10.1016/j.jep.2023.117659

5. Chang Y, Wei W, Tong L, Liu Y, Zhou A, Chen J, et al. Weikangning therapy in functional dyspepsia and the protective role of Nrf2. *Exp Ther Med*. (2017) 14:2885–94. doi: 10.3892/etm.2017.4892

6. Halliwell B. Free radicals and antioxidants - quo vadis? *Trends Pharmacol Sci*. (2011) 32:125–30. doi: 10.1016/j.tips.2010.12.002

7. He Y, Yang C, Wang P, Yang L, Wu H, Liu H, et al. Child compound Endothelium corneum attenuates gastrointestinal dysmotility through regulating the homeostasis of brain-gut-microbiota axis in functional dyspepsia rats. *J Ethnopharmacol*. (2019) 240:111953. doi: 10.1016/j.jep.2019.111953

8. Stavely R, Ott LC, Sahakian L, Rashidi N, Sakkal S, Nurgali K. Oxidative stress and neural dysfunction in gastrointestinal diseases: can stem cells offer a solution? *Stem Cells Transl Med*. (2023) 12:801–10. doi: 10.1093/stcltm/szad063

9. Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. *Physiol Rev*. (2014) 94:329–54. doi: 10.1152/physrev.00040.2012

10. Li L, Jia Q, Wang X, Wang Y, Wu C, Cong J, et al. Chaihu Shugan San promotes gastric motility in rats with functional dyspepsia by regulating Drp-1-mediated ICC mitophagy. *Pharm Biol*. (2023) 61:249–58. doi: 10.1080/13880209.2023.2166966

11. Wang X, Liu X, Wang Y, Yang K, Yeertai Y, Jia Q, et al. Chaihu Shugan Powder inhibits interstitial cells of cajal mitophagy through USP30 in the treatment of functional dyspepsia. *J Ethnopharmacol*. (2024) 323:117695. doi: 10.1016/j.jep.2023.117695

12. Wauters L, Talley NJ, Walker MM, Tack J, Vanuytsel T. Novel concepts in the pathophysiology and treatment of functional dyspepsia. *Gut*. (2020) 69:591–600. doi: 10.1136/gutjnl-2019-318536

13. Yu J, Meng J, Qin Z, Yu Y, Liang Y, Wang Y, et al. Dysbiosis of gut microbiota inhibits NMNAT2 to promote neurobehavioral deficits and oxidative stress response in the 6-OHDA-lesioned rat model of Parkinson's disease. *J Neuroinflamm*. (2023) 20:117. doi: 10.1186/s12974-023-02782-1

14. Migeotte I, Communi D, Parmentier M. Formyl peptide receptors: a promiscuous subfamily of G protein-coupled receptors controlling immune responses. *Cytokine Growth Factor Rev*. (2006) 17:501–19. doi: 10.1016/j.cytogfr.2006.09.009

15. Wang Y, Wu Y, Wang Y, Xu H, Mei X, Yu D, et al. Antioxidant properties of probiotic bacteria. *Nutrients*. (2017) 9:521. doi: 10.3390/nu9050521

16. Cheng X, Huang F, Zhang K, Yuan X, Song C. Effects of none-steroidal anti-inflammatory and antibiotic drugs on the oral immune system and oral microbial composition in rats. *Biochem Biophys Res Commun*. (2018) 507:420–5. doi: 10.1016/j.bbrc.2018.11.054

17. Feng W, Ao H, Peng C, Yan D. Gut microbiota, a new frontier to understand traditional Chinese medicines. *Pharmacol Res*. (2019) 142:176–91. doi: 10.1016/j.phrs.2019.02.024

18. Gou H, Su H, Liu D, Wong CC, Shang H, Fang Y, et al. Traditional medicine pien tze huang suppresses colorectal tumorigenesis through restoring gut microbiota and metabolites. *Gastroenterology*. (2023) 165:1404–19. doi: 10.1053/j.gastro.2023.08.052

19. Kennedy EA, King KY, Baldridge MT. Mouse microbiota models: comparing germ-free mice and antibiotics treatment as tools for modifying gut bacteria. *Front Physiol*. (2018) 9:1534. doi: 10.3389/fphys.2018.01534

20. Chen Q, Ma X, Xing Z, Zhao X, Zu H, Guo Z, et al. Antibiotic conditioning shapes pseudosterile mouse models by deleting colonic microbes rather than small intestinal microbes. *Microbiol Spectr*. (2023) 11:e0081423. doi: 10.1128/spectrum.00814-23

21. Shao T, Hsu R, Hacein-Bey C, Zhang W, Gao L, Kurth MJ, et al. The evolving landscape of fecal microbial transplantation. *Clin Rev Allergy Immunol*. (2023) 65:101–20. doi: 10.1007/s12016-023-08958-0

22. Ekmekciu I, von Klitzing E, Fiebiger U, Escher U, Neumann C, Bacher P, et al. Immune responses to broad-spectrum antibiotic treatment and fecal microbiota transplantation in mice. *Front Immunol*. (2017) 8:397. doi: 10.3389/fimmu.2017.00397

23. Shao S, Jia R, Zhao L, Zhang Y, Guan Y, Wen H, et al. Xiao-Chai-Hu-Tang ameliorates tumor growth in cancer comorbid depressive symptoms via modulating gut microbiota-mediated TLR4/MyD88/NF-κB signaling pathway. *Phytomedicine*. (2021) 88:153606. doi: 10.1016/j.phymed.2021.153606

24. Festing MF. On determining sample size in experiments involving laboratory animals. *Lab Anim*. (2018) 52:341–50. doi: 10.1177/0023677217738268

25. Wauters L, Dickman R, Drug V, Mulak A, Serra J, Enck P, et al. United European Gastroenterology (UEG) and European Society for Neurogastroenterology and Motility (ESNM) consensus on functional dyspepsia. *United Eur Gastroenterol J*. (2021) 9:307–31. doi: 10.1002/ueg2.12061

26. Shangguan X, Ling J, Deng J, Zeng L, Zhang Y, Xie T, et al. Effect of Chaihushugansan onapoptosis of gastric smooth musclecell and expression of NF-KB portein in rats with functional dyspepsia. *Guangxi Med Univ*. (2017) 34:481–5.

27. Jang H LIX, Yan T, Hu Y, Feng Z. Effect of Bacillus cereus in the treatment of diarrhea with viable quadruple bifidobacterium tablet in mice. *Chin J Microecol*. (2017) 29:1150–3. doi: 10.13381/j.cnki.cjm.201710008

28. Hintze KJ, Cox JE, Rompato G, Benninghoff AD, Ward RE, Broadbent J, et al. Broad scope method for creating humanized animal models for animal health and disease research through antibiotic treatment and human fecal transfer. *Gut Microbes*. (2014) 5:183–91. doi: 10.4161/gmic.28403

29. Liu C, Zhao D, Ma W, Guo Y, Wang A, Wang Q, et al. Denitrifying sulfide removal process on high-salinity wastewaters in the presence of Halomonas sp. *Appl Microbiol Biotechnol*. (2016) 100:1421–6. doi: 10.1007/s00253-015-7039-6

30. Clarke KR. Non-parametric multivariate analysis of changes in community structure. *Austral Ecol*. (1993) 18:117–43. doi: 10.1111/j.1442-9993.1993.tb00438.x

31. Jiao X, Liu N, Xu Y, Qiao H. Perfluorononanoic acid impedes mouse oocyte maturation by inducing mitochondrial dysfunction and oxidative stress. *Reprod Toxicol*. (2021) 104:58–67. doi: 10.1016/j.reprotox.2021.07.002

32. Bhattacharyya A, Chattopadhyay R, Mitra S, Crowe SE. Oxidative stress: an essential factor in the pathogenesis of gastrointestinal mucosal diseases. *Physiol Rev*. (2014) 94:329–54. doi: 10.1152/physrev.00040.2012

33. Bedard K, Krause KH. The NOX family of ROS-generating NADPH oxidases: physiology and pathophysiology. *Physiol Rev*. (2007) 87:245–313. doi: 10.1152/physrev.00044.2005

34. Mishina NM, Bogdanova YA, Ermakova YG, Panova AS, Kotova DA, Bilan DS, et al. Which antioxidant system shapes intracellular H_2O_2 gradients? *Antioxid Redox Signal*. (2019) 31:664–70. doi: 10.1089/ars.2018.7697

35. Panigrahi MK, Kaliaperumal V, Akella A, Venugopal G, Ramadass B. Mapping microbiome-redox spectrum and evaluating Microbial-Redox Index in chronic gastritis. *Sci Rep*. (2022) 12:8450. doi: 10.1038/s41598-022-12431-x

36. Wen Y, Zhan Y, Tang SY, Liu F, Wang QX, Kong PF, et al. Zhizhu decoction alleviates intestinal barrier damage via regulating SIRT1/foxO1 signaling pathway in slow transit constipation model mice. *Chin J Integr Med*. (2023) 29:809–17. doi: 10.1007/s11655-022-3539-2

37. Gamal NG, Abd-El-Salam RM, Gadelrub LN, Ahmed-Farid OA, Khayyal MT. The herbal preparation STW 5 affects serotonergic pathways in the brain and colon as well as stress parameters in experimental irritable bowel syndrome. *Neurogastroenterol Motil*. (2022) 34:e14301. doi: 10.1111/nmo.14301

38. Levy M, Kolodziejczyk AA, Thaiss CA, Elinav E. Dysbiosis and the immune system. *Nat Rev Immunol*. (2017) 17:219–32. doi: 10.1038/nri.2017.7

39. McCarthy J, O'Mahony L, O'Callaghan L, Sheil B, Vaughan EE, Fitzsimons N, et al. Double blind, placebo controlled trial of two probiotic strains in interleukin 10 knockout mice and mechanistic link with cytokine balance. *Gut*. (2003) 52:975–80. doi: 10.1136/gut.52.7.975

40. Bottacini F, Ventura M, van Sinderen D, O'Connell Motherway M. Diversity, ecology and intestinal function of bifidobacteria. *Microb Cell Fact*. (2014) 13 Suppl 1:S4. doi: 10.1186/1475-2859-13-S1-S4

41. Rakova EB, Nemichenko UM, Popkova SM, Serdyuk LV, Dolgikh VV, Ivanova EI, et al. The species characteristic of bifidobacteria in intestinal biotope of children with functional dyspepsia. *Klin Lab Diagn*. (2015) 60:50–3.

42. Petitfils C, Maurel S, Payros G, Hueber A, Agaiz B, Gazzo G, et al. Identification of bacterial lipopeptides as key players in IBS. *Gut*. (2023) 72:939–50. doi: 10.1136/gutjnl-2022-328084

43. Tuohongerbieke A, Wang H, Wu J, Wang Z, Dong T, Huang Y, et al. Xiao cheng qi decoction, an ancient Chinese herbal mixture, relieves loperamide-induced slow-transit constipation in mice: an action mediated by gut microbiota. *Pharm (Basel)*. (2024) 17:153. doi: 10.3390/ph17020153

44. Li J, Cui H, Cai Y, Lin J, Song X, Zhou Z, et al. Tong-Xie-Yao-Fang regulates 5-HT level in diarrhea predominant irritable bowel syndrome through gut microbiota modulation. *Front Pharmacol*. (2018) 9:1110. doi: 10.3389/fphar.2018.01110

45. Ebik B, Aslan N, Ekin N, Bacaksiz F, Arpa M, Neselioglu S, et al. Oxidative stress and the importance of H. pylori eradication in patients with functional dyspepsia. *Saudi J Gastroenterol*. (2022) 28:434–40. doi: 10.4103/sjg.sjg_114_22

46. Loffredo L, Ettorre E, Zicari AM, Inghilleri M, Nocella C, Perri L, et al. Oxidative stress and gut-derived lipopolysaccharides in neurodegenerative disease: role of NOX2. *Oxid Med Cell Longev*. (2020) 2020:8630275. doi: 10.1155/2020/8630275

47. Carnevale R, Nocella C, Petrozza V, Cammisotto V, Pacini L, Sorrentino V, et al. Localization of lipopolysaccharide from Escherichia Coli into human atherosclerotic plaque. *Sci Rep*. (2018) 8:3598. doi: 10.1038/s41598-018-22076-4

48. Liu N, Chen M, Song J, Zhao Y, Gong P, Chen X. Effects of *auricularia auricula* polysaccharides on gut microbiota composition in type 2 diabetic mice. *Molecules*. (2022) 27:6061. doi: 10.3390/molecules27186061

49. Yuan Y, Yang J, Zhuge A, Li L, Ni S. Gut microbiota modulates osteoclast glutathione synthesis and mitochondrial biogenesis in mice subjected to ovariectomy. *Cell Prolif*. (2022) 55:e13194. doi: 10.1111/cpr.13194

50. Yu S, Ma Q, Li Y, Zou J. Molecular and regulatory mechanisms of oxidative stress adaptation in Streptococcus mutans. *Mol Oral Microbiol*. (2023) 38:1–8. doi: 10.1111/omi.12388

51. Henningham A, Döhrmann S, Nizet V, Cole JN. Mechanisms of group A Streptococcus resistance to reactive oxygen species. *FEMS Microbiol Rev*. (2015) 39:488–508. doi: 10.1093/femsre/fuu009

52. Goodwin AC, Destefano Shields CE, Wu S, Huso DL, Wu X, Murray-Stewart TR, et al. Polyamine catabolism contributes to enterotoxigenic Bacteroides fragilis-induced colon tumorigenesis. *Proc Natl Acad Sci U S A*. (2011) 108:15354–9. doi: 10.1073/pnas.1010203108

53. Gao R, Tian S, Wang J, Zhu W. Galacto-oligosaccharides improve barrier function and relieve colonic inflammation via modulating mucosa-associated microbiota composition in lipopolysaccharides-challenged piglets. *J Anim Sci Biotechnol*. (2021) 12:92. doi: 10.1186/s40104-021-00612-z

54. Gao P, Zhang X, Huang X, Chen Z, Marietou A, Holmkvist L, et al. Genomic insight of sulfate reducing bacterial genus Desulfofaba reveals their metabolic versatility in biogeochemical cycling. *BMC Genomics*. (2023) 24:209. doi: 10.1186/s12864-023-09297-2

55. Jiang N, Liu Z, Wang H, Zhang L, Li M, Li G, et al. Alterations in metabolome and microbiome: new clues on cathelicidin-related antimicrobial peptide alleviates acute ulcerative colitis. *Front Microbiol*. (2024) 15:1306068. doi: 10.3389/fmicb.2024.1306068

56. Santos SG, Diniz CG, Silva VL, Lima FL, Andrade HM, Chapeaurouge DA, et al. Differentially regulated proteins in Prevotella intermedia after oxidative stress analyzed by 2D electrophoresis and mass spectrometry. *Anaerobe*. (2012) 18:76–82. doi: 10.1016/j.anaerobe.2011.12.008

57. Zhang S, Lin L, Liu W, Zou B, Cai Y, Liu D, et al. Shen-Ling-Bai-Zhu-San alleviates functional dyspepsia in rats and modulates the composition of the gut microbiota. *Nutr Res*. (2019) 71:89–99. doi: 10.1016/j.nutres.2019.10.001

58. Zhai L, Yang W, Li D, Zhou W, Cui M, Yao P. Network pharmacology and molecular docking reveal the immunomodulatory mechanism of rhubarb peony decoction for the treatment of ulcerative colitis and irritable bowel syndrome. *J Pharm Pharm Sci*. (2023) 26:11225. doi: 10.3389/jpps.2023.11225

59. Yi X, Zhou K, Deng N, Cai Y, Peng X, Tan Z. Simo decoction curing spleen deficiency constipation was associated with brain-bacteria-gut axis by intestinal mucosal microbiota. *Front Microbiol*. (2023) 14:1090302. doi: 10.3389/fmicb.2023.1090302

60. Saffouri GB, Shields-Cutler RR, Chen J, Yang Y, Lekatz HR, Hale VL, et al. Small intestinal microbial dysbiosis underlies symptoms associated with functional gastrointestinal disorders. *Nat Commun*. (2019) 10:2012. doi: 10.1038/s41467-019-09964-7

61. Yarandi SS, Kulkarni S, Saha M, Sylvia KE, Sears CL, Pasricha PJ. Intestinal bacteria maintain adult enteric nervous system and nitrergic neurons via toll-like receptor 2-induced neurogenesis in mice. *Gastroenterology*. (2020) 159:200–213.e8. doi: 10.1053/j.gastro.2020.03.050

frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 17 March 2025
DOI 10.3389/fimmu.2025.1563450

Check for updates

OPEN ACCESS

EDITED BY
Nar Singh Chauhan,
Maharshi Dayanand University, India

REVIEWED BY
Manu Bhambi,
Zigzoo Marketing Services Pvt Ltd, India
Sohini Mukhopadhyay,
National Institute of Science Education and Research (NISER), India
Asmaa M. M. Mawad,
Assiut University, Egypt

*CORRESPONDENCE
Ruilai Jiang
ruilai_jiang@yeah.net
Zongxin Ling
lingzongxin@zju.edu.cn
Yongtao Gao
yongtao_gao@yeah.net
Jian Wu
wujianglinxing@163.com

†These authors have contributed equally to this work

RECEIVED 20 January 2025
ACCEPTED 25 February 2025
PUBLISHED 17 March 2025

CITATION
Ding W, Cheng Y, Liu X, Zhu Z, Wu L, Gao J, Lei W, Li Y, Zhou X, Wu J, Gao Y, Ling Z and Jiang R (2025) Harnessing the human gut microbiota: an emerging frontier in combatting multidrug-resistant bacteria. *Front. Immunol.* 16:1563450. doi: 10.3389/fimmu.2025.1563450

COPYRIGHT
© 2025 Ding, Cheng, Liu, Zhu, Wu, Gao, Lei, Li, Zhou, Wu, Gao, Ling and Jiang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Harnessing the human gut microbiota: an emerging frontier in combatting multidrug-resistant bacteria

Wenwen Ding[1,2†], Yiwen Cheng[3†], Xia Liu[4†], Zhangcheng Zhu[5], Lingbin Wu[6], Jie Gao[3], Wenhui Lei[7], Yating Li[3], Xin Zhou[8,9,10,11], Jian Wu[12*], Yongtao Gao[1,2*], Zongxin Ling[3*] and Ruilai Jiang[6*]

[1]Department of Anesthesiology, Affiliated Hospital of Nantong University, Nantong, Jiangsu, China, [2]Medical School of Nantong University, Nantong, Jiangsu, China, [3]Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, National Clinical Research Center for Infectious Diseases, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang, China, [4]Department of Intensive Care Unit, The First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, Zhejiang, China, [5]Department of Preventive Medicine, School of Public Health and Management, Wenzhou Medical University, Wenzhou, Zhejiang, China, [6]Department of Intensive Care Unit, Lishui Second People's Hospital, Lishui, Zhejiang, China, [7]Jinan Microecological Biomedicine Shandong Laboratory, Jinan, Shandong, China, [8]Department of Genetics, Stanford University School of Medicine, Stanford, CA, United States, [9]Stanford Center for Genomics and Personalized Medicine, Stanford, CA, United States, [10]Stanford Diabetes Research Center, Stanford, CA, United States, [11]The Jackson Laboratory for Genomic Medicine, Farmington, CT, United States, [12]Department of Clinical Laboratory, Suzhou Municipal Hospital, Suzhou, Jiangsu, China

Antimicrobial resistance (AMR) has become a major and escalating global health threat, undermining the effectiveness of current antibiotic and antimicrobial therapies. The rise of multidrug-resistant bacteria has led to increasingly difficult-to-treat infections, resulting in higher morbidity, mortality, and healthcare costs. Tackling this crisis requires the development of novel antimicrobial agents, optimization of current therapeutic strategies, and global initiatives in infection surveillance and control. Recent studies highlight the crucial role of the human gut microbiota in defending against AMR pathogens. A balanced microbiota protects the body through mechanisms such as colonization resistance, positioning it as a key ally in the fight against AMR. In contrast, gut dysbiosis disrupts this defense, thereby facilitating the persistence, colonization, and dissemination of resistant pathogens. This review will explore how gut microbiota influence drug-resistant bacterial infections, its involvement in various types of AMR-related infections, and the potential for novel microbiota-targeted therapies, such as fecal microbiota transplantation, prebiotics, probiotics, phage therapy. Elucidating the interactions between gut microbiota and AMR pathogens will provide critical insights for developing novel therapeutic strategies to prevent and treat AMR infections. While previous reviews have focused on the general impact of the microbiota on human health, this review will specifically look at the latest research on the interactions between the gut microbiota and the evolution and spread of AMR, highlighting potential therapeutic strategies.

KEYWORDS

gut microbiota, antimicrobial resistance, colonization resistance, bacteriophage, probiotics

1 Introduction

Antimicrobial resistance (AMR) is a global health crisis, largely driven by the overuse and misuse of antibiotics. This has led to a significant rise in drug-resistant infections, contributing to millions of deaths each year (1, 2). Alarmingly, projections suggest that by 2050, AMR could be responsible for 10 million deaths annually (3, 4). The situation is made worse by the slow pace at which new antibiotics are developed and the growing presence of ESKAPE pathogens—*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species—which are leading contributors to AMR-related mortality (2, 5). These pathogens, although typically harmless and existing in a symbiotic relationship with the host, can become opportunistic in the event of immune system disruptions. This leads to infections that result from disturbances in the gut microbiota. Alterations in microbial composition, changes in bacterial metabolic activity, and shifts in local bacterial distribution further exacerbate gut dysbiosis, increasing the risk of infections. Furthermore, the slow pace of new antimicrobial drug development has failed to keep up with the rapid rise in AMR. This growing gap between rising resistance and limited new antibiotics highlights the urgent need for innovative strategies to manage infections and combat AMR.

As a response to this urgent challenge, researchers are increasingly turning to the human gut microbiota as a promising ally in the fight against AMR. The gut microbiota plays a vital role in supporting the immune system and protecting the body from infections (6–8). It helps regulate immune responses and maintains a delicate balance of microorganisms. However, disruptions to this balance—often caused by antibiotics or illness—can allow harmful, antibiotic-resistant pathogens to overgrow (5). Recent studies highlight how microbiota-based therapies, such as fecal microbiota transplantation (FMT) and probiotics, can help restore a healthy microbial balance and fight off drug-resistant infections. For example, Kellogg et al. find that succinate-producing microbiota drive tuft cell hyperplasia to protect against *Clostridioides difficile* (9). FMT has shown promise in treating recurrent *C. difficile* infections by outcompeting harmful bacteria and re-establishing a balanced microbiota, with approval from the U.S. FDA (10). Beyond FMT, other microbiota-based strategies, including prebiotics, synbiotics, postbiotics, and bacteriophage, are also being explored as potential weapons in the fight against AMR. These therapies work in various ways, such as fostering the growth of beneficial microbes, enhancing immune function, and directly inhibiting the growth of harmful bacteria.

This review will delve into the molecular mechanisms through which the gut microbiota influences AMR. It will examine the role of metabolites produced by gut bacteria, immune modulation, and competitive inhibition in shaping the body's response to infections. Moreover, we will explore the diversity of the gut microbiota in different AMR bacterial infections and assess the potential of microbiota-targeting therapies as a promising approach to combatting AMR.

2 Mechanisms of gut microbiota in combating MDR infections

The human microbiota has established a mutualistic symbiosis with its host, contributing to overall health through metabolic support, immune modulation, and protection against pathogens (11, 12). A key mechanism by which the gut microbiota combats multidrug-resistant (MDR) infections is colonization resistance (CR), which prevents the colonization and overgrowth of both external pathogens and resident pathobionts (13). The gut microbiota employs a multifaceted defense strategy to protect the host, including nutritional competition, niche exclusion, contact-dependent inhibition, and the production of antimicrobial peptides and inhibitory metabolites (Figure 1). Furthermore, the microbiota contributes to mucosal barrier integrity, creates oxygen-limited environments that are inhospitable to many pathogens, and modulates immune responses to enhance immune tolerance and protection (13–15). Collectively, these mechanisms form a robust defense system that restricts the establishment and proliferation of harmful microorganisms, thereby safeguarding gut health and preventing MDR infections. A deeper understanding of how the gut microbiota limits pathogen colonization can inform the development of innovative therapeutic strategies to address the growing challenge of AMR. Harnessing the potential of the gut microbiota to enhance CR offers a promising avenue to combat MDR infections and mitigate the global threat of AMR.

2.1 Nutritional competition

The gut microbiota plays a critical role in preventing drug-resistant bacterial infections through nutritional competition, a mechanism that revolves around the depletion of essential resources required for bacterial growth. By efficiently consuming nutrients such as carbon sources, nitrogen sources, and metal ions, the gut microbiota limits their availability to potential pathogens, thereby restricting their growth and colonization. A diverse microbial community enhances this competitive edge, as highlighted by Spragge et al., who demonstrated that a rich microbial ecosystem intensifies nutrient competition, effectively outcompeting harmful pathogens (16). For example, the commensal bacterium *Klebsiella michiganensis* has been shown to outcompete *Escherichia coli in vitro* by depleting essential nutrients, limiting *E. coli*'s survival and growth (17). Similarly, commensal strains of *E. coli*, such as HS and Nissle 1917, inhibit the colonization of pathogenic *E. coli* O157:H7 by consuming sugars critical for the pathogen's survival (18, 19). Genetic studies have

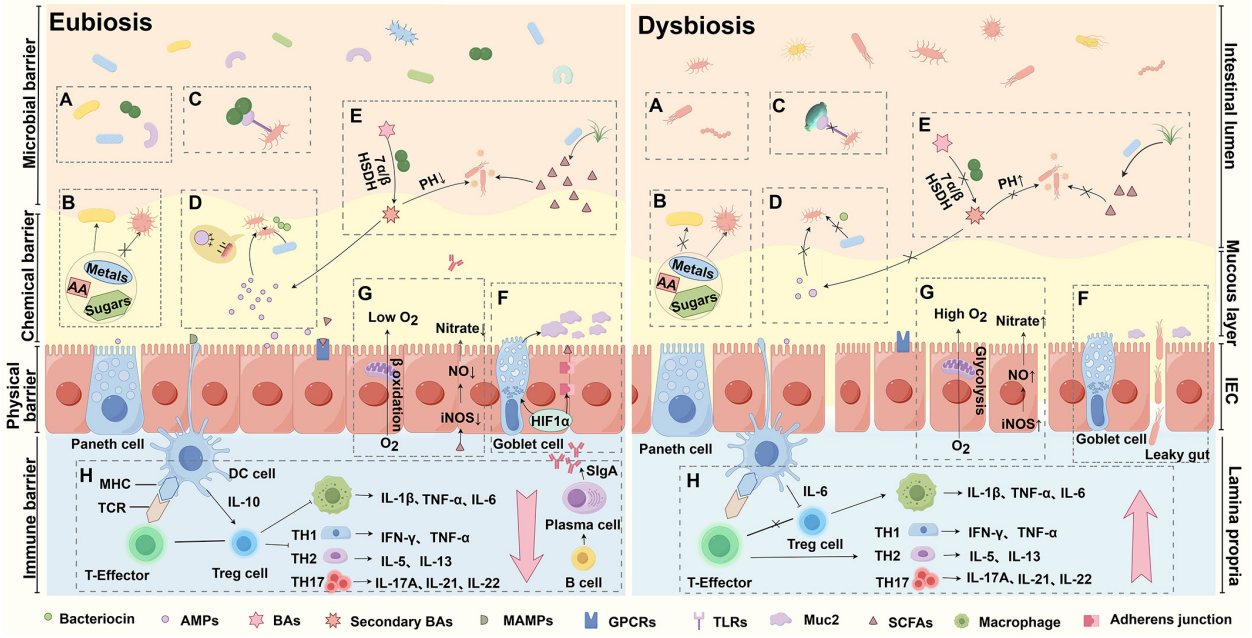


FIGURE 1
Mechanisms of gut microbiota in combating MDR infections. **(A)** Niche Exclusion: Dysbiosis reduces microbiota diversity, weakening pathogen exclusion. **(B)** Nutritional Competition: Healthy microbiota outcompetes pathogens for nutrients, thereby limiting the growth of pathogens by outcompeting them for resources. **(C)** Contact-Dependent Inhibition (CDI): Bacteria deliver toxins via cell contact, inhibiting or killing neighboring cells. **(D)** Antimicrobial Peptides (AMPs): Short-chain fatty acids (SCFAs) induce AMP synthesis, regulating microbiota and exerting antimicrobial effects. **(E)** Inhibitory Metabolites: SCFAs and secondary bile acids (BAs) inhibit pathogens or modulate immunity. **(F)** Mucosal Barrier: Dysbiosis reduces Muc2 production, compromising the intestinal barrier and increasing infection risk. **(G)** Oxygen Limitation: Inflammation, driven by iNOS and NADPH oxidase, limits oxygen availability during infection. **(H)** Immune Modulation: Microbiota activates Toll-like receptors (TLRs), crucial for immune signaling and defense.

further illuminated the role of nutrient competition in CR. For example, the gut microbiota's uptake of dietary amino acids, such as tryptophan and arginine, plays a crucial role in preventing infections by pathogens like *Citrobacter rodentium*. However, a high-protein diet can enhance *C. rodentium* colonization in mice, underscoring the influence of nutrient availability on microbial dynamics (20). This balance is also evident in the case of *C. difficile*, where the gut microbiota competes for essential amino acids, restricting the pathogen's proliferation (21). Interestingly, *C. difficile* attempts to counteract this by upregulating indole, a tryptophan metabolite, to promote its growth. Nevertheless, the microbiota's efficient nutrient utilization limits the pathogen's ability to exploit this strategy, further preventing its overgrowth (22). In addition to organic nutrients, metal ions such as iron, zinc, and manganese are critical for microbial survival and virulence. These metals are often scarce in the gut and are further sequestered by the host during inflammatory responses to limit pathogen access. Commensal bacteria, such as *E. coli* strain Nissle 1917, employ specialized mechanisms to acquire iron, providing protection against *Salmonella* infections (23). Conversely, pathogens like *Vibrio cholerae* and *Campylobacter jejuni* rely on efficient zinc uptake to survive and thrive in the gastrointestinal tract (24). By outcompeting pathogens for these vital nutrients, the gut microbiota establishes a robust defense mechanism, highlighting the critical role of nutritional competition in preventing infections and maintaining gut health. This understanding offers valuable insights into developing strategies to enhance CR and combat the growing threat of multidrug-resistant infections.

2.2 Niche exclusion

Niche exclusion is a critical strategy employed by the gut microbiota to limit pathogen colonization by occupying physical niches, depleting essential nutrients, or producing inhibitory metabolites. These competitive interactions are essential for maintaining gut homeostasis and preventing infections. Microbial species, particularly those genetically similar, often engage in direct competition. For instance, *K. pneumoniae*, *Citrobacter*, and *Clostridium* species inhibit the colonization of more pathogenic relatives through competitive exclusion (25–27). The "nutrient ecotope" hypothesis, introduced by Freter et al., provides a framework for understanding microbial competition in the gut. It posits that the availability of specific nutrients shapes the abundance and distribution of microbial species, with gut microbes actively consuming resources to define their niche (28). For example, Munehiro et al. isolated a symbiotic bacterial complex from healthy human fecal samples that inhibited Enterobacteriaceae, particularly *Klebsiella*, by regulating the availability of gluconates, a key nutrient for bacterial growth (19).

Beyond nutrient competition, microbial species can coexist by acquiring novel traits. Sweeney et al. demonstrated that two closely related *E. coli* strains coexisted in the same niche by acquiring a high-affinity gluconate transporter, enabling them to outcompete other bacteria for the same carbon source (29). This highlights how adaptive resource uptake can shift competitive dynamics. Adhesion to mucosal surfaces is another crucial aspect of niche exclusion, as it prevents the colonization of exogenous pathogens. Kasper et al. showed that the IgA response facilitates *Bacteroides fragilis* in occupying a stable mucosal niche, where it inhibits harmful bacteria (30). This underscores the interplay between microbial communities and host immune responses in mediating niche occupancy. Additionally, some microbial species modify their environment to enhance colonization while excluding competitors (30, 31). For example, symbiotic bacteria limit *Salmonella typhimurium* colonization by occupying mucosal space and depleting nutrients like carbon sources and oxygen (32). Collectively, niche exclusion mechanisms—through resource competition, adhesion site occupation, and environmental modification—form a robust defense system that maintains microbial homeostasis and protects the host from infections. These insights highlight the intricate competitive dynamics within the gut microbiota and their role in preventing pathogen colonization.

2.3 Contact-dependent inhibition

Contact-dependent inhibition (CDI) is a sophisticated bacterial defense mechanism that enables bacteria to outcompete rivals through direct cell-to-cell interactions. This system involves the delivery of toxic effectors into neighboring cells, leading to growth inhibition or cell death. Initially discovered in *E. coli* strain EC93 in 2005, the CDI locus was found to contain three genes—*CdiB*, *CdiA*, and *CdiI*—which are sufficient to confer CDI activity to *E. coli* K-12 (33). Since then, CDI systems have been identified in various bacteria, including *Enterobacteriaceae*, *Moraxellaceae*, *Pasteurellaceae*, *Neisseria meningitidis*, *Yersinia pestis*, *Dickeya dadantii*, *Enterobacter cloacae* and *Photorhabdus luminescens* (34–37). The CDI system is centered on the CdiA protein, which acts as the effector by disrupting cellular processes, such as DNA degradation, in target cells (38). CdiB facilitates the transport of CdiA across the bacterial membrane, while CdiI provides immunity to the CDI^+ bacterium by protecting it from its own toxins (39). Upon contact, the CdiB/CdiA two-partner secretion system delivers toxins directly into neighboring cells, inhibiting their growth or causing cell death (40). This mechanism allows bacteria to outcompete rivals in shared environments, such as during infection or biofilm formation (41). Consequently, the CDI system is increasingly viewed as a bacterial "weapon" system used to dominate microbial ecosystems (42, 43).

Another critical antibacterial mechanism is the type VI secretion system (T6SS), employed by many Gram-negative pathogens to inject toxic proteins into neighboring cells (44–47). 6SS-mediated interactions play a vital role in interbacterial competition, particularly among *Bacteroides* species in the gastrointestinal tract, where they compete for space and resources (48, 49). For example, *B. fragilis* inhibits *B. polymorphicus* growth *in vitro* through a T6SS-dependent mechanism (45) and exhibits competitive resistance against pathogenic strains (50, 51). These findings highlight the T6SS's role in maintaining gut homeostasis and protecting against pathogenic invasion. Other secretion systems, such as Type IV and Type VII, also mediate antibacterial interactions through protein translocation and cell killing (52, 53), highlighting the importance of antagonistic interactions in the gut. Collectively, these systems demonstrate how bacteria have evolved complex strategies to shape microbial ecosystems and promote their survival. The CDI and T6SS systems represent potential targets for novel antimicrobial therapies aimed at disrupting toxin-delivery pathways, offering promising avenues to combat MDR infections and restore microbial balance in the gut (Table 1).

2.4 Antimicrobial peptides

Antimicrobial peptides (AMPs) are essential components of the innate immune system, produced by host epithelial cells, neutrophils, and other immune cells such as mast cells and Paneth cells (54, 55). These peptides exhibit bactericidal, anti-inflammatory, and anti-endotoxin properties, serving as a first line of defense against microbial threats (56). AMPs disrupt microbial membranes and inhibit pathogen growth through diverse mechanisms. For example, nisin, produced by *Lactococcus lactis*, targets lipid II (a cell wall precursor) in pathogenic bacteria, forming pores in their membranes, causing cell content leakage, and disrupting membrane integrity (57). Similarly, RegIIIβ and RegIIIγ contribute to gut defense by interacting with the G protein-coupled receptor GPR43 (58, 59). AMPs also inhibit bacterial growth through specific molecular interactions. Microcin J25 targets *E. coli* RNA polymerase, suppressing bacterial replication (60), while colicins, produced by *E. coli*, degrade tRNA or disrupt proton gradients to inhibit pathogenic metabolism (61). Beyond their direct antimicrobial effects, AMPs regulate the gut microbiota, preventing the overgrowth of opportunistic pathogens and supporting immune homeostasis (62). For instance, oral administration of AMPs has been shown to alleviate intestinal inflammation induced by Enterohemorrhagic *E. coli* and modulate the gut microbiota (63). Bacteriocins, a subclass of AMPs produced by bacteria, selectively target closely related species to inhibit their growth or induce cell death, promoting competitive exclusion (64–66). For example, nisin is effective against Gram-positive bacteria like *S. aureus* and *Listeria monocytogenes* (67, 68), while class II bacteriocins such as Actifencin and Bacteroidetocin A selectively inhibit *Lactobacillus* and *Bacteroides* species, respectively, preserving microbiota diversity (69). Bacteriocins also enhance the resistance of beneficial bacteria, such as *E. coli* Nissle, against harmful microbes (70, 71). This specificity within microbial communities

TABLE 1 CDI protein and its mechanism of action.

Strains	CDI	Action mechanism	References
Firmicutes	LXG polymorphic toxin	•LXG toxins in *Streptococcus intermedius* inhibit Firmicutes via cell contact and Esx secretion	(52)
Group B Streptococcus	T7SSs	•GBS secretes LXG toxins via T7SS, killing or inhibiting neighboring bacteria by disrupting cell walls or metabolic pathways, gaining a competitive edge in microbial communities •GBS encodes an immune protein that pairs with LXG toxins, protecting itself from self-harm	(53)
E. coli	CdiA-CT	•N-terminal "entry" domains hijack membrane proteins to facilitate toxin assembly into the lipid bilayer. •CDI ionophores in *E. coli* isolates are grouped into six major classes based on entry domain structures •Ionophore domains show significant intra-group sequence variation, especially at CdiI interaction sites	(39)
E. coli EC93	CdiA	•BamA-CdiA interaction relies on a few non-conserved amino acids on BamA's extracellular surface, extending near its lateral gate •BamA's lateral gate can open without disrupting CdiA interaction	(38)
Acinetobacter baumannii, *Escherichia coli*	CdiB/CdiA	•CdiB transporters adopt a TpsB fold, with a 16-stranded β-barrel occluded by an N-terminal α-helix and extracellular loop 6, which exhibit distinct conformations •H1 and the DxxG motif are present in CdiB/TpsB transporters but absent in BamA/TamA proteins	(40)

CDI, Contact-dependent inhibition; LXG,Leu-X-Gly; T7SSs,Type VII secretion system; Tps, Type Vb secretion system.

helps maintain balance and offers therapeutic potential, such as protecting against *L. monocytogenes* infections via strains like *L. salivarius* UCC 118 (72, 73). Therefore, AMPs play a dual role in defending against infections and shaping the gut microbial landscape. Their ability to modulate microbial abundance and diversity is critical in preventing pathogen overgrowth and maintaining gut homeostasis (74). These properties highlight the potential of AMPs as therapeutic agents to combat infections and restore microbial balance.

2.5 Inhibitory metabolites

The gut microbiota produces a variety of inhibitory metabolites, including short-chain fatty acids (SCFAs) and secondary bile acids (BAs), which play critical roles in directly inhibiting pathogen growth and modulating host immune responses to maintain gut health (75, 76). SCFAs, primarily derived from the fermentation of dietary fibers by the gut microbiota (75), exhibit direct antimicrobial effects by disrupting bacterial membrane integrity and intracellular pH homeostasis. This mechanism inhibits pathogens such as *E. coli* and *Salmonella* under acidic conditions (77). Beyond their antimicrobial activity, SCFAs exert immunomodulatory effects, including promoting the differentiation of regulatory T cells (Tregs), which help mitigate excessive inflammation (78). For example, butyrate increases $Foxp3^+$ Tregs in the spleen and lymph nodes of antibiotic-treated mice (79), while propionate suppresses IL-17 production in intestinal γδT cells (80). SCFAs also enhance the intestinal epithelial barrier by promoting the integrity and function of epithelial cells, thereby strengthening mucosal immunity and preventing pathogen adhesion (81, 82). Additionally, SCFAs provide energy to intestinal epithelial cells, supporting normal cellular respiration and preventing the establishment of pathogens like *Citrobacter* (83). A key mechanism by which SCFAs maintain gut homeostasis is through the regulation of hypoxia-inducible factor 1-alpha (HIF-1α). Butyrate stabilizes HIF-1α by inhibiting histone deacetylases (HDACs) and activating G-protein-coupled receptors such as GPR43 and GPR109A. This process upregulates tight junction proteins like occludin and claudin-1, enhancing intestinal barrier function and reducing permeability (84). HIF-1α activation also promotes the secretion of β-defensins by intestinal epithelial cells, inhibiting the overgrowth of *E. coli* and *Salmonella* while supporting beneficial bacteria like *Lactobacillus* and *Bifidobacterium* (85).

Secondary BAs, such as deoxycholic acid (DCA) and lithocholic acid (LCA), are produced by specific gut microbiota species through the metabolism of primary BAs. These metabolites exhibit antimicrobial properties, inhibiting pathogens like *C. difficile* (86, 87) and *Salmonella* while selectively promoting beneficial bacteria (88, 89). DCA exerts bactericidal effects by lowering intracellular pH and disrupting bacterial membrane integrity (90, 91), while LCA indirectly enhances gut defense by stimulating the transcription of antimicrobial peptides like LL-37 (92). Secondary BAs also modulate immune responses, with certain *Bacteroidetes* species promoting Treg differentiation through BA modification, further supporting immune homeostasis (93, 94). Disruptions to the gut microbiota, such as those caused by antibiotics or dietary changes, can impair the production of these protective metabolites, increasing susceptibility to infections and pathogen overgrowth (95). Therefore, maintaining a balanced gut microbiota is essential for ensuring the continuous production of inhibitory metabolites that limit pathogen growth and support immune homeostasis. These insights highlight the therapeutic potential of modulating SCFAs and secondary BAs to combat infections and promote gut health.

2.6 Mucosal barrier

The gut mucosal barrier is a complex, multi-layered defense system that integrates physical, microbiological, immune, and chemical components to protect the gastrointestinal tract from pathogens. The intestinal epithelial barrier, including its mucus layer, serves as a physical shield against harmful microorganisms (96). Pathogens like *E. coli* can attach to epithelial cells to initiate infections, but commensal microorganisms strengthen the mucosal barrier to prevent this. Mucin 2 (Muc2), a key component of the mucus layer, is central to maintaining gut barrier integrity. Germ-free mice, which lack a normal gut microbiota, produce less Muc2 and are more susceptible to infections (97, 98). HIF-1α, a transcription factor responsive to low oxygen conditions, plays a critical role in regulating Muc2 secretion by goblet cells. This forms a protective mucus layer that prevents direct pathogen contact with epithelial cells (99). *E. coli* can also stimulate Muc2 production, highlighting the microbiota's role in host defense. Muc2-deficient mice are more vulnerable to pathogen colonization and experience severe infections, such as those caused by *L. monocytogenes* (100). Diet significantly influences mucosal barrier integrity. Mice fed a fiber-free diet develop a thinner mucus layer, increasing susceptibility to pathogens like *L. citrobacter* (101, 102). Additionally, L-fucose, a sugar produced by gut bacteria, reduces the virulence of pathogens like *Burkholderia citriodora*, further demonstrating the microbiota's role in modulating immune responses and pathogen defense (103). HIF-1α also regulates tight junctions between intestinal epithelial cells, enhancing barrier function. It upregulates tight junction proteins such as occludin, claudin-1, and ZO-1, preventing the penetration of pathogens and toxins (84). Disruption of these junctions by pathogens like *C. difficile*, which produces toxins TcdA and TcdB, increases intestinal permeability and facilitates pathogen invasion (104, 105). This underscores the critical role of the gut microbiota in maintaining epithelial barrier function. The gut microbiota further supports the mucosal barrier by producing metabolites that enhance immune function and inhibit pathogenic microbes (76). HIF-1α promotes energy metabolism in intestinal epithelial cells under hypoxic conditions, upregulating genes like GLUT1 and LDHA to reinforce barrier integrity (84). In summary, the mucosal barrier relies on a combination of mechanisms, including Muc2 secretion, tight junction regulation, and energy metabolism, to maintain gut homeostasis and protect against infections. These processes highlight the intricate interplay between the gut microbiota and host defenses in preserving intestinal health.

2.7 Oxygen-limited conditions

The gut is characterized by oxygen-limited conditions, with oxygen levels sharply decreasing in the deeper layers of the mucosal surface. This anaerobic environment is critical in shaping the composition and function of the gut microbiota. Pathogens that thrive in higher oxygen environments, such as *E. coli*, *Salmonella*, and *C. difficile*, struggle to establish themselves in this setting, while commensal bacteria, well-adapted to low oxygen, outcompete pathogens for space and nutrients (13, 106). Oxygen-limited conditions promote the growth of microbial species that produce SCFAs, which support mucosal immunity and strengthen the gut barrier. However, during infections, the oxygen balance can shift, creating a more favorable environment for pathogens. Infections trigger an inflammatory response, primarily through the activation of inducible nitric oxide synthase (iNOS) and NADPH oxidase. iNOS, encoded by the *Nos2* gene, produces nitric oxide by oxidizing L-arginine, while NADPH oxidase generates reactive oxygen species (ROS), contributing to inflammation (15, 107). Pathogens such as *S. typhimurium* and *E. coli* can induce inflammation and dysbiosis, increasing oxygen and nitrate availability in the intestinal lumen. These molecules serve as terminal electron acceptors for pathogenic bacteria, promoting their survival and growth (108, 109). Commensal bacteria play a vital role in maintaining the gut's hypoxic state, which limits pathogen expansion. For example, commensals that produce butyrate via β-oxidation reduce oxygen levels by stimulating aerobic respiration in intestinal cells (13), creating an oxygen-poor environment that hinders pathogen growth. However, in conditions of dysbiosis, where butyrate production is diminished, oxygen levels rise, supporting the growth of pathogens like *S. typhimurium* (110, 111). Inflammation and dysbiosis can further reduce butyrate-producing bacteria, exacerbating oxygen levels and promoting the growth of pathogenic anaerobes (112, 113). Therefore, oxygen-limited conditions are essential for maintaining a healthy gut microbiota and providing CR against pathogens.

2.8 Immune modulation

The gut is a central hub of the immune system, housing approximately 70–80% of the body's immune cells (114). It acts as a critical interface between the body and external pathogens, with the gut microbiota playing a pivotal role in maintaining immune homeostasis. This intricate interplay between the microbiota and the mucosal immune system is essential for both immune tolerance and defense mechanisms. The gut microbiota influences the development, maturation, and function of the immune system, impacting both innate and adaptive immunity (114). It educates the immune system to distinguish between harmful pathogens and harmless substances, such as food and beneficial microbes. A balanced microbiota promotes a tolerogenic immune response, preventing excessive activation that could lead to chronic inflammation or autoimmune diseases. One key mechanism by which the gut microbiota interacts with the immune system is through the activation of pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) (115). These receptors trigger the release of antimicrobial peptides, cytokines, and chemokines, initiating inflammatory responses to combat infections (116, 117). Additionally, the microbiota stimulates the production of IgA, an antibody that neutralizes pathogens, controls harmful bacterial colonization, and supports beneficial microbes (118). The absence of secretory IgT, an ancient immunoglobulin involved in mucosal

immunity, disrupts the microbiota in species like rainbow trout, making them more susceptible to infections (119). This highlights the evolutionary importance of immune responses in maintaining gut microbiota balance. The gut microbiota also plays a crucial role in the differentiation of Tregs, which suppress excessive immune reactions and prevent inflammation (120). Conversely, it can stimulate the production of Th17 cells, which defend against extracellular pathogens but, when overactivated, contribute to inflammatory conditions. A balanced microbiota maintains the equilibrium between Tregs (anti-inflammatory) and Th17 cells (pro-inflammatory), supporting gut homeostasis. Beneficial microbes produce SCFAs, such as butyrate, propionate, and acetate, which possess anti-inflammatory properties and enhance Treg function (121, 122). SCFAs also contribute to immune regulation by promoting the survival of memory T cells and maintaining gut barrier integrity, preventing "leaky gut" and systemic infections (78). Overall, the gut microbiota is essential for immune homeostasis. Dysbiosis can increase susceptibility to pathogen colonization, infections, and inflammatory or autoimmune disorders.

3 Roles of gut microbiota in different types of MDR infections

The escalating global threat of AMR, fueled by the widespread misuse and overuse of antimicrobials, has underscored the urgent need for innovative solutions. Multidrug-resistant (MDR) pathogens, including *C. difficile* and the ESKAPE (ESKAPCE) group, pose significant challenges to modern healthcare systems (123). These pathogens exhibit resistance to conventional antibiotics, leading to treatment failures, recurrent infections, prolonged hospitalizations, and increased mortality rates. The scarcity of effective therapeutic options exacerbates this crisis (124). Emerging evidence highlights the pivotal role of the gut microbiota in modulating the spread and progression of AMR (124). The gut microbiota, a complex microbial ecosystem, can either facilitate or impede the colonization of drug-resistant pathogens. Understanding the intricate interactions between the gut microbiota and MDR bacteria is critical for developing novel strategies to combat resistant infections (Figure 2, Table 2).

3.1 E. faecium

E. faecium, a Gram-positive facultative anaerobe, is a commensal member of the human gut microbiota, playing a role in digestion and homeostasis (149). However, under antibiotic pressure, it can transform into a pathogenic organism, particularly in immunocompromised or hospitalized individuals (150). Antibiotic-resistant strains, such as vancomycin-resistant *E. faecium* (VRE), pose significant clinical challenges, causing infections like urinary tract infections (UTIs), bacteremia, and endocarditis. The acquisition of resistance genes through horizontal gene transfer exacerbates treatment difficulties, leading to increased morbidity, prolonged hospital stays, and higher healthcare costs.

Studies have demonstrated that reduced expression of antimicrobial peptides RegIIIβ and RegIIIγ enhances susceptibility to VRE infections (151). Antibiotic-induced disruption of the gut microbiota diminishes RegIIIγ production, impairing VRE control. However, stimulation with the TLR7 ligand riquimod (R848) has been shown to restore RegIIIγ levels, facilitating VRE clearance in antibiotic-treated mice (152). Additionally, Kim et al. identified a four-strain *Blautia producta* consortium that restores resistance to VRE post-antibiotic treatment by producing a lantibiotic similar to nisin-A, which inhibits VRE growth (125). High lantibiotic gene abundance in at-risk patients correlates with reduced *E. faecium* levels, and lantibiotic-producing strains prevent VRE colonization in germ-free mice, highlighting their potential as probiotics. Further research has revealed that *L. murinus* Y74 and *L. plantarum* HT121 reduce VRE colonization and restore microbiota diversity in infected mice (126). *Barnesiella* spp. have also been effective in eliminating VRE colonization and improving survival by reshaping the gut microbiota (153). Butyrate-producing bacteria contribute to microbiota restoration and VRE suppression (127). FMT has emerged as a promising intervention, with studies reporting successful VRE decolonization in numerous patients (154–156). Moreover, *Enterococcus* species can exacerbate *C. difficile* pathogenicity by altering gut metabolism and supporting its growth through amino acids such as leucine and ornithine (157). Thus, modulating the gut microbiota and its metabolites presents a promising strategy for preventing and treating *E. faecium* infections.

3.2 *S. aureus*

S. aureus remains a leading cause of severe, life-threatening infections (158, 159), with methicillin-resistant *S. aureus* (MRSA) posing a particularly significant threat. MRSA is associated with high morbidity and mortality rates, especially among hospitalized adults (2, 160). MRSA frequently colonizes the gut, particularly in patients exposed to antibiotics or with critical illnesses, disrupting the normal gut microbiota. Its virulence factors—including enzymes, toxins, and biofilm formation—facilitate tissue invasion, induce inflammation, and impair immune responses, exacerbating infection severity and complicating treatment (161).

MRSA's ecological adaptations and nutritional competitiveness enhance its ability to colonize the gut and initiate infections. Genetic mutations and structural changes enable MRSA to efficiently metabolize carbohydrates, synthesize its cell wall, and produce energy under low-oxygen conditions, allowing it to thrive in the gut environment. However, the presence of beneficial bacteria can counteract its growth (162). Probiotics have demonstrated potential in reducing *S. aureus* enterotoxin production by altering the gut environment without significantly affecting bacterial growth. For instance, *S. lugdunensis* produces lugdunin, which directly inhibits *S. aureus* growth (128, 163). In MRSA-infected mice, a decline in butyrate-producing bacteria correlates with

10.3389/fimmu.2025.1563450

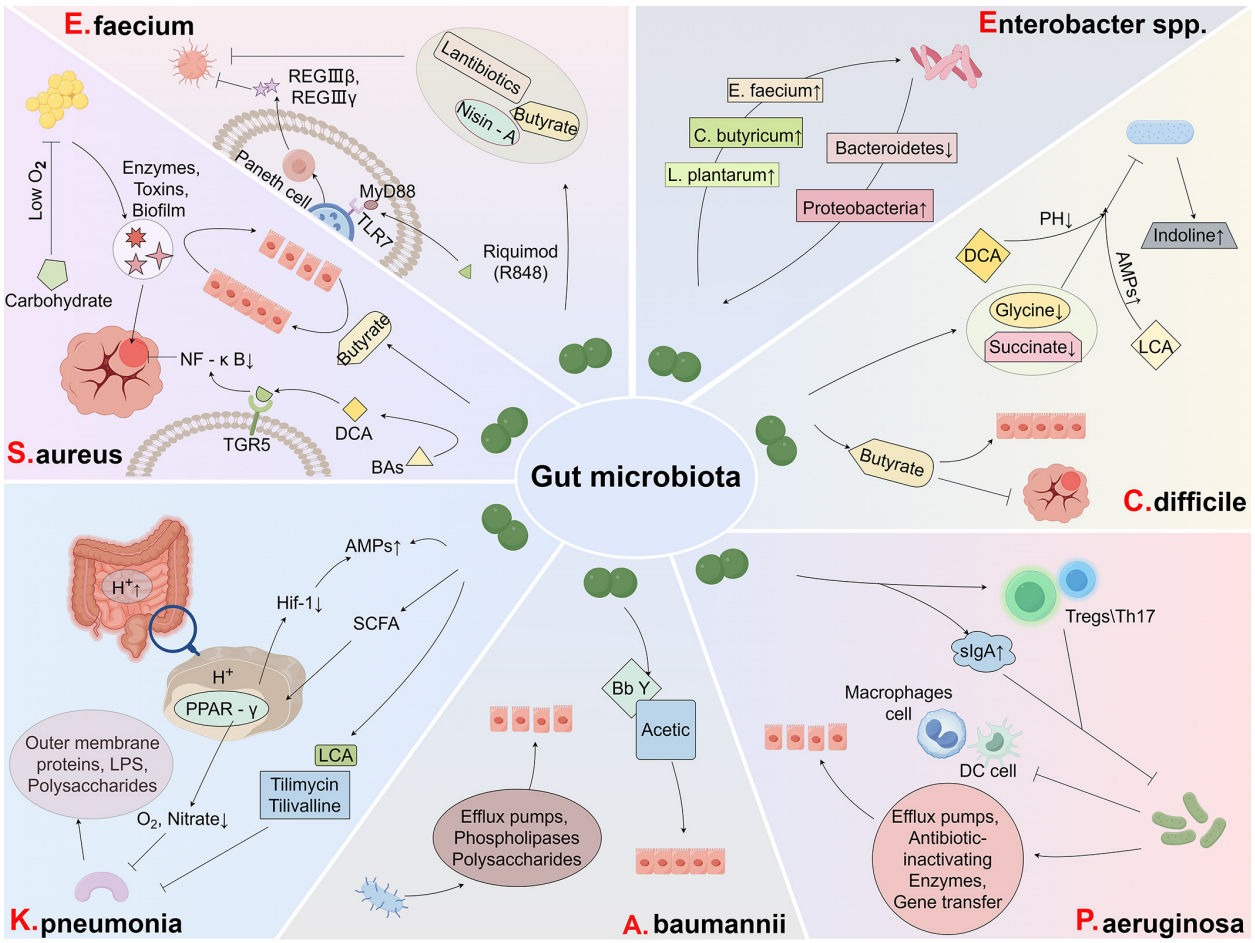


FIGURE 2
Roles of gut microbiota in different types of MDR infections. *Enterococcus faecium*: The gut microbiota resists *E. faecium* by secreting antibacterial substances that inhibit its growth. *Staphylococcus aureus*: The gut microbiota counters *S. aureus* by restoring the mucosal barrier and suppressing inflammation, thereby limiting bacterial colonization. *Klebsiella pneumoniae*: The release of beneficial metabolites and induction of intracellular acidification by the gut microbiota inhibit the growth of *K. pneumoniae*, curbing its spread. *Acinetobacter baumannii*: Restoration of the mucosal barrier by the gut microbiota prevents the invasion and colonization of *A. baumannii*, enhancing host defense. *Pseudomonas aeruginosa*: Through immune modulation, the gut microbiota strengthens the host's immune response, effectively suppressing *P. aeruginosa* infections. *Clostridium difficile*: The gut microbiota mitigates *C. difficile* infections by reducing inflammation, regulating microbial metabolites, and restoring mucosal barrier integrity. *Enterobacter* spp.: A diverse gut microbiota inhibits *Enterobacter* species, contributing to the prevention of infections and maintaining microbial balance.

TABLE 2 Roles of gut microbiota in different types of MDR infections.

Drug-resistant bacteria	Gut microbiota/ metabolites	Action mechanism	Major findings	References
E. faecium	*E.coli* Nissle 1917	Antimicrobial peptides	•*E.coli* Nissle 1917 expression Bacteroidetocin A (#22) effectively inhibited *Enterococcus faecalis* ATCC 19433.	(69)
	Clostridium bolteae, *Blautia producta* (BP_{SCSK}), *Bacteroides sartorii* and *Parabacteroides distasonis*	Antimicrobial peptides	•↑BPS_{CSK} lantibiotic •Inhibited VRE *in vitro* •Without disturb the gut microbiota	(125)
	Two *Lactobacillus* strains (Y74 and HT121)	Niche exclusion, antimicrobial peptides	•Defense-related genes (defensin α, Apoa1, and RegIII)	(126)

(Continued)

TABLE 2 Continued

Drug-resistant bacteria	Gut microbiota/ metabolites	Action mechanism	Major findings	References
			•Restore the diversity of gut microbiota	
	Bacteroides	Inhibitory metabolites	•↑butyric acid •gut microbiota changes	(127)
S. aureus	*Staphylococcus lugdunensis*	Nutritional competition	•*Staphylococcus ludunensis* exploits *S. aureus*-secreted iron carriers, depriving *S. aureus* of iron and inhibiting its growth	(128)
	NaB	Inhibitory metabolites	•↓inflammatory cell infiltration, airway wall cell hyperplasia, and alveolar thickening •↓IL-1β, TNF-α, IL-6 •↑Arg-1 •restore the imbalanced gut microbiota •↑α-diversity and β-diversity •inhibit the phosphorylation of STAT1 in MH-S cells •promote macrophage polarization toward M2 phenotype	(129)
	Clostridium scindens	Immune Modulation, Inhibitory metabolites	•enhance epithelial barrier integrity •suppress *S. aureus* enterotoxin production	(130)
	Deoxycholic acid (DCA)	Inhibitory metabolites	•↓TNF-α, IL-1β, IL-6, MPO •↓mammary damage, inflammatory parameters •↑ZO-1, Occludin, Claudin-3 •TGR5-cAMP-PKA-NF-κB/NLRP3 pathways	(131)
	Lactomodulin	Antimicrobial peptides	•↓lactomodulin toxicity •↓IL-6, IL-1β, TNF-α	(132)
K. pneumonia	*K. oxytoca strain* MK01	Nutritional competition	•They share similar spatial niches and are therefore likely to compete for nutrients. •↓beta-glycosides • CasA enables *K. oxytoca* to outcompete *K. pneumoniae* for beta-glucosides *in vivo*.	(27)
	F18-mix	Nutritional competition	•↑microbial diversity •Gnt-k-dependent gluconic acid metabolism is involved in regulation. •↓gluconate	(19)
	Normal microbiota	Inhibitory metabolites	•↓PH, intracellular acidification •↑acetate, propionate and butyrate	(77)
	high-fiber (HF) dietary carbohydrates	Nutritional competition	•↑microbiome diversity •↑*Lactobacillus johnsonii, Bifidobacterium*	(133)

(Continued)

TABLE 2 Continued

Drug-resistant bacteria	Gut microbiota/ metabolites	Action mechanism	Major findings	References
			pseudolongum and *Lachnoclostridium* •↓Inflammation score	
	Secondary bile acids	Inhibitory metabolites	•Binge-on-chronic alcohol consumption altered the intestinal microbiota •Binge-on-chronic alcohol consumption altered the fecal metabolic profile •Secondary bile acids inhibited *K. pneumoniae* growth •Lithocholic acid inhibited the adhesion of *K. pneumoniae* to Caco-2 cells	(134)
	Taurine	Inhibitory metabolites	•remodel the microbiota •↑α-diversity, β-diversity •↑ *Deltaproteobacteria* •↑bile acid-directed activity •Taurine-derived sulfide inhibits pathogen respiration	(135)
	Lactobacillus	Niche exclusion	•↓PH •Lactulose/isomalto-oligosaccharide/inulin and fructo-oligosaccharide can enhance the inhibitory effect of *Lactobacillus* strains against KPC001	(136)
A. baumannii	*Bifidobacterium* breve strain Yakult (BbY)	Mucosal barrier	•↓weight loss and mortality •↑acetic acid •↓PH •↓endotoxin levels •↑claudin-1, occludin, and ZO-1	(137)
P. aeruginosa	Normal microbiota	Immune Modulation	•remodel the microbiota •improved metabolism and function •↓TNF-α, IFN-γ, IL-6, IL-2, IL-17 •↑Foxp3, IL-10, TGFβ1 •restore Treg/Th17 cell balance	(138)
	Marine prebiotic fucoidans	CDI	•two-partner secretion (TPS) family proteins (TpsA1/CdiA2 and TpsA2/CdiA1) •↑Bacteroides, Enterobacteriaceae and Enterococcaceae	(139)
	L. plantarum spp	CDI	•reduce biofilm formation by pathogenic bacteria •disrupt preformed biofilms	(140)
	Normal microbiota	Inhibitory metabolites	•↓Disease score, lung injury •↑Percent survival, cDC1, cDC2 •↑*Muribaculaceae*, α-diversity, β-diversity •↑SCFAs (acetate, butyrate and propionate)	(95)

(Continued)

TABLE 2 Continued

Drug-resistant bacteria	Gut microbiota/ metabolites	Action mechanism	Major findings	References
C. difficile	*B.thetaiotaomicron*, *B.vulgatus, P.copri*	Inhibitory metabolites-succinate	•SUCNR1 activation by succinic acid triggers cluster cell proliferation and IL-25 release. •↑IL-25, IL-13 •Type 2 immune response activation enhances the repair and barrier function of the intestinal epithelium	(9)
	Akkermansia muciniphila Muc, *Ruthenibacterium lactatiformans* 585-1, *Alistipes timonensis* JC136, *Muribaculum intestinale* YL27, and *Bacteroides* sp. FP24	Nutritional competition	•↓mucosal sugar (NeuAc and GlcNAc) •↑Acetic Acid, Propanoic Acid •↓PH	(21)
	C. difficile strain 630	Niche exclusion	•Non-pathogenic *C. difficile* strains inhibit the germination of pathogenic *C. difficile* spores by competing with the cogermination factor glycine rather than with nutritional competition •↓glycine	(25)
	Bifidobacterium pseudocatenulatum INIA P815, *Enterococcus faecium* FUA027, and *Streptococcus thermophilus* FUA329	Antimicrobial peptides	•UroA •↓IL-1β, IL-6, TNF-α •↑ZO-1, Occludin, Claudin-4	(105)
	Phascolarctobacterium	Nutritional competition, Immune Modulation	•↓succinate •↑IL-22 •↑host N-glycosylation	(141)
	Butyrate	Inhibitory metabolites-succinate	•Inulin, maltodextrin, and xanthan gum are purified MACs that consistently suppress CDI •*C. difficile* fitness is most consistently impacted by butyrate, rather than the other two prominent SCFAs (acetate and propionate)	(142)
	Key butyrate-producing Firmicutes bacteria	Inhibitory metabolites	•Depletion of Firmicutes butyricogenes reduced gut microbiota diversity	(143)
	Ursodeoxycholic Acid (UDCA)	Inhibitory metabolites	•↓Spore germination and outgrowth, growth, and toxin activity •Fecal bile acid metabolome changed significantly •Minimal change to the microbiota •↓IL-1 R1, TLR •FXR/FGF15 pathway	(144)
	Bifidobacteria	Niche exclusion	•↓TcdA, TcdB •↓PH •↓IL-6, IL-17, IFN-γ, TNF-α •↑IL-10	(145)
	Clostridium butyricum	Inhibitory metabolites, Immune Modulation, Mucosal barrier	•↓succinate •↓TNF-α	(146)

(Continued)

TABLE 2 Continued

Drug-resistant bacteria	Gut microbiota/ metabolites	Action mechanism	Major findings	References
			•↑IL-17A, CD4+ • enhance the gut epithelial barrier function	
Enterobacter spp.	*Lactiplantibacillus plantarum* NWAFU-BIO-BS29	Antimicrobial peptides, Mucosal barrier	•↑Plantaricin Bio-LP1 •↓TNF-α, IL-6 and IL-β •TLR4 signaling-pathway •Increased the relative abundance of beneficial-intestinal-bacteria •Improve the intestinal mucosal barrier •↑SCFAs	(74)
	Normal microbiota	Inhibitory metabolites	•↑acetate, propionate, butyrate •↓PH, intracellular acidification •↓use of O2 or NO3	(77)
	Normal microbiota	Inhibitory metabolites, Immune Modulation	•↑l-tryptophan, butyrate, TMA, 3,4-TMAB, 4-TMAP, UDCA, GCA and benzoate • Microbiome metabolites as cytotoxic stressors, inducers of apoptosis and inhibitors of mitochondrial function	(82)
	Butyrate-producing bacteria	Inhibitory metabolites	•↑weight, prolonged survival •↑SCFAs (acetate, butyrate and propionate) •Delayed colonization in SPF-R mice is most prominent in the cecum •↑α-diversity •SCFA producing bacteria within the Firmicutes phylum were significantly elevated	(83)
	Butyricicoccus, Faecalibacterium, Ruminococcus, Collinsella, and *Coriobacterium*	Inhibitory metabolites	•↑UDCA •↓TGR5-NF-κB axis •↑SCFAs (acetate) •improve intestinal homeostasis •↓IL-6, TNF-α, IL-1β •↑IL-10 •↑tight-junction-related protein, occludin	(89)
	A mixture of formate, acetate, propionate, butyrate, valerate, isobutyrate, isovalerate, lactate, 5-aminovalerate and ethanol	Inhibitory metabolites	•↓PH •Antibiotic-reduced metabolites (acetate, propionate, butyrate, valerate) inhibit carbapenem-resistant *E. coli*	(147)
	Supernatants of *B. thetaiotaomicron* and *B. adolescentis*	Inhibitory metabolites	•↑SCFAs (acetate, butyrate and propionate) •All bacterial strains were maximally inhibited at pH 5.75	(148)

3,4-TMAB, 3-methyl-4-(trimethylammonio)butanoate; 4-TMAP, 4-(trimethylammonio)pentanoate; Arg-1, arginase-1; cAMP, cyclic adenosine monophosphate; CD, cluster of differentiation; cDC, conventional dendritic cells; CDI, contact-dependent inhibition; FGF15, fibroblast growth factor 15; Foxp3, forkhead box protein P3; FXR, farnesoid X receptor; GCA, glycocholic acid; GlcNAc, N-acetylglucosamine; Gnt-k, gluconate kinase; IFN, interferon; IL, interleukin; MACs, microbiota-accessible carbohydrates; MPO, myeloperoxidase; NaB, sodium butyrate; NeuAc, N-Acetylneuraminic Acid; NF-κB, nuclear factor kappa-B; NLRP3, NOD-like receptor thermal protein domain associated protein 3; PH, potential of hydrogen; PKA, protein kinase A; Reg, regenerating family member; SCFAs, short-chain fatty acid; SPF, specific pathogen-free; STAT1, signal transducer and activator of transcription 1; SUCNR1, succinate receptor 1 Curated; Tcd, toxin C. difficile; TGF, transforming growth factor; TGR5, takeda G-protein-coupled receptor 5; Th, T helper cell; TLR, Toll-like receptors; TMA, trimethylamine; TNF, tumor necrosis factor; Treg, regulatory T cell; UDCA, ursodeoxycholic acid; UroA, urolithin A; ZO-1, zonula occludens-1.
↓: downregulation; ↑: upregulation.

reduced butyrate levels in the gut and bloodstream. Butyrate supplementation has been shown to restore gut mucosal integrity and enhance immune function, offering a promising therapeutic strategy (129). Additionally, *Clostridium scindens* converts primary bile acids (BAs) to secondary BAs, such as DCA, which may enhance the antimicrobial properties of cell membranes (164, 165). Co-culturing *C. scindens* with colonic cells improves cell viability and strengthens the gut barrier, mitigating damage from *S. aureus* infections (130). DCA has also been shown to reduce *S. aureus*-induced mastitis in mice by activating the TGR5 receptor, which suppresses inflammatory pathways like NF-κB and NLRP3 (131). Dysbiosis impairs DCA production and TGR5 activation, worsening MRSA infections. However, restoring the microbiota with beneficial bacteria, such as *C. scindens* or segmented filamentous bacteria, improves infection outcomes (166). Furthermore, targeting the microbiota through genetic modulation, such as CYP1A1 knockdown, reduces harmful metabolites like cadaverine, offering protection against MRSA-induced sepsis (167). This evidence highlights the crucial role of the gut microbiota in both preventing and treating MRSA infections, offering new therapeutic possibilities beyond antibiotics.

3.3 K. pneumonia

K. pneumoniae is a highly virulent, antibiotic-resistant Gram-negative bacterium that causes severe infections, particularly in immunocompromised individuals (168). Its pathogenicity is driven by several factors: outer membrane proteins that facilitate adhesion and immune evasion, lipopolysaccharides that induce inflammation and septic shock, and polysaccharides that prevent engulfment by immune cells. These mechanisms complicate infection management and contribute to its persistence. The *K. oxytoca* species complex, a component of the human microbiome, produces enterotoxins such as tilimycin and tilivalline and plays a role in antibiotic resistance (169). Studies by Osbelt et al. have shown that certain *K. oxytoca* strains can reduce gut colonization by MDR *K. pneumoniae* in antibiotic-treated and gnotobiotic mouse models. This effect is largely attributed to competition for carbohydrates, such as beta-glucosides (133), which are critical for promoting resistance. These findings suggest that *K. oxytoca* strains may serve as next-generation probiotics to decolonize *K. pneumoniae* and protect against infections (27). Further research by Shen et al. demonstrated that high concentrations of LCA inhibit *K. pneumoniae* growth and reduce its adhesion to Caco-2 cells (134). Sorbara et al. highlighted the role of the gut microbiota in suppressing MDR *K. pneumoniae* by acidifying the proximal colon, which triggers SCFA-mediated intracellular acidification (77). This process activates PPAR-γ in host epithelial cells, reducing oxygen and nitrate availability, impairing the pathogen's respiration, and stabilizing HIF-1, which promotes antimicrobial peptide synthesis (110, 170). A recent study revealed that the gut microbiota exposed to *K. pneumoniae* produces sulfide via the taurine pathway, decreasing host cell respiration and preventing pathogen invasion (135). These findings highlight the crucial role of the microbiota in combating *K. pneumoniae* and point to potential therapeutic strategies.

3.4 *A. baumannii*

A. baumannii is a formidable opportunistic pathogen, particularly in hospital settings, where its ability to acquire multidrug resistance (MDR) poses a significant threat. It is a leading cause of infections in critically ill patients, including urinary tract infections, bloodstream infections, and ventilator-associated pneumonia, contributing to increased morbidity and mortality (171). While community-acquired infections are less frequently MDR, they can still result in severe outcomes (172).

A. baumannii typically colonizes the upper respiratory tract and skin, harboring a wide array of genes that confer MDR, such as those encoding carbapenemases and broad-spectrum β-lactamases. Carbapenem-resistant *A. baumannii* (CRAB) is particularly problematic in intensive care units (ICUs), where it is strongly associated with ventilator-associated pneumonia (171). The CRAB genome is equipped with numerous resistance genes and virulence factors, including efflux pumps, iron acquisition systems, secretion systems, phospholipases, and polysaccharides, which enhance its survival and colonization capabilities (173). CRAB infections are linked to prolonged ICU stays, elevated healthcare costs, and increased antibiotic use (174). The limited treatment options for widespread MDR strains prompted the World Health Organization (WHO) to classify *A. baumannii* as a "Priority Pathogen" in 2018, emphasizing the urgent need for novel antibiotics (175). Antibiotic use increases the risk of *A. baumannii* colonization and infection, likely due to the disruption of commensal bacteria that compete for ecological niches (176). Modulating the gut microbiota may offer a promising strategy to restore microbial balance and limit *A. baumannii* infections. For example, Asahara et al. demonstrated that continuous oral administration of *B. breve* strain Yakult (BbY) improved survival rates and inhibited CRAB growth in infected mice. BbY also helped mitigate intestinal environmental disruptions and maintained barrier function (137). Additionally, a positive correlation between CRAB levels and acetic acid production suggests that gut microbiota metabolites play a critical role in preserving intestinal barrier integrity (177). These findings highlight the potential of probiotic interventions to reduce *A. baumannii* infections, improve patient outcomes, and decrease reliance on antibiotics.

3.5 *P. aeruginosa*

P. aeruginosa is a Gram-negative opportunistic pathogen responsible for healthcare-associated infections (HAIs), including chronic obstructive pulmonary disease (COPD), cystic fibrosis, cancer, trauma, burns, sepsis, and ventilator-associated pneumonia (178, 179). It is a leading cause of HAIs globally, and the emergence of antimicrobial-resistant *P. aeruginosa* has led the WHO to classify it as a critical priority pathogen (180). This bacterium develops multidrug resistance (MDR) through mechanisms such as altered outer membrane permeability, efflux pump activity, production of antibiotic-inactivating enzymes, and horizontal gene transfer, making infections increasingly difficult to

treat (123, 181). Carbapenem antibiotics, such as imipenem and meropenem, are commonly used against *P. aeruginosa*, but the rise of carbapenem-resistant *P. aeruginosa* (CRPA) poses a significant threat (182). CRPA infections are associated with nearly 700,000 deaths annually, with resistance rates in Europe reaching 12.9% (183), underscoring the urgent need for novel therapeutic strategies.

A key factor in *P. aeruginosa* infections is intestinal colonization prior to ICU admission. Gomez-Zorrilla et al. found that pre-admission intestinal colonization by *P. aeruginosa* increases the risk of subsequent infection by nearly 15-fold (184). Disruption of the gut microbiota, which normally provides colonization resistance through antimicrobial compounds, facilitates pathogen invasion. Gut dysbiosis can impair host immune function, disrupt the intestinal barrier, and increase susceptibility to infections. The gut microbiota plays a critical role in immune modulation, with secretory IgA (sIgA) being a key component of mucosal immunity. sIgA, produced at mucosal surfaces such as the intestines and lungs, protects against pathogens like *P. aeruginosa* (185, 186). However, antimicrobial treatment can reduce IgA levels in the lungs, increasing the risk of respiratory infections (187). Research has shown that certain gut bacteria, such as segmented filamentous bacteria, enhance IgA production and protect against *P. aeruginosa* infection (188). Asymptomatic colonization of the gut by carbapenemase-producing Enterobacterales can lead to dysbiosis, exacerbating the severity of *P. aeruginosa* lung infections by reducing immune cells such as alveolar macrophages and conventional dendritic cells, which are critical for fighting respiratory infections (95). Microbial ecosystem treatment (MET-2), aimed at restoring a healthy gut microbiota, has shown promise in reducing *P. aeruginosa* load and promoting the growth of beneficial bacteria (189). Additionally, the gut-lung axis further illustrates the influence of gut microbiota on lung immunity. Th17 cells, a subset of T-helper cells, play a vital role in protecting the lungs from *P. aeruginosa* infections (190). Wen et al. demonstrated that transplanting host intestinal commensal bacteria restores the balance of Tregs and Th17 cells, enhancing their metabolic functions and providing protection against *P. aeruginosa* pneumonia (138). Thus, restoring gut eubiosis offers promising strategies for treating *P. aeruginosa* infections, potentially improving patient outcomes.

3.6 *C. difficile*

C. difficile is a spore-forming, toxin-producing anaerobic bacterium and a leading cause of hospital-acquired diarrhea and pseudomembranous colitis (191). It poses a significant healthcare burden, with over 500,000 infections and approximately 30,000 deaths annually, costing over $1.5 billion each year (192). The primary risk factor is antibiotic use, which disrupts the gut microbiota, promoting *C. difficile* spore germination and increasing infection risk (193, 194). While most patients respond to initial antibiotic treatments, up to 25% experience recurrence, with over 60% of relapsed patients suffering further episodes (195). This recurrent nature complicates management, as antibiotics further disrupt the microbiota, increasing relapse risk.

The gut microbiota plays a central role in regulating *C. difficile* infections through ecological competition and nutrient availability. Non-toxigenic *C. difficile* strains can inhibit toxigenic strains by depleting essential metabolites like glycine (25). Similarly, *Caulobacter* spp. inhibit *C. difficile* growth by reducing luminal succinate, a key metabolite for its proliferation (141). SCFAs, particularly butyrate, particularly butyrate, are critical in protecting against *C. difficile* infection. Butyrate strengthens the intestinal mucosal barrier and reduces inflammation (142, 196). A deficiency in butyrate has been linked to poor outcomes in FMT for recurrent infections (143). BAs, regulated by the gut microbiota, also influence *C. difficile* spore germination and resistance. Farnesoid X receptor (FXR) agonists like obeticholic acid reduce infection severity, while ursodeoxycholic acid (UCA) enhances immune responses against *C. difficile* (144, 197). These findings highlight the potential of BA modulation in managing infections. Emerging therapies targeting the microbiota show promise. Bacteriocins from *Bacillus thuringiensis* and AMPs from *Lactobacillus* species inhibit *C. difficile* growth and spore germination (198, 199). Additionally, *Enterococcus* species, often abundant in *C. difficile* patients, worsen outcomes by promoting toxin production through inflammatory effects (157). These findings suggest that modulation of the microbiota, either through direct interventions such as probiotics or through indirect approaches like BAs manipulation, could offer promising new strategies for preventing and treating *C. difficile* infections.

3.7 *Enterobacter* spp.

The genus *Enterobacter*, comprising facultative anaerobic Gram-negative bacilli within the *Enterobacteriaceae* family (200), includes the *E. cloacae* complex (ECC), a significant cause of nosocomial infections, particularly in healthcare settings (123). Carbapenem-resistant *Enterobacterales* (CRE) are especially concerning due to their resistance to broad-spectrum antibiotics, including carbapenems. Risk factors for CRE infections include prior CRE colonization, broad-spectrum antibiotic use, ICU admission, mechanical ventilation, prolonged hospital stays, and indwelling catheters (201). CRE infections are associated with higher in-hospital mortality and longer hospital stays compared to carbapenem-susceptible strains, highlighting the urgent need for effective interventions.

The gut microbiota plays a pivotal role in the development and persistence of *Enterobacter* infections. Studies show that CRE-positive individuals exhibit distinct microbiota profiles, characterized by reduced diversity and increased dominance of *Enterococcus*, *Sphingomonas*, and *Staphylococcus* (202). CRE carriers also show elevated Proteobacteria and reduced Bacteroidetes levels compared to non-carriers (203). These shifts may facilitate CRE establishment and persistence in the gut. Broad-spectrum antibiotics, commonly used to treat infections, disrupt CR, enabling CRE expansion. Antibiotics deplete microbial metabolites that suppress CRE growth while enriching nutrients that support CRE proliferation, perpetuating dysbiosis and

infection spread (147). Promisingly, certain probiotics and metabolites have shown potential in mitigating CRE colonization. Supernatants from *C. butyricum*, *E. faecium*, and *L. plantarum* suppress CRE growth in a dose-dependent manner, while those from *B. fragilis* and *B. longum* are less effective (204). Furthermore, SCFAs also differentially inhibit *Enterobacteriaceae* species, suggesting microbial metabolites play a key role in modulating CRE growth (148). These findings underscore the critical role of the gut microbiota in combating *Enterobacter* infections. The potential for microbiota-based therapies, such as probiotics or the restoration of microbial diversity, open an exciting avenue for future research. As our understanding of the gut's role in resisting *Enterobacter* spp. and other pathogens deepens, such therapies could play a key role in reducing the burden of CRE infections and improving patient outcomes.

4 Gut microbiota-based therapies for MDR infections

Advances in microbiome research, powered by omics technologies such as genomics, metagenomics, and metabolomics, have significantly enhanced our understanding of host-microbiota interactions (205). These insights highlight the potential of modulating the gut microbiota to combat MDR infections. By altering the composition and activity of the microbiota, it is possible to reduce the colonization and spread of drug-resistant pathogens, restore microbial balance, and improve gut health. Emerging strategies, including probiotics, prebiotics, synbiotics, postbiotics, FMT, and bacteriophages, are gaining traction as promising tools to address MDR infections (206–208) (Figure 3, Table 3).

4.1 Probiotics

Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits to the host by restoring or maintaining a healthy gut microbiota (212, 213). Common probiotic strains, such as *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces*, exert their effects through the production of antimicrobial substances (e.g., lactic acid, hydrogen peroxide), nutrient competition, and immune modulation (214). These mechanisms reduce infection risk, making probiotics valuable in

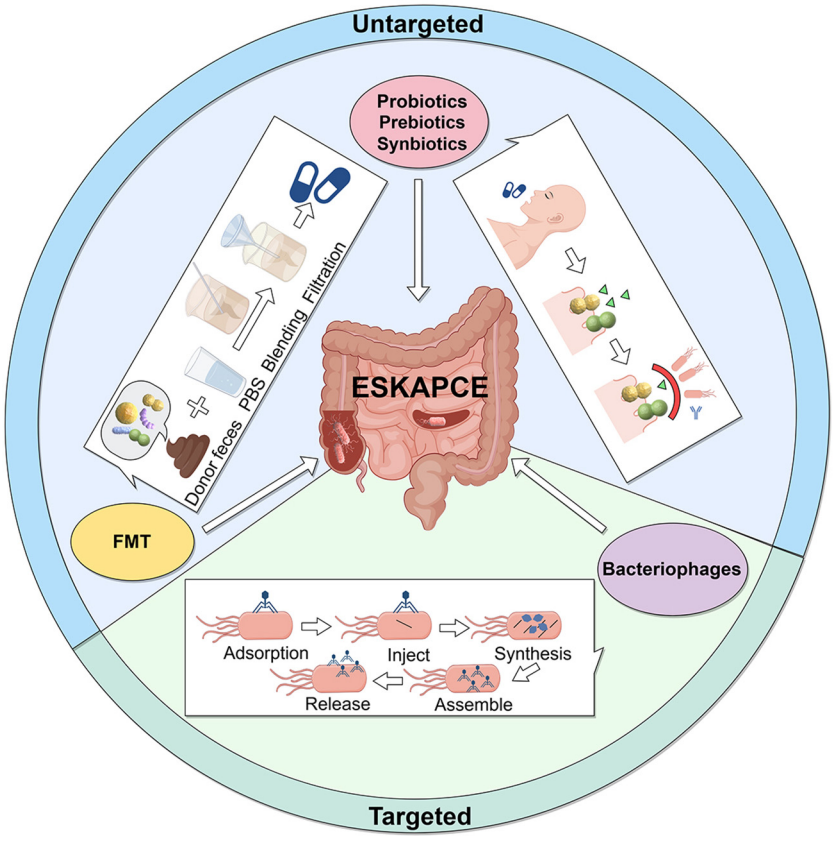


FIGURE 3
Gut microbiota-based therapies for MDR infections. Probiotics and Prebiotics: Administered in appropriate doses, they prevent pathogen colonization by competing for resources and binding sites, maintaining gut equilibrium, and enhancing immune responses. Fecal Microbiota Transplantation (FMT): Feces from healthy donors is processed and transplanted into patients to restore a balanced gut microbiota. Bacteriophages: Phages kill bacteria through a five-stage process: adsorption, injection, synthesis, assembly, and release, offering a targeted approach to treat bacterial infections.

10.3389/fimmu.2025.1563450

TABLE 3 Probiotics, prebiotics, synbiotics and postbiotics and their mechanisms of action.

Therapies		Pathogenic bacteria	Action mechanism	Major findings	References
Probiotics	*E.coli* Nissle 1917	*Enterococcus faecalis* ATCC 19433	Antimicrobial peptides	• ↑ Bacteroidetocin A (#22), Actifencin (#13) • ↓*Bacteroides, Lactobacillus* • ↑Microbial diversity	(69)
	E.coli Nissle 1917	*Enterobacteriaceae*	Antimicrobial peptides	• ↑EcN's microcins (mcmA, mchB) • The absence of mcmA, mchB resulted in significant changes in the intestinal microbial community structure	(70)
	Ligilactobacillus salivarius 7247	*Enteritidis* (SE), *Typhimurium* (ST)	Antimicrobial peptides	• ↑lactic acid • Lactic acid produced by the LS7247 strain increases the permeability of *Salmonella* strains' outer membrane. • ↑Enterolysin A, Metalloendopeptidase • ↑lantibiotic nisin S, bacteriocin (class IIb) • ATP Leakage	(73)
	Akkermansia muciniphila	*Citrobacter rodentium*	Mucosal barrier	• *A. muciniphila* confers infection resistance under fiber-rich conditions and in the absence of other mucin degraders • *A. muciniphila* prevents pathogen invasion by renewing the mucous layer and enhancing tight junction protein expression	(102)
	Bifidobacterial	*C. difficile*	Niche exclusion	• ↓TcdA,TcdB • ↓PH • ↓IL-6, IL-17, IFN-γ, TNF-α • ↑IL-10	(145)
	Clostridium butyricum	*C. difficile*	Inhibitory metabolites, Immune Modulation, Mucosal barrier	• ↓succinate • ↓TNF-α • ↑IL-17A, CD4+ • enhanced the gut epithelial barrier function	(146)
	L. plantarum spp	MDR *Pseudomonas aeruginosa, Staphylococcus aureus* and *Escherichia coli*	Contact-dependent inhibition(CDI)	• reduce biofilm formation by pathogenic bacteria • disrupt preformed biofilms	(140)
Prebiotics	Marine prebiotic fucoidans	*Pseudomonas aeruginosa*	Contact-dependent inhibition (CDI)	• two-partner secretion (TPS) family proteins (TpsA1/CdiA2 and TpsA2/CdiA1) • ↑Bacteroides, Enterobacteriaceae and Enterococcaceae	(139)
	Chitooligosaccharides	Pathogenic *Klebsiella*	Inhibitory metabolites	• ↑acetic acid • ↓propionic, butyric acids • ↓total bacterial population • It did not affect diversity and richness of the gut microbiota. • ↑Bacteroidetes • ↓Proteobacteria, Actinobacteria, Firmicutes/Bacteroidetes	(209)
	Green banana flour	/	/	• ↑Coriobacteriaceae_UCG-002, Turicibacter, Parasutterella, Gastranaerophilales_ge, and RF39_ge • amino acid biosynthesis and secondary metabolite biosynthesis	(210)

(Continued)

10.3389/fimmu.2025.1563450

TABLE 3 Continued

Therapies		Pathogenic bacteria	Action mechanism	Major findings	References
	Galacto-Oligosaccharide	*E. coli* O157	Mucosal barrier	• ↓IL-6, IL-1β, TNF-α • ↑MUC2, ZO1, Claudin and Occludin • ↑SCFAs • ↑Microbial diversity	(211)
Synbiotics	*Bifidobacterium breve* strain Yakult (BbY), galactooligosaccharides (GOS)	Multidrug-Resistant *Acinetobacter baumannii*	Mucosal barrier	• ↓weight loss and mortality • ↑acetic acid • ↓PH • ↓endotoxin levels • ↑claudin-1, occludin, and ZO-1	(137)
	Lactobacillus with lactulose/isomalto-oligosaccharide/inulin and fructo-oligosaccharide	*K. pneumoniae*	Niche exclusion	• ↓PH • Lactulose/isomalto-oligosaccharide/inulin and fructo-oligosaccharide can enhance the inhibitory effect of *Lactobacillus* strains against KPC001	(136)
Postbiotics	Lactomodulin	MRSA,VRE	Antimicrobial peptides	• lactomodulin toxicity is minimal • ↓IL-6, IL-1β, TNF-α	(132)
	Butyrate	*S. aureus*	Inhibitory metabolites	• ↓lungs displayed inflammatory cell infiltration, airway wall cell hyperplasia, and alveolar thickening • ↓IL-1β, TNF-α, IL-6 • ↑Arg-1 • restored the imbalanced gut microbiota • ↑α-diversity and β-diversity • inhibit the phosphorylation of STAT1 in MH-S cells • promote macrophage polarization toward M2 phenotype	(129)
	Deoxycholic acid (DCA)	*S. aureus*	Inhibitory metabolites	• ↓TNF-α, IL-1β, IL-6, MPO • ↓mammary damage, inflammatory parameters • ↑ZO-1, Occludin, Claudin-3 • TGR5-cAMP-PKA-NF-κB/NLRP3 pathways	(131)
	Lactomodulin	*S. aureus*	Antimicrobial peptides	• lactomodulin toxicity is minimal • ↓IL-6, IL-1β, TNF-α	(132)
	Secondary bile acids	*K. pneumonia*	Inhibitory metabolites	• Binge-on-chronic alcohol consumption altered the intestinal microbiota. • Binge-on-chronic alcohol consumption altered the fecal metabolic profile. • Secondary bile acids inhibited *K. pneumoniae* growth • Lithocholic acid inhibited the adhesion of *K. pneumoniae* to Caco-2 cells	(134)
	Taurine	*K. pneumonia*	Inhibitory metabolites	• remodel the microbiota • ↑alpha diversity, beta diversity • ↑Deltaproteobacteria • ↑bile acid-directed activity • Taurine-derived sulfide inhibits pathogen respiration.	(135)

Arg-1, arginase-1; ATP, Adenosine triphosphate; cAMP, Cyclic adenosine monophosphate; CD, Cluster of Differentiation; EcN, Escherichia coli Nissle; IFN, interferon; IL, interleukin; mcm, microcin M; MPO, Myeloperoxidase; MUC, Mucin; NF-κB, nuclear factor kappa-B; NLRP3, NOD-like receptor thermal protein domain associated protein 3; PH, Potential of hydrogen; PKA, protein kinase A; SCFAs, Short-chain fatty acid; STAT1, signal transducer and activator of transcription 1; Tcd, Toxin; TGR5, Takeda G-protein-coupled receptor 5; TNF, tumor necrosis factor; ZO-1, Zonula occludens-1.
↓: downregulation; ↑: upregulation.

both clinical and dietary approaches to health promotion and infection prevention.

Lactobacilli species, such as *L. plantarum* and *L. rhamnosus*, exhibit antimicrobial activity against pathogens like critical role in infection prevention, with strains like MRSA (215) and VRE (156, 216). These strains enhance immune responses and promote the production of beneficial SCFAs (217, 218). For example, *L. plantarum* increases butyrate-producing bacteria, reduces proinflammatory cytokines, and strengthens intestinal barriers, lowering infection risks (219). Additionally, *L. rhamnosus* produces lactomodulin, which has bactericidal effects on resistant pathogens like MRSA and VRE (132). Certain *Lactobacillus* strains also alleviate antibiotic-associated diarrhea and *C. difficile* infections (124, 220, 221). *Bifidobacterium* species, particularly *B. longum*, improve intestinal barrier function and regulate immune responses, suppressing pathogen growth. For example, *B. longum* JDM301 inhibits toxigenic *C. difficile* growth (145). Probiotic combinations, such as *S. boulardii* with *Lactobacillus* and *Bifidobacterium*, effectively prevent the colonization of MDR pathogens like extended-spectrum beta-lactamase (ESBL)-producing bacteria (222). Pre-treatment with combinations like LactoLevure® (*L. plantarum*, *L. acidophilus*, *S. boulardii* and *B. lactis*) improves survival in rodent models infected with MDR *P. aeruginosa* (223). *C. butyricum*, a butyrate-producing bacterium, plays a key role in gut health by promoting beneficial bacteria and inhibiting pathogens (143). For example, *C. butyricum* 588 enhances antibacterial efficacy against *C. difficile* through immune modulation and reinforcement of gut mucosal barriers (146). Despite their potential, the effectiveness of probiotics varies depending on factors such as strain, dosage, and individual microbiota composition. Challenges in their broader application include unclear molecular mechanisms, strain-specific effects, antibiotic resistance, and stability issues. Metabiotic components may offer solutions to these limitations, advancing the field of microbiota-based therapies.

4.2 Prebiotics, synbiotics and postbiotics

Prebiotics are non-digestible dietary compounds that promote the growth of beneficial microorganisms, enhancing gut health (224). By supporting beneficial bacteria like *Bifidobacterium* and *Lactobacillus*, prebiotics help restore microbial balance and suppress MDR pathogens (209). Common prebiotics include human milk oligosaccharides (HMOs), inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), and dietary fibers like β-glucan, pectin, and resistant starch (55). These compounds maintain a diverse microbiota, inhibiting pathogen growth through competitive exclusion (210). For instance, GOS reduces *E. coli* adhesion by 70% *in vitro* (211, 225), while fucoidan, a marine prebiotic, promotes *Bacteroides* growth and eliminates *P. aeruginosa* in mice (139). Additionally, 1,5-anhydro-d-fructose (1,5-AF), derived from starch and glycogen, exhibits antioxidant and antibacterial properties, reduces cytokine production, and boosts *F. prausnitzii* growth and NAD biosynthesis genes (226).

Synbiotics combine prebiotics and probiotics to synergistically enhance the survival and colonization of beneficial microbes, improving gut health (224, 227). This approach enhances the efficacy of both components: probiotics utilize prebiotics as growth substrates, while prebiotics are more effectively utilized by targeted microbes. For instance, pre-cultivating *L. plantarum* with xylitol reduces *C. difficile* spore germination and decreases mortality in infected mice from 44% to 22% (228). Similarly, combining *Lactobacillus* with prebiotics like lactulose or isomalto-oligosaccharides prevents colonization by KPC-2-producing *K. pneumoniae* (136). *In vitro*, synbiotics show superior antibacterial activity against MDR pathogens like *A. baumannii* and *E. faecalis*, outperforming probiotics alone (137, 229).

Postbiotics, derived from nonviable bacteria or their metabolic by-products, include bacterial components (e.g., cell walls, enzymes, SCFAs, vitamins, peptides) and paraprobiotics (e.g., peptidoglycan, surface proteins) (213, 230). These components provide immunological benefits, with bacteriocins and AMPs disrupting bacterial membranes. For example, Nisin, produced by *Lactobacillus*, inhibits cell wall formation and enhances penetration when complexed with nanoparticles (231–233). SCFAs from fiber fermentation and secondary BAs inhibit *C. difficile* growth and boost immune defense (234, 235). Lactic acid bacteria-derived cell-free supernatants also inhibit biofilm formation, offering a potential strategy against MDR pathogens like *P. aeruginosa*, *S. aureus*, and *E. coli* (140).

Together, prebiotics, synbiotics, and postbiotics offer a powerful approach to managing MDR infections and restoring gut health. Prebiotics nourish beneficial microbes, synbiotics combine probiotics and prebiotics for synergistic effects, and postbiotics provide antimicrobial and anti-inflammatory benefits. These strategies collectively suppress harmful bacteria, enhance immune responses, and maintain gut microbial balance.

4.3 FMT

FMT is emerging as a promising strategy to combat MDR bacterial infections. It involves transferring gut microbiota from a healthy donor to a recipient to restore a balanced, functional microbial community. This restoration enhances CR to MDR pathogens through mechanisms like competitive inhibition, bacteriocin production, and immune modulation (206, 236). FMT has demonstrated significant success in treating recurrent *C. difficile* infections and severe gut dysbiosis (237, 238), with a meta-analysis reporting a 90% success rate for recurrent *C. difficile* infections (239). The FDA-approved FMT product Rebyota (RBX2660) has shown a 78.9% therapeutic success rate in this patient group (240). The success of FMT in *C. difficile* infections has spurred interest in its application to other MDR pathogens, including VRE, MRSA, and carbapenemase-producing *Enterobacter* spp (241, 242). FMT restores immune functions and enhances CR, improving pathogen clearance. For example, FMT has reversed lethal sepsis by restoring butyrate-producing Bacteroidetes (243) and eradicated VRE in both animal models and human patients (153, 244, 245). It has also

successfully cleared NDM-1 *K. pneumoniae* and other CREs (246). Additionally, FMT has treated nosocomial MRSA enteritis (247) and recurrent UTIs caused by ESBL-producing *K. pneumoniae* (248). FMT holds considerable promise for managing MDR infections and restoring gut health, particularly in patients with recurrent infections and gut dysbiosis. By improving the gut microbiota, FMT modulates the immune system, enhances pathogen clearance, and provides systemic benefits. However, challenges remain (249, 250), including identifying optimal donor profiles (251), standardizing stool processing and administration protocols, addressing long-term safety concerns, and understanding the mechanisms behind FMT's efficacy. Clear regulatory guidelines and ethical donor selection are also essential. Overcoming these challenges will enhance FMT's effectiveness and safety, solidifying its role as a key therapeutic strategy in modern medicine.

4.4 Bacteriophages

Bacteriophages (phages) are viruses that specifically infect and lyse bacterial cells, offering a targeted alternative to traditional antibiotics (252, 253). Their specificity minimizes disruption to beneficial microbiota, as they often target only certain bacterial strains (254). After eradicating their bacterial hosts, phages naturally die off, reducing accumulation and toxicity risks. Unlike antibiotics, phages do not affect human cells, avoiding harm to healthy tissue. Additionally, bacterial resistance to phages develops more slowly than to antibiotics, making phage therapy a promising tool against MDR infections.

Phage therapy has evolved into various forms to combat MDR bacteria. Personalized phage therapy isolates specific phages tailored to the infecting bacterial strain, offering customized treatment for MDR infections (255). Phage cocktails, combining multiple phages targeting the same pathogen, enhance efficacy and reduce resistance risks. Phage-driven antibiotics explore how phages can complement traditional antibiotics by weakening bacterial defenses or disrupting protective biofilms (256). Clinical trials and case studies highlight the potential of phage therapy against MDR infections caused by *S. aureus*, *P. aeruginosa*, and *A. baumannii*. For example, a six-phage combination (including E215, E217, PAK_P1, PYO2, DEV, and PAK_P4) targeting *P. aeruginosa* outperformed single-phage treatments (257, 258), while a four-phage mix achieved over 98% efficacy against *S. aureus* and reduced the minimum inhibitory concentrations (MICs) of antibiotics like vancomycin in MRSA biofilms (259, 260). This synergy between phages and antibiotics effectively eliminates persistent infections while preserving beneficial gut microbiota (261).

Phages can also support beneficial bacteria. For example, *E. faecalis* V583 carries a phage that eliminates competing strains (262). Phages like ΦCD27 reduce *A. baumannii* bacterial load without harming non-pathogenic bacteria (194, 263). Phage P3CHA inhibits *P. aeruginosa* biofilm formation in mice (264, 265), though repeated exposure can lead to biofilm resistance (266). A four-phage combination disrupts *C. difficile* biofilms *in vitro* (259, 267). Recent studies highlight phages targeting *K. pneumoniae*, including capsular mutants, showing enhanced lethality in combination therapies (268). Corbellino et al. successfully eradicated MDR *K. pneumoniae* using a custom lytic phage preparation (269). These findings underscore the potential of phage therapy as an effective tool for treating infections caused by MDR bacteria. However, there are still challenges to overcome. Immune responses and bacterial resistance can limit the effectiveness of phage therapy. The immunogenicity of phage capsid proteins may lead to rapid clearance, and bacterial resistance complicates treatment outcomes. Strategies to optimize phage combinations and delivery methods are crucial, and further research is needed to establish standardized protocols and evaluate the long-term safety of phage therapy. Despite these challenges, phage therapy remains a promising approach in the fight against antibiotic-resistant infections.

5 Conclusion

The gut microbiota plays a critical yet complex role in combating MDR bacterial infections, presenting both significant challenges and untapped potential. The resistance of MDR pathogens to multiple antibiotics limits the efficacy of conventional therapies, while the intricate variability of the microbiota complicates the development of targeted treatments. The widespread overuse of antibiotics disrupts the microbiome's balance, promoting the spread of resistance genes and increasing infection risks. Additionally, our incomplete understanding of microbiota-host immune interactions hinders the creation of effective microbiota-based therapies. Despite these challenges, leveraging the gut microbiota has emerged as a pivotal strategy in addressing MDR infections and the growing threat of AMR. A range of therapeutic approaches—including probiotics, prebiotics, synbiotics, postbiotics, FMT, bacteriophage therapy, and CRISPR-Cas-based engineered strains—show promise in modulating the microbiota to enhance host resistance. These interventions aim to restore microbial balance, strengthen protective functions, and mitigate AMR effects.

However, the complexity of the microbiota and the need for precision medicine necessitate further research into personalized microbiota-based therapies and the development of novel antibacterial agents. Future studies should focus on tailoring interventions to individual microbiome profiles, ensuring the long-term safety of microbiota modulation, and integrating microbiome-based approaches with new antibacterial compounds. Advanced technologies, such as artificial intelligence and big data analytics, can enable more precise and effective treatments for MDR infections. Ultimately, overcoming AMR requires a collaborative, multidisciplinary approach to translate innovative strategies into scalable, safe, and practical solutions. By harnessing the full potential of the gut microbiota, we can pave the way for sustainable and effective approaches to managing resistant infections, improving public health outcomes, and addressing the global antibiotic resistance crisis.

Author contributions

WD: Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. YC: Funding acquisition, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. XL: Data curation, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. ZZ: Data curation, Investigation, Methodology, Writing – original draft. LW: Data curation, Investigation, Methodology, Writing – original draft. JG: Data curation, Investigation, Methodology, Writing – original draft. WL: Data curation, Formal Analysis, Methodology, Writing – original draft. YL: Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft. XZ: Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Conceptualization, Writing – review & editing. JW: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. YG: Data curation, Formal Analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. ZL: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. RJ: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This present work was funded by the grants of the National S&T Major Project of China (2023YFC2308400), the Jiangsu Provincial Research Hospital (YJXYY202204-YSB31), the Zhejiang Provincial Natural Science Foundation of China (LQ24H090005 and LQ22H030013), Shandong Provincial Laboratory Project (SYS202202), the Fundamental Research Funds for the Central Universities (2022ZFJH003), the Taishan Scholar Foundation of Shandong Province (tsqn202103119), and the Foundation of China's State Key Laboratory for Diagnosis and Treatment of Infectious Diseases (ZZ202316 and ZZ202319).

Acknowledgments

Figures are drawn using Figdraw (https://www.figdraw.com).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author(s) declared that they were an editorial board member of Frontiers, at the time of submission. This had no impact on the peer review process and the final decision.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Naddaf M. 40 million deaths by 2050: toll of drug-resistant infections to rise by 70. *Nature*. (2024) 633:747–48. doi: 10.1038/d41586-024-03033-w

2. Antimicrobialresistancecollaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *Lancet*. (2022) 399:629–55. doi: 10.1016/s0140-6736(21)02724-0

3. Blackman LD, Sutherland TD, De Barro PJ, Thissen H, Locock KES. Addressing a future pandemic: how can non-biological complex drugs prepare us for antimicrobial resistance threats? *Mater Horiz*. (2022) 9:2076–96. doi: 10.1039/d2mh00254j

4. Gbd2021antimicrobialresistancecollaborators. Global burden of bacterial antimicrobial resistance 1990-2021: a systematic analysis with forecasts to 2050. *Lancet*. (2024) 404:1199–226. doi: 10.1016/s0140-6736(24)01867-1

5. Luo Q, Lu P, Chen Y, Shen P, Zheng B, Ji J, et al. ESKAPE in China: epidemiology and characteristics of antibiotic resistance. *Emerg Microbes Infect*. (2024) 13:2317915. doi: 10.1080/22221751.2024.2317915

6. Carvalho MJ, Sands K, Thomson K, Portal E, Mathias J, Milton R, et al. Antibiotic resistance genes in the gut microbiota of mothers and linked neonates with or without sepsis from low- and middle-income countries. *Nat Microbiol*. (2022) 7:1337–47. doi: 10.1038/s41564-022-01184-y

7. Zheng XQ, Wang DB, Jiang YR, Song CL. Gut microbiota and microbial metabolites for osteoporosis. *Gut Microbes*. (2025) 17:2437247. doi: 10.1080/19490976.2024.2437247

8. Song P, Peng Z, Guo X. Gut microbial metabolites in cancer therapy. *Trends Endocrinol Metab*. (2025) 36:55–69. doi: 10.1016/j.tem.2024.06.016

9. Kellogg TD, Ceglia S, Mortzfeld BM, Tanna TM, Zeamer AL, Mancini MR, et al. Succinate-producing microbiota drives tuft cell hyperplasia to protect against Clostridioides difficile. *J Exp Med*. (2025) 222:e20232055. doi: 10.1084/jem.20232055

10. Ghani R, Chrysostomou D, Roberts LA, Pandiaraja M, Marchesi JR, Mullish BH. Faecal (or intestinal) microbiota transplant: a tool for repairing the gut microbiome. *Gut Microbes*. (2024) 16:2423026. doi: 10.1080/19490976.2024.2423026

11. Pickard JM, Zeng MY, Caruso R, Núñez G. Gut microbiota: Role in pathogen colonization, immune responses, and inflammatory disease. *Immunol Rev*. (2017) 279:70–89. doi: 10.1111/imr.12567

12. Yang W, Cong Y. Gut microbiota-derived metabolites in the regulation of host immune responses and immune-related inflammatory diseases. *Cell Mol Immunol*. (2021) 18:866–77. doi: 10.1038/s41423-021-00661-4

13. Caballero-Flores G, Pickard JM, Núñez G. Microbiota-mediated colonization resistance: mechanisms and regulation. *Nat Rev Microbiol*. (2023) 21:347–60. doi: 10.1038/s41579-022-00833-7

14. Granato ET, Meiller-Legrand TA, Foster KR. The evolution and ecology of bacterial warfare. *Curr Biol*. (2019) 29:R521–r37. doi: 10.1016/j.cub.2019.04.024

15. Sorbara MT, Pamer EG. Interbacterial mechanisms of colonization resistance and the strategies pathogens use to overcome them. *Mucosal Immunol*. (2019) 12:1–9. doi: 10.1038/s41385-018-0053-0

16. Spragge F, Bakkeren E, Jahn MT, E BNA, Pearson CF, Wang X, et al. Microbiome diversity protects against pathogens by nutrient blocking. *Science*. (2023) 382:eadj3502. doi: 10.1126/science.adj3502

17. Oliveira RA, Ng KM, Correia MB, Cabral V, Shi H, Sonnenburg JL, et al. *Klebsiella michiganensis* transmission enhances resistance to Enterobacteriaceae gut invasion by nutrition competition. *Nat Microbiol*. (2020) 5:630–41. doi: 10.1038/s41564-019-0658-4

18. Maltby R, Leatham-Jensen MP, Gibson T, Cohen PS, Conway T. Nutritional basis for colonization resistance by human commensal *Escherichia coli* strains HS and Nissle 1917 against *E. coli* O157:H7 in the mouse intestine. *PLoS One*. (2013) 8:e53957. doi: 10.1371/journal.pone.0053957

19. Furuichi M, Kawaguchi T, Pust MM, Yasuma-Mitobe K, Plichta DR, Hasegawa N, et al. Commensal consortia decolonize Enterobacteriaceae via ecological control. *Nature*. (2024) 633:878–86. doi: 10.1038/s41586-024-07960-6

20. Caballero-Flores G, Pickard JM, Fukuda S, Inohara N, Núñez G. An enteric pathogen subverts colonization resistance by evading competition for amino acids in the gut. *Cell Host Microbe*. (2020) 28:526–33.e5. doi: 10.1016/j.chom.2020.06.018

21. Pereira FC, Wasmund K, Cobankovic I, Jehmlich N, Herbold CW, Lee KS, et al. Rational design of a microbial consortium of mucosal sugar utilizers reduces Clostridiodes difficile colonization. *Nat Commun*. (2020) 11:5104. doi: 10.1038/s41467-020-18928-1

22. Sinha AK, Laursen MF, Brinck JE, Rybtke ML, Hjørne AP, Procházková N, et al. Dietary fibre directs microbial tryptophan metabolism via metabolic interactions in the gut microbiota. *Nat Microbiol*. (2024) 9:1964–78. doi: 10.1038/s41564-024-01737-3

23. Behnsen J, Zhi H, Aron AT, Subramanian V, Santus W, Lee MH, et al. Siderophore-mediated zinc acquisition enhances enterobacterial colonization of the inflamed gut. *Nat Commun*. (2021) 12:7016. doi: 10.1038/s41467-021-27297-2

24. Gielda LM, Dirita VJ. Zinc competition among the intestinal microbiota. *mBio*. (2012) 3:e00171–12. doi: 10.1128/mBio.00171-12

25. Leslie JL, Jenior ML, Vendrov KC, Standke AK, Barron MR, O'brien TJ, et al. Protection from Lethal Clostridioides difficile Infection via Intraspecies Competition for Cogerminant. *mBio*. (2021) 12:e00522-21. doi: 10.1128/mBio.00522-21

26. Mullineaux-Sanders C, Carson D, Hopkins EGD, Glegola-Madejska I, Escobar-Zepeda A, Browne HP, et al. *Citrobacter amalonaticus* Inhibits the Growth of *Citrobacter rodentium* in the Gut Lumen. *mBio*. (2021) 12:e0241021. doi: 10.1128/mBio.02410-21

27. Osbelt L, Wende M, Almási É, Derksen E, Muthukumarasamy U, Lesker TR, et al. *Klebsiella oxytoca* causes colonization resistance against multidrug-resistant K. pneumoniae in the gut via cooperative carbohydrate competition. *Cell Host Microbe*. (2021) 29:1663–79.e7. doi: 10.1016/j.chom.2021.09.003

28. Freter R, Brickner H, Botney M, Cleven D, Aranki A. Mechanisms that control bacterial populations in continuous-flow culture models of mouse large intestinal flora. *Infect Immun*. (1983) 39:676–85. doi: 10.1128/iai.39.2.676-685.1983

29. Sweeney NJ, Klemm P, Mccormick BA, Moller-Nielsen E, Utley M, Schembri MA, et al. The *Escherichia coli* K-12 gntP gene allows E. coli F-18 to occupy a distinct nutritional niche in the streptomycin-treated mouse large intestine. *Infect Immun*. (1996) 64:3497–503. doi: 10.1128/iai.64.9.3497-3503.1996

30. Donaldson GP, Ladinsky MS, Yu KB, Sanders JG, Yoo BB, Chou WC, et al. Gut microbiota utilize immunoglobulin A for mucosal colonization. *Science*. (2018) 360:795–800. doi: 10.1126/science.aaq0926

31. Bry L, Falk PG, Midtvedt T. and Gordon, J.I. A model of host-microbial interactions in an open mammalian ecosystem. *Science*. (1996) 273:1380–3. doi: 10.1126/science.273.5280.1380

32. Kamada N, Kim YG, Sham HP, Vallance BA, Puente JL, Martens EC, et al. Regulated virulence controls the ability of a pathogen to compete with the gut microbiota. *Science*. (2012) 336:1325–9. doi: 10.1126/science.1222195

33. Aoki SK, Pamma R, Hernday AD, Bickham JE, Braaten BA, Low DA. Contact-dependent inhibition of growth in *Escherichia coli*. *Science*. (2005) 309:1245–8. doi: 10.1126/science.1115109

34. Aoki SK, Diner EJ, De Roodenbeke CT, Burgess BR, Poole SJ, Braaten BA, et al. A widespread family of polymorphic contact-dependent toxin delivery systems in bacteria. *Nature*. (2010) 468:439–42. doi: 10.1038/nature09490

35. Beck CM, Morse RP, Cunningham DA, Iniguez A, Low DA, Goulding CW, et al. CdiA from Enterobacter cloacae delivers a toxic ribosomal RNase into target bacteria. *Structure*. (2014) 22:707–18. doi: 10.1016/j.str.2014.02.012

36. Willett JL, Gucinski GC, Fatherree JP, Low DA, Hayes CS. Contact-dependent growth inhibition toxins exploit multiple independent cell-entry pathways. *Proc Natl Acad Sci U S A*. (2015) 112:11341–6. doi: 10.1073/pnas.1512124112

37. Webb JS, Nikolakakis KC, Willett JL, Aoki SK, Hayes CS, Low DA. Delivery of CdiA nuclease toxins into target cells during contact-dependent growth inhibition. *PLoS One*. (2013) 8:e57609. doi: 10.1371/journal.pone.0057609

38. Curley CL, Fedrigoni TP, Flaherty EM, Woodilla CJ, Hagan CL. Bacterial contact-Dependent inhibition protein binds near the open lateral gate in BamA prior to toxin translocation. *Biochemistry*. (2021) 60:2956–65. doi: 10.1021/acs.biochem.1c00337

39. Halvorsen TM, Schroeder KA, Jones AM, Hammarlöf D, Low DA, Koskiniemi S, et al. Contact-dependent growth inhibition (CDI) systems deploy a large family of polymorphic ionophoric toxins for inter-bacterial competition. *PLoS Genet*. (2024) 20:e1011494. doi: 10.1371/journal.pgen.1011494

40. Guerin J, Botos I, Zhang Z, Lundquist K, Gumbart JC, Buchanan SK. Structural insight into toxin secretion by contact-dependent growth inhibition transporters. *Elife*. (2020) 9:e58100. doi: 10.7554/eLife.58100

41. Ocasio AB, Cotter PA. CDI/CDS system-encoding genes of Burkholderia Thailandensis are located in a mobile genetic element that defines a new class of transposon. *PLoS Genet*. (2019) 15:e1007883. doi: 10.1371/journal.pgen.1007883

42. Hayes CS, Koskiniemi S, Ruhe ZC, Poole SJ, Low DA. Mechanisms and biological roles of contact-dependent growth inhibition systems. *Cold Spring Harb Perspect Med*. (2014) 4:a010025. doi: 10.1101/cshperspect.a010025

43. Boopathi S, Liu D, Jia AQ. Molecular trafficking between bacteria determines the shape of gut microbial community. *Gut Microbes*. (2021) 13:1959841. doi: 10.1080/19490976.2021.1959841

44. Coyne MJ, Roelofs KG, Comstock LE. Type VI secretion systems of human gut Bacteroidales segregate into three genetic architectures, two of which are contained on mobile genetic elements. *BMC Genomics*. (2016) 17:58. doi: 10.1186/s12864-016-2377-z

45. Russell AB, Wexler AG, Harding BN, Whitney JC, Bohn AJ, Goo YA, et al. A type VI secretion-related pathway in Bacteroidetes mediates interbacterial antagonism. *Cell Host Microbe*. (2014) 16:227–36. doi: 10.1016/j.chom.2014.07.007

46. Silverman JM, Brunet YR, Cascales E, Mougous JD. Structure and regulation of the type VI secretion system. *Annu Rev Microbiol*. (2012) 66:453–72. doi: 10.1146/annurev-micro-121809-151619

47. Motta EVS, Lariviere PJ, Jones KR, Song Y, Moran NA. Type VI secretion systems promote intraspecific competition and host interactions in a bee gut symbiont. *Proc Natl Acad Sci U S A*. (2024) 121:e2414882121. doi: 10.1073/pnas.2414882121

48. Chatzidaki-Livanis M, Geva-Zatorsky N, Comstock LE. Bacteroides fragilis type VI secretion systems use novel effector and immunity proteins to antagonize human gut Bacteroidales species. *Proc Natl Acad Sci U S A*. (2016) 113:3627–32. doi: 10.1073/pnas.1522510113

49. Coyne MJ, Comstock LE. Type VI secretion systems and the gut microbiota. *Microbiol Spectr*. (2019) 7:10.1128. doi: 10.1128/microbiolspec.PSIB-0009-2018

50. Hecht AL, Casterline BW, Earley ZM, Goo YA, Goodlett DR, Bubeck Wardenburg J. Strain competition restricts colonization of an enteric pathogen and prevents colitis. *EMBO Rep*. (2016) 17:1281–91. doi: 10.15252/embr.201642282

51. Cianciotto NP. The type II secretion system as an underappreciated and understudied mediator of interbacterial antagonism. *Infect Immun*. (2024) 92:e0020724. doi: 10.1128/iai.00207-24

52. Whitney JC, Peterson SB, Kim J, Pazos M, Verster AJ, Radey MC, et al. A broadly distributed toxin family mediates contact-dependent antagonism between gram-positive bacteria. *Elife*. (2017) 6:e26938. doi: 10.7554/eLife.26938

53. Job AM, Doran KS, Spencer BL. A group B streptococcal type VII-secreted LXG toxin mediates interbacterial competition and colonization of the murine female genital tract. *mBio*. (2024) 15:e0208824. doi: 10.1128/mbio.02088-24

54. Ji S, An F, Zhang T, Lou M, Guo J, Liu K, et al. Antimicrobial peptides: An alternative to traditional antibiotics. *Eur J Med Chem*. (2024) 265:116072. doi: 10.1016/j.ejmech.2023.116072

55. Wiertsema SP, Van Bergenhenegouwen J, Garssen J, Knippels LMJ. The interplay between the gut microbiome and the immune system in the context of infectious diseases throughout life and the role of nutrition in optimizing treatment strategies. *Nutrients*. (2021) 13:886. doi: 10.3390/nu13030886

56. Marissen J, Reichert L, Härtel C, Fortmann MI, Faust K, Msanga D, et al. Antimicrobial peptides (AMPs) and the microbiome in preterm infants: consequences and opportunities for future therapeutics. *Int J Mol Sci*. (2024) 25:6684. doi: 10.3390/ijms25126684

57. Cotter PD, Ross RP, Hill C. Bacteriocins - a viable alternative to antibiotics? *Nat Rev Microbiol*. (2013) 11:95–105. doi: 10.1038/nrmicro2937

58. Zhao Y, Chen F, Wu W, Sun M, Bilotta AJ, Yao S, et al. GPR43 mediates microbiota metabolite SCFA regulation of antimicrobial peptide expression in intestinal epithelial cells via activation of mTOR and STAT3. *Mucosal Immunol*. (2018) 11:752–62. doi: 10.1038/mi.2017.118

59. Moniri NH, Farah Q. Short-chain free-fatty acid G protein-coupled receptors in colon cancer. *Biochem Pharmacol*. (2021) 186:114483. doi: 10.1016/j.bcp.2021.114483

60. Nguyen HA, Dunham CM. Digging the tunnel for chemical space. *Nat Chem Biol*. (2017) 13:1061–62. doi: 10.1038/nchembio.2480

61. Cascales E, Buchanan SK, Duché D, Kleanthous C, Lloubès R, Postle K, et al. Colicin biology. *Microbiol Mol Biol Rev*. (2007) 71:158–229. doi: 10.1128/mmbr.00036-06

62. Luo Y, Song Y. Mechanism of antimicrobial peptides: antimicrobial, anti-inflammatory and antibiofilm activities. *Int J Mol Sci*. (2021) 22:11401. doi: 10.3390/ijms222111401

63. Zhao XQ, Wang L, Zhu CL, Xue XH, Xia XJ, Wu XL, et al. Oral administration of the antimicrobial peptide mastoparan X alleviates enterohemorrhagic *Escherichia coli*-induced intestinal inflammation and regulates the gut microbiota. *Probiotics Antimicrob Proteins*. (2024) 16:138–51. doi: 10.1007/s12602-022-10013-x

64. Heilbronner S, Krismer B, Brötz-Oesterhelt H, Peschel A. The microbiome-shaping roles of bacteriocins. *Nat Rev Microbiol*. (2021) 19:726–39. doi: 10.1038/s41579-021-00569-w

65. Peng Z, Wang D, He Y, Wei Z, Xie M, Xiong T. Gut distribution, impact factor, and action mechanism of bacteriocin-producing beneficial microbes as promising antimicrobial agents in gastrointestinal infection. *Probiotics Antimicrob Proteins*. (2024) 16:1516–27. doi: 10.1007/s12602-024-10222-6

66. Gu Q, Yan J, Lou Y, Zhang Z, Li Y, Zhu Z, et al. Bacteriocins: Curial guardians of gastrointestinal tract. *Compr Rev Food Sci Food Saf*. (2024) 23:e13292. doi: 10.1111/1541-4337.13292

67. Asaduzzaman SM, Sonomoto K. Lantibiotics: diverse activities and unique modes of action. *J Biosci Bioeng*. (2009) 107:475–87. doi: 10.1016/j.jbiosc.2009.01.003

68. Wu M, Ma Y, Dou X, Zohaib Aslam M, Liu Y, Xia X, et al. A review of potential antibacterial activities of nisin against Listeria monocytogenes: the combined use of nisin shows more advantages than single use. *Food Res Int*. (2023) 164:112363. doi: 10.1016/j.foodres.2022.112363

69. Elisa Heesemann Rosenkilde C, Olsen Lützhøft D, Vazquez-Uribe R, Otto Alexander Sommer M. Heterologous expression and antimicrobial potential of class II bacteriocins. *Gut Microbes*. (2024) 16:2369338. doi: 10.1080/19490976.2024.2369338

70. Sassone-Corsi M, Nuccio SP, Liu H, Hernandez D, Vu CT, Takahashi AA, et al. Microcins mediate competition among Enterobacteriaceae in the inflamed gut. *Nature*. (2016) 540:280–83. doi: 10.1038/nature20557

71. Cherrak Y, Salazar MA, Yilmaz K, Kreuzer M, Hardt WD. Commensal *E. coli* limits *Salmonella* gut invasion during inflammation by producing toxin-bound siderophores in a tonB-dependent manner. *PLoS Biol*. (2024) 22:e3002616. doi: 10.1371/journal.pbio.3002616

72. Corr SC, Li Y, Riedel CU, O'toole PW, Hill C, Gahan CG. Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118. *Proc Natl Acad Sci U S A*. (2007) 104:7617–21. doi: 10.1073/pnas.0700440104

73. Abramov VM, Kosarev IV, Machulin AV, Deryusheva EI, Priputnevich TV, Panin AN, et al. *Ligilactobacillus salivarius* 7247 strain: probiotic properties and anti-*Salmonella* effect with prebiotics. *Antibiotics (Basel)*. (2023) 12:1535. doi: 10.3390/antibiotics12101535

74. Ismael M, Qayyum N, Gu Y, Zhezhe Y, Cui Y, Zhang Y, et al. Protective effect of plantaricin bio-LP1 bacteriocin on multidrug-resistance *Escherichia Coli* infection by alleviate the inflammation and modulate of gut-microbiota in BALB/c mice model. *Int J Biol Macromol*. (2023) 246:125700. doi: 10.1016/j.ijbiomac.2023.125700

75. Mann ER, Lam YK, Uhlig HH. Short-chain fatty acids: linking diet, the microbiome and immunity. *Nat Rev Immunol*. (2024) 24:577–95. doi: 10.1038/s41577-024-01014-8

76. Pan Y, Zhang H, Li M, He T, Guo S, Zhu L, et al. Novel approaches in IBD therapy: targeting the gut microbiota-bile acid axis. *Gut Microbes*. (2024) 16:2356284. doi: 10.1080/19490976.2024.2356284

77. Sorbara MT, Dubin K, Littmann ER, Moody TU, Fontana E, Seok R, et al. Inhibiting antibiotic-resistant Enterobacteriaceae by microbiota-mediated intracellular acidification. *J Exp Med*. (2019) 216:84–98. doi: 10.1084/jem.20181639

78. Bachem A, Makhlouf C, Binger KJ, De Souza DP, Tull D, Hochheiser K, et al. Microbiota-derived short-chain fatty acids promote the memory potential of antigen-activated $CD8^+$ T cells. *Immunity*. (2019) 51:285–97.e5. doi: 10.1016/j.immuni.2019.06.002

79. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, Deroos P, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature*. (2013) 504:451–5. doi: 10.1038/nature12726

80. Dupraz L, Magniez A, Rolhion N, Richard ML, Da Costa G, Touch S, et al. Gut microbiota-derived short-chain fatty acids regulate IL-17 production by mouse and human intestinal γδ T cells. *Cell Rep*. (2021) 36:109332. doi: 10.1016/j.celrep.2021.109332

81. Zheng L, Kelly CJ, Battista KD, Schaefer R, Lanis JM, Alexeev EE, et al. Microbial-derived butyrate promotes epithelial barrier function through IL-10 receptor-dependent repression of claudin-2. *J Immunol*. (2017) 199:2976–84. doi: 10.4049/jimmunol.1700105

82. Adams L, Li X, Burchmore R, Goodwin RJA, Wall DM. Microbiome-derived metabolite effects on intestinal barrier integrity and immune cell response to infection. *Microbiol (Reading)*. (2024) 170:001504. doi: 10.1099/mic.0.001504

83. Osbelt L, Thiemann S, Smit N, Lesker TR, Schröter M, Gálvez EJC, et al. Variations in microbiota composition of laboratory mice influence *Citrobacter rodentium* infection via variable short-chain fatty acid production. *PLoS Pathog*. (2020) 16:e1008448. doi: 10.1371/journal.ppat.1008448

84. Saeedi BJ, Liu KH, Owens JA, Hunter-Chang S, Camacho MC, Eboka RU, et al. Gut-resident *lactobacilli* activate hepatic nrf2 and protect against oxidative liver injury. *Cell Metab*. (2020) 31:956–68.e5. doi: 10.1016/j.cmet.2020.03.006

85. Zhang S, Zhao J, Xie F, He H, Johnston LJ, Dai X, et al. Dietary fiber-derived short-chain fatty acids: A potential therapeutic target to alleviate obesity-related nonalcoholic fatty liver disease. *Obes Rev*. (2021) 22:e13316. doi: 10.1111/obr.13316

86. Sato Y, Atarashi K, Plichta DR, Arai Y, Sasajima S, Kearney SM, et al. Novel bile acid biosynthetic pathways are enriched in the microbiome of centenarians. *Nature*. (2021) 599:458–64. doi: 10.1038/s41586-021-03832-5

87. Mcmillan AS, Theriot CM. Bile acids impact the microbiota, host, and *C. difficile* dynamics providing insight into mechanisms of efficacy of FMTs and microbiota-focused therapeutics. *Gut Microbes*. (2024) 16:2393766. doi: 10.1080/19490976.2024.2393766

88. Guzior DV, Quinn RA. Review: microbial transformations of human bile acids. *Microbiome*. (2021) 9:140. doi: 10.1186/s40168-021-01101-1

89. He Z, Ma Y, Yang S, Zhang S, Liu S, Xiao J, et al. Gut microbiota-derived ursodeoxycholic acid from neonatal dairy calves improves intestinal homeostasis and colitis to attenuate extended-spectrum β-lactamase-producing enteroaggregative *Escherichia coli* infection. *Microbiome*. (2022) 10:79. doi: 10.1186/s40168-022-01269-0

90. Sannasiddappa TH, Lund PA, Clarke SR. *In vitro* antibacterial activity of unconjugated and conjugated bile salts on Staphylococcus aureus. *Front Microbiol*. (2017) 8:1581. doi: 10.3389/fmicb.2017.01581

91. Watanabe M, Fukiya S, Yokota A. Comprehensive evaluation of the bactericidal activities of free bile acids in the large intestine of humans and rodents. *J Lipid Res*. (2017) 58:1143–52. doi: 10.1194/jlr.M075143

92. Termén S, Tollin M, Rodriguez E, Sveinsdóttir SH, Jóhannesson B, Cederlund A, et al. PU.1 and bacterial metabolites regulate the human gene CAMP encoding antimicrobial peptide LL-37 in colon epithelial cells. *Mol Immunol*. (2008) 45:3947–55. doi: 10.1016/j.molimm.2008.06.020

93. Li W, Hang S, Fang Y, Bae S, Zhang Y, Zhang M, et al. A bacterial bile acid metabolite modulates T(reg) activity through the nuclear hormone receptor NR4A1. *Cell Host Microbe*. (2021) 29:1366–77.e9. doi: 10.1016/j.chom.2021.07.013

94. Campbell C, Mckenney PT, Konstantinovsky D, Isaeva OI, Schizas M, Verter J, et al. Bacterial metabolism of bile acids promotes generation of peripheral regulatory T cells. *Nature*. (2020) 581:475–79. doi: 10.1038/s41586-020-2193-0

95. Le Guern R, Grandjean T, Stabler S, Bauduin M, Gosset P, Kipnis É, et al. Gut colonisation with multidrug-resistant *Klebsiella pneumoniae* worsens Pseudomonas aeruginosa lung infection. *Nat Commun*. (2023) 14:78. doi: 10.1038/s41467-022-35767-4

96. Renga G, Nunzi E, Stincardini C, Pariano M, Puccetti M, Pieraccini G, et al. CPX-351 exploits the gut microbiota to promote mucosal barrier function, colonization resistance, and immune homeostasis. *Blood*. (2024) 143:1628–45. doi: 10.1182/blood.2023021380

97. Schroeder BO, Birchenough GMH, Ståhlman M, Arike L, Johansson MEV, Hansson GC, et al. *Bifidobacteria* or fiber protects against diet-induced microbiota-mediated colonic mucus deterioration. *Cell Host Microbe*. (2018) 23:27–40.e7. doi: 10.1016/j.chom.2017.11.004

98. Di Vincenzo F, Del Gaudio A, Petito V, Lopetuso LR, Scaldaferri F. Gut microbiota, intestinal permeability, and systemic inflammation: a narrative review. *Intern Emerg Med*. (2024) 19:275–93. doi: 10.1007/s11739-023-03374-w

99. Pral LP, Fachi JL, Corrêa RO, Colonna M, Vinolo M. Hypoxia and HIF-1 as key regulators of gut microbiota and host interactions. *Trends Immunol*. (2021) 42:604–21. doi: 10.1016/j.it.2021.05.004

100. Zhang T, Sasabe J, Hullahalli K, Sit B, Waldor MK. Increased *Listeria monocytogenes* dissemination and altered population dynamics in muc2-deficient mice. *Infect Immun*. (2021) 89:e00667-20. doi: 10.1128/iai.00667-20

101. Neumann M, Steimle A, Grant ET, Wolter M, Parrish A, Willieme S, et al. Deprivation of dietary fiber in specific-pathogen-free mice promotes susceptibility to the intestinal mucosal pathogen. *Citrobacter rodentium*. *Gut Microbes*. (2021) 13:1966263. doi: 10.1080/19490976.2021.1966263

102. Wolter M, Grant ET, Boudaud M, Pudlo NA, Pereira GV, Eaton KA, et al. Diet-driven differential response of *Akkermansia muciniphila* modulates pathogen susceptibility. *Mol Syst Biol*. (2024) 20:596–625. doi: 10.1038/s44320-024-00036-7

103. Pickard JM, Maurice CF, Kinnebrew MA, Abt MC, Schenten D, Golovkina TV, et al. Rapid fucosylation of intestinal epithelium sustains host-commensal symbiosis in sickness. *Nature*. (2014) 514:638–41. doi: 10.1038/nature13823

104. Nusrat A, Von-Eichel-Streiber C, Turner JR, Verkade P, Madara JL, Parkos CA. *Clostridium difficile* toxins disrupt epithelial barrier function by altering membrane microdomain localization of tight junction proteins. *Infect Immun*. (2001) 69:1329–36. doi: 10.1128/iai.69.3.1329-1336.2001

105. Ghosh S, Erickson D, Chua MJ, Collins J, Jala VR. The microbial metabolite urolithin A reduces *Clostridioides difficile* toxin expression and toxin-induced epithelial damage. *mSystems*. (2024) 9:e0125523. doi: 10.1128/msystems.01255-23

106. Fachi JL, Pral LP, Assis HC, Oliveira S, Rodovalho VR, Dos Santos J, et al. Hyperbaric oxygen augments susceptibility to C. difficile infection by impairing gut microbiota ability to stimulate the HIF-1α-IL-22 axis in ILC3. *Gut Microbes*. (2024) 16:2297872. doi: 10.1080/19490976.2023.2297872

107. Zhao T, Liu X, Chu Z, Zhao J, Jiang D, Dong X, et al. L-arginine loading porous PEEK promotes percutaneous tissue repair through macrophage orchestration. *Bioact Mater*. (2024) 40:19–33. doi: 10.1016/j.bioactmat.2024.05.025

108. Winter SE, Winter MG, Xavier MN, Thiennimitr P, Poon V, Keestra AM, et al. Host-derived nitrate boosts growth of E. coli in the inflamed gut. *Science*. (2013) 339:708–11. doi: 10.1126/science.1232467

109. Baldelli V, Scaldaferri F, Putignani L, Del Chierico F. The role of enterobacteriaceae in gut microbiota dysbiosis in inflammatory bowel diseases. *Microorganisms*. (2021) 9:697. doi: 10.3390/microorganisms9040697

110. Byndloss MX, Olsan EE, Rivera-Chávez F, Tiffany CR, Cevallos SA, Lokken KL, et al. Microbiota-activated PPAR-γ signaling inhibits dysbiotic Enterobacteriaceae expansion. *Science*. (2017) 357:570–75. doi: 10.1126/science.aam9949

111. Rivera-Chávez F, Lopez CA, Bäumler AJ. Oxygen as a driver of gut dysbiosis. *Free Radic Biol Med*. (2017) 105:93–101. doi: 10.1016/j.freeradbiomed.2016.09.022

112. Litvak Y, Byndloss MX, Tsolis RM, Bäumler AJ. Dysbiotic Proteobacteria expansion: a microbial signature of epithelial dysfunction. *Curr Opin Microbiol*. (2017) 39:1–6. doi: 10.1016/j.mib.2017.07.003

113. Shelton CD, Byndloss MX. Gut epithelial metabolism as a key driver of intestinal dysbiosis associated with noncommunicable diseases. *Infect Immun*. (2020) 88:e00939-19. doi: 10.1128/iai.00939-19

114. Ziaka M, Exadaktylos A. Gut-derived immune cells and the gut-lung axis in ARDS. *Crit Care*. (2024) 28:220. doi: 10.1186/s13054-024-05006-x

115. Sun Y, Ho CT, Zhang X. Neuroprotection of food bioactives in neurodegenerative diseases: role of the gut microbiota and innate immune receptors. *J Agric Food Chem*. (2023) 71:2718–33. doi: 10.1021/acs.jafc.2c07742

116. Maynard CL, Elson CO, Hatton RD, Weaver CT. Reciprocal interactions of the intestinal microbiota and immune system. *Nature*. (2012) 489:231–41. doi: 10.1038/nature11551

117. Chen L, Zhang L, Hua H, Liu L, Mao Y, Wang R. Interactions between toll-like receptors signaling pathway and gut microbiota in host homeostasis. *Immun Inflammation Dis*. (2024) 12:e1356. doi: 10.1002/iid3.1356

118. Ost KS, O'meara TR, Stephens WZ, Chiaro T, Zhou H, Penman J, et al. Adaptive immunity induces mutualism between commensal eukaryotes. *Nature*. (2021) 596:114–18. doi: 10.1038/s41586-021-03722-w

119. Xu Z, Takizawa F, Casadei E, Shibasaki Y, Ding Y, Sauters TJC, et al. Specialization of mucosal immunoglobulins in pathogen control and microbiota homeostasis occurred early in vertebrate evolution. *Sci Immunol*. (2020) 5:eaay3254. doi: 10.1126/sciimmunol.aay3254

120. Ramanan D, Pratama A, Zhu Y, Venezia O, Sassone-Corsi M, Chowdhary K, et al. Regulatory T cells in the face of the intestinal microbiota. *Nat Rev Immunol*. (2023) 23:749–62. doi: 10.1038/s41577-023-00890-w

121. Zhou P, Chen C, Patil S, Dong S. Unveiling the therapeutic symphony of probiotics, prebiotics, and postbiotics in gut-immune harmony. *Front Nutr*. (2024) 11:1355542. doi: 10.3389/fnut.2024.1355542

122. Hays KE, Pfaffinger JM, Ryznar R. The interplay between gut microbiota, short-chain fatty acids, and implications for host health and disease. *Gut Microbes*. (2024) 16:2393270. doi: 10.1080/19490976.2024.2393270

123. Miller WR, Arias CA. ESKAPE pathogens: antimicrobial resistance, epidemiology, clinical impact and therapeutics. *Nat Rev Microbiol*. (2024) 22:598–616. doi: 10.1038/s41579-024-01054-w

124. Rahman MN, Barua N, Tin MCF, Dharmaratne P, Wong SH, Ip M. The use of probiotics and prebiotics in decolonizing pathogenic bacteria from the gut; a systematic review and meta-analysis of clinical outcomes. *Gut Microbes*. (2024) 16:2356279. doi: 10.1080/19490976.2024.2356279

125. Kim SG, Becattini S, Moody TU, Shliaha PV, Littmann ER, Seok R, et al. Microbiota-derived lantibiotic restores resistance against vancomycin-resistant *Enterococcus*. *Nature*. (2019) 572:665–69. doi: 10.1038/s41586-019-1501-z

126. Li X, Song L, Zhu S, Xiao Y, Huang Y, Hua Y, et al. Two strains of lactobacilli effectively decrease the colonization of VRE in a mouse model. *Front Cell Infect Microbiol*. (2019) 9:6. doi: 10.3389/fcimb.2019.00006

127. Mu A, Carter GP, Li L, Isles NS, Vrbanac AF, Morton JT, et al. Microbe-metabolite associations linked to the rebounding murine gut microbiome postcolonization with vancomycin-resistant *Enterococcus faecium*. *mSystems*. (2020) 5:e00452-20. doi: 10.1128/mSystems.00452-20

128. Rosenstein R, Torres Salazar BO, Sauer C, Heilbronner S, Krismer B, Peschel A. The Staphylococcus aureus-antagonizing human nasal commensal *Staphylococcus lugdunensis* depends on siderophore piracy. *Microbiome*. (2024) 12:213. doi: 10.1186/s40168-024-01913-x

129. Zhao Y, Sun H, Chen Y, Niu Q, Dong Y, Li M, et al. Butyrate protects against MRSA pneumonia via regulating gut-lung microbiota and alveolar macrophage M2 polarization. *mBio*. (2023) 14:e0198723. doi: 10.1128/mbio.01987-23

130. Wang H, Kim R, Wang Y, Furtado KL, Sims CE, Tamayo R, et al. *In vitro* co-culture of *Clostridium scindens* with primary human colonic epithelium protects the epithelium against *Staphylococcus aureus*. *Front Bioeng Biotechnol*. (2024) 12:1382389. doi: 10.3389/fbioe.2024.1382389

131. Zhao C, Wu K, Hao H, Zhao Y, Bao L, Qiu M, et al. Gut microbiota-mediated secondary bile acid alleviates *Staphylococcus aureus*-induced mastitis through the TGR5-cAMP-PKA-NF-κB/NLRP3 pathways in mice. *NPJ Biofilms Microbiomes*. (2023) 9:8. doi: 10.1038/s41522-023-00374-8

132. Mousa WK, Ghemrawi R, Abu-Izneid T, Ramadan A, Al-Marzooq F. Discovery of lactomodulin, a unique microbiome-derived peptide that exhibits dual anti-inflammatory and antimicrobial activity against multidrug-resistant pathogens. *Int J Mol Sci*. (2023) 24:6901. doi: 10.3390/ijms24086901

133. Hecht AL, Harling LC, Friedman ES, Tanes C, Lee J, Firrman J, et al. Dietary carbohydrates regulate intestinal colonization and dissemination of *Klebsiella pneumoniae*. *J Clin Invest*. (2024) 134:e174726. doi: 10.1172/jci174726

134. Shen M, Zhao H, Han M, Su L, Cui X, Li D, et al. Alcohol-induced gut microbiome dysbiosis enhances the colonization of *Klebsiella pneumoniae* on the mouse intestinal tract. *mSystems*. (2024) 9:e0005224. doi: 10.1128/msystems.00052-24

135. Stacy A, Andrade-Oliveira V, Mcculloch JA, Hild B, Oh JH, Perez-Chaparro PJ, et al. Infection trains the host for microbiota-enhanced resistance to pathogens. *Cell*. (2021) 184:615–27.e17. doi: 10.1016/j.cell.2020.12.011

136. Tang HJ, Chen CC, Lu YC, Huang HL, Chen HJ, Chuang YC, et al. The effect of *Lactobacillus* with prebiotics on KPC-2-producing Klebsiella pneumoniae. *Front Microbiol*. (2022) 13:1050247. doi: 10.3389/fmicb.2022.1050247

137. Asahara T, Takahashi A, Yuki N, Kaji R, Takahashi T, Nomoto K. Protective Effect of a Synbiotic against Multidrug-Resistant *Acinetobacter baumannii* in a Murine Infection Model. *Antimicrob Agents Chemother*. (2016) 60:3041–50. doi: 10.1128/aac.02928-15

138. Wen L, Shi L, Kong XL, Li KY, Li H, Jiang DX, et al. Gut Microbiota Protected Against *pseudomonas aeruginosa* Pneumonia via Restoring Treg/Th17 Balance and Metabolism. *Front Cell Infect Microbiol*. (2022) 12:856633. doi: 10.3389/fcimb.2022.856633

139. Janapatla RP, Dudek A, Chen CL, Chuang CH, Chien KY, Feng Y, et al. Marine prebiotics mediate decolonization of *Pseudomonas aeruginosa* from gut by inhibiting secreted virulence factor interactions with mucins and enriching *Bacteroides* population. *J BioMed Sci*. (2023) 30:9. doi: 10.1186/s12929-023-00902-w

140. Zamani H, Rahbar S, Garakoui SR, Afsah Sahebi A, Jafari H. Antibiofilm potential of *Lactobacillus plantarum* spp. cell free supernatant (CFS) against multidrug resistant bacterial pathogens. *Pharm Biomed Res*. (2017) 3:39–44. doi: 10.29252/pbr.3.2.39

141. Nagao-Kitamoto H, Leslie JL, Kitamoto S, Jin C, Thomsson KA, Gillilland MG 3rd, et al. Interleukin-22-mediated host glycosylation prevents *Clostridioides difficile* infection by modulating the metabolic activity of the gut microbiota. *Nat Med*. (2020) 26:608–17. doi: 10.1038/s41591-020-0764-0

142. Pensinger DA, Fisher AT, Dobrila HA, Van Treuren W, Gardner JO, Higginbottom SK, et al. Butyrate differentiates permissiveness to *Clostridioides difficile* infection and influences growth of diverse C. *difficile Isolates Infect Immun*. (2023) 91:e0057022. doi: 10.1128/iai.00570-22

143. Tian H, Cui J, Ye C, Zhao J, Yang B, Xu Y, et al. Depletion of butyrate-producing microbes of the Firmicutes predicts nonresponse to FMT therapy in patients with recurrent *Clostridium difficile* infection. *Gut Microbes*. (2023) 15:2236362. doi: 10.1080/19490976.2023.2236362

144. Winston JA, Rivera AJ, Cai J, Thanissery R, Montgomery SA, Patterson AD, et al. Ursodeoxycholic Acid (UDCA) Mitigates the Host Inflammatory Response during *Clostridioides difficile* Infection by Altering Gut Bile Acids. *Infect Immun*. (2020) 88:e00045-20. doi: 10.1128/iai.00045-20

145. Wei Y, Yang F, Wu Q, Gao J, Liu W, Liu C, et al. Protective effects of bifidobacterial strains against toxigenic *Clostridium difficile*. *Front Microbiol*. (2018) 9:888. doi: 10.3389/fmicb.2018.00888

146. Hagihara M, Ariyoshi T, Kuroki Y, Eguchi S, Higashi S, Mori T, et al. *Clostridium butyricum* enhances colonization resistance against *Clostridioides difficile* by metabolic and immune modulation. *Sci Rep*. (2021) 11:15007. doi: 10.1038/s41598-021-94572-z

147. Yip AYG, King OG, Omelchenko O, Kurkimat S, Horrocks V, Mostyn P, et al. Antibiotics promote intestinal growth of carbapenem-resistant Enterobacteriaceae by enriching nutrients and depleting microbial metabolites. *Nat Commun*. (2023) 14:5094. doi: 10.1038/s41467-023-40872-z

148. Chang KC, Nagarajan N, Gan YH. Short-chain fatty acids of various lengths differentially inhibit Klebsiella pneumoniae and Enterobacteriaceae species. *mSphere*. (2024) 9:e0078123. doi: 10.1128/msphere.00781-23

149. Krawczyk B, Wityk P, Gałęcka M, Michalik M. The many faces of *enterococcus* spp.-commensal, probiotic and opportunistic pathogen. *Microorganisms*. (2021) 9 (9):1900. doi: 10.3390/microorganisms9091900

150. Xu L, Wu Y, Yang X, Pang X, Wu Y, Li X, et al. The Fe-S cluster biosynthesis in *Enterococcus faecium* is essential for anaerobic growth and gastrointestinal colonization. *Gut Microbes*. (2024) 16:2359665. doi: 10.1080/19490976.2024.2359665

151. Brandl K, Plitas G, Mihu CN, Ubeda C, Jia T, Fleisher M, et al. Vancomycin-resistant *enterococci* exploit antibiotic-induced innate immune deficits. *Nature*. (2008) 455:804–7. doi: 10.1038/nature07250

152. Abt MC, Buffie CG, Sušac B, Becattini S, Carter RA, Leiner I, et al. TLR-7 activation enhances IL-22-mediated colonization resistance against vancomycin-resistant *enterococcus*. *Sci Transl Med*. (2016) 8:327ra25. doi: 10.1126/scitranslmed.aad6663

153. Ubeda C, Bucci V, Caballero S, Djukovic A, Toussaint NC, Equinda M, et al. Intestinal microbiota containing Barnesiella species cures vancomycin-resistant *Enterococcus faecium* colonization. *Infect Immun*. (2013) 81:965–73. doi: 10.1128/iai.01197-12

154. Dubberke ER, Mullane KM, Gerding DN, Lee CH, Louie TJ, Guthertz H, et al. Clearance of Vancomycin-Resistant *Enterococcus* Concomitant With Administration of a Microbiota-Based Drug Targeted at Recurrent *Clostridium difficile* Infection. *Open Forum Infect Dis*. (2016) 3:ofw133. doi: 10.1093/ofid/ofw133

155. Davido B, Batista R, Fessi H, Michelon H, Escaut L, Lawrence C, et al. Fecal microbiota transplantation to eradicate vancomycin-resistant *enterococci* colonization in case of an outbreak. *Med Mal Infect*. (2019) 49:214–18. doi: 10.1016/j.medmal.2018.11.002

156. Hyun J, Lee SK, Cheon JH, Yong DE, Koh H, Kang YK, et al. Faecal microbiota transplantation reduces amounts of antibiotic resistance genes in patients with multidrug-resistant organisms. *Antimicrob Resist Infect Control*. (2022) 11:20. doi: 10.1186/s13756-022-01064-4

157. Smith AB, Jenior ML, Keenan O, Hart JL, Specker J, Abbas A, et al. *Enterococci* enhance *Clostridioides difficile* pathogenesis. *Nature*. (2022) 611:780–86. doi: 10.1038/s41586-022-05438-x

158. Dantes R, Mu Y, Belflower R, Aragon D, Dumyati G, Harrison LH, et al. National burden of invasive methicillin-resistant *Staphylococcus aureus* infections, United States, 2011. *JAMA Intern Med*. (2013) 173:1970–8. doi: 10.1001/jamainternmed.2013.10423

159. Piewngam P, Otto M. *Staphylococcus aureus* colonisation and strategies for decolonisation. *Lancet Microbe*. (2024) 5:e606–e18. doi: 10.1016/s2666-5247(24)00040-5

160. Bitrus AA, Zunita Z, Khairani-Bejo S, Othman S, Ahmad Nadzir NA. Staphylococcal cassette chromosome mec (SCCmec) and characterization of the attachment site (attB) of methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin susceptible *Staphylococcus aureus* (MSSA) isolates. *Microb Pathog*. (2018) 123:323–29. doi: 10.1016/j.micpath.2018.07.033

161. Idrees M, Sawant S, Karodia N, Rahman A. *Staphylococcus aureus* biofilm: morphology, genetics, pathogenesis and treatment strategies. *Int J Environ Res Public Health*. (2021) 18:7602. doi: 10.3390/ijerph18147602

162. Zhou C, Pawline MB, Pironti A, Morales SM, Perault AI, Ulrich RJ, et al. Microbiota and metabolic adaptation shape Staphylococcus aureus virulence and antimicrobial resistance during intestinal colonization. *bioRxiv*. (2024). doi: 10.1101/2024.05.11.593044 (Preprint)

163. Zipperer A, Konnerth MC, Laux C, Berscheid A, Janek D, Weidenmaier C, et al. Human commensals producing a novel antibiotic impair pathogen colonization. *Nature*. (2016) 535:511–6. doi: 10.1038/nature18634

164. Kumar Y, Yadav R, Bhatia A. Can natural detergent properties of bile acids be used beneficially in tackling coronavirus disease-19? *Future Virol*. (2020) 15:779–82. doi: 10.2217/fvl-2020-0210

165. Ridlon JM, Harris SC, Bhowmik S, Kang DJ. and Hylemon, P.B. Consequences of bile salt biotransformations by intestinal bacteria. *Gut Microbes*. (2016) 7:22–39. doi: 10.1080/19490976.2015.1127483

166. Liu A, Garrett S, Hong W, Zhang J. *Staphylococcus aureus* infections and human intestinal microbiota. *Pathogens*. (2024) 13:276. doi: 10.3390/pathogens13040276

167. Ma X, Jin H, Chu X, Dai W, Tang W, Zhu J, et al. The host CYP1A1-microbiota metabolic axis promotes gut barrier disruption in methicillin-resistant *Staphylococcus aureus*-induced abdominal sepsis. *Front Microbiol*. (2022) 13:802409. doi: 10.3389/fmicb.2022.802409

168. Ali S, Alam M, Hasan GM, Hassan MI. Potential therapeutic targets of *Klebsiella pneumoniae*: a multi-omics review perspective. *Brief Funct Genomics*. (2022) 21:63–77. doi: 10.1093/bfgp/elab038

169. Osbelt L, Almási É DH, Wende M, Kienesberger S, Voltz A, Lesker TR, et al. *Klebsiella oxytoca* inhibits *Salmonella* infection through multiple microbiota-context-dependent mechanisms. *Nat Microbiol*. (2024) 9:1792–811. doi: 10.1038/s41564-024-01710-0

170. Hu J, Tong Y, Shen Z, Li Y, Cheng C, Au R, et al. Gegen Qinlian decoction ameliorates murine colitis by inhibiting the expansion of Enterobacteriaceae through activating PPAR-γ signaling. *BioMed Pharmacother*. (2022) 154:113571. doi: 10.1016/j.biopha.2022.113571

171. Yoon JG, Lim S, Hyun HJ, Seong H, Noh JY, Song JY, et al. Respiratory microbiome and clinical course of carbapenem-resistant *Acinetobacter baumannii* pneumonia in critically Ill patients. *Med (Baltimore)*. (2024) 103:e38988. doi: 10.1097/md.0000000000038988

172. Ren X, Palmer LD. *Acinetobacter* metabolism in infection and antimicrobial resistance. *Infect Immun*. (2023) 91:e0043322. doi: 10.1128/iai.00433-22

173. Uppalapati SR, Sett A, Pathania R. The outer membrane proteins ompA, carO, and oprD of *Acinetobacter baumannii* confer a two-pronged defense in facilitating its success as a potent human pathogen. *Front Microbiol*. (2020) 11:589234. doi: 10.3389/fmicb.2020.589234

174. Ejaz H, Ahmad M, Younas S, Junaid K, Abosalif KOA, Abdalla AE, et al. Molecular Epidemiology of Extensively-Drug Resistant *Acinetobacter baumannii* Sequence Type 2 Co-Harboring bla (NDM) and bla (OXA) From Clinical Origin. *Infect Drug Resist*. (2021) 14:1931–39. doi: 10.2147/idr.S310478

175. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis*. (2018) 18:318–27. doi: 10.1016/s1473-3099(17)30753-3

176. Ren X, Clark RM, Bansah DA, Varner EN, Tiffany CR, Jaswal K, et al. Amino acid competition shapes Acinetobacter baumannii gut carriage. *bioRxiv*. (2024). doi: 10.1101/2024.10.19.619093

177. Lin HR, Shu HY, Lin GH. Biological roles of indole-3-acetic acid in *Acinetobacter baumannii*. *Microbiol Res*. (2018) 216:30–9. doi: 10.1016/j.micres.2018.08.004

178. Jurado-Martín I, Sainz-Mejías M, Mcclean S. *Pseudomonas aeruginosa*: an audacious pathogen with an adaptable arsenal of virulence factors. *Int J Mol Sci*. (2021) 22:3128. doi: 10.3390/ijms22063128

179. Rossi E, La Rosa R, Bartell JA, Marvig RL, Haagensen J, Sommer LM, et al. *Pseudomonas aeruginosa* adaptation and evolution in patients with cystic fibrosis. *Nat Rev Microbiol*. (2021) 19:331–42. doi: 10.1038/s41579-020-00477-5

180. Horcajada JP, Montero M, Oliver A, Sorlí L, Luque S, Gómez-Zorrilla S, et al. Epidemiology and treatment of multidrug-resistant and extensively drug-resistant *Pseudomonas aeruginosa* infections. *Clin Microbiol Rev*. (2019) 32:e00031-19. doi: 10.1128/cmr.00031-19

181. Breidenstein EB, de-la-Fuente-Núñez C, Hancock RE. *Pseudomonas aeruginosa*: all roads lead to resistance. *Trends Microbiol*. (2011) 19:419–26. doi: 10.1016/j.tim.2011.04.005

182. Wheatley R, Diaz Caballero J, Kapel N, De Winter FHR, Jangir P, Quinn A, et al. Rapid evolution and host immunity drive the rise and fall of carbapenem resistance during an acute *Pseudomonas aeruginosa* infection. *Nat Commun*. (2021) 12:2460. doi: 10.1038/s41467-021-22814-9

183. Botelho J, Grosso F, Peixe L. Antibiotic resistance in *Pseudomonas aeruginosa* - Mechanisms, epidemiology and evolution. *Drug Resist Update*. (2019) 44:100640. doi: 10.1016/j.drup.2019.07.002

184. Gómez-Zorrilla S, Camoez M, Tubau F, Cañizares R, Periche E, Dominguez MA, et al. Prospective observational study of prior rectal colonization status as a predictor for subsequent development of *Pseudomonas aeruginosa* clinical infections. *Antimicrob Agents Chemother*. (2015) 59:5213–9. doi: 10.1128/aac.04636-14

185. Kubinak JL, Petersen C, Stephens WZ, Soto R, Bake E, O'connell RM, et al. MyD88 signaling in T cells directs IgA-mediated control of the microbiota to promote health. *Cell Host Microbe*. (2015) 17:153–63. doi: 10.1016/j.chom.2014.12.009

186. Zhou Y, Zhang H, Verma P, Samanta D, Verma MK, Kumar K. Validation of secretory Immunoglobulin A (IgA) for early and efficient diagnosis of *Pseudomonas aeruginosa* lung infection. *Cell Mol Biol (Noisy-le-grand)*. (2023) 69:35–9. doi: 10.14715/cmb/2023.69.7.6

187. Robak OH, Heimesaat MM, Kruglov AA, Prepens S, Ninnemann J, Gutbier B, et al. Antibiotic treatment-induced secondary IgA deficiency enhances susceptibility to *Pseudomonas aeruginosa* pneumonia. *J Clin Invest*. (2018) 128:3535–45. doi: 10.1172/jci97065

188. Lécuyer E, Rakotobe S, Lengliné-Garnier H, Lebreton C, Picard M, Juste C, et al. Segmented filamentous bacterium uses secondary and tertiary lymphoid tissues to induce gut IgA and specific T helper 17 cell responses. *Immunity*. (2014) 40:608–20. doi: 10.1016/j.immuni.2014.03.009

189. Rooney AM, Cochrane K, Fedsin S, Yao S, Anwer S, Dehmiwal S, et al. A microbial consortium alters intestinal *Pseudomonadota* and antimicrobial resistance genes in individuals with recurrent *Clostridioides difficile* infection. *mBio*. (2023) 14:e0348222. doi: 10.1128/mbio.03482-22

190. Lou TL, Ji T, Peng X, Ji WW, Yuan LX, Wang J, et al. Extract From Tetrastigma hemsleyanum Leaf Alleviates *Pseudomonas aeruginosa* Lung Infection: Network Pharmacology Analysis and Experimental Evidence. *Front Pharmacol*. (2021) 12:587850. doi: 10.3389/fphar.2021.587850

191. Di Bella S, Sanson G, Monticelli J, Zerbato V, Principe L, Giuffrè M, et al. *Clostridioides difficile* infection: history, epidemiology, risk factors, prevention, clinical manifestations, treatment, and future options. *Clin Microbiol Rev*. (2024) 37:e0013523. doi: 10.1128/cmr.00135-23

192. Bouwknegt M, Van Dorp S, Kuijper E. Burden of *Clostridium difficile* infection in the United States. *N Engl J Med*. (2015) 372:2368. doi: 10.1056/NEJMc1505190

193. Theriot CM, Koenigsknecht MJ, Carlson PE Jr., Hatton GE, Nelson AM, Li B, et al. Antibiotic-induced shifts in the mouse gut microbiome and metabolome increase susceptibility to *Clostridium difficile* infection. *Nat Commun*. (2014) 5:3114. doi: 10.1038/ncomms4114

194. Bratkovič T, Zahirović A, Bizjak M, Rupnik M, Štrukelj B, Berlec A. New treatment approaches for *Clostridioides difficile* infections: alternatives to antibiotics and fecal microbiota transplantation. *Gut Microbes*. (2024) 16:2337312. doi: 10.1080/19490976.2024.2337312

195. Louie T, Golan Y, Khanna S, Bobilev D, Erpelding N, Fratazzi C, et al. VE303, a defined bacterial consortium, for prevention of recurrent *Clostridioides difficile* infection: A randomized clinical trial. *JAMA*. (2023) 329:1356–66. doi: 10.1001/jama.2023.4314

196. Gurung B, Stricklin M, Wang S. Gut microbiota-gut metabolites and *Clostridioides difficile* infection: approaching sustainable solutions for therapy. *Metabolites*. (2024) 14:74. doi: 10.3390/metabo14010074

197. Jose S, Mukherjee A, Horrigan O, Setchell KDR, Zhang W, Moreno-Fernandez ME, et al. Obeticholic acid ameliorates severity of *Clostridioides difficile* infection in

high fat diet-induced obese mice. *Mucosal Immunol*. (2021) 14:500–10. doi: 10.1038/s41385-020-00338-7

198. Rea MC, Dobson A, O'sullivan O, Crispie F, Fouhy F, Cotter PD, et al. Effect of broad- and narrow-spectrum antimicrobials on *Clostridium difficile* and microbial diversity in a model of the distal colon. *Proc Natl Acad Sci U S A*. (2011) 108 Suppl 1:4639–44. doi: 10.1073/pnas.1001224107

199. Lay CL, Dridi L, Bergeron MG, Ouellette M, Fliss IL. Nisin is an effective inhibitor of *Clostridium difficile* vegetative cells and spore germination. *J Med Microbiol*. (2016) 65:169–75. doi: 10.1099/jmm.0.000202

200. Davin-Regli A, Lavigne JP, Pagès JM. *Enterobacter* spp.: update on taxonomy, clinical aspects, and emerging antimicrobial resistance. *Clin Microbiol Rev*. (2019) 32: e00002-19. doi: 10.1128/cmr.00002-19

201. Palacios-Baena ZR, Giannella M, Manissero D, Rodríguez-Baño J, Viale P, Lopes S, et al. Risk factors for carbapenem-resistant Gram-negative bacterial infections: a systematic review. *Clin Microbiol Infect*. (2021) 27:228–35. doi: 10.1016/j.cmi.2020.10.016

202. Sindi AA, Alsayed SM, Abushoshah I, Bokhary DH, Tashkandy NR. Profile of the gut microbiome containing carbapenem-Resistant enterobacteriaceae in ICU patients. *Microorganisms*. (2022) 10:1309. doi: 10.3390/microorganisms10071309

203. Baek MS, Kim S, Kim WY, Kweon MN, Huh JW. Gut microbiota alterations in critically Ill patients with carbapenem-resistant Enterobacteriaceae colonization: A clinical analysis. *Front Microbiol*. (2023) 14:1140402. doi: 10.3389/fmicb.2023.1140402

204. Kunishima H, Ishibashi N, Wada K, Oka K, Takahashi M, Yamasaki Y, et al. The effect of gut microbiota and probiotic organisms on the properties of extended spectrum beta-lactamase producing and carbapenem resistant Enterobacteriaceae including growth, beta-lactamase activity and gene transmissibility. *J Infect Chemother*. (2019) 25:894–900. doi: 10.1016/j.jiac.2019.04.021

205. Van Hul M, Cani PD, Petitfils C, De Vos WM, Tilg H, El-Omar EM. What defines a healthy gut microbiome? *Gut*. (2024) 73:1893–908. doi: 10.1136/gutjnl-2024-333378

206. Yadegar A, Bar-Yoseph H, Monaghan TM, Pakpour S, Severino A, Kuijper EJ, et al. Fecal microbiota transplantation: current challenges and future landscapes. *Clin Microbiol Rev*. (2024) 37:e0006022. doi: 10.1128/cmr.00060-22

207. Bonnet M, Eckert C, Tournebize R. Decolonization of asymptomatic carriage of multi-drug resistant bacteria by bacteriophages? *Front Microbiol*. (2023) 14:1266416. doi: 10.3389/fmicb.2023.1266416

208. Campos-Madueno EI, Moradi M, Eddoubaji Y, Shahi F, Moradi S, Bernasconi OJ, et al. Intestinal colonization with multidrug-resistant Enterobacterales: screening, epidemiology, clinical impact, and strategies to decolonize carriers. *Eur J Clin Microbiol Infect Dis*. (2023) 42:229–54. doi: 10.1007/s10096-023-04548-2

209. Liu W, Li X, Zhao Z, Pi X, Meng Y, Fei D, et al. Effect of chitooligosaccharides on human gut microbiota and antiglycation. *Carbohydr Polym*. (2020) 242:116413. doi: 10.1016/j.carbpol.2020.116413

210. Baek GH, Kim YJ, Lee Y, Jung SC, Seo HW, Kim JS. Prebiotic potential of green banana flour: impact on gut microbiota modulation and microbial metabolic activity in a murine model. *Front Nutr*. (2023) 10:1249358. doi: 10.3389/fnut.2023.1249358

211. Zou Y, Wang J, Wang Y, Peng B, Liu J, Zhang B, et al. Protection of Galacto-Oligosaccharide against E. coli O157 Colonization through Enhancing Gut Barrier Function and Modulating Gut Microbiota. *Foods*. (2020) 9:1710. doi: 10.3390/foods9111710

212. Gao J, Zhao L, Cheng Y, Lei W, Wang Y, Liu X, et al. Probiotics for the treatment of depression and its comorbidities: A systemic review. *Front Cell Infect Microbiol*. (2023) 13:1167116. doi: 10.3389/fcimb.2023.1167116

213. Nataraj BH, Ali SA, Behare PV, Yadav H. Postbiotics-parabiotics: the new horizons in microbial biotherapy and functional foods. *Microb Cell Fact*. (2020) 19:168. doi: 10.1186/s12934-020-01426-w

214. Clemente JC, Manasson J, Scher JU. The role of the gut microbiome in systemic inflammatory disease. *BMJ*. (2018) 360:j5145. doi: 10.1136/bmj.j5145

215. Bhattacharya S, Dutta A, Khanra PK, Gupta N, Dutta R, Tzvetkov NT, et al. In silico exploration of 4(α-l-rhamnosyloxy)-benzyl isothiocyanate: A promising phytochemical-based drug discovery approach for combating multi-drug resistant Staphylococcus aureus. *Comput Biol Med*. (2024) 179:108907. doi: 10.1016/j.compbiomed.2024.108907

216. Zelante T, Iannitti RG, Cunha C, De Luca A, Giovannini G, Pieraccini G, et al. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. *Immunity*. (2013) 39:372–85. doi: 10.1016/j.immuni.2013.08.003

217. Marco ML, Heeney D, Binda S, Cifelli CJ, Cotter PD, Foligné B, et al. Health benefits of fermented foods: microbiota and beyond. *Curr Opin Biotechnol*. (2017) 44:94–102. doi: 10.1016/j.copbio.2016.11.010

218. Qoms MS, Arulrajah B, Wan Ibadullah WZ, Ramli NS, Chau DM, Sarbini SR, et al. Performance of Azolla pinnata fern protein hydrolysates as an emulsifier and nutraceutical ingredient in an O/W emulsion system and their effect on human gut microbiota and mammalian cells. *Food Funct*. (2024) 15:6578–96. doi: 10.1039/d4fo00377b

219. Wang J, Ji H, Wang S, Liu H, Zhang W, Zhang D, et al. Probiotic *Lactobacillus plantarum* promotes intestinal barrier function by strengthening the epithelium and modulating gut microbiota. *Front Microbiol*. (2018) 9:1953. doi: 10.3389/fmicb.2018.01953

220. Ma Y, Yang JY, Peng X, Xiao KY, Xu Q, Wang C. Which probiotic has the best effect on preventing *Clostridium difficile*-associated diarrhea? A systematic review and network meta-analysis. *J Dig Dis*. (2020) 21:69–80. doi: 10.1111/1751-2980.12839

221. Alberda C, Marcushamer S, Hewer T, Journault N, Kutsogiannis D. Feasibility of a *Lactobacillus casei* Drink in the Intensive Care Unit for Prevention of Antibiotic Associated Diarrhea and Clostridium difficile. *Nutrients*. (2018) 10:539. doi: 10.3390/nu10050539

222. Wieërs G, Verbelen V, Van Den Driessche M, Melnik E, Vanheule G, Marot JC, et al. Do probiotics during in-hospital antibiotic treatment prevent colonization of gut microbiota with multi-drug-resistant bacteria? A randomized placebo-controlled trial comparing *Saccharomyces* to a mixture of *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces*. *Front Public Health*. (2020) 8:578089. doi: 10.3389/fpubh.2020.578089

223. Machairas N, Pistiki A, Droggiti DI, Georgitsi M, Pelekanos N, Damoraki G, et al. Pre-treatment with probiotics prolongs survival after experimental infection by multidrug-resistant *Pseudomonas aeruginosa* in rodents: an effect on sepsis-induced immunosuppression. *Int J Antimicrob Agents*. (2015) 45:376–84. doi: 10.1016/j.ijantimicag.2014.11.013

224. Swanson KS, Gibson GR, Hutkins R, Reimer RA, Reid G, Verbeke K, et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. *Nat Rev Gastroenterol Hepatol*. (2020) 17:687–701. doi: 10.1038/s41575-020-0344-2

225. Shoaf K, Mulvey GL, Armstrong GD, Hutkins RW. Prebiotic galactooligosaccharides reduce adherence of enteropathogenic *Escherichia coli* to tissue culture cells. *Infect Immun*. (2006) 74:6920–8. doi: 10.1128/iai.01030-06

226. Meng X, Kawahara K, Matsushita K, Nawa Y, Shrestha B, Kikuchi K, et al. Attenuation of LPS-induced iNOS expression by 1,5-anhydro-d-fructose. *Biochem Biophys Res Commun*. (2009) 387:42–6. doi: 10.1016/j.bbrc.2009.06.108

227. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol*. (2017) 14:491–502. doi: 10.1038/nrgastro.2017.75

228. Goldenberg JZ, Yap C, Lytvyn L, Lo CK, Beardsley J, Mertz D, et al. Probiotics for the prevention of *Clostridium difficile*-associated diarrhea in adults and children. *Cochrane Database Syst Rev*. (2017) 12:Cd006095. doi: 10.1002/14651858.CD006095.pub4

229. Fooks LJ, Gibson GR. *In vitro* investigations of the effect of probiotics and prebiotics on selected human intestinal pathogens. *FEMS Microbiol Ecol*. (2002) 39:67–75. doi: 10.1111/j.1574-6941.2002.tb00907.x

230. Salminen S, Collado MC, Endo A, Hill C, Lebeer S, Quigley EMM, et al. The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. *Nat Rev Gastroenterol Hepatol*. (2021) 18:649–67. doi: 10.1038/s41575-021-00440-6

231. Le Lay C, Fernandez B, Hammami R, Ouellette M, Fliss I. On *Lactococcus lactis* UL719 competitivity and nisin (Nisaplin(®)) capacity to inhibit Clostridium difficile in a model of human colon. *Front Microbiol*. (2015) 6:1020. doi: 10.3389/fmicb.2015.01020

232. Vukomanović M, Žunič V, Kunej Š, Jančar B, Jeverica S, Podlipec R, et al. Nano-engineering the antimicrobial spectrum of *Lantibiotics*: activity of nisin against gram negative bacteria. *Sci Rep*. (2017) 7:4324. doi: 10.1038/s41598-017-04670-0

233. Brunati C, Thomsen TT, Gaspari E, Maffioli S, Sosio M, Jabes D, et al. Expanding the potential of NAI-107 for treating serious ESKAPE pathogens: synergistic combinations against Gram-negatives and bactericidal activity against non-dividing cells. *J Antimicrob Chemother*. (2018) 73:414–24. doi: 10.1093/jac/dkx395

234. Gregory AL, Pensinger DA, Hryckowian AJ. A short chain fatty acid-centric view of Clostridioides difficile pathogenesis. *PLoS Pathog*. (2017) 17:e1009959. doi: 10.1371/journal.ppat.1009959

235. Sorg JA, Sonenshein AL. Bile salts and glycine as cogerminants for *Clostridium difficile* spores. *J Bacteriol*. (2008) 190:2505–12. doi: 10.1128/jb.01765-07

236. Van Der Waaij D, Vossen JM, Altes CK, Hartgrink C. Reconventionalization following antibiotic decontamination in man and animals. *Am J Clin Nutr*. (1977) 30:1887–95. doi: 10.1093/ajcn/30.11.1887

237. Green JE, Davis JA, Berk M, Hair C, Loughman A, Castle D, et al. Efficacy and safety of fecal microbiota transplantation for the treatment of diseases other than *Clostridium difficile* infection: a systematic review and meta-analysis. *Gut Microbes*. (2020) 12:1–25. doi: 10.1080/19490976.2020.1854640

238. Baunwall SMD, Andreasen SE, Hansen MM, Kelsen J, Høyer KL, Rågård N, et al. Faecal microbiota transplantation for first or second *Clostridioides difficile* infection (EarlyFMT): a randomised, double-blind, placebo-controlled trial. *Lancet Gastroenterol Hepatol*. (2022) 7:1083–91. doi: 10.1016/s2468-1253(22)00276-x

239. Orenstein R, Dubberke ER, Khanna S, Lee CH, Yoho D, Johnson S, et al. Durable reduction of *Clostridioides difficile* infection recurrence and microbiome restoration after treatment with RBX2660: results from an open-label phase 2 clinical trial. *BMC Infect Dis*. (2022) 22:245. doi: 10.1186/s12879-022-07256-y

240. Walter J, Shanahan F. Fecal microbiota-based treatment for recurrent *Clostridioides difficile* infection. *Cell*. (2023) 186:1087. doi: 10.1016/j.cell.2023.02.034

241. Benech N, Barbut F, Fitzpatrick F, Krutova M, Davies K, Druart C, et al. Update on microbiota-derived therapies for recurrent *Clostridioides difficile* infections. *Clin Microbiol Infect*. (2024) 30:462–68. doi: 10.1016/j.cmi.2023.12.007

242. Manges AR, Steiner TS, Wright AJ. Fecal microbiota transplantation for the intestinal decolonization of extensively antimicrobial-resistant opportunistic pathogens: a review. *Infect Dis (Lond)*. (2016) 48:587–92. doi: 10.1080/23744235.2016.1177199

243. Kim SM, Defazio JR, Hyoju SK, Sangani K, Keskey R, Krezalek MA, et al. Fecal microbiota transplant rescues mice from human pathogen mediated sepsis by restoring systemic immunity. *Nat Commun*. (2020) 11:2354. doi: 10.1038/s41467-020-15545-w

244. Dinh A, Fessi H, Duran C, Batista R, Michelon H, Bouchand F, et al. Clearance of carbapenem-resistant Enterobacteriaceae vs vancomycin-resistant *enterococci* carriage after faecal microbiota transplant: a prospective comparative study. *J Hosp Infect*. (2018) 99:481–86. doi: 10.1016/j.jhin.2018.02.018

245. Shin J, Lee JH, Park SH, Cha B, Kwon KS, Kim H, et al. Efficacy and safety of fecal microbiota transplantation for clearance of multidrug-resistant organisms under multiple comorbidities: A prospective comparative trial. *Biomedicines*. (2022) 10:2404. doi: 10.3390/biomedicines10102404

246. Bilinski J, Grzesiowski P, Sorensen N, Madry K, Muszynski J, Robak K, et al. Fecal microbiota transplantation in patients with blood disorders inhibits gut colonization with antibiotic-resistant bacteria: results of a prospective, single-center study. *Clin Infect Dis*. (2017) 65:364–70. doi: 10.1093/cid/cix252

247. Wei Y, Gong J, Zhu W, Guo D, Gu L, Li N, et al. Fecal microbiota transplantation restores dysbiosis in patients with methicillin resistant Staphylococcus aureus enterocolitis. *BMC Infect Dis*. (2015) 15:265. doi: 10.1186/s12879-015-0973-1

248. Bier N, Hanson B, Jiang ZD, Dupont HL, Arias CA, Miller WR. A case of successful treatment of recurrent urinary tract infection by extended-spectrum β-lactamase producing *Klebsiella pneumoniae* using oral lyophilized fecal microbiota transplant. *Microb Drug Resist*. (2023) 29:34–8. doi: 10.1089/mdr.2022.0031

249. Defilipp Z, Bloom PP, Torres Soto M, Mansour MK, Sater MRA, Huntley MH, et al. Drug-resistant E. coli bacteremia transmitted by fecal microbiota transplant. *N Engl J Med*. (2019) 381:2043–50. doi: 10.1056/NEJMoa1910437

250. Pamer EG. Fecal microbiota transplantation: effectiveness, complexities, and lingering concerns. *Mucosal Immunol*. (2014) 7:210–4. doi: 10.1038/mi.2013.117

251. Danne C, Rolhion N, Sokol H. Recipient factors in faecal microbiota transplantation: one stool does not fit all. *Nat Rev Gastroenterol Hepatol*. (2021) 18:503–13. doi: 10.1038/s41575-021-00441-5

252. Anand T, Virmani N, Kumar S, Mohanty AK, Pavulraj S, Bera BC, et al. Phage therapy for treatment of virulent *Klebsiella pneumoniae* infection in a mouse model. *J Glob Antimicrob Resist*. (2020) 21:34–41. doi: 10.1016/j.jgar.2019.09.018

253. Li Z, Li W, Ma W, Ding Y, Zhang Y, Yang Q, et al. Characterization and application of a lytic phage D10 against multidrug-resistant. *Salmonella Viruses*. (2021) 13:1626. doi: 10.3390/v13081626

254. Weber-Dąbrowska B, Żaczek M, Łobocka M, Łusiak-Szelachowska M, Owczarek B, Orwat F, et al. Characteristics of Environmental *Klebsiella pneumoniae* and *Klebsiella oxytoca* Bacteriophages and Their Therapeutic Applications. *Pharmaceutics*. (2023) 15:434. doi: 10.3390/pharmaceutics15020434

255. Wang L, Tkhilaishvili T, Jiang Z, Pirlar RF, Ning Y, Millán Laleona A, et al. Phage-liposome nanoconjugates for orthopedic biofilm eradication. *J Control Release*. (2024) 376:949–60. doi: 10.1016/j.jconrel.2024.09.049

256. Pirnay JP, Djebara S, Steurs G, Griselain J, Cochez C, De Soir S, et al. Personalized bacteriophage therapy outcomes for 100 consecutive cases: a multicentre, multinational, retrospective observational study. *Nat Microbiol*. (2024) 9:1434–53. doi: 10.1038/s41564-024-01705-x

257. Forti F, Roach DR, Cafora M, Pasini ME, Horner DS, Fiscarelli EV, et al. Design of a broad-range bacteriophage cocktail that reduces *Pseudomonas aeruginosa* biofilms and treats acute infections in two animal models. *Antimicrob Agents Chemother*. (2018) 62:e02573-17. doi: 10.1128/aac.02573-17

258. Alves DR, Perez-Esteban P, Kot W, Bean JE, Arnot T, Hansen LH, et al. A novel bacteriophage cocktail reduces and disperses *Pseudomonas aeruginosa* biofilms under static and flow conditions. *Microb Biotechnol*. (2016) 9:61–74. doi: 10.1111/1751-7915.12316

259. Liu S, Hon K, Bouras GS, Psaltis AJ, Shearwin K, Wormald PJ, et al. APTC-C-SA01: A novel bacteriophage cocktail targeting *Staphylococcus aureus* and MRSA biofilms. *Int J Mol Sci*. (2022) 23:6116. doi: 10.3390/ijms23116116

260. Joo H, Wu SM, Soni I, Wang-Crocker C, Matern T, Beck JP, et al. Phage and antibiotic combinations reduce *Staphylococcus aureus* in static and dynamic biofilms grown on an implant material. *Viruses*. (2023) 15:460. doi: 10.3390/v15020460

261. Mu A, Mcdonald D, Jarmusch AK, Martino C, Brennan C, Bryant M, et al. Assessment of the microbiome during bacteriophage therapy in combination with systemic antibiotics to treat a case of staphylococcal device infection. *Microbiome*. (2021) 9:92. doi: 10.1186/s40168-021-01026-9

262. Duerkop BA, Clements CV, Rollins D, Rodrigues JL, Hooper LV. A composite bacteriophage alters colonization by an intestinal commensal bacterium. *Proc Natl Acad Sci U S A*. (2012) 109:17621–6. doi: 10.1073/pnas.1206136109

263. Badawy S, Pajunen MI, Haiko J, Baka Z, Abou-Dobara MI, El-Sayed AKA, et al. Identification and functional analysis of temperate siphoviridae bacteriophages of *Acinetobacter baumannii*. *Viruses*. (2020) 12:604. doi: 10.3390/v12060604

264. Morello E, Saussereau E, Maura D, Huerre M, Touqui L, Debarbieux L. Pulmonary bacteriophage therapy on *Pseudomonas aeruginosa* cystic fibrosis strains: first steps towards treatment and prevention. *PLoS One*. (2011) 6:e16963. doi: 10.1371/journal.pone.0016963

265. Fong SA, Drilling A, Morales S, Cornet ME, Woodworth BA, Fokkens WJ, et al. Activity of bacteriophages in removing biofilms of *Pseudomonas aeruginosa* isolates from chronic rhinosinusitis patients. *Front Cell Infect Microbiol*. (2017) 7:418. doi: 10.3389/fcimb.2017.00418

266. Martinet MG, Lohde M, Higazy D, Brandt C, Pletz MW, Middelboe M, et al. Diversification of *Pseudomonas aeruginosa* Biofilm Populations under Repeated Phage Exposures Decreases the Efficacy of the Treatment. *Microorganisms*. (2024) 12:1880. doi: 10.3390/microorganisms12091880

267. Nath A, Bhattacharjee R, Nandi A, Sinha A, Kar S, Manoharan N, et al. Phage delivered CRISPR-Cas system to combat multidrug-resistant pathogens in gut microbiome. *BioMed Pharmacother*. (2022) 151:113122. doi: 10.1016/j.biopha.2022.113122

268. Fang Q, Feng Y, Mcnally A, Zong Z. Characterization of phage resistance and phages capable of intestinal decolonization of carbapenem-resistant *Klebsiella pneumoniae* in mice. *Commun Biol*. (2022) 5:48. doi: 10.1038/s42003-022-03001-y

269. Corbellino M, Kieffer N, Kutateladze M, Balarjishvili N, Leshkasheli L, Askilashvili L, et al. Eradication of a multidrug-resistant, carbapenemase-producing *Klebsiella pneumoniae* isolate following oral and intra-rectal therapy with a custom made, lytic bacteriophage preparation. *Clin Infect Dis*. (2020) 70:1998–2001. doi: 10.1093/cid/ciz782

frontiers | Frontiers in Immunology

TYPE Original Research
PUBLISHED 21 March 2025
DOI 10.3389/fimmu.2025.1553757

Check for updates

OPEN ACCESS

EDITED BY
Nar Singh Chauhan,
Maharshi Dayanand University, India

REVIEWED BY
Catherine Chaput,
NEC Laboratories Europe, Germany
Pankaj Jha,
Amrita Research Center Delhi-NCR, India

*CORRESPONDENCE
Yanling Wei
lingzi016@tmmu.edu.cn
Dongfeng Chen
chendf@tmmu.edu.cn

RECEIVED 31 December 2024
ACCEPTED 04 March 2025
PUBLISHED 21 March 2025

CITATION
Chen L, Ruan G, Zhao X, Yi A, Xiao Z, Tian Y, Cheng Y, Chen D and Wei Y (2025) *Pseudomonas aeruginosa* enhances anti-PD-1 efficacy in colorectal cancer by activating cytotoxic CD8$^+$ T cells. *Front. Immunol.* 16:1553757. doi: 10.3389/fimmu.2025.1553757

COPYRIGHT
© 2025 Chen, Ruan, Zhao, Yi, Xiao, Tian, Cheng, Chen and Wei. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Pseudomonas aeruginosa enhances anti-PD-1 efficacy in colorectal cancer by activating cytotoxic CD8$^+$ T cells

Lu Chen, Guangcong Ruan, Xuefei Zhao, Ailin Yi, Zhifeng Xiao, Yuting Tian, Yi Cheng, Dongfeng Chen* and Yanling Wei*

Department of Gastroenterology, Chongqing Key Laboratory of Digestive Malignancies, Daping Hospital, Army Medical University (Third Military Medical University), Chongqing, China

Background: Immune checkpoint therapy for colorectal cancer (CRC) has been found to be unsatisfactory for clinical treatment. Fecal microbiota transplantation (FMT) has been shown to remodel the intestinal flora, which may improve the therapeutic effect of αPD-1. Further exploration of key genera that can sensitize cells to αPD-1 for CRC treatment and preliminary exploration of immunological mechanisms may provide effective guidance for the clinical treatment of CRC.

Methods: In this study, 16S rRNA gene sequencing was analyzed in the fecal flora of both responders and no-responders to αPD-1 treatment, and the therapeutic effect was experimentally verified.

Results: *Pseudomonas aeruginosa* was found to be highly abundant in the fecal flora of treated mice, and *Pseudomonas aeruginosa* mannose-sensitive hemagglutinin (PA-MSHA) in combination with αPD-1 was effective in the treatment of CRC through the induction of CD8$^+$ T-cell immunological effects.

Conclusion: The clinical drug PA-MSHA can be used in combination with αPD-1 for the treatment of CRC as a potential clinical therapeutic option.

KEYWORDS

αPD-1, *Pseudomonas aeruginosa*, PA-MSHA, CRC, CD8$^+$T cell

1 Introduction

In recent years, colorectal cancer (CRC) has become the third most common form of malignant tumor, following only lung and stomach cancer, and CRC has the second highest mortality rate (1). The preferred method of treatment for CRC is surgical resection combined with radiotherapy and chemotherapy; however, immune checkpoint therapy has recently been incorporated as a supplementary therapy (2). Nevertheless, only approximately 20% of patients with advanced CRC respond to anti-programmed cell death protein 1 antibody (αPD-1) immunotherapy (3, 4). Increasing research revealed that

the microbiome plays a pivotal role in tumorigenesis and progression by influencing these processes through inflammatory and immune pathways (1, 5–10). Furthermore, the microbiome has been demonstrated to exerts an essential regulatory influence on the anti-PD-1 response in tumors, including melanoma (11).

A favorable association exists between gut microbiota and immune checkpoint blockade (ICB) efficacy, but the composition of beneficial microbiota may vary across cancer types. *Helicobacter pylori* and *Fusobacterium nucleatum* promote an unfavorable immune response against colon cancer (8, 12, 13). In contrast, *Roseburia intestinalis* and *Lactobacillus johnsonii* play active roles in colon cancer treatment, although their clinical applicability rates are low (14, 15). Fecal microbiota transplantation (FMT) and probiotic transplantation are effective methods for directly improving the gut microbiome. Studies have shown that FMT in combination with αPD-1 is effective against solid tumors, such as melanoma (11, 13, 16). The objective of this study was to investigate the potential of FMT as a biological agent for synergistic αPD-1 therapy in the treatment of CRC. Additionally, this study aimed to identify the most efficacious flora to provide a reference and expand the possibilities for clinical treatment.

In this study, we analyzed fecal samples collected from model mice treated with αPD-1 to assess the flora. We also investigated the ability of the intestinal flora to promote the effects of αPD-1 therapy in patients with CRC through animal model experiments. Our findings suggest that *Pseudomonas* may be a key genus involved in regulating the effects of αPD-1.

2 Materials and methods

2.1 Mouse models

Male C57BL/6 and BALB/c mice were purchased from Hunan SJA Laboratory Animal Co., Ltd (Changsha, Hunan, China). The mice were housed and reared under specific pathogen-free (SPF) conditions at the Army Specialty Medical Center. All procedures involving animals were performed in accordance with protocols approved by the Chongqing Animal Care and Use Committee.

The mice received antibiotics (ABX) for 7 days. The ABX were a mixture of ampicillin (A9518; Sigma-Aldrich, USA), streptomycin (5711; Sigma-Aldrich, USA), mucomycin (P1004; Sigma-Aldrich, USA), and vancomycin (94747; Sigma-Aldrich). In this study, we developed a mouse model of colon cancer induced by the carcinogens oxidized azomethane (AOM) and dextran sulfate sodium salt (DSS). The mice were anesthetized via an intraperitoneal (i.p.) injection of a mixture of 10 mg/kg AOM (MP Biomedicals). One week after the AOM challenge, the mice received drinking water containing 2.5% DSS for 7 days, followed by a normal diet and drinking water for the next 2 weeks. The DSS challenge was repeated two more times (for a total of 3 cycles of DSS), and the protocol duration was 10 weeks. During third cycle of modeling, mice started to receive FMT treatment daily. Upon completion of the model, the mice were administered an anti-mouse PD-1 monoclonal antibody (BP0146, clone number: RMP1-14, Bio X Cell) or control IgG (BP0089, clone number: 2A-3, Bio X Cell) by intraperitoneal injection (100 μg per mouse, 3 injections in total) every 3 days.

For the subcutaneous mouse model, the MSI-high CRC cell line MC38 (1×10^6/100 μL of cells per mouse) was inoculated subcutaneously into 8-week-old male C57BL/6 mice, and the CT26 cell line (1×10^6/100 μL of cells per mouse) was inoculated subcutaneously into 8-week-old male BALB/c mice. Three days after tumor implantation, the mice received daily FMT. One week after implantation, the mice were administered an anti-mouse PD-1 monoclonal antibody or control IgG by intraperitoneal injection (100 μg per mouse, 3 injections total) every 3 days.

For the PA-MSHA (Beijing Wante'er Biological Pharmaceutical Co. Ltd) combined with αPD-1 therapy, approximately 1×10^6 cells were injected subcutaneously into 6–8-week-old male mice. A total of 24 mice were equally divided into four groups: the control group (PBS), αPD-1 (100 μg), PA-MSHA (4×10^8 pcs/mL) and αPD-1(100 μg) plus PA-MSHA (4×10^8 pcs/mL) groups (17). One week after tumor implantation, PA-MSH was injected every five days. Tumor growth was monitored every 2 days by measuring tumor length (L) and width (W). Tumor volume (V) was then calculated via the formula, $V = 1/2 \times L \times W \times H$. Tumors were collected for pharmacodynamic analysis on day 27. The survival endpoint of tumor-bearing mice was reached when the primary tumor volume exceeded 1000 mm^3, when the animal demonstrated signs of severe pain and discomfort, or when the animal died because of disease progression.

2.2 T-cell isolation and activation

Mouse T cells were isolated from the spleens of naïve mice using the Mouse T-Cell Isolation Kit (19851, Thermo Fisher Scientific) and then cultured in mouse CD3 (14-0032-81, eBioscience), CD28 (14-0281-82, eBioscience) antibody-coated culture flasks with RPMI-1640 medium containing 30 U/mL recombinant mouse IL-2 (51061-MNAE, Sino Biological). T cell differentiation was induced for 48 hours in settings containing different concentrations of PA-MSHA (1×10^6 pcs/ml, 5×10^6 pcs/ml, 1×10^7 pcs/ml, 5×10^7 pcs/ml, or 1×10^8 pcs/ml).

2.3 16S rRNA gene sequencing

Fecal samples were collected from both the responder (n=4) and non-responder (n=4) groups of mice. Genomic DNA was extracted from the fecal samples, and its concentration and purity were evaluated using electrophoresis and the NanoDrop 2000 spectrophotometer. PCR amplification was performed on the extracted DNA using Phusion® High-Fidelity PCR Master Mix with GC Buffer (New England Biolabs). Universal 16S rRNA primers targeting the V4 region were utilized for the PCR reaction: 520F (5′-AYTGGGYDTAAAGNG-3′) and 802R (5′-TACNVGGGTATCTAATCC-3′). The resulting PCR products were sequenced on the NovaSeq 6000 platform. Prior to clustering, sequences with quality scores below 20, ambiguous bases, or improper primers were excluded. Chimeric sequences

were also identified and removed during the clustering process. High-quality sequences were clustered into operational taxonomic units (OTUs) at a 97% similarity threshold. The similarity in microbial community structure among samples was assessed using principal coordinate analysis (PCoA) based on the Bray-Curtis distance algorithm.

2.4 Flow cytometry

Male C57BL/6 mice were sacrificed via cervical dislocation. The tumor tissues were dissected into 1 mm^3 pieces and digested with collagenase IV (Worthington) and DNase II (Sigma) for 50 min at 37°C . Digested tumor extracts were filtered through 70 μm cell filters and centrifuged at 1650 rpm for 8 min. Cellular precipitates were collected and resuspended into single-cell suspensions for subsequent antibody labeling.

Antibodies against CD45 (103140), CD69 (104513), CD4 (100509), CD8 (100707), Ly6G (127605), CD11c (117309), CD86 (105007), CD206 (141707) and PD-1 (135229) were purchased from BioLegend. CD3 (45-0036-42), CD19 (12-0193-82) and Granzyme B (396414) were purchased from eBioscience. CD11b (557396), F4/80 (565411), MHC-II (562363), and FoxP3 (562996) antibodies were purchased from BD Biomedicals. The Zombie Aqua™ Fixable Viability Kit was purchased from BioLegend. The tumor cell suspensions were subjected to surface staining with fluorescently labeled antibodies according to the manufacturer's instructions. Cell viability was assessed using the Zombie Aqua™ Fixable Viability Kit (BioLegend). The cells were subsequently permeabilized using the Transcription Factor Buffer Set (BioLegend) and stained for FoxP3.The cells were analyzed on a CytoFLEX flow cytometer (Beckman Coulter, United States). Flow cytometry data were analyzed using FlowJo software (FlowJo, Ashland, OR, United States).

2.5 Statistical analyses

Statistical analysis was performed using GraphPad Prism 9 (GraphPad Software, CA, United States). Mann-Whitney t test was used for comparisons of two groups. Survival data were analyzed via the log-rank (Mantel-Cox) test. The values are expressed as the means ± standard errors of the means (SEM). Two-sided $P < 0.05$ was considered statistically significant ($*P \leq 0.05$, $**P \leq 0.01$, $***P < 0.001$). The numbers of animals used in each experiment are indicated in the figure legends.

3 Results

3.1 Different therapeutic effects of αPD-1 in the treatment of CRC

CRC is characterized by significantly altered microbiota that regulates the tumor microenvironment. Improving the flora structure is a viable adjuvant option for the treatment of colon cancer. Using the MC38 cell line (18), we modeled aPD-1 therapy for CRC (Figure 1A). Tumor volume changes were measured consecutively for 30 days and no significant differences were found between the IgG group and the αPD-1 group (Figure 1B). The excised tumor tissue was visually documented, and its mass was recorded (Figures 1C, D). The experimental cohorts were subsequently stratified based on the size and mass of tumor tissue, as well as its response to αPD-1. Tumor growth curves and time points were used to divide the mice into two groups, namely, the "no-responder" group and the "responder" group (Figure 1E). The tumor weights of no-responsive mice were 4.7 times greater than those of responsive mice. In addition, the tumor growth curves and tumor weights significantly differed between no-responsive mice with rapid tumor growth and responsive mice in the IgG control treatment group, with nonresponsive mice exhibiting 2.18 times greater tumor weights (Figure 1F). In addition, tumor tissue growth or weight did not significantly differ between the αPD-1 group and the IgG mAb-treated group (Figure 1G). In contrast, tumor tissue growth in responsive mice was inhibited by αPD-1, and the final weight was 0.5 times greater than that in the IgG mAb treatment group (Figure 1H).

αPD-1-responsive tumor tissues exhibited a notable decrease in Ki-67 and a notable increase in p53-positive cells (Figure 1I). These values were 0.3 times and 1.4 times greater than those of the nonresponsive group, respectively (Figure 1J). These data suggest that αPD-1 therapy for CRC is effective, but the observed differences were not significant. This outcome could be attributed to the mice utilized in the study. Certain mice may exhibit characteristics similar to those of αPD-1 treatment-responsive mice, leading to poor growth of subcutaneously implanted colon cancer cells, thereby affecting the T cell-associated immune response in the tumor immune microenvironment.

3.2 Fecal flora transplantation from αPD-1-responsive mice in combination with αPD-1 treatment improves survival in a mouse model of CRC

Previous studies have shown that gut microbiota plays an important role in supporting immune checkpoint therapies for tumors such as melanoma (5). The role of gut microbiota in promoting tumor progression and improving the efficacy of αPD-1 therapy in CRC needs to be further explored. Sufficient fecal samples were collected from healthy mice before and after tumor inoculation, after which the mice were treated with αPD-1. The mice were then separated into effective and noneffective groups on the basis of their therapeutic response. αPD-1 was combined with fecal microbiota transplantation (FMT) in both effective and noneffective mice to treat MC38-loaded tumor-bearing mice (Figure 2A).

FMT intervention in responsive mice (Re-FMT) effectively suppressed the tumor growth and significantly differed from FMT intervention in nonresponsive mice (No-FMT) (Figure 2B).

10.3389/fimmu.2025.1553757

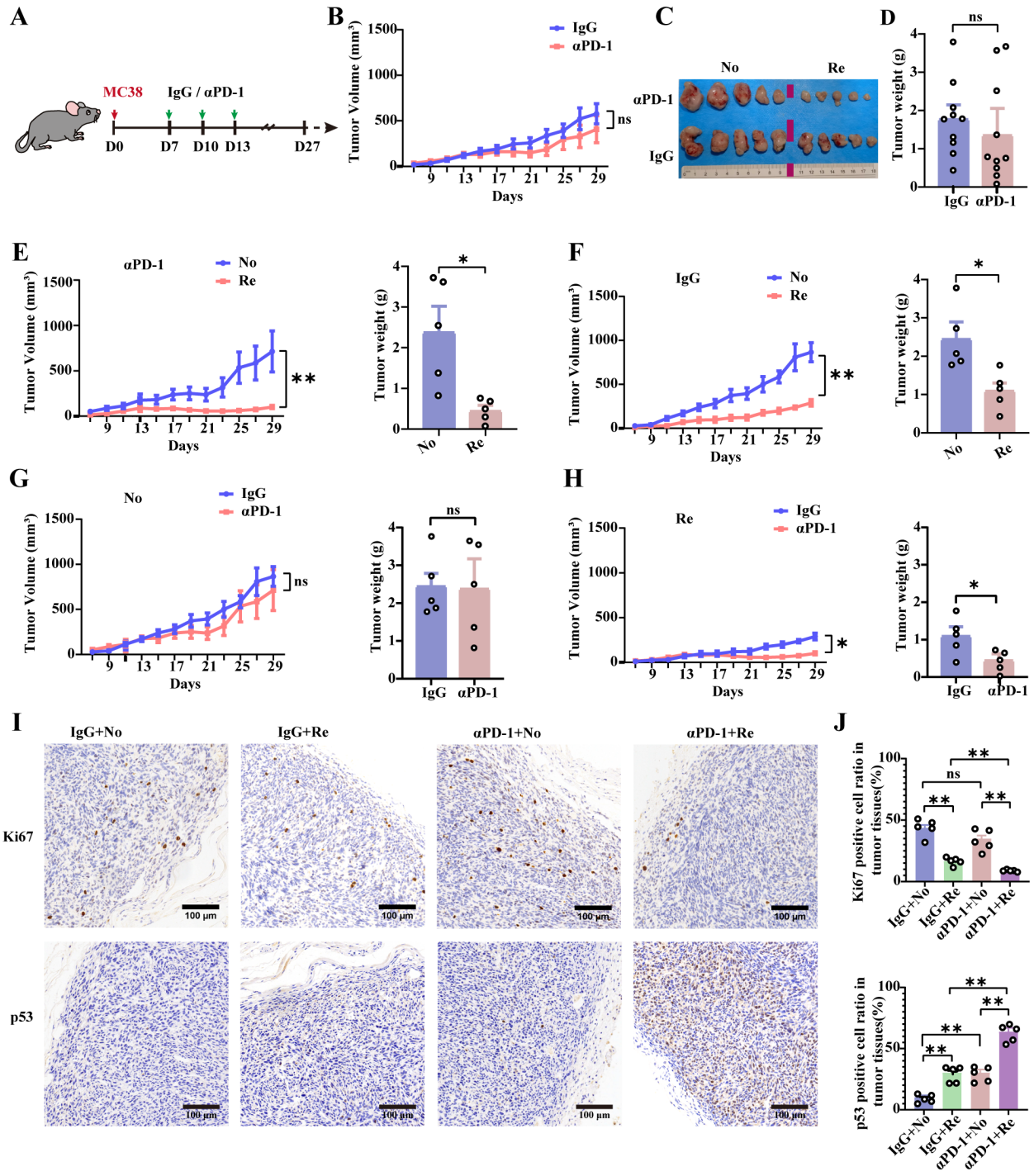


FIGURE 1
Differential therapeutic effects of αPD-1 treatment in colon cancer model mice. **(A)** Schematic showing the experimental design and schedule. **(B)** Tumor growth curves of αPD-1-treated MC38 hormone-treated mice. On the 27th day after tumor inoculation, tumor tissues were taken for imaging **(C)**, and tumor weight data were obtained **(D)**. n =10 mice per group. Tumor growth curves (left) and tumor loads (right) were counted according to tumor size (RE or NO) for αPD-1 **(E)** or IgG **(F)** treatment effects. Comparison of the therapeutic response of No **(G)** or Re **(H)** mice to αPD-1 therapy based on tumor growth curves (left) as well as tumor loads (right). n =5 mice per group. **(I)** Representative images of Ki-67 as well as p53 IHC staining after αPD-1 treatment (scale bar = 200 μm). **(J)** Statistics of the percentage of Ki67- as well as p53- positive cells. n =5 mice per group. Data represent mean ± SEM., the *P* value was determined by a Mann-Whitney *t* test. ns, not significant; *$P < 0.05$; **$P < 0.01$.

Moreover, the survival rate of the mice was significantly enhanced by the combination of Re-FMT and αPD-1 (Figure 2C). Figure 2D shows an example of tumor tissue from the 27th day after tumor inoculation in the two groups of treated mice. Furthermore, the tumor tissues were weighed, which revealed that the tumor weight of the Re-FMT + αPD-1 combination treatment group was 0.4 times higher than that of the No-FMT + αPD-1 combination treatment group (Figure 2E). Moreover, Ki67 was significantly underexpressed, and P53 was significantly overexpressed in the Re-FMT with αPD-1 combination treatment group (Figures 2F, G).

A mouse model of primary colon cancer induced by both AOM and DSS validated the promotional role of Re-FMT in αPD-1 tumor therapy (Figure 2H). Compared with treatment with No-FMT in combination with αPD-1, Re-FMT in combination with αPD-1 significantly increased the efficiency of targeted therapy (Figure 2I) and reduced the number and load of primary colon cancer tumors (Figures 2J, K). In addition, mice treated with Re-FMT in combination with αPD-1 also had lower developmental abnormality scores, significantly lower Ki-67 levels in the tumor and non-tumor colon tissues, significantly more P53-positive cells in tumor and non-tumor colon tissues (Figures 2L-N), and significantly increased protein expression of the tight junction protein ZO-1 and the mucin MUC2 (Supplementary Figure 1A, B). FMT is an important factor in the αPD-1 treatment of CRC and selecting the appropriate donor for FMT will likely support adjuvant αPD-1 colon cancer treatment.

3.3 The host intestinal flora modulates the tumor immune microenvironment to influence the αPD-1 response

It is imperative to discuss whether FMT could play a key role in promoting tumor progression and improving PD-1 treatment outcomes in patients with colon cancer by modulating the tumor immune microenvironment. The intestinal flora of the mice was modified with ABX and mouse-derived Re-FMT or No-FMT, and the mice were inoculated with MC38 cells and treated with αPD-1 7 days later (Figure 3A). Flow cytometry was used to detect immune cell infiltration and the activation of tumor tissue in experimental mice. Compared with αPD-1 and No-FMT treatment, the combination of αPD-1 and Re-FMT significantly inhibited the recruitment of $CD4^+$ T cells (especially Tregs) and enhanced the recruitment of $CD8^+$ T cells, B cells, and neutrophils in the subcutaneous tumor model (Figure 3B). The activated phenotype of $CD4^+$ T cells, CD69, was inhibited to some extent by the combination of αPD-1 and Re-FMT (Figure 3C). CD69 and Granzyme B were highly expressed in $CD8^+$ T cells in the αPD-1 -combined Re-FMT group; compared with the PD1mAb-combined No-FMT, IgG-combined No-FMT and IgG-combined Re-FMT groups, CD69 expression was 1.24-, 1.75- and 1.02-fold higher and Granzyme B expression was 1.95-, 2.22- and 1.81-fold higher, respectively (Figures 3D, F). We additionally examined the effects of PD-1 expression on the surface of $CD8^+$ T cells induced by αPD-1 and FMT intervention. The experimental results revealed that αPD-1 significantly down-regulated PD-1 expression, whereas Re-FMT intervention further mediated the down-regulation of PD-1 expression on $CD8^+$ T cells (Figure 3F). Immunofluorescence was used to identify $CD4^+$ and $CD8^+$ T cell infiltration in the parenchyma and margins of the tumor tissue. Results revealed no significant differences in $CD4^+$ T-cell presence at the tumor margins, whereas $CD8^+$ T-cell infiltration was considerably higher under αPD-1-responsive conditions (Figures 3G, I). Under PD1-responsive conditions, $CD4^+$ T-cell infiltration of the tumor parenchyma was reduced, whereas $CD8^+$ T-cell infiltration was significantly increased, but there was no significant difference in either case (Figures 3J–L).

Regional specificity determines immunological differences between primary colon cancer and subcutaneous tumor models. Therefore, we used a mouse primary colon cancer model to validate the sensitizing effect of Re-FMT on αPD-1 (Supplementary Figure 2A). Flow cytometry revealed that compared with αPD-1 combined with No-FMT, αPD-1 combined with Re-FMT inhibited $CD4^+$ T cell recruitment to mesenteric lymph nodes, whereas $CD8^+$ T cell and B cell recruitment increased (Supplementary Figure 2B). In primary colon cancer, the expression of CD69, an indicator of $CD4^+$ T-cell activation, was not significantly affected by αPD-1 or Re-FMT (Supplementary Figure 2C). However, αPD-1 in combination with Re-FMT significantly up-regulated both activated CD69 and cytotoxic Granzyme B in $CD8^+$ T-cell assays (Supplementary Figures 2D, E). The expression of PD1 and the effect of αPD-1 were not affected by Re-FMT or No-FMT (Supplementary Figure 2F). Consistent with the flow cytometry results, the immunofluorescence results revealed that αPD-1 combined with Re-FMT treatment inhibited $CD4^+$ T cell recruitment in colon cancer tissues while promoting $CD8^+$ T cell infiltration (Supplementary Figures 2G-I). These findings suggest that FMT may assist in tumor treatment by modifying the colon cancer immune microenvironment in response to tumor tolerance. Furthermore, the effectiveness of $CD8^+$ T cells against tumor cells was increased by combining FMT therapy with αPD-1.

3.4 *Pseudomonas* abundance in the host directly correlates with colon cancer resistance and αPD-1 sensitivity

Numerous experimental results suggest that FMT can be an effective companion for αPD-1 therapy in CRC, improving subcutaneous and primary CRC by remodeling the tumor microenvironment to up-regulate αPD-1 therapeutic response. However, the results also suggest that not all FMTs effectively increase αPD-1 efficacy. The selection of appropriate FMT is a key issue in adjuvant αPD-1 therapy for CRC in the clinic.

Fresh fecal samples were collected from all mice and analyzed using 16S rRNA high-throughput sequencing. The mice were categorized into responders and non-responders based on their therapeutic efficacy. Three distinct time points were established according to tumor load and treatment progression: D0 (baseline, prior to tumor cell inoculation), D7 (load, 7 days post-tumor

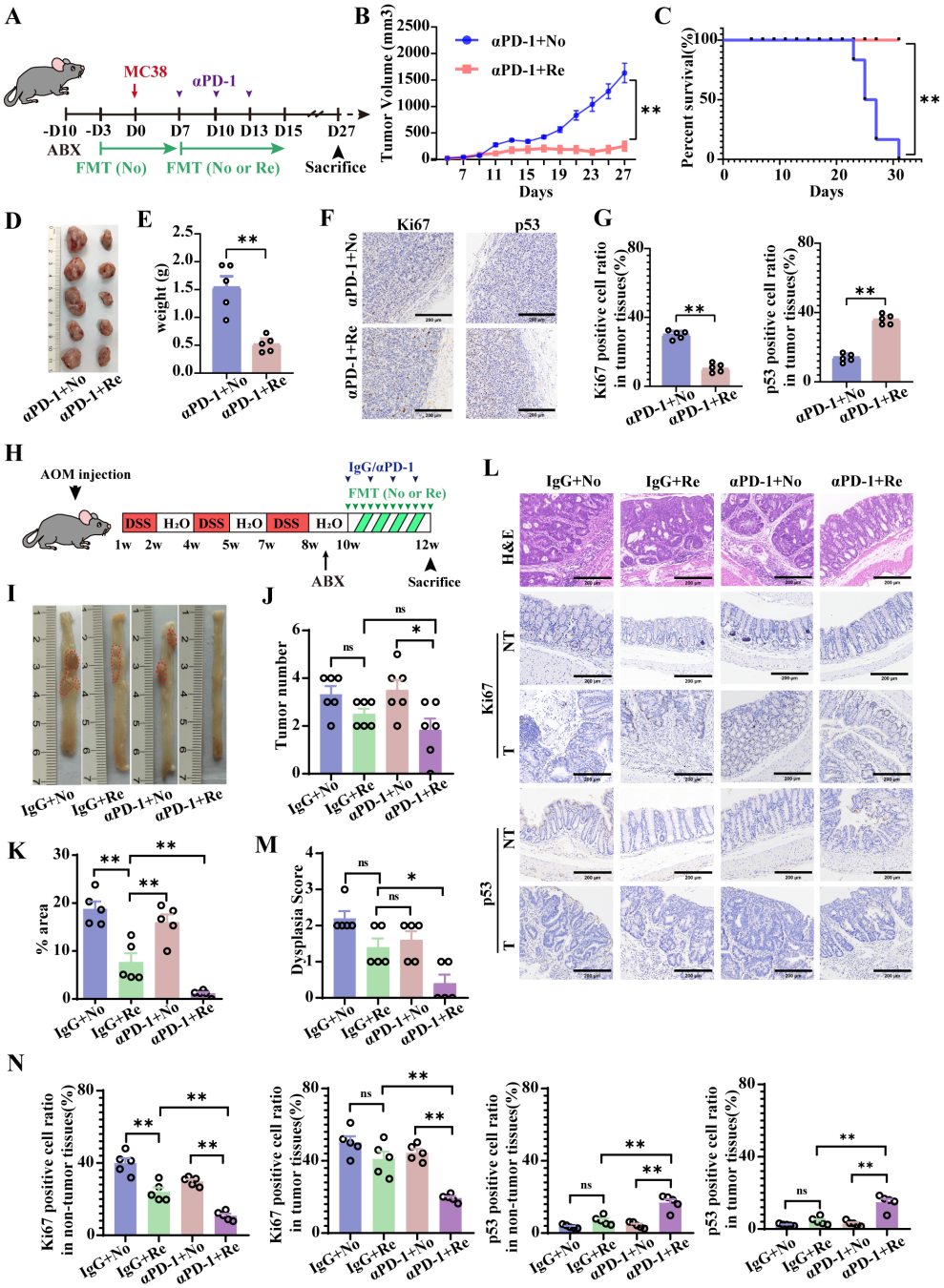


FIGURE 2
FMT from mice responsive to αPD-1 (Re-FMT) increases the therapeutic potential of αPD-1 in a mouse colon cancer model. **(A)** Schematic showing the experimental design and schedule of the subcutaneous tumor-inoculated mice. Tumor growth curves **(B)** as well as survival curves **(C)** of MC38 hormonal model mice treated with αPD-1 in combination with FMT. On the 27th day after tumor inoculation, tumor tissues were taken for imaging **(D)** as were tumor weight statistics **(E)**. **(F)** Representative imaging of Ki-67and p53 IHC staining after αPD-1 treatment (scale bar = 200 μm). **(G)** Statistics of the percentage of Ki67- and p53-positive cells. n =5 mice per group. **(H)** Schematic showing the experimental design and schedule of primary colon cancer mice. **(I)** Diagram of a representative jointed colon. Number of colon tumors **(J)** and tumor load (total tumor area, mm^2) **(K)** in model mice. **(L)** Representative H&E staining images and Ki-67 and p53 immunohistochemical (IHC) staining (scale bar = 200 μm). Histopathologic scoring **(M)** of the colon as well as histochemical scoring **(N)**. n =5 mice per group. Data represent mean ± SEM., the *P* value was determined by a Mann-Whitney *t* test. $^{*}P < 0.05$; $^{**}P < 0.01$.

inoculation), and D15 (treatment, following three rounds of therapy) (Figure 4A). Principal coordinate analysis (PCoA) revealed significant differences in β-diversity between D0 and D7 (Figure 4B). Furthermore, colony composition analysis at the genus level revealed 33 species of differential genera, with 16 species that exhibited high abundance in healthy mice, including *Alistipes*, *Parabacteroides* and *Pseudomonas* (Figure 4C). *Alistipes* and *Parabacteroides* have been demonstrated to increase αPD-1

10.3389/fimmu.2025.1553757

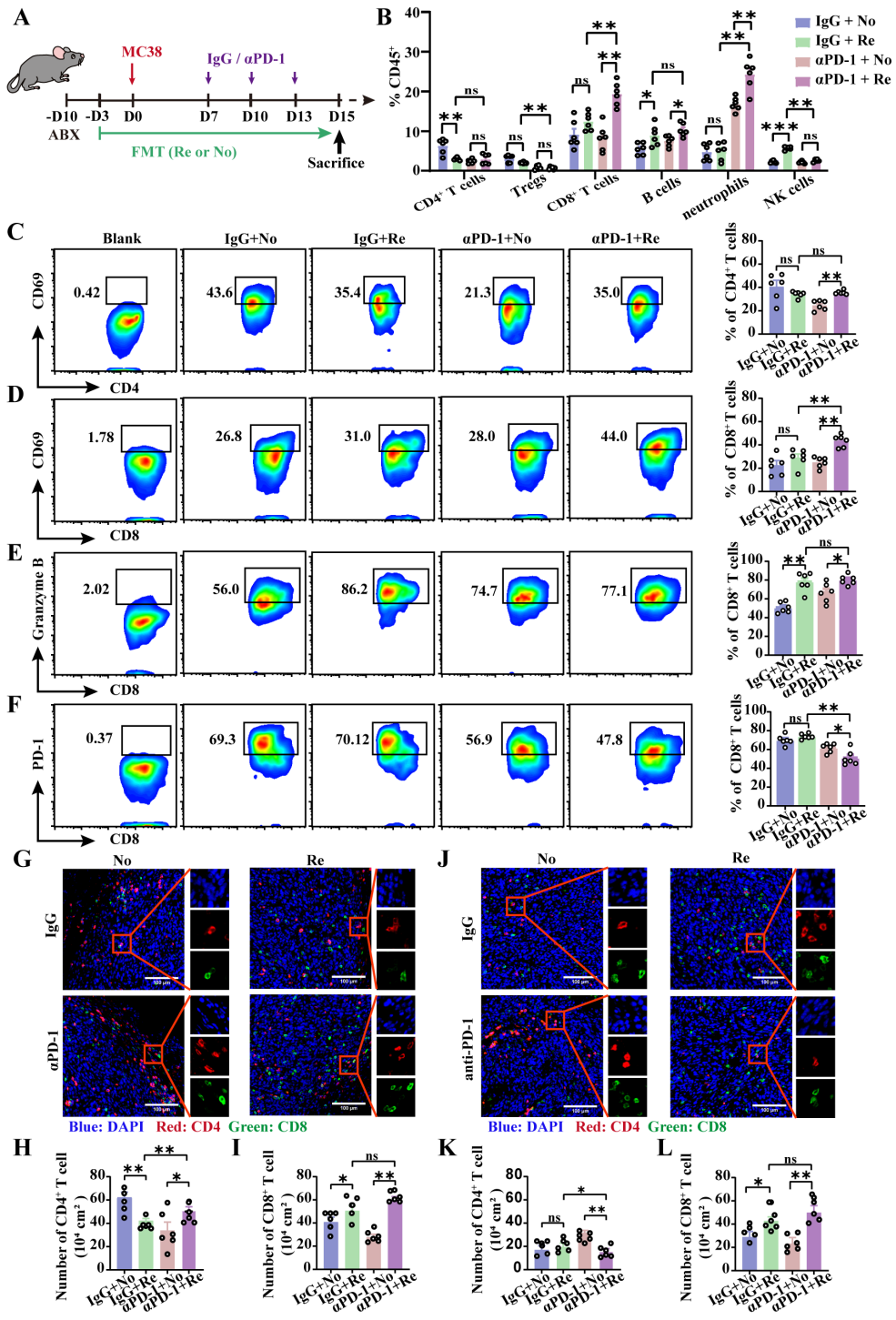


FIGURE 3
Re-FMT modulates the tumor immune microenvironment in subcutaneous colon cancer. **(A)** Schematic showing the experimental design and schedule of the subcutaneous tumor grafted mice. **(B)** Flow cytometry detection of immune cell recruitment to the tumor microenvironment. **(C)** Representative graphs of CD69 expression in $CD4^+$ T cells and positive statistics. Representative graphs of CD69 **(D)**, Granzyme B **(E)**, and PD-1 **(F)** expression in $CD8^+$ T cells and positive statistics. **(G)** Representative map of T cell infiltration at the tumor margin. The numbers of $CD4^+$ T cells **(H)** and $CD8^+$ T cells **(I)** at the tumor margin were counted. **(J)** Representative image of T cell infiltration in the tumor parenchyma. Counts of $CD4^+$ T cells **(K)** and $CD8^+$ T cells **(L)** in the tumor parenchyma. n =6 mice per group. Data represent mean ± SEM., the *P* value was determined by a Mann-Whitney *t* test. ns, not significant; $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

resistance and influence the prognosis of CRC treatment (19–21). *Pseudomonas* has been shown to possess anti-tumor properties (21), which may be a key factor by which healthy mouse feces can be employed as an adjunct to ICB for CRC.

We compared the differential effects of FMT on αPD-1 and found no significant difference in beta diversity between the Re and No groups at D0, D7, or D15 (Figure 4D-F). The intersection of genera with two-fold differences in mean values at each time point was extracted, and a heatmap presented the expression of each genus in the samples (Figures 4G, H). The results revealed a high abundance of *Pseudomonas* in the Regroup at all time points (Figure 4I, Supplementary Figures 3A, B). In addition, the cross-comparison between the two groups (Re and No) for tumor growth differences in the IgG group did not correspond to *Pseudomonas* (Supplementary Figures 3C-H). These results suggest that elevating the relative abundance of *Pseudomonas* in the host intestinal microbiota could potentially improve the therapeutic outcomes of αPD-1 treatment for CRC.

3.5 *Pseudomonas aeruginosa* mannose-sensitive hemagglutinin combined with αPD-1 effectively treats CRC

Pseudomonas aeruginosa (*P. aeruginosa*) *is a common subtype of Pseudomonas* and is usually considered to be associated with clinical infections (22, 23). Recent studies have suggested that *P. aeruginosa* and its secreted cupredoxin azurin are involved in tumor immunity (21). *P. aeruginosa mannose-sensitive-hemagglutinin* (PA-MSHA) is a drug developed based on the anti-tumor properties of *P. aeruginosa* for the treatment of clinical malignancies (17, 24–26). In this study, PA-MSHA was used instead of *P. aeruginosa* to explore its immune effect on αPD-1 in the treatment of CRC.

The therapeutic effect of PA-MSHA in combination with αPD-1 dosing on CRC was evaluated in MC38 and CT26 models. Compared with monotherapy, serial tumor volume measurements demonstrated that αPD-1 in combination with PA-MSHA exhibited a pronounced inhibitory effect on tumor growth (Figures 5A, D) and notably increased the survival of MC38- and CT26-loaded mice (Figures 5B, E).The tumor tissues from the MC38-loaded mice were photographed on day 25, and the tumor weights were determined, which showed that the tumor masses in the αPD-1 combined with PA-MSHA treatment group were 0.203 and 0.235 times higher than those in the αPD-1 or PA-MSHA alone groups, respectively (Figure 5C). Similarly, in the CT26 model, the tumor mass in the αPD-1 combined with PA-MSHA treatment group was 0.15 and 0.09 times higher than those in the αPD-1 or PA-MSHA alone group, respectively (Figure 5F). These findings suggest that PA-MSHA significantly enhances the therapeutic effect of αPD-1 on CRC.

Activated $CD8^+$ T cells were induced using different concentrations of PA-MSHA, as evidenced by the flow cytometry assay. The expression of the T-cell activation marker CD69 and the cytotoxicity marker Granzyme B was significantly elevated in response to PA-MSHA, with the optimal induction occurring at a concentration of 10^7 pcs/mL (Figures 5G, H). The relevant results further demonstrated that PA-MSHA could enhance the therapeutic effects of αPD-1 in CRC by further mediating $CD8^+$ T cell activation as well as Granzyme B expression (Figure 5I).

4 Discussions

FMT can sensitize αPD-1 to therapeutic effects in a wide range of solid tumors (11, 13–16). Here, we demonstrate the therapeutic effect of FMT-sensitizing PD-1 in CRC and emphasize the significant role of a high abundance of *Pseudomonas* in sensitizing PD-1, thereby advancing our understanding of FMT-assisted PD-1 therapy for CRC. *Pseudomonas* is a genus of gram-negative aerobic or slightly aerobic bacteria belonging to *Proteobacteria*, described in 1894. The most studied species is *P. aeruginosa* (27). In recent years, the worldwide spread of so-called high-risk clones of multidrug-resistant or extensively drug-resistant *P. aeruginosa* has become a public health threat (28, 29). *P. aeruginosa* causes a wide range of acute and chronic infections through the secretion of many cellular immunity-associated virulence factors, and it is multidrug resistant (30–33). The clinical morbidity and mortality rates associated with *P. aeruginosa* infections can reach 40%, with an attributable mortality rate of 13.5%, even when appropriate treatment is administered (34–36).

P. aeruginosa traditionally induces apoptosis in cancer cells by secreting the cupredoxin azurin (37–39). Researchers have developed an inactivating agent for tumor therapy based on the cellular immune-inducing properties of *P. aeruginosa*. PA-MSHA is safe and effective as an adjuvant treatment for lung cancer, liver cancer, breast cancer, bladder cancer, and lymphoma (40–45). Zhang et al., based on clinical observations, demonstrated that PA-MSHA-induced cytokine-induced killer (CIK) cells promote the progression of chemotherapy in malignant tumors by mediating CIK cell proliferation and the secretion of pro-inflammatory factors such as IFN-γ (25). In our study, the low abundance of *P. aeruginosa* in the gut microbiota may contribute to the suboptimal efficacy of αPD-1 therapy in CRC, a phenomenon not previously elucidated. Further investigations revealed that the inactivated *P. aeruginosa* preparation, PA-MSHA, enhances the therapeutic effect of αPD-1 by promoting $CD8^+$ T cell activity and Granzyme B release. By exploring the role of PA-MSHA in both chemotherapy and immune checkpoint therapy, our findings collectively suggest that PA-MSHA may serve as a promising adjuvant in future clinical cancer treatments. It should be noted that although therapeutic effects were observed in animal models, their clinical translatability necessitates further investigation. Additionally, we have not conducted a thorough evaluation of the potential side effects or consequences associated with the combination therapy.

Recent studies have reported the role of various microorganisms in CRC treatment, including *Roseburia intestinalis* and *Lactococcus lactis*. *Roseburia intestinalis* has been shown to enhance the efficacy of αPD-1 treatment for CRC by

10.3389/fimmu.2025.1553757

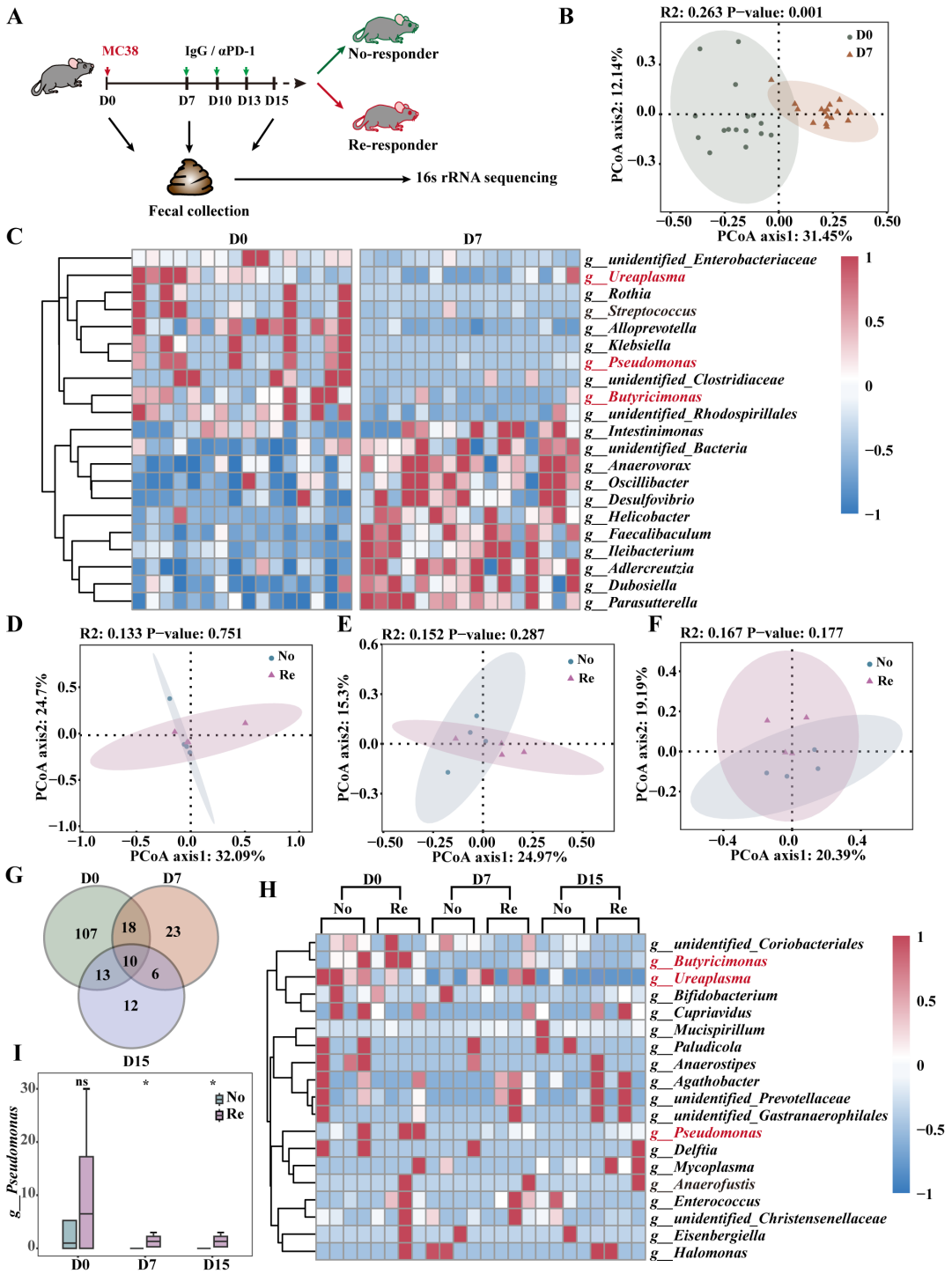


FIGURE 4
High abundance of *Pseudomonas* in therapeutically effective FMT. **(A)** Schematic diagram showing the process of αPD-1 immunotherapy in the subcutaneous MC38 xenograft tumor model. **(B)** PCoA of 16S rRNA gene sequencing of fecal samples from D0 and D7 mice at the operational taxonomic unit (OTU)level. n =16 mice per group. **(C)** Heatmap demonstrating P<0.05 differential bacterial expression in fecal samples from healthy and tumor-bearing mice. PCoA of 16S rRNA gene sequencing of D0 **(D)**, D7 **(E)** and D15 **(F)** fecal samples from responder mice and no-responder mice at the OTU level. n =4 mice per group. Intersecting genera with more than 2-fold differences in D0, D7 and D15 day means **(G)** and a heatmap of the expression of each genus in the samples **(H)**. The color bar represents the abundance. The relative abundance of *Pseudomonas* **(I)** in the FMT of αPD-1-treated mice was subjected to statistical analysis. n =4 mice per group. Data represent mean ± SEM., the *P* value was determined by a Mann-Whitney *t* test. ns, not significant; *$P < 0.05$.

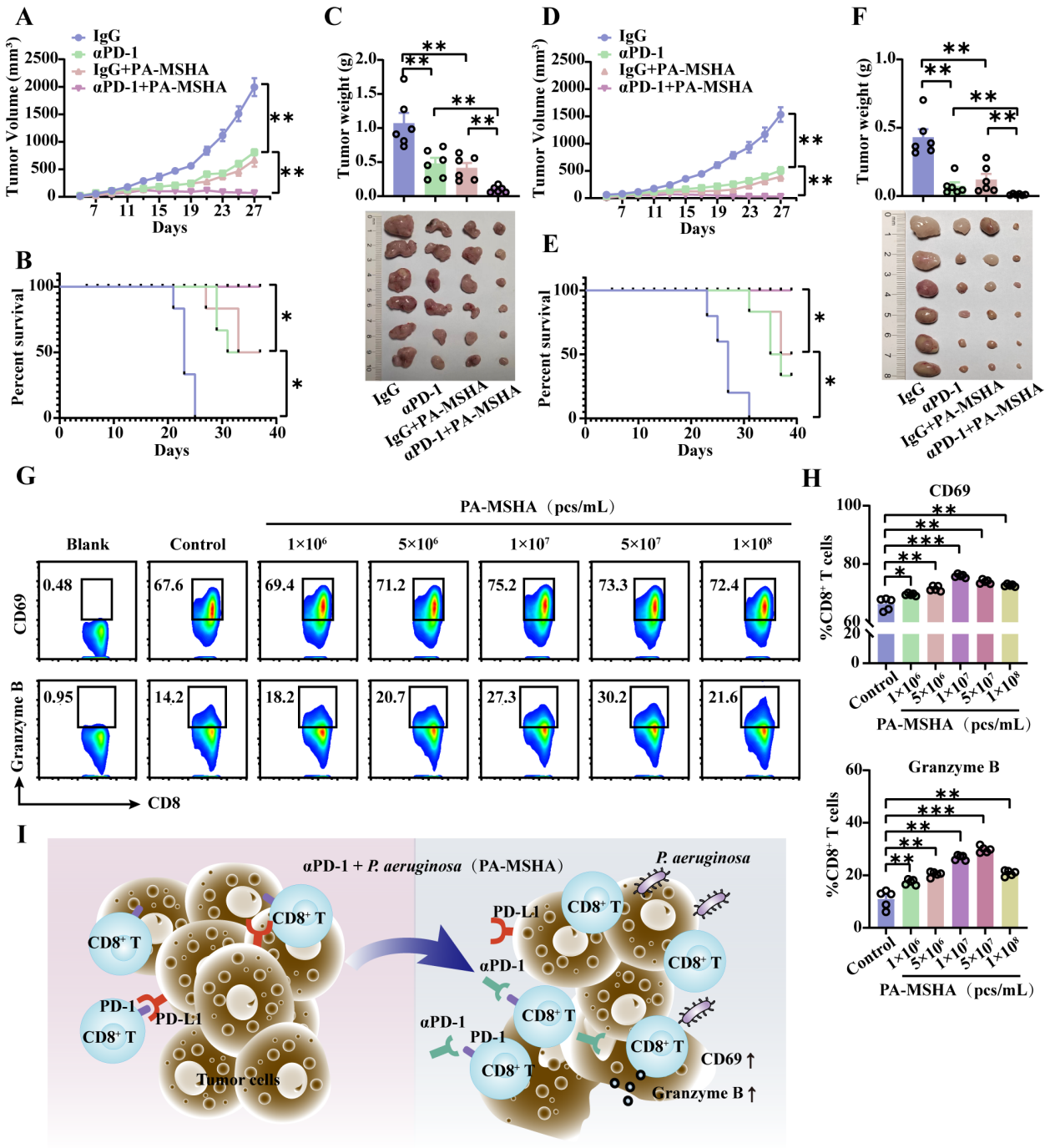


FIGURE 5
PA-MSHA sensitized cells to αPD-1 for CRC treatment by inducing CD8$^+$ T cell activation and cytotoxicity. Tumor growth curves **(A)** and survival curves **(B)** of MC38 hormonal model mice treated with PA-MSHA in combination with αPD-1. **(C)** On day 25, tumors were excised from MC38-loaded mice and subjected to statistical analysis. Tumor growth curves **(D)** and survival curves **(E)** of CT26 hormonal model mice treated with PA-MSHA in combination with αPD-1. **(F)** On day 25, tumors were excised from CT26-loaded mice and subjected to statistical analysis. n =6 mice per group. After PA-MSHA induced CD8$^+$ T cells *in vitro* for 48 h, the CD8$^+$ T cell activation marker DC69 and the cytotoxicity marker Granzyme B **(G)** were detected by flow cytometry and quantified **(H)**. n=5 per group. **(I)** *Pseudomonas aeruginosa* and its preparation PA-MSHA mediate the therapeutic effects of αPD-1 on CRC by increasing CD8$^+$ T cell activation and cytotoxicity. Data represent mean ± SEM., the *P* value was determined by a Mann-Whitney *t* test. $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

inducing functional CD8$^+$ T-cell immunity through the metabolite butyrate (14). *Lactococcus lactis* inhibits CRC progression through its production of α-mannosidase (46). Notably, researchers have focused their attention on the role of beneficial bacteria. Indeed, among the FMTs for which αPD-1 treatment was effective, the most significant variability was observed in *P. aeruginosa*, despite its status as a more commonly presumed causative organism in clinical infections.

In conclusion, our findings demonstrate that *P. aeruginosa* sensitizes cells to αPD-1 for the treatment of CRC mediated anti-tumor immune responses by activating cytotoxic $CD8^+$ T cells and upregulating Granzyme B expression. Furthermore, PA-MSHA has already received approval from the FDA as a pharmaceutical, suggesting that this novel combination therapy holds promise as an alternative approach to enhancing treatment outcomes, particularly for colorectal cancer patients who have not responded favorably to immunotherapy. This study presents an innovative strategy for integrating bacterial drugs with immune checkpoint inhibitors, offering potential benefits for individuals undergoing cancer immunotherapy.

Data availability statement

The data presented in the study are deposited in the China National GeneBank DataBase (CNGBdb, https://db.cngb.org/) repository, accession number CNP0006126.

Ethics statement

The animal study was approved by Laboratory Animal Welfare and Ethics Committee of Third Military Medical University. The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

LC: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. GR: Data curation, Investigation, Methodology, Writing – review & editing. XZ: Data curation, Software, Writing – review & editing. AY: Methodology, Writing – review & editing. ZX: Investigation, Writing – review & editing. YT: Investigation, Resources, Writing – review & editing. YC: Methodology, Writing – review & editing. DC: Writing – review & editing. YW: Funding acquisition, Project administration, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This work was supported by the National Natural Science Foundation of China, Grant/Award Numbers: 82200614; the National Natural Science Foundation of China, Grant/Award Numbers: 82070571; the Program of Chongqing Innovation and Entrepreneurship Leading Talent, Grant/Award Numbers: CQYC20220303576; the Program of Chongqing Natural Science Foundation, Grant/Award Numbers: CSTB2024NSCQ-MSX0001; and the Research Project Fund of Army Medical University, Grant/Award Numbers: 2018XLC2028.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2025.1553757/full#supplementary-material

SUPPLEMENTARY FIGURE 1
FMT derived from αPD-1-treated mice affects αPD-1 treatment-mediated intestinal barrier function in primary colon cancer. **(A)** Representative MUC2 and ZO-1 immunohistochemical staining images (scale bar = 200 μm). **(B)** MUC2- and ZO-1- positive area statistics. n =5 mice per group. Data represent mean ± SEM., the *P* value was determined by a Mann-Whitney *t* test. ***$P < 0.001$; ****$P < 0.0001$.

SUPPLEMENTARY FIGURE 2
Re-FMT modulates the tumor immune microenvironment in primary colon cancer. **(A)** Schematic showing the experimental design and schedule of primary colon cancer mice. **(B)** Flow cytometry detection of immune cell recruitment in mesenteric lymph nodes. **(C)** Representative graphs of CD69 expression in $CD4^+$ T cells and positive statistics. Representative graphs of CD69 **(D)**, Granzyme B **(E)**, and PD-1 **(F)** expression in $CD8^+$ T cells. **(G)** Representative map of T cell infiltration in the tumor region of colon tissue. Statistics of $CD4^+$ T cells **(H)** as well as $CD8^+$ T cells **(I)**. n =6 mice per group. Data represent mean ± SEM., the *P* value was determined by a Mann-Whitney *t* test. ns, not significant; * $P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

SUPPLEMENTARY FIGURE 3
Pseudomonas was significantly different in the αPD-1-treated group but not in the IgG-treated group. The relative abundances of *Butyricimonas* **(A)** and *Ureaplasma* **(B)** in the FMT of αPD-1-treated mice were statistically analyzed. PCoA of 16S rRNA gene sequencing of D0 **(C)**, D7 **(D)**, and D15 **(E)** fecal samples from IgG-responder mice and no-responder mice at the OTU level; n =4 mice per group. Intersecting genera with more than 2-fold differences in D0, D7, and D15 day means **(F)** and heatmaps of the expression of each genus in the samples **(G)**. The color bar represents the abundance, n =4 mice per group. Data represent mean ± SEM., the *P* value was determined by a Mann-Whitney *t* test. ns, not significant; * $P < 0.05$.

References

1. Wong SH, Yu J. Gut microbiota in colorectal cancer: mechanisms of action and clinical applications. *Nat Rev Gastroenterol Hepatol*. (2019) 16:690–704. doi: 10.1038/s41575-019-0209-8

2. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace. Colorectal cancer. Lancet MB. (2019) 394:1467–80. doi: 10.1016/S0140-6736(19)32319-0

3. Andre T, Shiu KK, Kim TW, Jensen BV, Jensen LH, Punt C, et al. Pembrolizumab in microsatellite-instability-high advanced colorectal cancer. *N. Engl J Med*. (2020) 383:2207–18. doi: 10.1056/NEJMoa2017699

4. Overman MJ, McDermott R, Leach JL, Lonardi S, Lenz HJ, Morse MA, et al. Nivolumab in patients with metastatic DNA mismatch repair-deficient or microsatellite instability-high colorectal cancer (CheckMate 142): an open-label, multicentre, phase 2 study. *Lancet Oncol*. (2017) 18:1182–91. doi: 10.1016/S1470-2045(17)30422-9

5. Sepich-Poore GD, Zitvogel L, Straussman R, Hasty J, Wargo JA, Knight R. The microbiome and human cancer. *Science*. (2021) 371:eabc4552. doi: 10.1126/science.abc4552

6. Zitvogel L, Daillere R, Roberti MP, Routy B, Kroemer G. Anticancer effects of the microbiome and its products. *Nat Rev Microbiol*. (2017) 15:465–78. doi: 10.1038/nrmicro.2017.44

7. Lin Y, Lau HC, Liu Y, Kang X, Wang Y, Ting NL, et al. Altered mycobiota signatures and enriched pathogenic aspergillus rambellii are associated with colorectal cancer based on multicohort fecal metagenomic analyses. *Gastroenterology*. (2022) 163:908–21. doi: 10.1053/j.gastro.2022.06.038

8. Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/beta-catenin signaling via its FadA adhesin. *Cell Host Microbe*. (2013) 14:195–206. doi: 10.1016/j.chom.2013.07.012

9. Janney A, Powrie F, Mann EH. Host-microbiota maladaptation in colorectal cancer. *Nature*. (2020) 585:509–17. doi: 10.1038/s41586-020-2729-3

10. Bell HN, Rebernick RJ, Goyert J, Singhal R, Kuljanin M, Kerk SA, et al. Reuterin in the healthy gut microbiome suppresses colorectal cancer growth through altering redox balance. *Cancer Cell*. (2022) 40:185–200 e6. doi: 10.1016/j.ccell.2021.12.001

11. Routy B, Le Chatelier E, Derosa L, Duong CPM, Alou MT, Daillere R, et al. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science*. (2018) 359:91–7. doi: 10.1126/science.aan3706

12. Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, et al. Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe*. (2013) 14:207–15. doi: 10.1016/j.chom.2013.07.007

13. Oster P, Vaillant L, Riva E, McMillan B, Begka C, Truntzer C, et al. Helicobacter pylori infection has a detrimental impact on the efficacy of cancer immunotherapies. *Gut*. (2022) 71:457–66. doi: 10.1136/gutjnl-2020-323392

14. Kang X, Liu C, Ding Y, Ni Y, Ji F, Lau HCH, et al. Roseburia intestinalis generated butyrate boosts anti-PD-1 efficacy in colorectal cancer by activating cytotoxic CD8(+) T cells. *Gut*. (2023) 72:2112–22. doi: 10.1136/gutjnl-2023-330291

15. Jia D, Wang Q, Qi Y, Jiang Y, He J, Lin Y, et al. Microbial metabolite enhances immunotherapy efficacy by modulating T cell stemness in pan-cancer. *Cell*. (2024) 187:1651–1665 e21. doi: 10.1016/j.cell.2024.02.022

16. Davar D, Dzutsev AK, McCulloch JA, Rodrigues RR, Chauvin JM, Morrison RM, et al. Fecal microbiota transplant overcomes resistance to anti-PD-1 therapy in melanoma patients. *Science*. (2021) 371:595–602. doi: 10.1126/science.abf3363

17. Huang M, He F, Li D, Xie YJ, Jiang ZB, Huang JM, et al. PA-MSHA induces inflamed tumor microenvironment and sensitizes tumor to anti-PD-1 therapy. *Cell Death Dis*. (2022) 13:931. doi: 10.1038/s41419-022-05368-6

18. Olson B, Li Y, Lin Y, Liu ET, Patnaik A. Mouse models for cancer immunotherapy research. *Cancer Discovery*. (2018) 8:1358–65. doi: 10.1158/2159-8290.CD-18-0044

19. Messaoudene M, Pidgeon R, Richard C, Ponce M, Diop K, Benlaifaoui M, et al. A natural polyphenol exerts antitumor activity and circumvents anti-PD-1 resistance through effects on the gut microbiota. *Cancer Discovery*. (2022) 12:1070–87. doi: 10.1158/2159-8290.CD-21-0808

20. Huang J, Liu D, Wang Y, Liu L, Li J, Yuan J, et al. Ginseng polysaccharides alter the gut microbiota and kynurenine/tryptophan ratio, potentiating the antitumour effect of antiprogrammed cell death 1/programmed cell death ligand 1 (anti-PD-1/PD-L1) immunotherapy. *Gut*. (2022) 71:734–45. doi: 10.1136/gutjnl-2020-321031

21. Hajjar R, Gonzalez E, Fragoso G, Oliero M, Alaoui AA, Calve A, et al. Gut microbiota influence anastomotic healing in colorectal cancer surgery through modulation of mucosal proinflammatory cytokines. *Gut*. (2023) 72:1143–54. doi: 10.1136/gutjnl-2022-328389

22. Twigg MS, Adu SA, Sugiyama S, Marchant R, Banat IM. Mono-rhamnolipid biosurfactants synthesized by pseudomonas aeruginosa detrimentally affect colorectal cancer cells. *Pharmaceutics*. (2022) 14:2799. doi: 10.3390/pharmaceutics14122799

23. Wu T, Zhu J. Recent development and optimization of pseudomonas aeruginosa exotoxin immunotoxins in cancer therapeutic applications. *Int Immunopharmacol*. (2021) 96:107759. doi: 10.1016/j.intimp.2021.107759

24. Liu X, Yan C, Yang A, Yu E, Yu J, Zhou C, et al. Efficacy of anti-programmed cell death protein 1 monoclonal antibody combined with bevacizumab and/or Pseudomonas aeruginosa injection in transplanted tumor of mouse forestomach carcinoma cell gastric cancer in mice and its mechanism in regulating tumor immune microenvironment. *Clin Exp Immunol*. (2023) 213:328–38. doi: 10.1093/cei/uxad069

25. Zhang C, Zhang Z, Wang L, Han J, Li F, Shen C, et al. Pseudomonas aeruginosa-mannose sensitive hemagglutinin injection treated cytokine-induced killer cells combined with chemotherapy in the treatment of Malignancies. *Int Immunopharmacol*. (2017) 51:57–65. doi: 10.1016/j.intimp.2017.08.003

26. Bozic D, Zivanovic J, Zivancevic K, Baralic K, Dukic-Cosic D. Trends in anti-tumor effects of pseudomonas aeruginosa mannose-sensitive-hemagglutinin (PA-MSHA): an overview of positive and negative effects. *Cancers (Basel)*. (2024) 16:524. doi: 10.3390/cancers16030524

27. Chevalier S, Bouffartigues E, Bodilis J, Maillot O, LesouHaitier O, Feuilloley MGJ, et al. Structure, function and regulation of Pseudomonas aeruginosa porins. *FEMS Microbiol Rev*. (2017) 41:698–722. doi: 10.1093/femsre/fux020

28. Zhao Y, Lin Q, Zhang T, Zhen S, Wang J, Jiang E, et al. Pseudomonas aeruginosa bloodstream infection in patients with hematological diseases: Clinical outcomes and prediction model of multidrug-resistant infections. *J Infect*. (2023) 86:66–117. doi: 10.1016/j.jinf.2022.08.037

29. Horcajada JP, Montero M, Oliver A, Sorli L, Luque S, Gomez-Zorrilla S, et al. Epidemiology and treatment of multidrug-resistant and extensively drug-resistant pseudomonas aeruginosa infections. *Clin Microbiol Rev*. (2019) 32:e00031-19. doi: 10.1128/CMR.00031-19

30. Newsom SW. Carbenicillin-resistant pseudomonas. *Lancet*. (1969) 2:1141. doi: 10.1016/s0140-6736(69)90742-9

31. Jurado-Martin I, Sainz-Mejias M, McClean S. Pseudomonas aeruginosa: an audacious pathogen with an adaptable arsenal of virulence factors. *Int J Mol Sci*. (2021) 22:3128. doi: 10.3390/ijms22063128

32. Manner C, Dias Teixeira R, Saha D, Kaczmarczyk A, Zemp R, Wyss F, et al. A genetic switch controls Pseudomonas aeruginosa surface colonization. *Nat Microbiol*. (2023) 8:1520–33. doi: 10.1038/s41564-023-01403-0

33. Tenover FC, Nicolau DP, Gill CM. Carbapenemase-producing Pseudomonas aeruginosa -an emerging challenge. *Emerg Microbes Infect*. (2022) 11:811–4. doi: 10.1080/22221751.2022.2048972

34. Dimopoulos G, Akova M, Rello J, Poulakou G. Understanding resistance in pseudomonas. *Intensive Care Med*. (2020) 46:350–2. doi: 10.1007/s00134-019-05905-6

35. Simonis A, Kreer C, Albus A, Rox K, Yuan B, Holzmann D, et al. Discovery of highly neutralizing human antibodies targeting Pseudomonas aeruginosa. *Cell*. (2023) 186:5098–5113 e19. doi: 10.1016/j.cell.2023.10.002

36. Qin S, Xiao W, Zhou C, Pu Q, Deng X, Lan L, et al. Pseudomonas aeruginosa: pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. *Signal Transduct. Target Ther*. (2022) 7:199. doi: 10.1038/s41392-022-01056-1

37. Abdelaziz AA, Kamer AMA, Al-Monofy KB, Al-Madboly LA. A purified and lyophilized Pseudomonas aeruginosa derived pyocyanin induces promising apoptotic and necrotic activities against MCF-7 human breast adenocarcinoma. *Microb Cell Fact*. (2022) 21:262. doi: 10.1186/s12934-022-01988-x

38. Shouman H, Said HS, Kenawy HI, Hassan R. Molecular and biological characterization of pyocyanin from clinical and environmental Pseudomonas aeruginosa. *Microb Cell Fact*. (2023) 22:166. doi: 10.1186/s12934-023-02169-0

39. Taglialegna A. Pseudomonas against cancer. *Nat Rev Microbiol*. (2023) 21:131. doi: 10.1038/s41579-023-00856-8

40. Li Z, Hao D, Li L, Zhou X, Ren L, Yang Y, et al. A clinical study on PA-MSHA vaccine in adjuvant therapy of lung cancer. *J. Zhongguo Fei Ai Za Zhi*. (1999) 2:20–2. doi: 10.3779/j.issn.1009-3419.1999.01.07

41. Cao Z, Shi L, Li Y, Wang J, Wang D, Wang G, et al. Pseudomonas aeruginosa: mannose sensitive hemagglutinin inhibits the growth of human hepatocarcinoma cells via mannose-mediated apoptosis. *Dig Dis Sci*. (2009) 54:2118–27. doi: 10.1007/s10620-008-0603-5

42. Liu ZB, Hou YF, Zhu J, Hu DL, Jin W, Ou ZL, et al. Inhibition of EGFR pathway signaling and the metastatic potential of breast cancer cells by PA-MSHA mediated by type 1 fimbriae via a mannose-dependent manner. *Oncogene*. (2010) 29:2996–3009. doi: 10.1038/onc.2010.70

43. Li T, Dong ZR, Guo ZY, Wang CH, Zhi XT, Zhou JW, et al. Mannose-mediated inhibitory effects of PA-MSHA on invasion and metastasis of hepatocellular carcinoma via EGFR/Akt/IkappaBbeta/NF-kappaB pathway. *Liver Int*. (2015) 35:1416–29. doi: 10.1111/liv.12644

44. Zhang X, Pei Z, Ren J, Shi J, Lu W, Shui Y, et al. PA-MSHA improves prognosis of patients undergoing radical cystectomy: a retrospective cohort study using inverse probability of treatment weighting. *Front Immunol*. (2024) 15:1403302. doi: 10.3389/fimmu.2024.1403302

45. Wang B, He Z, Yu H, Ou Z, Chen J, Yang M, et al. Intravesical Pseudomonas aeruginosa mannose-sensitive Hemagglutinin vaccine triggers a tumor-preventing immune environment in an orthotopic mouse bladder cancer model. *Cancer Immunol Immunother*. (2022) 71:1507–17. doi: 10.1007/s00262-021-03063-7

46. Su ACY, Ding X, Lau HCH, Kang X, Li Q, Wang X, et al. Lactococcus lactis HkyuLL 10 suppresses colorectal tumourigenesis and restores gut microbiota through its generated alpha-mannosidase. *Gut*. (2024) 73:1478–88. doi: 10.1136/gutjnl-2023-330835

frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 29 April 2025
DOI 10.3389/fimmu.2025.1570233

Check for updates

OPEN ACCESS

EDITED BY
Nar Singh Chauhan,
Maharshi Dayanand University, India

REVIEWED BY
Hongfei Li,
Zhejiang Ocean University, China
Talwinder Kaur,
Guru Nanak Dev University, India
Avinash Lomash,
Medanta The Medicity Hospital, India
Manar Bani Mfarrej,
Zayed University, United Arab Emirates

*CORRESPONDENCE
Asiya Nazir
Asiya.nazir@adu.ac.ae

RECEIVED 03 February 2025
ACCEPTED 24 March 2025
PUBLISHED 29 April 2025

CITATION
Nazir A, Hussain FHN, Nadeem Hussain TH, Al Dweik R and Raza A (2025) Therapeutic targeting of the host-microbiota-immune axis: implications for precision health. *Front. Immunol.* 16:1570233. doi: 10.3389/fimmu.2025.1570233

COPYRIGHT
© 2025 Nazir, Hussain, Nadeem Hussain, Al Dweik and Raza. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Therapeutic targeting of the host-microbiota-immune axis: implications for precision health

Asiya Nazir[1]*, Fathima Hasnain Nadeem Hussain[1], Tuahir Hassan Nadeem Hussain[2], Rania Al Dweik[1] and Afsheen Raza[1]

[1]Department of Biomedical Sciences, College of Health Sciences, Abu Dhabi University, Abu Dhabi, United Arab Emirates, [2]College of Medicine and Health Sciences, Khalifa University, Abu Dhabi, United Arab Emirates

The human body functions as a complex ecosystem, hosting trillions of microbes that collectively form the microbiome, pivotal in immune system regulation. The host-microbe immunological axis maintains homeostasis and influences key physiological processes, including metabolism, epithelial integrity, and neural function. Recent advancements in microbiome-based therapeutics, including probiotics, prebiotics and fecal microbiota transplantation, offer promising strategies for immune modulation. Microbial therapies leveraging microbial metabolites and engineered bacterial consortia are emerging as novel therapeutic strategies. However, significant challenges remain, including individual microbiome variability, the complexity of host-microbe interactions, and the need for precise mechanistic insights. This review comprehensively examines the host microbiota immunological interactions, elucidating its mechanisms, therapeutic potential, and the future directions of microbiome-based immunomodulation in human health. It will also critically evaluate challenges, limitations, and future directions for microbiome-based precision medicine.

KEYWORDS

gut microbiota, microbiota-immune interactions, microbiota therapeutics, probiotics, fecal microbiota transplantation, precision medicine and microbiome, microbial dysbiosis

1 Introduction

The human body is a complex ecosystem, housing trillions of microbes, including bacteria, viruses, fungi, and archaea, that collectively form the human microbiome (1). These microbial communities, residing predominantly in the gastrointestinal tract but also on the skin, respiratory tract (2, 3), and other mucosal surfaces, have evolved in a highly regulated relationship with the host immune system. This dynamic interplay, referred to as the host-microbe immunological axis, is essential for maintaining homeostasis, influencing not only immune system development and function but also a broad spectrum of physiological

processes, including metabolism, epithelial integrity, and neural function. Disruptions to this delicate balance have been implicated in the pathogenesis of a range of diseases, from autoimmune disorders to metabolic syndromes and even neurodegenerative diseases. Recent advances in microbiome research have underscored the importance of microbial composition and diversity in shaping host immune responses. It is now well-established that the gut microbiota is a central player in modulating innate and adaptive immunity (4). The host immune system, in turn, regulates microbial composition, creating a bidirectional axis that is fundamental for health. Dysbiosis, or an imbalance in the microbial community, can trigger immune dysregulation, leading to chronic inflammation and disease pathogenesis. The immune system's recognition of microbial signals through pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs), and its capacity to generate appropriate immune responses to microbeshave emerged as central themes in the study of host-microbe interactions (5, 6). In this context, the modulation of the host-microbe immunological axis presents a promising therapeutic approach for a wide range of human health conditions. Therapeutic strategies aimed at restoring or maintaining microbial balance (7), such as probiotics, prebiotics, dietary interventions, and fecal microbiota transplantation (FMT) (8, 9) have shown potential in the management of diseases such as inflammatory bowel disease (IBD), allergies, and even metabolic disorders (10). More recently, the exploration of microbiome-based immunotherapies has gained traction, with microbial products or metabolites being considered as potential modulators of immune responses (8). Similarly, in obesity, an altered gut microbiome composition has been linked to increased energy harvest, systemic inflammation, and metabolic dysregulation, contributing to insulin resistance and other comorbidities (11). Understanding dysbiosis and its role in these conditions is crucial for developing microbiome-targeted therapeutic strategies. These interventions hold the promise of targeted treatments that address the symptoms of diseases and their underlying immunological causes (12). However, while the potential for modulating the host-microbe immunological axis for therapeutic benefit is vast, significant challenges remain. The heterogeneity of microbiome composition across individuals, the complex nature of host-microbe interactions, and the potential for unintended consequences complicate the development of standardized, effective therapies (13). Moreover, a deeper understanding of the mechanisms by which microbes influence the immune system ranging from immune cell differentiation and activation to cytokine production and antigen presentation, is still needed (14–18). Additionally, the role of the microbiome in regulating immune responses in tissues beyond the gut, including the skin, lungs (19), and brain, is an area of intense research with significant implications for systemic health (12). This review aims to provide a comprehensive overview of the current state of knowledge on the modulation of the host-microbe immunological axis in human health. It explored the bidirectional relationship between the immune system and the microbiota, focusing on the mechanisms by which microbes influenced immune responses and how these interactions were modulated for therapeutic benefit. The review also examined emerging therapeutic strategies that targeted microbiome (20) and immune system, including probiotic and prebiotic interventions, microbiome-based drug development, and the potential use of microbiota-derived metabolites in immune modulation. Finally, the review will address the challenges and future directions in this rapidly evolving field, providing insight into the potential for microbiome-based immunomodulation in treating and preventing immune-related diseases.

Precision medicine in microbiome-based therapies involves tailoring interventions to an individual's unique microbial composition, genetic background, and immune response (20). By leveraging advanced sequencing technologies and computational tools, precision medicine aims to develop targeted microbial interventions, including probiotics, prebiotics, and microbiota-derived metabolites, to modulate the immune system effectively (21). This personalized approach enhances treatment efficacy, minimizes adverse effects, and holds promise for managing a range of immune-related disorders, from inflammatory diseases to cancer immunotherapy (22, 23).

2 Technological advances in understanding the immunological axis

Recent technological advancements, particularly high-throughput sequencing technologies, have significantly improved our ability to study microbiota-immune system interactions. Metagenomic, metatranscriptomic, and metabolomic techniques have provided valuable insights into the functional capabilities of microbial communities, including their ability to produce bioactive metabolites that can influence host immune responses (7). Additionally, single-cell RNA sequencing (scRNA-seq) has enabled the detailed examination of immune cell heterogeneity and the identification of specific immune cell subsets that interact with the microbiome, offering a deeper understanding of the cellular dynamics involved in immune modulation. Moreover, advances in high-resolution imaging techniques, such as flow cytometry and multiplex immunofluorescence, have facilitated the real-time analysis of immune cell activation, migration, and cytokine production in response to microbial stimuli (Table 1). These technologies along with germ-free and gnotobiotic animal models have provided critical insights into the causal relationships between microbiome composition and immune system function (32). Furthermore, the development of organ-on-a-chip models and 3D tissue cultures has allowed for studying host-microbe interactions in more physiologically relevant contexts, bridging the gap between *in vitro* and *in vivo* models. These technological breakthroughs have significantly enhanced our ability to investigate the dynamic nature of the host-microbe immunological axis. They are paving the way for more targeted, personalized therapeutic interventions aimed at modulating the microbiome for immune modulation (19).

3 Mechanisms of communication between microbiota and the immune system

The communication between microbiota and the immune system is mediated through microbial metabolites, pattern recognition receptors (PRRs), and immune signaling pathways Figure 1. These interactions play a crucial role in maintaining immune homeostasis and protecting the host from pathogens.

3.1 Pattern recognition receptors

Pattern recognition receptors (PRRs) are a crucial component of the immune system that enables the host to detect and respond to microbial invaders and danger signals. These receptors are found on various immune cells and are responsible for recognizing conserved molecular structures called pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs). The recognition of these patterns initiates immune responses that are essential for maintaining immune homeostasis and protecting the host from infections, tissue damage, and diseases such as cancer. PRRs are classified into several types, each specialized for recognizing different microbial or host-derived patterns. The main categories of PRRs include Toll-like receptors (TLRs), NOD-like receptors (NLRs), C-type lectin receptors (CLRs), and the various PAMPs and DAMPs recognized by these receptors (33, 34).

3.1.1 Toll-like receptors

TLRs, a subset of PRRs, are transmembrane receptors primarily expressed on immune cells such as dendritic cells, macrophages, and neutrophils. Each TLR specializes in recognizing distinct microbial signatures, for example, TLR4 detects lipopolysaccharide (LPS) from Gram-negative bacteria, while TLR3 recognizes double-stranded RNA from viruses (35, 36). Upon ligand binding, TLRs activate intracellular signaling pathways that stimulate pro-inflammatory cytokine secretion, recruit immune cells, and initiate adaptive immune responses (37).

3.1.2 NOD-like receptors

NLRs are intracellular PRRs that detect bacterial PAMPs, such as peptidoglycans, and activate the inflammasome, which processes cytokines such as IL-1β (38). NLR activation is crucial for immune system homeostasis, but dysregulation can lead to chronic inflammatory conditions (39). Certain NLRs also regulate autophagy, aiding intracellular pathogen clearance and maintaining cellular balance (40).

TABLE 1 Technological advances in deciphering the host-microbe immunological axis.

Technological Approach	Description	Key Insights	Strengths	Limitations	References
Metagenomics	Sequencing microbial genomes to assess functional potential.	Revealed SCFA production (e.g., butyrate) crucial for Treg differentiation and epithelial integrity.	Provides a broad view of microbial community composition and function.	Limited strain-level resolution due to reliance on reference databases.	(24, 25)
Metabolomics	Analyzing microbial metabolites and linking them to host immune pathways.	Identified metabolites (e.g., indoles) activating AhR to modulate inflammation.	Directly connects microbiome function to immune responses.	Attribution of metabolites to specific taxa remains challenging.	(26)
Transcriptomics	Captures changes in host gene expression during microbial interactions.	Elucidated TLR-mediated pathways involved in gut immune regulation.	Provides dynamic insights into host-microbe signaling.	Limited temporal resolution without longitudinal studies.	(27)
Single-Cell RNA Sequencing	High-resolution analysis of immune cell subsets and responses.	Identified distinct immune cell plasticity in conditions like IBD.	Offers unparalleled resolution of immune cell heterogeneity.	Loses spatial context of immune interactions within tissues.	(28)
Spatial Transcriptomics	Maps immune and microbial interactions within intact tissues.	Revealed disrupted cytokine gradients in IBD tissue regions with microbial dysbiosis.	Retains spatial context, enabling localized analysis of host-microbe interactions.	Resolution remains suboptimal, and data integration is computationally demanding.	(29)
Machine Learning (ML)	AI algorithms for integrating complex datasets and predicting patterns in immune responses.	Identified microbiome-based biomarkers and predicted immune-modulating microbial metabolites.	Enables large-scale data integration, biomarker discovery, and therapeutic design.	Often functions as a "black box," with limited biological explainability.	(30, 31)
Reinforcement Learning (AI)	Optimizes synthetic microbiota for desired immunological effects.	Designed microbial consortia for anti-inflammatory responses and precision therapies.	Supports personalized medicine through patient stratification.	Requires extensive preclinical and clinical validation to ensure efficacy.	(31)

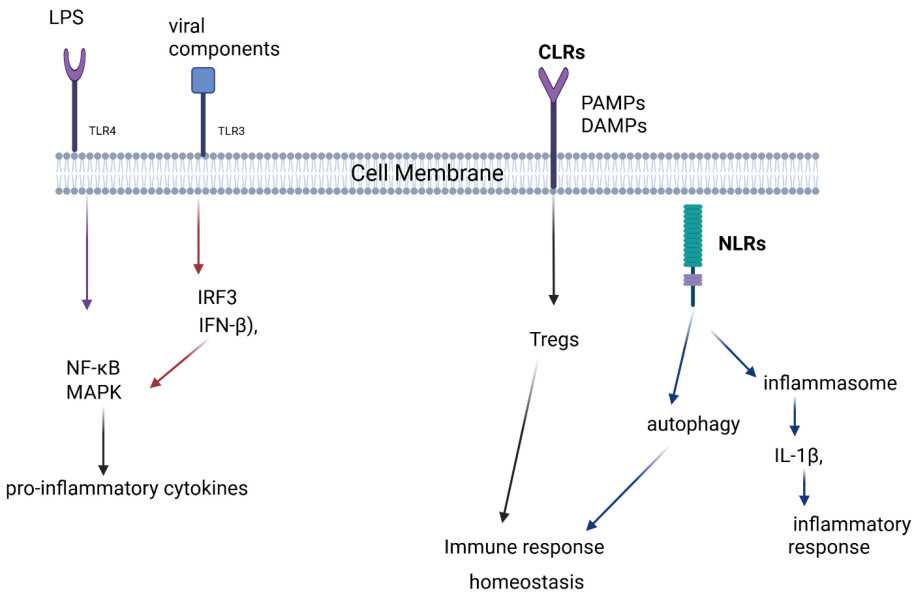


FIGURE 1
Pattern Recognition Receptors (PRRs) and Immune Signaling Pathways. This figure depicts how different pattern recognition receptors (TLRs, CLRs, and NLRs) recognize microbial components, leading to immune responses. TLR4 and TLR3 activate pro-inflammatory signaling via NF-κB, MAPK, and IFN-β, while CLRs contribute to immune homeostasis through Tregs. NLRs regulate autophagy and inflammasome activation, influencing inflammatory responses.

Some NLRs also detect non-microbial danger signals and form large cytoplasmic structures known as inflammasomes. These inflammasomes link the detection of microbial products and metabolic stress to the activation of proinflammatory cytokines such as IL-1beta and IL-18. The NALP3 complex has been implicated in several autoinflammatory diseases, including gout (41).

3.1.3 C-type lectin receptors

CLRs specialize in recognizing carbohydrate structures on pathogens, primarily targeting fungi, bacteria, and viruses. They are essential for immune activation, antigen uptake, and pathogen recognition, particularly in fungal infections (42, 43).

CLRs are involved in modulating immune responses to fungi, bacteria, and viruses, and play a role in pathogen recognition, antigen uptake, and immune activation (44). CLRs also influence T-helper cell polarization and regulatory T cell (Treg) development, promoting immune homeostasis (45).

3.1.4 PAMPs and DAMPs

PRRs recognize both pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) to differentiate between infection and tissue damage (46). DAMPs, such as high-mobility group box 1 (HMGB1), are released during cellular injury and can trigger inflammation via receptors like TLR4 (47). This dual recognition system balances pathogen clearance with tissue repair (48).

3.2 Short-chain fatty acids

The gut microbiota's production of SCFAs, including acetate, propionate, and butyrate, plays a fundamental role in maintaining immune tolerance, gut health, and host metabolism (49, 50). Figure 2 illustrates how gut microbiota ferments dietary fibers to produce SCFAs, including acetate, butyrate, and propionate. These SCFAs play a crucial role in epithelial cell growth, immune response, and metabolic regulation. These microbial metabolites are primarily produced in the colon through distinct bacterial pathways. Acetate is synthesized via the pyruvate pathway by *Akkermansia muciniphila*, *Bacteroides* spp., *Bifidobacterium* spp., and *Ruminococcus* spp. (51, 52). Propionate is generated through the succinate, acrylate, and propanediol pathways by species such as *Bacteroides* spp., *Veillonella* spp., and *Roseburia inulinivorans* (53). Butyrate a key immunomodulatory SCFA, is predominantly via the phosphotransbutyrylase/butyrate kinase and butyryl-CoA:acetate CoA-transferase pathwaysinvolving *Coprococcus catus*, *Faecalibacterium prausnitzii*, and *Eubacterium rectale* (54, 55).

Among butyrate-producing bacteria, *Faecalibacterium prausnitzii* plays a crucial role due to its ability to modulate gut immune responses and reinforce epithelial integrity (55). This bacterium exerts anti-inflammatory effects by inhibiting NF-κB activation and stimulating IL-10 production, thereby reducing gut inflammation and maintaining homeostasis (56). Additionally, butyrate enhances the expression of tight junction proteins such as ZO-1 and occludin, essential for maintaining gut barrier function (57). Disruptions in *F. prausnitzii* abundance have been linked to inflammatory bowel disease (IBD) and colorectal cancer,

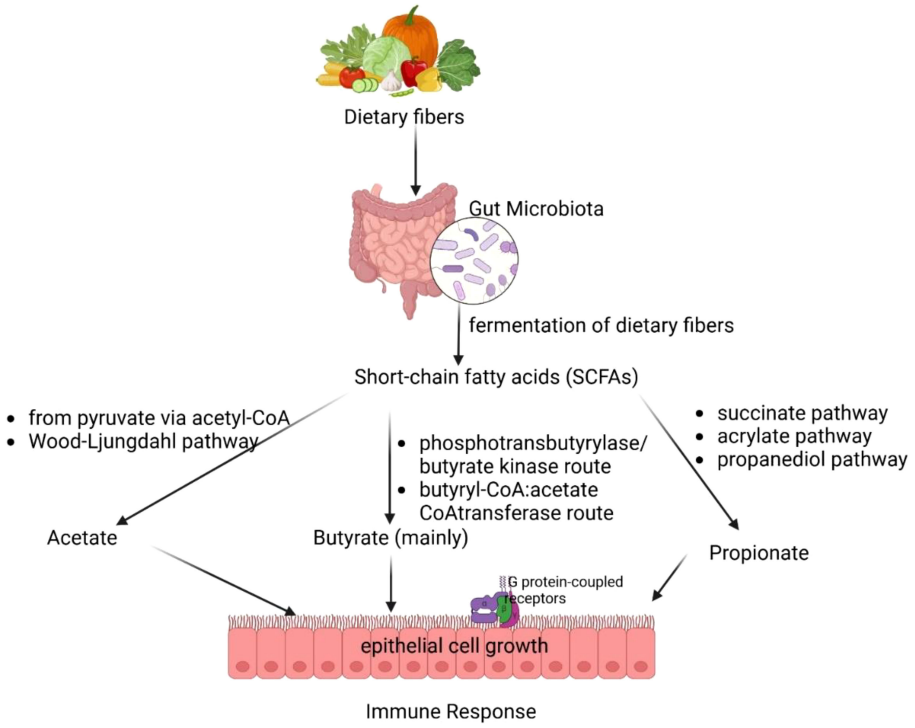


FIGURE 2
Gut microbial fermentation of dietary fibers and short-chain fatty acid production.

emphasizing its potential as a probiotic candidate for microbiota-targeted therapies (58).

SCFAs exert systemic effects by modulating immune function and metabolism. Acetate, the most abundant SCFA in circulation, can cross the blood-brain barrier to regulate appetite via central homeostatic mechanisms (59). Propionate, predominantly metabolized in the liver, contributes to lipid and glucose metabolism but remains at low peripheral concentrations (50). Butyrate plays a key role in reinforcing gut barrier integrity, reducing inflammation, and promoting regulatory T cell (Treg) differentiation, which is crucial for immune tolerance and the suppression of excessive inflammation (60–62). It also directly modulates dendritic cells and macrophages, enhancing their ability to recognize pathogen-associated molecular patterns (PAMPs) and regulating pro-inflammatory cytokine secretion (63).

A significant mechanism underlying butyrate's immunomodulatory effects is its inhibition of histone deacetylases (HDACs), leading to epigenetic modifications that regulate gene expression associated with immune homeostasis (64–66). This process fosters anti-inflammatory signaling, supports tissue repair, and balances immune responses in conditions such as infections and cancer. Mammals rely on gut bacteria to break down indigestible dietary components, such as fiber, generating SCFAs as metabolic byproducts that contribute to host health and disease prevention (11). The dynamic interplay between diet, microbial metabolism, and immune regulation highlights the potential of SCFAs as therapeutic targets for inflammatory and metabolic disorders.

3.3 Cytokine signaling pathways

Cytokine signaling pathways are fundamental to immune regulation, with the microbiome playing a key role in modulating both pro-inflammatory and anti-inflammatory responses. Pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, and IFN-γ, are critical in initiating inflammatory reactions and serve as primary mediators of immune responses during infection or injury (67). Microbial components, including lipopolysaccharides (LPS), activate signaling pathways such as the nuclear factor NF-κB pathway through Toll-like receptors, particularly TLR4, TLR3 and TLR5. The recognition of LPS by TLR4 triggers NF-κB activation, activator protein-1 (AP-1), and mitogen-activated protein kinase (MAPK) (68). These pathways stimulate the release of proinflammatory cytokines and chemokines, including interleukin (IL)-1, tumor necrosis factor (TNF)-α, and IL-6 (44, 69, 70). Cytokines are versatile proteins that play a key role in regulating osteoclast development and bone resorption, altering vascular endothelial permeability, and attracting immune cells to sites of inflammation (44, 71). The recognition of viral components by TLR3 triggers the TRIF-mediated signaling pathway, leading to the activation of Interferon Regulatory Factor 3 (IRF3) and the subsequent production of Interferon Beta (IFN-β), which plays a crucial role in inhibiting viral replication (72). Additionally, TLR3 stimulation activates the MAP kinase and NF-κB signaling pathways, promoting the production of pro-inflammatory cytokines (73).

This cytokine signaling process involves a highly regulated molecular cascade that is crucial for maintaining immunological homeostasis. Immune cells, such as dendritic cells and macrophages, recognize PAMPs from microbes or host-derived signals, such as SCFAs produced by microbiota. Upon recognition, these cells release cytokines, which can either promote or suppress inflammation, depending on the signals received (66). Cytokines bind to specific receptors on target cells, initiating intracellular signaling pathways involving kinases and transcription factors like NF-κB or STAT proteins. Once activated, these transcription factors translocate to the nucleus and regulate the expression of genes critical for immune responses, thereby enhancing T and B cell functions to combat infections and malignancies (74).

For instance, in inflammatory bowel disease (IBD), an overactive cytokine response leads to chronic inflammation in the gut, driven by excessive production of TNF-α, IL-1β, and IL-6, which contribute to tissue damage and immune dysregulation (75). Similarly, in rheumatoid arthritis, dysregulated cytokine signaling—particularly involving TNF-α and IL-6—results in persistent joint inflammation and autoimmunity (76). In contrast, in cancer, certain cytokines such as IL-10 and TGF-β suppress immune responses, allowing tumor cells to evade immune detection and promote tumor progression (77). These disease models illustrate the dual role of cytokines in either driving or suppressing immune responses, emphasizing the necessity of maintaining a precise balance between pro-inflammatory and anti-inflammatory signals.

To prevent excessive immune activation and inflammation, the immune system carefully balances pro-inflammatory and anti-inflammatory signals, ensuring a controlled response that maintains systemic homeostasis while addressing challenges such as infections, cancer, and interactions with the microbiota (34).

4 Distinction between symbiotic and pathogenic interactions

Host-microbe interactions can be broadly classified into symbiotic and pathogenic relationships, depending on their immunological effects and the impact on host health. While pathogenic interactions often result in infection and disease, symbiotic relationships include both mutualistic and commensal associations, which help maintain host homeostasis and immune balance.

4.1 Pathogenic interactions: disrupting host integrity

Pathogenic interactions occur when microorganisms invade the host, triggering an immune response that aims to eliminate the threat but can also lead to inflammation and tissue damage. Pro-inflammatory cytokines like IL-6, TNF-α, and IFN-γ are released to recruit immune cells and amplify the response (78). Pathogens are detected by pattern recognition receptors (PRRs) such as TLRs and NLRs, which recognize microbial-associated molecular patterns and initiate immune signaling (79). The adaptive immune system further engages by activating effector T cells and stimulating antibody production (2). However, excessive immune activation can lead to collateral damage and worsen disease outcomes (80). For example, *Salmonella enterica* triggers IL-6-mediated gut inflammation (81), *Mycobacterium tuberculosis* evades immune detection while inducing chronic inflammation (82), and SARS-CoV-2 provokes a severe cytokine storm, causing lung injury and systemic complications (83).

4.2 Symbiotic interactions: a balanced coexistence

Symbiotic microbes establish stable, non-harmful relationships with the host, offering benefits. These interactions can be mutualistic, where both the host and microbe benefit or commensal, where the microbe benefits without harming the host (84). In mutualistic interactions, microbes regulate immune responses, enhance barrier protection, and support immune homeostasis. For instance, Bacteroides fragilis produces polysaccharide A (PSA), which promotes regulatory T cells and prevents colitis (85). *Lactobacillus reuteri* secretes antimicrobial peptides that help protect the mucosal lining and prevent pathogen colonization (86). Additionally, mutualistic microbes stimulate anti-inflammatory cytokines like IL-10 and TGF-β, promoting immune tolerance and preventing excessive inflammation (87). They also strengthen epithelial integrity, as seen with *Lactobacillus* and *Bifidobacterium*, and foster immune balance through regulatory T cell activity (2).

4.3 Commensal relationships: coexistence without harm

In contrast, commensal relationships, where microbes coexist with the host without causing harm, are typically associated with a more regulated immune response. The immune system often promotes the production of anti-inflammatory cytokines, such as IL-10, and enhances processes supporting immune tolerance, including the activity of regulatory T cells (Tregs). For example, *Staphylococcus epidermidis* on the skin produces antimicrobial molecules that protect against *Staphylococcus aureus* infections, preventing harmful overgrowth (88). Similarly, the gut microbiome maintains a balance between beneficial and opportunistic bacteria, reducing the risk of infections like *Clostridioides difficile* (89). These mechanisms work to prevent unnecessary immune activation and inflammation, allowing commensals to persist in a balanced state without triggering excessive inflammatory responses (87). This delicate equilibrium between immune activation and regulation is crucial for maintaining host homeostasis while permitting the coexistence of beneficial microorganisms without compromising immune system integrity (90, 91).

5 Immunological changes during various illness

Immunological alterations during disease progression vary significantly based on factors such as the nature of the disease, its underlying etiology, and the extent to which it influences immune function. These changes can be categorized into two primary components: innate immunity, which provides the body's initial, rapid defense against pathogens, and adaptive immunity, which generates a more specific, durable immune response. The interplay between these systems can lead to the activation, suppression, or dysregulation of immune responses, depending on the pathophysiological context of the illness (Table 2). Dysregulation may occur when the immune system's normal functioning is disrupted, resulting in inadequate responses, excessive inflammation, or autoimmunity, thus exacerbating the disease and complicating recovery (92).

5.1 Immunological targets for better health

Immunological interventions aimed at promoting health encompass enhancing immune function, preventing infections, and managing chronic diseases. Targeting cytokines such as TNF-α and IL-6 has been effective in reducing inflammation in autoimmune disorders like rheumatoid arthritis. Likewise, immune checkpoint inhibitors targeting PD-1, PD-L1, and CTLA-4 have revolutionized cancer therapy by reactivating T cells to combat malignant cells. Another significant target is regulatory T cells (Tregs), which can be modulated to restore immune tolerance in autoimmune diseases or to enhance anti-tumor immunity (93). The gut microbiota plays a crucial role in immune modulation, with probiotics (94), prebiotics, and dietary interventions demonstrating therapeutic potential for conditions such as inflammatory bowel disease and obesity (95). Nutritional strategies (96), including supplementation with vitamin D, zinc, and omega-3 fatty acids, are also critical for maintaining optimal immune function (97). Additionally, targeting inflammasomes which are the key components responsible for the production of pro-inflammatory cytokines like IL-1β and IL-18—holds promise for treating diseases such as gout and autoimmune conditions (98). Vaccination remains a cornerstone of public health, offering cost-effective protection against infectious diseases and enhancing immunological memory. Technological advances, particularly mRNA vaccine platforms (99), have significantly improved immunity against pathogens such as influenza, HPV, and COVID-19 (100, 101). Toll-like receptors essential in pathogen recognition, are also being explored for their potential to boost immune responses against infections and cancers while mitigating excessive inflammation in autoimmune diseases (102, 103). Monoclonal antibodies and Janus kinase (JAK) inhibitors represent advanced therapeutic modalities for treating autoimmune disorders, cancers, and chronic inflammatory diseases by specifically targeting immune components. Furthermore, enhancing immune processes like antibody-dependent cellular cytotoxicity (ADCC) is being investigated for its potential to treat cancer and infections (104). By strategically modulating these immunological targets, it is possible to enhance immune function, improve disease management, and promote overall health (105). By strategically modulating these immunological targets, it is possible to enhance immune function, improve disease management, and promote overall health (106). Notably, microbiota-driven interventions hold immense promise in shaping immune responses by influencing T cell differentiation, mucosal immunity, and systemic inflammation. Harnessing microbial-derived metabolites, such as SCFAs, and engineered probiotics provides novel avenues for immunotherapy and personalized medicine (23). Advancing research in microbiota-immune interactions could lead to breakthroughs in disease prevention and therapeutic strategies, reinforcing the pivotal role of the gut microbiome in sustaining immune balance and health (107).

5.2 Immunotherapy targets

Monoclonal antibodies are sophisticated biological therapeutics that target specific antigens, thereby inducing their destruction or facilitating their elimination through mechanisms such as antibody-

TABLE 2 Role of Short-chain fatty acids (SCFAs) and Indole derivates in human health.

	SCFAs	Indole derivatives and tryptophan metabolism
Mechanism	• Produced when the gut bacteria ferments dietary fibers • Preserves the gut barrier • Activates T cells • Suppresses pro-inflammatory cytokines like TNF-α.	• Activates AhR • Stimulates Tregs • Help gut bacteria convert tryptophan into indole derivatives.
Benefits	• Boosts immunological and metabolic function • Decreases chronic inflammation • Improves gut health.	• Regulates inflammation • Maintains immunological balance • Strengthen the gut barrier • Boosts mucosal immunity

dependent cellular cytotoxicity (ADCC) and complement activation. These agents have demonstrated efficacy in reducing inflammation and joint damage in rheumatoid arthritis by neutralizing TNF-α and alleviating the inflammatory symptoms of psoriasis by targeting IL-17 and IL-23 (105). In oncology, monoclonal antibodies that block the PD-1/PD-L1 interaction restore T-cell functionality in non-small cell lung cancer, enhancing the immune system's capacity to target and eliminate tumors. Similarly, anti-CD20 monoclonal antibodies in hematological malignancies promote B cell depletion through ADCC and complement activation. In the context of inflammatory bowel disease integrin α4β7 inhibitors limit the migration of lymphocytes to the gut mucosa, thereby reducing intestinal inflammation (108). In parallel, JAK inhibitors, which are small molecule agents, interfere with the JAK-STAT signaling pathway which is a central regulator of pro-inflammatory cytokines and cellular growth factors. By inhibiting specific JAK isoforms such as JAK1, JAK2, and JAK3, these inhibitors reduce cytokine signaling and inflammation (109). For instance, JAK1 and JAK3 inhibitors are effective in managing cytokine-driven inflammatory responses in conditions like rheumatoid arthritis and ulcerative colitis. Targeting JAK2 specifically addresses abnormal cytokine signaling and mitigates tissue fibrosis in myelofibrosis. Additionally, JAK inhibitors have been shown to reduce skin inflammation in atopic dermatitis by modulating dysregulated cytokine pathways. In the context of graft-versus-host disease (GVHD), these inhibitors play a critical role in limiting immune-mediated tissue damage, thus providing essential immunological control following transplantation (Table 3) (115). Collectively, these therapeutic agents, both monoclonal antibodies and JAK inhibitors, represent significant advances in the treatment of a variety of inflammatory and immune-mediated disorders, leveraging distinct but complementary mechanisms of action to modulate immune system activity and reduce pathological inflammation.

6 Immunological axis modulation strategies: case studies and applications in each domain

Immunological axis modulation involves the regulation of the complex interactions between the immune system and microorganisms, such as gut microbiota, to promote health and prevent disease. This section highlights case studies and applications of various strategies in modulating immune responses, with a focus on probiotics, prebiotics, and other therapeutic approaches that influence immune homeostasis and inflammation.

6.1 Probiotics and prebiotics in immune modulation

Probiotics are live microorganisms that confer a health benefit on the host when administered in adequate amounts (Table 4). They are primarily found in fermented foods and supplements, and their therapeutic potential is linked to their ability to modulate immune responses, particularly in the gut (124). A prominent example is *Lactobacillus rhamnosus* GG, a probiotic strain that has been used in clinical trials for treating inflammatory bowel diseases (IBD), such as ulcerative colitis (116). *L. rhamnosus* GG has

TABLE 3 Immunological axis in the onset of various physiological and metabolic diseases.

	Feature	Immune mechanism	References
Cancer	Tumors create immunosuppressive microenvironments, evading immune detection through reduced antigen presentation or inhibitory molecules like PD-L1.	Tumors restrict T cell activation, whereas immune evasion limits identification and eradication.	(110)
Autoimmune Diseases	The immune system attacks self-cells and damages organs. Resulting from genetic, environmental, and regulatory disturbances. Central and peripheral tolerance inhibit self-reactivity.	Tolerance breakdown activates autoreactive cells. Pro-inflammatory cytokines contribute to inflammation. Cytokine treatments show potential.	111)
Metabolic and Chronic Diseases	Linked with immunological disorders. Diabetes: High blood sugar affects neutrophil activity and T cell responses, increasing the infection risk. Obesity causes low-grade inflammation from higher pro-inflammatory cytokines (IL-6, TNF-α).	Systemic inflammation speeds up the course of illness; obesity-related impaired cytokine control results in compromised immune function.	(112, 113)
Infectious Diseases	Pro-inflammatory cytokines (IL-6, TNF-α) are released by neutrophils and macrophages in response to bacterial infections. Interferons and cytotoxic reactions are brought on by viral infections. Immune exhaustion is brought on by persistent illnesses, such as HIV.	T cells and antibodies can be used to activate adaptive immunity against bacterial infections. Parasites: TH1, TH2, and TH17 pathways - Viral infections: cytotoxic cell activation and interferon production.	(34, 114)
Allergic Disorders	Excessive response of the immune system to harmless stimuli (like pollen)- Inflammation caused by mast cell histamine release. Exhibiting a TH2-skewed immunological response.	IgE-mediated mast cell activation. Eosinophilic inflammation and imbalanced TH2 responses are features of chronic allergy-related conditions.	(114)

10.3389/fimmu.2025.1570233

TABLE 4 Probiotics: composition, duration, target diseases, and efficacy.

Probiotic	Composition	Duration of Therapy	Target Diseases	Efficacy	Reference
Lactobacillus rhamnosus GG	Single strain probiotic	4–8 weeks	Inflammatory Bowel Disease (IBD), Diarrhea, Allergies	Reduces gut inflammation, strengthens mucosal immunity	(116)
Bifidobacterium breve	Single strain probiotic	6–12 weeks	Necrotizing Enterocolitis (NEC), Infant Colic	Enhances gut barrier function, reduces colic symptoms	(1)
Saccharomyces boulardii	Yeast probiotic	4–6 weeks	Antibiotic-associated diarrhea, Clostridioides difficile infection	Reduces diarrhea incidence, restores gut flora balance	(117)
Lactobacillus acidophilus & Bifidobacterium bifidum	Multi-strain probiotic	8–12 weeks	Irritable Bowel Syndrome (IBS), Atopic Dermatitis	Alleviates bloating, improves gut microbiota composition	(118)
Streptococcus thermophilus	Single strain probiotic	6–10 weeks	Lactose Intolerance, Immune modulation	Enhances lactose digestion, boosts gut immunity	(119)
VSL#3	Multi-strain probiotic blend (8 bacterial strains)	12+ weeks	Ulcerative Colitis, Pouchitis	Reduces inflammation, supports remission maintenance	(120)
Escherichia coli Nissle 1917	Single strain probiotic	6–12 weeks	Ulcerative Colitis, Irritable Bowel Syndrome	Supports gut homeostasis, reduces inflammation	(49)
Lactobacillus reuteri	Single strain probiotic	4–6 weeks	Infant Colic, H. pylori Infection	Reduces colic symptoms, inhibits pathogen growth	(121)
Bacillus coagulans	Spore-forming probiotic	6–8 weeks	Rheumatoid Arthritis, Gastrointestinal Infections	Reduces inflammation, enhances immune response	(122)
Clostridium butyricum	Butyrate-producing probiotic	8–12 weeks	Inflammatory Bowel Disease, Metabolic Disorders	Improves gut barrier integrity, modulates immune function	(123)

been shown to enhance gut mucosal immunity by promoting anti-inflammatory cytokines like IL-10 and reducing pro-inflammatory cytokines such as TNF-α. Other probiotic strains, including *Lactobacillus* and *Bifidobacterium* species, are known for their immunomodulatory effects, promoting a balanced immune response by stimulating the production of regulatory T cells (Tregs) and strengthening mucosal immunity (117, 125). These probiotics also improve gut barrier function and reduce intestinal inflammation, helping prevent infections and alleviating the severity of autoimmune diseases and allergies (94). The beneficial effects of probiotics on immune regulation are also supported by their ability to increase gut microbiota diversity, which has been shown to help restore immune tolerance and mitigate disease pathology (118).

In addition to probiotics, prebiotics have gained significant attention as a tool for modulating immune responses (119). Prebiotics are non-digestible fibers that selectively stimulate the growth of beneficial gut bacteria (Table 5). These fibers influence immune function by fostering a gut microbiota composition that promotes immune tolerance, particularly in preventing and treating allergic diseases (126). One key case study involves the supplementation of inulin, a prebiotic, in infants at high risk for allergies. The intervention resulted in a reduced incidence of atopic dermatitis, along with an increase in beneficial bacteria like

TABLE 5 List of available prebiotics, their sources, mechanism of action, duration of therapy, target diseases, and efficacy.

Prebiotic	Source	Mechanism of Action	Duration of Therapy	Target Diseases	Efficacy
Inulin	Chicory root, bananas, onions	Stimulates *Bifidobacterium* growth	8–12 weeks	Atopic Dermatitis, Gut Dysbiosis	Reduces allergic symptoms and enhances gut microbiota composition (49)
Oligofructose	Wheat, garlic, asparagus	Modulates Th2/ Treg balance	6–10 weeks	Allergic Rhinitis, Asthma	Reduces IgE-mediated responses and inflammation (121)
Galacto-oligosaccharides (GOS)	Dairy products, legumes	Promotes beneficial gut bacteria	4–8 weeks	Infant Gut Health, Immune Support	Enhances gut barrier integrity and immune function (126)
Resistant Starch	Whole grains, potatoes	Increases SCFA production	10–16 weeks	Type 2 Diabetes, Obesity	Enhances butyrate production and improves insulin sensitivity (127)
Arabinoxylan	Whole wheat, rice bran	Supports *Bacteroides* species	8–12 weeks	Gut Health, Metabolic Disorders	Improves microbial diversity and reduces inflammation (128)

Bifidobacterium and *Lactobacillus*, which support immune regulation (49). Similarly, oligofructose supplementation in children with allergic rhinitis demonstrated a reduction in allergen-specific IgE production, which is linked to allergic responses, and improved the balance between pro-inflammatory Th2 cells and Tregs (122). This suggests that prebiotics can help alleviate allergic symptoms by modulating the immune system and fostering a more balanced, less inflammatory immune response.

A key mediator of both probiotic and prebiotic effects on immune regulation is SCFAs, particularly butyrate (128). Butyrate plays a crucial role in maintaining intestinal barrier integrity, enhancing the regeneration of epithelial cells, and reducing intestinal permeability, which is often implicated in inflammatory diseases (129). Butyrate also activates Tregs, which are essential for immune tolerance, and inhibits the production of pro-inflammatory cytokines such as TNF-α, further promoting a balanced immune response (122). The production of SCFAs, including butyrate, is influenced by the gut microbiota's fermentation of dietary fibers, underscoring the importance of a balanced microbiome in producing these metabolites (127). Beyond their gut health benefits, SCFAs like butyrate exert systemic effects, modulating immune responses and potentially protecting against chronic diseases, including obesity, type 2 diabetes, and autoimmune disorders (130, 131). Figure 3. illustrates the contrasting effects of symbiotic and pathogenic microbial interactions on the immune response

Together, probiotics and prebiotics work synergistically to enhance immune health by improving gut microbiota diversity, promoting the production of anti-inflammatory cytokines, and regulating immune responses through metabolites like butyrate. These effects are essential for reducing inflammation, preventing infections, and mitigating the risks associated with autoimmune diseases and allergies (94, 120).

6.2 Fecal microbiota transplantation

Fecal microbiota transplantation (FMT) has emerged as a revolutionary immunological strategy for modulating the host-microbe axis, particularly in cases of diseases where the gut microbiota is disrupted. FMT involves transferring fecal material from a healthy donor into the gastrointestinal tract of a recipient, thereby restoring microbial diversity and balance (132). One of the most well-documented and successful applications of FMT is in the treatment of recurrent *Clostridium difficile* (*C. difficile*) infection, which often arises after antibiotic treatments disrupt the natural gut microbiota. A landmark case study demonstrated that FMT achieved a 90% success rate in curing recurrent *C. difficile* infections (133). Patients who underwent FMT experienced significant restoration of gut microbiota diversity, which helped re-establish immune homeostasis. Safety and efficacy of fecal microbiota transplantation (FMT) as a modern adjuvant therapy in various diseases and disorders (134). The restored microbiota was able to suppress the overgrowth of *C. difficile* and reduced intestinal inflammation, offering a direct example of how microbiota modulation can enhance immune function and control infection (135). Beyond infectious diseases, FMT has shown promise in autoimmune and inflammatory disorders. For instance, in patients with ulcerative colitis (UC), a form of inflammatory bowel disease (IBD), FMT has been explored as a means of reducing inflammation and modulating the immune system (136). A study involving UC patients who received FMT showed improvements in clinical symptoms, including reduced disease activity and inflammation. This therapeutic effect was attributed to changes in the gut microbiota that promoted a shift from pro-inflammatory to anti-inflammatory immune responses, including an increase in regulatory T cells (Tregs) that are crucial for immune tolerance (137). Additionally, FMT has been

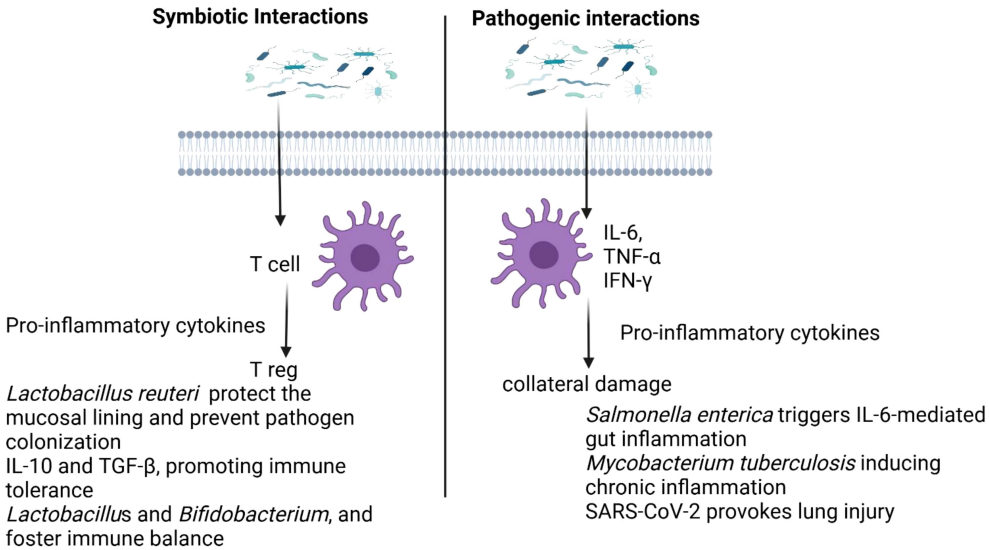


FIGURE 3
Differing impacts of symbiotic and pathogenic microbial interactions on immune regulation.

investigated in the context of multiple sclerosis (MS), an autoimmune disease where the immune system attacks the central nervous system (138). Preliminary clinical studies in MS patients receiving FMT have shown promising results, with some patients experiencing reduced disease progression and fewer relapses, potentially due to changes in gut microbiota composition that impact the peripheral immune system (134). These case studies highlight FMT's potential not only for treating infections but also for modulating the immune system in autoimmune and inflammatory diseases by restoring microbial diversity and re-establishing immune balance, suggesting a new frontier for therapeutic interventions in immunology.

6.3 Dietary interventions in inflammatory diseases

Dietary components provide not only essential nutrients for the body's physiological processes but also substrates that nourish the microbiota within the gastrointestinal tract, collectively known as the gut microbiome (139). This symbiotic relationship between the host and the microbiome is critical for maintaining health Table 5. Key dietary elements such as dietary fibers (140), polyphenols, and fatty acids play significant roles in modulating the gut microbiota and supporting immune function. Their combined effects are essential in maintaining a balanced immune system and improving overall health, as demonstrated in (Figure 4) (141). Gut bacteria metabolize dietary fibers into SCFAs, including butyrate, which contribute to the reinforcement of the intestinal epithelial barrier, reduce inflammatory responses, and promote the differentiation and activity of regulatory T cells (142). These SCFAs are essential for maintaining gut health and preventing dysregulated immune responses. Some of the compounds such as polyphenols, found abundantly in fruits, vegetables, and beverages such as tea, act as prebiotics. These compounds possess potent antioxidant and anti-inflammatory properties and facilitate the growth of beneficial gut bacteria, including *Lactobacillus* and *Bifidobacterium* (143). These bacteria, in turn, help maintain a balanced microbial ecosystem and modulate immune responses, thereby supporting systemic health. Additionally, omega-3 fatty acids, which are present in fatty fish and certain plant oils, exert anti-inflammatory effects and modulate immune cell activity, further contributing to the resolution of chronic inflammation and the maintenance of immune homeostasis (144).

Dietary interventions are crucial in managing various inflammatory diseases, including neuroinflammatory, gastrointestinal, autoimmune, and cardiovascular diseases. In neuroinflammatory conditions, dietary fibers, polyphenols, and

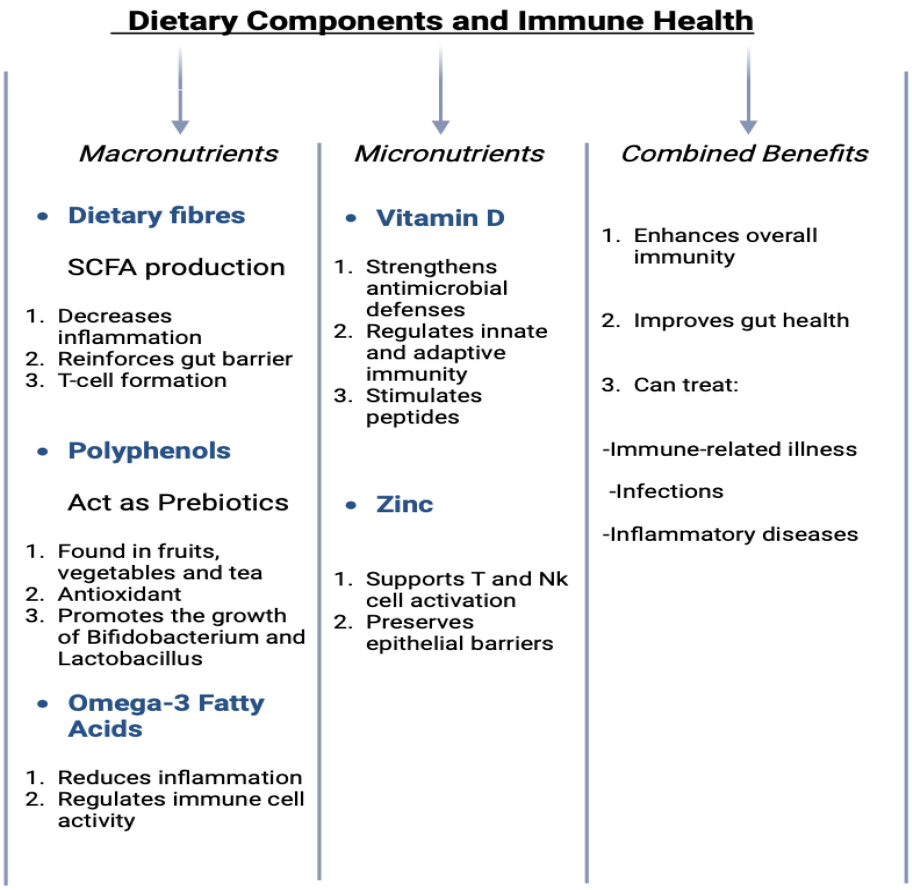


FIGURE 4
Role of various dietary components in human's immunological health.

omega-3 fatty acids help modulate the gut microbiota, reduce inflammation, and improve immune function, with clinical evidence suggesting benefits in conditions like Alzheimer's and multiple sclerosis (145). For gastrointestinal diseases like Crohn's and ulcerative colitis, high-fiber diets and polyphenols enhance gut barrier integrity and reduce inflammation, showing promise in clinical trials (146). In autoimmune diseases such as rheumatoid arthritis and lupus, omega-3 fatty acids and polyphenols have demonstrated significant anti-inflammatory effects, improving symptoms and immune regulation (147). Furthermore, in cardiovascular diseases, diets rich in omega-3s and antioxidants have been shown to reduce oxidative stress.

By supporting both the gut microbiota and immune function, these nutrients provide a holistic approach to managing inflammation and immune dysregulation. In animal models, the ketogenic diet led to reduced systemic inflammation, improved brain function, and decreased levels of pro-inflammatory cytokines in the central nervous system. Furthermore, the diet modulated immune cell populations, particularly by reducing the activation of microglia, the resident immune cells in the brain. These findings suggest that dietary interventions can modulate the gut-brain immune axis and provide therapeutic benefits in conditions that involve neuro-inflammation (21). Tryptophan, an essential amino acid, plays a critical role in maintaining normal growth, health, and metabolic functions in both humans and animals. Beyond its fundamental physiological functions, tryptophan has a significant impact on modulating the gut-brain axis (GBA) through the metabolites produced during its metabolism by gut microbiota. Microbial communities in the gut can convert tryptophan into a range of bioactive compounds, most notably indole and its derivatives. These metabolites have profound effects on immune regulation and the maintenance of gut homeostasis (148). Indole derivatives, in particular, interact with the aryl hydrocarbon receptor a ligand-activated transcription factor present on immune and epithelial cells. Activation of AhR by these metabolites influences various immune cell functions, including the differentiation and expansion of regulatory T cells (Tregs), which are critical for maintaining immune tolerance and controlling inflammatory responses (149). This interaction not only influences immune cell activity but also modulates cytokine production, promoting an anti-inflammatory profile. By facilitating the differentiation of Tregs, indole derivatives help regulate local gut immunity and systemic immune responses, thus balancing the production of pro-inflammatory and anti-inflammatory signals. Additionally, these metabolites play a crucial role in gut barrier function, enhancing epithelial cell integrity and strengthening the intestinal mucosal barrier. This contributes to preventing the translocation of pathogens and reducing intestinal permeability, further supporting immune function and gut health (149). The metabolic products of tryptophan metabolism thus serve as a key link between gut microbiota activity and immune system regulation. Through their interaction with AhR, indole derivatives connect microbiome function with immune responses, ultimately enhancing both gut health and systemic immunity. This integration underscores the importance of the gut microbiota in modulating immune processes and highlights the therapeutic potential of tryptophan-derived metabolites in managing gastrointestinal (150) and systemic inflammatory conditions (117).

6.4 Immunomodulation via microbiome-derived metabolites in cancer therapy

A growing area of research is the use of microbiome-derived metabolites to modulate the immune system in the context of cancer therapy (Table 6).

Recent evidence suggests that the gut microbiome plays a crucial role in influencing responses to immune checkpoint inhibitors (ICIs), such as anti-PD-1 and anti-CTLA-4 therapies, which are widely used in cancer treatment. Among the microbial taxa associated with enhanced ICI efficacy, Firmicutes, particularly *Faecalibacterium prausnitzii*, *Ruminococcus* spp., and *Clostridium cluster XIVa*, have been identified as key contributors to a favorable immunological landscape (154).

Mechanistically, Firmicutes influence ICI efficacy through several pathways. *Faecalibacterium prausnitzii* produces butyrate, a short-chain fatty acid that plays a crucial role in regulatory T cell (Treg) differentiation while simultaneously supporting CD8+ T cell activation in the tumor microenvironment. This dual effect reduces chronic inflammation while enhancing anti-tumor immunity (23). Additionally, certain Firmicutes species promote dendritic cell maturation and MHC class I expression, which in turn enhances

TABLE 6 The role of dietary and microbiome-derived components in gut microbiota modulation and immune function.

Mechanism	Role in ICI Efficacy	Key References
SCFA Production & Treg Modulation	*Faecalibacterium prausnitzii* produces butyrate, which promotes regulatory T cell (Treg) differentiation while enhancing CD8+ T cell activation in the tumor microenvironment. This reduces inflammation and boosts anti-tumor immunity.	(151)
Increased Antigen Presentation	Firmicutes enhance dendritic cell maturation and MHC class I expression, improving tumor antigen presentation and activation of cytotoxic T cells.	(152)
PD-1/PD-L1 Pathway Modulation	Higher levels of *Firmicutes* correlate with increased CD8+ T cell infiltration, enhancing PD-1 blockade efficacy.	(8)
Gut Barrier Integrity & Systemic Immunity	*Firmicutes* upregulate tight junction proteins (e.g., occludin, ZO-1), preventing microbial translocation and systemic inflammation, which could otherwise suppress anti-tumor responses.	(153)

tumor antigen presentation and activation of cytotoxic T cells (155). Higher levels of *Firmicutes* have been correlated with increased infiltration of cytotoxic CD8+ T cells, which improves the effectiveness of PD-1 blockade therapies (156). Furthermore, *Firmicutes* reinforce gut barrier integrity by upregulating tight junction proteins such as occludin and ZO-1, thereby preventing microbial translocation and systemic inflammation, both of which are known to suppress anti-tumor responses (22, 157, 158).

Clinical studies have provided compelling evidence supporting the association between Firmicutes and ICI response. A landmark study by Routy et al. (8) demonstrated that cancer patients with a higher abundance of *Firmicutes* in their gut microbiome had improved progression-free survival and overall response rates to anti-PD-1 therapy. Similarly, Gopalakrishnan et al. (159) found that melanoma patients who responded well to ICIs had increased levels of *Firmicutes*, particularly *Ruminococcus* and *Faecalibacterium*, compared to non-responders. Fecal microbiota transplantation (FMT) studies further validated this association, as transplanting microbiota from ICI responders into germ-free mice restored anti-tumor immunity and enhanced PD-1 blockade efficacy (8).

These findings underscore the potential for modulating the gut microbiota through probiotics, dietary interventions, or FMT as a strategy to optimize ICI therapy outcomes. Future research should focus on identifying specific microbial signatures that could serve as predictive biomarkers for immunotherapy response and exploring the potential of microbiome-targeted interventions in cancer treatment (160).

7 Challenges and ethical considerations

Despite the promising therapeutic potential of fecal microbiota transplantation (FMT), several significant challenges remain that must be addressed before its widespread adoption in clinical practice (161). One of the primary issues is the variability in outcomes, which can arise from differences in the donor microbiota as well as the methods used in stool preparation and administration. These variations can result in inconsistent therapeutic effects, making it difficult to predict patient responses (95). Standardizing the entire FMT process, including donor screening, stool preparation, and administration techniques, is essential to ensure reliable and safe results (162). Fecal microbiota transplantation: An update on clinical practice (162) This standardization would help to minimize variability and improve the reproducibility of FMT's outcomes (132). Additionally, stringent donor screening is essential to prevent the transmission of infectious agents, antibiotic-resistant bacteria, or other undesirable genetic material through FMT, which could pose serious health risks to recipients (163, 164).

Similarly, while probiotics and prebiotics have demonstrated significant immunomodulatory and gut health benefits, their efficacy and stability remain key concerns. The viability of probiotics is influenced by factors such as storage conditions, gastrointestinal survival, and their ability to colonize the gut effectively. Many probiotic strains exhibit strain-specific effects, meaning their therapeutic potential is not always universally applicable (128). Additionally, the stability of probiotics in commercial formulations often poses challenges, as exposure to heat, moisture, or gastric acidity can reduce their potency before they reach the intestine (127). Prebiotics, on the other hand, rely on selective fermentation by beneficial gut microbes to exert their effects, but individual variations in gut microbiota composition can impact their effectiveness, leading to differential immune and metabolic responses among individuals (119).

Beyond efficacy, long-term clinical studies are needed to assess the safety and durability of FMT, probiotics, and prebiotics. While these interventions show promise in managing conditions like inflammatory bowel disease, allergies, and metabolic disorders, their long-term impact on the host microbiome remains uncertain. Potential risks include microbiome imbalances, unintended immune reactions, or the emergence of dysbiosis-related diseases over time (134). Addressing these challenges through rigorous research, improved formulation technologies, and personalized approaches will be essential to fully harness the benefits of microbiome-based therapies while minimizing risks.

Furthermore, challenges include the standardization of personalized microbiome-based treatments and the need for long-term safety evaluations to ensure the stability and predictability of therapeutic outcomes. These factors must be considered to optimize the clinical translation of microbiome-based interventions.

8 Current status of research in this area

In recent decades, research on the host-microbe immunological axis has experienced significant growth, with studies emerging from diverse geographical regions worldwide (165).

Microbiome research has predominantly been led by North America, Europe, and Asia, regions with substantial funding and advanced research infrastructure. A study analyzing over 444,000 human microbiome samples found that more than 71% originated from Europe, the United States, and Canada, with the U.S. alone accounting for 46.8% (166). However, there is a growing interest in microbiome research from developing regions. In Africa, microbiome studies have been conducted in 61% of the countries, with South Africa, Kenya, and Uganda leading the efforts (167). This field has advanced considerably with the advent of cutting-edge technologies. The application of multi-omics techniques, such as transcriptomics, metabolomics, and metagenomics (168), has enabled comprehensive studies of microbiomes, revealing both microbial diversity and functional roles at unprecedented levels (169). Single-cell and spatial transcriptomics have provided detailed insights into the complex interactions between immune cells and microbiota (170). Additionally, machine learning and artificial intelligence (AI) have revolutionized data analysis by generating predictive models and uncovering microbial biomarkers, thus enhancing our understanding of microbial ecosystems and

facilitating the development of personalized therapeutic interventions (171). The exploration of the complex relationships between immunity and the microbiota is crucial for advancing our knowledge of human health and disease. This endeavor employs a variety of methodological approaches, including in silico techniques, animal models, and next-generation *in vitro* systems, each of which has its own advantages and limitations (170). Animal models provide in-depth biological context but present ethical and logistical challenges. Modern *in vitro* systems offer solutions to these concerns by reducing animal use, though they come at a higher cost and require specialized materials. In silico methods, while offering potential for resource efficiency and sustainability, also need ongoing refinement and rigorous experimental validation to ensure their reliability (172). Together, these integrative approaches support a more holistic understanding of the microbiome-immunity axis, paving the way for future research that is both sustainable and ethically responsible (173).

9 Conclusion

The host-microbe immunological axis plays a crucial role in maintaining human health and regulating immune responses. Advances in technologies like machine learning, single-cell analysis, and multi-omics have provided deeper insights into this complex relationship, paving the way for novel therapeutic approaches. Interventions such as probiotics, prebiotics, and fecal microbiota transplantation show promise in treating immune-related disorders and microbial imbalances. However, challenges remain, including ethical concerns, technological standardization, and understanding the long-term effects of microbial therapies. Future research should focus on personalized treatments, address these challenges, and further explore the potential of microbial modulation in promoting human health.

10 Future directions

Future advancements in microbiome therapy will focus on individualized treatments tailored to each patient's unique microbiome composition, driven by innovations in machine learning, sequencing technologies, and a deeper understanding of microbiome dynamics. This will include therapies such as fecal microbiota transplants, dietary interventions, prebiotics, probiotics, and Microbiome-derived therapeutics. However, challenges remain in addressing population variability, predicting outcomes in complex microbial ecosystems, and ensuring the affordability and accessibility of personalized treatments. Ethical concerns regarding informed consent, data privacy, and equitable access must be addressed, along with the development of standardized regulatory frameworks to ensure safety and efficacy. Moreover, ongoing research is needed to explore the stability of microbial-immune interactions, assess long-term effects, and evaluate potential risks, such as ecological shifts or resistance. By addressing these scientific, ethical, and regulatory hurdles, microbiome-based therapies have the potential to revolutionize human health and offer targeted interventions for immune-related disorders. These therapies could have a transformative impact on diseases with immune-related components, such as Inflammatory Bowel Disease (IBD), autoimmune diseases like rheumatoid arthritis, asthma and allergies, cancer, metabolic disorders (e.g., obesity and type 2 diabetes), infectious diseases (e.g., HIV and tuberculosis), chronic fatigue syndrome, and neurodegenerative diseases (e.g., Alzheimer's and Parkinson's). In these conditions, microbiome modulation could offer a novel approach to restore immune balance, reduce inflammation, enhance treatment efficacy, and provide long-term benefits to patients. A more detailed discussion on the challenges of microbiome-based therapies, including standardization, regulatory hurdles, and ethical concerns, is essential to fully elucidate the complexities of their clinical application and the role of AI and precision medicine in overcoming these barriers.

Author contributions

AN: Conceptualization, Supervision, Validation, Writing – original draft, Writing – review & editing. FH: Writing – original draft, Writing – review & editing. TN: Conceptualization, Visualization, Writing – review & editing. RA: Conceptualization, Writing – review & editing. AR: Conceptualization, Visualization, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. The Office of Research and Sponsored Programs (ORSP) as a Faculty Research Incentive Grant, Abu Dhabi University supported this work.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Yadav M, Kumar T, Kanakan A, Maurya R, Pandey R, Chauhan NS. Isolation and characterization of human intestinal bacteria cytobacillus oceanisediminis NB2 for probiotic potential. *Front Microbiol*. (2022) 13:932795. doi: 10.3389/fmicb.2022.932795

2. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. *Cell*. (2014) 157:121–41. doi: 10.1016/j.cell.2014.03.011

3. Marchesi JR, Adams DH, Fava F, Hermes GDA, Hirschfield GM, Hold GL. The gut microbiota and host health: A new clinical frontier. *Gut*. (2016) 65:330–9. doi: 10.1136/gutjnl-2015-309990

4. Yadav M, Pandey R, Chauhan NS. Catabolic machinery of the human gut microbes bestow resilience against vanillin antimicrobial nature. *Front Microbiol*. (2020) 11:588545. doi: 10.3389/fmicb.2020.588545

5. Fusco W, Lorenzo MB, Cintoni M, Porcari S, Rinninella E, Kaitsas F, et al. Short-chain fatty-acid-producing bacteria: Key components of the human gut microbiota. *Nutrients*. (2023) 15:2211. doi: 10.3390/nu15092211

6. Rooks MG, Garrett WS. Gut microbiota, metabolites, and host immunity. *Nat Rev Immunol*. (2016) 16:341–52. doi: 10.1038/nri.2016.42

7. Kumar Mondal A, Kumar J, Pandey R, Gupta S, Kumar M, Bansal G, et al. Comparative genomics of host–symbiont and free-living oceanobacillus species. *Genome Biol Evol*. (2017) 9:1175–82. doi: 10.1093/gbe/evx076

8. Routy B, Le Chatelier E, Derosa L, Alou MT, Gopalakrishnan V. Gut microbiome influences efficacy of PD-1-based immunotherapy against epithelial tumors. *Science*. (2018) 359:91–7. doi: 10.1126/science.aan3706

9. Hesse S, O'Hanlon M, Cohen L. Phage therapy in the era of multi-drug resistance: Antibiotics and bacteriophages. *Microbial Cell Factories*. (2019) 18:54. doi: 10.1186/s12934-019-1104-8

10. Hooper LV, Littman DR, Macpherson AJ. Interactions between the microbiota and the immune system. *Science*. (2012) 336:1268–73. doi: 10.1126/science.1223490

11. Ley RE, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell*. (2006) 124:837–48. doi: 10.1016/j.cell.2006.02.017

12. Carreto-Binaghi LE, Sztein MB, Booth JS. Role of cellular effectors in the induction and maintenance of IgA responses leading to protective immunity against enteric bacterial pathogens. *Front Immunol*. (2024) 15:1446072. doi: 10.3389/fimmu.2024.1446072

13. Zelante T, Iannitti R, Romani L. Tryptophan catabolites from microbiota engage AhR to modulate mucosal immunity. *Cell Host Microbe*. (2013) 13:664–75. doi: 10.1016/j.chom.2013.05.013

14. Belkaid Y, Harrison OJ. Homeostatic immunity and the microbiota. *Immunity*. (2017) 46:562–76. doi: 10.1016/j.immuni.2017.04.008

15. Stienstra R, Joosten LAB, Riksen NP. The inflammasome mediates obesity and metabolic syndrome. *Cell Metab*. (2012) 15:10–8. doi: 10.1016/j.cmet.2011.12.009

16. Nazir A, Hussain FHN, Raza A. Advancing microbiota therapeutics: The role of synthetic biology in engineering microbial communities for precision medicine. *Front Bioengineering Biotechnol*. (2024) 12:1511149. doi: 10.3389/fbioe.2024.1511149

17. Lynch SV, Pedersen O. The human intestinal microbiome in health and disease. *New Engl J Med*. (2016) 375:2369–79. doi: 10.1056/NEJMra1600266

18. Pluta R, Sowa-Kucma M, Maciejewski R. Gut microbiota as a tool in neuroprotection and neurodegeneration. *Neurobiol Dis*. (2021) 153:105303. doi: 10.1016/j.nbd.2021.105303

19. Chauhan NS, Mukerji M, Gupta S. Editorial: Role of microbiome in diseases diagnostics and therapeutics. *Front Cell Infect Microbiol*. (2022) 12:1025837. doi: 10.3389/fcimb.2022.1025837

20. Chauhan NS, Kumar S. *Microbiome Therapeutics: Personalized Therapy Beyond Conventional Approaches*. San Diego: Elsevier Science & Technology (2023). pp. 1–514. doi: 10.1016/C2021-0-01533-9.

21. Yadav M, Chauhan NS. Microbiome therapeutics: exploring the present scenario and challenges. *Gastroenterol Rep*. (2022) 0:goab046. doi: 10.1093/gastro/goab046

22. Tsai Y, Tai W, Liang C, Wu C, Tsai M, Hu W, et al. Alterations of the gut microbiota and the Firmicutes/Bacteroidetes ratio after biologic treatment in inflammatory bowel disease. *J Microbiology Immunol Infection*. (2025) 58:62–9. doi: 10.1016/j.jmii.2024.09.006

23. Kang X, Cheuk-Hay Lau H, Yu J. Modulating gut microbiome in cancer immunotherapy: Harnessing microbes to enhance treatment efficacy. *Cell Rep Med*. (2024) 5:101478. doi: 10.1016/j.xcrm.2024.101478

24. Tan J, Lee SP, Yang Y. The role of short-chain fatty acids in health and disease. *Cell Metab*. (2021) 33:707–17. doi: 10.1016/j.cmet.2021.01.017

25. Quince C, Ijaz UZ, Loman NJ, Willis AD. Shotgun metagenomics, from sampling to analysis. *Nat Methods*. (2017) 14:833–44. doi: 10.1038/nmeth.4172

26. Losol P, Wolska M, Wypych TP, Yao L, O'Mahony L, Sokolowska M. A cross talk between microbial metabolites and host immunity: Its relevance for allergic diseases. *Clin Transl Allergy*. (2024) 14(2):e12339. doi: 10.1002/clt2.12339

27. Jandhyala SM, Talukdar R, Subramanyam C, Sreenivas V, Madhusudhan KS. Role of the normal gut microbiota. *Gut Pathog*. (2015) 7:23. doi: 10.1186/s13099-015-0071-7

28. Stubbington MJ, Lukk M, Regev A. Single-cell transcriptomics to explore immune cell heterogeneity. *Nat Rev Mol Cell Biol*. (2017) 18:472–85. doi: 10.1038/nrm.2017.16

29. Xiao H, Ye S, Chen Y. Mapping immune-microbiota interactions in IBD using spatial transcriptomics. *Immunity*. (2023) 56:489–501. doi: 10.1016/j.immuni.2023.02.005

30. Asgari E, Zeynizadeh B, Gharibi T, Rezaei N, Eslami H. AI-driven insights into microbiome-host interactions: Applications and future perspectives. *BMC Bioinf*. (2021) 22:113. doi: 10.1186/s12859-021-04058-w

31. Topol EJ. High-performance medicine: The convergence of human and artificial intelligence. *Cell*. (2019) 177:5–10. doi: 10.1016/j.cell.2019.01.003

32. Kumar T, Chauhan NS. Microbiome therapeutics: A path toward sustainable healthcare. In: *Comprehensive Gut Microbiota*. Amsterdam, Netherlands: Elsevier (2022) 234–45. doi: 10.1016/B978-0-12-819265-8.00010-3

33. Kumar T, Pandey R, Chauhan NS. Hypoxia inducible factor-1α: the curator of gut homeostasis. *Front Cell Infect Microbiol*. (2020) 10:227. doi: 10.3389/fcimb.2020.00227

34. Arango Duque G, Descoteaux A. Macrophage cytokines: involvement in immunity and infectious diseases. *Front Immunol*. (2014) 5:491. doi: 10.3389/fimmu.2014.00491

35. Jang JH, Shin HW, Lee JM, Lee HW, Kim EC, Park SH. An overview of pathogen recognition receptors for innate immunity in dental pulp. *Mediators Inflammation*. (2015) 2015:794143. doi: 10.1155/2015/794143

36. Oliveira-Nascimento L, Massari P, Wetzler LM. The role of TLR2 in infection and immunity. *Front Immunol*. (2012) 3:79. doi: 10.3389/fimmu.2012.00079

37. Lee EY, Lee MW, Wong GCL. Modulation of toll-like receptor signaling by antimicrobial peptides. *Semin Cell Dev Biol*. (2019) 88:173–84. doi: 10.1016/j.semcdb.2018.02.002

38. Franchi L, Warner N, Viani K, Nuñez G. Function of Nod-like receptors in microbial recognition and host defense. *Immunol Rev*. (2009) 227:106–28. doi: 10.1111/j.1600-065X.2008.00734.x

39. Martinon F, Mayor A, Tschopp J. The inflammasomes: Guardians of the body. *Annu Rev Immunol*. (2009) 27:229–65. doi: 10.1146/annurev.immunol.021908.132715

40. Biasizzo M, Kopitar-Jerala N. Interplay between NLRP3 inflammasome and autophagy. *Front Immunol*. (2020) 11:591803. doi: 10.3389/fimmu.2020.591803

41. Xu Z, Kombe Kombe AJ, Deng S, Zhang H, Wu S, Ruan J, et al. NLRP inflammasomes in health and disease. *Mol Biomedicine*. (2024) 5:14. doi: 10.1186/s43556-024-00179-x

42. Hale RC, Morais D, Chou J, Stowell SR. The role of glycosylation in clinical allergy and immunology. *J Allergy Clin Immunol*. (2024) 153:55–66. doi: 10.1016/j.jaci.2023.09.003

43. Scur M, Parsons BD, Dey S, Makrigiannis AP. The diverse roles of C-type lectin-like receptors in immunity. *Front Immunol*. (2023) 14:1126043. doi: 10.3389/fimmu.2023.1126043

44. Kawai T, Akira S. The roles of TLRs, RLRs and NLRs in pathogen recognition. *Int Immunol*. (2009) 21:317–37. doi: 10.1093/intimm/dxp017

45. Speakman EA, Dambuza IM, Salazar F, Brown GD. T cell antifungal immunity and the role of C-type lectin receptors*Trends Immunol*. (2019) 41(1):61––76. doi: 10.1016/j.it.2019.11.007

46. Li D, Wu M. Pattern recognition receptors in health and diseases. *Signal Transduc Targeted Ther*. (2021) 6(1):1–24. doi: 10.1038/s41392-021-00687-0

47. Raymond SL, Holden DC, Mira JC, Stortz JA, Loftus TJ, Mohr AM, et al. Microbial recognition and danger signals in sepsis and trauma. *Biochim Biophys Acta (BBA) - Mol Basis Dis*. (2017) 1863:2564–73. doi: 10.1016/j.bbadis.2017.01.013

48. Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. *J Leukoc Biol*. (2007) 81(1):1–5. doi: 10.1189/jlb.0306164

49. Silva YP, Bernardi A, Frozza RL. The role of short-chain fatty acids from gut microbiota in gut-brain communication. *Front Endocrinol*. (2020) 11:25. doi: 10.3389/fendo.2020.00025

50. Cummings JH, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut*. (1987) 28:1221–7. doi: 10.1136/gut.28.10.1221

51. Louis P, Hold GL, Flint HJ. The gut microbiota, bacterial metabolites, and colorectal cancer. *Nat Rev Microbiol*. (2014) 12:661–72. doi: 10.1038/nrmicro3344

52. Rey FE, Faith JJ, Bain J, Muehlbauer MJ, Stevens RD, Newgard CB, et al. Dissecting the *in vivo* metabolic potential of two human gut acetogens. *J Biol Chem*. (2010) 285:22082–90. doi: 10.1074/jbc.M110.117713

53. Scott KP, Martin JC, Campbell G, Mayer CD, Flint HJ. Whole-genome transcription profiling reveals genes upregulated by growth on fucose in the human

gut bacterium *Roseburia inulinivorans*. *J Bacteriology*. (2006) 188:4340–9. doi: 10.1128/JB.00223-06

54. Duncan SH, Barcenilla A, Stewart CS, Pryde SE, Flint HJ. Acetate utilization and butyryl CoA:acetate CoA-transferase in butyrate-producing bacteria from the human large intestine. *Appl Environ Microbiol*. (2002) 68:5186–90. doi: 10.1128/AEM.68.10.5186-5190.2002z

55. Bourriaud C, Robins RJ, Martin L, Kozlowski F, Tenailleau E, Cherbut C, et al. Lactate is mainly fermented to butyrate by human intestinal microflora but inter-individual variation exists. *J Appl Microbiol*. (2005) 99:201–12. doi: 10.1111/j.1365-2672.2005.02605.x

56. He X, Zhao S, Li Y. Faecalibacterium prausnitzii: A next-generation probiotic in gut disease improvement. *Can J Infect Dis Med Microbiol*. (2020) 2021:6666114. doi: 10.1155/2021/6666114

57. Ghosh S, Whitley CS, Haribabu B, Jala VR. Regulation of intestinal barrier function by microbial metabolites. *Cell Mol Gastroenterol Hepatol*. (2020) 11:1463–82. doi: 10.1016/j.jcmgh.2021.02.007

58. Effendi RMRA, Anshory M, Kalim H, Dwiyana RF, Suwarsa O, Pardo LM, et al. *Akkermansia muciniphila* and *Faecalibacterium prausnitzii* in Immune-Related Diseases. *Microorganisms*. (2022) 10:2382. doi: 10.3390/microorganisms10122382

59. Frost G, Sleeth ML, Lizarbe B, Cerdan S, Brody L, Anastasovska J, et al. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nature Commun*. (2014) 5(1):1–11. doi: 10.1038/ncomms4611

60. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature*. (2013) 504:451–5. doi: 10.1038/nature12726

61. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature*. (2013) 504:446–50. doi: 10.1038/nature12721

62. Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohlooly-Y M, et al. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science*. (2013) 341:569–73. doi: 10.1126/science.1241165

63. Liao T, Wang N, Jia Y, Li C, Zhang X. Regulation of short-chain fatty acids in the immune system. *Front Immunol*. (2023) 14:1186892. doi: 10.3389/fimmu.2023.1186892

64. Davie JR. Inhibition of histone deacetylase activity by butyrate. *J Nutr*. (2003) 133:2485S–93S. doi: 10.1093/jn/133.7.2485S

65. Yan Q, Jia S, Li D, Yang J. The role and mechanism of action of microbiota-derived short-chain fatty acids in neutrophils: From activation to becoming potential biomarkers. *Biomedicine Pharmacotherapy*. (2023) 169:115821. doi: 10.1016/j.biopha.2023.115821

66. Ullah H, Arbab S, Tian Y, Chen Y, Liu C, Li Q, et al. Crosstalk between gut microbiota and host immune system and its response to traumatic injury. *Front Immunol*. (2024) 15:1413485. doi: 10.3389/fimmu.2024.1413485

67. Schirmer M, Smeekens SP, Vlamakis H, Jaeger M, Oosting M, Franzosa EA, et al. Linking the human gut microbiome to inflammatory cytokine production capacity. *Cell*. (2016) 167:1125–1136.e8. doi: 10.1016/j.cell.2016.10.020

68. Kany S, Vollrath JT, Relja B. Cytokines in inflammatory disease. *Int J Mol Sci*. (2019) 20:6008. doi: 10.3390/ijms20236008

69. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell*. (2006) 124:783–801. doi: 10.1016/j.cell.2006.02.015

70. He W, Qu T, Yu Q, Wang Z, Lv H, Zhang J, et al. LPS induces IL-8 expression through TLR4, MyD88, NF-kappaB and MAPK pathways in human dental pulp stem cells. *Int endodontic J*. (2013) 46:128–36. doi: 10.1111/j.1365-2591.2012.02096.x

71. Lee SI, Kim GT, Kim HJ, Park SH, Kim EC. NOD2 mediates odontoblast differentiation and RANKL expression. *J Dental Res*. (2014) 93:678–84. doi: 10.1177/0022034514535214

72. Finoti LS, Nepomuceno R, Pigossi SC, Corbi SCT, Secolin R, Scarel-Caminaga RM. Association between interleukin-8 levels and chronic periodontal disease: A PRISMA-compliant systematic review and meta-analysis. *Med (Baltimore)*. (2017) 96:e6932. doi: 10.1097/MD.0000000000006932

73. Slots J. Oral viral infections of adults. *Periodontology 2000*. (2009) 49:60–86. doi: 10.1111/j.1600-0757.2008.00291.x

74. Chen S, Saeed AFUH, Liu Q, Jiang Q, Xu H, Xiao GG, et al. Macrophages in immunoregulation and therapeutics. *Signal Transduction Targeted Ther*. (2023) 8:1–35. doi: 10.1038/s41392-023-01388-6

75. Giambra V, Pagliari D, Rio P, Totti B, Nunzio CD, Bosi A, et al. Gut microbiota, inflammatory bowel disease, and cancer: The role of guardians of innate immunity. *Cells*. (2023) 12:2654. doi: 10.3390/cells12222654

76. Ding Q, Hu W, Wang R, Yang Q, Zhu M, Li M, et al. Signaling pathways in rheumatoid arthritis: Implications for targeted therapy. *Signal Transduction Targeted Ther*. (2023) 8:68. doi: 10.1038/s41392-023-01331-9

77. Jarnicki AG, Lysaght J, Todryk S, Mills KHG. Suppression of antitumor immunity by IL-10 and TGF-β-producing T cells infiltrating the growing tumor: Influence of tumor environment on the induction of CD4+ and CD8+ regulatory T cells. *J Immunol*. (2006) 177:896–904. doi: 10.4049/jimmunol.177.2.896

78. Hosseinian K, Gerami A, Bral M, Venketaraman V. *Mycobacterium tuberculosis*-human immunodeficiency virus infection and the role of T cells in protection. *Vaccines*. (2024) 12:730. doi: 10.3390/vaccines12070730

79. Medzhitov R. Recognition of microorganisms and activation of the immune response. *Nature*. (2007) 449:819–26. doi: 10.1038/nature06246

80. Schroeder BO, Bäckhed F. Signals from the gut microbiota to distant organs in physiology and disease. *Nat Med*. (2016) 22:1079–89. doi: 10.1038/nm.4185

81. Rivera-Chávez F, Bäumler AJ. The pyRomaniac inside you: Salmonella metabolism in the host gut. *Annu Rev Microbiol*. (2015) 69:31–48. doi: 10.1146/annurev-micro-091014-104230

82. Cambier CJ, Takaki KK, Larson RP, Hernandez RE, Tobin DM, Urdahl KB, et al. Mycobacteria manipulate macrophage recruitment through coordinated use of membrane lipids. *Nature*. (2014) 505:218–22. doi: 10.1038/nature12799

83. Merad M, Martin JC. Pathological inflammation in patients with COVID-19: A key role for monocytes and macrophages. *Nat Rev Immunol*. (2020) 20:355–62. doi: 10.1038/s41577-020-0331-4

84. Chow J, Lee SM, Shen Y, Khosravi A, Mazmanian SK. Host-bacterial symbiosis in health and disease. *Adv Immunol*. (2010) 107:243–74. doi: 10.1016/B978-0-12-381300-8.00008-3

85. Telesford KM, Yan W, Ochoa-Reparaz J, Pant A, Kircher C, Christy MA, et al. A commensal symbiotic factor derived from Bacteroides fragilis promotes human CD39 +Foxp3+ T cells and Treg function. *Gut Microbes* (2015) 6(4):234–42. doi: 10.1080/19490976.2015.1056973

86. Yu Z, Chen J, Liu Y, Meng Q, Liu H, Yao Q, et al. The role of potential probiotic strains Lactobacillus reuteri in various intestinal diseases: new roles for an old player. *Front Microbiol*. (2023) 14:1095555. doi: 10.3389/fmicb.2023.1095555

87. Pang Z, Mao X, Zhou S, Yu S, Liu G, Lu C, et al. Microbiota-mediated nitrogen fixation and microhabitat homeostasis in aerial root-mucilage. *Microbiome*. (2023) 11:85. doi: 10.1186/s40168-023-01525-x

88. Cogen AL, Nizet V, Gallo RL. Skin microbiota: A source of disease or defense? *Br J Dermatol*. (2008) 158:442–55. doi: 10.1111/j.1365-2133.2008.08437.x

89. Sehgal K, Khanna S. Gut microbiome and *Clostridioides difficile* infection: A closer look at the microscopic interface. *Ther Adv Gastroenterol*. (2021) 14:1756284821994736. doi: 10.1177/1756284821994736

90. Overstreet RM, Lotz JM. Host–symbiont relationships: understanding the change from guest to pest. In: *The Rasputin Effect: When Commensals and Symbionts Become Parasitic*, vol. 3. Cham, Switzerland: Springer International Publishing (2016). p. 27–64. doi: 10.1007/978-3-319-28170-4_2

91. Drew GC, Stevens EJ, King KC. Microbial evolution and transitions along the parasite-mutualist continuum. *Nat Rev Microbiol*. (2021) 19:623–38. doi: 10.1038/s41579-021-00550-7

92. Aristizábal B, González Á. Innate immune system. In: Anaya JM, Shoenfeld Y, Rojas-Villarraga A, et al, editors. *Autoimmunity: From Bench to Bedside*. El Rosario University Press, Bogota (Colombia (2013). Available at: https://www.ncbi.nlm.nih.gov/books/NBK459455/.

93. Venkatesha SH, Dudics S, Acharya B, Moudgil KD. Cytokine-modulating strategies and newer cytokine targets for arthritis therapy. *Int J Mol Sci*. (2014) 16:887–906. doi: 10.3390/ijms16010887

94. Yadav M, Kumar T, Maurya R, Pandey R, Chauhan NS. Characterization of Cellulomonas sp. HM71 as potential probiotic strain for human health. *Front Cell Infect Microbiol*. (2023) 12:1082674. doi: 10.3389/fcimb.2022.1082674

95. Hitch TCA, Hall LJ, Walsh SK, Leventhal GE, Slack E, de Wouters T, et al. Microbiome-based interventions to modulate gut ecology and the immune system. *Mucosal Immunol*. (2022) 15:1095–113. doi: 10.1038/s41385-022-00564-1

96. Chauhan NS. Personalized nutrition, personalized medicine, and microbiome therapeutics. In: *Microbiome Therapeutics*. Amsterdam, Netherlands: Elsevier (2023). p. 387–99. doi: 10.1016/B978-0-323-99336-4.00017-3

97. Shakoor H, Feehan J, Al Dhaheri AS, Ali HI, Platat C, Ismail LC, et al. Immune-boosting role of vitamins D, C, E, zinc, selenium and omega-3 fatty acids: Could they help against COVID-19? *Maturitas*. (2021) 143:1–9. doi: 10.1016/j.maturitas.2020.08.003

98. Kim SK. The mechanism of the NLRP3 inflammasome activation and pathogenic implication in the pathogenesis of gout. *J rheumatic Dis*. (2022) 29:140–53. doi: 10.4078/jrd.2022.29.3.140

99. Chauhan NS, Monika, Kumar T, Sharma P, Goyal K. RNA beyond humans. In: *Rna-Based Regulation in Human Health and Disease*. London, United Kingdom: Elsevier (2020). p. 117–38. doi: 10.1016/B978-0-12-817193-6.00005-4

100. Saifi S, Ravi V, Sharma S, Swaminathan A, Chauhan NS, Pandey R. SARS-CoV-2 VOCs, Mutational diversity and clinical outcome: Are they modulating drug efficacy by altered binding strength? *Genomics*. (2022) 114:110466. doi: 10.1016/j.ygeno.2022.110466

101. Ravi V, Shamim U, Khan MA, Swaminathan A, Mishra P, Singh R, et al. Unraveling the genetic evolution of SARS-CoV-2 Recombinants using mutational dynamics across the different lineages. *Front Med*. (2024) 10:1294699. doi: 10.3389/fmed.2023.1294699

102. Ghattas M, Dwivedi G, Lavertu M, Alameh MG. Vaccine technologies and platforms for infectious diseases: current progress, challenges, and opportunities. *Vaccines*. (2021) 9:1490. doi: 10.3390/vaccines9121490

103. Verdecia M, Kokai-Kun JF, Kibbey M, Acharya S, Venema J, Atouf F. COVID-19 vaccine platforms: Delivering on a promise? *Hum Vaccines immunotherapeutics*. (2021) 17:2873–93. doi: 10.1080/21645515.2021.1911204

104. Jung SM, Kim WU. Targeted immunotherapy for autoimmune disease. *Immune network*. (2022) 22:e9. doi: 10.4110/in.2022.22.e9

105. Zahavi D, Weiner L. Monoclonal antibodies in cancer therapy. *Antibodies (Basel Switzerland)*. (2020) 9:34. doi: 10.3390/antib9030034

106. Julier Z, Park AJ, Briquez PS, Martino MM. Promoting tissue regeneration by modulating the immune system. *Acta Biomaterialia*. (2017) 53:1–12. doi: 10.1016/j.actbio.2017.01.056

107. Dong TS, Mayer E. Advances in brain–gut–microbiome interactions: A comprehensive update on signaling mechanisms, disorders, and therapeutic implications. *Cell Mol Gastroenterol Hepatol*. (2023) 18:1–13. doi: 10.1016/j.jcmgh.2024.01.024

108. Qian K, Li G, Zhang S, Zou Y, Ai H, Zheng X, et al. The application of antibody-based agents in cancer therapy based on their mechanisms of action. *Fundam Res*. (2024) In press. doi: 10.1016/j.fmre.2024.02.021

109. Schwartz DM, Kanno Y, Villarino A, Ward M, Gadina M, O'Shea JJ. JAK inhibition as a therapeutic strategy for immune and inflammatory diseases. *Nat Rev Drug Discovery*. (2017) 17:78. doi: 10.1038/nrd.2017.267

110. Cui J, Li Y, Yang Y, Yang H, Dong J, Xiao Z, et al. Tumor immunotherapy resistance: Revealing the mechanism of PD-1 / PD-L1-mediated tumor immune escape. *Biomed Pharmacother*. (2024) 171:116203. doi: 10.1016/j.biopha.2024.116203

111. Yasmeen F, Pirzada RH, Ahmad B, Choi B, Choi S. Understanding autoimmunity: mechanisms, predisposing factors, and cytokine therapies. *Int J Mol Sci*. (2024) 25:7666. doi: 10.3390/ijms25147666

112. Rohm TV, Meier DT, Olefsky JM, Donath MY. Inflammation in obesity, diabetes, and related disorders. *Immunity*. (2022) 55:31–55. doi: 10.1016/j.immuni.2021.12.013

113. Thimmappa PY, Vasishta S, Ganesh K, Nair AS, Joshi MB. Neutrophil (dys) function due to altered immuno-metabolic axis in type 2 diabetes: implications in combating infections. *Hum Cell*. (2023) 36:1265–82. doi: 10.1007/s13577-023-00905-7

114. Marshall JS, Warrington R, Watson W, Kim HL, Antonetti FR. An introduction to immunology and immunopathology. *Allergy Asthma Clin Immunol*. (2018) 14:49. doi: 10.1186/s13223-018-0278-1

115. Shawky AM, Almalki FA, Abdalla AN, Abdelazeem AH, Gouda AM. A comprehensive overview of globally approved JAK inhibitors. *Pharmaceutics*. (2022) 14:1001. doi: 10.3390/pharmaceutics1405100

116. Pagnini C, Di Paolo MC, Urgesi R, Pallotta L, Fanello G, Graziani MG, et al. Safety and potential role of *Lactobacillus rhamnosus* GG administration as monotherapy in ulcerative colitis patients with mild–moderate clinical activity. *Microorganisms*. (2023) 11:1381. doi: 10.3390/microorganisms11061381

117. Liu J, Tan Y, Cheng H, Zhang D, Feng W, Peng C. Functions of gut microbiota metabolites, current status and future perspectives. *Aging Dis*. (2022) 13:1106–26. doi: 10.14336/AD.2022.0104

118. Mazziotta C, Tognon M, Martini F, Torreggiani E, Rotondo JC. Probiotics mechanism of action on immune cells and beneficial effects on human health. *Cells*. (2023) 12:184. doi: 10.3390/cells12010184

119. Al-Habsi N, Al-Khalili M, Haque SA, Elias M, Olqi NA, Al Uraimi T. Health benefits of prebiotics, probiotics, synbiotics, and postbiotics. *Nutrients*. (2024) 16:3955. doi: 10.3390/nu16223955

120. Wang J, Zhu N, Su X, Gao Y, Yang R. Gut-microbiota-derived metabolites maintain gut and systemic immune homeostasis. *Cells*. (2023) 12:793. doi: 10.3390/cells12050793

121. den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. *J Lipid Res*. (2013) 54:2325–40. doi: 10.1194/jlr.R036012

122. Ramos Meyers G, Samouda H, Bohn T. Short chain fatty acid metabolism in relation to gut microbiota and genetic variability. *Nutrients*. (2022) 14:5361. doi: 10.3390/nu14245361

123. Tang X. Probiotic roles of *Clostridium butyricum* in piglets: Considering aspects of intestinal barrier function. *Animals*. (2024) 14:1069. doi: 10.3390/ani14071069

124. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol*. (2014) 11:506–14. doi: 10.1038/nrgastro.2014.66

125. Yadav M, Chauhan NS, Prasher B, Mukerji M. Dissecting human microbiome for personalized therapy. In: *Comprehensive Gut Microbiota*. Elsevier (2022). p. 274–85. doi: 10.1016/B978-0-12-819265-8.00024-3

126. Davani-Davari D, Negahdaripour M, Karimzadeh I, Seifan M, Mohkam M, Masoumi SJ, et al. Prebiotics: Definition, types, sources, mechanisms, and clinical applications. *Foods*. (2019) 8:92. doi: 10.3390/foods8030092

127. Hsu C, Khachatryan LG, Younis NK, Mustafa MA, Ahmad N, Athab ZH, et al. Microbiota-derived short chain fatty acids in pediatric health and diseases: From gut development to neuroprotection. *Front Microbiol*. (2024) 15:1456793. doi: 10.3389/fmicb.2024.1456793

128. Markowiak-Kopeć P, Śliżewska K. The effect of probiotics on the production of short-chain fatty acids by human intestinal microbiome. *Nutrients*. (2020) 12:1107. doi: 10.3390/nu12041107

129. Salvi PS, Cowles RA. Butyrate and the intestinal epithelium: Modulation of proliferation and inflammation in homeostasis and disease. *Cells*. (2021) 10:1775. doi: 10.3390/cells10071775

130. Akhtar M, Chen Y, Ma Z, Zhang X, Shi D, Khan JA, et al. Gut microbiota-derived short chain fatty acids are potential mediators in gut inflammation. *Anim Nutr (Zhongguo xu mu shou yi xue hui)*. (2021) 8:350–60. doi: 10.1016/j.aninu.2021.11.005

131. Morrison DJ, Preston T. Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism. *Gut Microbes*. (2016) 7:189–200. doi: 10.1080/19490976.2015.1134082

132. Kumar J, Kumar M, Gupta S, Ahmed V, Bhambi M, Pandey R, et al. An improved methodology to overcome key issues in human fecal metagenomic DNA extraction. *Genomics Proteomics Bioinf*. (2016) 14:371–8. doi: 10.1016/j.gpb.2016.06.002

133. Nivet C, Duhalde V, Beaurain M, Delobel P, Quelven I, Alric L. Fecal microbiota transplantation for refractory *Clostridioides difficile* infection is effective and well tolerated even in very old subjects: A real-life study. *J Nutrition Health Aging*. (2022) 26:290. doi: 10.1007/s12603-022-1756-1

134. Karimi M, Shirsalimi N, Hashempour Z, Salehi Omran H, Sedighi E, Beigi F, et al. Safety and efficacy of fecal microbiota transplantation (FMT) as a modern adjuvant therapy in various diseases and disorders: A comprehensive literature review. *Front Immunol*. (2024) 15:1439176. doi: 10.3389/fimmu.2024.1439176

135. Buonomo EL. The microbiota and immune response during Clostridium difficile infection. *Anaerobe*. (2016) 41:79. doi: 10.1016/j.anaerobe.2016.05.009

136. Fanizzi F, D'Amico F, Zanotelli Bombassaro I, Zilli A, Furfaro F, Parigi TL, et al. The role of fecal microbiota transplantation in IBD. *Microorganisms*. (2024) 12:1755. doi: 10.3390/microorganisms12091755

137. Zhang H, Jin Y, Yang H, Zhang Y, Ma H, Guan J, et al. Fecal microbiota transplantation ameliorates active ulcerative colitis by downregulating pro-inflammatory cytokines in mucosa and serum. *Front Microbiol*. (2022) 13:818111. doi: 10.3389/fmicb.2022.818111

138. Yang R, Chen Z, Cai J. Fecal microbiota transplantation: Emerging applications in autoimmune diseases. *J Autoimmun*. (2023) 141:103038. doi: 10.1016/j.jaut.2023.103038

139. Verma MK, Ahmed V, Gupta S, Kumar J, Pandey R, Mandhan V, et al. Functional metagenomics identifies novel genes ABCTPP, TMSRP1 and TLSRP1 among human gut enterotypes. *Sci Rep*. (2018) 8:1397. doi: 10.1038/s41598-018-19862-5

140. Adjele JJB, Devi P, Kumari P, Yadav A, Tchuenchieu Kamgain AD, Mouafo HT, et al. Exploring the influence of age and diet on gut microbiota development in children during the first 5 years: a study from Yaoundé, Cameroon. *Front Microbiol*. (2024) 15:1512111. doi: 10.3389/fmicb.2024.1512111

141. Makki K, Deehan EC, Walter J, Bäckhed F. The impact of dietary fiber on gut microbiota in host health and disease. *Cell Host Microbe*. (2018) 23:705–15. doi: 10.1016/j.chom.2018.05.012

142. Shin Y, Han S, Kwon J, Ju S, Choi TG, Kang I, et al. Roles of short-chain fatty acids in inflammatory bowel disease. *Nutrients*. (2023) 15:4466. doi: 10.3390/nu15204466

143. Aizawa E, Tsuji H, Asahara T, Takahashi T, Teraishi T, Yoshida S, et al. Possible association of Bifidobacterium and Lactobacillus in the gut microbiota of patients with major depressive disorder. *J Affect Disord*. (2016) 202:254–7. doi: 10.1016/j.jad.2016.05.038

144. Aziz T, Hussain N, Hameed Z, Lin L. Elucidating the role of diet in maintaining gut health to reduce the risk of obesity, cardiovascular and other age-related inflammatory diseases: Recent challenges and future recommendations. *Gut Microbes*. (2024) 16:2297864. doi: 10.1080/19490976.2023.2297864

145. McGrattan AM, McGuinness B, McKinley MC, Kee F, Passmore P, Woodside JV, et al. Diet and inflammation in cognitive ageing and Alzheimer's disease. *Curr Nutr Rep*. (2019) 8:53. doi: 10.1007/s13668-019-0271-4

146. Gyriki D, Nikolaidis C, Stavropoulou E, Bezirtzoglou I, Tsigalou C, Vradelis S, et al. Exploring the gut microbiome's role in inflammatory bowel disease: Insights and interventions. *J Personalized Med*. (2024) 14:507. doi: 10.3390/jpm14050507

147. Mackay CR. Diet, the gut microbiome, and autoimmune diseases. In: *The Autoimmune Diseases*, *Sixth Edition* . Cambridge, Massachusetts, USA: Academic Press (2019). p. 331–42. doi: 10.1016/B978-0-12-812102-3.00019-1

148. Gao K, Mu CL, Farzi A, Zhu WY. Tryptophan metabolism: A link between the gut microbiota and brain. *Adv Nutr (Bethesda Md.)*. (2020) 11:709–23. doi: 10.1093/advances/nmz127

149. Paeslack N, Mimmler M, Becker S, Gao Z, Khuu MP, Mann A, et al. Microbiota-derived tryptophan metabolites in vascular inflammation and cardiovascular disease. *Amino Acids*. (2022) 54:1339–56. doi: 10.1007/s00726-022-03161-5

150. Kumar J, Verma MK, Kumar T, Gupta S, Pandey R, Yadav M, et al. S9A serine protease engender antigenic gluten catabolic competence to the human gut microbe. *Indian J Microbiol*. (2018) 58:294–300. doi: 10.1007/s12088-018-0732-2

151. Zhou L, Zhang M, Wang Y, Dorfman RG, Liu H, Yu T, et al. Faecalibacterium prausnitzii Produces Butyrate to Maintain Th17/Treg Balance and to Ameliorate

Colorectal Colitis by Inhibiting Histone Deacetylase 1. *Inflamm Bowel Dis*. (2018) 24 (9):1926–40. doi: 10.1093/ibd/izy182

152. Mazzoccoli L, Liu B. Dendritic cells in shaping anti-tumor T cell response. *Cancers* (2023) 16(12):2211. doi: 10.3390/cancers16122211

153. Kuo WT, Odenwald MA, Turner JR, Zuo L. Tight junction proteins occludin and ZO-1 as regulators of epithelial proliferation and survival. *Ann N Y Acad Sci*. (2022) 1514(1):21–33. doi: 10.1111/nyas.14798

154. Ma J, Wei Q, Cheng X, Zhang J, Zhang Z, Su J. Potential role of gut microbes in the efficacy and toxicity of immune checkpoints inhibitors. *Front Pharmacol*. (2023) 14:1170591. doi: 10.3389/fphar.2023.1170591

155. Embgenbroich M, Burgdorf S. Current concepts of antigen cross-presentation. *Front Immunol*. (2018) 9:1643. doi: 10.3389/fimmu.2018.01643

156. Xia L, Zhu X, Wang Y, Lu S. The gut microbiota improves the efficacy of immune-checkpoint inhibitor immunotherapy against tumors: From association to cause and effect. *Cancer Lett*. (2024) 598:217123. doi: 10.1016/j.canlet.2024.217123

157. Zhang Y, Zhu X, Yu X, Novák P, Gui Q, Yin K. Enhancing intestinal barrier efficiency: A novel metabolic diseases therapy. *Front Nutr*. (2023) 10:1120168. doi: 10.3389/fnut.2023.1120168

158. Yadav M, Chauhan NS. Microbiome therapeutics: Exploring the present scenario and challenges. *Gastroenterol Rep*. (2021) 10:goab046. doi: 10.1093/gastro/goab046

159. Gopalakrishnan V, Helmink BA, Spencer CN, Reuben A, Wargo JA. The influence of the gut microbiome on cancer, immunity, and cancer immunotherapy. *Cancer Cell*. (2018) 33:570. doi: 10.1016/j.ccell.2018.03.015

160. Oh B, Boyle F, Pavlakis N, Clarke S, Eade T, Hruby G, et al. The gut microbiome and cancer immunotherapy: can we use the gut microbiome as a predictive biomarker for clinical response in cancer immunotherapy? *Cancers*. (2021) 13:4824. doi: 10.3390/cancers13194824

161. Chauhan NS. Microbiome, therapeutics application and epigenetics: conclusion and future prospective. In: *Comprehensive Gut Microbiota*. Elsevier (2022). p. 418–27. doi: 10.1016/B978-0-12-819265-8.00025-5

162. Kim KO, Gluck M. Fecal microbiota transplantation: An update on clinical practice. *Clin Endoscopy*. (2019) 52:137–43. doi: 10.5946/ce.2019.009

163. Mehta P, Yadav M, Ahmed V, Goyal K, Pandey R, Chauhan NS. Culture-independent exploration of the hypersaline ecosystem indicates the environment-specific microbiome evolution. *Front Microbiol*. (2021) 12:686549. doi: 10.3389/fmicb.2021.686549

164. Seida I, Al Shawaf M, Mahroum N. Fecal microbiota transplantation in autoimmune diseases - An extensive paper on a pathogenetic therapy. *Autoimmun Rev*. (2024) 23:103541. doi: 10.1016/j.autrev.2024.103541

165. Zhou Y, Zhang L, Li J, Li Y. A bibliometric analysis of global research on gut microbiota and host immune response: 2011–2021. *Front Microbiol*. (2022) 13:932197. doi: 10.3389/fmicb.2022.932197

166. Abdill RJ, Adamowicz EM, Blekhman R. Public human microbiome data are dominated by highly developed countries. *PloS Biol*. (2022) 20:e3001536. doi: 10.1371/journal.pbio.3001536

167. Allali I, Abotsi RE, Tow LA, Thabane L, Zar HJ, Mulder NM, et al. Human microbiota research in Africa: A systematic review reveals gaps and priorities for future research. *Microbiome*. (2021) 9:241. doi: 10.1186/s40168-021-01195-7

168. Kumar M, Kumar J, Chauhan NS. Metagenomics: A systemic approach to explore microbial world. In: Kalia VC, editor. *Microbial Factories*. Springer India, New Delhi (2015). p. 281–98. doi: 10.1007/978-81-322-2595-9_18

169. Chauhan NS. Metagenome analysis and interpretation. In: *Data Processing Handbook for Complex Biological Data Sources*. London, United Kingdom: Academic Press (2019). p. 139–60. doi: 10.1016/B978-0-12-816548-5.00010-1

170. Bertorello S, Cei F, Fink D, Niccolai E, Amedei A. The future exploring of gut microbiome-immunity interactions: From *in vivo*/vitro models to in silico innovations. *Microorganisms*. (2024) 12:1828. doi: 10.3390/microorganisms12091828

171. D'Urso F, Broccolo F. Applications of artificial intelligence in microbiome analysis and probiotic interventions—An overview and perspective based on the current state of the art. *Appl Sci*. (2024) 14:8627. doi: 10.3390/app14198627

172. Mukherjee P, Roy S, Ghosh D, Nandi SK. Role of animal models in biomedical research: A review. *Lab Anim Res*. (2022) 38:18. doi: 10.1186/s42826-022-00128-1

173. Kamel M, Aleya S, Alsubih M, Aleya L. Microbiome dynamics: A paradigm shift in combatting infectious diseases. *J Personalized Med*. (2024) 14:217. doi: 10.3390/jpm14020217

frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 08 May 2025
DOI 10.3389/fimmu.2025.1559480

Check for updates

OPEN ACCESS

EDITED BY
Nar Singh Chauhan,
Maharshi Dayanand University, India

REVIEWED BY
Monika Yadav,
Sanjay Ghodawat University, India
Asiya Nazir,
Abu Dhabi University, United Arab Emirates

*CORRESPONDENCE
Valentyn Oksenych
valentyn.oksenych@uib.no
Hamed Tahmasebi
h.tahmasebi87@yahoo.com

RECEIVED 12 January 2025
ACCEPTED 16 April 2025
PUBLISHED 08 May 2025

CITATION
Eslami M, Naderian R, Bahar A, Babaeizad A, Rezanavaz Gheshlagh S, Oksenych V and Tahmasebi H (2025) Microbiota as diagnostic biomarkers: advancing early cancer detection and personalized therapeutic approaches through microbiome profiling.
Front. Immunol. 16:1559480.
doi: 10.3389/fimmu.2025.1559480

COPYRIGHT
© 2025 Eslami, Naderian, Bahar, Babaeizad, Rezanavaz Gheshlagh, Oksenych and Tahmasebi. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Microbiota as diagnostic biomarkers: advancing early cancer detection and personalized therapeutic approaches through microbiome profiling

Majid Eslami[1], Ramtin Naderian[2], Aisa Bahar[3], Ali Babaeizad[4], Solaleh Rezanavaz Gheshlagh[4], Valentyn Oksenych[5]* and Hamed Tahmasebi[6]*

[1]Cancer Research Center, Semnan University of Medical Sciences, Semnan, Iran, [2]Clinical Research Development Unit, Kowsar Educational, Research and Therapeutic Hospital, Semnan University of Medical Sciences, Semnan, Iran, [3]Department of Biochemistry, School of Medicine, Iran University of Medical Sciences, Tehran, Iran, [4]Student Research Committee, Semnan University of Medical Sciences, Semnan, Iran, [5]Faculty of Medicine, University of Bergen, Bergen, Norway, [6]School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran

The important function of microbiota as therapeutic modulators and diagnostic biomarkers in cancer has been shown by recent developments in microbiome research. The intricate interplay between the gut microbiota and the development of cancer, especially in colorectal and breast cancers, emphasizes how microbial profiling may be used for precision treatment and early diagnosis. Important microbial signatures, including *Bacteroides fragilis* and *Fusobacterium nucleatum*, have been linked to the development and progression of cancer, providing important information on the processes behind carcinogenesis. Additionally, the influence of microbiota on the effectiveness of treatments such as immunotherapy and chemotherapy highlights its dual function in improving treatment outcomes and reducing side effects. To optimize treatment results, strategies including dietary changes and fecal microbiota transplantation (FMT) are being investigated. Despite these developments, there are still issues, such as individual variations in microbial composition, a lack of standardized procedures, and the requirement for reliable biomarkers. Integrating microbiome-based diagnostics with conventional approaches, such as liquid biopsies and machine learning algorithms, could revolutionize cancer detection and management. This review provides an overview of the current understanding of the host–microbe immunological axis and discusses emerging therapeutic strategies centered on microbiota modulation to support human health. Further research is essential to overcome existing challenges and fully realize the promise of microbiota-driven innovations in oncology.

KEYWORDS

biomarkers, cancer, microbiota, microbiome profiling, personalized medicine

1 Current research on microbiota in early cancer detection

Complex mechanisms that rely on the host's tolerance are essential for preserving the symbiosis between the host and microbes in the intestine, which can involve the physical barriers of the gut tissues or the release of antimicrobial peptides and antibodies (1). Nevertheless, this interdependent relationship relies on a delicate and ever-changing balance, and disruptions in the communication between the body and the gut bacteria have been linked to various long-lasting disease like cancer (2).

The relevance and novelty of this review article its in-depth analysis of microbiota as both diagnostic and therapeutic biomarkers in oncology, with a specific focus on colorectal cancer (CRC), breast cancer, lung cancer, pancreatic cancer and prostate cancer. The manuscript integrates recent advances in microbiome research, it highlights how microbial signatures could transform early cancer detection and personalized treatment strategies (3). In terms of relevance, the study emphasizes the critical need for non-invasive, accurate, and tailored diagnostic methods in cancer care. It leverages the growing body of evidence linking microbiota composition to carcinogenesis, treatment outcomes, and disease prognosis. By integrating microbial biomarkers with conventional diagnostic methodologies such as liquid biopsies and machine learning algorithms, the paper offers a forward-looking perspective for addressing diagnostic and therapeutic challenges in oncology. Regarding novelty, the manuscript provides valuable insights into the dual role of microbiota in both tumor development and therapeutic outcomes. It not only revisits well-known microbial links, such as the association of *Fusobacterium nucleatum* with CRC, but also examines underexplored areas like the influence of microbiota on immunotherapy and chemotherapy responses (4). Furthermore, it explores innovative concepts, such as fecal microbiota transplantation (FMT) as a strategy to boost treatment efficacy, positioning it as a breakthrough avenue in precision oncology. The discussion surrounding the integration of microbiome profiling with AI-based diagnostic technology further strengthens the manuscript's innovative contribution (5).

1.1 Contribution of the microbiota to cancer

Evidence is mounting to support the idea that the gut microbiome might affect the beginning and progression of tumor development. The gut microbiome is likely to impact the risk of cancer in multiple ways, including its involvement in cancer onset, advancement, dissemination, and reaction to treatments, as disturbances in host-microbiome equilibrium have been connected to several well-known cancer characteristics (1). Overall, these defining features consist of irregularities in the host's metabolism and immune response, disturbances in the body's equilibrium processes that result in persistent inflammation, and support for genetic instability and alterations (6). Building on this intricate relationship, the following section delves into how microbial exposure during early life shapes immune development and sustains homeostasis.

While a balanced microbiota contributes to immune homeostasis, its disruption—termed dysbiosis—has been increasingly recognized as a hallmark of several diseases. The microbiota significantly influences the development, progression, and treatment response across various malignancies, including breast, lung, pancreatic, and prostate cancers. The intricate relationship between microbial communities and oncogenic processes highlights the potential of microbiome profiling as a transformative tool in cancer diagnostics and therapeutics. In breast cancer, microbiota is recognized for its role at both local tissue and systemic levels in tumorigenesis. Research suggests that the breast microbiome modulates immune responses and metabolic processes (7). Beneficial bacterial strains, such as *Lactobacillus* and *Bifidobacterium*, are linked to anti-inflammatory effects that may provide protection against malignancy. Conversely, harmful species like *Escherichia coli* and *Staphylococcus aureus* can drive chronic inflammation and genomic instability (8).

The gut microbiota further influences breast cancer risk by regulating estrogen metabolism through the estrobolome, which manages systemic estrogen levels. Disruptions in the gut microbiome may elevate estrogen levels, promoting tumor growth. As a result, interventions targeting the microbiota such as probiotics, dietary modifications, and FMT are being explored as complementary strategies to enhance treatment success and minimize adverse effects (9). The research into xanthohumol anticancer properties complements the ongoing exploration of how dietary elements impact cancer progression and treatment via microbiome modulation. Similar to microbiome-targeted strategies like probiotics and dietary adjustments that enhance therapeutic outcomes, xanthohumol bioactive potential may play a role in anticancer effects by influencing gut microbiota and tumor microenvironments. This underscores the potential for a more personalized approach in oncology care (10). The research on hydrolyzed protein formulas and their impact on microbiota changes highlights the broader conversation about how dietary adjustments shape gut microbiome composition and contribute to overall health. Similar to how microbiome-focused nutritional approaches can boost the effectiveness of cancer therapies and strengthen immune functions, promoting microbial balance through personalized diets like hydrolyzed protein formulas underscores the promising role of microbiome-centered strategies in disease management and personalized treatment (11).

Lung cancer has also been linked to microbiota alterations, particularly through the gut-lung axis. The lung microbiome composition varies significantly between healthy individuals and those with lung cancer, with elevated levels of *Streptococcus* and *Veillonella* correlating with poor prognosis. Gut microbiota dysbiosis impacts immune surveillance by influencing inflammatory pathways and altering the production of short-chain fatty acids (SCFAs), crucial regulators of immune function (12). The effectiveness of immune checkpoint inhibitors (ICIs), such as anti-PD-1/PD-L1 therapies, has been shown to depend on gut microbiota composition. Certain bacteria, like *Akkermansia*

muciniphila and *Bifidobacterium*, are associated with better immunotherapy responses, while microbial imbalances contribute to resistance. Consequently, microbiome profiling has emerged as a potential biomarker to predict which patients may benefit most from immunotherapy (13).

Research conducted with *in vitro* and murine models has indicated that *Fusobacterium nucleatum* may stimulate the proliferation of CRC cells and boost tumor growth rates. A number of explanations have been suggested to account for these findings. Through the interaction of FadA adhesin, *F. nucleatum* boosts cell division by binding to E-cadherin on the surface of CRC cells and initiates the oncogenic Wnt/β-catenin signaling cascade (3, 14). In addition to structural components of microbes, their metabolic byproducts—such as SCFAs and tryptophan derivatives—play pivotal roles in modulating host immune responses. SCFAs, such as butyrate, propionate, and acetate, are produced through the fermentation of dietary fibers and support the differentiation of regulatory T cells (Tregs) by promoting histone acetylation and increasing FOXP3 expression. Butyrate also strengthens the integrity of the intestinal epithelial barrier and reduces the production of pro-inflammatory cytokines, thereby supporting mucosal immune balance. Furthermore, tryptophan-derived indole compounds activate the aryl hydrocarbon receptor (AhR) on innate lymphoid cells (ILC3), leading to enhanced secretion of interleukin-22 (IL-22), which is vital for protecting mucosal immunity. Bile acid metabolites further influence immune cell signaling through receptors like the farnesoid X receptor (FXR) and TGR5, affecting dendritic cell function and the inflammatory response. Recent research has associated disturbances in these metabolite pathways with cancer progression, autoimmune disorders, and resistance to immune checkpoint inhibitors, underscoring their importance as diagnostic biomarkers and potential therapeutic targets. *F. nucleatum* promotes the growth of CRC by using Fap2 adhesin to attach to a specific sugar pattern and changing the behavior of immune cells in the tumor microenvironment through interaction with the TIGIT receptor. Activation of autophagy in CRC cells by *F. nucleatum* through LPS and Toll-like receptor 4 contributes to chemotherapy resistance (15). It is clear from the previously mentioned instances that variations in the gut microbiome and its potential association with CRC could offer a promising explanation for certain unexplained patterns in the disease's occurrence. The causative relationship between bacterial traits in the gut microbiota and CRC is still unclear, and the evidence on the potential mechanisms involved is not strong enough to draw firm conclusions due to limitations (1, 16) (Figure 1).

For pancreatic cancer, particularly pancreatic ductal adenocarcinoma (PDAC), distinct microbiota signatures have been identified that impact disease progression and resistance to treatment. Salivary and gut microbiota analyses have identified increased levels of *Porphyromonas gingivalis* in pancreatic cancer patients suggest its use as a non-invasive biomarker for early detection. Chronic infections with *Helicobacter pylori* also appear to drive pancreatic carcinogenesis by sustaining inflammation and altering oncogenic pathways like Wnt/β-catenin signaling. In addition, the gut microbiota modulates chemotherapy response by affecting drug metabolism and immune system activity, highlighting the need for microbiome-based therapeutic innovations to improve treatment outcomes (17).

Prostate cancer progression has also been linked to microbiota dysbiosis, particularly regarding its effects on androgen metabolism. The gut microbiota plays a role in regulating systemic androgen levels, influencing tumor progression and the efficacy of androgen deprivation therapy (ADT) (18). Pro-inflammatory bacterial genera, such as *Bacteroides* and *Clostridium*, have been associated with disease progression, while protective effects have been linked to *Lactobacillus*. Microbial composition also affects the outcomes of hormonal therapy and radiotherapy, making it a promising target for therapeutic optimization. In summary, microbiota plays a crucial role across multiple cancer types by modulating oncogenic pathways, immune responses, and treatment efficacy. The growing body of microbiome research opens doors for early cancer

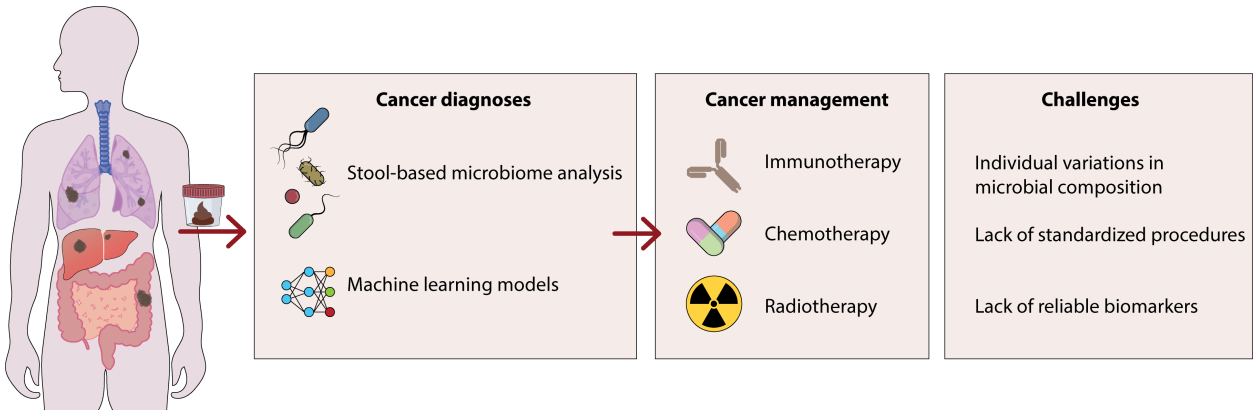


FIGURE 1
This figure shows the role of certain bacteria in the development of gastrointestinal cancer. These bacteria enhance the production of inflammatory cytokines including IL-6, iNOS and TGF-β through the activation of β-catenin/WNT signaling, which can lead to the development of colon cancers. Additionally, by producing DNA-damaging substances, these bacteria can lead to carcinogenic damage to the host DNA.

detection, personalized therapy, and innovative treatment modalities. Future research should focus on integrating microbiome profiling into traditional diagnostic techniques, standardizing methodologies, and exploring the mechanistic pathways through which microbiota influence cancer progression and treatment outcomes. By establishing a deeper understanding of microbiota-cancer interactions, precision oncology can advance toward more individualized and effective cancer management strategies (19). Manipulating the microbiota presents substantial therapeutic opportunities, especially for boosting the effectiveness of cancer immunotherapy and chemotherapy. Strategies such as fecal microbiota transplantation (FMT), tailored probiotics, and diet alteration have demonstrated potential benefits. For instance, transferring microbiota from immunotherapy responders to non-responders has enhanced the effectiveness of checkpoint inhibitors in mouse models. Furthermore, particular probiotics, including *Akkermansia muciniphila* and *Bifidobacterium longum*, have been linked to better clinical results in patients undergoing PD-1 blockade treatment. Given these immunomodulatory effects, therapeutic modulation of the microbiota—through probiotics, prebiotics, or FMT—has gained considerable attention in clinical research.

2 Microbiota and cancer screening

2.1 Colorectal cancer screening and gut microbiome

CRC remains a leading cause of cancer-related deaths globally, with early detection playing a critical role in improving patient outcomes. Conventional screening methods such as colonoscopy, fecal occult blood tests (FOBT), and fecal immunochemical tests (FIT) are widely used to identify precancerous lesions and tumors. Despite their importance, these approaches come with notable drawbacks, including invasiveness, patient discomfort, and variability in sensitivity. Recent advancements in microbiome research suggest that analyzing gut microbial profiles could offer a complementary, less invasive tool for CRC screening (20).

The gut microbiome has emerged as a key player in the initiation and progression of CRC. Dysbiosis, or an imbalance in the microbial composition of the gut, has been associated with chronic inflammation, DNA damage, and immune system dysfunction factors that contribute to colorectal tumor development (21). Several bacterial species have been identified as potential biomarkers for CRC. *Fusobacterium nucleatum*, for example, fosters tumor growth by activating β-catenin signaling, modulating the immune response, and increasing resistance to chemotherapy. *Bacteroides fragilis* produces enterotoxins that can induce DNA damage and inflammation, elevating CRC risk. *Escherichia coli* (pks+ strain) harbors the pks pathogenicity island, responsible for producing colibactin, a compound linked to genomic instability. *Peptostreptococcus anaerobius* contributes to tumor progression by altering the tumor microenvironment, promoting cell proliferation, and triggering inflammation. These microbial markers not only provide insight into CRC development but also hold promise for enhancing early detection strategies (22).

Given the strong link between gut microbes and CRC, several microbiome-based diagnostic tools are under investigation. One promising approach is stool microbiome analysis a non-invasive method that identifies microbial signatures associated with CRC and demonstrates greater sensitivity and specificity than traditional fecal blood tests. This technique can detect key bacterial markers like *Fusobacterium nucleatum* and *Bacteroides fragilis* with precision. Another innovative option is liquid biopsy coupled with circulating microbial DNA (cmDNA) analysis, which involves detecting microbial DNA fragments in blood samples. This method allows for real-time monitoring of CRC progression and treatment efficacy while potentially being combined with circulating tumor DNA (ctDNA) analysis to enhance diagnostic accuracy. Additionally, the integration of machine learning with microbiome-based predictive models is revolutionizing CRC diagnostics (23).

2.2 Breast cancer screening and microbiota

Traditional breast cancer screening methods, including mammography, ultrasound, and MRI, dominate current practice. However, growing research highlights the potential of microbiota signatures as reliable biomarkers for early detection and risk categorization. Distinct microbial profiles in breast tissue, nipple aspirate fluid, and systemic circulation have been shown to differentiate between healthy individuals and those with breast cancer (24). Patients with breast cancer often exhibit microbial imbalances, marked by reduced levels of beneficial bacteria like *Lactobacillus* and *Bifidobacterium*, alongside an overrepresentation of pro-inflammatory and potentially pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and *Fusobacterium nucleatum*. These microbial disruptions may drive carcinogenesis by development chronic inflammation, oxidative stress, and immune system evasion (25). Additionally, the gut microbiota influences estrogen metabolism through the estrobolome, which can elevate systemic estrogen levels and heighten the risk of hormone receptor-positive breast cancer. Circulating microbial DNA (cmDNA) is emerging as a promising biomarker for non-invasive breast cancer detection. Metagenomic analyses of breast tissue and nipple aspirate fluid have identified unique microbial patterns that could signal malignancy at its earliest stages. Also, computational algorithms that combine microbiome profiles with clinical data are being refined to enhance diagnostic accuracy. There is also growing interest in microbiome-focused preventive strategies, such as probiotics and diet modifications, to reduce breast cancer risk (26).

2.3 Lung cancer screening and the respiratory microbiome

Low-dose computed tomography (LDCT) remains the cornerstone of lung cancer screening, but advancements in

microbiome research suggest a complementary avenue for improved diagnostic precision. Alterations in the lung microbiome, heavily influenced by the gut-lung axis, are evident in lung cancer patients. Elevated occurrences of microbial genera such as *Streptococcus*, *Veillonella*, and *Prevotella* have been detected in the sputum and bronchoalveolar lavage fluid of affected individuals. These shifts offer distinct microbial markers for early disease identification. Gut microbiota imbalances also play a role in modulating systemic immune responses and the tumor microenvironment, impacting lung cancer progression. Liquid biopsy techniques that analyze microbial DNA from blood or respiratory secretions are emerging as non-invasive options to optimize screening efforts (25).

2.4 Pancreatic cancer screening and the oral-gut microbiome axis

The early detection of pancreatic cancer remains a significant challenge, primarily due to the lack of highly specific biomarkers. However, disruptions in the oral-gut microbiome axis have emerged as a potential diagnostic way, showing strong associations with pancreatic ductal adenocarcinoma (PDAC). Elevated levels of microbial species such as *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Fusobacterium nucleatum* have been identified in saliva samples from pancreatic cancer patients (27). Additionally, fecal microbiota analyses have highlighted an altered *Bacteroides*-to-*Firmicutes* ratio in these individuals. These microbial patterns could play a transformative role in advancing non-invasive screening methods for pancreatic cancer. Recent research suggests that bacterial metabolites, including lipopolysaccharides (LPS) and short-chain fatty acids (SCFAs), may contribute to the carcinogenesis of the pancreas by promoting chronic inflammation and immune evasion. The oral-gut microbiome axis also influences the tumor microenvironment by modulating pathways such as nuclear factor-kappa B (NF-κB) and Toll-like receptor (TLR) signaling, both of which are key promoters of tumor progression in PDAC. Salivary microbiota profiling, integrated with next-generation sequencing (NGS) and metagenomic analysis, shows promise for improving pancreatic cancer screening accuracy in terms of both sensitivity and specificity (28).

2.5 Prostate cancer screening and gut microbiota

The gut microbiota has also been closely linked to the development and diagnosis of prostate cancer. Dysbiosis, characterized by elevated levels of bacteria such as *Bacteroides*, *Clostridium*, and *Escherichia coli*, has been consistently observed in prostate cancer patients, alongside a decline in beneficial microbes like *Lactobacillus* and *Faecalibacterium*. Evaluating stool and urine microbiomes could complement traditional screening tools, such as prostate-specific antigen (PSA) testing and multiparametric MRI, offering improved diagnostic precision and risk assessment capabilities (29). Emerging evidence suggests the role of gut microbiota in regulating androgen metabolism and systemic inflammation, two critical factors in prostate cancer pathophysiology. The gut microbiome influences androgen bioavailability by modulating steroid hormone metabolism, potentially driving the initiation and progression of prostate tumors. Moreover, microbial-derived metabolites such as secondary bile acids and trimethylamine-N-oxide (TMAO) have been implicated in inflammatory pathways that may fuel tumorigenesis. Advancements in machine learning and artificial intelligence (AI) have facilitated the development of predictive models integrating microbiome data with conventional clinical markers. These models aim to enhance risk stratification and diagnostic accuracy. Future research should focus on microbiome-based interventions, including prebiotics, probiotics, and FMT, as promising approaches to address gut dysbiosis and improve prostate cancer prevention and treatment effectiveness (30).

3 Advancements in microbiome profiling for cancer therapy

3.1 Chemotherapy

Various research projects have explored how systemic cancer treatment impacts the makeup of gut bacteria in various cancer forms. Different types of cancer, including those affecting the gastrointestinal system and other areas, were examined in these studies, along with various chemotherapy treatments and settings (3). Systemic chemotherapy led to a rise in *Faecalibacterium prausnitzii* levels in midgut NET patients, as noted in those with neuroendocrine tumors. Even though this study utilized FISH to target specific species, recent articles employ sequencing-based methods to thoroughly analyze bacterial species composition (31).

The intestinal microbiota composition underwent a notable transformation following a five-day high-dose chemotherapy regimen for bone marrow transplantation, as revealed by sequencing the 16S rRNA gene, according to studies (14). A notable decrease was noted in the number of different microbe's present, the estimated variety of microbes, and the diversity of microbes, suggesting a decrease in α-diversity as a result of chemotherapy. Therefore, it can be inferred that intensive chemotherapy led to a significant reduction in the variety of microorganisms and altered the composition of the microbial population (32). *Bacteroidetes* and *Proteobacteria* showed higher numbers at the phylum level, whereas *Firmicutes* and *Actinobacteria* displayed lower levels. Post-chemotherapy samples exhibited significantly higher levels of *Bacteroides* and *Escherichia* on the genus level when compared to pre-chemotherapy samples (33).

Additionally, there was a notable change from Gram-positive to Gram-negative bacteria observed in patients undergoing chemotherapy. It is worth noting that this research also

mentioned that a few fewer common types of bacteria emerged following chemotherapy. Following chemotherapy, there was a reduction in *Blautia*, *Faecalibacterium*, *Roseburia*, and *Bifidobacterium*, which are known to promote health and have anti-inflammatory properties (3, 34). During induction chemotherapy, AML patients experienced significant alterations in their intestinal microbiota composition, as noted by Galloway-Peña et al. Through the analysis of the 16S rRNA gene, a notable decline in the diversity of microorganisms was detected, along with a reduction in the presence of the anaerobic bacteria *Blautia*. Conversely, chemotherapy led to a rise in the levels of *Lactobacillus* (35). One intriguing result of chemotherapy was the heightened occurrence of intestinal domination, where over 30% of intestinal bacteria are associated with a particular taxon. Opportunistic pathogenic bacteria, which are recognized for causing bacteremia, were responsible for 50% of the domination occurrences post-chemotherapy (32, 36). Moreover, a rise in opportunistic pathogenic genera was linked to elevated temporal variability within individual patients. Therefore, it was determined that the therapy caused a change towards a microbial composition closely resembling that of the gut microbiome in individuals without health issues. Youssef et al. (2018) obtained stool samples from individuals undergoing treatment for gastrointestinal tumors and healthy participants, contradicting previous studies that showed a decline in gut microbiota rather than an improvement. Tumors found in the stomach, small intestine, or rectum are classified as gastrointestinal neoplasms (37).

3.2 Immunotherapy

Certain research papers in human clinical trials were discovered that discussed shifts in the human gut microbiome while undergoing immunotherapy and using longitudinal sampling. Treatment with anti-PD-1, anti-CTLA-4, or interferon alpha-2b was given to patients diagnosed with metastatic or unresectable melanoma, renal cell carcinoma (RCC), non-small cell lung cancer (NSCLC), or neuroendocrine tumors (NET) (2, 16).

Moreover, there were no alterations in Shannon and Simpson α-diversity indices while undergoing ipilimumab therapy, indicating that the gut microbiota remained unaffected by the treatment. On the other hand, it is essential to point out that the quantity of fecal samples studied dropped to four over time. Although ipilimumab did not have a direct impact on the gut microbiota in this research, alterations in the gut microbiota were noted when colitis developed while on ipilimumab treatment. Hence, stool samples from seven colitis patients were obtained and juxtaposed with their original samples. Notable discrepancies in microbiota structure were detected among different family and genus categories (16).

The occurrence of ipilimumab-induced colitis in metastatic melanoma patients was linked to a notable decrease in the relative abundance of seven key genera (*Lachnospiracea incertae sedis*, *Ruminococcus, Clostridium* IV, *Blautia, Pseudoflavonifracto, Eubacterium*, unclassified and *Lachnospiraceae*). Moreover, a significant reduction in other bacteria, predominantly *Firmicutes*, was observed. A correlation was found between the microbiome in the human gut and the occurrence of colitis from systemic cancer therapy, which was linked to reduced α-diversity. Nevertheless, the disruptions in the microbes were probably due to the colitis rather than the treatment (6, 16).

Surprisingly, studies have demonstrated that mouse tumors treated with ipilimumab exhibit improved responses to FMT from cluster C individuals rather than cluster B individuals. It is suggested that ipilimumab could potentially transform the enterotype to the more advantageous cluster C. In addition to the research conducted by Routy, Chaput, and Vetizou, three studies carried out extended monitoring of stool samples from a small group of melanoma patients undergoing immunotherapy (16) (Figure 2).

3.3 Hormonal therapy

There are other research projects that have looked into alterations in the human gut microbiome caused by hormonal treatment. Fecal samples were obtained from patients with neuroendocrine tumors who were undergoing treatment with somatostatin analogs, as documented by Puhalla et al. The abundance of certain bacterial groups in these subjects remained unchanged despite the administration of somatostatin analogs (38).

In a study by Sfanos et al. (2018), the intestinal microbiota of prostate cancer patients receiving androgen axis-targeted treatments was compared to those who were not on hormonal medication. In the realm of androgen axis-targeted therapies, treatment methods like gonadotropin-releasing hormone (GNRH) or therapies targeting the androgen receptor axis were utilized. Healthy controls, benign tumor patients, and untreated prostate cancer patients were part of the group not taking hormonal medication. Prostate cancer patients, whether on hormonal medication or not, exhibited similar α-diversity levels according to this study. Compared to the GNRH group and the group without hormonal medication, the ATT group displayed the lowest β-diversity (39). The findings suggest that the gut microbiota of patients on ATT was more alike compared to those not taking any medication. Moreover, ATT was linked to a reduction in β-diversity. There were notable changes in the types and amounts of bacteria in the stool samples of men who were on oral ATT in comparison to those not using any medication. Microbiome's role in cancer therapy shown in Table 1.

4 Linking microbiota to cancer detection and progression

4.1 The role of the microbiome in pancreatic ductal adenocarcinoma pathogenesis

Considering the established connection between infectious agents, chronic inflammation, and cancer, it is not far-fetched to think of the microbiome as a factor in PDAC pathogenesis.

10.3389/fimmu.2025.1559480

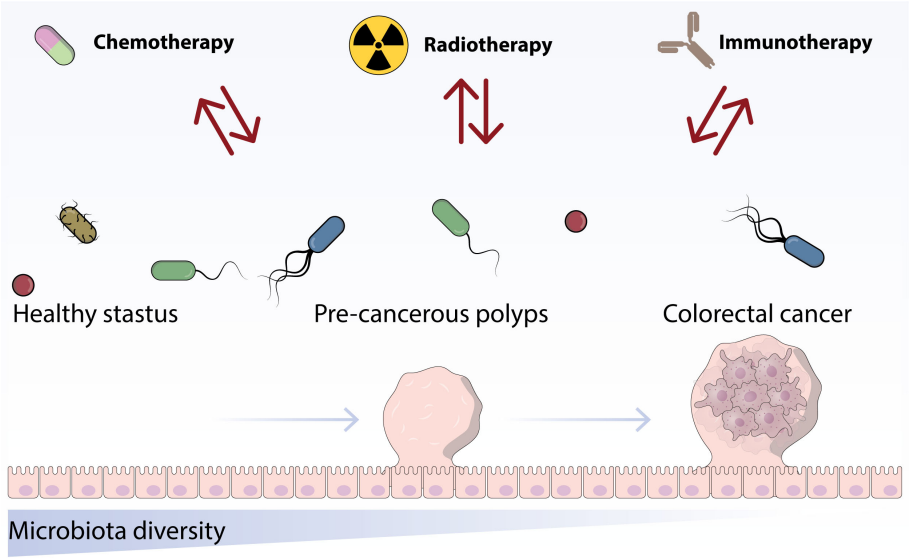


FIGURE 2
This figure indicates the role of chemotherapy and immunotherapy treatments on the structure and proportion of the established microbiota.

Additionally, the absence of such microbes in a normal pancreas suggests that this idea could be valid (40, 41). A significant portion of cancer diagnoses, exceeding 16%, can be attributed to pathogen infections, with detailed descriptions available for most of the mechanisms. Eleven different types of "oncomicrobes" have been identified as causing cancer. Nevertheless, certain microorganisms implicated in the development of different types of cancer, like through the disruption of the Wnt/β-catenin signaling pathway, were similarly identified in PDAC, such as *Clostridium, Bacteroides*, and *E. coli*. Alterations in the Wnt/β-catenin signaling pathway are prominent in PDAC, presenting advantageous prospects for studying the microbiome's involvement in PDAC tumor formation (40).

TABLE 1 Microbiome's role in cancer therapy.

Therapy Type	Microbiome Influence	Example Microbes	Potential Applications
Chemotherapy	Affects drug metabolism and resistance	*Fusobacterium nucleatum, Enterococcus hirae*	Enhancing drug response, reducing toxicity
Immunotherapy	Modulates immune checkpoint responses	*Akkermansia muciniphila, Bifidobacterium*	Predicting response to immune checkpoint inhibitors (ICIs)
Radiotherapy	Alters inflammation and tissue repair	*Faecalibacterium prausnitzii, Bacteroidetes*	Reducing radiation-induced toxicity
Hormonal Therapy	Impacts androgen and estrogen metabolism	*Lactobacillus, Clostridium*	Enhancing hormone therapy efficacy
FMT	Restores beneficial microbiota	Diverse gut microbiota	Improving immunotherapy and chemotherapy outcomes

Beyond the triggering of oncogenic signaling, there are other viable pathways by which microbes could be involved in the development and maintenance of PDAC. Mutagenesis can lead to direct cancer-causing effects. Similarly, to oncogenic signaling, there is a parallel that supports the idea that microbes could have a hand in PDAC (40). The speculation is that *F. nucleatum* contributes to the carcinogenic and oncogenic processes of oral squamous cell carcinoma cells by activating the Ku70/p53 pathway and causing DNA double strand breaks. Indirectly, chronic inflammation could contribute to carcinogenic effects. Research has indicated a link between the development of prostate cancer and the connection between the oral microbiome, and periodontitis (40). According to another investigation, lipopolysaccharide (LPS), which is also known as microbe-associated molecular patterns, plays a role in promoting PC. In colonic cancer, microbial metabolites like short-chain fatty acids (SCFA) have been proven to transmit indirect oncogenic effects. It is crucial to reconsider the interaction between the microbiome and the immune system in terms of reshaping the immunogenic TME. Microbial infiltration has been identified in research as a possible cause of cancer development through the alteration of the immune system (40–42).

4.2 The impact of microbiome on cancer diagnostics

Detecting cancer at an early stage has been proven to reduce the chances of negative results and increase the chances of successful

therapy. Different levels of invasiveness are seen in the current techniques used for cancer diagnosis, and a significant number of diagnoses continue to depend on invasive biopsies for verification. Research efforts have been directed towards identifying less intrusive approaches that can still ensure a high level of sensitivity (such as in cancer instances) and specificity (excluding non-cancer instances). Current research has emphasized the use of microbiomes for diagnosis, especially in cancer cases, where samples such as saliva, stool, and plasma can be collected more conveniently than other diagnostic approaches (15).

4.2.1 Salivary microbiota

The analysis of the bacteria in saliva, known as salivary microbiota, is a highly studied diagnostic technique that can be conducted using noninvasive samples of saliva. Cancers in various parts of the body, such as the mouth, pancreas, and lungs, have been linked to the bacteria in saliva. The salivary microbiome of individuals with oral squamous cell carcinoma (OSCC) contains a unique mix of *Capnocytophaga gingivalis*, *Prevotella melaninogenica*, and *Streptococcus mitis*, which can be utilized as a diagnostic marker with an accuracy of 80% sensitivity and 83% specificity in contrast to healthy controls (43). Despite being considered commensal organisms, these three bacterial species were found to be notably increased in cancer patients. Recent research has suggested that *C. gingivalis* may have a role in promoting tumors, while the mechanisms for the other two species are not yet understood. The combination of certain oral bacteria, such as *Neisseria elongata* and *S. mitis*, can be used as a diagnostic tool for pancreatic cancer, distinguishing it from pancreatitis with good accuracy. Another study, more recent in nature, examined saliva samples from PDAC patients using advanced sequencing techniques, finding no significant variations in previously identified oral microbes like *P. gingivalis*, *N. elongate*, and *S. mitis*, raising doubts about their utility as diagnostic indicators (44).

One significant drawback of using the salivary microbiota for diagnosis is the uncertainty surrounding whether variations in microbes are a consequence or trigger of cancer. To clarify, these bacteria could exhibit a change in response to cancer only after its development, making them less reliable for detecting cancer in its early stages, although more research is necessary to unravel this link. Furthermore, the composition of the oral microbiome can vary significantly depending on the age, race/ethnicity, dietary habits, and lifestyle choices of individuals, emphasizing the importance of studying larger and more diverse patient cohorts to ensure precise diagnostic accuracy across all demographics (45).

4.2.2 Fecal microbiota

The fecal microbiome, much like the oral microbiome, has been the subject of in-depth research and can be explored through noninvasive collection methods involving stool samples. Most studies focusing on using the fecal microbiome as a diagnostic indicator have primarily centered on CRC (46). The fecal microbiome, much like the oral microbiome, has been the subject of in-depth research and can be explored through noninvasive collection methods involving stool samples. The main focus of research on using the fecal microbiome as a diagnostic tool has been on CRC (3).

In addition to this, the fecal microbiome has been employed to examine biomarkers for different forms of cancer, albeit to a lesser extent. In the gut microbiome of lung cancer patients, a study using 16S rRNA analysis revealed a reduction in certain bacterial genera and an increase in 11 bacterial genera. Further investigation is necessary to uncover the rationale behind why these categories might act as gut markers for lung cancer and to validate this concept with larger groups of patients (45).

4.2.3 Plasma cell-free DNA

A new trend has emerged in the field of cancer diagnosis, shifting the focus towards the utilization of cell-free DNA from human plasma, departing from the traditional methods used in microbially-rich environments. Liquid biopsies, or cell-free DNA tests, have experienced a rapid surge in application, transitioning from their initial role in prenatal examinations to broader health issue assessments (47).

Nevertheless, these examinations concentrate on the use of circulating tumor DNA found in the bloodstream. Recent studies have delved into the presence of microbial DNA sequences in plasma as a potential diagnostic tool, following earlier research that established a link between microbes and cancer. Microbial DNA was identified in the plasma of three early-onset breast cancer patients through next-generation sequencing, with the majority coming from bacteria, along with contributions from fungi and viruses (33, 45). The bacterial DNA detected in the blood of cancer patients differed significantly from that of the control group, showing higher levels of *Pseudomonas* spp. and *Sphingomonas* spp. in cancer patients, while *Acinetobacter* spp. were more prevalent in the controls. The authors suggest that more extensive studies with a larger sample size are necessary before any definitive conclusions can be made. Further investigations have revealed alterations in microbial patterns in the bloodstream of cancer patients without genetic mutations, with researchers noting the potential presence of microbial sequences in various blood components apart from plasma (48).

Even though there have been advancements in utilizing various microbiomes for cancer detection in experiments, it is probable that these microbiome-centered diagnostic strategies will be employed in conjunction with other established methods like imaging or biopsies, rather than as standalone diagnostic tools. Detecting cancer in its early stages and reducing the need for invasive tests could be significantly enhanced by these techniques. It is anticipated that diagnostic strategies centered on the microbiome could emerge as autonomous tools as the scientific community delves deeper into the intricate connections between microbiomes and cancer (48). Diagnostic methods involving microbiota shown in Table 2.

5 Microbiota as biomarkers for early cancer diagnosis

Finding the perfect biomarkers for various diseases, such as certain forms of cancer, is seen as a challenging endeavor. Most current sampling techniques for cancer tissues do not have the

TABLE 2 Diagnostic methods involving microbiota.

Diagnostic Method	Sample Type	Cancer Type	Sensitivity & Specificity	Advantages	Limitations
Stool Microbiome Analysis	Stool	Colorectal, Pancreatic, Prostate	High for CRC (≥90%)	Non-invasive, easy collection	Affected by diet and lifestyle
Salivary Microbiome Profiling	Saliva	Pancreatic, Oral, Lung	Moderate	Easy to collect, low-cost	Microbiome varies over time
Blood Microbial DNA (cmDNA)	Plasma	Breast, Lung, Colorectal	Emerging	Minimally invasive	Low microbial DNA concentration
Bronchoalveolar Lavage (BAL) Microbiome	BAL Fluid	Lung	Moderate	Direct tumor site sampling	Invasive procedure required
Urinary Microbiome Analysis	Urine	Prostate	Emerging	Non-invasive	Requires further validation

capability to distinguish patients who will not respond to therapy and face challenges in accurately categorizing cancer subtypes because of the variability in tumors both within and among patients. It is important for a biomarker to be readily quantifiable, non-intrusive, and inexpensive (3, 44).

The human microbiome, specifically the gut microbiome, offers a non-intrusive method for pinpointing disease biomarkers that have the potential to detect various illnesses in their initial phases. Also, the utilization of microbiome-related biomarkers, in conjunction with clinical data and other biomarkers, can boost the accuracy of disease classification. To illustrate, some microbes are recognized for their contribution to the shift from adenoma to carcinoma in certain cancers, such as CRC. Microorganisms like these can serve as useful indicators for the effectiveness of treatments for CRC (3, 36, 47).

Besides microbiome-derived biomarkers, mast cells (MCs), microRNAs (miRNAs), imaging methods, and machine-learning models are also gaining attention as non-invasive biomarkers for disease diagnosis and prognosis, offering a glimpse into the future of precision medicine. Occasionally, the human microbiota interacts with different genetic or chemical markers. Changes in small RNA patterns found in feces of individuals with CRC are indicative of the types of bacteria present in their stool samples. Therefore, the utilization of several interlinked biomarkers in a network could enhance the efficiency of current biomarkers (46, 49). Infectious diseases were replaced by cancer and cardiovascular diseases as the leading causes of death on a global scale, due to an epidemiological shift. Currently, there are 19 million new cancer cases and 10 million fatalities globally, with an expected 50% surge in the next 20 years, especially in countries with low levels of human development (49, 50).

5.1 Colorectal cancer

The microbiome has emerged as an essential biomarker for cancer detection, prognosis, and treatment response across multiple types, including colorectal, breast, lung, pancreatic, and prostate cancers. In colorectal cancer, specific microbial signatures such as *Fusobacterium nucleatum*, *Bacteroides fragilis*, and *Escherichia coli* containing the pks pathogenicity island are linked to tumorigenesis through mechanisms like chronic inflammation, DNA damage, and immune modulation (51). Among them, *Fusobacterium nucleatum* is extensively studied for its role in activating the β-catenin signaling pathway and promoting immune evasion, making it a highly promising non-invasive biomarker detectable in stool and tissue samples. Recent advancements in microbiome-based stool tests, when combined with standard methods like the fecal immunochemical test (FIT), have notably improved diagnostic precision. Moreover, circulating microbial DNA (cmDNA) in plasma is being explored as a liquid biopsy biomarker for colorectal cancer. The microbiome also significantly influences treatment outcomes, with *Fusobacterium nucleatum* enrichment linked to resistance against fluoropyrimidine-based chemotherapy. In contrast, beneficial microbes such as *Akkermansia muciniphila* are associated with better responses to immune checkpoint inhibitors (52, 53).

5.2 Breast cancer

In breast cancer, the microbiome contributes to tumor development through both local and systemic mechanisms, particularly its impact on estrogen metabolism via the gut-associated estrobolome. Research findings indicate that breast cancer patients exhibit distinct microbial compositions, including reduced levels of anti-inflammatory species like *Lactobacillus* and *Bifidobacterium*, alongside elevated levels of pro-inflammatory and oncogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, and *Fusobacterium nucleatum* (54). This disruption of gut microbial diversity alters estrogen metabolism, increasing systemic estrogen levels and elevating the risk of hormone receptor-positive breast cancer. Metagenomic analyses of breast tissue, nipple aspirate fluid, and blood samples have successfully identified microbial DNA that differentiates malignant from benign conditions, supporting their potential as biomarkers for early cancer detection. Additionally, gut microbiota composition appears to modulate the efficacy of endocrine therapies such as selective estrogen receptor modulators and aromatase inhibitors where higher microbial diversity correlates with more effective drug metabolism and treatment responses (55).

5.3 Lung cancer

In lung cancer, research into the gut-lung axis has highlighted its role in shaping immune responses and tumor dynamics. Patients with lung cancer exhibit pronounced alterations in both pulmonary and gut microbiota. For example, elevated levels of species such as *Streptococcus*, *Veillonella*, and *Prevotella* in bronchoalveolar lavage fluid and sputum have been proposed as potential diagnostic markers. Gut microbiota also influences the efficacy of immune checkpoint inhibitors like PD-1/PD-L1 therapies; an abundance of beneficial microbes such as *Akkermansia muciniphila* and *Bifidobacterium* enhances immunotherapy responses, whereas dysbiosis involving species like *Clostridium* and *Ruminococcus* correlates with resistance. Additionally, circulating microbial DNA in plasma is being investigated as a non-invasive diagnostic tool for lung cancer. Using machine learning algorithms to integrate microbiome data has further improved diagnostic accuracy (56).

5.4 Pancreatic cancer

In pancreatic cancer, the microbiome significantly influences disease development and progression. Microbial signatures from oral, gut, and pancreatic tumor microbiota show promise as diagnostic biomarkers. Increased levels of *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, and *Fusobacterium nucleatum* in saliva samples are associated with pancreatic ductal adenocarcinoma (PDAC), pointing to an oral-gut microbiome axis involved in tumorigenesis (27). Gut dysbiosis characterized by a higher *Bacteroides*-to-*Firmicutes* ratio has also been linked to pancreatic cancer progression. Microbial metabolites such as lipopolysaccharides and short-chain fatty acids contribute to carcinogenesis by driving chronic inflammation and immune evasion. Importantly, certain bacteria like *Gammaproteobacteria* produce enzymes capable of inactivating gemcitabine, a primary chemotherapy drug for pancreatic cancer, thereby contributing to drug resistance. Strategies aimed at modifying the microbiota such as antibiotics or FMT are being explored to enhance chemotherapy effectiveness (57).

5.5 Prostate cancer

The gut microbiome has been increasingly implicated in the progression of prostate cancer through its effects on androgen metabolism and systemic inflammation. Individuals with prostate cancer exhibit distinct microbial alterations in stool and urine samples, characterized by an overabundance of *Bacteroides*, *Clostridium*, and *Escherichia coli* microbes linked to chronic inflammation and tumor growth alongside a reduction in beneficial species such as *Lactobacillus* and *Faecalibacterium*. Current research indicates that the composition of the gut microbiota can influence the effectiveness of androgen deprivation therapy (ADT), with certain bacteria capable of metabolizing androgens, potentially impacting treatment outcomes. Additionally, microbiome-targeted strategies, such as probiotics and dietary interventions, are being explored as promising therapeutic approaches to enhance prostate cancer management (58). Microbial biomarkers across cancer types shown in Table 3.

6 Personalized cancer treatment through microbiome insight

The human microbiome plays a pivotal role in shaping how individuals respond to cancer treatments, influencing drug metabolism, immune system activity, and tumor behavior. While traditional cancer therapies often adhere to standardized approaches, research increasingly highlights the microbiome's capacity to significantly affect treatment outcomes. By integrating microbiome insights, researchers and clinicians aim to create personalized cancer therapies that not only improve effectiveness but also reduce side effects (59). Every person has a unique microbial ecosystem that interacts intricately with their immune system and metabolic processes, shaping their response to cancer treatments. Gut bacteria, for instance, affect the absorption and breakdown of chemotherapy drugs. Some microbes produce enzymes that degrade or deactivate these drugs, leading to treatment resistance. In CRC, *Fusobacterium nucleatum* has been

TABLE 3 Microbial biomarkers across cancer types.

Cancer Type	Key Microbial Biomarkers	Role in Carcinogenesis	Detection Method
Colorectal Cancer	*Fusobacterium nucleatum, Bacteroides fragilis, Escherichia coli (pks+)*	Promotes inflammation, immune evasion, and DNA damage	Stool microbiome analysis, liquid biopsy
Breast Cancer	*Lactobacillus, Bifidobacterium, Escherichia coli, Staphylococcus aureus*	Alters estrogen metabolism and immune modulation	Nipple aspirate fluid, blood microbiome
Lung Cancer	*Streptococcus, Veillonella, Prevotella*	Affects immune surveillance and inflammation	Bronchoalveolar lavage, sputum analysis
Pancreatic Cancer	*Porphyromonas gingivalis, Fusobacterium nucleatum*	Induces chronic inflammation and immune suppression	Salivary microbiome, stool analysis
Prostate Cancer	*Bacteroides, Clostridium, Lactobacillus*	Influences androgen metabolism and immune modulation	Urine microbiome, stool analysis

identified as a contributor to chemoresistance, while *Akkermansia muciniphila* has shown promise in boosting chemotherapy effectiveness by enhancing immune activation. Similarly, the success of ICIs like PD-1 and CTLA-4 inhibitors is linked to specific gut bacteria. Patients with high levels of *Bifidobacterium* and *Faecalibacterium prausnitzii* often exhibit improved responses to immunotherapy, whereas those suffering from dysbiosis may experience diminished treatment benefits. In the case of radiotherapy, certain beneficial microbes such as species of *Lactobacillus* help reduce radiation-induced inflammation and maintain gut barrier integrity, mitigating toxicity during treatment (60).

Beyond observing the microbiome's impact, researchers are now investigating ways to actively modify it to optimize cancer treatment outcomes. Emerging innovations include synthetic probiotics engineered to deliver therapeutic compounds, prebiotic-based solutions aimed at fostering beneficial microbial populations, and CRISPR-powered technologies to alter bacterial genes involved in drug metabolism. Live biotherapeutic products (LBPs) genetically modified microbes designed to enhance immune modulation and chemotherapy responses are also under development. These approaches signal a transformative shift toward incorporating microbiome engineering as a key component of precision oncology (25). Advancements in sequencing technologies, such as metagenomic and shotgun sequencing, now enable real-time tracking of a patient's microbiome during treatment. This development allows clinicians to monitor microbial dynamics, predict potential drug resistance, and tailor personalized interventions as needed. Additionally, machine learning tools are being integrated with microbiome datasets to identify predictive biomarkers, categorize patients based on treatment responses, and fine-tune therapeutic planning. By identifying ARPC1A as a prognostic biomarker in low-grade glioma, reinforcing the move toward personalized cancer treatment. By integrating microbiome and genetic insights, clinicians can design therapies tailored to an individual's unique tumor profile, moving away from the limitations of a one-size-fits-all approach (61).

However, the promise of microbiome-driven cancer therapy, several challenges remain. Microbiome diversity across individuals shaped by diet, genetics, antibiotics, and other factors complicates the creation of universally effective interventions. Furthermore, the absence of standardized protocols for microbiome sampling, sequencing methods, and data interpretation poses challenges for clinical implementation. Regulatory hurdles compound these issues, particularly for novel therapies like FMT and engineered probiotics, both of which require comprehensive validation in large-scale clinical trials before achieving broader approval in oncology settings (62). Utilizing the microbiota to enhance cancer treatments has emerged as a new approach in personalized medicine. While current results are promising, challenges remain including a limited understanding of how microbiota affects therapy, undefined microbial biomarkers, and no consensus on the best modulation methods. Moreover, most research focuses on bacteria, overlooking the roles of commensal viruses, fungi, and archaea in cancer. Modern strategies integrating microbiome in cancer treatment shown in Table 4.

TABLE 4 Modern strategies integrating microbiome in cancer treatment.

Cancer Type	Microbiome-Related Strategies
Colorectal Cancer	• Antibiotic targeting of *Fusobacterium nucleatum* • Prebiotics/Probiotics to enhance chemotherapy • FMT for immunotherapy improvement • Stool-based microbiota screening
Breast Cancer	• Estrobolome-targeting probiotics to optimize endocrine therapy • Gut microbiota modulation for reducing chemotherapy toxicity • Liquid biopsy for microbial DNA analysis
Lung Cancer	• *Akkermansia muciniphila*-driven immunotherapy enhancement • Selective antibiotic use to prevent ICI resistance • Microbial profiling in BAL fluid for early detection
Pancreatic Cancer	• Antibiotic targeting of *Gammaproteobacteria* to improve gemcitabine response • Salivary microbiota biomarkers for early diagnosis • FMT for gut microbiota restoration
Prostate Cancer	• Gut microbiome modulation for androgen deprivation therapy • Urinary microbiota analysis for non-invasive screening • High-fiber diets and probiotics for treatment support

7 Microbiota's role in predictive oncology and custom therapies

The microbiota has emerged as a critical factor in oncology, influencing cancer development, progression, and treatment response. Its predictive role is being increasingly studied across several aspects of cancer care. Certain microbiota, particularly in the gut, have been linked to cancer development. For example, certain strains of *E. coli* possess a pathogenicity island known as *pks*, which contains genes responsible for producing the genotoxic compound colibactin. This metabolite could potentially be used as a biomarker to predict the risk of colon cancer. Approximately 16% of cancer types have been caused by microbial organisms, *Helicobacter pylori* is known to play a role in the development of adenocarcinoma in gastric and duodenal epithelial cells, as well as gastric cancer (63, 64). *Streptococcus*, *Haemophillus*, and *Bifidobacterium* have been identified as oral cancer biomarkers, using sequence-based techniques (63). However, the precise ways bacteria contribute to cancer development are still unclear, likely due to the complexity of bacterial communities and their interaction with living cells, so developing non-invasive tests to analyze microbiome composition could help predict cancer risk and prognosis in real-time (19, 63).

The composition of a patient's microbiota can also predict their response to cancer treatment. Chemoresistance caused by *F. nucleatum* in mice may also be seen in human CRC patients. Higher levels of *F. nucleatum* in tumors are associated with quicker cancer recurrence and can predict CRC recurrence more accurately than the traditional cancer staging system (65, 66). Meanwhile, as the relationship between CDD-L, found in gram-negative bacteria, and gemcitabine chemoresistance is well known, a clinical study exhibited the potential role of intratumoral

lipopolysaccharide, a cell wall component of Gram-negative bacteria, to be a negative predictor of gemcitabine efficacy in advanced pancreatic cancer (67). So, antibiotics targeting the CDD-L-producing bacteria improve gemcitabine response in patients with pancreatic ductal adenocarcinoma (PDAC) (65, 67). Preclinical studies indicated that prebiotics inulin and oligofructose increase the cytotoxic effects of 5-fluorouracil and CTX. Prebiotic consumption may enrich immune-effective bacteria. Clinical studies on the impact of prebiotics on chemotherapy are lacking So, Future investigations are essential to address the clinical safety of prebiotics (65).

Previous studies investigated the role of microbiota on the clinical response of ICIs and tried to elucidate the principle mechanism of dysbiosis of microbiota in the immune response. The microbiota that is effective in cancer immunotherapy response is not the same in clinical studies. This variation may be due to not having standard methods across studies. However, some of the bacteria identified from human studies, including *A. muciniphila*, *B. intestinihominis*, and *B. thetaiotaomicron*, were shown to improve therapeutic response through immunomodulation (65). As mentioned before *F. nucleatum* has been linked to poor prognosis in CRC, but it enhances the efficacy of immunotherapy. Thus, *F. nucleatum* is a potential bacterial biomarker for CRC and also immunotherapy response (63). Various therapeutic approaches have been tailored to modulate the microbiome to enhance the effectiveness of immunotherapy, based on recent discoveries in this field. FMT treatment is the transplantation of an individual's gut microbiome usually from responders to non-responders. Its key benefit is that it can alter the microbiome, even without a clear understanding of the underlying mechanisms. Human and animal studies indicated that FMT can boost both immunotherapy response and irAEs, such as colitis (65, 68). Dietary modulation like high fiber diet in metastatic non-small cell lung carcinoma (NSCLC) patients receiving ICI, increased *Bifidobacterium* species which are another immunogenic bacteria associated with improved anticancer immunity (65). In addition, choosing specific antibiotic regimens can potentially have indirect anticancer benefits and help minimize complications during cancer therapies by targeting harmful microorganisms that either contribute to cancer development or lead to adverse events (69, 70). The research on immunoadjuvant-functionalized metal–organic frameworks (MOFs) complements the growing understanding of how microbiome insights can improve cancer immunotherapy. Similar to how beneficial gut microbes such as *Akkermansia muciniphila* and *Bifidobacterium* enhance the efficacy of immune checkpoint inhibitors, MOFs provide an innovative approach to modulating tumor immunity. This holds promise for advancing precision oncology and transcending conventional therapeutic methods (71).

The gut microbiome can be a double-edged sword for irAEs. Researches indicated that Checkpoint inhibitor colitis (CIC), the most frequently reported irAE, can be developed by *Faecalibacterium prausnitii* and *Bacteroidetes* or be protected by *B. fragilis* (68). In a clinical study, chaput et al. demonstrated that in metastatic melanoma patients receiving Ipilimumab, a higher abundance of *Faecalibacterium* and other *Firmicutes* was associated with better response with more colitis. Therefore, understanding the relationship between different microbes and side effects is particularly important for individualized palliation of these adverse events (69).

8 Challenges and future of microbiota in cancer diagnostics

Investigate the current challenges in using microbiota for cancer diagnostics, such as variability in microbial communities and standardization issues. Discuss potential future directions and solutions. Early diagnosis of cancer usually guarantees a better prognosis and increased treatment success rate. The available diagnosis techniques often involve invasive biopsies. This calls for research into less invasive techniques. Such techniques are those that have high sensitivity, which detects cancer patients, and high specificity, which rules out non-cancer patients. Recent studies have tested the ability of microbiomes to diagnose cancer through non-invasive means, using easily accessible samples such as saliva, stool, and plasma-a promising alternative to traditionally used invasive techniques. In fact, the use of microbiota for diagnostics to the cancer is mirrored by a remarkable promise combined with major challenges. Some limiting factors for broad use of microbiota-based diagnostics in cancer include variation in microbial communities, discrepancies in methods of approaches and lack of uniformity in approaches (72).

Challenges for microbiota-based diagnostics the composition of the microbiota varies between individuals and among populations and is highly modulated by diet, age, geographic location, medication use, and lifestyle (72, 73). These factors may mask any association between specific microbial signatures and cancer. Whereas detection of *Fusobacterium* enrichment in CRC lesions and stools has been reported in various populations, microbial community variation among individuals and populations complicates generalization. Such variability requires even finer control and adjustments in the design of studies to account for such variability (74).

Despite its potential, microbiome-driven cancer treatment faces significant challenges, mainly regarding standardization and reproducibility. One major issue is the natural variability of the microbiome across individuals, shaped by factors such as genetics, diet, geographic location, medication use (notably antibiotics), and underlying health conditions. This variability complicates efforts to create universally effective therapeutic approaches centered on the microbiome. A critical obstacle is the lack of standardized protocols for microbiome analysis. Variations in sampling techniques whether using stool, saliva, blood, or tumor tissue as well as differences in DNA extraction methods and sequencing technologies, contribute to inconsistent research outcomes. Additionally, the diverse range of bioinformatics tools employed to process microbiome data can lead to biases and conflicting interpretations. Without unified methodologies, it remains challenging to transition microbiome-focused discoveries from the research phase into practical clinical use (75).

10.3389/fimmu.2025.1559480

Another key challenge is the identification of clinically relevant microbial biomarkers. While studies often report links between specific bacterial species and cancer treatment outcomes, these associations are not always causative. The intricate dynamics of host-microbiome interactions make it difficult to determine whether particular microbes actively affect treatment responses or simply reflect other underlying factors. Large-scale, and carefully controlled clinical trials are essential to validate these findings before microbial biomarkers can inform routine clinical practices. Regulatory challenges add another layer of complexity to adoption of microbiome-based interventions. FMT, for example, has shown encouraging results in restoring microbial balance and improving responses to immunotherapy. However, concerns about long-term safety and potential transmission of harmful pathogens remain. Similarly, engineered probiotics and drugs designed to modulate the microbiome require rigorous safety evaluations before they can receive regulatory approval for use in cancer care (76).

Standardization issues: To overcome these challenges, the field must move toward standardized microbiome research frameworks that facilitate reproducibility and enable clinical application. Establishing universal guidelines for microbiome sampling, sequencing, and data analysis will significantly enhance the comparability of findings across studies. Moreover, integrating machine learning and AI to analyze microbiome data may help identify microbial signatures that can predict treatment responses more accurately. Another critical area for future development is the creation of personalized microbiome-based therapies personalized to the unique needs of each patient. Innovations in microbiome engineering, such as developing synthetic probiotics and microbiota-specific drugs, hold promise for delivering more precise therapeutic options. Additionally, integrating microbiome analysis into multimodal cancer treatment plans by combining it with data from genomics, proteomics, and metabolomics can pave the way for a fully individualized approach to cancer care (77–79). Personalized cancer treatment strategies shown in Table 5.

TABLE 5 Personalized cancer treatment strategies.

Cancer Type	Microbiome-Based Strategy	Mechanism of Action	Expected Benefit
Colorectal Cancer	Antibiotics targeting *Fusobacterium nucleatum*	Reducing tumor-promoting bacteria	Enhancing chemotherapy response
Breast Cancer	Probiotics for estrobolome modulation	Regulating estrogen metabolism	Improving hormone therapy efficacy
Lung Cancer	*Akkermansia muciniphila* enhancement via diet	Boosting immune response	Enhancing ICI effectiveness
Pancreatic Cancer	Targeting *Gammaproteobacteria* to improve chemotherapy	Reducing drug inactivation	Enhancing gemcitabine response
Prostate Cancer	High-fiber diet and probiotics	Modulating androgen metabolism	Improving androgen deprivation therapy outcomes

9 Combining microbiome data with traditional cancer tests

Explain how integrating microbiome data with conventional diagnostic methods can improve cancer detection accuracy. Provide examples of combined approaches and their benefits. The human microbiome communities of bacteria, viruses, fungi, and other microorganisms residing in and on our bodies has been shown to be linked with several cancer types. For instance, particular microbial signatures, such as the overrepresentation of *F. nucleatum* in CRC, have been consistently observed in both tissue and stool samples (80).

Microbial cell-free DNA (cfDNA) from liquid biopsies is emerging as a minimally invasive approach for early cancer detection. Traditionally, cancer detection using liquid biopsies targets tumor-derived markers like circulating tumor DNA (ctDNA). However, ctDNA can be difficult to detect, especially in early-stage cancers due to low concentrations. Recent advances leverage non-human cfDNA from the microbiome, analyzed via whole-genome sequencing (WGS), to detect cancer. Microbial cfDNA, released by tumor-associated bacteria, viruses, and fungi, offers an additional layer of diagnostic potential, with studies demonstrating its ability to distinguish between cancer and non-cancer cases using machine learning models (81).

Although, the use of stool-based microbiome analysis in conjunction with traditional CRC screening methods has shown great promise. In a study that combined a fecal immunochemical test (FIT), which detects blood hidden in the stool, with microbiome analysis, researchers found a significant improvement in the accuracy of CRC diagnosis. The sensitivity of FIT alone for detecting CRC is approximately 79%, but when combined with microbiome markers such as *Fusobacterium* or *Porphyromonas*, the sensitivity improved to more than 90% (82).

Also, Machine learning (ML) models use feature vectors to make predictions, where the input vector consists of M features, and the model predicts a target value based on a decision function. The primary goal of ML models is to minimize the loss function, which measures the discrepancy between predicted and actual outcomes. These tasks are categorized into classification or regression, depending on whether the target variable is categorical or numerical. In cancer-related microbiome research, classification is the most common approach, focusing on cancer diagnosis or tumor type identification. Regression tasks, though less frequent, are used for outcomes such as predicting survival time or tumor growth in experimental models.

9.1 Support vector machines

Support vector machines are popular for cancer-related microbiome research, particularly for identifying biomarkers. They work by defining a decision boundary (hyperplane) and,

using techniques like the "kernel trick," can handle non-linear data (83). SVMs have shown good performance in CRC prediction but are less interpretable than other models like Random Forests (84).

9.2 Decision tree-based models

Decision tree-based models especially Random Forests, are widely used in cancer-microbiome studies. Random Forests reduce variability by averaging the predictions of multiple trees, providing better generalization than single Decision Tree (85). They have successfully identified various cancers, such as colorectal and lung cancer (85). Boosting further improves performance by building models sequentially and adjusting for errors, with models like Explainable Boosting Machines (EBMs) enhancing interpretability (86).

9.3 Logistic regression

Logistic regression is a simple yet effective model, often used for feature selection in microbiome studies. While typically outperformed by more complex models, it remains useful due to its interpretability, especially when identifying potential cancer biomarkers. Regularization methods like LASSO and Ridge help reduce overfitting, making it an efficient choice for small datasets (87) (Figure 3).

10 Microorganisms and cancer: drivers or companions

The intricate relationship between microorganisms and cancer has been widely studied, but it remains a topic of ongoing debate whether microbes function as drivers (actively contributing to the initiation and progression of cancer) or as companions (simply coexist with tumors without directly causing them). To resolve this ambiguity, researchers must investigate into the biological mechanisms connecting microbes to cancer, distinguish correlation from causation, analyze experimental findings, and address the limitations in current studies. Microorganisms influence cancer development in several ways, predominantly through mechanisms like chronic inflammation, DNA damage, metabolic disturbances, and immune modulation (88). Persistent infections by certain microbes often provoke inflammatory responses, releasing ROS and pro-inflammatory cytokines that can cause DNA mutations and foster tumor growth. For instance, *Helicobacter pylori* plays a role in gastric cancer by inducing chronic inflammation, while *Fusobacterium nucleatum* has been linked to colorectal cancer through immune modulation and enhanced tumor cell survival (89). Additionally, some bacteria, such as *Escherichia coli* strains carrying the pks pathogenicity island, produce genotoxins like colibactin, which generates DNA double-strand breaks, leading to genomic instability. Beyond direct genetic damage, microbial metabolites reshape the tumor microenvironment in diverse ways. While certain bacterial byproducts, such as SCFAs, may exhibit

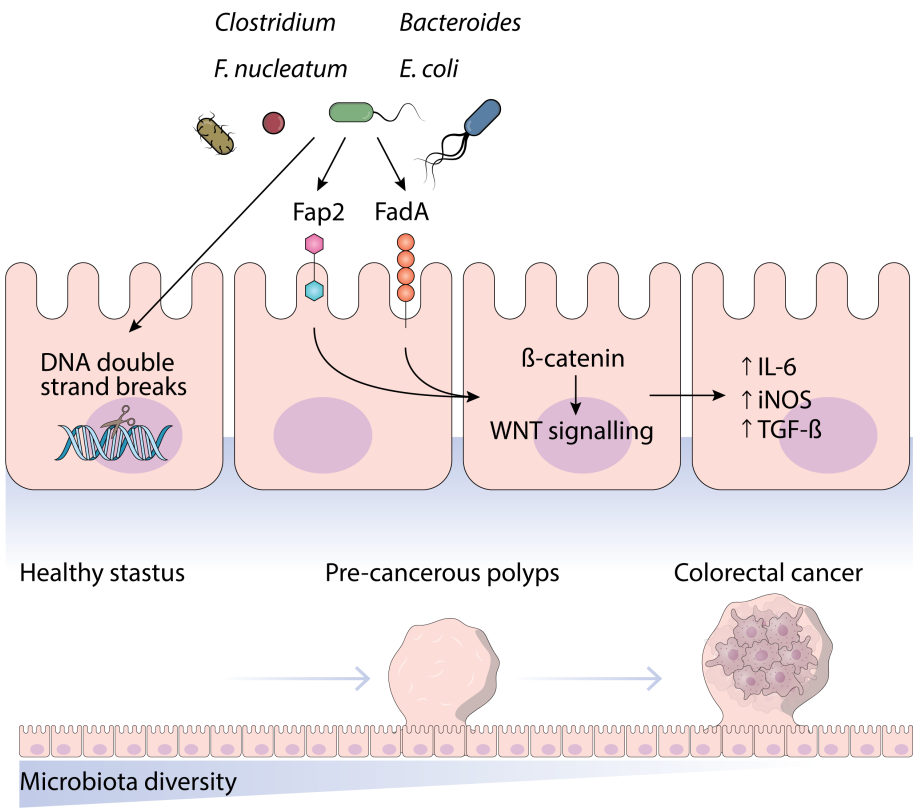


FIGURE 3
This figure highlights the role of microbiota in cancer diagnosis and treatment selection and its challenges.

protective effects against cancer, others can stimulate tumor proliferation. A case in point is *Fusobacterium nucleatum*, which influences the metabolic dynamics of CRC by increasing glucose uptake in tumor cells, thereby accelerating their growth (90).

A significant challenge in microbiome-oncology research is differentiating between correlation and action, despite evident biological associations. Many microbes linked to cancer may not initiate tumor formation but rather opportunistic colonizers in the tumor microenvironment. To determine their roles, it is critical to compare microbial presence in early versus advanced tumor stages. Microorganisms consistently found in pre-cancerous lesions prior to tumor development are more likely to be drivers of cancer. Conversely, those primarily detected in late-stage tumors are more likely opportunistic participants. Tumors often create unique conditions such as hypoxia and immune suppression that encourage microbial proliferation, further complicating efforts to determine whether microbes cause cancer or simply adapt to tumor-associated conditions. Longitudinal studies that follow individuals over time are instrumental in unraveling this relationship. For instance, research showing that eradication of *H. pylori* lowers gastric cancer risk provides robust evidence of its role as a driver. However, for many other microbes, such long-term data is lacking (91).

Experimental models offer additional insights into the microbiome's role in cancer. Germ-free mouse models, which are without of microorganisms, develop fewer spontaneous tumors, indicating microbes may play a part in carcinogenesis. Introducing specific cancer-associated bacteria into these models often accelerates tumor growth. For example, introducing *Fusobacterium nucleatum* into mice predisposed to colorectal cancer significantly enhances tumor progression, supporting its role as a driver. Other experimental interventions, such as antibiotic treatments or microbiome transplants, provide further evidence. When antibiotics targeting specific microbes reduce tumor burden, it suggests those microbes have a direct role in cancer progression. Similarly, transplanting a cancer-associated microbiome into germ-free mice and observing increased tumor incidence supports microbial involvement in cancer initiation. Human cohort studies also contribute valuable evidence. Epidemiological links between *H. pylori* and gastric cancer and between *F. nucleatum* and colorectal cancer progression underscore their potential roles as cancer drivers. However, practical and ethical limitations restrict the extent of direct experimental work possible in human subjects (88) (Figure 4).

11 Conclusion

In conclusion, a deeper understanding of the host–microbiota immune axis lays the groundwork for innovative, personalized, and microbiota-targeted therapies that may transform human health management. Using the potential to revolutionize precision medicine, the integration of microbiome research into oncology has created new opportunities for cancer diagnoses and treatment. The host's and microbiota dynamic interactions highlight how important

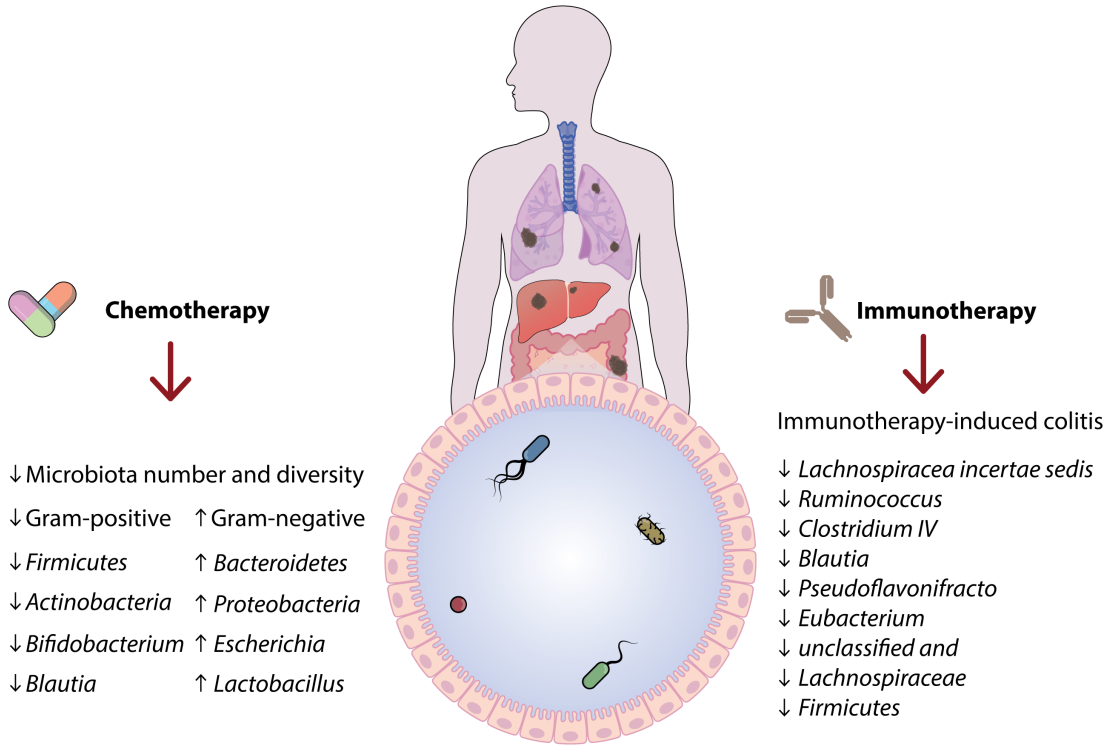


FIGURE 4
A schematic representation illustrates the two-way interaction between the microbiota and the host immune system. Microbial metabolites like short-chain fatty acids, indoles, and bile acids affect immune responses through pathways including AHR and G-protein coupled receptors. These signals influence immune cell populations, such as regulatory T cells and Th17 cells, playing a role in maintaining immune balance, protecting against chronic inflammation, and potentially impacting cancer development.

they are to the development, progression, and response to cancer therapy. In contrast dietary modification and FMT have become cutting-edge strategies to improve therapeutic efficacy and reduce side effects, microbial biomarkers, such as *F. nucleatum* and *B. fragilis*, demonstrate promising in early cancer detection, especially in colorectal and breast cancers. Significant challenges remain in spite of these developments, such as the inherent variability of microbiota composition between individuals and populations, the lack of standardized procedures, and the requirement for complete verification of biomarkers obtained from microbiota. There is potential for increasing diagnosis precision and prediction treatment results through the creation of integrated diagnostic frameworks that integrate microbiome data with conventional techniques like liquid biopsies and advanced machine learning algorithms. Future research should focus on addressing these challenges by establishing standardized protocols, expanding diverse patient cohorts, and exploring the roles of underrepresented microbial domains, such as viruses and fungi, in cancer biology. Deciphering the intricacies of the interaction between microbiota and cancer will help the field reach its full potential and eventually result in lower-invasive, more individualized, and more successful cancer care approaches.

Author contributions

ME: Conceptualization, Investigation, Project administration, Supervision, Visualization, Writing – review & editing. RN: Data curation, Investigation, Resources, Writing – original draft. AiB: Formal Analysis, Investigation, Writing – original draft. AlB: Software, Writing – original draft. SG: Investigation, Writing – original draft. VO: Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Visualization, Writing – review & editing. HT: Formal Analysis, Investigation, Methodology, Resources, Writing – original draft.

Funding

The author(s) declare that no financial support was received for the research and/or publication of this article.

Acknowledgments

Cancer Research Center, Semnan University of Medical Sciences, Semnan, Iran.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Wu WKK, Yu J. Microbiota-based biomarkers and therapeutics for cancer management. *Nat Rev Gastroenterol Hepatol*. (2024) 21:72–3. doi: 10.1038/s41575-023-00879-9

2. Union-Caballero A, Meroño T, Zamora-Ros R, Rostgaard-Hansen AL, Miñarro A, Sánchez-Pla A, et al. Metabolome biomarkers linking dietary fibre intake with cardiometabolic effects: results from the Danish Diet, Cancer and Health-Next Generations MAX study. *Food Funct*. (2024) 15:1643–54. doi: 10.1039/D3FO04763F

3. Zwezerijnen-Jiwa FH, Sivov H, Paizs P, Zafeiropoulou K, Kinross J. A systematic review of microbiome-derived biomarkers for early colorectal cancer detection. *Neoplasia*. (2023) 36:100868. doi: 10.1016/j.neo.2022.100868

4. Mahmoudian F, Gheshlagh SR, Hemati M, Farhadi S, Eslami M. The influence of microbiota on the efficacy and toxicity of immunotherapy in cancer treatment. *Mol Biol Rep*. (2025) 52:86. doi: 10.1007/s11033-024-10188-2

5. Lin A, Jiang A, Huang L, Li Y, Zhang C, Zhu L. From chaos to order: optimizing fecal microbiota transplantation for enhanced immune checkpoint inhibitors efficacy. *Gut Microbes*. (2025) 17:2452277. doi: 10.1080/19490976.2025.2452277

6. Nath AR, Natarajan J. Gut metagenomic analysis of gastric cancer patients reveals Akkermansia, Gammaproteobacteria, and Veillonella microbiota as potential non-invasive biomarkers. *Genomics Inform*. (2024) 22:1. doi: 10.1186/s44342-024-00001-8

7. Eslami M, Naderian R, Ahmadpour A, Shushtari A, Maleki S, Mohammadian P, et al. Microbiome structure in healthy and pregnant women and importance of vaginal dysbiosis in spontaneous abortion. *Front Cell Infection Microbiol*. (2025) 14:1401610. doi: 10.3389/fcimb.2024.1401610

8. Cristofori F, Naderian R, Ahmadpour A, Shushtari A, Maleki S, Mohammadian P, et al. Anti-inflammatory and immunomodulatory effects of probiotics in gut inflammation: a door to the body. *Front Immunol*. (2021) 12:578386. doi: 10.3389/fimmu.2021.578386

9. Ghasemian A, Dargenio VN, Dargenio C, Miniello VL, Barone M, Francavilla R, et al. Probiotics and their increasing importance in human health and infection control. *Rev Res Med Microbiol*. (2018) 29:153–8. doi: 10.1097/MRM.0000000000000147

10. Jiang C-H, Eslami M, Shafiei M, Najafipour S, Rajabi A, et al. Anticancer activity and mechanism of xanthohumol: a prenylated flavonoid from hops (Humulus lupulus L.). *Front Pharmacol*. (2018) 9:530. doi: 10.3389/fphar.2018.00530

11. Bai M, Sun TL, Xiang DX, Wei SS, Li WQ. Hydrolyzed protein formula improves the nutritional tolerance by increasing intestinal development and altering cecal microbiota in low-birth-weight piglets. *Front Nutr*. (2024) 11:1439110. doi: 10.3389/fnut.2024.1439110

12. Bou Zerdan M, Liu H, Yan Y, Duan S, Szeto IMY, He J, et al. The lung microbiota and lung cancer: A growing relationship. *Cancers (Basel)*. (2022) 14:4813. doi: 10.3390/cancers14194813

13. Eslami M, Alibabaei F, Babaeizad A, Banihashemian SZ, Mazandarani M, Hoseini A, et al. The importance of gut microbiota on choline metabolism in neurodegenerative diseases. *Biomolecules*. (2024) 14:1345. doi: 10.3390/biom14111345

14. Negrut RL, Cote A, Maghiar AM. Exploring the potential of oral microbiome biomarkers for colorectal cancer diagnosis and prognosis: A systematic review. *Microorganisms*. (2023) 11:1586. doi: 10.3390/microorganisms11061586

15. Merz V, Mangiameli D, Zecchetto C, Quinzii A, Pietrobono S, Messina C, et al. Predictive biomarkers for a personalized approach in resectable pancreatic cancer. *Front Surg*. (2022) 9:866173. doi: 10.3389/fsurg.2022.866173

16. Ning L, Hong J. Gut microbiome ecological topology as next-generation biomarkers for cancer immunotherapy. *Cell*. (2024) 187:3231–2. doi: 10.1016/j.cell.2024.04.044

17. Salek Farrokhi A, Darabi N, Yousefi B, Askandar RH, Shariati M, Eslami M, et al. Is it true that gut microbiota is considered as panacea in cancer therapy? *J Cell Physiol*. (2019) 234:14941–50. doi: 10.1002/jcp.28333

18. Zha C, Peng Z, Huang K, Tang K, Wang Q, Zhu L, et al. Potential role of gut microbiota in prostate cancer: immunity, metabolites, pathways of action? *Front Oncol*. (2023) 13:1196217. doi: 10.3389/fonc.2023.1196217

19. Hemmati MA, Monemi M, Asli S, Mohammadi S, Foroozanmehr B, Haghmorad D, et al. Using new technologies to analyze gut microbiota and predict cancer risk. *Cells*. (2024) 13:1987. doi: 10.3390/cells13231987

20. Beniwal SS, Lamo P, Kaushik A, Lorenzo-Villegas DL, Liu Y, MohanaSundaram A. Current status and emerging trends in colorectal cancer screening and diagnostics. *Biosensors (Basel)*. (2023) 13:926. doi: 10.3390/bios13100926

21. Kim J, Lee HK. Potential role of the gut microbiome in colorectal cancer progression. *Front Immunol*. (2021) 12:807648. doi: 10.3389/fimmu.2021.807648

22. Ibeanu GC, Rowaiye AB, Okoli JC, Eze DU. Microbiome differences in colorectal cancer patients and healthy individuals: implications for vaccine antigen discovery. *Immunotargets Ther*. (2024) 13:749–74. doi: 10.2147/ITT.S486731

23. Chen G, Ren Q, Zhong Z, Li Q, Huang Z, Zhang C, et al. Exploring the gut microbiome's role in colorectal cancer: diagnostic and prognostic implications. *Front Immunol*. (2024) 15:1431747. doi: 10.3389/fimmu.2024.1431747

24. Abu Abeelh E, AbuAbeileh Z. Comparative effectiveness of mammography, ultrasound, and MRI in the detection of breast carcinoma in dense breast tissue: A systematic review. *Cureus*. (2024) 16:e59054. doi: 10.7759/cureus.59054

25. Yaqub MO, Jain A, Joseph CE, Edison LK. Microbiome-driven therapeutics: from gut health to precision medicine. *Gastrointestinal Disord*. (2025) 7:7. doi: 10.3390/gidisord7010007

26. Ruan Y, Yuan PP, Li PY, Chen Y, Fu Y, Gao LY, et al. Tingli Dazao Xiefei Decoction ameliorates asthma *in vivo* and *in vitro* from lung to intestine by modifying NO–CO metabolic disorder mediated inflammation, immune imbalance, cellular barrier damage, oxidative stress and intestinal bacterial disorders. *J Ethnopharmacol*. (2023) 313:116503. doi: 10.1016/j.jep.2023.116503

27. Pourali G, Kazemi D, Chadeganipour AS, Arastonejad M, Kashani SN, Pourali R, et al. Microbiome as a biomarker and therapeutic target in pancreatic cancer. *BMC Microbiol*. (2024) 24:16. doi: 10.1186/s12866-023-03166-4

28. Karbalaei M, Keikha M, Yousefi B, Ali-Hassanzadeh M, Eslami M. Contribution of aging oral microbiota in getting neurodegenerative diseases. *Rev Res Med Microbiol*. (2021) 32:90–4. doi: 10.1097/MRM.0000000000000245

29. Bahman Y, Maryam M, Aisa B, Falalyeyeva T, Kobyliak N, Majid E. Immunomodulatory role of Faecalibacterium prausnitzii in obesity and metabolic disorders. *Minerva Biotechnol Biomolecular Res*. (2021) 33:76–85. doi: 10.23736/S2724-542X.21.02759-2

30. Huang L, Li Y, Tang R, Yang P, Zhuo Y, Jiang X, et al. Bile acids metabolism in the gut-liver axis mediates liver injury during lactation. *Life Sci*. (2024) 338:122380. doi: 10.1016/j.lfs.2023.122380

31. Del Giudice T, Staropoli N, Tassone P, Tagliaferri P, Barbieri V. Gut microbiota are a novel source of biomarkers for immunotherapy in non-small-cell lung cancer (NSCLC). *Cancers (Basel)*. (2024) 16:1806. doi: 10.3390/cancers16101806

32. Li H, Sheng D, Jin C, Zhao G, Zhang L. Identifying and ranking causal microbial biomarkers for colorectal cancer at different cancer subsites and stages: a Mendelian randomization study. *Front Oncol*. (2023) 13:1224705. doi: 10.3389/fonc.2023.1224705

33. Hou W, Yi C, Zhu H. Predictive biomarkers of colon cancer immunotherapy: Present and future. *Front Immunol*. (2022) 13:1032314. doi: 10.3389/fimmu.2022.1032314

34. Parent P, Marcq G, Adeleke S, Turpin A, Boussios S, Rassy E, et al. Predictive biomarkers for immune checkpoint inhibitor response in urothelial cancer. *Ther Adv Med Oncol*. (2023) 15:17588359231192402. doi: 10.1177/17588359231192402

35. Galloway-Peña JR, Smith DP, Sahasrabhojane P, Ajami NJ, Wadsworth WD, Daver NG, et al. The role of the gastrointestinal microbiome in infectious complications during induction chemotherapy for acute myeloid leukemia. *Cancer*. (2016) 122:2186–96. doi: 10.1002/cncr.v122.14

36. Kolisnik T, Sulit AK, Schmeier S, Frizelle F, Purcell R, Smith A, et al. Identifying important microbial and genomic biomarkers for differentiating right- versus left-sided colorectal cancer using random forest models. *BMC Cancer*. (2023) 23:647. doi: 10.1186/s12885-023-10848-9

37. Youssef O, Lahti L, Kokkola A, Karla T, Tikkanen M, Ehsan H, et al. Stool microbiota composition differs in patients with stomach, colon, and rectal neoplasms. *Dig Dis Sci*. (2018) 63:2950–8. doi: 10.1007/s10620-018-5190-5

38. Puhalla S, Bhattacharya S, Davidson NE. Hormonal therapy in breast cancer: a model disease for the personalization of cancer care. *Mol Oncol*. (2012) 6:222–36. doi: 10.1016/j.molonc.2012.02.003

39. Murphy CC, Bartholomew LK, Carpentier MY, Bluethmann SM, Vernon SW. Adherence to adjuvant hormonal therapy among breast cancer survivors in clinical practice: a systematic review. *Breast Cancer Res Treat*. (2012) 134:459–78. doi: 10.1007/s10549-012-2114-5

40. Miyabayashi K, Ijichi H, Fujishiro M. The role of the microbiome in pancreatic cancer. *Cancers (Basel)*. (2022) 14:4479. doi: 10.3390/cancers14184479

41. Tijeras-Raballand A, Hilmi M, Astorgues-Xerri L, Nicolle R, Bièche I, Neuzillet C. Microbiome and pancreatic ductal adenocarcinoma. *Clin Res Hepatol Gastroenterol*. (2021) 45:101589. doi: 10.1016/j.clinre.2020.101589

42. Eslami M, Bahar A, Hemati M, Rasouli Nejad Z, Mehranfar F, Karami S, et al. Dietary pattern, colonic microbiota and immunometabolism interaction: new frontiers for diabetes mellitus and related disorders. *Diabetic Med*. (2021) 38:e14415. doi: 10.1111/dme.14415

43. Sukmana BI, Saleh RO, Najim MA, Al-Ghamdi HS, Achmad H, Al-Hamdani MM, et al. Oral microbiota and oral squamous cell carcinoma: a review of their relation and carcinogenic mechanisms. *Front Oncol*. (2024) 14:1319777. doi: 10.3389/fonc.2024.1319777

44. Thomas AM, Fidelle M, Routy B, Kroemer G, Wargo JA, Segata N, et al. Gut OncoMicrobiome Signatures (GOMS) as next-generation biomarkers for cancer immunotherapy. *Nat Rev Clin Oncol*. (2023) 20:583–603. doi: 10.1038/s41571-023-00785-8

45. Rezasoltani S, Aghdaei HA, Jasemi S, Gazouli M, Dovrolis N, Sadeghi A, et al. Oral microbiota as novel biomarkers for colorectal cancer screening. *Cancers (Basel)*. (2022) 15:192. doi: 10.3390/cancers15010192

46. Fan JQ, Zhao WF, Lu QW, Zha FR, Lv LB, Ye GL, et al. Fecal microbial biomarkers combined with multi-target stool DNA test improve diagnostic accuracy for colorectal cancer. *World J Gastrointest Oncol*. (2023) 15:1424–35. doi: 10.4251/wjgo.v15.i8.1424

47. Helissey C, Cavallero S, Mondot S, Parnot C, Yssaad H, Becherirat S, et al. Correlation between serum and urine biomarkers and the intensity of acute radiation cystitis in patients treated with radiation therapy for localized prostate cancer: protocol for the radiotoxicity bladder biomarkers (RABBIO) study. *JMIR Res Protoc*. (2023) 12:e38362. doi: 10.2196/38362

48. Pietrzak B, Kawacka I, Olejnik-Schmidt A, Schmidt M. Circulating microbial cell-free DNA in health and disease. *Int J Mol Sci*. (2023) 24:3051. doi: 10.3390/ijms24033051

49. Zidi O, Souai N, Raies H, Ben Ayed F, Mezlini A, Mezrioui S, et al. Fecal metabolic profiling of breast cancer patients during neoadjuvant chemotherapy reveals potential biomarkers. *Molecules*. (2021) 26:2266. doi: 10.3390/molecules26082266

50. Hemmati M, Kashanipoor S, Mazaheri P, Alibabaei F, Babaeizad A, Asli S, et al. Importance of gut microbiota metabolites in the development of cardiovascular diseases (CVD). *Life Sci*. (2023) 329:121947. doi: 10.1016/j.lfs.2023.121947

51. Qasem HH, El-Sayed WM. The bacterial microbiome and cancer: development, diagnosis, treatment, and future directions. *Clin Exp Med*. (2024) 25:12. doi: 10.1007/s10238-024-01523-9

52. Kobyliak N, Falalyeyeva T, Kyriachenko Y, Tseyslyer Y, Kovalchuk O, Hadiliia O, et al. Akkermansia muciniphila as a novel powerful bacterial player in the treatment of metabolic disorders. *Minerva Endocrinol*. (2022) 47:242–52. doi: 10.23736/S2724-6507.22.03752-6

53. Eslami M, Yousefi B, Kokhaei P, Hemati M, Nejad ZR, Arabkari V, et al. Importance of probiotics in the prevention and treatment of colorectal cancer. *J Cell Physiol*. (2019) 234:17127–43. doi: 10.1002/jcp.v234.10

54. Mir R, Albarqi SA, Albalawi W, Alatwi HE, Alatawy M, Bedaiwi RI. Emerging role of gut microbiota in breast cancer development and its implications in treatment. *Metabolites*. (2024) 14:683. doi: 10.3390/metabo14120683

55. Zeng M, Zou Y, Shi Z, Wang J, Yang Y, Bai Y, et al. A broad-spectrum broth rapidly and completely repairing the sublethal injuries of Escherichia coli caused by freezing and lactic acid alone or in combination for accurate enumeration. *LWT*. (2024) 201:116219. doi: 10.1016/j.lwt.2024.116219

56. Eslami M, Sadrifar S, Karbalaei M, Keikha M, Kobyliak NM, Yousefi B. Importance of the microbiota inhibitory mechanism on the Warburg effect in colorectal cancer cells. *J gastrointestinal Cancer*. (2020) 51:738–47. doi: 10.1007/s12029-019-00329-3

57. Pickard JM, Zeng MY, Caruso R, Núñez G. Gut microbiota: Role in pathogen colonization, immune responses, and inflammatory disease. *Immunol Rev*. (2017) 279:70–89. doi: 10.1111/imr.2017.279.issue-1

58. Zeng J, Li Y, Zou Y, Yang Y, Yang T, Zhou Y. Intestinal toxicity alleviation and efficacy potentiation through therapeutic administration of Lactobacillus paracasei GY-1 in the treatment of gout flares with colchicine. *Food Funct*. (2024) 15:1671–88. doi: 10.1039/D3FO04858F

59. Farhadi Rad H, Tahmasebi H, Javani S, Hemati M, Zakerhamidi D, Hosseini M, et al. Microbiota and cytokine modulation: innovations in enhancing anticancer immunity and personalized cancer therapies. *Biomedicines*. (2024) 12:2776. doi: 10.3390/biomedicines12122776

60. Eslami M, Yousefi B. Akkermansia Muciniphila as novel powerful bacterial player in colorectal cancer biotherapeutics. *Rev Res Med Microbiol*. (2024) 35:18–22. doi: 10.1097/MRM.0000000000000328

61. Dai J, Gao J, Dong H. Prognostic relevance and validation of ARPC1A in the progression of low-grade glioma. *Aging (Albany NY)*. (2024) 16:11162. doi: 10.18632/aging.205952

62. Schupack DA, Mars RA, Voelker DH, Abeykoon JP, Kashyap PC. The promise of the gut microbiome as part of individualized treatment strategies. *Nat Rev Gastroenterol Hepatol*. (2022) 19:7–25. doi: 10.1038/s41575-021-00499-1

63. Shirazi MSR, Al-Alo KZK, Al-Yasiri MH, Lateef ZM, Ghasemian A. Microbiome dysbiosis and predominant bacterial species as human cancer biomarkers. *J gastrointestinal Cancer*. (2020) 51:725–8. doi: 10.1007/s12029-019-00311-z

64. Eslami M, Yousefi B, Kokhaei P, Arabkari V, Ghasemian A. Current information on the association of Helicobacter pylori with autophagy and gastric cancer. *J Cell Physiol*. (2019) 234:14800–11. doi: 10.1002/jcp.v234.9

65. Ting NL-N, Lau HC-H, Yu J. Cancer pharmacomicrobiomics: targeting microbiota to optimise cancer therapy outcomes. *Gut*. (2022) 71:1412–25. doi: 10.1136/gutjnl-2021-326264

66. Li J, Chen Y, Zhang S, Zhao Y, Gao D, Xing J, et al. Purslane (Portulaca oleracea L.) polysaccharide attenuates carbon tetrachloride-induced acute liver injury by modulating the gut microbiota in mice. *Genomics*. (2025) 117:110983. doi: 10.1016/j.ygeno.2024.110983

67. Guenther M, Haas M, Heinemann V, Kruger S, Westphalen CB, von Bergwelt-Baildon M, et al. Bacterial lipopolysaccharide as negative predictor of gemcitabine efficacy in advanced pancreatic cancer–translational results from the AIO-PK0104 Phase 3 study. *Br J Cancer*. (2020) 123:1370–6. doi: 10.1038/s41416-020-01029-7

68. Zhang M, Liu J, Xia Q. Role of gut microbiome in cancer immunotherapy: from predictive biomarker to therapeutic target. *Exp Hematol Oncol*. (2023) 12:84. doi: 10.1186/s40164-023-00442-x

69. Zhao L-Y, Mei JX, Yu G, Lei L, Zhang WH, Liu K, et al. Role of the gut microbiota in anticancer therapy: from molecular mechanisms to clinical applications. *Signal Transduction Targeted Ther*. (2023) 8:201. doi: 10.1038/s41392-023-01406-7

70. Firouzi Amandi A, Jokar E, Eslami M, Dadashpour M, Rezaie M, Yazdani Y, et al. Enhanced anti-cancer effect of artemisinin-and curcumin-loaded niosomal nanoparticles against human colon cancer cells. *Med Oncol*. (2023) 40:170. doi: 10.1007/s12032-023-02032-7

71. Zhao C, Song W, Wang J, Tang X, Jiang Z, et al. Immunoadjuvant-functionalized metal-organic frameworks:Synthesis and applications in tumor immune modulation. *Chem Commun*. (2025) 61:1962–77. doi: 10.1039/d4cc06510g

72. Tito RY, Verbandt S, Aguirre Vazquez M, Lahti L, Verspecht C, Lloréns-Rico V, et al. Microbiome confounders and quantitative profiling challenge predicted microbial targets in colorectal cancer development. *Nat Med*. (2024) 30:1339–48. doi: 10.1038/s41591-024-02963-2

73. Yousefi B, Kokhaei P, Mehranfar F, Bahar A, Abdolshahi A, Emadi A, et al. The role of the host microbiome in autism and neurodegenerative disorders and effect of epigenetic procedures in the brain functions. *Neurosci Biobehavioral Rev*. (2022) 132:998–1009. doi: 10.1016/j.neubiorev.2021.10.046

74. Young C, Wood HM, Fuentes Balaguer A, Bottomley D, Gallop N, Wilkinson L, et al. Microbiome analysis of more than 2,000 NHS bowel cancer screening programme samples shows the potential to improve screening accuracy. *Clin Cancer Res*. (2021) 27:2246–54. doi: 10.1158/1078-0432.CCR-20-3807

75. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. *Nature*. (2012) 489:220–30. doi: 10.1038/nature11550

76. Wang R, Li W, Cao H, Zhang L. Decoding the tumor-associated microbiota: from origins to nanomedicine applications in cancer therapy. *Biology*. (2025) 14:243. doi: 10.3390/biology14030243

77. Vandeputte D, Falony G, Vieira-Silva S, Tito RY, Joossens M, Raes J. Stool consistency is strongly associated with gut microbiota richness and composition, enterotypes and bacterial growth rates. *Gut*. (2016) 65:57–62. doi: 10.1136/gutjnl-2015-309618

78. Huang L, Luo S, Liu S, Jin M, Wang Y, Zong X. Comparative multiomics analyses reveal the breed effect on the colonic host–microbe interactions in pig. *iMetaOmics*. (2024) 1:e8. doi: 10.1002/imo2.v1.1

79. Han Y, Zhang Y, Yang Z, Zhang Q, He X, Song Y, et al. Improving aerobic digestion of food waste by adding a personalized microbial inoculum. *Curr Microbiol*. (2024) 81:277. doi: 10.1007/s00284-024-03796-5

80. Wong SH, Yu J. Gut microbiota in colorectal cancer: mechanisms of action and clinical applications. *Nat Rev Gastroenterol hepatol*. (2019) 16:690–704. doi: 10.1038/s41575-019-0209-8

81. Kataria R, Shoaie S, Grigoriadis A, Wan JC. Leveraging circulating microbial DNA for early cancer detection. *Trends Cancer*. (2023) 9:879–82. doi: 10.1016/j.trecan.2023.08.001

82. Baxter NT, Koumpouras CC, Rogers MA, Ruffin IV, MT, Schloss PD. DNA from fecal immunochemical test can replace stool for microbiota-based colorectal cancer screening. *bioRxiv*. (2016) p:048389. doi: 10.1101/048389

83. Rossi M, Aspromonte SM, Kohlhapp FJ, Newman JH, Lemenze A, Pepe RJ, et al. Gut microbial shifts indicate melanoma presence and bacterial interactions in a murine model. *Diagnostics*. (2022) 12:958. doi: 10.3390/diagnostics12040958

84. Yuan B, Ma B, Yu J, Meng Q, Du T, Li H, et al. Fecal bacteria as non-invasive biomarkers for colorectal adenocarcinoma. *Front Oncol*. (2021) 11:664321. doi: 10.3389/fonc.2021.664321

85. Friedman J. *The elements of statistical learning: Data mining, inference, and prediction*. Springer Series in Statistics, Second Edition. (2009).

86. Topçuoğlu BD, Lesniak NA, Ruffin IV, MT, Wiens J, Schloss PD. A framework for effective application of machine learning to microbiome-based classification problems. *mBio*. (2020) 11:10–1128. doi: 10.1128/mBio.00434-20

87. Ranstam J, Cook JA. LASSO regression. *Br J Surg*. (2018) 105:1348–8. doi: 10.1002/bjs.10895

88. Rahman MM, Islam MR, Shohag S, Ahasan MT, Sarkar N, Khan H, et al. Microbiome in cancer: Role in carcinogenesis and impact in therapeutic strategies. *Biomed Pharmacother*. (2022) 149:112898. doi: 10.1016/j.biopha.2022.112898

89. Salek Farrokhi A, Mohammadlou M, Abdollahi M, Eslami M, Yousefi B. Histone deacetylase modifications by probiotics in colorectal cancer. *J Gastrointestinal Cancer*. (2020) 51:754–64. doi: 10.1007/s12029-019-00338-2

90. Chen S, Long S, Liu Y, Wang S, Hu Q, Fu L. Evaluation of a three-gene methylation model for correlating lymph node metastasis in postoperative early gastric cancer adjacent samples. *Front Oncol*. (2024) 14:1432869. doi: 10.3389/fonc.2024.1432869

91. Bhatt AP, Redinbo MR, Bultman SJ. The role of the microbiome in cancer development and therapy. *CA Cancer J Clin*. (2017) 67:326–44. doi: 10.3322/caac.21398

frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 12 June 2025
DOI 10.3389/fimmu.2025.1595977

Check for updates

OPEN ACCESS

EDITED BY
Nar Singh Chauhan,
Maharshi Dayanand University, India

REVIEWED BY
Yean Kong Yong,
Malaysia, Malaysia
Avinash Lomash,
Medanta The Medicity Hospital, India
Rohit Patel,
Harvard Medical School, United States

*CORRESPONDENCE
Xuejun Guo
pygxj@163.com
Wenxue Ma
wma@health.ucsd.edu

RECEIVED 19 March 2025
ACCEPTED 26 May 2025
PUBLISHED 12 June 2025

CITATION
Guo X, Wang K, Liu Q, Baran N and Ma W (2025) The gut-immune axis in primary immune thrombocytopenia (ITP): a paradigm shifts
in treatment approaches.
Front. Immunol. 16:1595977.
doi: 10.3389/fimmu.2025.1595977

COPYRIGHT
© 2025 Guo, Wang, Liu, Baran and Ma. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

The gut-immune axis in primary immune thrombocytopenia (ITP): a paradigm shifts in treatment approaches

Xuejun Guo[1*], Ke Wang[1], Qianhui Liu[1], Natalia Baran[2,3] and Wenxue Ma[4*]

[1]Department of Hematology, Puyang Oilfield General Hospital Affiliated with Xinxiang Medical College, Puyang, Henan, China, [2]Department of Hematology and Central Hematology Laboratory, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland, [3]Institute of Hematology and Transfusion, Department of Experimental Hematology, Warsaw, Poland, [4]Department of Medicine, Sanford Stem Cell Institute, Moores Cancer Center, University of California San Diego, San Diego, CA, United States

Primary immune thrombocytopenia (ITP) is an autoimmune disorder characterized by platelet destruction and impaired production, leading to bleeding risk. While immunosuppressive therapies are standard, many patients experience relapses or refractory disease, highlighting the need for novel approaches. Emerging evidence suggests the gut microbiota plays a role in immune regulation, yet its impact on ITP remains unclear. Dysbiosis has been linked to immune dysfunction in other autoimmune diseases, but whether it drives or results from immune dysregulation in ITP is debated. This review explores the gut-immune axis in ITP, focusing on microbiota-driven immune modulation, cytokine signaling, and platelet homeostasis. We assess microbiota-targeted interventions, including fecal microbiota transplantation (FMT), probiotics, and dietary modifications, while addressing key controversies and knowledge gaps. Advances in microbiome sequencing and artificial intelligence may facilitate personalized interventions. Standardizing microbiota-based diagnostics and validating their efficacy in clinical trials are crucial for their integration into ITP management. Bridging these gaps may lead to microbiota-driven strategies that enhance immune regulation and improve patient outcomes.

KEYWORDS

primary immune thrombocytopenia (ITP), gut-immune axis, immune dysregulation, microbiota-based therapy, platelet homeostasis

Highlights

- The gut-immune axis influences ITP pathogenesis and platelet homeostasis.
- Dysbiosis disrupts immune regulation and drives disease progression.
- FMT, probiotics, and dietary interventions offer potential ITP therapies.

- Microbiome sequencing and AI may advance personalized treatments.
- Standardization and clinical validation are crucial for microbiota-based strategies.

1 Introduction

Primary immune thrombocytopenia (ITP) is an acquired autoimmune disorder characterized by a persistent low platelet count due to immune-mediated platelet destruction and impaired platelet production (1, 2). While immunosuppressive therapies, such as corticosteroids and intravenous immunoglobulin (IVIG), are commonly used, their effectiveness varies, and long-term management remains challenging (3, 4). In particular, the frequent need for alternative therapeutic strategies in refractory cases underscores the need for a deeper understanding of ITP pathogenesis and the development of novel treatment approaches.

Traditionally, ITP is associated with autoantibody-mediated platelet destruction, where antibodies target platelet surface glycoproteins such as GPIIb/IIIa and GPIb/IX (5–7). However, emerging evidence reveals a more complex pathophysiology involving dysregulated T-cell responses, pro-inflammatory cytokines, and defective megakaryopoiesis. An imbalance between regulatory T cells (Tregs) and effector T cells (Th1 and Th17) drives persistent inflammation and immune-mediated platelet destruction, underscoring the multifaceted nature of ITP pathogenesis (8, 9). Notably, a reduction or dysfunction of Tregs is associated with the induction of ITP, whereas their expansion or restoration is considered immunoprotective, restoring immune tolerance and suppressing autoreactive responses against platelets (10).

Beyond these immune mechanisms, recent research suggests that gut microbiota plays a crucial role in shaping immune responses in ITP (11). The gut-immune axis, which governs interactions between intestinal microbiota and systemic immunity, has been implicated in various autoimmune diseases, including systemic lupus erythematosus and rheumatoid arthritis (RA) (12–14). Dysbiosis, an imbalance in gut microbial composition, has been shown to drive immune dysregulation, promote inflammation, and influence hematologic conditions (15–17). However, the precise role of the gut microbiota in ITP pathogenesis remains unclear, presenting a significant knowledge gap.

A key controversy center on whether alterations in the gut microbiota are a consequence of immune dysfunction in ITP or an independent driver of disease progression (18). Additionally, while microbiota-targeted interventions such as fecal microbiota transplantation (FMT) and probiotics have shown promise in other autoimmune conditions, their therapeutic potential in ITP remains largely unexplored (19, 20). These gaps highlight the need for further investigation into the mechanisms linking gut dysbiosis to ITP pathogenesis and treatment response.

This review therefore comprehensively analyzes the gut-immune axis in ITP, critically evaluating current knowledge and identifying key unanswered questions. We explore the potential of microbiota-targeted therapies to restore immune homeostasis, highlight areas of consensus and controversy, and propose directions for future research. By synthesizing available evidence and pinpointing knowledge gaps, we aim to advance the understanding of gut microbiota's role in ITP and its implications for novel therapeutic strategies.

Abbreviations: AhR, aryl hydrocarbon receptor; AI, artificial intelligence; Bregs, regulatory B cells; FMT, fecal microbiota transplantation; GALT, gut-associated lymphoid tissue; GPIb/IX, glycoprotein Ib/IX; GPIIb/IIIa, glycoprotein IIb/IIIa; IBD, inflammatory bowel disease; IFN-γ, interferon-gamma; IL-6, interleukin-6; ITP, immune thrombocytopenia; IVIG, intravenous immunoglobulin; MAMPs, microbial-associated molecular patterns; NK, natural killer; PRRs, pattern recognition receptors; RA, rheumatoid arthritis; SCFA, short-chain fatty acid; SLE, systemic lupus erythematosus; Th1, T helper 1 cells; Th17, T helper 17 cells; TLRs, toll-like receptors; TME, tumor microenvironment; TNF-α, tumor necrosis factor-alpha; TPO-RA, thrombopoietin receptor agonists; Tregs, regulatory T cells.

2 The gut-immune axis: a new perspective in ITP pathogenesis

Recent advancements in immunology and microbiome research have revealed the intricate interplay between the gut microbiota and systemic immune regulation (21, 22). This relationship is particularly relevant in autoimmune disorders such as primary ITP, where immune dysregulation leads to platelet destruction. The gut-immune axis serves as a dynamic interface between microbial communities and immune homeostasis, influencing inflammation and hematologic balance (22). Although disruptions in this axis have been implicated in several autoimmune diseases, their precise role in hematologic diseases like ITP remains poorly understood, underscoring significant gaps in current knowledge (19, 23).

2.1 Overview of the gut microbiota and immune system interactions

The gut microbiota, comprising a diverse and dynamic ecosystem of bacteria, viruses, fungi, and archaea, interacts closely with the immune system to maintain immune homeostasis. Gut-associated lymphoid tissue (GALT) plays a pivotal role in sensing microbial antigens and orchestrating immune responses (24). Dendritic cells (DCs) sample microbial metabolites and antigens, directing the differentiation of naïve T cells into Tregs or effector T cells (Th1 and Th17) (25, 26). This interaction promotes immune tolerance while maintaining a controlled inflammatory response.

However, the precise role of the gut microbiota in modulating hematologic immune responses remains controversial (22, 27). While some studies suggest that gut microbial communities influence immune regulation through cytokine production, others propose that the immune system primarily shapes microbiota composition (28, 29). This bidirectional relationship underscores the complexity of gut-immune interactions and necessitates further investigation in the context of ITP.

The intricate interactions between the gut microbiota and the immune system, including the influence of both commensal and pathogenic microbes in shaping immune tolerance, modulating inflammatory responses, and regulating systemic immune regulation, is critical to understanding hematologic disorders such as ITP. These mechanisms are visually summarized in Figure 1.

2.2 Mechanisms of gut microbiota in immune homeostasis and the role of B cells in ITP

The gut microbiota plays a pivotal role in immune regulation through multiple interconnected mechanisms, influencing both systemic and hematologic immune responses (12). Key pathways include microbial metabolite production, pattern recognition receptor (PRR) signaling, and maintenance of intestinal barrier integrity, each impacting immune homeostasis and ITP disease progression (30).

One essential pathway involves the production of microbial metabolites, particularly short-chain fatty acids (SCFAs) such as butyrate, propionate, and acetate, generated by commensal bacteria fermenting dietary fiber (31). SCFAs enhance regulatory T cell (Treg) differentiation, suppress inflammatory cytokines (IL-6, TNF-α, IFN-γ), and promote anti-inflammatory responses both systemically and within the gut (32). A reduction in SCFA-producing bacteria has been linked to impaired immune tolerance, heightened inflammation, and increased risk of autoimmunity, suggesting a potential link between dysbiosis and ITP pathogenesis (33, 34). Importantly, diminished Treg frequency or function is associated with ITP induction, whereas restoration or expansion of Tregs has been shown to confer protective effects by

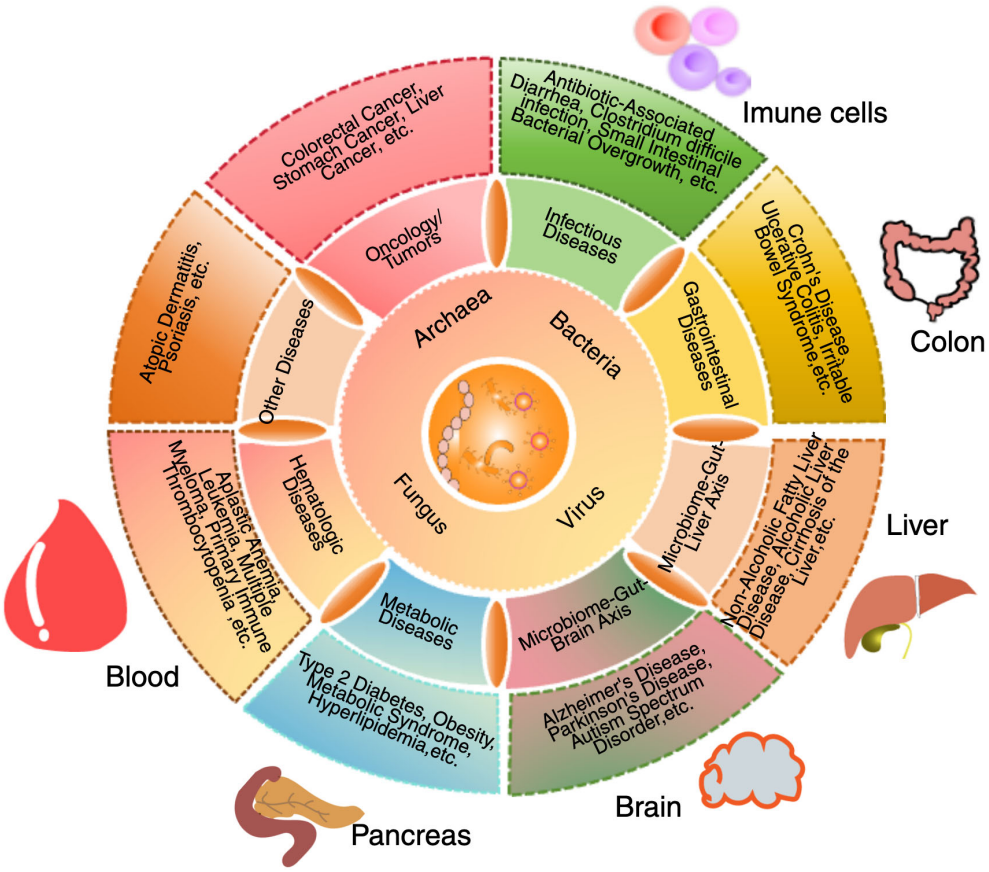


FIGURE 1
Interactions between gut microbiota, systemic organs and the immune system. The gut microbiota plays a central role in regulating immune function, metabolism, and overall systemic health through interactions with multiple organ systems. This schematic illustration depicts how bacteria, viruses, fungi, and archaea influence host physiology by modulating immune responses, metabolic processes, and disease development. Dysbiosis has been implicated in various conditions, including autoimmune diseases, neurodegenerative disorders, metabolic syndromes, and inflammatory diseases. Key organ systems affected by alterations in microbiota include the immune system, colon, liver, and pancreas. In immune system, the gut microbiota regulates Tregs, Th1/Th17 responses, and inflammation. In the colon, microbial communities influence gut barrier integrity and are associated with inflammatory bowel diseases. In the liver, the gut-derived metabolites affect metabolic conditions such as non-alcoholic fatty liver disease (NAFLD). In the pancreas, microbiota alterations have been associated with type 2 diabetes and other metabolic disorders. In the bloodstream, microbial dysbiosis is linked to hematologic conditions, including immune thrombocytopenia (ITP). In the brain, microbiota-derived metabolites have been implicated in neurological conditions such as Alzheimer's disease, depression, and neuroinflammation. By modulating microbial composition and function, the gut microbiota exerts local and systemic effects that contribute to immune homeostasis, disease pathogenesis, and potential therapeutic interventions.

re-establishing immune homeostasis and attenuating platelet destruction (35).

Another critical mechanism is PRR signaling, where Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) recognize microbe-associated molecular patterns (MAMPs) to distinguish commensal bacteria from pathogens (36, 37). This interaction normally regulates immune tolerance by limiting unnecessary immune activation (38). However, in gut dysbiosis, aberrant PRR signaling may trigger chronic immune activation, disrupting the balance between Tregs/effector T cells (Th1/Th17) and promoting a pro-inflammatory milieu that exacerbates platelet destruction in ITP (39, 40).

Furthermore, the gut microbiota maintains intestinal barrier integrity, preventing the translocation of microbial components into systemic circulation (41). Tight junction proteins (occludin, claudin, zonulin) regulate intestinal permeability, preventing leakage of bacterial endotoxins e.g., lipopolysaccharides (LPS) into the bloodstream (41, 42). Dysbiosis-induced compromise of the gut barrier can elevate circulating LPS levels, trigger systemic inflammation, and aberrantly activate monocytes and DCs factors that may contribute to immune dysregulation and platelet destruction in ITP (11, 43).

Although these mechanisms highlight the immunomodulatory potential of the gut microbiota, key controversies remain regarding its specific role in ITP (12, 44). For instance, SCFA's effects on immune tolerance appear to be context-dependent, varying with disease state, microbial composition, and host genetic factors (45, 46). Similarly, while PRR signaling is crucial for immune surveillance, its dysbiosis-related overactivation has been associated with both immune suppression and chronic inflammation, resulting in conflicting findings in hematologic disorders (22). This lack of consensus underscores the need for further research to clarify how gut microbiota alterations influence platelet homeostasis and immune regulation in ITP (19, 47).

In addition to T cell-mediated pathways, B cells, particularly regulatory B cells (Bregs) have emerged as important players in maintaining immune tolerance in autoimmune disorders, including ITP (48). Bregs exert their immunosuppressive effects primarily through IL-10 production, which inhibits pro-inflammatory T cell responses and promotes Treg development (49). Dysregulation of Bregs has been observed in ITP patients, suggesting that impaired Breg function may contribute to loss of peripheral tolerance and heightened platelet destruction (50). Gut microbiota has been shown to modulate Breg development and function, likely via microbial metabolites and PRR signaling. Thus, altered microbial composition in ITP may impair Breg-mediated suppression of autoimmunity, further implicating the gut-immune axis in disease pathogenesis (51).

2.3 Dysbiosis and its impact on autoimmunity and hematologic diseases

Dysbiosis, defined as a disruption in gut microbial composition, has been implicated in numerous autoimmune and hematologic disorders (16, 52). Studies have demonstrated that conditions such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and multiple sclerosis (MS) exhibit characteristic microbial imbalances, often marked by an overrepresentation of pro-inflammatory species (e.g., Prevotella, Ruminococcus) and a concurrent depletion of beneficial bacteria (e.g., Bifidobacterium, Lactobacillus) (53, 54). These alterations are associated with immune dysregulation, excessive cytokine production, and chronic inflammation, all of which contribute to disease progression (55).

In hematologic disorders, gut dysbiosis has been increasingly associated with the breakdown of immune tolerance, platelet dysregulation, and systemic inflammation (19, 52). Certain bacterial taxa, such as *Enterobacteriaceae* and *Prevotella*, have been associated with an elevated pro-inflammatory Th17 response, promoting systemic inflammation and disrupting hematologic homeostasis (41, 56). Conversely, commensal bacteria like *Bifidobacterium* and *Lactobacillus* have been shown to enhance regulatory T cell (Treg) activity, mitigate excessive immune activation, and support immune balance (57, 58). The depletion of these beneficial microbes in patients with ITP suggests a potential role of the gut microbiota in modulating platelet homeostasis; however, direct causal relationships have yet to be confirmed (19, 47).

Beyond immune cell modulation, dysbiosis is associated with metabolic shifts that further influence immune responses (59, 60). A decrease in SCFA-producing bacteria, such as *Bacteroides* and *Firmicutes*, correlates with reduced Treg activity, impaired immune regulation, and increased inflammation, all of which are observed in autoimmune conditions (32, 61). Additionally, altered bile acid metabolism and tryptophan catabolism can modulate T cell differentiation, cytokine production, and systemic immune responses, potentially exacerbating platelet destruction in ITP (32, 62). However, whether these microbial and metabolic changes are a cause or consequence of immune dysregulation in ITP remains unclear, necessitating longitudinal studies and mechanistic research to establish causality.

A growing body of evidence suggests that distinct microbiota alterations are shared among various autoimmune diseases, highlighting common patterns of dysbiosis that may drive immune dysregulation across multiple conditions (12, 44). Table 1 summarizes key microbial alterations in autoimmune diseases, including ITP, and their associated immune effects.

2.4 Evidence linking gut microbiota alterations to ITP development and severity

Emerging research suggests that alterations in gut microbiota may contribute to the pathogenesis of ITP; however, findings remain inconsistent (18, 69). Comparative studies of the gut microbiomes of ITP patients and healthy controls have identified notable microbial differences (52, 70). Specifically, a reduction in SCFA-producing bacteria, such as *Bacteroides* and *Firmicutes*, may impair immune tolerance mechanisms, potentially exacerbating autoimmune

TABLE 1 Key microbiota alterations in autoimmune diseases and ITP.

Disease	Microbial changes	Immune impact	References
Systemic lupus erythematosus (SLE)	Decreased *firmicutes*, increased *bacteroidetes*	Elevated Th17 responses, decreased Tregs	(60, 63)
Rheumatoid arthritis (RA)	Increased *Prevotella copri*, reduced SCFA-producing bacteria	Pro-inflammatory cytokine production (IL-6, TNF-α)	(64, 65)
Multiple sclerosis (MS)	Reduced *akkermansia*, increased pro-inflammatory taxa	Enhanced Th1/Th17 responses	(66, 67)
Primary immune thrombocytopenia (ITP)	Decreased *bacteroides* and *firmicutes*, increased *enterobacteriaceae*	Impaired immune tolerance, platelet destruction	(19, 68)

responses (12, 46). Concurrently, an increase in pro-inflammatory taxa, including *Escherichia coli* and *Enterobacteriaceae*, has been associated with heightened Th17 responses and systemic inflammation (71). Additionally, experimental models suggest that antibiotic-induced dysbiosis can alter platelet counts and immune responses, reinforcing a potential link between gut microbiota and ITP progression (19, 72).

Despite these compelling findings, several critical gaps remain in the literature (11, 47, 72–74). Many studies are limited by small sample sizes, cross-sectional designs, and a lack of longitudinal analyses, making it difficult to establish causality between gut dysbiosis and ITP development (74). To clarify the causal relationship between gut dysbiosis and ITP, recent proposals emphasize the need for prospective longitudinal studies that track microbiota composition before and after disease onset or treatment (72). Additionally, mechanistic investigations using gnotobiotic mouse models may help elucidate how specific microbial taxa and metabolites influence immune regulation and platelet homeostasis (75, 76). Furthermore, while microbiota-targeted therapies such as FMT and probiotics have shown promise in other autoimmune diseases, their efficacy and applicability in ITP remain largely unexplored (77). Emerging studies indicate that microbiota-driven systemic metabolic changes can influence immune regulation in diseases beyond the gut, as seen in brain metastasis, where alterations in the gut microbiome affect tumor progression via the gut-to-brain axis (78).

To address these knowledge gaps, future research should focus on three key areas. First, longitudinal studies are needed to investigate how changes in gut microbiota correlate with the onset, severity, and treatment response of ITP over time (19). Second, mechanistic studies should explore the causal relationships between specific microbial taxa, microbial metabolites, immune dysregulation, and platelet homeostasis (47). Lastly, controlled clinical trials are essential to assess the therapeutic potential of microbiota-targeted interventions, including probiotics, prebiotics, dietary modifications, and FMT, in the management of ITP (11, 79).

By addressing these critical gaps, researchers can determine whether modulating the gut microbiota represents a viable therapeutic avenue for improving immune regulation and clinical outcomes in patients with ITP (80).

3 Gut microbiota modulation in ITP: a novel therapeutic strategy

Given the emerging evidence linking gut microbiota dysbiosis to immune dysregulation in ITP, researchers have begun exploring microbiota-targeted interventions as potential therapeutic strategies (19, 81). By restoring gut microbial balance, these approaches aim to modulate immune responses, promote immune tolerance, and mitigate the pathogenic mechanisms underlying ITP. Several microbiota-based interventions, including fecal FMT, probiotics, dietary modifications, and microbiome-targeted pharmacologic strategies, have shown promise in modulating the gut-immune axis (82, 83). However, the clinical translation of these therapies remains challenging due to a limited mechanistic understanding and the need for well-designed clinical trials.

Various microbiota-targeted therapies have been proposed as potential interventions for ITP, aiming to restore microbial balance and modulate immune function (11, 84). These strategies include FMT, probiotics, prebiotics, SCFA supplementation, and bile acid modulation (85, 86). While some have shown promise in autoimmune diseases, their application in ITP remains underexplored. The following Table 2 summarizes the mechanisms, current evidence, and challenges associated with these microbiota-based therapies.

3.1 Fecal microbiota transplantation: mechanisms, clinical applications, and emerging evidence in ITP

FMT has gained attention as a promising approach to modulating gut microbiota composition (87). This procedure involves transferring fecal material from a healthy donor to a recipient, aiming to restore microbial diversity and improve immune homeostasis. Studies suggest that FMT can replenish beneficial taxa such as *Bacteroides* and *Firmicutes*, which are associated with immune tolerance (88). Originally developed for treating recurrent *Clostridioides difficile* infections, FMT has shown potential in various autoimmune and inflammatory diseases, including ITP (81).

TABLE 2 Potential microbiota-targeted therapies for ITP.

Therapeutic Approach	Mechanism of Action	Current Evidence	Challenges & Limitations
Fecal Microbiota Transplantation (FMT)	Restores microbial diversity, promotes immune tolerance	Small studies suggest platelet improvement in ITP	Donor variability, safety concerns, regulatory issues
Probiotics	Enhances Treg activity, reduces inflammatory cytokines	Effective in other autoimmune diseases; underexplored in ITP	Strain specificity, inconsistent responses
Prebiotics	Supports beneficial bacterial growth, increases SCFA production	Some evidence in metabolic disorders and autoimmunity	Need for targeted prebiotics in ITP
SCFA Supplementation	Directly modulates immune responses via gut-immune axis	Butyrate shows promise in reducing inflammation	Bioavailability and dosing challenges
Bile Acid Modulation	Regulates T-cell differentiation and inflammatory pathways	Early-stage research in gut-immune interactions	Requires further validation in hematologic diseases

Restoring gut microbial diversity and modulating immune responses through fecal microbiota transplantation (FMT) has emerged as a promising therapeutic strategy to improve disease outcomes, as illustrated in Figure 2. This schematic highlights the key mechanisms through which FMT may contribute to immune homeostasis, including shifts in microbial composition, an increase in the activity of Tregs, and a reduction in systemic inflammation.

FMT modulates immune responses through several mechanisms, including the enhancement of Tregs, increased *Bifidobacterium* abundance, and suppression of pro-inflammatory Th1 and Th17 responses, as observed in autoimmune conditions such as multiple sclerosis and inflammatory bowel disease (IBD) (12, 46). Additionally, microbial metabolites such as SCFAs contribute to immune modulation by strengthening the intestinal barrier and suppressing excessive immune activation (89, 90).

The gut microbiota also influences systemic immune function, as demonstrated by recent findings on the gut-brain axis, where microbiota-derived metabolites, such as kynurenic acid, impact immune interactions at distant sites. This highlights the broader systemic effects of gut microbiota and underscores the need for further investigation into microbiota-targeted therapies in autoimmune conditions like ITP (78).

Emerging evidence suggests that gut microbiota may directly influence platelet regulation. Certain microbial metabolites, including SCFAs and secondary bile acids, have been shown to modulate platelet function by affecting megakaryocyte differentiation and platelet activation (11, 91). Moreover, gut dysbiosis has been associated with increased platelet aggregation and altered hemostatic balance in other diseases, suggesting that restoring microbial equilibrium through FMT could contribute to improved platelet homeostasis (92). While direct evidence of FMT's effect on platelet regulation in ITP is limited, case reports and small clinical studies have observed platelet count improvement following microbiota restoration therapies (19, 72).

Despite these promising findings, several challenges remain in translating FMT into a standardized treatment for ITP. Donor microbiota variability and long-term engraftment pose significant challenges, as microbial compositions differ significantly between donors and recipients, potentially affecting therapeutic outcomes. Additionally, safety concerns include the risk of pathogen transmission, unintended immune activation, and unpredictable long-term effects (93). Therefore, well-controlled, randomized clinical trials are necessary to confirm the efficacy and safety of FMT in ITP management and to determine optimal protocols for donor selection, microbiota preparation, and recipient response monitoring. Addressing these issues is critical for integrating FMT into mainstream ITP treatment (88).

3.2 Probiotics and prebiotics: potential to restore gut balance and regulate immune responses

Probiotics and prebiotics represent another avenue for modulating gut microbiota in ITP (21, 47). Probiotics, including specific *Lactobacillus* and *Bifidobacterium* strains, enhance beneficial bacterial populations, while prebiotics, such as inulin and fructooligosaccharides, selectively stimulate microbial growth, promoting gut-immune homeostasis (94). However, the immunomodulatory effects of probiotics are highly strain-dependent, with some strains exerting potent anti-inflammatory properties, while others may have limited or even opposing effects in different individuals (95).

Clinical studies suggest that *Lactobacillus rhamnosus* and *Bifidobacterium breve* increase Treg activity while reducing IL-6 and TNF-α, leading to decreased systemic inflammation in autoimmune diseases (96). Additionally, *Lactobacillus plantarum* has been shown to enhance gut barrier integrity by upregulating tight junction proteins, thereby preventing bacterial translocation and reducing systemic immune activation (97). Similarly, prebiotics, including inulin and fructooligosaccharides, serve as metabolic substrates for beneficial bacteria, fostering a gut environment that supports immune homeostasis (98). The combination of specific probiotic strains with targeted prebiotic supplementation (synbiotics) may offer enhanced therapeutic potential by optimizing microbial colonization and metabolic activity (99).

10.3389/fimmu.2025.1595977

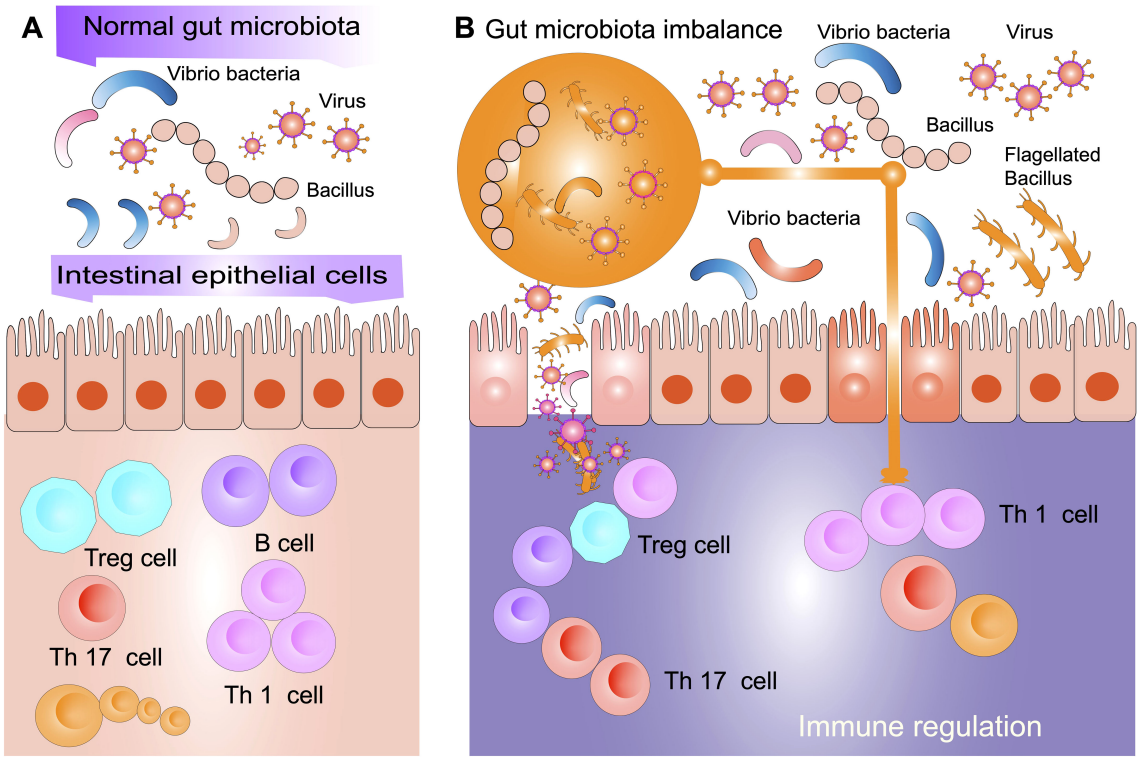


FIGURE 2
Gut microbiota balance and immune modulation. **(A)** In a normal gut microbiota environment, commensal bacteria such as *Vibrio* and *Bacillus* interact with intestinal epithelial cells to maintain immune homeostasis. This balance promotes the differentiation of regulatory T cells (Treg) and immune tolerance while preventing excessive inflammation driven by Th1 and Th17 cells. A balanced gut microbiota contributes to a well-regulated immune system by enhancing gut barrier integrity and modulating host immune responses. **(B)** In a state of gut microbiota imbalance (known as dysbiosis), there is an overrepresentation of pathogenic bacteria and viruses, along with a reduction in beneficial microbial populations. This microbial shift disrupts gut barrier function, leading to increased bacterial translocation and heightened immune activation. Dysbiosis skews immune regulation by reducing Treg activity and increasing Th1 and Th17 responses, promoting systemic inflammation and immune dysregulation. Such alterations in the gut microbiota may contribute to autoimmune conditions, including ITP, by exacerbating inflammatory pathways and impairing immune tolerance.

Despite promising preclinical and clinical evidence supporting probiotic use in other autoimmune diseases, their application in ITP remains underexplored (100). One of the major challenges is the variability in probiotic efficacy, which stems from strain-specific effects, inter-individual differences in microbiota composition, and inconsistencies in host immune responses (101). For instance, while *Bifidobacterium adolescentis* has been shown to reduce Th17-mediated inflammation in rheumatoid arthritis, similar effects have not been confirmed in ITP (102). Additionally, the interaction between probiotics and endogenous microbial communities can lead to variable colonization success, limiting the predictability of therapeutic outcomes (103).

Future research should focus on defining optimal probiotic formulations, identifying microbial signatures predictive of response, and evaluating their impact on platelet homeostasis and immune regulation (69). Advancements in microbiome sequencing and metabolomic profiling may enable the development of personalized microbiota-based interventions, tailoring probiotic and prebiotic therapies to individual ITP patients for improved efficacy (104).

3.3 Dietary and metabolomic interventions: role of SCFAs, bile acids, and tryptophan metabolites in immune modulation

Dietary interventions play a pivotal role in shaping gut microbiota composition and function (87). Specific dietary components influence the production of key microbial metabolites, which, in turn, modulate immune responses (105). Among these, SCFAs, bile acids, and tryptophan-derived metabolites are particularly significant in maintaining immune homeostasis and resolving inflammation (105). However, while their immunoregulatory effects have been well characterized in autoimmune diseases, their specific impact on platelet regulation in ITP remains an emerging area of research.

SCFAs, including butyrate and propionate, are microbial fermentation products that exert profound immunomodulatory effects (53). These metabolites enhance regulatory T cell (Treg) differentiation and inhibit pro-inflammatory cytokines such as IL-6, TNF-α, and IFN-γ, thereby reducing autoimmune activity (106). Butyrate, in particular, acts as a histone deacetylase inhibitor,

promoting Treg expansion and suppressing Th17-mediated inflammation, mechanism relevant to platelet destruction in ITP (69, 107). Additionally, SCFAs strengthen gut barrier integrity by upregulating tight junction proteins, thereby reducing microbial translocation and systemic immune activation factors that may contribute to excessive immune responses in ITP (62, 69).

Recent studies suggest that lower SCFA levels in autoimmune conditions are linked to immune dysregulation and altered thrombopoiesis (108, 109). Butyrate has been shown to reduce megakaryocyte apoptosis, potentially impacting platelet production and turnover (110). Additionally, SCFA supplementation has demonstrated protective effects on platelet homeostasis in inflammatory conditions, indicating a possible therapeutic avenue for ITP (32, 108).

Bile acids, traditionally recognized for their role in lipid metabolism, have recently emerged as key modulators of immune responses, influencing T-cell differentiation via interactions with gut microbiota (111). Secondary bile acids, such as deoxycholic acid and lithocholic acid, regulate immune pathways through host receptors, including the Farnesoid X receptor (FXR) and Takeda G-protein-coupled receptor 5 (TGR5) (112). FXR activation suppresses Th17 cell differentiation, thereby reducing inflammatory cytokine production, a process that could mitigate platelet autoantibody formation in ITP (113). TGR5 signaling has been shown to enhance Treg function, contributing to immune tolerance and reducing autoimmunity in conditions such as SLE and RA (8, 114).

Alterations in bile acid metabolism have been identified in ITP patients, suggesting a potential role in immune dysregulation (47). Recent studies indicate that bile acid supplementation can modulate thrombopoiesis, potentially linking gut microbiota-derived bile acids to platelet production and function (115). These findings support the investigation of bile acid-targeted therapies in ITP as a novel immunomodulatory approach (11).

Tryptophan metabolism also plays a crucial role in immune homeostasis, with its metabolites modulating immune responses via the aryl hydrocarbon receptor (AhR) and indoleamine 2,3-dioxygenase (IDO) pathways (116). Kynurenine, an AhR ligand, has been shown to promote Treg differentiation, enhancing immune tolerance and reducing autoimmune activity (117). Indole derivatives regulate Th17 differentiation, thereby controlling inflammatory responses that may drive platelet destruction in ITP (2, 118). Additionally, serotonin, a tryptophan metabolite, has been implicated in platelet aggregation, further underscoring the potential gut-immune-thrombosis axis in ITP (11, 43). Dysregulation of tryptophan metabolism in autoimmune conditions suggests a possible link to ITP pathophysiology, with recent findings highlighting microbiota-mediated tryptophan metabolism as a key factor in hematologic disorders (119).

Despite growing interest in dietary interventions, their role in ITP remains speculative (120). Limited clinical data exist regarding the effects of specific dietary modifications on platelet counts and immune function in ITP patients (121). Additionally, dietary interventions may have variable effects depending on individual microbiota composition and metabolic responses (122). Future research should prioritize characterizing microbial metabolite profiles in ITP patients to identify potential therapeutic targets, investigating the roles of SCFAs, bile acids, and tryptophan metabolites in modulating platelet regulation and immune responses. It should also assess the clinical efficacy of dietary interventions, including probiotic and prebiotic supplementation, as well as bile acid modulation, through well-designed randomized controlled trials (47, 123). By integrating microbiome sequencing and metabolomic profiling, researchers can better define the role of microbial metabolites in platelet function and immune modulation, leading to potential novel therapeutic approaches in ITP (52, 79).

3.4 Pharmacologic strategies targeting the gut: microbiome-based drug development

Pharmacologic approaches to modulating the gut microbiota are emerging as a potential strategy for treating autoimmune diseases, including ITP (12). These approaches include microbiome-based small molecules, postbiotics, and engineered probiotics designed to selectively modulate microbial communities and immune function (21).

Postbiotics, which are bioactive compounds produced by beneficial bacteria, have shown promise in immune modulation without the need for live microorganisms (124). Microbiome-based small molecules are being developed to target specific microbial metabolic pathways that influence immune responses (125). Additionally, engineered probiotics are being designed to deliver immunomodulatory molecules directly within the gut, offering a targeted approach to restoring immune balance (126).

While microbiome-based drug development is still in its early stages, these strategies hold great potential for providing precise and effective treatments for ITP (127). However, key challenges include ensuring microbial stability, understanding long-term safety, and optimizing drug delivery systems (128). Future studies should investigate the pharmacokinetics and pharmacodynamics of these microbiome-based therapies in ITP, as well as their potential for integration with standard treatments (129).

4 Challenges and limitations in gut microbiota therapy for ITP

Despite the growing interest in microbiota-targeted therapies for ITP, several challenges and limitations must be addressed before these strategies can be effectively translated into clinical practice. While preclinical and early clinical studies suggest that microbiota modulation may help restore immune tolerance and regulate platelet homeostasis (130), inconsistencies in research findings, methodological limitations, and unresolved safety concerns remain key barriers. This section critically examines the current knowledge, debates ongoing controversies, and identifies gaps that future research should address.

4.1 Variability in gut microbiota composition among individuals

One of the fundamental challenges in microbiota-based therapies is the high degree of inter-individual variability in gut microbial composition (131). Factors such as genetics, diet, medication history (including prior antibiotic use), and environmental influences contribute to significant differences in microbial diversity and function (132). This variability complicates the standardization of microbiota-based interventions, as a treatment effective for one patient may not yield similar benefits for another (133). Additionally, baseline microbiota differences may influence therapeutic responses, highlighting the need for patient-specific approaches. Future research should focus on stratifying patient populations based on microbiome profiling to optimize treatment efficacy (134).

4.2 Standardization and safety concerns in FMT and microbiota-based interventions

FMT has shown promise as a microbiota-based intervention in autoimmune diseases, but its application in ITP remains largely experimental (135). One major concern is the lack of standardization in FMT protocols, including donor selection, preparation methods, and delivery routes (87). Donor microbiota composition can vary significantly, leading to inconsistent therapeutic outcomes (136). Additionally, potential risks associated with FMT include the transmission of infectious agents, unintended immune activation, and long-term alterations in gut microbiota that may have unpredictable consequences (137).

Beyond FMT, the safety profile of probiotics and prebiotics in ITP patients has not been rigorously evaluated (69). While some probiotic strains exhibit immunomodulatory properties, others may provoke excessive immune activation or lead to bacterial overgrowth, particularly in immunocompromised individuals (138). To address these safety concerns, further clinical trials with well-defined protocols are needed to assess the risks and benefits of microbiota-targeted therapies in ITP.

4.3 Need for robust clinical trials and biomarker discovery in ITP-microbiota research

Although emerging studies suggest a link between gut dysbiosis and ITP, most existing research relies on small-scale, cross-sectional studies with limited statistical power (139). Longitudinal studies are needed to investigate how changes in gut microbiota correlate with the onset, severity, and treatment response of ITP over time. Second, mechanistic studies should explore the causal relationships determine whether gut microbiota alterations precede ITP onset or arise as a consequence of the disease and its treatments (18). Furthermore, the identification of reliable microbial biomarkers for disease progression and treatment response remains a significant gap in current research (140). Developing standardized methods for microbiome analysis, including metagenomic sequencing and metabolomic profiling, could help establish microbiota-based diagnostic and prognostic tools for ITP (141).

Clinical trials evaluating microbiota-targeted interventions in ITP are also lacking (52). While probiotics, prebiotics, dietary modifications, and FMT have been explored in other autoimmune conditions, few studies have directly assessed their impact on platelet counts and immune regulation in ITP patients. Large-scale, randomized controlled trials are essential to determine the efficacy, safety, and durability of these interventions in a hematologic context (142).

4.4 Ethical and regulatory considerations in applying microbiota therapies to hematologic disorders

The integration of microbiota-based therapies into hematologic disease management raises several ethical and regulatory challenges (143). Unlike conventional pharmacologic agents, microbiota-based interventions involve live organisms, making it difficult to define consistent dosing, manufacturing processes, and quality control standards (69). Regulatory agencies, such as the FDA and EMA, currently classify FMT as an investigational therapy, necessitating rigorous oversight before its widespread adoption in ITP treatment (77).

Additionally, ethical concerns related to FMT donor selection, consent processes, and long-term safety monitoring must be addressed (87). Patients undergoing microbiota-based treatments should be informed of potential risks, including unforeseen immune complications or persistent microbiome alterations (144). Establishing regulatory frameworks that balance innovation with patient safety will be crucial in advancing microbiota-targeted therapies in ITP (129).

To overcome these limitations, future research should focus on developing standardized microbiota-based protocols by establishing guidelines for donor screening, sample preparation, and treatment administration to improve the reproducibility and safety of microbiota-based therapies (93). Additionally, personalizing microbiota interventions through microbiome sequencing and precision medicine approaches can help tailor treatments based on individual microbiota profiles, thereby increasing therapeutic efficacy (145). Conducting large-scale, controlled clinical trials will be essential for evaluating the long-term impact of microbiota-targeted therapies in ITP and ensuring their clinical adoption (84). Furthermore, clarifying the mechanisms of action through further research is necessary to elucidate how specific microbial taxa and metabolites influence platelet regulation and immune responses in ITP (11). Addressing these aspects will provide a stronger foundation for the integration of microbiota-based therapies into ITP management (96). By tackling these challenges and knowledge gaps, microbiota-targeted interventions may become a viable and evidence-based approach for managing ITP, complementing

existing immunomodulatory treatments and improving patient outcomes.

5 Future directions and clinical translation

While significant strides have been made in understanding the gut-immune axis in ITP, translating these findings into effective clinical applications remains a challenge (18). Several critical gaps must be addressed, including the lack of standardized microbiota profiling in clinical practice, variability in treatment responses, and the need for robust clinical trials to validate microbiota-based interventions (146). This section highlights the key areas of future research that could bridge these gaps and facilitate the integration of microbiota-targeted therapies into mainstream ITP management.

5.1 Integrating microbiota profiling in ITP diagnosis and prognosis

One promising avenue for advancing ITP management is the incorporation of microbiota profiling into diagnostic and prognostic assessments. Given the increasing evidence that gut microbiota composition influences immune responses and disease severity, recent studies have identified microbial signatures linked to hematologic diseases, where altered *Bacteroides* and *Enterobacteriaceae* profiles predict disease progression, suggesting potential biomarker applications for ITP (19, 147).

Recent advancements in microbiome sequencing have facilitated the identification of microbial alterations linked to systemic disease progression (140). For instance, studies in brain metastases have demonstrated how gut microbiota composition can influence disease dynamics, underscoring the potential of microbiota profiling as a valuable tool for diagnosing and monitoring immune-mediated disorders such as ITP (78). Metagenomic sequencing enables high-resolution characterization of microbial taxa, revealing microbiota-related risk factors predictive of autoimmune disease severity (148).

An overview of microbiota-based biomarkers and diagnostic approaches in ITP, including key microbial alterations, sequencing methodologies, and their potential clinical applications, is presented in Figure 3. This schematic highlights how microbiota profiling could be integrated into routine diagnostic workflows to enhance precision medicine approaches in ITP.

Despite these advances, specific microbial signatures distinguishing ITP from other autoimmune and hematologic diseases remain unclear. While some studies suggest that gut dysbiosis may contribute to platelet regulation and immune modulation in ITP, no definitive microbial biomarkers have been validated for ITP diagnosis or disease stratification (11, 19). Furthermore, the high inter-individual variability in gut microbiota composition complicates the reproducibility and clinical application of microbiome-based diagnostics (12, 131).

The application of microbiota profiling in ITP diagnosis presents both opportunities and challenges (18, 19). Table 3 summarizes the advantages and limitations of various microbiome-based diagnostic approaches, highlighting their potential role in disease monitoring and personalized treatment strategies.

While microbiota profiling holds promise, its clinical relevance in ITP remains uncertain. Studies suggest an association between gut dysbiosis and platelet regulation in ITP, but causal mechanisms remain unclear, necessitating longitudinal studies to establish microbiota-driven disease modulation (19, 47). Additionally, the standardization of sampling methods, data interpretation, and validation across different populations is needed before microbiota profiling can be reliably incorporated into clinical practice (149).

To advance microbiota profiling as a diagnostic tool in ITP, future research should focus on identifying robust microbial biomarkers specific to ITP that distinguish it from other hematologic and autoimmune disorders (150); conducting large-scale, multi-cohort microbiome studies to evaluate consistent microbial signatures across diverse populations (151); integrating microbiome data with clinical parameters and multi-omics approaches (e.g., metabolomics, proteomics) to refine diagnostic accuracy (152); and developing standardized guidelines for microbiome-based clinical diagnostics to ensure reproducibility and regulatory approval for ITP diagnosis (153).

5.2 Personalized microbiota-based therapies for ITP management

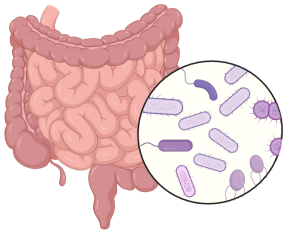
The concept of personalized microbiota-based therapies is gaining traction as an alternative or adjunctive strategy for ITP management (19). Given the variability in microbiota composition among individuals, tailoring interventions based on a patient's specific microbial profile may enhance treatment efficacy (82). Personalized approaches may include selective probiotic formulations, prebiotic-enriched diets, or customized FMT protocols designed to restore microbial balance and immune homeostasis (154).

However, significant challenges remain. The efficacy of probiotics and prebiotics in ITP has not been rigorously tested, and the optimal strains or formulations for modulating immune responses remain undefined (138). Moreover, inter-individual differences in microbiota composition may influence therapeutic outcomes, necessitating a precision medicine approach (145). Future studies should focus on characterizing microbiota profiles associated with positive treatment responses and developing predictive models to guide personalized microbiome-based interventions (155).

5.3 Advances in microbiome sequencing and artificial intelligence for targeted interventions

Technological advancements in microbiome sequencing and artificial intelligence (AI) are revolutionizing the development of

(A) Microbial signatures in ITP



Feature	Healthy gut microbiota	ITP-associated microbiota
Microbial diversity	High microbial diversity, balanced ecosystem	Reduced microbial diversity, dysbiosis
Dominant bacterial taxa	Firmicutes and Bacteroides support immunity	Increased enterobacteriaceae, reduced firmicutes and Bacteroides
Pathogenic bacteria	Low levels of enterobacteriaceae and prevotella	Increased enterobacteriaceae and Prevotella
SCFA-producing bacteria	High Bifidobacterium, Lactobacillus, Faecalibacterium	Decreased SCFA producers, leading to inflammation
Inflammatory bacterial taxa	Low abundance of pro-inflammatory bacteria	High Enterobacteriaceae, Prevotella (Th1/Th17 activation)
Gut barrier integrity	Strong tight junctions, reduced permeability	Weakened barrier, increased permeability
Impact on immune system	Balanced immune response, active Tregs	Increased Th1/Th17 activation, reduced Tregs
Cytokine profile	Normal IL-10, TGF-β (immune regulation)	High IL-6, TNF-α, IL-17 (inflammation)
Association with platelet regulation	Stable platelet homeostasis	Possible role in platelet destruction, ITP progression

(B) Diagnostic tools



Diagnostic approach	Benefits	Challenges and limitations
Metagenomic sequencing	Identifies specific microbial signatures; high-resolution analysis	Costly, requires advanced bioinformatics
16S rRNA sequencing	Rapid microbial composition analysis; relatively cost-effective	Lower resolution than whole-genome sequencing
Microbiota biomarker panels	Potential for early detection and prognosis	Need for standardization and validation
Multi-omics integration	Combines microbiota data with metabolomics and proteomics	Complex analysis; requires large datasets
Microbiota analysis (predictive modeling)	Personalized risk assessment, predictive modeling	Requires extensive training data and validation

(C) Clinical applications and future perspectives



Component	Description	Clinical Relevance
Predictive models	Uses microbiota biomarkers to detect early ITP-related microbial changes	Helps in early diagnosis and risk assessment
Personalized treatment strategies	Tailors interventions like probiotics, diet, and immunotherapy based on microbiota profiling	Improves treatment precision and reduces side effects
Longitudinal monitoring	Tracks microbiota changes over time to evaluate disease progression and therapy response	Aids in adaptive disease management and treatment refinement

FIGURE 3
Overview of microbiota-based biomarkers and diagnostic approaches in ITP. This figure illustrates the role of microbiota profiling in the diagnosis and management of immune thrombocytopenia (ITP). **(A)** Microbial signatures in ITP highlight key alterations in bacterial taxa, including decreased *Bacteroides* and *Firmicutes*, and increased *Enterobacteriaceae*, which are associated with immune dysregulation and platelet destruction. **(B)** A comparison of diagnostic tools—metagenomic sequencing, 16S rRNA sequencing, microbiota biomarker panels, and multi-omics integration—highlighting their benefits and limitations for clinical application in ITP. **(C)** Clinical applications and future perspectives include predictive models for early detection, personalized treatment strategies integrating probiotics and dietary interventions, and longitudinal monitoring to assess disease progression and therapeutic responses. This schematic underscores the potential of microbiota-based diagnostics in improving precision medicine approaches for ITP management.

microbiota-targeted interventions. High-throughput sequencing techniques, such as 16S rRNA sequencing and shotgun metagenomics, allow for comprehensive profiling of gut microbial communities, facilitating the identification of microbial alterations linked to ITP (156). These tools could be leveraged to refine microbiota-based diagnostics and therapeutic strategies.

AI-driven approaches further enhance our ability to analyze complex microbiota datasets, predict treatment responses, and develop targeted therapeutic interventions (157). Machine learning models can identify microbial patterns associated with disease states and recommend tailored microbiota-based therapies based on an individual's gut microbiota composition. Integrating AI with microbiome research holds great potential for optimizing precision medicine approaches in ITP and improving clinical outcomes (158).

5.4 Potential for gut microbiota manipulation in combination with standard ITP therapies

Combining microbiota-targeted therapies with conventional ITP treatments represents an exciting avenue for improving

TABLE 3 Advantages and challenges of microbiota profiling in ITP diagnosis and prognosis.

Diagnostic Tool	Benefits	Challenges & limitations
Metagenomic Sequencing	Identifies specific microbial signatures, high-resolution analysis	Costly, requires specialized bioinformatics
16S rRNA Sequencing	Rapid microbial composition analysis, relatively cost-effective	Lower resolution than whole-genome sequencing
Microbiota Biomarker Panels	Potential for early detection and prognosis	Need for standardization and validation
Microbiota Analysis	Personalized risk assessment, predictive modeling	Requires extensive training data and validation

patient outcomes (159). Current standard therapies, such as corticosteroids, thrombopoietin receptor agonists, and immunosuppressive agents, exhibit variable efficacy and often associated with significant side effects (160). Modulating the gut microbiota may serve as a complementary strategy to enhance treatment efficacy, reduce immune-related side effects, and improve long-term disease management (69).

Certain microbiota-targeted interventions, such as probiotics and SCFA-based dietary strategies, may help regulate immune responses, reduce inflammation, and promote platelet production, thereby decreasing reliance on long-term immunosuppressive therapies (108). Additionally, microbiota-based strategies may facilitate immune tolerance in refractory ITP cases, improving the likelihood of sustained remission. However, the challenge remains in identifying the most effective microbiota-based combinations and understanding their interactions with existing therapies. Future clinical trials should investigate the synergistic effects of microbiota-targeted therapies with current ITP treatments to optimize patient care (129).

Future directions should prioritize robust Phase II/III clinical trials, standardized microbiome diagnostics, longitudinal microbiota-immune tracking, and integration of AI-driven predictive models to personalize treatment. These efforts will advance precision microbiome therapeutics for ITP and improve long-term outcomes.

While microbiota-targeted therapies hold great promise for ITP, several obstacles must be addressed before they can be effectively implemented in clinical practice (161). Future research should prioritize large-scale, randomized controlled trials to validate the efficacy of microbiota-based interventions, establish standardized diagnostic protocols, and further explore the mechanistic pathways linking gut dysbiosis to ITP pathogenesis (162). Integrating microbiota profiling into precision medicine approaches and leveraging AI-driven strategies may pave the way for innovative and personalized treatment options (145).

Robust Phase II/III clinical trials are urgently needed to validate the clinical efficacy of microbiota-targeted interventions, including fecal microbiota transplantation, SCFA supplementation, and strain-specific probiotics (163). These studies should incorporate standardized microbiome profiling and immune monitoring to establish reproducible outcomes (164). In parallel, recent proposals emphasize the importance of prospective longitudinal studies and gnotobiotic mouse models to establish causality and elucidate the underlying microbial mechanisms that regulate immune responses and platelet homeostasis in ITP (165).

By addressing these challenges, the field can move toward more targeted and effective microbiota-based therapies for ITP, ultimately improving patient outcomes and transforming disease management (127).

6 Conclusion

The growing recognition of the gut-immune axis in primary ITP represents a paradigm shift in understanding and managing this autoimmune disorder (19). Traditional treatment strategies have primarily focused on immunosuppressive approaches; however, emerging evidence highlights the pivotal role of gut microbiota in immune regulation and disease progression (12). The interplay between gut microbiota, immune tolerance, and platelet homeostasis offers new avenues for therapeutic intervention, shifting the focus toward microbiota-targeted strategies.

Microbiota-based therapies, including FMT, probiotics, prebiotics, and dietary interventions, hold significant promise in modulating immune responses and restoring microbial balance in ITP (166). These approaches can enhance immune tolerance, reduce inflammation, and complement existing treatment modalities (167). However, while early studies provide compelling insights, further clinical trials are essential to validate the safety, efficacy, and long-term effects of these interventions (168).

Despite these promising developments, several challenges must be addressed before microbiota-targeted therapies can be fully integrated into clinical practice. Standardizing microbiome profiling methods, identifying reliable microbial biomarkers, and optimizing therapeutic strategies tailored to individual microbiota compositions remain critical research priorities (154, 169). Importantly, clarifying the temporal relationship between dysbiosis and immune dysfunction in ITP requires longitudinal microbiome studies and mechanistic experiments, including the use of gnotobiotic animal models (165). Additionally, long-term studies are needed to evaluate the durability of microbiota modulation and its sustained effects on disease outcomes.

Continued research is crucial for translating gut-immune insights into effective clinical strategies. Advancements in microbiome sequencing, artificial intelligence-driven microbiota profiling, and biomarker discovery may facilitate the development of personalized, precision-based therapies for ITP (104). Integrating microbiota modulation with standard immunosuppressive therapies and thrombopoietin receptor agonist therapies may lead to synergistic treatment effects and improved patient outcomes (82).

In conclusion, leveraging the gut-immune axis for ITP management represents an exciting frontier in autoimmune disease research. As our understanding of gut microbiota expands, microbiota-targeted interventions may pave the way for more effective, sustainable, and personalized treatment strategies for ITP, ultimately improving patients' quality of life and long-term disease management.

Author contributions

XG: Writing – original draft, Data curation, Resources, Writing – review & editing, Funding acquisition, Investigation, Validation, Project administration, Visualization, Conceptualization, Supervision. WK: Writing – original draft, Methodology, Formal Analysis, Software, Investigation, Data curation, Validation. QL: Formal Analysis, Data curation, Methodology, Writing – original draft, Investigation. NB: Funding acquisition, Formal Analysis, Writing – review & editing, Validation, Investigation. WM: Validation, Data curation, Resources, Visualization, Formal Analysis, Project administration, Conceptualization, Software,

Methodology, Writing – review & editing, Supervision, Writing – original draft, Investigation.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. Henan Provincial Science and Technology Research Project in Biomedicine (Grant No. 182102311233, X.G.)National Science Centre, Poland (Grant No. 2021/43/B/NZ5/03345, N.B.).

Conflict of interest

WM is a scientific and medical advisor at Calidi Biotherapeutics Inc. San Diego, CA, USA.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

WM holds the position of associate editor of *Frontiers in Immunology* at the time of submission. This had no impact on the peer review process or the final decision.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. David P, Santos GM, Patt YS, Orsi FA, Shoenfeld Y. Immune thrombocytopenia (Itp) - could it be part of autoimmune/inflammatory syndrome induced by adjuvants (Asia)? *Autoimmun Rev*. (2024) 23:103605. doi: 10.1016/j.autrev.2024.103605

2. Liu XG, Hou Y, Hou M. How we treat primary immune thrombocytopenia in adults. *J Hematol Oncol*. (2023) 16:4. doi: 10.1186/s13045-023-01401-z

3. Danieli MG, Antonelli E, Gammeri L, Longhi E, Cozzi MF, Palmeri D, et al. Intravenous immunoglobulin as a therapy for autoimmune conditions. *Autoimmun Rev*. (2025) 24:103710. doi: 10.1016/j.autrev.2024.103710

4. Wang Y, Sheng L, Han F, Guo Q, Zhang Z, Hou Y, et al. Efficacy and safety of treatments in newly diagnosed adult primary immune thrombocytopenia: A systematic review and network meta-analysis. *EClinicalMedicine*. (2023) 56:101777. doi: 10.1016/j.eclinm.2022.101777

5. Roeser A, Lazarus AH, Mahévas M. B cells and antibodies in refractory immune thrombocytopenia. *Br J Haematol*. (2023) 203:43–53. doi: 10.1111/bjh.18773

6. Schoenaker JM, Nelson VS, Henderickx JGE, Terveer EM, Jansen AJG, Porcelijn L, et al. The intestinal flora: the key to unraveling heterogeneity in immune thrombocytopenia? *Blood Rev*. (2025) 69:101252. doi: 10.1016/j.blre.2024.101252

7. Skoric D, Krcunovic J, Svorcan J, Krstovski N, Rodic P, Lazic J, et al. Pediatric immune thrombocytopenia: the impact of antithyroid antibodies on the treatment outcomes. *Clin Pediatr (Phila)*. (2025) 64:264–70. doi: 10.1177/00099228241262173

8. Hardtke-Wolenski M, Landwehr-Kenzel S. Tipping the balance in autoimmunity: are regulatory T cells the cause, the cure, or both? *Mol Cell Pediatr*. (2024) 11:3. doi: 10.1186/s40348-024-00176-8

9. Mackie J, Suan D, McNaughton P, Haerynck F, O'Sullivan M, Guerin A, et al. Functional validation of a novel Stat3 'Variant of unknown significance' Identifies a new case of Stat3 Gof syndrome and reveals broad immune cell defects. *Clin Exp Immunol*. (2025) 219(1):uxaf005. doi: 10.1093/cei/uxaf005

10. Elsayh KI, Saad K, Osman NS, Mahmoud KH, Ahmad FA, Khalaf SM, et al. Regulatory T-lymphocyte subsets in children with chronic immune thrombocytopenia after high-dose of dexamethasone. *Pediatr Res*. (2022) 92:1432–6. doi: 10.1038/s41390-022-01978-0

11. Zhu G, Yan L, Fang L, Fan C, Sun H, Zhou X, et al. Possible immune mechanisms of gut microbiota and its metabolites in the occurrence and development of immune thrombocytopenia. *Front Microbiol*. (2024) 15:1426911. doi: 10.3389/fmicb.2024.1426911

12. Wang X, Yuan W, Yang C, Wang Z, Zhang J, Xu D, et al. Emerging role of gut microbiota in autoimmune diseases. *Front Immunol*. (2024) 15:1365554. doi: 10.3389/fimmu.2024.1365554

13. Qi P, Chen X, Tian J, Zhong K, Qi Z, Li M, et al. The gut homeostasis-immune system axis: novel insights into rheumatoid arthritis pathogenesis and treatment. *Front Immunol*. (2024) 15:1482214. doi: 10.3389/fimmu.2024.1482214

14. Li J, Xie Z, Yang L, Guo K, Zhou Z. The impact of gut microbiome on immune and metabolic homeostasis in type 1 diabetes: clinical insights for prevention and treatment strategies. *J Autoimmun*. (2025) 151:103371. doi: 10.1016/j.jaut.2025.103371

15. Lu Q, Zhu R, Zhou L, Zhang R, Li Z, Xu P, et al. Gut dysbiosis contributes to the development of Budd-Chiari syndrome through immune imbalance. *mSystems*. (2024) 9:e0079424. doi: 10.1128/msystems.00794-24

16. Fakharian F, Thirugnanam S, Welsh DA, Kim WK, Rappaport J, Bittinger K, et al. The role of gut dysbiosis in the loss of intestinal immune cell functions and viral pathogenesis. *Microorganisms*. (2023) 11:1849. doi: 10.3390/microorganisms11071849

17. Yang X, Huang J, Peng J, Wang P, Wong FS, Wang R, et al. Gut microbiota from B-cell-specific Tlr9-deficient nod mice promote Il-10(+) Breg cells and protect against T1d. *Front Immunol*. (2024) 15:1413177. doi: 10.3389/fimmu.2024.1413177

18. Wang JG, Dou HH, Liang QY. Impact of gut microbiota and inflammatory cytokines on immune thrombocytopenia. *Eur J Haematol*. (2025) 114:120–8. doi: 10.1111/ejh.14310

19. Sun H, Yan L, Fang L, Shi Z. A novel approach to immune thrombocytopenia intervention: modulating intestinal homeostasis. *BMC Immunol*. (2024) 25:71. doi: 10.1186/s12865-024-00660-w

20. Napiorkowska-Baran K, Bilinski J, Pujanek M, Halakuc P, Pietryga A, Szymczak B, et al. Fecal microbiota transplantation in a patient with chronic diarrhea and primary and secondary immunodeficiency (Common variable immunodeficiency and splenectomy). *Front Cell Infect Microbiol*. (2024) 14:1456672. doi: 10.3389/fcimb.2024.1456672

21. Zhou P, Chen C, Patil S, Dong S. Unveiling the therapeutic symphony of probiotics, prebiotics, and postbiotics in gut-immune harmony. *Front Nutr*. (2024) 11:1355542. doi: 10.3389/fnut.2024.1355542

22. Li Z, Xiong W, Liang Z, Wang J, Zeng Z, Kołat D, et al. Critical role of the gut microbiota in immune responses and cancer immunotherapy. *J Hematol Oncol*. (2024) 17:33. doi: 10.1186/s13045-024-01541-w

23. Schifferli A, Cavalli F, Godeau B, Liebman HA, Recher M, Imbach P, et al. Understanding immune thrombocytopenia: looking out of the box. *Front Med (Lausanne)*. (2021) 8:613192. doi: 10.3389/fmed.2021.613192

24. Chakraborty P, Banerjee D, Majumder P, Sarkar J. Gut microbiota nexus: exploring the interactions with the brain, heart, lungs, and skin axes and their effects on health. *Med Microecol*. (2024) 20:100104. doi: 10.1016/j.medmic.2024.100104

25. Ma S, Ming Y, Wu J, Cui G. Cellular metabolism regulates the differentiation and function of T-cell subsets. *Cell Mol Immunol*. (2024) 21:419–35. doi: 10.1038/s41423-024-01148-8

26. Kedmi R, Littman DR. Antigen-presenting cells as specialized drivers of intestinal T cell functions. *Immunity*. (2024) 57:2269–79. doi: 10.1016/j.immuni.2024.09.011

27. Xie Y, Liu F. The role of the gut microbiota in tumor, immunity, and immunotherapy. *Front Immunol*. (2024) 15:1410928. doi: 10.3389/fimmu.2024.1410928

28. Colella M, Charitos IA, Ballini A, Cafiero C, Topi S, Palmirotta R, et al. Microbiota revolution: how gut microbes regulate our lives. *World J Gastroenterol*. (2023) 29:4368–83. doi: 10.3748/wjg.v29.i28.4368

29. Han K, Xu J, Xie F, Crowther J, Moon JJ. Engineering strategies to modulate the gut microbiome and immune system. *J Immunol*. (2024) 212:208–15. doi: 10.4049/jimmunol.2300480

30. Wilhelm G, Mertowska P, Mertowski S, Przysucha A, Struzyna J, Grywalska E, et al. The crossroads of the coagulation system and the immune system: interactions and connections. *Int J Mol Sci*. (2023) 24:12563. doi: 10.3390/ijms241612563

31. Zhang D, Jian Y-P, Zhang Y-N, Li Y, Gu L-T, Sun H-H, et al. Short-chain fatty acids in diseases. *Cell Communication Signaling*. (2023) 21:212. doi: 10.1186/s12964-023-01219-9

32. Du Y, He C, An Y, Huang Y, Zhang H, Fu W, et al. The role of short chain fatty acids in inflammation and body health. *Int J Mol Sci*. (2024) 25:7379. doi: 10.3390/ijms25137379

33. Yan Q, Jia S, Li D, Yang J. The role and mechanism of action of microbiota-derived short-chain fatty acids in neutrophils: from the activation to becoming potential biomarkers. *Biomedicine Pharmacother*. (2023) 169:115821. doi: 10.1016/j.biopha.2023.115821

34. He M, Wei W, Zhang Y, Xiang Z, Peng D, Kasimumali A, et al. Gut microbial metabolites Scfas and chronic kidney disease. *J Trans Med*. (2024) 22:172. doi: 10.1186/s12967-024-04974-6

35. Bu S, Liu M, Yang L, Lee P, Miller H, Park CS, et al. The function of T cells in immune thrombocytopenia. *Front Immunol*. (2025) 16:1499014. doi: 10.3389/fimmu.2025.1499014

36. Wicherska-Pawlowska K, Wrobel T, Rybka J. Toll-like receptors (Tlrs), nod-like receptors (Nlrs), and rig-I-like receptors (Rlrs) in innate immunity. Tlrs, Nlrs, and Rlrs ligands as immunotherapeutic agents for hematopoietic diseases. *Int J Mol Sci*. (2021) 22:13397. doi: 10.3390/ijms222413397

37. Sundaram B, Tweedell RE, Prasanth Kumar S, Kanneganti T-D. The Nlr family of innate immune and cell death sensors. *Immunity*. (2024) 57:674–99. doi: 10.1016/j.immuni.2024.03.012

38. Kawai T, Ikegawa M, Ori D, Akira S. Decoding toll-like receptors: recent insights and perspectives in innate immunity. *Immunity*. (2024) 57:649–73. doi: 10.1016/j.immuni.2024.03.004

39. Ullah H, Arbab S, Tian Y, Chen Y, Liu CQ, Li Q, et al. Crosstalk between gut microbiota and host immune system and its response to traumatic injury. *Front Immunol*. (2024) 15:1413485. doi: 10.3389/fimmu.2024.1413485

40. Kato R, Yamamoto T, Ogata H, Miyata K, Hayashi S, Gershon MD, et al. Indigenous gut microbiota constitutively drive release of ciliary neurotrophic factor from mucosal enteric glia to maintain the homeostasis of enteric neural circuits. *Front Immunol*. (2024) 15:1372670. doi: 10.3389/fimmu.2024.1372670

41. Di Vincenzo F, Del Gaudio A, Petito V, Lopetuso LR, Scaldaferri F. Gut microbiota, intestinal permeability, and systemic inflammation: A narrative review. *Intern Emerg Med*. (2024) 19:275–93. doi: 10.1007/s11739-023-03374-w

42. Dmytriv TR, Storey KB, Lushchak VI. Intestinal barrier permeability: the influence of gut microbiota, nutrition, and exercise. *Front Physiol*. (2024) 15:1380713. doi: 10.3389/fphys.2024.1380713

43. Yan M, Wang Z, Qiu Z, Cui Y, Xiang Q. Platelet signaling in immune landscape: comprehensive mechanism and clinical therapy. *biomark Res*. (2024) 12:164. doi: 10.1186/s40364-024-00700-y

44. Zhou H, Balint D, Shi Q, Vartanian T, Kriegel MA, Brito I. Lupus and inflammatory bowel disease share a common set of microbiome features distinct from other autoimmune disorders. *Ann Rheumatic Dis*. (2024) 84(1):93–105. doi: 10.1136/ard-2024-225829

45. Van Hul M, Cani PD, Petitfils C, De Vos WM, Tilg H, El-Omar EM. What defines a healthy gut microbiome? *Gut*. (2024) 73:1893–908. doi: 10.1136/gutjnl-2024-333378

46. Sadeghpour Heravi F. Gut microbiota and autoimmune diseases: mechanisms, treatment, challenges, and future recommendations. *Curr Clin Microbiol Rep*. (2024) 11:18–33. doi: 10.1007/s40588-023-00213-6

47. Hong Y, Zhang C, Shen K, Dong X, Chen B. Genetically predicted plasma metabolites mediate the causal relationship between gut microbiota and primary immune thrombocytopenia (Itp). *Front Microbiol*. (2024) 15:1447729. doi: 10.3389/fmicb.2024.1447729

48. Zhu Q, Rui K, Wang S, Tian J. Advances of regulatory B cells in autoimmune diseases. *Front Immunol*. (2021) 12:592914. doi: 10.3389/fimmu.2021.592914

49. Zhang Q, Liao J, Liu Z, Song S, Tian L, Wang Y. The immune tolerance role of Bregs in inhibiting human inflammatory diseases, with a focus on diabetes mellitus. *Front Immunol*. (2025) 16:1565158. doi: 10.3389/fimmu.2025.1565158

50. Provan D, Semple JW. Recent advances in the mechanisms and treatment of immune thrombocytopenia. *EBioMedicine*. (2022) 76:103820. doi: 10.1016/j.ebiom.2022.103820

51. He L, Li X, Jiang S, Ou Y, Wang S, Shi N, et al. The influence of the gut microbiota on B cells in autoimmune diseases. *Mol Med*. (2025) 31:149. doi: 10.1186/s10020-025-01195-5

52. Saki N, Hadi H, Keikhaei B, Mirzaei A, Purrahman D. Gut microbiome composition and dysbiosis in immune thrombocytopenia: A review of literature. *Blood Rev*. (2024) 67:101219. doi: 10.1016/j.blre.2024.101219

53. Kim CH. Complex regulatory effects of gut microbial short-chain fatty acids on immune tolerance and autoimmunity. *Cell Mol Immunol*. (2023) 20:341–50. doi: 10.1038/s41423-023-00987-1

54. Gui L, Zuo X, Feng J, Wang M, Chen Z, Sun Y, et al. Outgrowth of Escherichia is susceptible to aggravation of systemic lupus erythematosus. *Arthritis Res Ther*. (2024) 26:191. doi: 10.1186/s13075-024-03413-7

55. Lathakumari RH, Vajravelu LK, Satheesan A, Ravi S, Thulukanam J. Antibiotics and the gut microbiome: understanding the impact on human health. *Med Microecol*. (2024) 20:100106. doi: 10.1016/j.medmic.2024.100106

56. Singh S, Giron LB, Shaikh MW, Shankaran S, Engen Phillip A, Bogin ZR, et al. Distinct intestinal microbial signatures linked to accelerated systemic and intestinal biological aging. *Microbiome*. (2024) 12:31. doi: 10.1186/s40168-024-01758-4

57. Bhutta NK, Xu X, Jian C, Wang Y, Liu Y, Sun J, et al. Gut microbiota mediated T cells regulation and autoimmune diseases. *Front Microbiol*. (2024) 15:1477187. doi: 10.3389/fmicb.2024.1477187

58. Li C, Peng K, Xiao S, Long Y, Yu Q. The role of lactobacillus in inflammatory bowel disease: from actualities to prospects. *Cell Death Discov*. (2023) 9:361. doi: 10.1038/s41420-023-01666-w

59. Thapa R, Magar AT, Shrestha J, Panth N, Idrees S, Sadaf T, et al. Influence of gut and lung dysbiosis on lung cancer progression and their modulation as promising therapeutic targets: A comprehensive review. *MedComm (2020)*. (2024) 5:e70018. doi: 10.1002/mco2.70018

60. Alagiakrishnan K, Morgadinho J, Halverson T. Approach to the diagnosis and management of dysbiosis. *Front Nutr*. (2024) 11:1330903. doi: 10.3389/fnut.2024.1330903

61. Qu S, Gao Y, Ma J, Yan Q. Microbiota-derived short-chain fatty acids functions in the biology of B lymphocytes: from differentiation to antibody formation. *Biomedicine Pharmacother*. (2023) 168:115773. doi: 10.1016/j.biopha.2023.115773

62. Druszczynska M, Sadowska B, Kulesza J, Gasienica-Gliwa N, Kulesza E, Fol M. The intriguing connection between the gut and lung microbiomes. *Pathogens*. (2024) 13. doi: 10.3390/pathogens13111005

63. Yao K, Xie Y, Wang J, Lin Y, Chen X, Zhou T. Gut microbiota: A newly identified environmental factor in systemic lupus erythematosus. *Front Immunol*. (2023) 14:1202850. doi: 10.3389/fimmu.2023.1202850

64. Chasov V, Gilyazova E, Ganeeva I, Zmievskaya E, Davletshin D, Valiullina A, et al. Gut microbiota modulation: A novel strategy for rheumatoid arthritis therapy. *Biomolecules*. (2024) 14:1653. doi: 10.3390/biom14121653

65. Taneja V. Gut microbes as the major drivers of rheumatoid arthritis: our microbes are our fortune! *Microorganisms*. (2025) 13:255. doi: 10.3390/microorganisms13020255

66. Campagnoli LIM, Marchesi N, Varesi A, Morozzi M, Mascione L, Ricevuti G, et al. New therapeutic avenues in multiple sclerosis: is there a place for gut microbiota-based treatments? *Pharmacol Res*. (2024) 209:107456. doi: 10.1016/j.phrs.2024.107456

67. Turner T-A, Lehman P, Ghimire S, Shahi SK, Mangalam A. Game of microbes: the battle within – gut microbiota and multiple sclerosis. *Gut Microbes*. (2024) 16:2387794. doi: 10.1080/19490976.2024.2387794

68. Rui X, Fu Y, Cai J, Zhang Y, Fu Q, He C. Gut microbiota were altered with platelet count and red blood cell count in immune thrombocytopenia patients with different treatments. *Front Cell Infect Microbiol*. (2023) 13:1168756. doi: 10.3389/fcimb.2023.1168756

69. Zhao L-Y, Mei J-X, Yu G, Lei L, Zhang W-H, Liu K, et al. Role of the gut microbiota in anticancer therapy: from molecular mechanisms to clinical applications. *Signal Transduction Targeted Ther*. (2023) 8:201. doi: 10.1038/s41392-023-01406-7

70. Saini S, Koh AY, Zia A. Identifying gut microbiota and immune host factors associated with bleeding risk in children with immune thrombocytopenic purpura. *Blood*. (2024) 144:2551–. doi: 10.1182/blood-2024-199928

71. Gilliland A, Chan JJ, De Wolfe TJ, Yang H, Vallance BA. Pathobionts in inflammatory bowel disease: origins, underlying mechanisms, and implications for clinical care. *Gastroenterology*. (2024) 166:44–58. doi: 10.1053/j.gastro.2023.09.019

72. Guo D, Chen Q, Wang G, Li C, FinnGen c. Causal relationship between gut microbiota and immune thrombocytopenia: A Mendelian randomization study of two samples. *Front Microbiol*. (2023) 14:1190866. doi: 10.3389/fmicb.2023.1190866

73. Wang H, Bi H, Yang M, Wang X, Song C, Yang W, et al. Intestinal flora altered and correlated with interleukin-2/4 in patients with primary immune thrombocytopenia. *Hematology*. (2023) 28:2277501. doi: 10.1080/16078454.2023.2277501

74. Mei Z, Wang F, Bhosle A, Dong D, Mehta R, Ghazi A, et al. Strain-specific gut microbial signatures in type 2 diabetes identified in a cross-cohort analysis of 8,117 metagenomes. *Nat Med*. (2024) 30:2265–76. doi: 10.1038/s41591-024-03067-7

75. Park JC, Im SH. Of men in mice: the development and application of a humanized gnotobiotic mouse model for microbiome therapeutics. *Exp Mol Med*. (2020) 52:1383–96. doi: 10.1038/s12276-020-0473-2

76. Mars RAT, Yang Y, Ward T, Houtti M, Priya S, Lekatz HR, et al. Longitudinal multi-omics reveals subset-specific mechanisms underlying irritable bowel syndrome. *Cell*. (2020) 182:1460–73.e17. doi: 10.1016/j.cell.2020.08.007

77. Al-Samkari H. 2025 Update on clinical trials in immune thrombocytopenia. *Am J Hematol*. (2024) 99:2178–90. doi: 10.1002/ajh.27448

78. Massara M, Ballabio M, Dolfi B, Morad G, Wischnewski V, Lamprou E, et al. The bacterial microbiome modulates the initiation of brain metastasis by impacting the gut-to-brain axis. *iScience*. (2025) 28:111874. doi: 10.1016/j.isci.2025.111874

79. Jiang C, Deng S, Ma X, Song J, Li J, Yuan E. Mendelian randomization reveals association of gut microbiota with Henoch–Schönlein Purpura and immune thrombocytopenia. *Int J Hematol*. (2024) 120:50–9. doi: 10.1007/s12185-024-03777-1

80. Simpson RC, Shanahan ER, Scolyer RA, Long GV. Towards modulating the gut microbiota to enhance the efficacy of immune-checkpoint inhibitors. *Nat Rev Clin Oncol*. (2023) 20:697–715. doi: 10.1038/s41571-023-00803-9

81. Ebrahimi R, Farsi Y, Nejadghaderi SA. Fecal microbiota transplantation for glaucoma; a potential emerging treatment strategy. *Curr Res Microb Sci*. (2024) 7:100314. doi: 10.1016/j.crmicr.2024.100314

82. Kang X, Lau HC, Yu J. Modulating gut microbiome in cancer immunotherapy: harnessing microbes to enhance treatment efficacy. *Cell Rep Med*. (2024) 5:101478. doi: 10.1016/j.xcrm.2024.101478

83. Loh JS, Mak WQ, Tan LKS, Ng CX, Chan HH, Yeow SH, et al. Microbiota-gut-brain axis and its therapeutic applications in neurodegenerative diseases. *Signal Transduct Target Ther*. (2024) 9:37. doi: 10.1038/s41392-024-01743-1

84. Li J, Li J, Liu Y, Zeng J, Liu Y, Wu Y. Large-scale bidirectional Mendelian randomization study identifies new gut microbiome significantly associated with immune thrombocytopenic purpura. *Front Microbiol*. (2024) 15:1423951. doi: 10.3389/fmicb.2024.1423951

85. Qin L, Fan B, Zhou Y, Zheng J, Diao R, Wang F, et al. Targeted gut microbiome therapy: applications and prospects of probiotics, fecal microbiota transplantation and natural products in the management of type 2 diabetes. *Pharmacol Res*. (2025), 107625. doi: 10.1016/j.phrs.2025.107625

86. Gao Y, Li W, Huang X, Lyu Y, Yue C. Advances in gut microbiota-targeted therapeutics for metabolic syndrome. *Microorganisms*. (2024) 12:851. doi: 10.3390/microorganisms12050851

87. Yadegar A, Bar-Yoseph H, Monaghan TM, Pakpour S, Severino A, Kuijper EJ, et al. Fecal microbiota transplantation: current challenges and future landscapes. *Clin Microbiol Rev*. (2024) 37:e0006022. doi: 10.1128/cmr.00060-22

88. Karimi M, Shirsalimi N, Hashempour Z, Salehi Omran H, Sedighi E, Beigi F, et al. Safety and efficacy of fecal microbiota transplantation (Fmt) as a modern adjuvant therapy in various diseases and disorders: A comprehensive literature review. *Front Immunol*. (2024) 15:1439176. doi: 10.3389/fimmu.2024.1439176

89. Rasouli-Saravani A, Jahankhani K, Moradi S, Gorgani M, Shafaghat Z, Mirsanei Z, et al. Role of microbiota short-chain fatty acids in the pathogenesis of autoimmune diseases. *Biomedicine Pharmacother*. (2023) 162:114620. doi: 10.1016/j.biopha.2023.114620

90. Facchin S, Bertin L, Bonazzi E, Lorenzon G, De Barba C, Barberio B, et al. Short-chain fatty acids and human health: from metabolic pathways to current therapeutic implications. *Life*. (2024) 14:559. doi: 10.3390/life14050559

91. Zheng K, Wei Z, Li W. Ecological insights into hematopoiesis regulation: unraveling the influence of gut microbiota. *Gut Microbes*. (2024) 16:2350784. doi: 10.1080/19490976.2024.2350784

92. Singh P, Meenatchi R, Ahmed ZHT, Thacharodi A, R M, Kumar RRS, et al. Implications of the gut microbiome in cardiovascular diseases: association of gut microbiome with cardiovascular diseases, therapeutic interventions and multi-omics approach for precision medicine. *Med Microecol*. (2024) 19:100096. doi: 10.1016/j.medmic.2023.100096

93. Rasmussen TS, Mao X, Forster S, Larsen SB, Von Münchow A, Tranæs KD, et al. Overcoming donor variability and risks associated with fecal microbiota transplants through bacteriophage-mediated treatments. *Microbiome*. (2024) 12:119. doi: 10.1186/s40168-024-01820-1

94. Yoo S, Jung SC, Kwak K, Kim JS. The role of prebiotics in modulating gut microbiota: implications for human health. *Int J Mol Sci*. (2024) 25:4834. doi: 10.3390/ijms25094834

95. Mazziotta C, Tognon M, Martini F, Torreggiani E, Rotondo JC. Probiotics mechanism of action on immune cells and beneficial effects on human health. *Cells*. (2023) 12:184. doi: 10.3390/cells12010184

96. Zeng L, Yang K, He Q, Zhu X, Long Z, Wu Y, et al. Efficacy and safety of gut microbiota-based therapies in autoimmune and rheumatic diseases: A systematic review and meta-analysis of 80 randomized controlled trials. *BMC Med*. (2024) 22:110. doi: 10.1186/s12916-024-03303-4

97. Chandrasekaran P, Weiskirchen S, Weiskirchen R. Effects of probiotics on gut microbiota: an overview. *Int J Mol Sci*. (2024) 25:6022. doi: 10.3390/ijms25116022

98. Kumari A, GR K, Sudhakaran VA, Warrier AS, Singh NK. Unveiling the health benefits of prebiotics: A comprehensive review. *Indian J Microbiol*. (2024) 64:376–88. doi: 10.1007/s12088-024-01235-4

99. Al-Habsi N, Al-Khalili M, Haque SA, Elias M, Olqi NA, Al Uraimi T. Health benefits of prebiotics, probiotics, synbiotics, and postbiotics. *Nutrients*. (2024) 16:3955. doi: 10.3390/nu16223955

100. Fneish FH, Abd El Galil KH, Domiati SA. Evaluation of single and multi-strain probiotics with gentamicin against E. Coli O157:H7: insights from *in vitro* and *in vivo* studies. *Microorganisms*. (2025) 13:460. doi: 10.3390/microorganisms13020460

101. Tufail MA, Schmitz RA. Exploring the probiotic potential of Bacteroides spp. Within one health paradigm. *Probiotics Antimicrob Proteins*. (2024) 17(2):681–704. doi: 10.1007/s12602-024-10370-9

102. Jia X, Wang J, Ren D, Zhang K, Zhang H, Jin T, et al. Impact of the gut microbiota-Th17 cell axis on inflammatory depression. *Front Psychiatry*. (2024) 15:1509191. doi: 10.3389/fpsyt.2024.1509191

103. Wang X, Peng J, Cai P, Xia Y, Yi C, Shang A, et al. The emerging role of the gut microbiota and its application in inflammatory bowel disease. *Biomedicine Pharmacother*. (2024) 179:117302. doi: 10.1016/j.biopha.2024.117302

104. Manrique P, Montero I, Fernandez-Gosende M, Martinez N, Cantabrana CH, Rios-Covian D. Past, present, and future of microbiome-based therapies. *Microbiome Res Rep*. (2024) 3:23. doi: 10.20517/mrr.2023.80

105. Arifuzzaman M, Collins N, Guo CJ, Artis D. Nutritional regulation of microbiota-derived metabolites: implications for immunity and inflammation. *Immunity*. (2024) 57:14–27. doi: 10.1016/j.immuni.2023.12.009

106. Cao S, Budina E, Raczy MM, Solanki A, Nguyen M, Beckman TN, et al. A serine-conjugated butyrate prodrug with high oral bioavailability suppresses autoimmune arthritis and neuroinflammation in mice. *Nat Biomed Eng*. (2024) 8:611–27. doi: 10.1038/s41551-024-01190-x

107. Zhang C, Liu X, Gu C, Su Y, Lv J, Liu Y, et al. Histone deacetylases facilitate Th17-cell differentiation and pathogenicity in autoimmune uveitis via Cdk6/Id2 axis. *J Advanced Res*. (2024). doi: 10.1016/j.jare.2024.07.029

108. Liu XF, Shao JH, Liao YT, Wang LN, Jia Y, Dong PJ, et al. Regulation of short-chain fatty acids in the immune system. *Front Immunol*. (2023) 14:1186892. doi: 10.3389/fimmu.2023.1186892

109. Zhang W, Mackay CR, Gershwin ME. Immunomodulatory effects of microbiota-derived short-chain fatty acids in autoimmune liver diseases. *J Immunol*. (2023) 210:1629–39. doi: 10.4049/jimmunol.2300016

110. Hua T, Yao F, Wang H, Liu W, Zhu X, Yao Y. Megakaryocyte in sepsis: the trinity of coagulation, inflammation and immunity. *Crit Care*. (2024) 28:442. doi: 10.1186/s13054-024-05221-6

111. Larabi AB, Masson HLP, Baumler AJ. Bile acids as modulators of gut microbiota composition and function. *Gut Microbes*. (2023) 15:2172671. doi: 10.1080/19490976.2023.2172671

112. Fleishman JS, Kumar S. Bile acid metabolism and signaling in health and disease: molecular mechanisms and therapeutic targets. *Signal Transduction Targeted Ther*. (2024) 9:97. doi: 10.1038/s41392-024-01811-6

113. Spivak I, Fluhr L, Elinav E. Local and systemic effects of microbiome-derived metabolites. *EMBO Rep*. (2022) 23:e55664. doi: 10.15252/embr.202255664

114. Yoon JH, Do JS, Velankanni P, Lee CG, Kwon HK. Gut microbial metabolites on host immune responses in health and disease. *Immune Netw*. (2023) 23:e6. doi: 10.4110/in.2023.23.e6

115. Zhang Z, Lv T, Wang X, Wu M, Zhang R, Yang X, et al. Role of the microbiota–gut–heart axis between bile acids and cardiovascular disease. *Biomedicine Pharmacother*. (2024) 174:116567. doi: 10.1016/j.biopha.2024.116567

116. Yan J, Chen D, Ye Z, Zhu X, Li X, Jiao H, et al. Molecular mechanisms and therapeutic significance of tryptophan metabolism and signaling in cancer. *Mol Cancer*. (2024) 23:241. doi: 10.1186/s12943-024-02164-y

117. Liu X, Yang M, Xu P, Du M, Li S, Shi J, et al. Kynurenine-Ahr reduces T-cell infiltration and induces a delayed T-cell immune response by suppressing the Stat1-Cxcl9/Cxcl10 axis in tuberculosis. *Cell Mol Immunol*. (2024) 21:1426–40. doi: 10.1038/s41423-024-01230-1

118. Moulinet T, Moussu A, Pierson L, Pagliuca S. The many facets of immune-mediated thrombocytopenia: principles of immunobiology and immunotherapy. *Blood Rev*. (2024) 63:101141. doi: 10.1016/j.blre.2023.101141

119. Xu X, Zhang J, Xing H, Han L, Li X, Wu P, et al. Identification of metabolism-related key genes as potential biomarkers for pathogenesis of immune thrombocytopenia. *Sci Rep*. (2024) 14:9040. doi: 10.1038/s41598-024-59493-7

120. Wiggins RW, Woo J, Cauba JN, Mito S. Evaluating the potential of herbal extracts as treatment in immune thrombocytopenia: A review of evidence and limitations. *Appl Biosci*. (2025) 4:1. doi: 10.3390/applbiosci4010001

121. Kuter DJ. The treatment of immune thrombocytopenia (Itp)—Focus on thrombopoietin receptor agonists. *Ann Blood*. (2021) 6:7. doi: 10.21037/aob-21-23

122. Ben-Yacov O, Godneva A, Rein M, Shilo S, Lotan-Pompan M, Weinberger A, et al. Gut microbiome modulates the effects of a personalised postprandial-targeting (Ppt) diet on cardiometabolic markers: A diet intervention in pre-diabetes. *Gut*. (2023) 72:1486–96. doi: 10.1136/gutjnl-2022-329201

123. Datta S, Pasham S, Inavolu S, Boini KM, Koka S. Role of gut microbial metabolites in cardiovascular diseases-current insights and the road ahead. *Int J Mol Sci*. (2024) 25:10208. doi: 10.3390/ijms251810208

124. Zhao X, Liu S, Li S, Jiang W, Wang J, Xiao J, et al. Unlocking the power of postbiotics: A revolutionary approach to nutrition for humans and animals. *Cell Metab*. (2024) 36:725–44. doi: 10.1016/j.cmet.2024.03.004

125. Yang X, Hang HC. Chemical genetic approaches to dissect microbiota mechanisms in health and disease. *Science*. (2024) 386:eado8548. doi: 10.1126/science.ado8548

126. Heavey MK, Hazelton A, Wang Y, Garner M, Anselmo AC, Arthur JC, et al. Targeted delivery of the probiotic saccharomyces Boulardii to the extracellular matrix enhances gut residence time and recovery in murine colitis. *Nat Commun*. (2024) 15:3784. doi: 10.1038/s41467-024-48128-0

127. Madkhali MA. Recent advances in the management of immune thrombocytopenic purpura (Itp): A comprehensive review. *Med (Baltimore)*. (2024) 103:e36936. doi: 10.1097/MD.0000000000036936

128. Ezike TC, Okpala US, Onoja UL, Nwike CP, Ezeako EC, Okpara OJ, et al. Advances in drug delivery systems, challenges and future directions. *Heliyon*. (2023) 9:e17488. doi: 10.1016/j.heliyon.2023.e17488

129. Lambert MP. On the horizon: upcoming new agents for the management of itp. *Hematol Am Soc Hematol Educ Program*. (2024) 2024:692–9. doi: 10.1182/hematology.2024000596

130. Brown JA, Bashir H, Zeng MY. Lifelong partners: gut microbiota-immune cell interactions from infancy to old age. *Mucosal Immunol*. (2025) 25. doi: 10.1016/j.mucimm.2025.01.006

131. Zhao Q, Chen Y, Huang W, Zhou H, Zhang W. Drug-microbiota interactions: an emerging priority for precision medicine. *Signal Transduction Targeted Ther*. (2023) 8:386. doi: 10.1038/s41392-023-01619-w

132. Huang H, Jiang J, Wang X, Jiang K, Cao H. Exposure to prescribed medication in early life and impacts on gut microbiota and disease development. *eClinicalMedicine*. (2024) 68:102428. doi: 10.1016/j.eclinm.2024.102428

133. Crudele L, Gadaleta RM, Cariello M, Moschetta A. Gut microbiota in the pathogenesis and therapeutic approaches of diabetes. *eBioMedicine*. (2023) 97:104821. doi: 10.1016/j.ebiom.2023.104821

134. Arnone AA, Ansley K, Heeke AL, Howard-McNatt M, Cook KL. Gut microbiota interact with breast cancer therapeutics to modulate efficacy. *EMBO Mol Med*. (2025) 17:219–34. doi: 10.1038/s44321-024-00185-0

135. Beyi AF, Wannemuehler M, Plummer PJ. Impacts of gut microbiota on the immune system and fecal microbiota transplantation as a re-emerging therapy for autoimmune diseases. *Antibiotics (Basel)*. (2022) 11:1093. doi: 10.3390/antibiotics11081093

136. Chen Q, Wu C, Xu J, Ye C, Chen X, Tian H, et al. Donor-recipient intermicrobial interactions impact transfer of subspecies and fecal microbiota transplantation outcome. *Cell Host Microbe*. (2024) 32:349–65.e4. doi: 10.1016/j.chom.2024.01.013

137. Porcari S, Benech N, Valles-Colomer M, Segata N, Gasbarrini A, Cammarota G, et al. Key determinants of success in fecal microbiota transplantation: from microbiome to clinic. *Cell Host Microbe*. (2023) 31:712–33. doi: 10.1016/j.chom.2023.03.020

138. Sarita B, Samadhan D, Hassan MZ, Kovaleva EG. A comprehensive review of probiotics and human health-current prospective and applications. *Front Microbiol*. (2024) 15:1487641. doi: 10.3389/fmicb.2024.1487641

139. Kaltsas A, Giannakodimos I, Markou E, Adamos K, Stavropoulos M, Kratiras Z, et al. The role of gut microbiota dysbiosis in erectile dysfunction: from pathophysiology to treatment strategies. *Microorganisms*. (2025) 13:250. doi: 10.3390/microorganisms13020250

140. Ma Z, Zuo T, Frey N, Rangrez AY. A systematic framework for understanding the microbiome in human health and disease: from basic principles to clinical translation. *Signal Transduct Target Ther*. (2024) 9:237. doi: 10.1038/s41392-024-01946-6

141. Kumar B, Lorusso E, Fosso B, Pesole G. A comprehensive overview of microbiome data in the light of machine learning applications: categorization, accessibility, and future directions. *Front Microbiol*. (2024) 15:1343572. doi: 10.3389/fmicb.2024.1343572

142. Han R, Acosta JN, Shakeri Z, Ioannidis JPA, Topol EJ, Rajpurkar P. Randomised controlled trials evaluating artificial intelligence in clinical practice: A scoping review. *Lancet Digital Health*. (2024) 6:e367–e73. doi: 10.1016/S2589-7500(24)00047-5

143. Guevara-Ramirez P, Cadena-Ullauri S, Paz-Cruz E, Ruiz-Pozo VA, Tamayo-Trujillo R, Cabrera-Andrade A, et al. Gut microbiota disruption in hematologic cancer therapy: molecular insights and implications for treatment efficacy. *Int J Mol Sci*. (2024) 25:10255. doi: 10.3390/ijms251910255

144. Zhang Y, Zhao X, Zhang J, Zhang Y, Wei Y. Advancements in the impact of human microbiota and probiotics on leukemia. *Front Microbiol*. (2024) 15:1423838. doi: 10.3389/fmicb.2024.1423838

145. Shukla V, Singh S, Verma S, Verma S, Rizvi AA, Abbas M. Targeting the microbiome to improve human health with the approach of personalized medicine: latest aspects and current updates. *Clin Nutr ESPEN*. (2024) 63:813–20. doi: 10.1016/j.clnesp.2024.08.005

146. Davoutis E, Gkiafi Z, Lykoudis PM. Bringing gut microbiota into the spotlight of clinical research and medical practice. *World J Clin cases*. (2024) 12:2293–300. doi: 10.12998/wjcc.v12.i14.2293

147. Hasan R, Kumar A, Bose S, Roy R, Arora A, Sharma P, et al. Novel microbial signatures in the faecal microbiome associated with severe alcoholic hepatitis: Bacteroides finegoldii and Veillonella dispar. *Open Microbiol J*. (2023) 17. doi: 10.2174/18742858-v17-e230803-2023-7

148. Zheng J, Sun Q, Zhang M, Liu C, Su Q, Zhang L, et al. Noninvasive, microbiome-based diagnosis of inflammatory bowel disease. *Nat Med*. (2024) 30:3555–67. doi: 10.1038/s41591-024-03280-4

149. Lee D, Maaskant A, Ngo H, Montijn RC, Bakker J, Langermans JAM, et al. A rapid, affordable, and reliable method for profiling microbiome biomarkers from fecal images. *iScience*. (2024) 27:111310. doi: 10.1016/j.isci.2024.111310

150. Kapur R. Broadening the horizon of immune thrombocytopenia through omics approaches. *Br J Haematol*. (2024) 204:2159–61. doi: 10.1111/bjh.19514

151. Chen Y-C, Su Y-Y, Chu T-Y, Wu M-F, Huang C-C, Lin C-C. Prelect: prevalence leveraged consistent feature selection decodes microbial signatures across cohorts. *NPJ Biofilms Microbiomes*. (2025) 11:3. doi: 10.1038/s41522-024-00598-2

152. Chetty A, Blekhman R. Multi-omic approaches for host-microbiome data integration. *Gut Microbes*. (2024) 16:2297860. doi: 10.1080/19490976.2023.2297860

153. Stumptner C, Stadlbauer V, O'Neil D, Gessner A, Hiergeist A, Zatloukal K, et al. The pre-analytical Cen/Ts standard for microbiome diagnostics-how can research and development benefit? *Nutrients*. (2022) 14:1976. doi: 10.3390/nu14091976

154. Kamel M, Aleya S, Alsubih M, Aleya L. Microbiome dynamics: A paradigm shift in combatting infectious diseases. *J Personalized Med*. (2024) 14:217. doi: 10.3390/jpm14020217

155. Barone M, Martucci M, Sciara G, Conte M, Medina LSJ, Iattoni L, et al. Towards a personalized prediction, prevention and therapy of insomnia: gut microbiota profile can discriminate between paradoxical and objective insomnia in post-menopausal women. *EPMA J*. (2024) 15:471–89. doi: 10.1007/s13167-024-00369-1

156. Bars-Cortina D, Ramon E, Rius-Sansalvador B, Guinó E, Garcia-Serrano A, Mach N, et al. Comparison between 16s Rrna and shotgun sequencing in colorectal cancer, advanced colorectal lesions, and healthy human gut microbiota. *BMC Genomics*. (2024) 25:730. doi: 10.1186/s12864-024-10621-7

157. D'Urso F, Broccolo F. Applications of artificial intelligence in microbiome analysis and probiotic interventions—an overview and perspective based on the current state of the art. *Appl Sci*. (2024) 14:8627. doi: 10.3390/app14198627

158. Danieli MG, Brunetto S, Gammeri L, Palmeri D, Claudi I, Shoenfeld Y, et al. Machine learning application in autoimmune diseases: state of art and future prospectives. *Autoimmun Rev*. (2024) 23:103496. doi: 10.1016/j.autrev.2023.103496

159. He J, Chen Y, Zhao H, Li Y. The interplay between gut bacteria and targeted therapies: implications for future cancer treatments. *Mol Med*. (2025) 31:58. doi: 10.1186/s10020-025-01108-6

160. Soulard M, Galicier L, Mahlaoui N, Fieschi C, Deshayes S, Gobert D, et al. Efficacy and safety of Tpo receptor agonists in treatment of Itp associated with predominantly antibody deficiencies. *Blood Adv*. (2024) 8:6171–82. doi: 10.1182/bloodadvances.2024014370

161. Gebrayel P, Nicco C, Al Khodor S, Bilinski J, Caselli E, Comelli EM, et al. Microbiota medicine: towards clinical revolution. *J Trans Med*. (2022) 20:111. doi: 10.1186/s12967-022-03296-9

162. LeVine DN, Goggs R, Kohn B, Mackin AJ, Kidd L, Garden OA, et al. Acvim consensus statement on the treatment of immune thrombocytopenia in dogs and cats. *J Vet Intern Med*. (2024) 38:1982–2007. doi: 10.1111/jvim.17079

163. Allegretti JR, Khanna S, Mullish BH, Feuerstadt P. The progression of microbiome therapeutics for the management of gastrointestinal diseases and beyond. *Gastroenterology*. (2024) 167:885–902. doi: 10.1053/j.gastro.2024.05.004

164. Metwaly A, Kriaa A, Hassani Z, Carraturo F, Druart C, Consortium I, et al. A consensus statement on establishing causality, therapeutic applications and the use of preclinical models in microbiome research. *Nat Rev Gastroenterol Hepatol*. (2025) 22:343–56. doi: 10.1038/s41575-025-01041-3

165. Darnaud M, De Vadder F, Bogeat P, Boucinha L, Bulteau AL, Bunescu A, et al. A standardized gnotobiotic mouse model harboring a minimal 15-member mouse gut microbiota recapitulates Sopf/Spf phenotypes. *Nat Commun*. (2021) 12:6686. doi: 10.1038/s41467-021-26963-9

166. Noguera-Fernandez N, Candela-Gonzalez J, Orenes-Pinero E. Probiotics, prebiotics, fecal microbiota transplantation, and dietary patterns in inflammatory bowel disease. *Mol Nutr Food Res*. (2024) 68:e2400429. doi: 10.1002/mnfr.202400429

167. Song Y, Li J, Wu Y. Evolving understanding of autoimmune mechanisms and new therapeutic strategies of autoimmune disorders. *Signal Transduction Targeted Ther*. (2024) 9:263. doi: 10.1038/s41392-024-01952-8

168. Kishon R, Modlin NL, Cycowicz YM, Mourtada H, Wilson T, Williamson V, et al. A rapid narrative review of the clinical evolution of psychedelic treatment in clinical trials. *NPJ Ment Health Res*. (2024) 3:33. doi: 10.1038/s44184-024-00068-9

169. Yaqub MO, Jain A, Joseph CE, Edison LK. Microbiome-driven therapeutics: from gut health to precision medicine. *Gastrointestinal Disord*. (2025) 7:7. doi: 10.3390/gidisord7010007

frontiers | Frontiers in Cellular and Infection Microbiology

TYPE Mini Review
PUBLISHED 12 June 2025
DOI 10.3389/fcimb.2025.1577108

Check for updates

OPEN ACCESS

EDITED BY
Nar Singh Chauhan,
Maharshi Dayanand University, India

REVIEWED BY
Maurizio Sanguinetti,
Catholic University of the Sacred Heart, Italy
Zeyad Khalil,
October 6 University, Egypt

*CORRESPONDENCE
Michelle Di Palma
michelle.dipalma@asu.edu

RECEIVED 15 February 2025
ACCEPTED 28 April 2025
PUBLISHED 12 June 2025

CITATION
Nieves L, Roach A, Hunter J, Smeh S, Islas A, Islas A, Blattman J and Di Palma M (2025) Harnessing the microbiome to improve clinical outcomes for cancer, transplant, and immunocompromised patients in the intensive care unit (ICU). *Front. Cell. Infect. Microbiol.* 15:1577108. doi: 10.3389/fcimb.2025.1577108

COPYRIGHT
© 2025 Nieves, Roach, Hunter, Smeh, Islas, Islas, Blattman and Di Palma. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Harnessing the microbiome to improve clinical outcomes for cancer, transplant, and immunocompromised patients in the intensive care unit (ICU)

Lizbeth Nieves[1], Alexandra Roach[2], Joseph Hunter[1], Sarah Smeh[1], Andrew Islas[1], Ariana Islas[1], Joseph Blattman[1] and Michelle Di Palma[1]*

[1]School of Life Sciences, Arizona State University, Tempe, AZ, United States, [2]Department of Medicine, University of Arizona College of Medicine, Phoenix, AZ, United States

In recent decades, there has been a growing emphasis on understanding how the architecture of the human microbiome can impact typical biological processes and patient clinical outcomes. In fact, microbiome modifications and modulations have not only been associated with impacts on general health and well-being but have also been shown to yield differences in patient responsiveness to vaccines, medications, and chemotherapeutic regimens. Much of this influence likely stems from how changes in the microbiome result in differences in microbial communities and the subsequent release of microbial-derived metabolites that can alter typical immunological processes. Understanding how microbial composition can impact patient responsiveness can be particularly important in the intensive care unit (ICU), where the efficacy of medications and treatments can result in negative patient outcomes if unsuccessful. Clinical scientists have further developed the concept of the pathobiome, a disease-promoting microbiome whose development can be associated with dysbiosis. Understanding how the microbiome and its associated components can impact patient responsiveness, especially in the ICU, must be further researched and understood. Here, we analyze what causes variances in the microbiome and pathobiome in significant immunocompromised populations, including cancer patients and transplant recipients, and how variances in the microbiome can impact patient outcomes in the ICU. Further, we detail potential future applications of how our understanding of what impacts the human microbiome during the treatment of these populations may be exploited to improve patient prognosis.

KEYWORDS

microbiome, dysbiosis, immunocompromised, cancer, transplant, pathobiome, intensive care unit, ICU

Introduction

The human microbiome is a collective of various microorganisms, including bacteria, fungi, viruses, and their associated byproducts. In a healthy microbiome, thousands of microorganisms interact to create a balanced and resilient ecosystem in both mucosal and epithelial regions. Recently, it has been found that the architecture of the microbiome can strongly impact host biological processes, with many of these impacts being linked not only to general human health but also to disease outcomes (Moloney et al., 2014; Manos, 2022). Thus, an emphasis on how microbiome modulation can impact human health and disease pathology has become of great interest to researchers and medical professionals. Modern advancements such as high-throughput genomic sequencing and metagenomic studies allow researchers to not only dissect the composition of the microbiome but also deepen their understanding of how the products from microbial species impact cellular and organ functions (Shi et al., 2022). Leveraging many of these advancements, the Human Microbiome Project revealed that healthy individuals have substantial diversity within their microbiome populations, highlighting the complexity of understanding its full impact (Huttenhower et al., 2012). Further studies indicate that products made by both the microbiome of the gut and of specific organs can modulate the function of both innate and adaptive immune cells and their subsequent impact on host organs and diseases (Russo et al., 2016; Thaiss et al., 2016). Disruptions to this system, particularly microbial depletion or loss of commensal diversity, can be especially detrimental in immunocompromised and cancer patients, whose immune systems are already burdened. Several studies have indicated that specific microbial compositions can impact disease severity, progression, and responsiveness to treatment for immunodeficient patients (Moloney et al., 2014).

Extreme dysbiosis is characterized by a significant loss of commensal microbes and a dominance of opportunistic pathogens, leading to an imbalance in gut bacteria. This can significantly impact critical illness, increasing susceptibility to nosocomial infection and organ failure (McDonald et al., 2016). Further insight into the formation of the pathobiome, a microbiome characterized by an overabundance of pathogenic microorganisms that advance disease, could deepen our knowledge of how Intensive Care Unit (ICU) patients are impacted by treatment (Munley et al., 2023). Broadening our understanding of the impact of microbial composition on immunodeficiency is essential if we hope to improve treatment strategies for immunocompromised patients. Here, we elucidate how the microbiome's composition can impact patient disease progression in the context of immunocompromised and cancer patients and disease outcomes for these patients in the ICU. Further, we explain findings from recent studies that have sought to determine potential modulations of the microbiome for patients living with compromised immune systems, with the future potential to exploit these findings to improve patient outcomes in the ICU.

How microbiome composition influences treatment outcomes in the ICU

Understanding how treatment outcomes for patients in the ICU can be impacted by the presence or absence of certain microbes is vital if clinicians hope to one day tailor treatments to patients to catalyze the best possible outcomes on an individual basis. Here, we detail known associations of specific microbes and microbial compositions with clinical and treatment outcomes in ICU patients.

General patients in the ICU

An estimated 5.7 million patients are admitted annually to ICU in the United States, with this number expected to grow due to the aging population and subsequent increasing chronic health conditions (Viglianti and Iwashyna, 2017). Within this population, the microbiome can be depleted to as few as four main species, which compete for dominance, heightening susceptibility to hospital-acquired infections (HAIs), multiorgan dysfunction, and ultimately poorer clinical outcomes (Miniet et al., 2021). Globally, alpha-diversity - the measure of number and spread of species within the microbiome - was slightly reduced in early ICU admission of adults and children. While this early measure was not associated with in-hospital mortality, the alpha-diversity was found to decline in ICU patients overtime (Evans et al., 2023). In addition to microbiome depletion, characterized by a reduction in microbial diversity and/or loss of commensal microbes, early evidence suggests that healthy hospital workers do not show increased colonization rates by bacteria associated with HAIs, implying that these organisms and a depleted and/or overtaxed immune system are necessary to create a pathological environment (Figure 1A). ICU patients have a higher susceptibility to HAIs due to interrelated factors: microbiome depletion from broad-spectrum antibiotics, immune dysfunction, and frequent barrier compromise from essential medical devices (Wohrley et al., 2018). Proposed mechanisms for this change include decreasing immunological barriers within the gut mucosa. This would include compromised mucosal epithelia, reduced secretory IgA, and impaired immune cell function, which normally would prevent the inward migration of pathogenic species and slow the removal of bacterial colonies from the gut. This dysregulation can heighten the host immune system's infection risk or inflammatory response, leading to organ system damage. A multifaceted approach is needed to analyze the various microbiota-related factors that contribute to HAIs to both prevent and manage them (Tozzo et al., 2022). For example, patients diagnosed with a *Clostridium difficile* infection are 70% more likely to be re-hospitalized with sepsis, highlighting the importance of minimizing dysbiosis in ICU patients (Nakov et al., 2020). These microbiome-related vulnerabilities are especially crucial in cancer patients, who face not only higher rates of ICU admission but often experience poorer

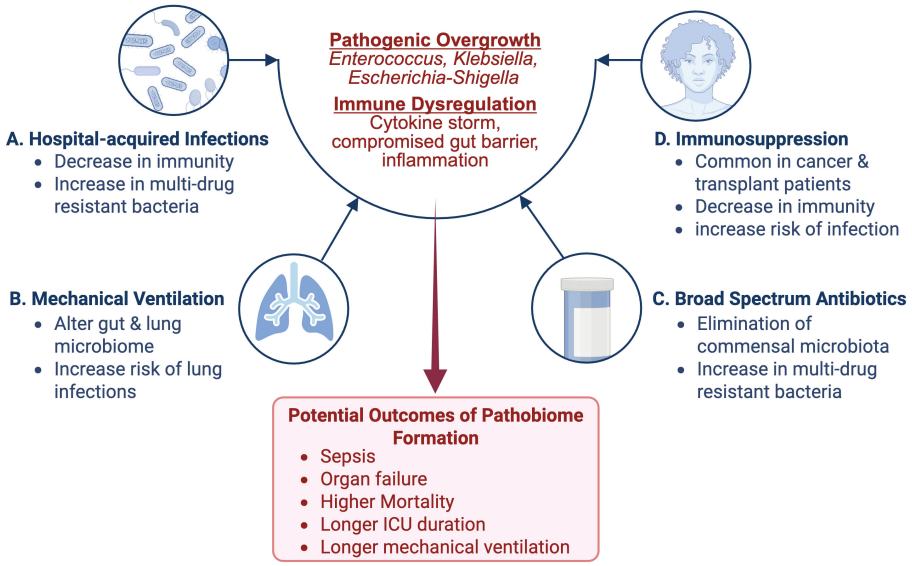


FIGURE 1
Contributing Factors to Microbiome Dysbiosis and Pathobiome Development in the ICU: Several factors specific to the ICU contribute to microbiome dysbiosis and subsequent pathobiome development. Among these factors are **(A)** the use of broad-spectrum antibiotics (ATB) can kill off commensal microbiota, allowing for resistant and potentially pathogenic microbiota to flourish; **(B)** the use of mechanical ventilation, which can alter the mucosal microbiome and increase the risk for lung infections; **(C)** Immunosuppression, which is common in the case of cancer and transplant patients, can lead to a decrease in immune defenses and increased infection risk **(D)** Hospital-acquired infections (HAIs), which can weaken immune defenses and introduce multidrug-resistant bacteria. Image created with BioRender.com.

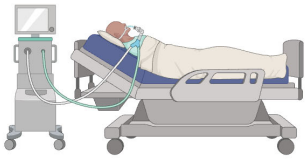
outcomes following intensive care unit interventions (Nazer et al., 2022).

Cancer patients in the ICU

Recent studies indicate that 5.2-6.4% of cancer patients will develop a critical illness that will result in an ICU visit within two to five years of diagnosis (Bos et al., 2015; Puxty et al., 2015). Lung and colorectal cancer rank as the most fatal, with lung and bronchus cancer accounting for 21% of mortalities (Siegel et al., 2023, 2024). Several studies report that lung cancer has the highest ICU mortality rate and poorest survival rate post-ICU admission, with an average ICU mortality rate of 40.1%. Notably, invasive mechanical ventilation, which is required for up to half of all ICU cancer patients, was a key factor linked to higher mortality (Soares et al., 2007; Andréjak et al., 2011; Puxty et al., 2014) (Figure 1B). Further, research suggests a link between microbial diversity and ICU outcomes, particularly during mechanical ventilation.

Recent studies have shown critically ill patients who underwent mechanical ventilation and didn't survive had significantly lower microbial α-diversity than survivors in their lung and gut, with noted migration of gut microbes to the lungs (Zhou et al., 2023). The diseased group also exhibited a significantly reduced concentration of fecal short-chain fatty acids (SFCAs): pentanoic acid, butyric acid, isobutyric acid, and isovaleric acid (Zhou et al., 2023). Along with a significantly increased amount of *Enterococcaceae* and *Enterobacteriaceae* within the gut and correlated with a 28-day mortality rate (Zhou et al., 2023). Additionally, 44% (27 patients) of the cohort died within 28 days and had significantly lower microbial diversity in both their lungs and gut ($P<0.05$) compared to the survivors (Zhou et al., 2023) (Figure 2). The microbiome of the survival group was enriched with commensal bacteria: *Streptococcus, Akkermansia, Lactobacillus*, and *Prevotella*. The deceased group showed decreased commensal bacteria and increased opportunistic bacteria: *Escherichia-Shigella, Klebsiella*, and *Enterococcus* (Figure 2). Further, patients with low lung α-diversity showed significantly higher mortality than those with high lung α-diversity ($P<0.01$) (Zhou et al., 2023). While this study provides key insights, note that it was completed in a small cohort and at a single center, highlighting the potentially limited application of these findings. Considering the significant link between microbial imbalances, respiratory infections, and poor outcomes in lung cancer patients on mechanical ventilation, similar investigations may be warranted in lung cancer patients with acute respiratory failure (ARF) (Yoo et al., 2013). These findings underscore the growing interest in how microbiome composition influences both respiratory outcomes and treatment efficacy in lung cancer patients.

Although immune checkpoint inhibitors (ICIs) have revolutionized the therapeutic landscape in cancers such as advanced non-small cell lung cancer (NSCLC), the microbiome has been shown to impact their efficacy. A recent study indicated that broad-spectrum antibiotics (ATB) significantly increased metastasis in ATB-treated ARF patients with NSCLC compared to non-ATB treated ($P<0.01$). These ATB-treated patients had a significant reduction in α-diversity within the gut microbiome, with notable shifts in phyla microbial







	Mechanical Ventilation	Broad Spectrum Antibiotics	Organ Transplant
Helpful Commensal Microbes	*Streptococcus* *Akkermansia* *Lactobacillus* *Prevotella*	*Actinobacteria* *Bifidobacteriaceae* *Coriobacteriaceae*	*Enterococcaceae* *Lactobacillaceae* *Clostridiaceae* *Ruminococcaceae* *Peptostreptococcaceae*
Harmful Microbes	*Enterococcaceae* *Enterobacteriaceae* *Escherichia-Shigella* *Klebsiella* *Enterococcus*	*Enterobacteriaceae* *Corynebacteriales* *Gammaproteobacteria*	*Clostridium difficile* *Bacteroidetes* *Enterobacteriaceae* *Streptococcaceae* *Bifidobacteriaceae*
Negative Health Impact	Increased mortality & days on mechanical ventilator	Increased metastasis in NSCLC & multidrug-resistant bacteria	Increased mortality, multidrug-resistant bacteria & acute cellular rejection
References	Zhou et al.	Xu et al.	Kato et al. Chan et al.

FIGURE 2
Helpful and harmful microbiota associated with treatment outcomes: A simplified overview of varying studies on ICU interventions and the microbes associated with helpful and harmful clinical outcomes. The gut microbiome is a complex network that has been shown to have drastic impacts on different treatments. Within the ICU, an increase in pathogenic and decrease in commensal bacteria can lead to increased mortality, increased metastasis in patients with NSCLC, and even acute cellular rejection. Image created with BioRender.com.

diversity. In the non-ATB group, the microbiome was enriched with *Actinobacteria*, *Bifidobacteriaceae*, and *Coriobacteriaceae*. In contrast, the ATB group contained *Enterobacteriaceae*, *Corynebacteriales*, and *Gammaproteobacteria* (Xu et al., 2022) (Figures 1C, 2). ATB can deplete bacterial colonies that are propionogenic, having the potential to produce short-chain fatty acids (SCFAs) essential for T-cell function, which are necessary for efficacy of ICIs and control of tumorgenesis (DiPalma and Blattman, 2023).

Microbiota diversity in patients significantly influences the immune response during tumorigenesis; individuals undergoing anticancer treatments have demonstrated a strong correlation between specific commensal bacteria and enhanced protective antitumor T-cell responses. Patients receiving PD-L1 inhibition therapies showed improved treatment efficacy when *Bifidobacteria* species were present in their microbiome (Gopalakrishnan et al., 2018). Patients treated with vancomycin exhibited more effective radiotherapy outcomes on tumor lesions when levels of immunosuppressive metabolites, specifically butyrate and propionate, derived from *Clostridiales*, were lowered post-antibiotic treatment (Sepich-Poore et al., 2021). This suggests that the concentration of propiongeneic species in the microbiome influences treatment efficacy. Lower abundances may impair immune cell function and contribute to treatment resistance. Since many therapies depend on functional T-cells, their dysregulation by microbiome shifts caused by ATB could directly undermine therapeutic outcomes.

It may seem counterintuitive that antibiotics are associated with a reduced efficacy of ICI in cancer therapy, given that decreases in microbial diversity are associated with increases in immune activation (Gopalakrishnan et al., 2018; Wilson et al., 2020). This paradox is likely explained by the loss of specific bacterial species, such as *A. muciniphila* and *Ruminococcaceae*, that are associated with improved ICI responses. Antibiotics may specifically reduce these "favorable" microbial species. Supporting this, the restoration of *A. muciniphila* to the microbiome can reinstate the efficacy of PD-1 blockade in a T cell-dependent manner in a mouse tumor model (Mager et al., 2020; Wang et al., 2024). Second, much like autoimmunity and HIV/AIDS progression, cancer is a long-term disease, and the rules for how alterations to the microbiome in acute versus chronic disease situations may be quite different. Decreased microbial diversity is associated with faster disease progression rather than improved prognosis. One may infer then that decreased microbial diversity and increases in circulating microbial products/metabolites may provide an advantage to the tumor rather than to the immune system.

Transplant and immunosuppressed patients in the ICU

Microbiome health is also critical in solid organ transplant recipients (SOTR), who face a heightened risk of severe infections

such as *C. difficile* infection (CDI) and recurrent CDI (rCDI), both linked to higher mortality, especially in liver transplant recipients (Rodig et al., 2023; Almohaya et al., 2024) (Figure 2). Studies in lung, kidney, and liver transplant patients have further shown that CDI and multidrug-resistant bacteria (MDRB) contribute to increased mortality (Chan et al., 2020; Ponholzer et al., 2024) (Figure 2). Often, these infections are treated with antibiotics and/or fecal transplants, which have been shown to restore the GI microbiome to a healthy state, implying a link between severe infection in SOTRs and their microbiome health. Further, SOTRs with gut dysbiosis are at an increased mortality risk (Swarte et al., 2022). As SOTR are already at increased risk of hospitalization, and individuals hospitalized in the ICU setting have a significantly increased dysbiosis due to various factors, understanding the relationship between microbiome health and SOTR mortality is principal (Donnelly et al., 2015; Szychowiak et al., 2022).

One study showed loss of microbial diversity in liver transplant patients was associated with acute cellular rejection (ACR) and bloodstream infections (BSI). In ACR, *Bacteroides, Enterobacteriaceae, Streptococcaceae*, and *Bifidobacteriaceae* were increased, whereas *Enterococcaceae, Lactobacillaceae*, *Clostridiaceae*, *Ruminococcaceae*, and *Peptostreptococcaceae* were decreased (Kato et al., 2017) (Figure 2). Within the Diaz et al. study evaluating the salivary microbiome of patients who underwent kidney and heart transplants, their microbiome was disrupted by opportunistic pathogenic species, including *Enterobacteriaceae, Pseudomonas* and *Acinetobacter* (Diaz et al., 2013). Certain species from the following families can be considered pathogenic *Bacteroides, Pseudomonas, Acinetobacter Enterobacteriaceae, Streptococcaceae*, and *Bifidobacteriaceae* (Kato et al., 2017). Even though some species of Bacteroides are among the dominant beneficial gut microbes, there are species that are considered pathogenic (Brown et al., 2019; Zafar and Saier, 2021). Commensal bacteria provide nutrients, reduce opportunistic microbes, assist in digestion, and modulate the immune system. Surgery, antibiotics, immunosuppressants, and other treatments can disrupt the microbiome in which pathogenic bacteria outcompete the commensal bacteria. Some of these opportunistic microbes, such as those within the *Enterobacteriaceae* family, can release immunogenic substances such as endotoxin lipopolysaccharide (LPS), which can induce an inflammatory response. Additionally, there is emerging evidence that has shown a relationship between the gut microbiome and solid organ transplant-associated pathogenic infections. One of the driving forces is associated with the interactions between the gut microbes and the host's immune system. Studies have shown that the microbiota can influence innate and adaptive immune responses. Multiple studies using mouse models found that broad-spectrum antibiotics worsened outcomes and reduced the effectiveness of cancer immunotherapy compared to the control group (Nelson et al., 2015; Zou et al., 2021; DiPalma and Blattman, 2023). The exact mechanism affecting the immune system via the elimination of the microbiome is still unknown. Many studies suggest that it's due to microbial-derived metabolites, such as short-chain fatty acids (SCFAs), that can influence the immune system (Gonçalves et al., 2018; DiPalma and Blattman, 2023). Additionally, other studies have shown that the microbiome can modulate T-cell homing (Yao et al., 2022). The reduction of beneficial microbes and increase in pathogenic microbes could potentially result in reduced T-cell homing, especially to mucosal sites (Yao et al., 2022). Overall, a healthy microbiome can reduce the number of infections in liver transplant recipients, a trend that may extend to other solid organ types (Chan et al., 2020).

In the context and heart transplantation, patients face a heightened risk of CDI and other nosocomial infections compared to recipients of other solid organs, highlighting a critical gap in our understanding (Donnelly et al., 2015). One potential clue to this increased risk of CDI is that in heart transplant recipients, immunosuppressive regimens have been associated with overgrowth of pathogenic microbial strains (Olek et al., 2023). The presence of pathogenic microbial strains in the mucosa could contribute to the etiology of harmful infections (Husebye, 2005). Shifts in microbiome composition in patients post-transplant and potentially in response to immunosuppressive therapy could result in other complications for patients. Lung transplant patients have been found to display a dysbiotic lung bacterial microbiome post-transplant and a higher chance of developing chronic lung allograft dysfunction (CLAD), which is characterized by hyperimmune activation in lung and airway tissues as well as an increased risk of allograft rejection (McGinniss et al., 2021; Wu et al., 2023).

The top five causes of death in kidney transplant recipients with allograft function is infectious complications (Chan et al., 2020) Several studies have shown a relationship between the pathogenesis of transplant associated infection due to the disruption of the microbiome post kidney transplant (Chan et al., 2020). Diarrhea is a common complication post kidney transplantation, which is thought to be due to the immunosuppressant mycophe-nolate mofetil (MMF). One study showed that within a cohort of 97 kidney transplant patients, 40 individuals who experienced post-transplant diarrhea had a significantly lower median Shannon diversity within the fecal specimens than the non-diarrhea group (Zhang et al., 2021). Additionally, *Ruminococcus*, *Bacteroides Dorea*, and *Coprococcus* were also significantly lower in the diarrhea group (Zhang et al., 2021). The aforementioned groups of genus contain species that are considered beneficial commensal bacteria within the gut, with *Bacteroides* being one of the most abundant within the gut.

In the case of hematopoietic stem cell transplants (HCSTs), those who receive allogeneic HCSTs with more diverse gut microbiomes have lower HCSTs-related mortality and increased overall survival compared with patients with lower microbial diversity (Gopalakrishnan et al., 2018). These studies suggest that greater microbial diversity is often associated with positive transplant patient outcomes and that more research is needed to determine if similar microbiome-driven survival benefits extend to lung and heart transplant recipients.

Differences in immunosuppression dosage across different transplant types may impact disparities of infection risk (Figure 1D). For example, maintenance dosing of tacrolimus, a commonly used immunosuppressant, in renal transplant recipients are typically 6–10 ng/mL trough levels, while cardiac transplant recipients are 10–15 ng/mL trough levels (Arnol et al., 2020; Lindenfeld et al., 2004).

A study using a mouse model, evaluated the effects of tacrolimus treatment which resulted in a significant shift in the abundance of *Bacteroides*, *Allobaculum* (P <.01), and *Lactobacillus* (P <.05), a decrease in *Clostridium* (P <.01), *Ruminococcus*, *Rikenella*, *Ruminococcaceae* (P <.05) as well as an increase in CD4 +CD25 hiFoxP3 + regulatory T cells in the blood and mucosa (Zhang et al., 2018). The significant difference was observed in both high dose tacrolimus treated and treatment via fecal transplant from a high dose tacrolimus-treated donor (Zhang et al., 2018). There was no significant change in the microbiota within the low dose (0.1 mg/kg) of tacromilus. Within a human study evaluating the impact of immunosuppressive treatments, everolimus plus mycophenolate mofetil (n = 9) vs tacrolimus plus mycophenolate mofetil (n = 11), on the gut microbiota within renal transplant patients showed no significant difference on the taxonomic level between the two groups but showed a difference in functional genes (Zaza et al., 2017). Within the Zaza et al. study, they did not have a control group without immunosuppressant treatment to compare to. Although there was no difference between the two groups treated with immunosuppressants, there may be a microbiome difference between patients receiving or not receiving immunosuppressants. Outside of the groups listed above, there is still a wide variety of patients with dysregulation of their immune systems from autoimmune diseases and treatments for these diseases. In mouse models, mice susceptible to developing rheumatoid arthritis (RA) showed a significant decrease in microbial diversity, even before disease onset. Within a human fecal genomic sequencing study, patients with RA were found to have increased concentration of pathogenic microbes and a concurrent decrease in commensal organisms (Lindenfeld et al., 2004). Current research indicates these changes increase the immune system's inflammatory responses, which can, therefore, degrade intestinal immune barriers and predispose a patient who is already prone to developing RA to develop more severe disease manifestations.

The causality of changes in the microbiome is less certain. Microbiomes of patients with Inflammatory Bowel Disease (IBD) show decreased diversity, including between areas of inflammation and without inflammation within the digestive tract (Shan et al., 2022). One mechanism by which this dysbiosis may be associated with the worsening of IBD presentation is that it can result in changes in the glycosylation of intestinal barrier cells, resulting in damage to the junctional proteins and upregulation of pro-inflammation gene transcription (Macfarlane et al., 2009).

Modulating the microbiome to alter health outcomes in the ICU

Another possibility was set forth by *Brenchley* et al (Brenchley et al., 2006), in which circulating microbial products are correlated with systemic immune activation in HIV patients and more rapid progression to AIDS. It may be inferred, then, that the microbiome may have systemic effects in another setting of immune activation and suggests that differences in the microbiome can impact immune function in the settings of transplant, autoimmunity, and responsiveness to immune checkpoint blockade for cancer. Research that has uncovered how the microbiome's composition can impact patients' outcomes in the ICU has also implied that alterations to it may improve treatment outcomes. Here, we describe potential modifications that may alter health outcomes and improve patient prognosis in ICU patients.

General patients in the ICU

One practical approach to ensuring positive outcomes for ICU patients is medical nutrition therapy (MNT), which involves nutritional interventions to manage critical conditions. For instance, a recent study demonstrated that administering a fiber-based diet in ICU patients receiving ATB allowed for colonizing bacteria that metabolize fiber into SCFAs associated with resistance to MDRB (Freedberg et al., 2020). However, the administration of MNT is not without risk; interventions must be approached with caution in critically ill patients, as impaired gut motility and barrier dysfunction could increase the risk of adverse events. More recent studies seek to characterize specific nutritional components that beneficially modulate the microbiome while minimizing possible adverse events, though these findings have yet to be published.

Cancer patients in the ICU

While the relationship between the microbiome and cancer progression is complex, much research suggests that its modulation can improve therapy outcomes. Promising interventions include probiotics, prebiotics, synbiotics, nutrition, and fecal microbiota transplant (FMT). Given the growing interest in these interventions, their potential application in critically ill cancer patients, including those requiring mechanical ventilation, has also gained attention.

In some cases, mechanical ventilation can lead to ventilator-associated pneumonia (VAP) within 48 hours, which is considered the most common complication in critically ill patients. The impact of probiotics and synbiotics on critically ill patients undergoing mechanical ventilation remains unclear. A meta-analysis evaluating 30 randomized clinical trials, where patients were administered with probiotics or synbiotics, suggested that within critically ill patients, probiotic intervention decreased the rate of infection by 20% and the rate of VAP by 25-30% (Manzanares et al., 2016) (Table 1). Additionally, within a randomized controlled trial (n = 72), evaluating the impact of synbiotics in reducing complications in mechanically ventilated patients with sepsis, the results indicated that the incidence of enteritis and VAP were significantly ($p < 0.05$) lower in patients administered with synbiotics (Shimizu et al., 2018) (Table 1). This study showed a significant increase in the total bacterial number of *Lactobacillus* and *Bifidobacterium* within the gut microbiome of the synbiotics group than the non-synbiotics group (Shimizu et al., 2018) (Table 1). Other factors, such as mortality rate and number of ventilator-free days at 28 days showed no statistical significance between the groups (Shimizu

TABLE 1 Study comparisons of ICU patients administered with probiotics or synbiotics.

Reference	Sample Size	Type of Probiotics or Synbiotics	Clinical Findings
Saikrishna et al.	n = 35	Probiotics: VSL#3® Capsule	No significant difference in prevalence of VAP and ventilator-free days between probiotic and non-probiotic groups. Statistical difference in microbiome composition between probiotic and non-probiotic groups, including the presence of *Lactobacillus paracasei, Lactobacillus rhamnosus*, and *Streptococcus thermophilus*
Knight et al.	n = 259	Synbiotics: Synbiotic 2000 FORTE®	No significant difference in the incidence of VAP or mortality between the synbiotic and non-synbiotic groups.
Shimizu et al.	n = 72	Synbiotics: *Bifidobacterium breve* strain *Yakult*, *Lactobacillus casei* strain *Shirota*, and galactooligosaccharides	Incidence of enteritis and VAP were significantly ($p < 0.05$) lower in patients administered with synbiotics. Significant increase in the total bacterial number of Lactobacillus and Bifidobacterium within the gut microbiome of the synbiotics group. Mortality rate and number of ventilator-free days at 28 days showed no statistical significance.
Manzanares et al.	Meta-analysis of 30 randomized clinical trials	Probiotics: *Lactobacillus plantarum*, *Lactobacillus rhamnosus* strain GG	Probiotic intervention decreased the rate of infection by 20% and the rate of VAP by 25-30%

et al., 2018) (Table 1). In contrary, a randomized, double-blind study (n = 259) evaluating the impact of Synbiotic 2000 FORTE® on VAP and mortality within mechanically ventilated ICU patients showed no significant difference in the incidence of VAP or mortality between the synbiotic and non-synbiotic groups (Knight et al., 2009) (Table 1). Note that the *Shimizu* et al. study evaluated mechanically ventilated patients with sepsis; meanwhile, the Knight et al. did not. An additional study by *Saikrishna* et al., evaluating the effects of probiotics on ICU patients (n = 35) and the prevalence of VAP and ventilator-free days also found no statistical difference between probiotic and non-probiotic groups (Saikrishna et al., 2023) (Table 1). Even though the aforementioned study showed a statistical difference in microbiome composition between probiotic and non-probiotic groups, including the presence of *Lactobacillus paracasei, Lactobacillus rhamnosus*, and *Streptococcus thermophilus* (Saikrishna et al., 2023), variations in these results could stem from sample size, duration, and type of probiotics/synbiotics, as well as a single-center study vs multi-center study and variation in ICU patient diagnosis, to name a few (Table 1). Within these studies, patients with cancer are included if they meet the study criteria, but they are not the sole study population. Further research is required to investigate the conditions in which probiotics and/or synbiotics are clinically effective for ICU patients. Understanding the conditions would further our knowledge of why studies have varying results and what factors maximize the benefits. Regardless once we further our understanding probiotics and synbiotics should be considered for clinical use, but this would require FDA approval and further research. Other than probiotics and synbiotic as a therapeutic option, nutrition and diet can be of therapeutic value since diet can modify the microbiome. The benefit of leveraging nutrition and diet is that this does not require FDA approval. Regardless, this would require further research to understand what types of nutrition and diet would benefit patients in the ICU.

Patients with cancer are prone to infection, leading to their admittance to the ICU and administration of antibiotics. A significant concern is MDRB linked to high mortality rates, such as carbapenemase-producing enterobacteria (CPE), extended-spectrum beta-lactamase-carrying strains, and vancomycin-resistant *Enterococcus* (VRE). As previously mentioned, cancer studies have indicated that antibiotic exposure can lead to dysbiosis and reduced treatment efficacy such as ICIs (Tsikala-Vafea et al., 2021). The effects of ATB can lead to dysbiosis, which can introduce pathogenic bacteria. Several proposed interventions include supplementing with probiotics/synbiotics, fecal transplants, and diet. Within a randomized double-blind, placebo-controlled trial (n = 120), patients were treated with amoxicillin-clavulanate antibiotics for 10 days and compared the effects of a 30-day intervention with placebo *Saccharomyces boulardii* CNCM I-745® and a probiotic treated group enriched with *Lactobacillus paracasei* Lpc-37, *Lactobacillus acidophilus* NCFM, *Bifidobacterium lactis* Bl-04, *Saccharomyces boulardii*, and *Bifidobacterium lactis* Bi-07 (Bactiol duo®) (Wieërs et al., 2021). The results showed a significant decrease in *Pseudomonas* after treatment with probiotics post-antibiotic treatment ($P < 0.05$) (Wieërs et al., 2021). Even though there was a transient increase in AmpC-producing enterobacteria after antibiotic treatment, the probiotic group had a significant decline ($P<0.05$) compared to the placebo group (Wieërs et al., 2021). The overall study claims an association of *Saccharomyces boulardii* paired with specific *Lactobacillus* and *Bifidobacterium* species decreasing the number of antibiotic-resistant pathogens within the gut and thus impacting antibiotic treatment (Wieërs et al., 2021).

Transplant and immunosuppressed patients

Noting that decreases in microbial diversity are associated with more severe impacts on transplant patients, several potential approaches to increasing gut microbiome diversity in patients have been investigated (Chan et al., 2020). These include

prebiotics (non-digestible food ingredients), probiotics (often bacteria and yeast-supplemented to increase microbial diversity), and nutrition-based interventions. While prebiotics have not been extensively studied for SOTR patients, there is evidence that hospitalization, especially in the ICU, can significantly alter dietary intake and lead to dysbiosis. Initial studies into SOTR probiotics have begun, focusing on liver transplant patients (Chan et al., 2020). Further, some studies suggest that critically ill patients who were given enteral nutrition with a high-protein diet enriched with arginine, fiber, and antioxidants had a significantly lower catheter-related sepsis rate than patients fed a standard high-protein diet. However, it is important to note that the use of microbiome-targeted interventions in SOTR patients remains limited by a lack of large-scale clinical trials. Resultantly, concerns about probiotic safety and variability in patient response still need to be further elucidated.

Beyond SOTRs, microbiome-related immune modulation has been implicated in other disease settings, including autoimmunity, HIV, and hematopoietic stem cell transplant (HSCT). In HSCT, in which patients that develop graft-versus-host disease (GVHD) have a higher propensity for gut microbiota dysbiosis (Bhatia et al., 2007; Taur et al., 2014). Notably, this dysbiosis mainly manifests as decreased microbial diversity, resulting in changes in microbial composition and differences in microbiome-derived metabolites, consistent with the model proposed by Brenchley et al. (2006) in HIV patients, may result in global increases in inflammation and immune activation resulting in predictors of poorer prognosis in GVHD patients. AS such, microbiome modulation strategies, such as fecal transplant or other methods described above, could significantly decrease GVHD in HSCT patients by concomitant decreases in inflammation and immune function.

Additional studies have shown that probiotics can reduce infection rates in liver transplant recipients (Zhang et al., 2013; Lederer et al., 2017). This indicates that including probiotics in the treatment regimen of SOTR and other immunocompromised individuals within the ICU setting may decrease infection rates. However, these solutions may be hindered by the lack of FDA-approved probiotics, creating a barrier to administer in a hospital or ICU setting as doctors cannot prescribe specific probiotics. Lack of regulation of probiotics may increase the risk of introducing unhealthy bacteria to patients due to contamination. On the other hand, prebiotics may be easily included by modifying the patient's diet under the guidance of a nutritionist. As our understanding of the microbiome expands, so must the treatment options for clinicians, including probiotics that may be prescribed, so that they can harness this expanded understanding for improved patient outcomes.

Discussion

Further research into how modulations of microbiome composition affect immunocompromised patients is essential for advancing current treatment modalities. Microbial imbalances can lead to adverse health outcomes, particularly in vulnerable populations such as ICU patients. Often, dysbiosis in these patients can be exacerbated by HAIs, among other factors. Studies have shown that microbial dysbiosis can result not only in complications such as infection, organ failure, and increased mortality in ICU patients but can be even more devastating for those with compromised immune systems. This can lead to increased toxicity, decreased effectiveness of anticancer agents, and adverse health outcomes in patients undergoing cellular therapy treatments (Gopalakrishnan et al., 2018; Sepich-Poore et al., 2021). Thus, studies that aim to reduce or reverse this dysbiosis need to be better understood to improve ICU patients' prognosis.

One area of research that holds the potential to skew dysbiosis in ICU patients is oral supplements that can be administered to patients before or during hospital admission to alter their microbiome and later bias those patients toward positive outcomes. For instance, using probiotics alone or combined with prebiotics in ICU patients prevented them from developing ventilator-associated pneumonia (VAP) (Batra et al., 2020). The use of prebiotics in the ICU to alter the microbiome's composition in patients holds much promise, as adding prebiotics or alternative nutrition that contains prebiotics can be easily changed and does not require FDA approval. Recent investigations have focused on nutrition's impact on modulating the gut microbiome's composition (Cresci, 2025). Some recent studies have indicated that applying fiber-rich diets increased the abundance of *Bifidobacterium* species, which are often involved in cross-feeding with other gut microbes (Oliver et al., 2021). Additionally, low-fiber diets promote the expansion of mucosa-degrading bacteria, while high-fiber diets can restore healthier microbiome composition (Desai et al., 2016). These findings suggest that targeted nutritional strategies could be an easy way to tailor the microbiome to shape the microbiome toward a more beneficial state.

Subsequent studies analyzed the impact of fecal microbiota transplantation on patients in the ICU with confirmed dysbiosis, demonstrating the reversal of dysbiosis post-transplant (Alagna et al., 2019). However, the lack of FDA regulation currently limits oral therapies that could potentially skew microbiome composition and prevent or reverse dysbiosis. They require more development before they can be applied clinically. It is also essential to consider the potential negative impacts of probiotics or microbiome transplantation before their administration to patients, as potential adverse effects can occur (Dailey et al., 2019). For instance, it is possible that gut flora associated with the pathobionts could be introduced into a patient's microbiome, especially post-fecal transplantation, potentially leading to adverse health outcomes and unintentional infections, such as the development of antibiotic-resistant bacteria, which has been seen in some patient cases post-fecal transplant (CH and CT, 2020). Additionally, standard methods of fecal microbiota transplant administration, such as colonoscopy or nasogastric tube delivery, can result in psychological stress to patients, causing them to meet with hesitation or resistance (Qu et al., 2022). Further, differences in donor-related variability could result in inconsistent outcomes, as there is currently no consensus on what an "ideal" donor looks like.

All these potential contradictions should be considered when deciding to administer FMT.

Another promising research avenue is gaining a deeper understanding of the pathobiome, which, if understood, could help us design treatments that eliminate pathogenic microbes and skew patient responses in a more favorable direction. When colonized with microbes that drive inflammatory responses, patients have been found to have an increased risk of complications associated with inflammation, including sepsis (Alverdy and Krezalek, 2017). Targeting specific inflammatory microbes in the pathobiome could be a potential intervention with the ICU, but more research is required to understand this complex dynamic. The interplay between the microbiome shift to a pathobiome, the gut environment, microbial byproducts, and the immune system is not well understood. Most current research investigates the reestablishment of a healthy microbiome via probiotics, synbiotics, nutrition, antibiotics, fecal microbiota transplant, and immune-boosting strategies, but there is a lack of research focusing on a therapeutic approach to targeting specifically the inflammation-causing pathobiome. Further studies that aim to identify which species are involved in negative or positive patient outcomes and learn which microbial species promote health may also be beneficial in developing future therapeutic interventions. Ultimately, they hold the potential to exploit these findings to improve patient outcomes in the ICU.

Author contributions

LN: Conceptualization, Data curation, Funding acquisition, Investigation, Writing – original draft, Writing – review & editing, Formal analysis, Project administration, Validation, Visualization. AR: Writing – original draft, Writing – review & editing, Data curation, Formal analysis, Investigation. JH: Data curation, Investigation, Writing – original draft, Writing – review & editing, Formal analysis. SS: Writing – review & editing. AnI: Formal analysis, Investigation, Writing – original draft, Writing – review & editing. ArI: Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing. JB: Writing – review & editing. MD: Conceptualization, Formal analysis, Investigation, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. Funding for this article was provided in part by the Graduate Student Government (GSG) at Arizona State University (ASU) in conjunction with the Graduate College at ASU.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Alagna, L., Haak, B. W., and Gori, A. (2019). Fecal microbiota transplantation in the ICU: perspectives on future implementations. *Intensive Care Med.* 45, 998–1001. doi: 10.1007/S00134-019-05645-7/TABLES/2

Almohaya, A., Fersovich, J., Weyant, R. B., Fernández García, O. A., Campbell, S. M., Doucette, K., et al. (2024). The impact of colonization by multidrug resistant bacteria on graft survival, risk of infection, and mortality in recipients of solid organ transplant: systematic review and meta-analysis. *Clin. Microbiol. Infection* 30, 1228–1243. doi: 10.1016/j.cmi.2024.03.036

Alverdy, J. C., and Krezalek, M. A. (2017). Collapse of the microbiome, emergence of the pathobiome, and the immunopathology of sepsis. *Crit. Care Med.* 45, 337–347. doi: 10.1097/CCM.0000000000002172

Andréjak, C., Terzi, N., Thielen, S., Bergot, E., Zalcman, G., Charbonneau, P., et al. (2011). Admission of advanced lung cancer patients to intensive care unit: A retrospective study of 76 patients. *BMC Cancer* 11. doi: 10.1186/1471-2407-11-159

Arnol, M., Naumovic, R., Dimitrov, E. P., Racki, S., Bucsa, C. A., Covic, A., et al. (2020). Immunosuppressive regimens following kidney transplantation in five European countries: The observational RECORD study. *Transplant. Rep.* 5, 100061. doi: 10.1016/J.TPR.2020.100061

Batra, P., Soni, K. D., and Mathur, P. (2020). Efficacy of probiotics in the prevention of VAP in critically ill ICU patients: an updated systematic review and meta-analysis of randomized control trials. *J. Intensive Care* 8, 1–14. doi: 10.1186/S40560-020-00487-8/FIGURES/14

Bhatia, S., Francisco, L., Carter, A., Sun, C. L., Scott Baker, K., Gurney, J. G., et al. (2007). Late mortality after allogeneic hematopoietic cell transplantation and functional status of long-term survivors: report from the Bone Marrow Transplant Survivor Study. *Blood* 110, 3784–3792. doi: 10.1182/BLOOD-2007-03-082933

Bos, M. M. E. M., Verburg, I. W. M., Dumaij, I., Stouthard, J., Nortier, J. W. R., Richel, D., et al. (2015). Intensive care admission of cancer patients: a comparative analysis. *Cancer Med.* 4. doi: 10.1002/cam4.430

Brenchley, J. M., Price, D. A., Schacker, T. W., Asher, T. E., Silvestri, G., Rao, S., et al. (2006). Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat. Med.* 12, 1365–1371. doi: 10.1038/nm1511

Brown, E. M., Ke, X., Hitchcock, D., Jeanfavre, S., Avila-Pacheco, J., Nakata, T., et al. (2019). Bacteroides-derived sphingolipids are critical for maintaining intestinal homeostasis and symbiosis. *Cell Host Microbe* 25, 668–680.e7. doi: 10.1016/J.CHOM.2019.04.002

CH, C., and CT, C. (2020). Drug-resistant bacteremia after fecal microbiota transplant. *N Engl. J. Med.* 382, 1960–1961. doi: 10.1056/NEJMC2002496

Chan, S., Hawley, C. M., Campbell, K. L., Morrison, M., Campbell, S. B., Isbel, N. M., et al. (2020). Transplant associated infections—The role of the gastrointestinal microbiota and potential therapeutic options. *Nephrology* 25, 5–13. doi: 10.1111/NEP.13670

Cresci, G. A. M. (2025). Understanding how foods and enteral feedings influence the gut microbiome. *Nutr. Clin. Pract.* doi: 10.1002/NCP.11285

Dailey, F. E., Turse, E. P., Daglilar, E., and Tahan, V. (2019). The dirty aspects of fecal microbiota transplantation: a review of its adverse effects and complications. *Curr. Opin. Pharmacol.* 49, 29–33. doi: 10.1016/J.COPH.2019.04.008

Desai, M. S., Seekatz, A. M., Koropatkin, N. M., Kamada, N., Hickey, C. A., Wolter, M., et al. (2016). A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell* 167, 1339–1353.e21. doi: 10.1016/J.CELL.2016.10.043/ATTACHMENT/44648B92-C9AB-4773-9950-347AF836AAF8/MMC7.XLSX

Diaz, P. I., Hong, B. Y., Frias-Lopez, J., Dupuy, A. K., Angeloni, M., Abusleme, L., et al. (2013). Transplantation-associated long-term immunosuppression promotes oral colonization by potentially opportunistic pathogens without impacting other members of the salivary bacteriome. *Clin. Vaccine Immunol.* 20. doi: 10.1128/CVI.00734-12

DiPalma, M. P., and Blattman, J. N. (2023). The impact of microbiome dysbiosis on T cell function within the tumor microenvironment (TME). *Front. Cell Dev. Biol.* 11. doi: 10.3389/FCELL.2023.1141215/BIBTEX

Donnelly, J. P., Wang, H. E., Locke, J. E., Mannon, R. B., Safford, M. M., and Baddley, J. W. (2015). Hospital-onset Clostridium difficile infection among solid organ transplant recipients. *Am. J. Transplant.* 15, 2970–2977. doi: 10.1111/AJT.13491

Evans, T., Ali, U., Anderton, R., Raby, E., Manning, L., and Litton, E. (2023). Lower gut dysbiosis and mortality in acute critical illness: a systematic review and meta-analysis. *Intensive Care Med. Exp.* 11, 1–17. doi: 10.1186/S40635-022-00486-Z/TABLES/3

Freedberg, D. E., Messina, M., Lynch, E., Tess, M., Miracle, E., Chong, D. H., et al. (2020). Impact of fiber-based enteral nutrition on the gut microbiome of ICU patients receiving broad-spectrum antibiotics: A randomized pilot trial. *Crit. Care Explor.* 2, E0135. doi: 10.1097/CCE.0000000000000135

Gonçalves, P., Araújo, J. R., and Di Santo, J. P. (2018). A cross-talk between microbiota-derived short-chain fatty acids and the host mucosal immune system regulates intestinal homeostasis and inflammatory bowel disease. *Inflammation Bowel Dis.* 24. doi: 10.1093/ibd/izx029

Gopalakrishnan, V., Helmink, B. A., Spencer, C. N., Reuben, A., and Wargo, J. A. (2018). The influence of the gut microbiome on cancer, immunity, and cancer immunotherapy. *Cancer Cell* 33, 570–580. doi: 10.1016/J.CCELL.2018.03.015

Husebye, E. (2005). The pathogenesis of gastrointestinal bacterial overgrowth. *Chemotherapy* 51, 1–22. doi: 10.1159/000081988

Huttenhower, C., Gevers, D., Knight, R., Abubucker, S., Badger, J. H., Chinwalla, A. T., et al. (2012). Structure, function and diversity of the healthy human microbiome. *Nature* 486, 207–214. doi: 10.1038/nature11234

Kato, K., Nagao, M., Miyamoto, K., Oka, K., Takahashi, M., Yamamoto, M., et al. (2017). Longitudinal analysis of the intestinal microbiota in liver transplantation. *Transplant. Direct* 3, E144. doi: 10.1097/TXD.0000000000000661

Knight, D. J. W., Gardiner, D., Banks, A., Snape, S. E., Weston, V. C., Bengmark, S., et al. (2009). Effect of synbiotic therapy on the incidence of ventilator associated pneumonia in critically ill patients: A randomised, double-blind, placebo-controlled trial. *Intensive Care Med.* 35. doi: 10.1007/s00134-008-1368-1

Lederer, A. K., Pisarski, P., Kousoulas, L., Fichtner-Feigl, S., Hess, C., and Huber, R. (2017). Postoperative changes of the microbiome: are surgical complications related to the gut flora? A systematic review. *BMC Surg.* 17, 125. doi: 10.1186/S12893-017-0325-8

Lindenfeld, J. A., Miller, G. G., Shakar, S. F., Zolty, R., Lowes, B. D., Wolfel, E. E., et al. (2004). Drug therapy in the heart transplant recipient part I: Cardiac rejection and immunosuppressive drugs. *Circulation* 110, 3734–3740. doi: 10.1161/01.CIR.0000149745.83186.89/ASSET/4FD0AC59-E843-4AEC-A3E8-BADD639C208D/ASSETS/GRAPHIC/20FF1.JPEG

Macfarlane, G., Blackett, K., Nakayama, T., Steed, H., and Macfarlane, S. (2009). The gut microbiota in inflammatory bowel disease. *Curr. Pharm. Des.* 15, 1528–1536. doi: 10.2174/138161209788168146

Mager, L. F., Burkhard, R., Pett, N., Cooke, N. C. A., Brown, K., Ramay, H., et al. (2020). Microbiome-derived inosine modulates response to checkpoint inhibitor immunotherapy. *Sci. (1979)* 369, 1481–1489. doi: 10.1126/SCIENCE.ABC3421/SUPPL_FILE/ABC3421_MDAR_REPRODUCIBILITY_CHECKLIST.PDF

Manos, J. (2022). The human microbiome in disease and pathology. *APMIS* 130, 690–705. doi: 10.1111/APM.13225

Manzanares, W., Lemieux, M., Langlois, P. L., and Wischmeyer, P. E. (2016). Probiotic and synbiotic therapy in critical illness: A systematic review and meta-analysis. *Crit. Care* 20. doi: 10.1186/s13054-016-1434-y

McDonald, D., Ackermann, G., Khailova, L., Baird, C., Heyland, D., Kozar, R., et al. (2016). Extreme dysbiosis of the microbiome in critical illness. *mSphere* 1, 199–215. doi: 10.1128/MSPHERE.00199-16/SUPPL_FILE/SPH004162139S1.DOCX

McGinniss, J. E., Whiteside, S. A., Simon-Soro, A., Diamond, J. M., Christie, J. D., Bushman, F. D., et al. (2021). The lung microbiome in lung transplantation. *J. Heart Lung Transplant.* 40, 733–744. doi: 10.1016/J.HEALUN.2021.04.014

Miniet, A. A., Grunwell, J. R., and Coopersmith, C. M. (2021). The microbiome and the immune system in critical illness. *Curr. Opin. Crit. Care* 27, 157–163. doi: 10.1097/MCC.0000000000000800

Moloney, R. D., Desbonnet, L., Clarke, G., Dinan, T. G., and Cryan, J. F. (2014). The microbiome: Stress, health and disease. *Mamm. Genome* 25, 49–74. doi: 10.1007/s00335-013-9488-5

Munley, J. A., Kelly, L. S., Pons, E. E., Kannan, K. B., Coldwell, P. S., Whitley, E. M., et al. (2023). Multicompartmental traumatic injury and the microbiome: Shift to a pathobiome. *J. Trauma Acute Care Surg.* 94, 15–22. doi: 10.1097/TA.0000000000003803

Nakov, R., Segal, J. P., Settanni, C. R. S., Bibb, S., Barrini, A. G., Cammarota, G., et al. (2020). Microbiome: what intensivists should know. *Minerva Anestesiol* 86, 777–785. doi: 10.23736/S0375-9393.20.14278-0

Nazer, L., Lopez-Olivo, M. A., Cuenca, J. A., Awad, W., Brown, A. R., Abusara, A., et al. (2022). All-cause mortality in cancer patients treated for sepsis in intensive care units: a systematic review and meta-analysis. *Supportive Care Cancer* 30, 10099–10109. doi: 10.1007/S00520-022-07392-W/METRICS

Nelson, M. H., Diven, M. A., Huff, L. W., and Paulos, C. M. (2015). Harnessing the microbiome to enhance cancer immunotherapy. *J. Immunol. Res.* 2015. doi: 10.1155/2015/368736

Olek, K., Kuczaj, A. A., Warwas, S., Hrapkowicz, T., Przybyłowski, P., and Tanasiewicz, M. (2023). Gut microbiome in patients after heart transplantation—Current state of knowledge. *Biomedicines* 11, 1588. doi: 10.3390/BIOMEDICINES11061588

Oliver, A., Chase, A. B., Weihe, C., Orchanian, S. B., Riedel, S. F., Hendrickson, C. L., et al. (2021). High-fiber, whole-food dietary intervention alters the human gut microbiome but not fecal short-chain fatty acids. *mSystems* 6. doi: 10.1128/MSYSTEMS.00115-21/SUPPL_FILE/MSYSTEMS.00115-21-ST001.XLSX

Ponholzer, F., Bogensperger, C., Krendl, F. J., Krapf, C., Dumfarth, J., Schneeberger, S., et al. (2024). Beyond the organ: lung microbiome shapes transplant indications and outcomes. *Eur. J. Cardio-Thoracic Surg.* 66. doi: 10.1093/EJCTS/EZAE338

Puxty, K., McLoone, P., Quasim, T., Kinsella, J., and Morrison, D. (2014). Survival in solid cancer patients following intensive care unit admission. *Intensive Care Med.* 40. doi: 10.1007/s00134-014-3471-9

Puxty, K., McLoone, P., Quasim, T., Sloan, B., Kinsella, J., and Morrison, D. S. (2015). Risk of critical illness among patients with solid cancers. *JAMA Oncol.* 1. doi: 10.1001/jamaoncol.2015.2855

Qu, Z., Tian, P., Yang, B., Zhao, J., Wang, G., and Chen, W. (2022). Fecal microbiota transplantation for diseases: Therapeutic potential, methodology, risk management in clinical practice. *Life Sci.* 304, 120719. doi: 10.1016/J.LFS.2022.120719

Rodig, N. M., Weatherly, M., Kaplan, A. L., Ballal, S. A., Elisofon, S. A., Daly, K. P., et al. (2023). Fecal microbiota transplant in pediatric solid organ transplant recipients. *Transplantation* 107, 2073–2077. doi: 10.1097/TP.0000000000004656

Russo, E., Taddei, A., Ringressi, M. N., Ricci, F., and Amedei, A. (2016). The interplay between the microbiome and the adaptive immune response in cancer development. *Therap Adv. Gastroenterol.* 9, 594–605. doi: 10.1177/1756283X16635082/ASSET/IMAGES/LARGE/10.1177_1756283X16635082-FIG1.JPEG

Saikrishna, K., Talukdar, D., Das, S., Bakshi, S., Chakravarti, P., Jana, P., et al. (2023). Study on effects of probiotics on gut microbiome and clinical course in patients with critical care illnesses. *Microb Ecol.* 86. doi: 10.1007/s00248-023-02224-8

Sepich-Poore, G. D., Zitvogel, L., Straussman, R., Hasty, J., Wargo, J. A., and Knight, R. (2021). The microbiome and human cancer. *Sci. (1979)* 371. doi: 10.1126/SCIENCE.ABC4552/ASSET/F710C098-B478-4A4D-836C-46346490BCF8/ASSETS/GRAPHIC/371_ABC4552_FA.JPEG

Shan, Y., Lee, M., and Chang, E. B. (2022). The gut microbiome and inflammatory bowel diseases. *Annu. Rev. Med.* 73, 455–468. doi: 10.1146/ANNUREV-MED-042320-021020

Shi, Y., Wang, G., Lau, H., Yu, C.-H., Metagenomic, J., Shi, Y., et al. (2022). Metagenomic sequencing for microbial DNA in human samples: emerging technological advances. *Int. J. Mol. Sci. 2022* 23, 2181. doi: 10.3390/IJMS23042181

Shimizu, K., Yamada, T., Ogura, H., Mohri, T., Kiguchi, T., Fujimi, S., et al. (2018). Synbiotics modulate gut microbiota and reduce enteritis and ventilator-associated pneumonia in patients with sepsis: a randomized controlled trial. *Crit. Care* 22. doi: 10.1186/s13054-018-2167-x

Siegel, R. L., Giaquinto, A. N., and Jemal, A. (2024). Cancer statistic. *CA Cancer J. Clin.* 74. doi: 10.3322/caac.21820

Siegel, R. L., Miller, K. D., Wagle, N. S., and Jemal, A. (2023). Cancer statistic. *CA Cancer J. Clin.* 73. doi: 10.3322/caac.21763

Soares, M., Darmon, M., Salluh, J. I. F., Ferreira, C. G., Thiéry, G., Schlemmer, B., et al. (2007). Prognosis of lung cancer patients with life-threatening complications. *Chest* 131. doi: 10.1378/chest.06-2244

Swarte, J. C., Li, Y., Hu, S., Björk, J. R., Gacesa, R., Vila, A. V., et al. (2022). Gut microbiome dysbiosis is associated with increased mortality after solid organ transplantation. *Sci. Transl. Med.* 14. doi: 10.1126/SCITRANSLMED.ABN7566/SUPPL_FILE/SCITRANSLMED.ABN7566_MDAR_REPRODUCIBILITY_CHECKLIST.PDF

Szychowiak, P., Villageois-Tran, K., Patrier, J., Timsit, J. F., and Ruppé, É. (2022). The role of the microbiota in the management of intensive care patients. *Ann. Intensive Care* 12, 1–16. doi: 10.1186/S13613-021-00976-5

Taur, Y., Jenq, R. R., Perales, M. A., Littmann, E. R., Morjaria, S., Ling, L., et al. (2014). The effects of intestinal tract bacterial diversity on mortality following allogeneic hematopoietic stem cell transplantation. *Blood* 124, 1174–1182. doi: 10.1182/BLOOD-2014-02-554725

Thaiss, C. A., Zmora, N., Levy, M., and Elinav, E. (2016). The microbiome and innate immunity. *Nature* 2016. doi: 10.1038/nature18847

Tozzo, P., Delicati, A., and Caenazzo, L. (2022). Human microbiome and microbiota identification for preventing and controlling healthcare-associated infections: a systematic review. *Front. Public Health* 10. doi: 10.3389/FPUBH.2022.989496

Tsikala-Vafea, M., Belani, N., Vieira, K., Khan, H., and Farmakiotis, D. (2021). Use of antibiotics is associated with worse clinical outcomes in patients with cancer treated with immune checkpoint inhibitors: A systematic review and meta-analysis. *Int. J. Infect. Dis.* 106, 142–154. doi: 10.1016/J.IJID.2021.03.063

Viglianti, E. M., and Iwashyna, T. J. (2017). Toward the ideal ratio of patients to intensivists: finding a reasonable balance. *JAMA Intern Med.* 177, 396. doi: 10.1001/JAMAINTERNMED.2016.8476

Wang, S., Xu, B., Zhang, Y., Chen, G., Zhao, P., Gao, Q., et al. (2024). The role of intestinal flora on tumorigenesis, progression, and the efficacy of PD-1/PD-L1 antibodies in colorectal cancer. *Cancer Biol. Med.* 21, 65–82. doi: 10.20892/J.ISSN.2095-3941.2023.0376

Wieërs, G., Verbelen, V., Van Den Driessche, M., Melnik, E., Vanheule, G., Marot, J. C., et al. (2021). Do probiotics during in-hospital antibiotic treatment prevent colonization of gut microbiota with multi-drug-resistant bacteria? A randomized placebo-controlled trial comparing saccharomyces to a mixture of lactobacillus, bifidobacterium, and saccharomyces. *Front. Public Health* 8. doi: 10.3389/fpubh.2020.578089

Wilson, B. E., Routy, B., Nagrial, A., and Chin, V. T. (2020). The effect of antibiotics on clinical outcomes in immune-checkpoint blockade: a systematic review and meta-analysis of observational studies. *Cancer Immunology Immunotherapy* 69, 343–354. doi: 10.1007/S00262-019-02453-2/FIGURES/4

Wohrley, J. D., Bartlett, A. H., Wohrley, J. D., and Bartlett, A. H. (2018). The role of the environment and colonization in healthcare-associated infections. *Healthcare-Associated Infections Children* 17. doi: 10.1007/978-3-319-98122-2_2

Wu, J., Li, C., Gao, P., Zhang, C., Zhang, P., Zhang, L., et al. (2023). Intestinal microbiota links to allograft stability after lung transplantation: a prospective cohort study. *Signal Transduction Targeted Ther.* 8, 1–9. doi: 10.1038/s41392-023-01515-3

Xu, K., Cai, J., Xing, J., Li, X., Wu, B., Zhu, Z., et al. (2022). Broad-spectrum antibiotics associated gut microbiome disturbance impairs T cell immunity and promotes lung cancer metastasis: a retrospective study. *BMC Cancer* 22. doi: 10.1186/s12885-022-10307-x

Yao, L., D'Agostino, G. D., Park, J., Hang, S., Adhikari, A. A., Zhang, Y., et al. (2022). A biosynthetic pathway for the selective sulfonation of steroidal metabolites by human gut bacteria. *Nat. Microbiol* 7. doi: 10.1038/s41564-022-01176-y

Yoo, H., Suh, G. Y., Jeong, B. H., Lim, S. Y., Chung, M. P., Jung Kwon, O., et al. (2013). Etiologies, diagnostic strategies, and outcomes of diffuse pulmonary infiltrates causing acute respiratory failure in cancer patients: A retrospective observational study. *Crit. Care* 17. doi: 10.1186/cc12829

Zafar, H., and Saier, M. H. (2021). Gut Bacteroides species in health and disease. *Gut Microbes* 13, 1–20. doi: 10.1080/19490976.2020.1848158

Zaza, G., Gassa, A. D., Felis, G., Granata, S., Torriani, S., and Lupo, A. (2017). Impact of maintenance immunosuppressive therapy on the fecal microbiome of renal transplant recipients: Comparison between an everolimus-and a standard tacrolimusbased regimen. *PloS One* 12. doi: 10.1371/journal.pone.0178228

Zhang, Y., Chen, J., Wu, J., Chalson, H., Merigan, L., and Mitchell, A. (2013). Probiotic use in preventing postoperative infection in liver transplant patients. *Hepatobiliary Surg. Nutr.* 2, 142–147. doi: 10.3978/J.ISSN.2304-3881.2013.06.05

Zhang, Z., Liu, L., Tang, H., Jiao, W., Zeng, S., Xu, Y., et al. (2018). Immunosuppressive effect of the gut microbiome altered by high-dose tacrolimus in mice. *Am. J. Transplant.* 18. doi: 10.1111/ajt.14661

Zhang, L. T., Westblade, L. F., Iqbal, F., Taylor, M. R., Chung, A., Satlin, M. J., et al. (2021). Gut microbiota profiles and fecal beta-glucuronidase activity in kidney transplant recipients with and without post-transplant diarrhea. *Clin. Transplant.* 35. doi: 10.1111/ctr.14260

Zhou, P., Zou, Z., Wu, W., Zhang, H., Wang, S., Tu, X., et al. (2023). The gut-lung axis in critical illness: microbiome composition as a predictor of mortality at day 28 in mechanically ventilated patients. *BMC Microbiol* 23. doi: 10.1186/s12866-023-03078-3

Zou, R., Wang, Y., Ye, F., Zhang, X., Wang, M., and Cui, S. (2021). Mechanisms of primary and acquired resistance to PD-1/PD-L1 blockade and the emerging role of gut microbiome. *Clin. Trans. Oncol.* 23. doi: 10.1007/s12094-021-02637-2

frontiers | Frontiers in Immunology

TYPE Original Research
PUBLISHED 18 June 2025
DOI 10.3389/fimmu.2025.1553411

Check for updates

OPEN ACCESS

EDITED BY
Nar Singh Chauhan,
Maharshi Dayanand University, India

REVIEWED BY
Monika Yadav,
Sanjay Ghodawat University, India
Shashank Gupta,
Norwegian University of Life Sciences, Norway
Asiya Nazir,
Abu Dhabi University, United Arab Emirates

*CORRESPONDENCE
Mekuriaw Belayneh
mekuriawbelayneh82@gmail.com
Bantayehu Addis Tegegne
Bantayehuaddis.90@gmail.com

†PRESENT ADDRESS
Yeshambel Belyhun,
Institute of Human Virology, School of Medicine, University of Maryland, Baltimore, MD, United States

RECEIVED 30 December 2024
ACCEPTED 28 May 2025
PUBLISHED 18 June 2025

CITATION
Belayneh M, Tegegne BA, Lemma M, Abay Z and Belyhun Y (2025) Clinical and biochemical characterization of hepatitis B surface antigen-positive patients with or without *Helicobacter pylori* co-infection at the University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia. *Front. Immunol.* 16:1553411. doi: 10.3389/fimmu.2025.1553411

COPYRIGHT
© 2025 Belayneh, Tegegne, Lemma, Abay and Belyhun. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Clinical and biochemical characterization of hepatitis B surface antigen-positive patients with or without *Helicobacter pylori* co-infection at the University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia

Mekuriaw Belayneh[1]*, Bantayehu Addis Tegegne[2]*, Mulualem Lemma[3], Zenahebezu Abay[4] and Yeshambel Belyhun[3†]

[1]Department of Medical Laboratory Sciences, College of Medicine and Health Sciences, Debremarkos University, Debre Markos, Ethiopia, [2]Department of Pharmacy, College of Medicine and Health Sciences, Debremarkos University, Debre Markos, Ethiopia, [3]Department of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia, [4]Department of Internal Medicine, College of Medicine and Health Sciences, University of Gondar, Gondar, Ethiopia

Background: Hepatitis B virus (HBV) is a major global public health issue and the most common etiology of chronic liver disease (CLD). The relationship between *Helicobacter pylori* and HBsAg+ patients was not well investigated and has attracted much scientific and clinical interest, although the relationship remains controversial.

Objective: This study aimed to assess the clinical and biochemical characteristics of HBsAg+ liver disease patients with and without *H. pylori* infection.

Methods: From April 1, 2021, to March 30, 2022, a hospital-based cross-sectional study was done at the University of Gondar Comprehensive Specialized Hospital on 384 known HBsAg+ liver disease patients recruited using a convenient sampling technique. All the HBsAg+ patients were tested for fecal *H. pylori* antigen, and blood specimens were analyzed for ALT, AST, ALP, ALB, TP, BILT, TG, and TChol tests using an automated biochemistry analyzer. GraphPad Prism 8.02 and SPSS 25 were used for data analysis, considering a statistically significant *P-value* of 0.05.

Results: *H. pylori* co-infection was found in 153 (39.8%) of HBsAg+ study participants. ALT, AST, and total cholesterol mean levels were significantly higher in patients co-infected with *H. pylori* ($p<0.04$). Portal hypertension (47.8%), variceal bleeding (60.7%), and hepatocellular carcinoma (HCC) (57.5%) were more common ($p< 0.01$) in patients with HBV and H. pylori co-infection.

Conclusions: ALT, AST, and TChol mean levels were higher in *H. pylori* co-infected HBsAg+ patients. Our findings showed that *H. pylori* has a role in the elevation of clinical and biochemical parameters in HBsAg+ liver diseases.

KEYWORDS

HBV, chronic liver disease, H. pylori, HBV/H. pylori co-infection, Ethiopia

Introduction

Hepatitis B virus (HBV) is a partially double-stranded hepatotropic DNA virus (1). It is an enveloped DNA virus that belongs to the family *Hepadnaviridae*, genus *Orthohepadnavirus* (2). The envelope surrounds an icosahedral nucleocapsid, which encloses a partially double-stranded, relaxed circular DNA (rcDNA) genome of ~3.2 kilobases (3). Four partially overlapping open reading frames (ORFs), termed P (polymerase), S (surface), C (core), and X (HBx protein), define the coding capacity of the HBV genome (4). Mutations in the HBV genome have been observed in all four ORFs in both acute and chronic HBV-infected individuals (5). A total of ten different genotypes of HBV (A-J) have been reported worldwide (6).

Acute HBV infection in healthy and immune-competent adults causes self-restrictive disease with less than 2-3% progression to serum hepatitis B surface antigen-positive (HBsAg +) chronic hepatitis B (CHB) infections (7). The reason behind the adaptive replication of HBV in hepatocytes is mainly due to a lack of functional innate DNA sensing pathways to recognize, control, and clear the virus (8). The adaptive immune response mediates both viral clearance and liver damage, but HBV appears to cause little or no innate immune activation (9). Both the inability of the immune system to resolve CHB and the unique replication strategy of HBV to form a stable covalently closed circular DNA (cccDNA) minichromosome in the hepatocyte nucleus enable infection persistence (10).

The natural course of CHB infection can be divided into four chronological phases based on virus-host interactions: The immune-tolerance phase is characterized by active replication of HBV, HBV e antigen (HBeAg) positivity, and normal alanine transferase (ALT) levels. In the immune clearance phase, HBeAg-positive patients have elevated serum ALT levels and fluctuating HBV-DNA levels. The third stage is the inactive carrier state, in which patients clear HBeAg and develop the corresponding antibody to HBeAg (HBeAg seroconversion), with the remission of liver disease (11). Approximately 20 −30% of individuals in the inactive carrier state may experience a viral relapse and enter the reactivation phase (fourth stage) during follow-up (12, 13).

HBV is not directly cytopathic for hepatocytes. Unlike other viruses, such as hepatitis C virus (HCV) or human immunodeficiency virus (HIV), which enter a rapid phase of propagation after infection, acute HBV infection is preceded by low HBV DNA and antigens in serum and liver for many weeks before the subsequent amplification and spreading phase of HBV infection (14). The virus adapts to different mechanisms for persistent infection and is thought to be a 'stealth virus', which poorly induces the expression of type 1 interferon (15).

Liver cirrhosis is a diffuse condition characterized by fibrosis and nodule formation, with CHB virus infection as one of the causes (16). The dysfunctional immune responses play an essential role in persistent HBV infection as well as liver inflammation (17). When comparing the characteristics of immune responses in acute and chronic hepatitis B, Chronic HBV infection develops due to the failure of HBV-specific immune responses (18). Chronic infection of HBV revealed impaired dendritic cell (DC) function, which is reflected by the weakness of both T-cell and B-cell virus-specific immune responses in CHB patients (19).

Persistent exposure of T cells to HBV antigens is crucial for maintaining depressed T cell functionality (20). The quantitative and functional deficiencies of the HBV-specific T-cell response are well-acknowledged as a primary contributor to viral persistence (21). The HBV-specific T cell response is modulated during HBV infection by multi-factorial mechanisms, including programmed cell death 1 (PD-1) expression, IL-10, arginase, myeloid suppressor cells, and T regulatory cells (22).

The humoral immune responses will hand over the virus after release from hepatocytes (23). The released antigens (HBsAg, HBeAg, and HBcAg) will induce the production of their respective antibodies. Hepatitis B core antigen (HBcAg) will not circulate in the blood as it is found in the core of the virus. Anti-HBc antibodies are used to differentiate between acute and chronic HBV infections (24).

H. pylori causes both active and chronic infection and can lead to several disorders, from chronic gastritis to gastric adenocarcinoma,

Abbreviations: ALB, Albumin; ALP, Alkaline Phosphatase; ALT, Alanine Transaminase; AST, Aspartate Transaminase; BILT, Bilirubin Total; cccDNA, Covalently Closed Circular DNA; CHB, Chronic Hepatitis B; CLD, Chronic Liver Disease; DBIL, Direct Bilirubin; ELISA, Enzyme-Linked Immunosorbent Assay; GI, Gastrointestinal; HCC, Hepatocellular Carcinoma; *H. Pylori*, *Helicobacter pylori;* INF, Interferon; MAMPs, Microbe-associated Molecular Patterns; NAFLD, Non-alcoholic Fatty Liver Disease; PRR, Pathogen Recognition Receptors; PUD, Peptic Ulcer Disease; PCR, Polymerase Chain Reaction; RcDNA, Relaxed Circular DNA; TLR, Toll-like Receptor; Treg, Regulatory T cells; TP, Total Protein.

and activates both innate and adaptive immune responses, but the response fails to eradicate the infection (25). Although it is believed that *H. pylori* is a type of 'commensal bacterium", it cannot be classified as normal flora because all patients with gastro-duodenal *H. pylori* colonization show histological gastroenteritis (26). To complete the colonization process and cause harm to the gastric mucosa, the bacterium must overcome the stomach acid barrier and infiltrate the mucus layer (27). Gastric epithelial cells (GECs) are a primary target for *H. pylori* infection and actively contribute to the development of acute and chronic inflammation (28).

H. pylori may induce immunosuppressive Tregs, dampening antiviral immune responses against HBV and facilitating viral persistence. Chronic *H. pylori* infection could also exacerbate T-cell exhaustion, reducing control of HBV replication and increasing liver damage (29, 30). The greatest feature of *H. pylori* is its ability to last for years in the gastric epithelium of the host (31). This adaptive property occurs due to a complex mechanism of *H. pylori* persistence mediated by proteins, glycoconjugates, and lipids exposed on the surface of this bacterium (32). Gastric innate immune effectors can either eliminate the bacteria or mobilize adaptive immune responses (e.g., Toll-like receptors (TLRs) and cytosolic DNA sensor/adaptor proteins) (33). Recent studies have shown that *H. pylori* infection most often results in M1 (Inflammatory) and Mreg (Regulatory) macrophage activation (34). The recruited macrophages at the site of infection can produce IL-12, which stimulates T-helper 1(Th1) cells and the production of cytokines such as IFN-γ (35).

H. pylori escapes identification by pattern recognition receptors (PRR) by multiple methods, including avoidance of recognition by TLRs and inhibition of c-type lectin and Dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin (DC-SIGN) mediated signaling (36). Although the acquired immune response to *H. pylori* is composed of both Th1- and Th2-type cells, cytokine profiles indicate a predominance of a Th1 response (37). Although *H. pylori* is an extracellular pathogen, its immune response is biased to the Th1 type (38).

In recent years, *H. pylori* has been reported to be associated with the development of a variety of extra-digestive manifestations, including type 2 diabetes, and cardiovascular and liver diseases (39). Chronic *H. pylori* infection induces a Th1-mediated inflammatory response, potentially contributing to systemic inflammation that could exacerbate liver injury. Virulence factors (e.g., CagA, VacA) may enter the bloodstream, promoting oxidative stress and pro-inflammatory cytokine release, which might accelerate liver fibrosis or carcinogenesis. This study tried to evaluate the burden and clinical contribution of *H. pylori* infection on the outcome of HBV-related liver diseases in Northwest Ethiopia.

The relationship between *H. pylori* and liver diseases remains controversial. Recent studies have been trying to investigate the association of *H. pylori* in the progression of diseases other than gastrointestinal diseases (40). The presence of *Helicobacter* species has been reported in the hepatic tissues of patients with different hepatic disorders. Chronic inflammation from H. pylori might promote fibrosis progression to cirrhosis and HCC. However, confounders like viral hepatitis or environmental toxins complicate these associations (41). *H. pylori* has been implicated in some extra-digestive diseases such as cardiovascular, neurologic, and hepatobiliary conditions (42). *H. pylori* has the induction potential of regulatory B cells, suggesting its role in diminishing the response of effector B cells during HBV infections (43).

H. pylori infection in patients with liver cirrhosis may impact the exacerbation of inflammatory injuries in the stomach, which could directly or indirectly lead to a deficiency of liver function (44). Research conducted in China also showed that the *H. pylori* positivity rate is high among patients with CHB, and *H. pylori* is a putative risk factor in the development of HBV-related complications. This is because *H. pylori* reaches the liver via the bloodstream or the biliary system and then becomes an independent etiological factor causing inflammation (45).

In *H. pylori*-infected individuals, a Th2 cell-related response induces IgG1 production while a Th1-related response contributes to IgG2 production through IL-2 and IFN-γ secretion (46). *Helicobacter* species are regarded as important in the development of hepatobiliary illnesses because they can produce inflammatory, fibrotic, and necrotic damage to the liver, which can progress to HCC (47). In the present study, we tried to assess the interplay of HBV and *H. pylori* infections on CHB-related liver disease progression.

Materials and methods

Study area, design, and period

The study was conducted at the University of Gondar Comprehensive Specialized Hospital, located in Gondar city, Amhara National Regional State, Northwest Ethiopia. Gondar City is 738km Northwest of Addis Ababa, the capital city of Ethiopia. Based on the Central Statistical Agency of Ethiopia (CSA), Gondar City had a total population of 500,788, of whom 300,000 were men and 200,788 were women (48). The University of Gondar Comprehensive Specialized Hospital is a tertiary care center serving approximately 7 million people in Northwest Ethiopia. A hospital-based cross-sectional study was conducted from April 1, 2021, to March 30, 2022. It was a single-center institution-based study.

Source and study populations

All HBsAg+ liver disease patients attending the University of Gondar Comprehensive Specialized Hospital. HBsAg+ patients presented at the University of Gondar Comprehensive Specialized Hospital during the study period and were eligible to study.

Inclusion criteria exclusion criteria

Patients who were positive for HBsAg and with the age of above 18 years old were included in the current study. We excluded patients with clinically confirmed schistosomiasis, and viral hepatitis other than HBV, HIV, and pregnancy.

Sample size and sampling technique

The sample size was determined using the single population proportion formula by considering the prevalence of *H. pylori* in HBsAg+ patients =50% (p=0.5), Z $\alpha/2$ = 1.96, and margin of error =5% (d=0.05). Finally, the total sample size became 384 HBsAg+ individuals who were recruited using a convenient sampling technique.

Data collection and laboratory methods

Demographic and clinical data collection

Demographic and clinical data were collected following the approval of the study protocol by the ethical review committee of the University of Gondar. At each data collection unit, trained nurses collected all relevant information (demographic, clinical) using a structured questionnaire in a face-to-face interview with the patient and from the patient's medical records. During clinical data collection, the patients were enrolled from the inpatient and outpatient departments of the gastroenterology clinic at the University of Gondar Comprehensive Specialized Hospital. The clinical parameters, such as portal hypertension, hepatocellular carcinoma (HCC), variceal bleeding, and hepatic decompensation, were defined based on the radiological (ultrasound, endoscopy/colonoscopy) and pathology (biopsy) findings.

Specimen collection and processing

We collected about eight milliliters (8 mL) of venous blood by serum separator tubes (SST) from the forearms of all eligible patients. The serum was isolated from the collected blood samples by centrifuging at 3500 rpm for 5 minutes in the serology laboratory and separated into different serum test tubes. The first serum tube (4 ml) was transported to the clinical chemistry lab for biochemical testing. For further serological and virological examination, the second serum was transferred to cryotubes and stored at -80°C.

In addition, a stool sample was collected from each CLD patient included in this study for the identification of the *H. pylori* fecal antigen (RAPID Hp StAR test).

Biochemical assays

The mean concentrations of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), serum albumin (ALB), total protein (TP), total bilirubin (BILT), total cholesterol (TChol) and triglycerides (TG) were analyzed by using automated DxC AU 700 Chemiluminescence assay (BECKMAN COULTER, Ireland Inc., Lismeehan, O'Callaghan's Mills, Co. Clare, Ireland) following the manufacturer's instructions. Both enzymatic and non-enzymatic assays were performed by the same automated biochemistry analyzer.

Data quality control

Study site assessment and pretest of the tools and assays were done before data collection to optimize the experimental setup. Data collectors were given appropriate training about data collection and related procedures. Positive and negative controls were run against HBsAg and *H. pylori* tests. Patient samples were collected, shipped, and stored under appropriate conditions and strict supervision. Standard operating procedures (SOPs) and optimization protocols were followed during serological and virological assays.

Data analysis

Data entry was done via Microsoft Excel 2013. Normally distributed data were expressed as mean ± standard deviation, and skewed data were expressed as median (interquartile range, IQR). Statistical analyses were performed using SPSS 25.0 (IBM, New York) or GraphPad Prism 8.0.2. Categorical variables were analyzed by the Chi-square test, and nonparametric tests (Mann-Whitney test) were used for non-normal continuous data, by taking a *P-value* of<0.05 as statistically significant.

Ethical considerations

The study protocol was reviewed ethically and approved by the ethical committee of the School of Biomedical and Laboratory Sciences (SBLS) at the University of Gondar. Ethical clearance with a reference number (Ref. No. SBMLS/2759) was obtained. Study participants were asked for their consent to be included in the study. Study participants who tested positive for HBsAg and/or *H. pylori* during screening were linked to healthcare providers of the UoG hospital. All the information obtained from the study participants was coded to maintain confidentiality.

Results

Demographic characteristics of the study participants

From a total of 384 HBsAg+ adults enrolled in this study, 153 participants were *H. pylori* co-infected (39.8%). 259 (67.4%) subjects were males, and 268 (69.8%) of them were rural residents. Overall, 47.1% of subjects were unable to read and write, and 221 (57.6%) of them were farmers. Overall, 317 (82.6%) participants were married. Three hundred thirty-nine (88.3%) of participants were not alcohol users (Table 1).

Clinical and biochemical features of study participants

Among 384 HBsAg+ study participants, 83 (21.6%) patients used traditional medicine in different forms or preparations. Regarding their HBV treatment status, 106 (27.6%) patients were treatment-naïve. The treatment status and traditional medicine use of HBsAg+ patients showed no significant differences between the two groups of participants. Portal hypertension (47.8%), variceal

TABLE 1 Socio-demographic characteristics of HBsAg+ study participants with and without *H. pylori* at University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia, 2022.

Characteristics		*H. pylori* status			P-value
		Positive, n (%)	Negative, n (%)	Total, n (%)	Sub-total
Sex	Male	99 (64.7)	160 (69.2)	259 (67.4)	0.02
	Female	54 (35.3)	71 (31.8)	125 (32.6)	
	Ratio (Male/female)	1:8:1	2.25: 1	2.07: 1	
Age (years)	≤ 24	14 (9.1)	32 (13.8)	46 (12)	0.14
	25-30	15 (9.8)	41 (17.7)	56 (14.5)	
	31-34	15 (9.8)	15 (64.9)	30 (7.8)	
	35-39	17 (11.1)	35 (15.1)	52 (13.5)	
	≥40	92 (60.1)	108 (46.7)	200 (52)	
Residence	Rural	113 (73.8)	155 (66.2)	268 (69.8)	0.03
	Urban	40 (26.2)	76 (33.8)	116 (30.2)	
Level of education	Unable to read and write	77 (50.3)	104 (45)	181 (47.1)	0.06
	Read and write	42 (27.4)	74 (32)	116 (30.2)	
	Primary school	14 (9.1)	29 (12.5)	43 (11.2)	
	Secondary school	13 (8.5)	12 (5.2)	25 (6.5)	
	College and above	7 (4.6)	12 (5.2)	19 (4.9)	
Marital status	Married	128 (83.7)	189 (81.8)	317 (82.6)	0.2
	Single	18 (11.7)	28 (12.1)	46 (12)	
	Divorced	5 (3.3)	10 (4.3)	15 (3.9)	
	Widowed	2 (1.3)	4 (1.7)	6 (1.5)	
Occupation	Farmer	93 (60.8)	128 (55.4)	221 (57.6)	
	Merchant	29 (18.9)	53 (22.9)	62 (16)	
	Housewife	12 (7.8)	24 (10.4)	36 (9.4)	0.04
	Employee	12 (7.8)	16 (6.9)	28 (7.3)	
	Student	7 (4.6)	10 (4.3)	17 (4.4)	
Smoking habit	Smoker	7 (4.6)	5 (2.2)	12 (4.4)	0.6
	Non-smoker	146 (95.4)	226 (97.8)	372 (95.6)	
Alcohol use	Ever used	23 (15)	22 (9.5)	45 (11.7)	0.06
	Not used	130 (85)	209 (90.5)	339 (88.3)	
Total		153 (39.8)	231 (60.2)	384 (100)	

bleeding (60.7%), and hepatocellular carcinoma (HCC) (57.5%) had a significant association with *H. pylori* co-infection ($p<0.01$) (Table 2).

Among the biochemical parameters, ALT, AST, and TChol levels were significantly higher in the *H. pylori co*-infected patient group ($p<0.01$). Although there was a difference in the mean value of ALP, ALB, TP, and BILT in the two groups, the difference was not statistically significant. Overall, the mean concentrations of liver enzymes and proteins were higher in the *H. pylori* co-infected group of patients (Table 3).

Discussions

Infections with HBV and *H. pylori* are two major public health issues in Ethiopia. Epidemiological studies in Ethiopia have reported a high prevalence of both infections, but there were no previously published reports on the relationship between HBV and *H. pylori* in liver disease patients and the contribution of *H. pylori* co-infection, particularly in Northwestern Ethiopia. Assessing the clinical and laboratory features of liver disease patients infected with these important pathogens is crucial for understanding and

TABLE 2 Clinical characteristics of 384 HBsAg+ study participants with and without *H. pylori* at University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia, 2022.

Characteristics		*H. pylori*+, n (%)	*H. pylori*-, n (%)	Total, n (%)	*P-value*
Ascites	Yes	81 (44.8)	100 (55.2)	181 (47.1)	0.64
	No	72 (35.5)	131 (64.5)	203 (52.9)	
Portal hypertension	Yes	77 (47.8)	84 (52.2)	161 (42.9)	0.007
	No	76 (34.1)	147 (65.9)	223 (57.1)	
Hepatic decompensation	Yes	14 (58.3)	10 (41.7)	24 (6.2)	0.056
	No	139 (38.6)	221 (61.4)	360 (93.8)	
Variceal bleeding	Yes	37 (60.7)	24 (39.3)	61 (15.9)	<0.001
	No	116 (38.7)	207 (61.3)	323 (84.1)	
HCC	Yes	23 (57.5)	17 (42.5)	40 (10.4)	0.01
	No	130 (37.8)	214 (62.2)	344 (89.6)	
Treatment status for HBV	Naïve	39 (36.8)	67 (63.2)	106 (27.6)	0.45
	TDF experienced	114 (41)	164 (59)	278 (72.4)	
Traditional medicine of HBV	Ever used	36 (43.4)	47 (56.6)	83 (21.6)	0.46
	Not used	117 (38.9)	184 (61.1)	301 (78.4)	
Total, N		153 (39.8)	231 (60.2)	384 (100)	

N, Number at each group; n, Number; %, Percentage; TDF, Tenofovir Disoproxil Fumarate; HCC, Hepatocellular Carcinoma; HBV, Hepatitis B virus.

managing the disease progression in HBV-liver disease. In the present study, we tried to characterize the clinical, serological, and virological features of HBsAg+ liver disease patients with and without *H. pylori* co-infection.

In the current study, the prevalence of *H. pylori* infection (39.8%) among HBsAg+ patients appeared lower than the overall pooled prevalence in the general Ethiopian population (52.2%) (49). However, the prevalence of *H. pylori* infection in the current study population was comparable to similar studies on HBV-related liver disease patients (40, 50). Previous studies reported a higher *H. pylori* prevalence in the general population than in liver disease patients. This discrepancy could be due to the repeated exposure of patients to different antibiotics during hospital visits, variations in the sensitivity of fecal *H. pylori* antigen tests, and the time trend of studies showing a decreasing pattern of *H. pylori* infection.

H. pylori colonization of the liver may occur through bacterial translocation from the stomach through the portal system,

TABLE 3 Comparison of Biochemical Tests in 384 HBsAg+ Study Participants with and without *H. pylori* co-infection at University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia, 2022.

Parameter (U/L)	*H. pylori*- mean levels	*H. pylori* +, mean levels	Reference range	*P-value*
ALT (U/L)	35	40	0-30	0.01
AST (U/L)	42	56	0-37	0.002
ALP (U/L)	104	120	40-106	0.146
ALB (g/dl)	3.7	3.8	3.4-5.4	0.93
TP (g/dl)	7.1	7.2	6-8.3	0.28
BILT (mg/dl)	1.2	1.2	0.1-1.2	0.99
TChol (mg/dl)	130	140	<200	0.04
TG (mg/dl)	92	97	<150	0.06
Total, N	231 (60.2)	153 (39.8)		384 (100)

N, Number at each group; ALT, Alanine transaminase; AST, Aspartate transaminase; ALP, Alkaline phosphatase; ALB, Albumin; TP, Total protein; BILT, Total bilirubin; TChol, Total Cholesterol; TG, Triglycerides. U/L, Unit per liters.

especially in the advanced stages of liver disease when portal hypertension develops. Furthermore, the bacteria can reach the liver via circulating phagocytes and macrophages. The present study was in agreement with previous studies that demonstrated that post-inflammatory liver cirrhosis complications were more frequent in *H. pylori*-infected patients than in the *H. pylori*-negative patient group, as *H. pylori* could upregulate the expression of various inflammatory factors (50–52). In our study population, HBV-induced complications of liver cirrhosis, such as portal hypertension, variceal bleeding, and HCC, were more prevalent in *H. pylori* co-infected patients than in the HBV-mono-infected group of patients. In this study, hepatic decompensation was also higher among the co-infected group, although it showed no significant difference. These clinical findings confirm our hypothesis that *H. pylori* co-infection has a synergistic effect on the progression of CHB-related liver diseases (50).

Liver enzymes and non-enzymatic biochemical markers of liver function had been increased in our study subjects who had *H. pylori* co-infection, in line with similar studies (53). In this study, ALT, AST, and TChol levels were significantly higher in the *H. pylori* co-infected group of patients, while TG and ALP levels were slightly elevated in the same group of patients. Our results support the theory that liver colonization with *H. pylori* bacteria promotes liver function deterioration via toxic injury and autoimmune inflammation. These findings align with studies reporting the role of *H. pylori* in CHB disease progression (54, 55).

There were several limitations in this study. First thing, we had no access to molecular, experimental, and immunological assays. We just used a cross-sectional study design with convenience sampling techniques. We did not use quantitative assays to measure the level of fecal antigen for the diagnosis of *H. pylori* infection. Consequently, the exact change of values might not be revealed in the HBV/*H. pylori* co-infected and HBV mono-infected patients. Saying all these, this study provided insight into the contribution of *H. pylori* infection among HBV-infected liver disease patients.

Conclusions and recommendations

The ALT, AST, and TChol levels were increased during *H. pylori* co-infection. Our data also showed that clinical parameters, including portal hypertension, variceal bleeding, and hepatocellular carcinoma (HCC), were more frequently detected in HBsAg/*H. pylori* co-infected patients than those with HBsAg mono-infection. From the findings of the current study, it can be concluded that *H. pylori* significantly enhances the clinical, biochemical, and serological parameters during the co-infection of *H. pylori* with HBsAg+ liver disease patients. Physicians who treat liver disease patients are highly recommended to screen and treat patients with *H. pylori* co-infection. We propose active screening for *H. pylori* in patients with CHB. Virological markers of HBV should also be closely monitored during the treatment of CHB-related liver diseases. We would also like to recommend that future researchers conduct large-scale studies that include advanced laboratory methods with an adequate sampling of patients to assess the accurate implications of all test parameters of CHB-related liver diseases co-infected with *H. pylori* bacteria. Besides, it would be imperative that future studies include molecular and immunological methods with a longitudinal cohort of patients and advanced statistical methods.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Ethics statement

The study protocol was reviewed ethically following the Helsinki Declaration and approved by the ethical committee of the School of Biomedical and Laboratory Sciences (SBLS) at the University of Gondar. Ethical clearance with a reference number (Ref. No. SBMLS/2759) was obtained. Study participants were asked for their consent to be included in the study. Study participants who tested positive for HBsAg and/or *H. pylori* during screening were linked to healthcare providers of the UoG hospital. All the information obtained from the study participants was coded to maintain confidentiality.

Author contributions

MB: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. BT: Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. ML: Conceptualization, Data curation, Formal Analysis, Resources, Supervision, Writing – original draft, Writing – review & editing. ZA: Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing. YB: Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research and/or publication of this article.

Acknowledgments

We would like to express our gratitude to the Department of Immunology and Molecular Biology, School of Biomedical and Laboratory Sciences (SBLS), College of Medicine and Health Sciences (CMHS), University of Gondar, for allowing me to perform this study in my field of interest.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Valaydon ZS, Locarnini SA. The virological aspects of hepatitis B. *Best Pract Res Clin Gastroenterol.* (2017) 31:257–64. doi: 10.1016/j.bpg.2017.04.013

2. Sajid M, Ullah H, Yan K, He M, Feng J, Shereen MA, et al. The functional and antiviral activity of interferon alpha-inducible IFI6 against hepatitis B virus replication and gene expression. *Front Immunol.* (2021) 12:1028. doi: 10.3389/fimmu.2021.634937

3. Xi J, Luckenbaugh L, Hu J. Multiple roles of PP2A binding motif in hepatitis B virus core linker and PP2A in regulating core phosphorylation state and viral replication. *PloS Pathog.* (2021) 17:e1009230. doi: 10.1371/journal.ppat.1009230

4. Yuan S, Liao G, Zhang M, Zhu Y, Wang K, Xiao W, et al. Translatomic profiling reveals novel self-restricting virus-host interactions during HBV infection. *J Hepatol.* (2021) 75:74–85. doi: 10.1016/j.jhep.2021.02.009

5. Adesina OA, Akanbi OA, Opaleye OO, Japhet MO, Wang B, Oluyege AO, et al. Detection of Q129H immune escape mutation in apparently healthy hepatitis B virus carriers in southwestern Nigeria. *Viruses.* (2021) 13:1273. doi: 10.3390/v13071273

6. Song H, Xu F, Xiao Q, Tan G. Hepatitis B virus X protein and its host partners. *Cell Mol Immunol.* (2021) 18:1345–6. doi: 10.1038/s41423-021-00674-z

7. Joshi SS, Coffin CS. Hepatitis B and pregnancy: virologic and immunologic characteristics. *Hepatol Commun.* (2020) 4:157–71. doi: 10.1002/hep4.1460

8. Golsaz-Shirazi F, Shokri F. Cross talk between hepatitis B virus and innate immunity of hepatocytes. *Rev Med Virol.* (2022) 32(1):e2256. doi: 10.1002/rmv.2256

9. Iannacone M, Guidotti LG. Immunobiology and pathogenesis of hepatitis B virus infection. *Nat Rev Immunol.* (2022) 22:19–32. doi: 10.1038/s41577-021-00549-4

10. Dandri M. Epigenetic modulation in chronic hepatitis B virus infection. *Semin Immunopathology.* (2020) 42:173–85. doi: 10.1007/s00281-020-00780-6

11. Block TM, Chang K-M, Guo J-T. Prospects for the global elimination of hepatitis B. *Annu Rev Virol.* (2021) 8:437–58. doi: 10.1146/annurev-virology-091919-062728

12. Cai Y, Yin W. The multiple functions of B cells in chronic HBV infection. *Front Immunol.* (2020) 11:582292. doi: 10.3389/fimmu.2020.582292

13. Campos-Valdez M, Monroy-Ramírez HC, Armendáriz-Borunda J, Sánchez-Orozco LV. Molecular mechanisms during hepatitis B infection and the effects of the virus variability. *Viruses.* (2021) 13:1167. doi: 10.3390/v13061167

14. Cheng X, Xia Y, Serti E, Block PD, Chung M, Chayama K, et al. Hepatitis B virus evades innate immunity of hepatocytes but activates cytokine production by macrophages. *Hepatology.* (2017) 66:1779–93. doi: 10.1002/hep.29348

15. Megahed FAK, Zhou X, Sun P. The interactions between HBV and the innate immunity of hepatocytes. *Viruses.* (2020) 12:285. doi: 10.3390/v12030285

16. Mehrabi S, Genco E, Maturi M, D'Onofrio M. Chronic hepatitis and liver fibrosis/cirrhosis. In: *Imaging of the liver and intra-hepatic biliary tract*. United States: Springer (2021). p. 281–93.

17. Saeidi A, Zandi K, Cheok YY, Saeidi H, Wong WF, Lee CYQ, et al. T-cell exhaustion in chronic infections: reversing the state of exhaustion and reinvigorating optimal protective immune responses. *Front Immunol.* (2018) 9:2569. doi: 10.3389/fimmu.2018.02569

18. Lebossé F, Testoni B, Fresquet J, Facchetti F, Galmozzi E, Fournier M, et al. Intrahepatic innate immune response pathways are downregulated in untreated chronic hepatitis B. *J Hepatol.* (2017) 66:897–909. doi: 10.1016/j.jhep.2016.12.024

19. Farag MM, Peschel G, Müller M, Weigand K. Characterization of the interaction between subviral particles of hepatitis B virus and dendritic cells–*in* vitro study. *Infection Drug resistance.* (2019) 12:3125. doi: 10.2147/IDR.S221294

20. Marotel M, Villard M, Drouillard A, Tout I, Besson L, Allatif O, et al. Peripheral natural killer cells in chronic hepatitis B patients display multiple molecular features of T cell exhaustion. *Elife.* (2021) 10:e60095. doi: 10.7554/eLife.60095.sa2

21. Kräutler NJ, Yermanos A, Pedrioli A, Welten SP, Lorgé D, Greczmiel U, et al. Quantitative and qualitative analysis of humoral immunity reveals continued and personalized evolution in chronic viral infection. *Cell Rep.* (2020) 30:997–1012. e1016. doi: 10.1016/j.celrep.2019.12.088

22. Sun Y, Yu M, Qu M, Ma Y, Zheng D, Yue Y, et al. Hepatitis B virus-triggered PTEN/β-catenin/c-Myc signaling enhances PD-L1 expression to promote immune evasion. *Am J Physiology-Gastrointestinal Liver Physiol.* (2020) 318:G162–73. doi: 10.1152/ajpgi.00197.2019

23. Danane J, Allali K, Hammouch Z. Mathematical analysis of a fractional differential model of HBV infection with antibody immune response. *Chaos Solitons Fractals.* (2020) 136:109787. doi: 10.1016/j.chaos.2020.109787

24. Fourati S, Pawlotsky J-M. Recent advances in understanding and diagnosing hepatitis B virus infection. *Virologie.* (2019) 23:23–34. doi: 10.12688/f1000research.8983.1

25. Darmadi D, Ruslie RH. *Immunology of helicobacter pylori infection*. Rijeka: IntechOpen (2022). doi: 10.5772/intechopen.104592

26. Gu H. Role of flagella in the pathogenesis of helicobacter pylori. *Curr Microbiol.* (2017) 74:863–9. doi: 10.1007/s00284-017-1256-4

27. Wizenty J, Tacke F, Sigal M. Responses of gastric epithelial stem cells and their niche to Helicobacter pylori infection. *Ann Trans Med.* (2020) 8:568. doi: 10.21037/atm.2020.02.178

28. Pachathundikandi SK, Tegtmeyer N, Arnold IC, Lind J, Neddermann M, Falkeis-Veits C, et al. T4SS-dependent TLR5 activation by Helicobacter pylori infection. *Nat Commun.* (2019) 10:1–11. doi: 10.1038/s41467-019-13506-6

29. Ofoezie EF, Ogbonna CA, Olisakwe SC, Anunobi CJ, George ET, Babarinde S, et al. Alisigwe CV et al: Role of infectious agents in cancer pathogenesis and therapy. *Microbe.* (2025) 6:100284. doi: 10.1016/j.microb.2025.100284

30. Roetman JJ, Apostolova MKI, Philip M. Viral and cellular oncogenes promote immune evasion. *Oncogene.* (2022) 41:921–9. doi: 10.1038/s41388-021-02145-1

31. Ailloud F, Didelot X, Woltemate S, Pfaffinger G, Overmann J, Bader RC, et al. Within-host evolution of Helicobacter pylori shaped by niche-specific adaptation, intragastric migrations and selective sweeps. *Nat Commun.* (2019) 10:1–13. doi: 10.1038/s41467-019-10050-1

32. Ansari S, Yamaoka Y. Helicobacter pylori virulence factors exploiting gastric colonization and its pathogenicity. *Toxins.* (2019) 11:677. doi: 10.3390/toxins11110677

33. Dooyema SD, Noto JM, Wroblewski LE, Piazuelo MB, Krishna U, Suarez G, et al. Helicobacter pylori actively suppresses innate immune nucleic acid receptors. *Gut Microbes.* (2022) 14:2105102. doi: 10.1080/19490976.2022.2105102

34. Bagheri N, Salimzadeh L, Shirzad H. The role of T helper 1-cell response in Helicobacter pylori-infection. *Microbial pathogenesis.* (2018) 123:1–8. doi: 10.1016/j.micpath.2018.06.033

35. da Silva EAW, da Silva NMJW, Rodrigues RR, Adad SJ, de Lima Pereira SA, Ribeiro BM, et al. Arginase-1 and treg profile appear to modulate inflammatory process in patients with chronic gastritis: IL-33 may be the alarm cytokine in H. pylori-positive patients. *Mediators Inflammation.* (2019) 2019:2536781. doi: 10.1155/2019/2536781

36. Mejías-Luque R, Gerhard M. Immune evasion strategies and persistence of Helicobacter pylori. *Mol Pathogenesis Signal Transduction by Helicobacter pylori.* (2017) 400:53–71. doi: 10.1007/978-3-319-50520-6_3

37. Xie W, Zhao W, Zou Z, Kong L, Yang L. Oral multivalent epitope vaccine, based on UreB, HpaA, CAT, and LTB, for prevention and treatment of Helicobacter pylori infection in C57BL/6 mice. *Helicobacter*. (2021) 26(3):e12807. doi: 10.1111/hel.12807

38. Jafarzadeh A, Larussa T, Nemati M, Jalapour S. T cell subsets play an important role in the determination of the clinical outcome of Helicobacter pylori infection. *Microbial pathogenesis*. (2018) 116:227–36. doi: 10.1016/j.micpath.2018.01.040

39. Jamali R, Mofid A, Vahedi H, Farzaneh R, Dowlatshahi S. The effect of helicobacter pylori eradication on liver fat content in subjects with non-alcoholic Fatty liver disease: a randomized open-label clinical trial. *Hepatitis monthly*. (2013) 13: e14679. doi: 10.5812/hepatmon.14679

40. Séhonou J, Kpossou A, Kanvi J. Helicobacter pylori infection in hepatitis B virus carriers in Cotonou: epidemiology and socio-demographic factors associated with the co-infection. *Gastroenterol Hepatol Open Access*. (2019) 10:14–7. doi: 10.15406/ghoa.2019.10.00350

41. Castaño-Rodríguez N, Mitchell HM, Kaakoush NO. NAFLD, Helicobacter species and the intestinal microbiome. *Best Pract Res Clin Gastroenterol*. (2017) 31:657–68. doi: 10.1016/j.bpg.2017.09.008

42. Mohammadifard M, Saremi Z, Rastgoo M, Akbari E. Relevance between helicobacter pylori infection and non-alcoholic fatty liver disease in Birjand, Iran. *J Med Life*. (2019) 12:168. doi: 10.25122/jml-2019-0012

43. Jansen K, Cevhertas L, Ma S, Satitsuksanoa P, Akdis M, van de Veen W. Regulatory B cells, A to Z. *Allergy*. (2021) 76:2699–715. doi: 10.1111/all.14763

44. Al-Amrani M, Al-Zaazaai A. The relation between helicobacter pylori infection, serum ammonia and hepatic encephalopathy in Yemeni cirrhotic patients. *Res Rep Gastroenterol*. (2020) 111:2. doi: 10.37532/rrg.2020.4(1).111

45. Cui L, Markou A, Stratton CW, Lianidou E. Diagnosis and assessment of microbial infections with host and microbial microRNA profiles. In: *Advanced techniques in diagnostic microbiology*. Switzerland: Springer (2018). p. 563–97.

46. Soudi H, Falsafi T, Gharavi S, Mahboubi M. The role of helicobacter pylori proinflammatory outer membrane protein and propolis in immunomodulation on U937 macrophage cell model. *Galen Med J*. (2020) 9:1687. doi: 10.31661/gmj.v9i0.1687

47. Fukui H. Role of gut dysbiosis in liver diseases: what have we learned so far? *Diseases*. (2019) 7:58. doi: 10.3390/diseases7040058

48. Yirsa T. Serological and molecular detection of Toxoplasma gondii among slaughtered domestic ruminants and pregnant women in Gondar town, Northwest Ethiopia. *Tsedalu Yirsa*. (2020) 15:2403–12. doi: 10.17485/IJST/v15i44.1810

49. Melese A, Genet C, Zeleke B, Andualem T. Helicobacter pylori infections in Ethiopia; prevalence and associated factors: a systematic review and meta-analysis. *BMC Gastroenterol*. (2019) 19:1–15. doi: 10.1186/s12876-018-0927-3

50. Wang J, Chen R-C, Zheng Y-X, Zhao S-S, Li N, Zhou R-R, et al. Helicobacter pylori infection may increase the risk of progression of chronic hepatitis B disease among the Chinese population: a meta-analysis. *Int J Infect Dis*. (2016) 50:30–7. doi: 10.1016/j.ijid.2016.07.014

51. Madala S, MacDougall K, Surapaneni BK, Park R, Girotra M, Kasi A. Coinfection of helicobacter pylori and hepatitis C virus in the development of hepatocellular carcinoma: A systematic review and meta-analysis. *J Clin Med Res*. (2021) 13:530. doi: 10.14740/jocmr4637

52. Abdel-Razik A, Mousa N, Elhelaly R, Elzehery R, Hasan AS, Abdelsalam M, et al. Helicobacter pylori as an initiating factor of complications in patients with cirrhosis: a single-center observational study. *Front Med*. (2020) 7:96. doi: 10.3389/fmed.2020.00096

53. Zhao B, Sheng Q, Qin Y, Wang X, Zhao H, Zhao N. Correlations of Helicobacter pylori with liver function, inflammatory factors and serum levels of FoxP3 and RORgammat in patients with hepatitis B cirrhosis. *Eur Rev Med Pharm Sci*. (2021) 25:459–65. doi: 10.26355/eurrev_202101_24415

54. Kazemifar AM, Shafikhani AA, HajiNoormohammadi E, Azarion Z, Hajiaghamohammadi A. Investigating effect of Helicobacter pylori treatment on improvement of non-alcoholic fatty liver parameters: a randomized trial. *Egyptian Liver J*. (2019) 9:1–5. doi: 10.1186/s43066-019-0001-z

55. Łapiński TW. The importance of H. pylori infection in liver diseases. In: *Helicobacter pylori-new approaches of an old human microorganism*. Rijeka: IntechOpen (2018).

frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 14 August 2025
DOI 10.3389/fimmu.2025.1642942

Check for updates

OPEN ACCESS

EDITED BY
Nar Singh Chauhan,
Maharshi Dayanand University, India

REVIEWED BY
Monika Yadav,
Sanjay Ghodawat University, India
Gulin Ozcan,
Atilim University School of Medicine, Türkiye
Naincy Rathee,
Maharshi Dayanand University, India

*CORRESPONDENCE
Fuju Wu
Wufj@jlu.edu.cn

RECEIVED 07 June 2025
ACCEPTED 23 July 2025
PUBLISHED 14 August 2025

CITATION
Deng Y, Li Y, Liu T and Wu F (2025) Interaction of vaginal microbiota and biomarkers in Premature rupture of membranes: from bench to beside. *Front. Immunol.* 16:1642942. doi: 10.3389/fimmu.2025.1642942

COPYRIGHT
© 2025 Deng, Li, Liu and Wu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Interaction of vaginal microbiota and biomarkers in Premature rupture of membranes: from bench to beside

Yudi Deng, Yang Li, Tiana Liu and Fuju Wu*

Department of Obstetrics and Gynecology, The Second Hospital of JilinUniversity, Changchun, Jilin, China

Preterm premature rupture of membranes (PROM) is a critical obstetric complication endangering maternal and neonatal health, with growing evidence linking vaginal microecology to its pathogenesis. This review synthesizes the relationship between vaginal microbiota and PROM risk, as well as microecology-targeted prevention and management strategies. A balanced vaginal microbiome, dominated by lactobacilli that maintain an acidic protective environment, is essential for reproductive health. Dysbiosis—marked by reduced lactobacilli and increased pathogens like Gardnerella and Atopobium—impairs local immunity, weakens fetal membranes, and elevates PROM risk, with bacterial vaginosis (BV) strongly associated with this condition. Pathogenic overgrowth activates inflammatory (via TLR-mediated IL-1β, TNF-α, IL-6 overproduction) and oxidative stress pathways: pro-inflammatory cytokines promote cervical ripening, induce matrix metalloproteinases (MMPs) to degrade fetal membrane collagen, while reactive oxygen species (ROS) directly damage structural proteins, compromising membrane integrity. Monitoring inflammatory/oxidative stress biomarkers (e.g., cytokine levels, ROS activity) enables early risk assessment. Potential interventions include probiotics to restore microbial balance, antioxidants/immunomodulators to counteract stress/inflammation, and MMP inhibitors to preserve membrane structure, all aiming to improve pregnancy outcomes. In conclusion, vaginal microecology plays a pivotal role in PROM development, underscoring the need for early microecological monitoring. Future research should dissect mechanistic complexities and develop precision tools for preterm labor management.

KEYWORDS

preterm premature rupture of membranes (PROM), vaginal microecology, bacterial vaginosis (BV), inflammation, oxidative stress

1 Introduction

Premature rupture of membranes (PROM), especially when it happens prior to 37 weeks of gestation, is a common and significant pregnancy complication that impacts a considerable number of expectant mothers globally (2020) (1). The incidence of preterm premature rupture of membranes (PPROM) frequently results in early labor, which may lead to various negative perinatal consequences, such as neonatal respiratory distress syndrome, sepsis, necrotizing enterocolitis, and developmental disorders in infants (2). These conditions significantly impact neonatal survival rates and may also lead to irreversible long-term developmental damage. Moreover, PPROM increases the risk of maternal infection, particularly when membranes are ruptured, as bacteria may ascend and invade the amniotic cavity, potentially causing severe infectious complications such as chorioamnionitis (3, 4). Therefore, it is clinically crucial to conduct a thorough investigation into the etiology of PPROM and its potential effects on the health of mothers and infants to reduce the rate of preterm birth and enhance the prognosis for both mothers and infants.

The intricate and diverse mechanisms contributing to PPROM are often linked to elements like physical harm to the fetal membranes, immune system reactions, and the presence of infections. Recent research has increasingly highlighted the importance of vaginal microbiota in preterm premature rupture of membranes (PPROM) (5, 6). A balanced vaginal microbiota is defined by a dominance of *Lactobacillus* species (7) which helps sustain the vagina's acidic conditions by generating lactic acid, thereby suppressing pathogen development. In contrast, an imbalance in the vaginal microbiota results in fewer lactobacilli and a rise in harmful bacteria (8). This imbalance can disturb the local immune response and compromise the membranes, which may elevate the risk of membrane rupture. Bacterial vaginosis (BV), commonly seen as a sign of vaginal microbial imbalance, has shown a significant correlation with an increased risk of PPROM (9). Moreover, BV is frequently associated with heightened levels of pro-inflammatory cytokines such as IL-6 and IL-8 (10), which contribute to membrane rupture by enhancing cervical ripening and stimulating uterine contractions. Furthermore, changes in certain biochemical markers are recognized as important indicators for the early identification of PPROM. In recent studies, fluctuations in the levels of these biochemical markers have been recognized as promising biomarkers for evaluating the cervicovaginal environment and predicting the risk of premature rupture of the membranes (11, 12). The objective of this paper is to conduct a comprehensive examination of the influence of vaginal microbiota and biochemical markers on PPROM. Additionally, the study will explore the potential for enhancing pregnancy outcomes through the early monitoring of these biomarkers. This research is of significant importance for elucidating the mechanisms underlying PPROM and for the development of effective intervention strategies.

2 Overview of vaginal microbiota

2.1 Normal vaginal microbiota composition

To comprehend the traits of both healthy and dysbiotic cervicovaginal microbiota, it is crucial to acknowledge the intricacies of this microbial ecosystem and its significant influence on female reproductive health. The phrase 'vaginal microbiota' pertains to the diverse microbial community that inhabits the lower genital tract of females. The makeup and equilibrium of this microbiota are essential for preserving reproductive health.Typically, the vaginal microbiota is highly specific and exhibits low diversity, with a predominance of certain beneficial bacterial species, particularly *Lactobacillus*. The combined activities of these microorganisms lead to the development of a stable micro-ecosystem, which is essential for preserving the health of the female reproductive system (13). In the vagina of a healthy woman, *Lactobacillus* is the predominant beneficial bacterial species, which maintains the acidic environment through the secretion of lactic acid and other metabolic products, thereby inhibiting the growth of harmful pathogens (14). *Lactobacillus* bacteria are the primary species within the vaginal microbiota, and their dominance is considered an indicator of vaginal health. Indeed, the cervicovaginal microbiota comprises at least five principal community state types (CSTs), each characterized by a dominant *Lactobacillus* species (15) (Table 1).

2.2 Other common commensal bacteria

In addition to *Lactobacillus*, the vagina is inhabited by a variety of other commensal bacteria.In a healthy state, these bacteria do not induce disease and play a role in sustaining the balance of the vaginal microecological environment to a certain degree (16). These commensal bacteria are typically found in microenvironments dominated by *Lactobacillus*. However, when there is a reduction in *Lactobacillus* numbers, these bacteria may proliferate uncontrollabl. For example, *Bifidobacterium* spp. are predominantly located within the gut, although they can also be identified in the vagina in certain instances. In BV patients, bifidobacteria can coexist alongside *Gardnerella* and anaerobic bacteria, but they cannot fulfil the core function of *lactobacilli*. They produce lactic acid and serve as a defense against potential pathogens, but the effect was not significant (20). As another illustration, *Gardnerella vaginalis* is less prevalent in individuals with optimal vaginal health; however, its abundance markedly increases in cases of BV (21). Although it is an important marker of vaginal dysbiosis, it may also be present as a normal commensal in some individuals. Additionally, an increase in the anaerobic bacteria *Prevotella* spp. and *Mobiluncus* spp. is typically observed in cases of vaginal dysbiosis, particularly in individuals diagnosed with BV. The presence of these organisms is directly contributes to an inflammatory response and an elevated vaginal pH level (15, 22).

TABLE 1 Five principal community state types of cervicovaginal microbiota in healthy woman.

CST type	Dominant bacteria	Function	Clinical significance	References
CST I	*Lactobacillus crispatus*	Dominant acidifier: Sustains optimal pH ≤ 4.5 barrier against pathogens	Associated with vaginal health, low infection and preterm birth risk	(16)
CST II	*Lactobacillus gasseri*	Dominant lactic acid producer: maintains an acidic environment, supports normal pregnancy, and reduces the risk of infection.	Associated with normal pregnancy, low infection risk	(17)
CST III	*Lactobacillus iners*	Weak acidifiers:have a weaker acidifying effect, and their clinical significance is controversial.associated with variable health outcomes, including the possibility of bacterial vaginosis	Controversial: Linked with both health and BV	(16)
CST IV	*Lactobacillus jensenii*	Lactic acid-producing acidifiers: maintain acidic pH levels, associated with low infection rates and favorable pregnancy outcomes.	Associated with low infection risk, good pregnancy outcomes	(18)
CST V	*L. iners & anaerobic bacteria (Gardnerella, Prevotella)*	Associated with dysbiosis and increased pathogenic activity	Linked with vaginal dysbiosis, increased infection risk	(19)

BV, Bacterial vaginosis.

2.3 Physiological functions of vaginal microbiota

2.3.1 Maintain a stable vaginal microenvironment

Lactobacillus is essential for preserving the stability of the vaginal microenvironment. Its main physiological role involves metabolizing glycogen to generate lactic acid, which helps to sustain the vagina's acidic environment (23). As a result, this process usually keeps the vaginal pH between 3.5 and 4.5 (24) a spectrum that has been demonstrated to effectively curb the proliferation of different pathogens. The primary functions of vaginal microbiota are to maintain the stability of the vaginal microenvironment and to defend against pathogens that invade the vagina. The metabolites produced by beneficial bacteria, such as *Lactobacillus*, along with their immunomodulatory mechanisms, enable vaginal microbiota to serve as a natural defense barrier for the female reproductive tract. The vast majority of women (35/36, 97%) who underwent vaginal delivery at term exhibited a vaginal microbiome characterised by >75% abundance of Lactobacillus spp. Furthermore, 83% (30/36) of these women demonstrated Lactobacillus spp. abundance levels exceeding 98%. Samples obtained prior to PPROM were comparatively enriched for intermediate or Lactobacillus spp. depleted communities (PPROM; 14/60, 23% vs. Control; 1/36, 3%, P Farrell = 0.011), decreased total Lactobacillus spp. abundance (PPROM; 79% vs. Control; 96%, P Farrell = 0.016) and increased richness (total number of species observed, PPROM; 65 vs. Control; 10, P Farrell = 0.0086) (25). Rupture of the amniotic membrane takes place in the middle to late stages of pregnancy (24-29 + 6 and 30-36 + 6 weeks of gestation), prompting researchers to label these two periods as the 'immune clock.' (25–27). Furthermore, Zheng et al. (28) and Juliana et al. (29) underscore the significance of preserving the natural equilibrium of the vaginal microbiota throughout the gestational period.

2.3.2 Resistance to pathogen invasion

An additional important function of the vaginal microbiome is its protective role against pathogens, achieved via various mechanisms. During pregnancy, increased concentrations of vaginal oestradiol and glycogen lead to greater vaginal acid levels, subsequently fostering the dominance of *Lactobacillus*. This genus is able to directly prevent the proliferation of harmful bacteria by influencing pH levels and producing antimicrobial compounds. Additionally, it enhances the host's immune response, thereby strengthening the body's defenses (30). The four principal mechanisms by which *Lactobacillus* resists pathogen invasion are as follows: the phenomenon of competitive exclusion, defined as *Lactobacilli* competing for adhesion sites by preferentially occupying receptor sites on vaginal epithelial cells, thus preventing the colonization of these cells by pathogens (31). The process of immunomodulation refers to the alteration of the immune system's response to external stimuli. *Lactobacillus* has the ability to stimulate the release of anti-inflammatory cytokines, including IL-10, and simultaneously reduces the overproduction of pro-inflammatory components through its interactions with vaginal epithelial cells, which helps to deter undesirable inflammatory responses (32, 33). Furthermore, *Lactobacilli* have been shown to enhance local immune defenses by stimulating the mucosal immune system (MALT) (34). The secretion of antimicrobial substances is a key defense mechanism employed by these microorganisms. In addition to lactic acid and hydrogen peroxide, *Lactobacillus* lactis is capable of secreting bacteriocins and other antimicrobial peptides. Antimicrobial agents possess the ability to directly suppress the proliferation of harmful bacteria or eliminate them by compromising their cell walls and membrane structures (35). Various antimicrobial substances linked to the protection of vaginal epithelial cells, such as neutrophil gelatinase-associated lipocalin, calcium-binding proteins, and hyaluronic acid, are selectively stimulated by *Lactobacillus casei*

L. iners (36). The symbiotic relationship between the host and microorganisms is sustained; the vaginal microbiota significantly contribute to maintaining the integrity of the mucosal barrier against external pathogens through their interaction with the host immune system. A dysbiotic vaginal microbiota facilitates the penetration of pathogens through the mucosal barrier, thereby precipitating infection and inflammation (37).

3 Biochemical markers associated with PROM of the fetal membranes

Recent studies have extensively examined the importance of biochemical markers in forecasting premature rupture of the membranes. Research indicates a strong connection between particular inflammatory factors, elements related to oxidative stress, and markers like matrix metalloproteinases with both the structural integrity of the membranes and the inflammatory response (38). The subsequent section will examine the particular roles of inflammation, oxidative stress, and additional biochemical markers in the context of premature rupture of the membranes.

3.1 Inflammation-related biochemical markers

The integrity of the fetal membrane relies on both its mechanical strength and the regulation of local and systemic inflammatory responses (39). A substantial body of evidence from numerous studies suggests that the inflammatory response plays a crucial role in the pathogenesis of premature rupture of membranes, particularly concerning the involvement of various inflammatory cytokines and mediators (40–42). A case-control study of patients in the FTB, PTB, PROM and pPROM groups ($n > 6$) revealed that: TNF-α, IL-6 and ADAMTS9 mRNA levels were significantly higher in the PROM and pPROM groups ($p < 0.001$). (42). Furthermore, evidence indicates that a reduction in *Lactobacillus* and an increase in pathogenic bacteria (e.g., *Sneathia* spp.) are associated with increased fragility of the fetal membranes and the onset of early neonatal sepsis (EONS) (42).

3.1.1 Various inflammatory cytokines

Research has shown that the microbiota associated with premature rupture of membranes, including *Gardnerella vaginalis* and Atopobium vaginae, are significantly linked to increased pro-inflammatory factors (40, 43–45). In particular, pro-inflammatory factors such as IL-1 and IL-6, which are markedly elevated in the inflammatory vaginal environment, exacerbate cervical ripening and fetal membrane fragility, thereby heightening the risk of PROM (46) (Table 2).

3.1.2 Mediator of inflammation

Prostaglandins (PGs) are biologically active lipid molecules metabolized from arachidonic acid found in cell membranes. They are essential in the body's inflammatory response and in facilitating uterine contraction (56). Prostaglandin E2 (PGE2) and prostaglandin F2α (PGF2α) are two prostaglandins closely associated with pregnancy and childbirth; additionally, they might play a role in the premature rupture of membranes.PGE2 is particularly significant in the onset of labor, primarily by regulating cervical softening and initiating uterine contractions (57). Research indicates that in instances of preterm premature rupture of membranes (PPROM), prostaglandin E2 (PGE2) exacerbates the inflammatory response by activating local immune cells and promoting the secretion of interleukin-1 (IL-1) and interleukin-6 (IL-6) (58). Concurrently, prostaglandin F2α (PGF2α) is essential in facilitating labor progression by inducing contractions of the uterine muscles. Importantly, the amniotic fluid from patients experiencing PPROM demonstrates significantly increased levels of PGF2α, highlighting its crucial function in the contractions occurring after membrane rupture (57, 59, 60).

TABLE 2 Inflammatory markers and PROM risk.

Inflammatory marker	Mechanism of action	Impact on PROM risk	References
IL-1	Key initiator of acute inflammatory response. Elevated during PROM, activates NF-κB signaling.	Increases inflammation, weakens membranes, raises PROM risk.	(43, 44, 46)
IL-6	Pro-inflammatory cytokine secreted by monocytes, macrophages, and fetal membrane cells.	Elevated in PPROM patients, early PROM marker, increases rupture risk.	(42, 47–49)
IL-1β	Regulates PI3K/AKT pathway, induces IL-6 production, activates NLRP3 inflammasome.	Increases cytokines, disrupts ECM, heightens PROM risk.	(41, 45, 50–53)
TNF-α	Produced by macrophages and T cells, key in infectious inflammation.	Promotes MMP expression, degrades ECM, increases membrane rupture risk.	(42, 54, 55)

IL-1, Interleukin 1. PROM, Preterm Rupture of Membranes. NF-κB, Nuclear Factor kappa-light-chain-enhancer of activated B cells.
IL-6, Interleukin 6; PPROM, Preterm Prerupture of Membranes; IL-1β, Interleukin 1 Beta; PI3K, Phosphoinositol-3 Kinase; AKT, Protein Kinase B; NLRP3, NOD-like Receptor Family Pyrin Domain Containing 3. ECM, Extracellular Matrix; TNF-α, Tumor Necrosis Factor Alpha; MMP, Matrix Metalloproteinase.

3.2 Oxidative stress-related biochemical markers

Oxidative stress refers to a physiological state that occurs due to an overproduction of reactive oxygen species or inadequate

performance of the antioxidant defense mechanisms during the body's metabolic activities (61). This condition has been identified as a major contributing element to the onset of preterm premature rupture of membranes, particularly in instances of preterm delivery (45, 62). This condition increases the vulnerability of the fetal membranes by disrupting their cellular structure and modulating the inflammatory response (63) (Figure 1).

Abbreviation: ECM, Extracellular Matrix. ROS, Reactive Oxygen Species. SOD, Superoxide Dismutase. CAT, Catalase. PI3K, Phosphoinositol-3 Kinase. AKT, Protein Kinase B. NF-κB, Nuclear Factor kappa-light-chain-enhancer of activated B cells. NLRP3, NOD-like Receptor Family Pyrin Domain Containing 3. Bcl-2, B-cell CLL/lymphoma 2. Caspase-1, Cysteinyl aspartate specific proteinase 1, IC-1β, Interleukin 1β.

3.2.1 Reactive oxygen species and antioxidant enzymes

ROS are naturally occurring by-products of cellular metabolism, including superoxide, hydrogen peroxide, and hydroxyl radicals (64). Under standard conditions, the human body has an antioxidant defense mechanism that can eliminate these reactive oxygen species. Nonetheless, the existence of a microbial community largely made up of *non-Lactobacillus* s*pecies* might increase ROS production, thereby worsening cellular and tissue harm (65). An excessive accumulation of ROS within the fetal membrane can lead to embrittlement and eventual rupture of the membrane structure. Antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), play a pivotal role in the scavenging of ROS. SOD converts superoxide radicals into hydrogen peroxide, which is then broken down into water and oxygen by CAT. These antioxidant enzymes are essential for maintaining cellular redox homeostasis through the regulation of ROS production and scavenging (66). In patients with PPROM, it has been demonstrated that the IL-1β-induced PI3K/AKT pathway can facilitate ROS generation, while the activity of antioxidant enzymes is significantly diminished. This evidence suggests that oxidative stress may be a critical mechanism underlying the fragility and rupture of the fetal membrane (67).

3.2.2 Oxidative stress

Reactive oxygen species (ROS) have the capability to directly damage collagen and elastin, which serve as structural proteins within the extracellular matrix (68). Collagen in the fetal membranes is essential for maintaining their strength and elasticity. ROS-mediated oxidative damage to collagen fibers results in their weakening, which subsequently increases the likelihood of membrane rupture (69). Concurrently, this process triggers the NLRP3 inflammatory vesicle, amplifying the inflammatory response and cytokine secretion within the fetal membranes (45, 51, 53). Such reactions further compromise the integrity of collagen fibers in the fetal membranes, thereby increasing their fragility. An excess of ROS may also activate

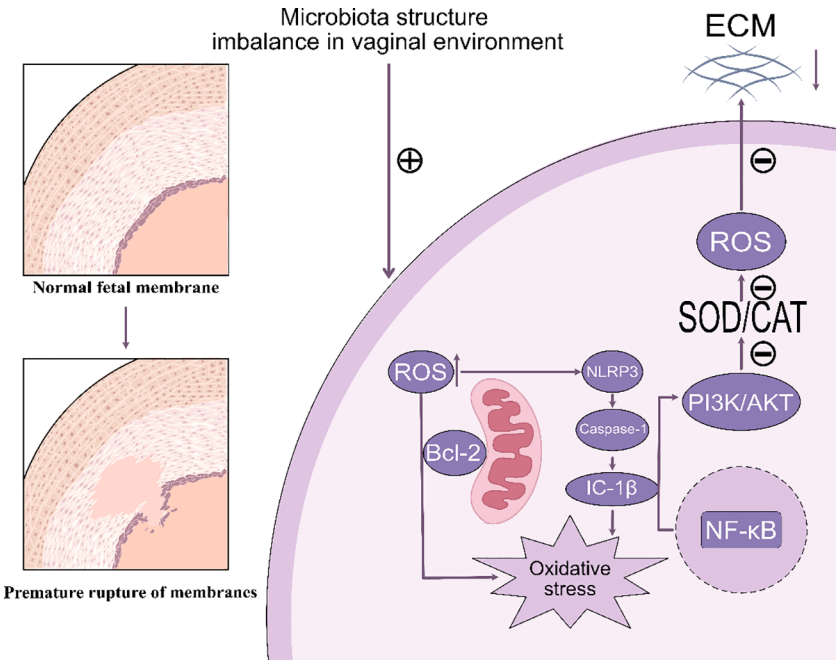


FIGURE 1
This image illustrates how microbial structural imbalances within the vaginal environment contribute to the transition from normal fetal membranes to premature rupture. The microbial imbalance generates reactive oxygen species (ROS), which activate NLRP3 inflammatory vesicles and promote the maturation of IL-1β via caspase-1. This process subsequently activates NF-κB and increases oxidative stress. Concurrently, ROS influence the PI3K/AKT and SOD/CAT pathways, resulting in damage to the extracellular matrix (ECM), which can ultimately lead to premature membrane rupture. ECM, Extracellular Matrix. ROS, Reactive Oxygen Species. SOD, Superoxide Dismutase. CAT, Catalase. PI3K, Phosphoinositol-3 Kinase. AKT, Protein Kinase B. NF-κB, Nuclear Factor kappa-light-chain-enhancer of activated B cells. NLRP3, NOD-like Receptor Family Pyrin Domain Containing 3. Bcl-2, B-cell CLL/lymphoma 2. Caspase-1, Cysteinyl aspartate specific proteinase 1, IC-1β, Interleukin 1β.

apoptotic pathways by damaging mitochondrial membranes. The accumulation of apoptotic cells within fetal membrane tissues can weaken the structural integrity of the membrane, thereby elevating the risk of PPROM (70).

3.3 Other relevant biochemical markers

In addition to biochemical markers associated with inflammation and oxidative stress, matrix metalloproteinases (MMPs) and their inhibitory factors (TIMPs), among others, have been identified as playing a significant role in premature rupture of the membranes.

3.3.1 Matrix metalloproteinases and their inhibitors

MMPs are a class of enzymes that degrade extracellular matrix proteins and play a crucial role in tissue remodeling and the renewal of the extracellular matrix (71). However, excessive activation of MMPs can disrupt the extracellular matrix, leading to an increased risk of premature rupture of membranes. MMP-9 is a particularly abundant matrix metalloproteinase found in fetal membrane tissue and is primarily responsible for the degradation of collagen and elastin. In patients with PPROM, there is a significant increase in MMP-9 activity, resulting in the degradation of collagen fibers and a reduction in membrane strength (72).

Tissue inhibitor of metalloproteinases (TIMP-1) serves as an endogenous inhibitor of MMPs preventing their excessive degradation of the extracellular matrix through binding interactions (71). A reduction in TIMP-1 activity may result in uncontrolled MMP activity, thereby exacerbating membrane degradation in the context of premature rupture of the membrane (73).

3.3.2 Fibronectin

Fibronectin, a multifunctional protein found within the extracellular matrix, is crucial for cell adhesion, migration, and tissue repair.The findings showed a notable rise in fibronectin concentrations in cervicovaginal secretions from patients who were undergoing premature rupture of the membranes. This finding implies that fibronectin may act as an early biochemical marker for membrane rupture (74). Evidence suggests that fibronectin is a crucial factor in preserving the structural integrity of fetal membranes due to its role in extracellular matrix remodeling and the fetal membrane repair processes (55, 75).

4 Direct interaction of vaginal microbiota with biochemical markers

The microbiota of the vagina is crucial for ensuring the health of the female reproductive system, especially in thwarting infections and maintaining the ecological balance within the vaginal environment. However, a dysbiotic microbiota—characterized by a reduction in *Lactobacillus* and an increase in pathogenic bacteria—can trigger a range of adverse biological responses that influence the expression levels of biochemical markers. These biochemical markers not only serve as diagnostic indicators of inflammatory and oxidative stress states but also interact with changes in vaginal microbiota, creating positive or negative feedback mechanisms that further impact reproductive health. This paragraph will examine the direct interactions between vaginal microbiota and biochemical markers, with a particular emphasis on the effects of dysbiosis on biochemical markers and the counteracting influences of these markers on the balance of vaginal microbiota.

4.1 Influence of vaginal microbiota on biochemical markers

The stabilisation of the vaginal microbiota is largely dependent on the presence of *Lactobacillus*, with strains such as *Lactobacillus crispatus* and *Lactobacillus gasseri* playing a particularly significant role.The lactic acid produced by these bacteria maintains the acidic environment of the vagina, which inhibits the multiplication of pathogenic bacteria.In the event of a dysbiotic vaginal microbiota, as observed in cases of bacterial vaginosis or anaerobic bacteria, a localised pro-inflammatory response is initiated, accompanied by a notable elevation in the levels of inflammatory biochemical markers, including IL-1, IL-6 and TNF-α.

4.1.1 Mechanisms of elevated inflammation-related biochemical markers due to dysbiosis

Antimicrobial peptides (AMPs) play a crucial role in maintaining the physiological barrier of the vagina. They contribute to microbiota stability by inhibiting pathogenic organisms (76). Human beta-defensin-2 (HBD-2) is a vital antimicrobial peptide that protects against microbial invasion through the innate immune system (77). Various research works indicate that levels of HBD-2 are markedly lower in individuals suffering from bacterial vaginosis (BV) while being heightened in healthy pregnant women (78). A decrease in HBD-2 levels has been observed in instances of dysbiotic vaginal microbiota, characterized by an imbalance in microbiota dominated by pathogenic bacteria such as *Gardnerella vaginalis*. Such imbalances indicate a disruption of vaginal microecology (79, 80). Moreover, reduced concentrations of HBD-2 have been demonstrated to hinder immune function and elevate the likelihood of premature rupture of membranes (PROM) as a result of an inflammatory reaction that raises pro-inflammatory biochemical indicators, such as IL-6 and TNF-α (81). Concurrently, the proliferation of pathogenic bacteria, such as *Gardnerella vaginalis* and Atopobium vaginae, which are indicative of dysbiotic vaginal microbiota, leads to the destruction of epithelial cell integrity and stimulates the release of pro-inflammatory factors by local immune cells (13, 82). Pro-inflammatory factors have been shown to activate inflammatory signals via the NF-κB and AP-1 pathways, thereby amplifying local inflammatory responses. For example, IL-1β increases the release of IL-6 by activating the PI3K/AKT signaling pathway via the NLRP3 inflammasome, which facilitates the maturation of IL-1β through the activation of

caspase-1 (83). As pro-inflammatory factor levels rise, there is a substantial influx of immune cells from local tissues, further disrupting the vaginal environment. The persistence of this inflammatory response leads to the disruption and increased fragility of the fetal membrane structure, consequently heightening the risk of premature rupture of the membranes. Additionally, in women experiencing premature rupture of the membranes, specific inflammatory factors such as CXCL10, CCL26, CCL22, and IL-16 are strongly associated with CST type IV vaginal microbiota, particularly pathogenic bacteria like *Sneathia sanguinegens* (83). The concentration of these pro-inflammatory mediators tends to rise as the non-*Lactobacillus*-dominated microbiota expands. This imbalance in gut microbiota initiates a localized inflammatory reaction, leading to heightened biochemical indicators linked to inflammation and a greater likelihood of premature rupture of the membranes (Figure 2).

4.1.2 Modulation of markers associated with oxidative stress

It has been demonstrated that the prevalence of *L. iners* fluctuates considerably during the early stages of pregnancy, with a notable increase observed as vaginal cleanliness declines (84). This indicates that the rise in biochemical markers linked to vaginal microbiota dysbiosis and oxidative stress may be attributable to the prevalence of *L. iners*, which facilitates the proliferation of pathogenic bacteria, resulting in elevated levels of ROS production. This, in turn, further exacerbates the imbalance in the vaginal environment and tissue damage (85, 86). In a usual situation, Lactobacilli hinder the growth of harmful bacteria by preserving an acidic environment and generating hydrogen peroxide (H_2O_2). When dysbiosis occurs, there is a decrease in HBD-2 levels, resulting in a rise of pathogenic bacteria in the vaginal area.The production of toxins and the induction of reactive oxygen species (ROS) by these bacteria lead to oxidative stress (87). ROS production results in damage and increased fragility of the fetal membrane structure, due to the attack on collagen fibres and other extracellular matrix proteins that occur within the fetal membrane (88). Concurrently, the activity of antioxidant enzymes (e.g. SOD, CAT) within the vaginal environment is diminished, thereby further compromising the capacity to avert oxidative stress (89). The available literature indicates a negative correlation between specific microbial types (e.g., *Gardnerella vaginalis* and *Atopobium vaginae*) and CXCL10 levels. This suggests that these pathogens may contribute to the disruption of vaginal microecological stability by triggering inflammation and oxidative stress (90).

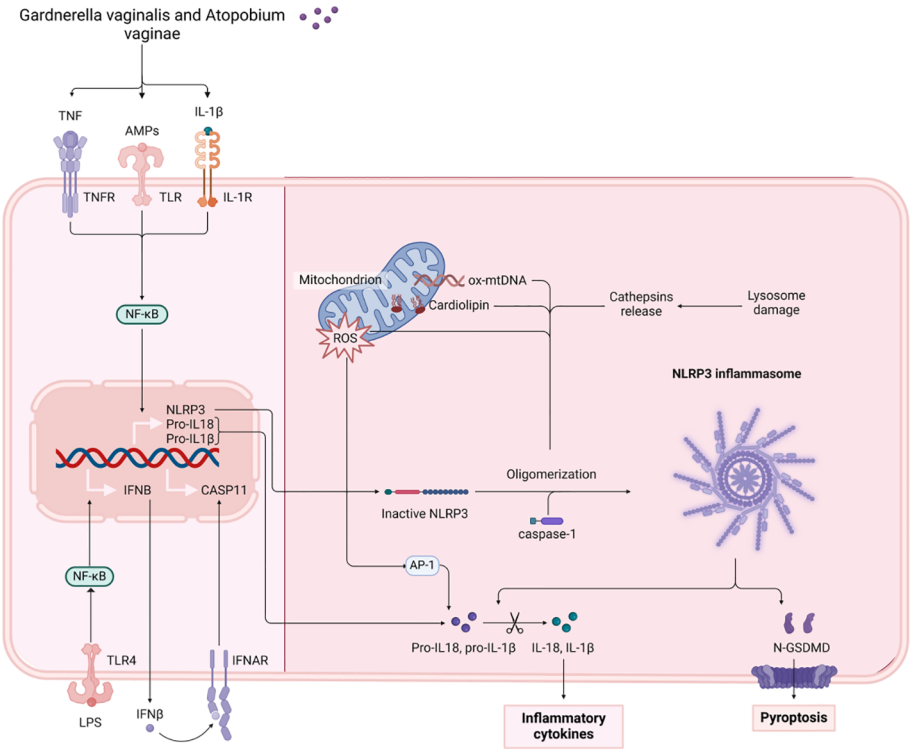


FIGURE 2
The figure illustrates the mechanisms by which *Gardnerella vaginalis* and *Atopobium vaginae* activate NLRP3 inflammatory vesicles through TNF, LPS, and other pathways. This activation results in the release of the inflammatory factors IL-1β and IL-18, subsequently triggering cellular pyroptosis. TNF, Tumor Necrosis Factor. NF-κB, Nuclear Factor kappa-light-chain-enhancer of activated B cells. LPS, Lipopolysaccharide. TNFR, Tumor Necrosis Factor Receptor. TLR, Toll-like Receptor. IFNβ, Interferon Beta. AMPs, Antimicrobial Peptides. IL-1β, Interleukin 1 Beta. CASP11, Caspase 11. IL-1R, Interleukin 1 Receptor. IFNAR, Interferon Alpha and Beta Receptor. ROS, Reactive Oxygen Species. NLRP3, NOD-like Receptor Family Pyrin Domain Containing 3. CARD, Caspase Recruitment Domain. ox-mtDNA, Oxidized Mitochondrial DNA. Cathepsins, Proteolytic enzymes found in lysosomes. N-GSDMD, N-terminal Gasdermin D. API, Apoptosis Inhibitor. IL-18, Interleukin 18.

Oxidative stress linked to dysbiosis increases the risk of fetal membrane rupture. Besides directly harming cellular structures, an overabundance of reactive oxygen species (ROS) further encourages the breakdown of the extracellular matrix through the activation of MMPs (90). It is evident that the inflammatory response and oxidative stress induced by dysbiosis not only increase the levels of pro-inflammatory cytokines but also enhance the expression of biochemical markers related to oxidative stress, thereby further accelerating the degradation of fetal membranes.

4.2 Reverse effects of biochemical markers on vaginal microbiota

4.2.1 Changes in the composition of the vaginal microbiota by the inflammatory environment

In non-pregnant women, the vaginal microbiota composition is less stable, marked by a higher percentage of pathogens. In contrast, a healthy pregnancy is associated with increased stability of the vaginal microbiota, showcasing a higher prevalence of beneficial bacteria like *Lactobacillus*. This shift may serve to address the protective needs of both the fetus and the mother (91). Notably, a microbiota dominated by a single strain of *Lactobacillus iners* during the early stages of pregnancy is significantly associated with the occurrence of preterm labor (6). The research revealed that L. iners was detected in merely 85% of women who underwent preterm deliveries, while only 16% of those with full-term pregnancies showed the presence of this strain (92). Furthermore, the pro-inflammatory cytokines IL-1β and TNF-α not only promote localized inflammation but also have a considerable impact on the makeup of the vaginal microbiota (93). As levels of inflammation increase, the quantity of *Lactobacillus* bacteria diminishes, whereas the presence of anaerobic pathogens, such as *Gardnerella vaginalis* and *Atopobium vaginae*, rises. This change exacerbates dysbiosis, thereby heightening the risk of premature rupture of membranes. In women of African descent, inflammatory conditions are more conducive to the colonization of anaerobic bacteria like *Gardnerella* and *Prevotella*, resulting in a transition of the vaginal microbiota from a state dominated by *Lactobacillus* to one defined by anaerobic dysbiosis (94). A strong correlation has been observed between vaginal cleanliness, leukocyte esterase levels, and the composition of the vaginal microbiota. Specifically, the presence of leukocyte esterase correlates with a notable increase in the abundance of *L. iners*, while the abundance of L. crispatus shows a significant decline (85). The *CST IV* microbiota, characterized by the dominance of *non-Lactobacillus bacteria* in the vagina during inflammatory states, has been associated with elevated levels of several pro-inflammatory biochemical markers (95). Pro-inflammatory agents, including IL-1β, not only enhance the synthesis of HBD2 within an inflammatory context but also modify the vaginal microbiota's composition, suppressing the growth of beneficial bacteria such as *Lactobacillus* while encouraging the increase of pathogenic bacterial populations. A study involving 317 patients diagnosed with bacterial vaginosis (BV) revealed that vaginal hBD-2 levels were 54.48% lower compared to those in the healthy control group ($p < 0.01$). Furthermore, a significant negative correlation was observed between Nugent scores and both hBD-1 (Spearman's rho = -0.2118; $p = 0.0001$) and hBD-2 (Spearman's rho = -0.2117; $p = 0.0001$) levels (78). It has been proposed that during the inflammatory process, the concentration of intravaginal AMPs, such as HBD2, increases, effectively inhibiting the reproduction of pathogenic bacteria and protecting beneficial microbiota (80, 90). This counterproductive mechanism suggests that biochemical markers play a crucial role in maintaining the equilibrium of vaginal microbiota, thereby facilitating the prevention of infection.

4.2.2 Adaptive changes in bacterial microbiota under oxidative stress conditions

In the context of oxidative stress, AMPs influence the composition of the vaginal microbiota in two principal ways: first, by directly inhibiting pathogenic bacteria, and second, by modulating the local inflammatory response (96). For instance, the overproduction of ROS and other oxidative stress-related markers can adversely affect the vaginal microbiota. ROS are not only toxic to host cells but also impact the environmental conditions necessary for microorganisms to survive and thrive (97). In the presence of oxidative stress, pathogenic bacteria capable of tolerating oxidative damage, such as *Sneathia* spp., tend to dominate the vaginal environment, further exacerbating the inflammatory response and tissue damage. The survival of *Lactobacillus* in this oxidative stress environment is compromised, leading to a reduction in its population. Consequently, this reduction results in an increase in vaginal pH, creating a more favorable environment for the colonization and proliferation of pathogenic bacteria. Oxidative stress promotes the adaptive growth of pathogenic bacteria by altering the vaginal microecology, thereby heightening the likelihood of an inflammatory response and membrane rupture. This feedback mechanism suggests that elevated biochemical markers are not merely a consequence of changes in the vaginal milieu; rather, they actively contribute to the perpetuation of microbiota dysbiosis, establishing a vicious cycle (98). For example, in the context of oxidative stress, *L. iners* exhibits enhanced resilience and the ability to persist in a dysbiotic vaginal environment (85). However, the presence of *L. iners* does not effectively inhibit the colonization of other harmful bacteria and may instead serve as a marker of vaginal microbiota dysbiosis (84).

5 Mechanism of synergy between vaginal microbiota and biochemical markers in PROM

Recent studies have demonstrated that the vaginal microbiota and biochemical markers exert a synergistic effect on the pathogenesis of premature rupture of membranes. This paragraph will provide a detailed discussion of the mechanisms underlying the synergy between vaginal microbiota and biochemical markers in the context of PROM.

5.1 Synergistic effects through inflammatory pathways

5.1.1 Vaginal microbiota triggers an inflammatory response and activates the expression of relevant biochemical markers

Changes in the vaginal microbiota have been demonstrated to play a pivotal role in the initiation of the inflammatory response. The usual vaginal microbiome is defined by a dominance of *Lactobacillus* species, which produce lactic acid to create an acidic setting, thereby effectively preventing the proliferation of harmful bacteria (23, 24). However, in the event of a dysbiotic vaginal microbiota, the proliferation of pathogenic bacteria, including *Gardnerella vaginalis*, *Prevotella* and *Atopobium vaginae*, is observed (99). The colonisation of pathogenic bacteria has been demonstrated to have a dual impact on the vaginal barrier function, leading to its destruction and the subsequent activation of the host's innate immune system. This occurs through direct interaction with host cells, which in turn triggers an inflammatory response (42, 82, 100). These pathogenic bacteria have been demonstrated to activate host immune cells via the TLR (Toll-like receptor) pathway, which results in the excessive release of pro-inflammatory cytokines (e.g. IL-1β, TNF-α, IL-6) (101, 102). The TLR2/TLR1 heterodimer has been demonstrated to recognize bacterial triacylglycerol lipopeptides, such as those derived from Mycoplasma, and to activate Th17-type immune responses, which subsequently lead to pro-inflammatory reactions. The normal vaginal microbiota, particularly Lactobacillus, has been shown to suppress TLR2 expression, thereby preventing excessive inflammatory responses against commensal bacteria. However, in instances where the microbiota is disrupted, TLR2/TLR1 heterodimers activated by pathogens like Streptococcus gordonii trigger Th17 responses, resulting in inflammation and pyroptosis (103). The presence of lipopolysaccharide (LPS) has been shown to induce the activation of Toll-like receptor 4 (TLR4), thereby stimulating the nuclear factor kappa B (NF-κB) pathway and promoting the secretion of pro-inflammatory mediators such as interleukin-6 (IL-6) and vascular endothelial growth factor-A (VEGF-A). During periods of microbial imbalance, the overgrowth of potentially pathogenic bacteria, such as those associated with aerobic vaginitis, can lead to increased secretion of LPS, which in turn activates the NF-κB pathway, disrupting the balance of vaginal microbiota and exacerbating inflammation (104). TLR9 has been found in the cytoplasm of cells and is capable of recognizing non-methylated CpG dinucleotides in bacterial DNA, for instance, in Staphylococcus. This recognition process is induced via the MyD88 pathway, resulting in the secretion of IFN-γ and IL-1β. During bacterial community dysregulation, the secretion of bacterial DNA through TLR9 is induced by antibiotic action (105).

It has been demonstrated that the levels of these inflammatory markers are markedly elevated in patients diagnosed with BV (19). The release of pro-inflammatory factors has been demonstrated to cause direct damage to fetal membrane cells (106), additionally, the expression of MMPs (e.g., MMP-8, MMP-9) is triggered (107), further disruption of the collagen fibre structure of the membranes results in an increased brittleness of the membranes (Figure 3).

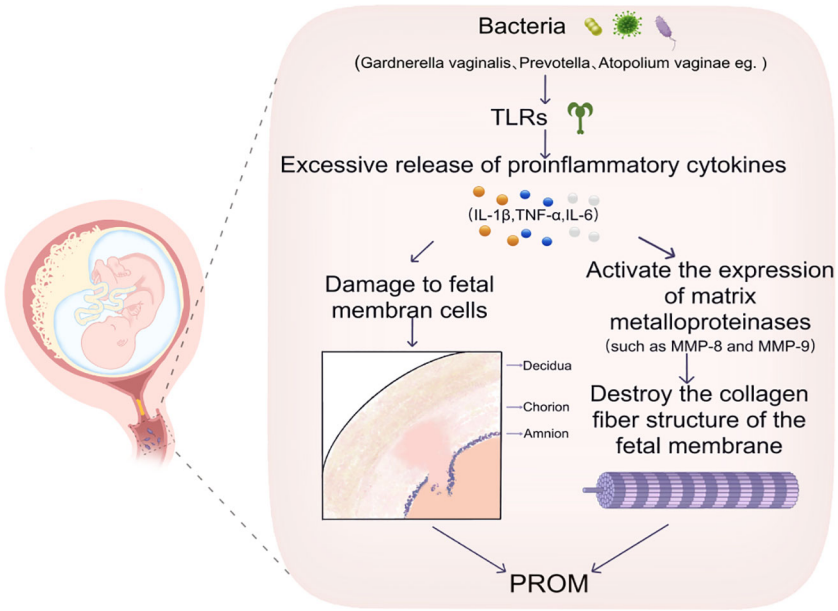


FIGURE 3
This image illustrates that certain bacteria, such as *Gardnerella vaginalis*, *Prevotella*, and *Atopobium vaginae*, induce an excessive release of inflammatory cytokines, including IL-1β, TNF-α, and IL-6, through the activation of TLRs. This activation results in damage to fetal membrane cells. Consequently, this damage triggers the expression of MMPs, specifically MMP-8 and MMP-9, which disrupts the collagen fiber structure of the fetal membranes and may ultimately contribute to PROM. TLRs, Toll-like Receptors. IL-1β,Interleukin 1 Beta. TNF-α, Tumor Necrosis Factor Alpha. IL-6, Interleukin 6.MMP-8, Matrix Metalloproteinase 8. MMP-9, Matrix Metalloproteinase 9. PROM, Preterm Rupture of Membranes.

5.1.2 Biochemical markers further exacerbate inflammation leading to premature rupture of membranes

Biochemical markers in the inflammatory response serve not only as indicators of inflammation but also have the potential to directly exacerbate fetal membrane damage. Matrix metalloproteinases (MMPs) represent a category of enzymes crucial for breaking down the extracellular matrix, which is vital for the proper remodeling of fetal membranes (62, 108). However, when activated by the inflammatory response, the overexpression of MMPs leads to structural damage to the membranes. Specifically, MMP-9 and MMP-8 are released in large quantities during inflammation and infection, resulting in the degradation of crucial structural components, such as collagen fibers and elastin, thereby weakening the strength and elasticity of the membranes (71). At the same time, pro-inflammatory cytokines such as IL-1β and IL-6 worsen the damage to fetal membranes by increasing the activity of MMPs (109). Studies have demonstrated that the levels of IL-6 in amniotic fluid are significantly elevated in women experiencing premature rupture of membranes (PROM) and are strongly correlated with increased MMP-9 activity. (110). The combined effects of these pro-inflammatory factors and MMPs contribute to the premature rupture of the fetal membranes due to accelerated degradation of the extracellular matrix.

5.2 Synergistic effects through oxidative stress pathways

Dysbiosis of the vaginal microbiota is associated with both an inflammatory response and exacerbation of fetal membrane damage via the oxidative stress pathway (111). A reduction in *Lactobacillus* levels correlates with a significant increase in oxidative stress, which facilitates the proliferation of pathogenic bacteria such as *Gardnerella* and *Prevotella*. These bacteria produce substantial quantities of ROS, which not only directly damage fetal membrane cells but also exacerbate inflammatory responses by activating pro-inflammatory pathways (99). Elevated levels of ROS in the vagina have been significantly linked to the disruption of fetal membranes and an increased risk of preterm labor (8, 112). For instance, *Gardnerella vaginalis*, *Prevotella*, and *Sneathia* can generate considerable amounts of ROS as a result of their metabolic processes. The overproduction of ROS leads to oxidative damage to fetal membrane cells and further enhances the release of pro-inflammatory cytokines by activating inflammatory pathways such as NF-κB. Research has shown that in patients with vaginal dysbiosis, ROS levels are markedly elevated, accompanied by a reduction in the antioxidant capacity of fetal membrane cells, resulting in increased oxidative damage to these cells (113, 114). The interplay between oxidative stress and vaginal microbiota dysbiosis significantly influences the structure and function of fetal membranes, resulting in cellular-level disruptions. Reactive oxygen species (ROS) not only inflict direct damage on lipids, proteins, and DNA inside the cells of the fetal membrane but also encourage the breakdown of the extracellular matrix through the activation of matrix metalloproteinases (MMPs) (115). In patients experiencing premature rupture of membranes, elevated levels of oxidative damage markers, including lipid peroxidation products (MDA), have been detected in fetal membrane tissues, underscoring the substantial role of oxidative stress in fetal membrane injury (116). Furthermore, oxidative stress adversely affects the signaling processes of fetal membrane cells, particularly through the activation of pathways such as NF-κB and MAPK. This stimulation increases the production of pro-inflammatory elements, which, in turn, heightens the inflammatory response and the functioning of MMPs. As a result, this establishes a harmful cycle that causes the swift degradation of membranes, stemming from the synergistic impact of oxidative stress and inflammation, ultimately culminating in the premature rupture of membranes (117, 118).

5.3 Other synergistic mechanisms

5.3.1 Co-regulation involving MMPs and others

MMPs play a crucial role in both the physiological remodeling and pathological degradation of the fetal membrane. The activation of MMPs occurs through inflammatory and oxidative stress pathways; however, their expression is also modulated by the microbiota and other metabolites (119). For instance, the vaginal microbiota, predominantly composed of *Lactobacillus* spp., not only maintains an acidic environment but also diminishes MMP activity by inhibiting the production of pro-inflammatory factors (24). Conversely, when the microbiota becomes dysbiotic, particularly with the prevalence of pathogens, MMP activity is heightened, leading to accelerated degradation of cell membranes and an increased risk of premature rupture of membranes (24, 120). Bacterial metabolites, including short-chain fatty acids (SCFAs) and lipopolysaccharides (LPS), may affect the activity of MMP through both direct and indirect pathways (121). In the context of vaginal dysbiosis, metabolites produced by pathogenic bacteria may further exacerbate fetal membrane degradation by activating MMPs (122, 123). Moreover, the degradation of fetal membranes is closely linked to the action of MMP inhibitors, such as tissue inhibitors of metalloproteinases (TIMPs). During healthy pregnancies, MMP activity is tightly regulated by TIMPs, which help maintain the structural integrity of the fetal membrane (73). However, in the presence of inflammatory and oxidative stress, TIMP expression is diminished, leading to the deregulation of MMP activity and further disruption of the fetal membrane.

5.3.2 Influence on metabolism and signalling in fetal membrane cells

Vaginal microbiota and their metabolites can regulate the function and structure of fetal membranes by influencing the metabolism and signaling of fetal membrane cells (74, 124). Metabolites produced by *Lactobacillus*, including lactic acid and hydrogen peroxide, are essential for preserving the balance of fetal membrane cells and for inhibiting harmful bacteria. However, a reduction in *Lactobacillus* populations may disrupt the metabolic pathways of fetal membrane cells.

The impact of vaginal microbiota imbalance on amniotic membrane cells extends beyond mere inflammation activation; it profoundly disrupts fundamental metabolic processes and crucial signaling pathways within the cells. Dysregulation associated with bacterial vaginosis, for instance, significantly affects amino acid, carbohydrate, and energy metabolic pathways, thereby impacting the nutrient supply and energy homeostasis of amniotic membrane cells. This disruption manifests as abnormal alterations in lipid metabolic pathways, including fatty acid oxidation and membrane lipid synthesis, closely linked to insulin resistance and impaired cellular signaling (125). Concurrently, the imbalance in the microbial community directly induces excessive levels of reactive oxygen species (ROS) in amniotic fluid cells via metabolic processes, inhibiting important antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPX), which disrupts the balance of oxidation-reduction processes. Furthermore, this oxidative stress state activates the NLRP3 inflammasome, promoting the maturation of the IL-1β inducer, leading to collagen degradation and extracellular matrix (ECM) damage, thus increasing the risk of amniotic membrane structural damage (126). Additionally, dysbiosis has been shown to interfere with crucial non-inflammatory signaling pathways, including the reduction of antimicrobial peptides such as human beta-defensin-2 (HBD-2), weakening the innate immune barrier function, and disrupting tryptophan metabolism (e.g., kynurenine accumulation), which affects the differentiation of amniotic membrane cells and the barrier repair function through the aryl hydrocarbon receptor (AhR) pathway. While this pathway has been extensively studied in the context of intestinal microbiota, it has received comparatively minimal attention concerning vaginal microbiota (127). Notably, these effects often occur independently of the classic inflammatory response.

Furthermore, substances produced by harmful bacteria, including lipopolysaccharides (LPS), have the capacity to trigger apoptosis and inflammatory reactions in fetal membrane cells through the activation of the TLR signaling pathway (128). The activation of the TLR pathway not only enhances the release of pro-inflammatory factors but also regulates gene expression.

In response to the negative effects of dysbiosis, metabolites of lactic acid bacteria establish a crucial protective signaling network. Lactic acid not only lowers vaginal pH to create a chemical barrier but also directly enhances the barrier function of the cervical epithelium. Clinical observations indicate that elevated levels of lactic acid are significantly associated with the upregulation of tight junction proteins, such as ZO-1 (129). Short-chain fatty acids (SCFAs), including acetate and propionate, are produced by Lactobacillus. These SCFAs activate G protein-coupled receptors, such as GPR43, thereby exerting potent anti-inflammatory effects. They promote IL-10 secretion, inhibit the NF-κB pathway, and alleviate oxidative damage (130). Simultaneously, SCFAs inhibit the assembly of the NLRP3 inflammasome and the maturation of IL-1β, thus protecting the extracellular matrix (ECM) from excessive proteolytic damage (131). Perhaps most importantly, Lactobacillus colonization can reverse the abnormalities in purine degradation and membrane lipid metabolism caused by dysbiosis through metabolic reprogramming. This process restores energy metabolism homeostasis and reduces the risk of insulin resistance. Its metabolites, such as nicotinamide, enhance the efficiency of the mitochondrial respiratory chain via the NAD+ pathway, inhibit excessive activation of p38 MAPK, and effectively maintain mitochondrial function. Consequently, this alleviates cellular stress damage and comprehensively enhances the defensive and reparative capabilities of the amniotic membrane (132).

6 Clinical implications based on the interaction of vaginal microbiota with biochemical markers

Recent studies have demonstrated that dysregulation of vaginal microbiota is closely linked to alterations in biochemical markers, including pro-inflammatory cytokines and MMPs. As a result, the combined evaluation and handling of vaginal microbiota paired with biochemical indicators offer novel possibilities for the clinical identification and management of premature rupture of membranes and preterm labor.

6.1 Diagnostic

6.1.1 Combined vaginal microbiota test and biochemical marker test

In clinical diagnosis, the combined assessment of vaginal microbiota and biochemical markers can enhance the early detection of premature rupture of membranes. Traditional diagnostic methods typically depend on clinical symptoms or the identification of individual pathogens; however, the dynamics of the vaginal microbiota, particularly the balance between microbiota such as *Lactobacillus crispatus* and *Gardnerella vaginalis*, are crucial for the onset of PROM (16, 133, 134). Studies have demonstrated that dysbiosis of the vaginal microbiota, characterized by a decrease in *Lactobacillus* and an increase in anaerobic bacteria, is frequently associated with elevated levels of pro-inflammatory factors (e.g., IL-6, TNF-α) and MMPs (e.g., MMP-8, MMP-9). These biochemical markers are integral to the process of premature rupture of membranes.

By integrating vaginal microbiota testing with biochemical markers, clinicians can more effectively identify patients at risk, facilitating timely interventions. For instance, in individuals diagnosed with bacterial vaginosis (BV), elevated levels of pathogenic bacteria, such as *Gardnerella vaginalis* and *Prevotella*, closely correlate with variations in inflammatory markers. These markers can serve as crucial reference points for the early diagnosis of premature rupture of membranes (135). The combination of vaginal microbiota assessment and biochemical markers not only enhances diagnostic sensitivity and specificity but also lays the groundwork for individualized treatment approaches (136). Furthermore, the detection of interactions between pro-inflammatory cytokines and vaginal fmicrobiota may prove valuable in evaluating the progression of fetal membrane rupture. Research indicates that the inflammatory response is a significant

trigger for premature rupture, with elevated inflammatory markers showing a positive correlation with structural damage to the fetal membranes (137). By routinely assessing these biochemical markers alongside microbiota status, clinicians can better predict a patient's risk of preterm rupture of membranes and implement timely interventions.

6.1.2 Exploration of new diagnostic markers

In addition to traditional markers of inflammation and MMPs, scientists have recently begun to investigate new diagnostic markers, such as the detection of vaginal metabolites and indicators of oxidative stress. For instance, the generation of ROS is closely linked to oxidative damage to fetal membranes, and certain oxidative stress markers, such as malondialdehyde (MDA), can indicate the extent of structural damage to these membranes (138, 139). Furthermore, specific metabolites, such as short-chain fatty acids (SCFAs), may reflect the relationship between microbial metabolic activity and imbalances in the vaginal environment, potentially providing a diagnostic basis for the early identification of high-risk patients (130). By exploring these novel markers, future diagnostic tools are expected to be more comprehensive and accurate, enabling effective differentiation between various types of microbial diseases and their corresponding biomarker changes, thereby offering personalized diagnostic solutions for diverse patient populations.

Novel diagnostic markers currently under investigation include specific metabolites identified through metabolomic analyses, such as lactic acid, acetic acid, and lactate (140). Dynamic changes in vaginal metabolites not only reflect the balance of the microbiota but can also serve as early warning signals for inflammatory responses and tissue destruction. Additionally, microbial genetic markers are increasingly utilized in diagnostics due to advancements in genomics. This novel testing approach can accurately identify potential pathogens and assess the risk of preterm labor and rupture of membranes by analyzing their metabolic activity (137, 141).

6.2 Therapeutic strategies

The present study proposes a therapeutic approach involving the concurrent administration of probiotics (e.g., Lactobacillus and Bifidobacterium) and antibiotics to regulate the vaginal microbiota (Figure 4).

6.2.1 Therapeutic strategies to modulate vaginal microbiota to influence biochemical markers

The regulation of vaginal microbiota has garnered significant attention in recent years as a potential therapeutic strategy for addressing premature rupture of membranes (74, 142). The application of probiotics, such as *Lactobacillus crispatus* and *Lactobacillus rhamnosus*, has been shown to restore *Lactobacillus* dominance in the vagina, thereby mitigating the inflammatory response and the overexpression of MMPs. Clinical studies indicate that regular probiotic use is associated with decreased levels of pro-inflammatory biochemical markers, reduced colonization by pathogenic bacteria, and a lower risk of preterm labor and PROM (143). Furthermore, antibiotic therapy may be employed to regulate vaginal microbiota, particularly in patients diagnosed with bacterial vaginosis or aerobic vaginitis (142). However, the use of antibiotics must be approached with caution, as they can disrupt normal microbiota and potentially exacerbate the inflammatory response. Consequently, a combined approach utilizing both probiotics and antibiotics, which targets pathogenic

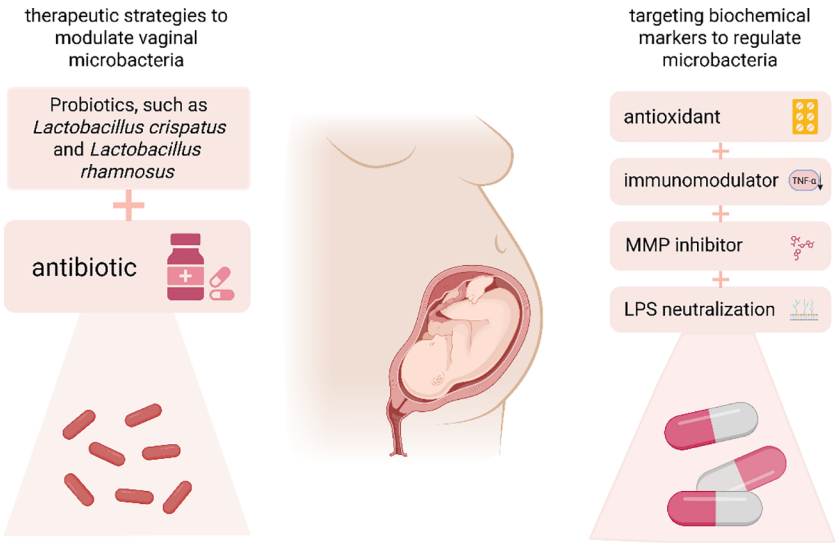


FIGURE 4
This study presents therapeutic strategies aimed at modulating vaginal microbiota through the use of probiotics, such as *Lactobacillus crispatus* and *Lactobacillus rhamnosus*, in conjunction with antibiotics. Additionally, it explores the targeting of biochemical markers using antioxidants, immunomodulators, MMP inhibitors, and lipopolysaccharides to further influence the vaginal microbiota. MMP, Matrix Metalloproteinase.

bacteria while promoting the restoration of beneficial microbiota, has emerged as a promising therapeutic strategy. Some studies have demonstrated that this combination therapy yields improved outcomes, with probiotics effectively reducing the expression of pro-inflammatory markers while restoring microbial balance. This dual approach not only addresses infections in the short term but also diminishes the risk of reinfection and inflammatory flare-ups by maintaining long-term microbiota stability (144). Additionally, personalized probiotic therapies tailored to individual microbiota differences are increasingly being investigated and may serve as a crucial tool in the prevention and treatment of preterm rupture in the future. However, its clinical application still faces multiple challenges. The primary challenge lies in the precise identification of key functional strains. Vaginal microbiota exhibit significant variability among individuals (e.g., classification based on community state type (CST)), and it remains unclear which combination of lactobacilli (e.g., a single strain of L. crispatus or a mixed population of L. gasseri and L. crispatus) is most effective in treating specific types of dysbiosis. Furthermore, the colonization, persistence, and stability of exogenous probiotics are inadequate, with vaginal colonization rates typically falling below 30%. This is partly due to competitive exclusion effects from the native microbiota (e.g., L. iners inhibits the proliferation of exogenous strains through metabolic products). The high heterogeneity of host responses further complicates matters—individual genetic backgrounds, immune states, and local microenvironmental factors (such as pH fluctuations and differences in cervical mucus composition) can significantly influence the metabolic activity and immunomodulatory efficacy of probiotics. The anti-inflammatory effects of the same strain can vary by up to fivefold across different hosts, further hindering the precision of interventions. These challenges collectively impede the standardized application and predictability of the efficacy of probiotic therapy in clinical settings.

6.2.2 Therapeutic strategies for targeting biochemical markers to regulate microbiota

The management of premature rupture of membranes heavily relies on the regulation of biochemical markers. This can be achieved by either modulating the inflammatory response or suppressing the overactivity of MMPs, the degradation of fetal membranes can be mitigated, thereby delaying or preventing premature rupture. Recent studies indicate that antioxidants can effectively lower levels of oxidative stress and diminish ROS-induced damage to fetal membranes. For instance, vitamins C and E, as antioxidants, can inhibit ROS generation and protect fetal membrane cells from oxidative stress-related damage (145, 146). In the realm of anti-inflammatory therapy, certain immunomodulatory drugs, such as TNF-α inhibitors, have demonstrated promising therapeutic effects (147). These agents reduce the release of pro-inflammatory factors, thereby interrupting the inflammatory cascade and decreasing MMP activity. Furthermore, biological drugs like MMP inhibitors, which specifically target MMP activity to reduce fetal membrane degradation, are currently under preclinical investigation (71, 121). Additionally, probiotic treatments that modulate microbiota can protect fetal membranes by diminishing the metabolites of pathogenic bacteria, such as LPS, and significantly reducing the aberrant expression of biochemical markers[(73, 123). By integrating multiple treatment modalities, including probiotics, anti-inflammatory agents, and antioxidants, future therapeutic strategies can become more comprehensive and individualized, providing a variety of options for patients at high risk of premature rupture of membranes and preterm labor.

6.2.3 Emerging microbiota-targeted therapies

In recent years, significant breakthroughs have been achieved in the prevention and treatment of vaginal microecological imbalances and premature rupture of membranes (PROM). The focus has centered on three main areas: probiotic intervention, vaginal microbiota transplantation (VMT), and biofilm-targeted therapy. A key advancement in probiotics has been the selection of specific strains (148). A study by Short et al. involving HIV-positive pregnant women demonstrated that the presence of Lactobacillus in the gut significantly reduces the expression of pro-inflammatory factors (IL-6 and TNF-α) and matrix metalloproteinases (MMP-9), thereby lowering the risk of preterm birth (149). Large-scale clinical trials have shown that probiotics can decrease the risk of recurrent preterm PROM in high-risk pregnant women by 30% to 40%, while also extending the average gestational age at pregnancy termination by two to three weeks (150). Further randomized clinical trials have confirmed that using probiotic preparations containing L. crispatus during mid-pregnancy can reduce the risk of recurrent preterm PROM ($P = 0.006$) (151). Prebiotics, such as oligosaccharides, enhance the acid barrier by promoting the growth of lactobacilli, which indirectly inhibits biofilm formation (152). For high-risk groups, such as those with a history of preterm birth or bacterial vaginosis (BV), probiotic treatment with L. rhamnosus GR-1 can normalize vaginal microbiota in mid-pregnancy and reduce GBS colonization by 40% (153). Treatment plans should be personalized and optimized based on microbial community genotyping (e.g., intervention for L. iners-dominant differentiation) and metabolomics (e.g., short-chain fatty acid levels).

Vaginal microbiota transplantation (VMT) is an emerging therapy that rapidly restores the microecological balance centered on lactobacilli by transplanting healthy donor microbiota (154). The first human clinical trial confirmed its effectiveness against recurrent bacterial vaginosis (BV). However, strict donor screening is essential to prevent the transmission of pathogenic bacteria, such as ensuring that the abundance of L. crispatus exceeds 70% (155). Current challenges include verifying long-term safety, standardizing transplantation methods (e.g., lyophilized preparations versus fresh samples), and establishing ethical guidelines.Biomembrane-targeted therapy represents an innovative solution to drug resistance. Enzymes such as lysozyme and DNase can degrade the extracellular polymers present in biomembranes (156). New anti-biofilm agents, including phages, can specifically lyse pathogenic bacteria without harming commensal bacteria (157). Furthermore, targeted delivery systems, such as pH-responsive nanogels, have been shown to enhance the penetration efficiency of drugs into the deeper layers of the vaginal epithelium (158, 159).

In summary, contemporary treatment strategies are increasingly integrating multimodal approaches: probiotics provide fundamental microecological regulation, VMT facilitates rapid reconstruction, and biofilm-targeted therapies overcome the challenges of drug resistance. In the future, it will be crucial to incorporate multi-omics technologies (such as spatial transcriptomics) to analyze the dynamic interactions between the microbiota and the host, promote individualized treatment plans and phased clinical trials, and fundamentally revolutionize the prevention and treatment system for PROM.

6.3 Preventive strategies and standardised assessment of vaginal health

6.3.1 Microbiota state typing (CST)-guided prevention

Different CST types necessitate strain-specific probiotic regimens. The optimal condition is CST-I (Lactobacillus crispatus-dominant), which can be sustained through the oral intake of Lactobacillus crispatus strains, such as L. crispatus DSM31983. This regimen maintains a low pH (approximately 4.0), inhibiting the adherence of pathogens and consequently reducing the risk of preterm birth (43). In the case of CST-III type, characterized by Lactobacillus acidophilus dominance, vaginal suppositories containing Lactobacillus rhamnosus GR-1 and Lactobacillus gasseri (10^9 CFU/day for 10 days) have been shown to significantly extend the gestational latency period in patients experiencing preterm PROM. This effect is attributed to the enhancement of local immune defensin HBD-2 secretion and the regulation of the placental TLR signaling pathway. For CST-IV/V type, which consists of a mixed dominance of anaerobic bacteria, a biofilm-targeted therapy is necessary: following the degradation of the biofilm matrix by lysozyme, Lactobacillus rhamnosus CA15 (10^10 CFU/ml) or PB01 strain (>10^8 CFU/ml) is administered sequentially for 10 days, resulting in an increase in the proportion of lactobacilli to 62 ± 8% while concurrently reducing pro-inflammatory factors IL-6/TNF-α (160). For women with a history of preterm birth or concurrent PROM, extending the probiotic treatment duration to 28 days significantly decreases the incidence of PROM (relative risk (RR) = 0.42, $p < 0.001$) (133, 160). It is crucial to acknowledge that the protective effects of orally administered probiotics on asymptomatic women remain contentious. Additionally, the probiotic dosage should be adjusted based on dynamic monitoring of vaginal pH (161, 162). There is a lack of consensus regarding the clinical significance of CST-III microbiota. Some researchers hypothesize that this condition may evolve into a mixed type (CST-IV). To achieve a more refined classification, it is essential to incorporate host immune markers, such as HBD-2 levels. Furthermore, while vaginal microbiota transplantation (VMT) has been shown to reduce the risk of PROM by 40% in animal models, the long-term safety of this procedure and its standardized methodology require further validation.

6.3.2 Dynamic monitoring of vaginal pH

Vaginal pH dynamic monitoring serves as a crucial tool for assessing the microecological balance of the female reproductive tract, with its fluctuations closely associated with pregnancy outcomes and the risk of PROM. A normal vaginal environment depends on a microecological balance predominantly maintained by lactobacilli, which ensure an acidic pH (≤4.5) that inhibits pathogen colonization through lactic acid metabolism. A sustained pH exceeding 4.7 indicates a microecological imbalance, such as bacterial vaginosis (BV), which is significantly correlated with an increased risk of PROM (163). When the pH level rises above 4.7, the population of lactobacilli diminishes, facilitating the proliferation of anaerobic bacteria, such as Gardnerella, and activating the TLR pathway. This activation leads to the release of pro-inflammatory factors, including IL-6 and TNF-α (133). These inflammatory responses induce oxidative stress (elevated reactive oxygen species, ROS), activating matrix metalloproteinase MMP-9, which degrades collagen in the amniotic membrane, thereby increasing its fragility. In early pregnancy, a pH level greater than 4.7, coupled with reduced levels of defensin HBD-2, heightens the risk of PROM by 2.3 times. Fluctuations in pH levels exceeding 0.5 units per week indicate a shift in the microbiota toward a high-risk type (CST-IV), necessitating a more refined classification based on immune markers (163). Smart tampon sensors facilitate real-time monitoring of vaginal pH at home, addressing the limitations of traditional single-visit clinic testing. Dynamic monitoring of vaginal pH is a key indicator for predicting PROM. By identifying early dysbiosis and guiding targeted probiotic interventions alongside inflammation marker analysis, these sensors provide precise strategies for improving pregnancy outcomes. Moving forward, it is essential to promote standardized home monitoring technologies and integrated prediction models that consider both microbiome and immune factors.

7 Challenges and prospects

7.1 Challenges

While the current study elucidates the mechanisms of interaction between vaginal microbiota and biochemical markers in PROM, several challenges remain in clinical application and research practice. First, the reproducibility and stability of study results require further attention. The high variability of vaginal microbiota among individuals, coupled with the complex array of factors influencing microbiota—such as genetic background, environmental influences, and lifestyle habits—often complicates the standardization of results across different studies. Thus, validating these findings in larger clinical trials and ensuring the reliability of the results represent significant challenges at present. Second, the difficulties associated with clinical translation must not be overlooked. The assessment of the safety and efficacy of treatments necessitates additional clinical trial data. For instance, although probiotic therapies have demonstrated potential in regulating vaginal microbiota, their long-term safety and optimal

use in conjunction with antibiotics and anti-inflammatory drugs warrant thorough investigation (16). In addition, therapies targeting biochemical markers, such as MMP inhibitors, require rigorous clinical validation to confirm their efficacy in patients at high risk of premature rupture.

7.2 Prospects

In the future, advancements in multi-omics research techniques (e.g., metabolomics, microbiomics, transcriptomics) will enable a more precise and in-depth study of the interactions between vaginal microbiota and biochemical markers. By integrating multi-omics approaches, we can achieve a comprehensive understanding of the metabolic functions of vaginal microbiota, microbe-host interactions, and the dynamics of biochemical markers. This comprehensive approach will enable the identification of novel pathological mechanisms linked to the premature rupture of membranes, thus establishing a basis for tailored diagnostic and therapeutic strategies. In terms of clinical implications, an improved comprehension of how vaginal microbiota interacts with biochemical markers could strengthen the application of personalized medicine in preventing and managing premature rupture of membranes. Future treatment trends may include combined probiotic therapy, marker-based targeted therapies, and the concurrent use of antioxidants and anti-inflammatory drugs for individuals at high risk of premature rupture. Furthermore, the development of convenient and efficient tools for testing vaginal microbiota and biochemical markers will significantly improve the accuracy of clinical diagnoses and the effectiveness of early interventions.

8 Conclusions

Research has highlighted the significant interaction between vaginal microbiota and biochemical markers in instances of premature rupture of membranes. Dysbiosis within the microbiota is strongly associated with changes in the expression of pro-inflammatory cytokines, matrix metalloproteinases (MMPs), and various other biochemical markers.These elements interact through inflammatory responses and oxidative stress pathways, ultimately influencing the stability of fetal membranes. Based on these findings, a comprehensive evaluation of vaginal microbiota along with biochemical indicators presents new strategies for the early identification and tailored management of premature rupture of membranes.Despite notable advancements in research, challenges persist, particularly regarding the reproducibility of results and the clinical applicability of findings. Looking ahead, the advancement of multi-omics technology promises to enhance our understanding of vaginal microbiota and biochemical markers, thereby providing more effective tools and strategies for the clinical management of preterm birth. Future research and clinical practice should prioritize improving the accuracy of diagnostic tools and tailoring treatments to enhance pregnancy outcomes and mitigate the effects of preterm birth on maternal and infant health.

Author contributions

YD: Writing – original draft, Writing – review & editing. YL: Writing – review & editing. TL: Investigation, Writing – review & editing. FW: Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research and/or publication of this article.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that Generative AI was used in the creation of this manuscript. The future perspectives section of this review was drafted with the assistance of GPT-4.0 (OpenAI), and the authors assume full responsibility for its final content, interpretation, and integration into the manuscript.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Prelabor rupture of membranes: ACOG practice bulletin, number 217. *Obstet Gynecol.* (2020) 135:e80-e97. doi: 10.1097/AOG.0000000000003700

2. Clark EA, Varner M. Impact of preterm PROM and its complications on long-term infant outcomes. *Clin Obstet Gynecol.* (2011) 54:358–69. doi: 10.1097/GRF.0b013e318217ee18

3. Zhang C, Cao J, Xu M, Wu D, Li W, Chang Y. The role of neutrophils in chorioamnionitis. *Front Immunol*. (2023) 14:1198831. doi: 10.3389/fimmu.2023.1198831

4. Jain VG, Willis KA, Jobe A, Ambalavanan N. Chorioamnionitis and neonatal outcomes. *Pediatr Res*. (2022) 91:289–96. doi: 10.1038/s41390-021-01633-0

5. Di Simone N, Santamaria Ortiz A, Specchia M, Tersigni C, Villa P, Gasbarrini A, et al. Recent insights on the maternal microbiota: impact on pregnancy outcomes. *Front Immunol*. (2020) 11:528202. doi: 10.3389/fimmu.2020.528202

6. Bayar E, Bennett PR, Chan D, Sykes L, Macintyre DA. The pregnancy microbiome and preterm birth. *Semin Immunopathol*. (2020) 42:487–99. doi: 10.1007/s00281-020-00817-w

7. France M, Alizadeh M, Brown S, Ma B, Ravel J. Towards a deeper understanding of the vaginal microbiota. *Nat Microbiol*. (2022) 7:367–78. doi: 10.1038/s41564-022-01083-2

8. Saraf VS, Sheikh SA, Ahmad A, Gillevet PM, Bokhari H, Javed S. Vaginal microbiome: normalcy vs dysbiosis. *Arch Microbiol*. (2021) 203:3793–802. doi: 10.1007/s00203-021-02414-3

9. Goodfellow L, Verwijs MC, Care A, Sharp A, Ivandic J, Poljak B, et al. Vaginal bacterial load in the second trimester is associated with early preterm birth recurrence: a nested case-control study. *Bjog*. (2021) 128:2061–72. doi: 10.1111/1471-0528.16816

10. Novak J, Ferreira CST, Golim MA, Silva MG, Marconi C. Covariates of vaginal microbiota and pro-inflammatory cytokine levels in women of reproductive age. *Benef Microbes*. (2023) 14:131–42. doi: 10.3920/BM2022.0060

11. Ronde E, Reiss IKM, Hankemeier T, De Meij TG, Frerichs N, Schoenmakers S. The potential of metabolomic analyses as predictive biomarkers of preterm delivery: A systematic review. *Front Endocrinol (Lausanne)*. (2021) 12:668417. doi: 10.3389/fendo.2021.668417

12. Shaffer Z, Romero R, Tarca AL, Galaz J, Arenas-Hernandez M, Gudicha DW, et al. The vaginal immunoproteome for the prediction of spontaneous preterm birth: A retrospective longitudinal study. *Elife*. (2024) 13. doi: 10.7554/eLife.90943

13. Kyrgiou M, Moscicki AB. Vaginal microbiome and cervical cancer. *Semin Cancer Biol*. (2022) 86:189–98. doi: 10.1016/j.semcancer.2022.03.005

14. Pendharkar S, Skafte-Holm A, Simsek G, Haahr T. Lactobacilli and their probiotic effects in the vagina of reproductive age women. *Microorganisms*. (2023) 11:636. doi: 10.3390/microorganisms11030636

15. Kroon SJ, Ravel J, Huston WM. Cervicovaginal microbiota, women's health, and reproductive outcomes. *Fertil Steril*. (2018) 110:327–36. doi: 10.1016/j.fertnstert.2018.06.036

16. Chee WJY, Chew SY, Than LTL. Vaginal microbiota and the potential of Lactobacillus derivatives in maintaining vaginal health. *Microb Cell Fact*. (2020) 19:203. doi: 10.1186/s12934-020-01464-4

17. Gudnadottir U, Debelius JW, Du J, Hugerth LW, Danielsson H, Schuppe-Koistinen I, et al. The vaginal microbiome and the risk of preterm birth: a systematic review and network meta-analysis. *Sci Rep*. (2022) 12:7926. doi: 10.1038/s41598-022-12007-9

18. Aldunate M, Srbinovski D, Hearps AC, Latham CF, Ramsland PA, Gugasyan R, et al. Antimicrobial and immune modulatory effects of lactic acid and short chain fatty acids produced by vaginal microbiota associated with eubiosis and bacterial vaginosis. *Front. Physiol*. (2015) 6:164. doi: 10.3389/fphys.2015.00164

19. Redelinghuys MJ, Geldenhuys J, Jung H, Kock MM. Bacterial vaginosis: current diagnostic avenues and future opportunities. *Front Cell Infect Microbiol*. (2020) 10:354. doi: 10.3389/fcimb.2020.00354

20. Tabatabaei N, Eren AM, Barreiro LB, Yotova V, Dumaine A, Allard C, et al. Vaginal microbiome in early pregnancy and subsequent risk of spontaneous preterm birth: a case-control study. *Bjog*. (2019) 126:349–58. doi: 10.1111/1471-0528.15299

21. Onderdonk AB, Delaney ML, Fichorova RN. The human microbiome during bacterial vaginosis. *Clin Microbiol Rev*. (2016) 29:223–38. doi: 10.1128/CMR.00075-15

22. Liu L, Yin T, Zhang X, Sun L, Yin Y. Temporal and spatial variation of the human placental microbiota during pregnancy. *Am J Reprod Immunol*. (2024) 92:e70023. doi: 10.1111/aji.70023

23. Gupta S, Kakkar V, Bhushan I. Crosstalk between vaginal microbiome and female health: A review. *Microb Pathog*. (2019) 136:103696. doi: 10.1016/j.micpath.2019.103696

24. Barrientos-Durán A, Fuentes-López A, De Salazar A, Plaza-Díaz J, García F. Reviewing the composition of vaginal microbiota: inclusion of nutrition and probiotic factors in the maintenance of eubiosis. *Nutrients*. (2020) 12:419. doi: 10.3390/nu12020419

25. Brown RG, Al-Memar M, Marchesi JR, Lee YS, Smith A, Chan D, et al. Establishment of vaginal microbiota composition in early pregnancy and its association with subsequent preterm prelabor rupture of the fetal membranes. *Transl Res*. (2019) 207:30–43. doi: 10.1016/j.trsl.2018.12.005

26. Peterson LS, Stelzer IA, Tsai AS, Ghaemi MS, Han X, Ando K, et al. Multiomic immune clockworks of pregnancy. *Semin Immunopathol*. (2020) 42:397–412. doi: 10.1007/s00281-019-00772-1

27. Liu L, Chen J, Chen Y, Jiang S, Xu H, Zhan H, et al. Characterization of Vaginal Microbiota in Third Trimester Premature Rupture of Membranes Patients through 16S rDNA Sequencing. *Pathogens*. (2022) 11:847. doi: 10.3390/pathogens11080847

28. Zheng N, Guo R, Yao Y, Jin M, Cheng Y, Ling Z. Lactobacillus iners is associated with vaginal dysbiosis in healthy pregnant women: A preliminary study. *BioMed Res Int*. (2019) 2019:6079734. doi: 10.1155/2019/6079734

29. Juliana NCA, Peters RPH, Al-Nasiry S, Budding AE, Morré SA, Ambrosino E. Composition of the vaginal microbiota during pregnancy in women living in sub-Saharan Africa: a PRISMA-compliant review. *BMC Pregnancy Childbirth*. (2021) 21:596. doi: 10.1186/s12884-021-04072-1

30. Pruski P, Correia GDS, Lewis HV, Capuccini K, Inglese P, Chan D, et al. Direct on-swab metabolic profiling of vaginal microbiome host interactions during pregnancy and preterm birth. *Nat Commun*. (2021) 12:5967. doi: 10.1038/s41467-021-26215-w

31. Qin D, Ma Y, Wang Y, Hou X, Yu L. Contribution of lactobacilli on intestinal mucosal barrier and diseases: perspectives and challenges of lactobacillus casei. *Life (Basel)*. (2022) 12:1910. doi: 10.3390/life12111910

32. Raheem A, Liang L, Zhang G, Cui S. Modulatory effects of probiotics during pathogenic infections with emphasis on immune regulation. *Front Immunol*. (2021) 12:616713. doi: 10.3389/fimmu.2021.616713

33. Hearps AC, Tyssen D, Srbinovski D, Bayigga L, Diaz DJD, Aldunate M, et al. Vaginal lactic acid elicits an anti-inflammatory response from human cervicovaginal epithelial cells and inhibits production of pro-inflammatory mediators associated with HIV acquisition. *Mucosal Immunol*. (2017) 10:1480–90. doi: 10.1038/mi.2017.27

34. Mazziotta C, Tognon M, Martini F, Torreggiani E, Rotondo JC. Probiotics mechanism of action on immune cells and beneficial effects on human health. *Cells*. (2023) 12:184. doi: 10.3390/cells12010184

35. Daba GM, Elkhateeb WA. Ribosomally synthesized bacteriocins of lactic acid bacteria: Simplicity yet having wide potentials - A review. *Int J Biol Macromol*. (2024) 256:128325. doi: 10.1016/j.ijbiomac.2023.128325

36. Linhares IM, Sisti G, Minis E, De Freitas GB, Moron AF, Witkin SS. Contribution of epithelial cells to defense mechanisms in the human vagina. *Curr Infect Dis Rep*. (2019) 21:30. doi: 10.1007/s11908-019-0686-5

37. Mallott EK, Amato KR. Host specificity of the gut microbiome. *Nat Rev Microbiol*. (2021) 19:639–53. doi: 10.1038/s41579-021-00562-3

38. Stefanovic V, Andersson S, Vento M. Oxidative stress - Related spontaneous preterm delivery challenges in causality determination, prevention and novel strategies in reduction of the sequelae. *Free Radic Biol Med*. (2019) 142:52–60. doi: 10.1016/j.freeradbiomed.2019.06.008

39. Menon R, Behnia F, Polettini J, Richardson LS. Novel pathways of inflammation in human fetal membranes associated with preterm birth and preterm pre-labor rupture of the membranes. *Semin Immunopathol*. (2020) 42:431–50. doi: 10.1007/s00281-020-00808-x

40. Menon R. Fetal inflammatory response at the fetomaternal interface: A requirement for labor at term and preterm. *Immunol Rev*. (2022) 308:149–67. doi: 10.1111/imr.13075

41. Zhou F, Li C, Zhang SY. NLRP3 inflammasome: a new therapeutic target for high-risk reproductive disorders? *Chin Med J (Engl)*. (2020) 134:20–7. doi: 10.1097/CM9.0000000000001214

42. Li W, Zhao X, Li S, Chen X, Cui H, Chang Y, et al. Upregulation of TNF-α and IL-6 induces preterm premature rupture of membranes by activation of ADAMTS-9 in embryonic membrane cells. *Life Sci*. (2020) 260:118237. doi: 10.1016/j.lfs.2020.118237

43. Kumar M, Murugesan S, Singh P, Saadaoui M, Elhag DA, Terranegra A, et al. Vaginal microbiota and cytokine levels predict preterm delivery in asian women. *Front Cell Infect Microbiol*. (2021) 11:639665. doi: 10.3389/fcimb.2021.639665

44. Sims JE, Smith DE. The IL-1 family: regulators of immunity. *Nat Rev Immunol*. (2010) 10:89–102. doi: 10.1038/nri2691

45. Xu L, Yang T, Wen M, Wen D, Jin C, An M, et al. Frontiers in the etiology and treatment of preterm premature rupture of membrane: from molecular mechanisms to innovative therapeutic strategies. *Reprod Sci*. (2024) 31:917–31. doi: 10.1007/s43032-023-01411-9

46. El-Shazly S, Makhseed M, Azizieh F, Raghupathy R. Increased expression of pro-inflammatory cytokines in placentas of women undergoing spontaneous preterm delivery or premature rupture of membranes. *Am J Reprod Immunol*. (2004) 52:45–52. doi: 10.1111/j.1600-0897.2004.00181.x

47. Liu B, Li M, Zhou Z, Guan X, Xiang Y. Can we use interleukin-6 (IL-6) blockade for coronavirus disease 2019 (COVID-19)-induced cytokine release syndrome (CRS)? *J Autoimmun*. (2020) 111:102452. doi: 10.1016/j.jaut.2020.102452

48. Mikołajczyk M, Wirstlein P, Adamczyk M, Skrzypczak J, Wender-Ożegowska E. Value of cervicovaginal fluid cytokines in prediction of fetal inflammatory response syndrome in pregnancies complicated with preterm premature rupture of membranes (pPROM). *J Perinat Med*. (2020) 48:249–55. doi: 10.1515/jpm-2019-0280

49. Habelrih T, Augustin TL, Mauffette-Whyte F, Ferri B, Sawaya K, Côté F, et al. Inflammatory mechanisms of preterm labor and emerging anti-inflammatory interventions. *Cytokine Growth Factor Rev*. (2024) 78:50–63. doi: 10.1016/j.cytogfr.2024.07.007

50. Chen K, Li Y, Zhang X, Ullah R, Tong J, Shen Y. The role of the PI3K/AKT signalling pathway in the corneal epithelium: recent updates. *Cell Death Dis*. (2022) 13:513. doi: 10.1038/s41419-022-04963-x

51. Wang Z, Zhang S, Xiao Y, Zhang W, Wu S, Qin T, et al. NLRP3 inflammasome and inflammatory diseases. *Oxid Med Cell Longev*. (2020) 2020:4063562. doi: 10.1155/2020/4063562

52. Miller AS, Hidalgo TN, Abrahams VM. Human fetal membrane IL-1β production in response to bacterial components is mediated by uric-acid induced NLRP3 inflammasome activation. *J Reprod Immunol*. (2022) 149:103457. doi: 10.1016/j.jri.2021.103457

53. Motomura K, Romero R, Galaz J, Tao L, Garcia-Flores V, Xu Y, et al. Fetal and maternal NLRP3 signaling is required for preterm labor and birth. *JCI Insight*. (2022) 7: e158238. doi: 10.1172/jci.insight.158238

54. Alam MS, Otsuka S, Wong N, Abbasi A, Gaida MM, Fan Y, et al. TNF plays a crucial role in inflammation by signaling via T cell TNFR2. *Proc Natl Acad Sci U.S.A*. (2021) 118:e2109972118. doi: 10.1073/pnas.2109972118

55. Shi JW, Lai ZZ, Yang HL, Yang SL, Wang CJ, Ao D, et al. Collagen at the maternal-fetal interface in human pregnancy. *Int J Biol Sci*. (2020) 16:2220–34. doi: 10.7150/ijbs.45586

56. Szczuko M, Kikut J, Komorniak N, Bilicki J, Celewicz Z, Ziętek M. The role of arachidonic and linoleic acid derivatives in pathological pregnancies and the human reproduction process. *Int J Mol Sci*. (2020) 21:9628. doi: 10.3390/ijms21249628

57. Li WJ, Lu JW, Zhang CY, Wang WS, Ying H, Myatt L, et al. PGE2 vs PGF2α in human parturition. *Placenta*. (2021) 104:208–19. doi: 10.1016/j.placenta.2020.12.012

58. Chopra A, Radhakrishnan R, Sharma M. Porphyromonas gingivalis and adverse pregnancy outcomes: a review on its intricate pathogenic mechanisms. *Crit Rev Microbiol*. (2020) 46:213–36. doi: 10.1080/1040841X.2020.1747392

59. Wood EM, Hornaday KK, Slater DM. Prostaglandins in biofluids in pregnancy and labour: A systematic review. *PLoS One*. (2021) 16:e0260115. doi: 10.1371/journal.pone.0260115

60. Šket T, Ramuta T, Starčič Erjavec M, Kreft ME. The role of innate immune system in the human amniotic membrane and human amniotic fluid in protection against intra-amniotic infections and inflammation. *Front Immunol*. (2021) 12:735324. doi: 10.3389/fimmu.2021.735324

61. Sena LA, Chandel NS. Physiological roles of mitochondrial reactive oxygen species. *Mol Cell*. (2012) 48:158–67. doi: 10.1016/j.molcel.2012.09.025

62. Menon R. Oxidative stress damage as a detrimental factor in preterm birth pathology. *Front Immunol*. (2014) 5:567. doi: 10.3389/fimmu.2014.00567

63. Lannon SM, Vanderhoeven JP, Eschenbach DA, Gravett MG, Adams Waldorf KM. Synergy and interactions among biological pathways leading to preterm premature rupture of membranes. *Reprod Sci*. (2014) 21:1215–27. doi: 10.1177/1933719114534535

64. Unsal V, Dalkiran T, Çiçek M, Kölükçü E. The role of natural antioxidants against reactive oxygen species produced by cadmium toxicity: A review. *Adv Pharm Bull*. (2020) 10:184–202. doi: 10.34172/apb.2020.023

65. Läsche M, Urban H, Gallwas J, Gründker C. HPV and other microbiota; who's good and who's bad: effects of the microbial environment on the development of cervical cancer-A non-systematic review. *Cells*. (2021) 10:714. doi: 10.3390/cells10030714

66. Dumanović J, Nepovimova E, Natić M, Kuča K, Jaćević V. The significance of reactive oxygen species and antioxidant defense system in plants: A concise overview. *Front Plant Sci*. (2020) 11:552969. doi: 10.3389/fpls.2020.552969

67. Yin N, Wang H, Zhang H, Ge H, Tan B, Yuan Y, et al. IL-27 induces a pro-inflammatory response in human fetal membranes mediating preterm birth. *Int Immunopharmacol*. (2017) 50:361–9. doi: 10.1016/j.intimp.2017.06.031

68. Kammeyer A, Luiten RM. Oxidation events and skin aging. *Ageing Res Rev*. (2015) 21:16–29. doi: 10.1016/j.arr.2015.01.001

69. Kim KK, Sheppard D, Chapman HA. TGF-β1 signaling and tissue fibrosis. *Cold Spring Harb Perspect Biol*. (2018) 10:a022293. doi: 10.1101/cshperspect.a022293

70. Menon R, Richardson LS. Preterm prelabor rupture of the membranes: A disease of the fetal membranes. *Semin Perinatol*. (2017) 41:409–19. doi: 10.1053/j.semperi.2017.07.012

71. Cabral-Pacheco GA, Garza-Veloz I, Castruita-De La Rosa C, Ramirez-Acuña JM, Perez-Romero BA, Guerrero-Rodriguez JF, et al. The roles of matrix metalloproteinases and their inhibitors in human diseases. *Int J Mol Sci*. (2020) 21:9739. doi: 10.3390/ijms21249739

72. Athayde N, Edwin SS, Romero R, Gomez R, Maymon E, Pacora P, et al. A role for matrix metalloproteinase-9 in spontaneous rupture of the fetal membranes. *Am J Obstet Gynecol*. (1998) 179:1248–53. doi: 10.1016/S0002-9378(98)70141-3

73. Carvalho NS, Moron AF, Witkin SS, Menon R, Cavalheiro S, Barbosa MM, et al. Histological response and expression of collagen, metalloproteinases MMP-1 and MMP-9 and tissue inhibitors of metalloproteinases TIMP-1 and TIMP-2 in fetal membranes following open intrauterine surgery: an experimental study. *J Matern Fetal Neonatal Med*. (2022) 35:1301–9. doi: 10.1080/14767058.2020.1752654

74. Liu L, Xu HJ, Chen JL, Chen Z, Zhan HY, Xu DX, et al. Detection of vaginal metabolite changes in premature rupture of membrane patients in third trimester pregnancy: a prospective cohort study. *Reprod Sci*. (2021) 28:585–94. doi: 10.1007/s43032-020-00338-9

75. Zuo G, Dong JX, Zhao FF, Chen Y. Expression of matrix metalloproteinase-9 and its substrate level in patients with premature rupture of membranes. *J Obstet Gynaecol*. (2017) 37:441–5. doi: 10.1080/01443615.2016.1250734

76. Son GH, Lee JJ, Kim Y, Lee KY. The role of antimicrobial peptides in preterm birth. *Int J Mol Sci*. (2021) 22:8905. doi: 10.3390/ijms22168905

77. Fusco A, Savio V, Perfetto B, Mattina R, Donnarumma G. Antimicrobial peptide human β-defensin-2 improves *in vitro* cellular viability and reduces pro-inflammatory effects induced by enteroinvasive Escherichia coli in Caco-2 cells by inhibiting invasion and virulence factors' expression. *Front Cell Infect Microbiol*. (2022) 12:1009415. doi: 10.3389/fcimb.2022.1009415

78. Noda-Nicolau NM, Silva MC, Bento GFC, Ferreira JSB, Novak J, Morales JAP, et al. Cervicovaginal levels of human beta defensins during bacterial vaginosis. *PLoS One*. (2021) 16:e0260753. doi: 10.1371/journal.pone.0260753

79. Plesniarski A, Siddik AB, Su RC. The microbiome as a key regulator of female genital tract barrier function. *Front Cell Infect Microbiol*. (2021) 11:790627. doi: 10.3389/fcimb.2021.790627

80. Das S, Konwar BK. Influence of connatural factors in shaping vaginal microflora and ensuring its health. *Arch Gynecol Obstet*. (2024) 309:871–86. doi: 10.1007/s00404-023-07200-8

81. Cieślik M, Bagińska N, Górski A, Jończyk-Matysiak E. Human β-defensin 2 and its postulated role in modulation of the immune response. *Cells*. (2021) 10:2991. doi: 10.3390/cells10112991

82. Balakrishnan SN, Yamang H, Lorenz MC, Chew SY, Than LTL. Role of vaginal mucosa, host immunity and microbiota in vulvovaginal candidiasis. *Pathogens*. (2022) 11:618. doi: 10.3390/pathogens11060618

83. Chen MY, Ye XJ, He XH, Ouyang DY. The signaling pathways regulating NLRP3 inflammasome activation. *Inflammation*. (2021) 44:1229–45. doi: 10.1007/s10753-021-01439-6

84. Zheng N, Guo R, Wang J, Zhou W, Ling Z. Contribution of lactobacillus iners to vaginal health and diseases: A systematic review. *Front Cell Infect Microbiol*. (2021) 11:792787. doi: 10.3389/fcimb.2021.792787

85. Artym J, Zimecki M. Antimicrobial and prebiotic activity of lactoferrin in the female reproductive tract: A comprehensive review. *Biomedicines*. (2021) 9:1940. doi: 10.3390/biomedicines9121940

86. Kumar L, Dwivedi M, Jain N, Shete P, Solanki S, Gupta R, et al. The female reproductive tract microbiota: friends and foe. *Life (Basel)*. (2023) 13:1313. doi: 10.3390/life13061313

87. Oyinloye BE, Adenowo AF, Kappo AP. Reactive oxygen species, apoptosis, antimicrobial peptides and human inflammatory diseases. *Pharm (Basel)*. (2015) 8:151–75. doi: 10.3390/ph8020151

88. Sowbhagya R, Muktha H, Ramakrishnaiah TN, Surendra AS, Sushma SM, Tejaswini C, et al. Collagen as the extracellular matrix biomaterials in the arena of medical sciences. *Tissue Cell*. (2024) 90:102497. doi: 10.1016/j.tice.2024.102497

89. Lu J, Wang Z, Cao J, Chen Y, Dong Y. A novel and compact review on the role of oxidative stress in female reproduction. *Reprod Biol Endocrinol*. (2018) 16:80. doi: 10.1186/s12958-018-0391-5

90. Dabee S, Passmore JS, Heffron R, Jaspan HB. The complex link between the female genital microbiota, genital infections, and inflammation. *Infect Immun*. (2021) 89. doi: 10.1128/IAI.00487-20

91. Gupta P, Singh MP, Goyal K. Diversity of vaginal microbiome in pregnancy: deciphering the obscurity. *Front Public Health*. (2020) 8:326. doi: 10.3389/fpubh.2020.00326

92. Zhou Q, Yu Y, Zhou J, Liu J, Gao J. Relationship of lactobacillus vaginal microbiota changes and the risk of preterm birth: A systematic review and meta-analysis. *J Womens Health (Larchmt)*. (2024) 33:228–38. doi: 10.1089/jwh.2023.0393

93. Bai B, Tuerxun G, Tuerdi A, Maimaiti R, Sun Y, Abudukerimu A. Analysis of vaginal flora diversity and study on the role of Porphyromonas asaccharolytica in promoting IL-1β in regulating cervical cancer. *Sci Rep*. (2024) 14:21731. doi: 10.1038/s41598-024-73146-9

94. Shen L, Zhang W, Yuan Y, Zhu W, Shang A. Vaginal microecological characteristics of women in different physiological and pathological period. *Front Cell Infect Microbiol*. (2022) 12:959793. doi: 10.3389/fcimb.2022.959793

95. Al-Nasiry S, Ambrosino E, Schlaepfer M, Morré SA, Wieten L, Voncken JW, et al. The interplay between reproductive tract microbiota and immunological system in human reproduction. *Front Immunol*. (2020) 11:378. doi: 10.3389/fimmu.2020.00378

96. Moretta A, Scieuzo C, Petrone AM, Salvia R, Manniello MD, Franco A, et al. Antimicrobial peptides: A new hope in biomedical and pharmaceutical fields. *Front Cell Infect Microbiol*. (2021) 11:668632. doi: 10.3389/fcimb.2021.668632

97. Sahu PK, Jayalakshmi K, Tilgam J, Gupta A, Nagaraju Y, Kumar A, et al. ROS generated from biotic stress: Effects on plants and alleviation by endophytic microbes. *Front Plant Sci*. (2022) 13:1042936. doi: 10.3389/fpls.2022.1042936

98. Greathouse KL, Faucher MA, Hastings-Tolsma M. The gut microbiome, obesity, and weight control in women's reproductive health. *West J Nurs Res*. (2017) 39:1094–119. doi: 10.1177/0193945917697223

99. Baldewijns S, Sillen M, Palmans I, Vandecruys P, Van Dijck P, Demuyser L. The role of fatty acid metabolites in vaginal health and disease: application to candidiasis. *Front Microbiol*. (2021) 12:705779. doi: 10.3389/fmicb.2021.705779

100. Li H, Zang Y, Wang C, Li H, Fan A, Han C, et al. The interaction between microorganisms, metabolites, and immune system in the female genital tract microenvironment. *Front Cell Infect Microbiol*. (2020) 10:609488. doi: 10.3389/fcimb.2020.609488

101. Federico S, Pozzetti L, Papa A, Carullo G, Gemma S, Butini S, et al. Modulation of the innate immune response by targeting toll-like receptors: A perspective on their agonists and antagonists. *J Med Chem*. (2020) 63:13466–513. doi: 10.1021/acs.jmedchem.0c01049

102. Xia P, Wu Y, Lian S, Yan L, Meng X, Duan Q, et al. Research progress on Toll-like receptor signal transduction and its roles in antimicrobial immune responses. *Appl Microbiol Biotechnol*. (2021) 105:5341–55. doi: 10.1007/s00253-021-11406-8

103. Gryaznova MV, Kozarenko ON, Ivannikova VM, Smirnova YD, Burakova IY, Altukhova OB, et al. Pattern-recognition receptors and cervical microbiome in patients with early miscarriages. *Int J Inflamm*. (2024) 2024:5320926. doi: 10.1155/2024/5320926

104. Oerlemans EFM, Wuyts S, Bellen G, Wittouck S, De Boeck I, Ruban K, et al. The dwindling microbiota of aerobic vaginitis, an inflammatory state enriched in pathobionts with limited TLR stimulation. *Diagnostics (Basel)*. (2020) 10:879. doi: 10.3390/diagnostics10110879

105. Zheng X, Hu N, Liu J, Zhao K, Li H, Wang J, et al. Cervicovaginal microbiota disorder combined with the change of cytosine phosphate guanine motif- toll like receptor 9 axis was associated with cervical cancerization. *J Cancer Res Clin Oncol*. (2023) 149:17371–81. doi: 10.1007/s00432-023-05453-z

106. Uchide N, Ohyama K, Bessho T, Takeichi M, Toyoda H. Possible roles of proinflammatory and chemoattractive cytokines produced by human fetal membrane cells in the pathology of adverse pregnancy outcomes associated with influenza virus infection. *Mediators Inflammation*. (2012) 2012:270670. doi: 10.1155/2012/270670

107. Jing M, Chen X, Qiu H, He W, Zhou Y, Li D, et al. Insights into the immunomodulatory regulation of matrix metalloproteinase at the maternal-fetal interface during early pregnancy and pregnancy-related diseases. *Front Immunol*. (2022) 13:1067661. doi: 10.3389/fimmu.2022.1067661

108. Strauss JF 3rd. Extracellular matrix dynamics and fetal membrane rupture. *Reprod Sci*. (2013) 20:140–53. doi: 10.1177/1933719111424454

109. Flores-Espinosa P, Olmos-Ortíz A, Granados-Cepeda M, Quesada-Reyna B, Vega-Sánchez R, Velázquez P, et al. Prolactin protects the structural integrity of human fetal membranes by downregulating inflammation-induced secretion of matrix metalloproteinases. *Immunol Invest*. (2022) 51:1313–29. doi: 10.1080/08820139.2021.1936012

110. Wang C, Chen Q, Wang Y. Correlation of amniotic fluid inflammatory markers with preterm birth: a meta-analysis. *J Obstet Gynaecol*. (2024) 44:2368764. doi: 10.1080/01443615.2024.2368764

111. Zhao F, Hu X, Ying C. Advances in research on the relationship between vaginal microbiota and adverse pregnancy outcomes and gynecological diseases. *Microorganisms*. (2023) 11:991. doi: 10.3390/microorganisms11040991

112. Vidal MS Jr., Lintao RCV, Severino MEL, Tantengco OAG, Menon R. Spontaneous preterm birth: Involvement of multiple feto-maternal tissues and organ systems, differing mechanisms, and pathways. *Front Endocrinol (Lausanne)*. (2022) 13:1015622. doi: 10.3389/fendo.2022.1015622

113. Han Y, Liu Z, Chen T. Role of vaginal microbiota dysbiosis in gynecological diseases and the potential interventions. *Front Microbiol*. (2021) 12:643422. doi: 10.3389/fmicb.2021.643422

114. Hussain T, Murtaza G, Metwally E, Kalhoro DH, Kalhoro MS, Rahu BA, et al. The role of oxidative stress and antioxidant balance in pregnancy. *Mediators Inflammation*. (2021) 2021:9962860. doi: 10.1155/2021/9962860

115. Bartosz G. Reactive oxygen species: destroyers or messengers? *Biochem Pharmacol*. (2009) 77:1303–15. doi: 10.1016/j.bcp.2008.11.009

116. Woods JR Jr. Reactive oxygen species and preterm premature rupture of membranes-a review. *Placenta*. (2001) 22 Suppl A:S38–44. doi: 10.1053/plac.2001.0638

117. Wang Y, Qi H, Liu Y, Duan C, Liu X, Xia T, et al. The double-edged roles of ROS in cancer prevention and therapy. *Theranostics*. (2021) 11:4839–57. doi: 10.7150/thno.56747

118. Pandey M, Awasthi S, Baranwal S. IL-6: An endogenous activator of MMP-9 in preterm birth. *J Reprod Immunol*. (2020) 141:103147. doi: 10.1016/j.jri.2020.103147

119. Wu Y, Li Y, Luo Y, Zhou Y, Wen J, Chen L, et al. Gut microbiome and metabolites: The potential key roles in pulmonary fibrosis. *Front Microbiol*. (2022) 13:943791. doi: 10.3389/fmicb.2022.943791

120. Zaga-Clavellina V, Garcia-Lopez G, Flores-Pliego A, Merchant-Larios H, Vadillo-Ortega F. *In vitro* secretion and activity profiles of matrix metalloproteinases, MMP-9 and MMP-2, in human term extra-placental membranes after exposure to Escherichia coli. *Reprod Biol Endocrinol*. (2011) 9:13. doi: 10.1186/1477-7827-9-13

121. Lu Y, Zhang Y, Zhao X, Shang C, Xiang M, Li L, et al. Microbiota-derived short-chain fatty acids: Implications for cardiovascular and metabolic disease. *Front Cardiovasc Med*. (2022) 9:900381. doi: 10.3389/fcvm.2022.900381

122. Daskalakis G, Psarris A, Koutras A, Fasoulakis Z, Prokopakis I, Varthaliti A, et al. Maternal infection and preterm birth: from molecular basis to clinical implications. *Children (Basel)*. (2023) 10:907. doi: 10.3390/children10050907

123. Zhu B, Tao Z, Edupuganti L, Serrano MG, Buck GA. Roles of the microbiota of the female reproductive tract in gynecological and reproductive health. *Microbiol Mol Biol Rev*. (2022) 86:e0018121. doi: 10.1128/mmbr.00181-21

124. Ansari A, Bose S, You Y, Park S, Kim Y. Molecular mechanism of microbiota metabolites in preterm birth: pathological and therapeutic insights. *Int J Mol Sci*. (2021) 22:8145. doi: 10.3390/ijms22158145

125. Hussain T, Tan B, Murtaza G, Metwally E, Yang H, Kalhoro MS, et al. Role of dietary amino acids and nutrient sensing system in pregnancy associated disorders. *Front Pharmacol*. (2020) 11:586979. doi: 10.3389/fphar.2020.586979

126. Martini S, Aceti A, Della Gatta AN, Beghetti I, Marsico C, Pilu G, et al. Antenatal and postnatal sequelae of oxidative stress in preterm infants: A narrative review targeting pathophysiological mechanisms. *Antioxidants (Basel)*. (2023) 12:422. doi: 10.3390/antiox12020422

127. Pero R, Brancaccio M, Laneri S, Biasi MG, Lombardo B, Scudiero O. A novel view of human helicobacter pylori infections: interplay between microbiota and beta-defensins. *Biomolecules*. (2019) 9:237. doi: 10.3390/biom9060237

128. Robertson SA, Hutchinson MR, Rice KC, Chin PY, Moldenhauer LM, Stark MJ, et al. Targeting Toll-like receptor-4 to tackle preterm birth and fetal inflammatory injury. *Clin Transl Immunol*. (2020) 9:e1121. doi: 10.1002/cti2.1121

129. Delgado-Diaz DJ, Jesaveluk B, Hayward JA, Tyssen D, Alisoltani A, Potgieter M, et al. Lactic acid from vaginal microbiota enhances cervicovaginal epithelial barrier integrity by promoting tight junction protein expression. *Microbiome*. (2022) 10:141. doi: 10.1186/s40168-022-01337-5

130. Moylan HEC, Nguyen-Ngo C, Lim R, Lappas M. The short-chain fatty acids butyrate and propionate protect against inflammation-induced activation of mediators involved in active labor: implications for preterm birth. *Mol Hum Reprod*. (2020) 26:452–68. doi: 10.1093/molehr/gaaa025

131. Meng L, Meng M, Zhang R, Wubulikasimu A, Peng H, Zhang L, et al. Microbiome-producing SCFAs are associated with preterm birth via trophoblast function modulation. *mBio*. (2024) 15:e0270224. doi: 10.1128/mbio.02702-24

132. Yan C, Hong F, Xin G, Duan S, Deng X, Xu Y. Alterations in the vaginal microbiota of patients with preterm premature rupture of membranes. *Front Cell Infect Microbiol*. (2022) 12:858732. doi: 10.3389/fcimb.2022.858732

133. Gorczyca K, Kozioł MM, Kimber-Trojnar Ż, Kępa J, Satora M, Rekowska AK, et al. Premature rupture of membranes and changes in the vaginal microbiome - Probiotics. *Reprod Biol*. (2024) 24:100899. doi: 10.1016/j.repbio.2024.100899

134. Basavaprabhu HN, Sonu KS, Prabha R. Mechanistic insights into the action of probiotics against bacterial vaginosis and its mediated preterm birth: An overview. *Microb Pathog*. (2020) 141:104029. doi: 10.1016/j.micpath.2020.104029

135. Paramel Jayaprakash T, Wagner EC, Van Schalkwyk J, Albert AY, Hill JE, Money DM. High diversity and variability in the vaginal microbiome in women following preterm premature rupture of membranes (PPROM): A prospective cohort study. *PLoS One*. (2016) 11:e0166794. doi: 10.1371/journal.pone.0166794

136. Shipitsyna E, Roos A, Datcu R, Hallén A, Fredlund H, Jensen JS, et al. Composition of the vaginal microbiota in women of reproductive age–sensitive and specific molecular diagnosis of bacterial vaginosis is possible? *PLoS One*. (2013) 8:e60670. doi: 10.1371/journal.pone.0060670

137. Noda-Nicolau NM, Tantengco OAG, Polettini J, Silva MC, Bento GFC, Cursino GC, et al. Genital mycoplasmas and biomarkers of inflammation and their association with spontaneous preterm birth and preterm prelabor rupture of membranes: A systematic review and meta-analysis. *Front Microbiol*. (2022) 13:859732. doi: 10.3389/fmicb.2022.859732

138. Zhang M, He S, Qin B, Jin X, Wang M, Ren C, et al. Exogenous melatonin reduces the inhibitory effect of osmotic stress on antioxidant properties and cell ultrastructure at germination stage of soybean. *PLoS One*. (2020) 15:e0243537. doi: 10.1371/journal.pone.0243537

139. Ibrahim A, Khoo MI, Ismail EHE, Hussain NHN, Zin AAM, Noordin L, et al. Oxidative stress biomarkers in pregnancy: a systematic review. *Reprod Biol Endocrinol*. (2024) 22:93. doi: 10.1186/s12958-024-01259-x

140. Gonzalez-Covarrubias V, Martínez-Martínez E, Del Bosque-Plata L. The potential of metabolomics in biomedical applications. *Metabolites*. (2022) 12:194. doi: 10.3390/metabo12020194

141. Romero R, Mazaki-Tovi S, Vaisbuch E, Kusanovic JP, Chaiworapongsa T, Gomez R, et al. Metabolomics in premature labor: a novel approach to identify patients at risk for preterm delivery. *J Matern Fetal Neonatal Med*. (2010) 23:1344–59. doi: 10.3109/14767058.2010.482618

142. Ma X, Wu M, Wang C, Li H, Fan A, Wang Y, et al. The pathogenesis of prevalent aerobic bacteria in aerobic vaginitis and adverse pregnancy outcomes: a narrative review. *Reprod Health*. (2022) 19:21. doi: 10.1186/s12978-021-01292-8

143. Moraes DV, Queiroz DB, Eleutério JJ. Vaginal probiotics on the outcomes of premature rupture of membranes: a pilot randomised clinical trial. *J Obstet Gynaecol*. (2022) 42:3527–30. doi: 10.1080/01443615.2022.2151349

144. Ji J, Yang H. Using probiotics as supplementation for helicobacter pylori antibiotic therapy. *Int J Mol Sci*. (2020) 21:1136. doi: 10.3390/ijms21031136

145. Saucedo R, Ortega-Camarillo C, Ferreira-Hermosillo A, Díaz-Velázquez MF, Meixueiro-Calderón C, Valencia-Ortega J. Role of oxidative stress and inflammation in gestational diabetes mellitus. *Antioxidants (Basel)*. (2023) 12:1812. doi: 10.3390/antiox12101812

146. Sebastiani G, Navarro-Tapia E, Almeida-Toledano L, Serra-Delgado M, Paltrinieri AL, García-Algar Ó, et al. Effects of antioxidant intake on fetal development and maternal/neonatal health during pregnancy. *Antioxidants (Basel)*. (2022) 11:648. doi: 10.3390/antiox11040648

147. Rabaan AA, Al-Ahmed SH, Muhammad J, Khan A, Sule AA, Tirupathi R, et al. Role of inflammatory cytokines in COVID-19 patients: A review on molecular mechanisms, immune functions, immunopathology and immunomodulatory drugs to counter cytokine storm. *Vaccines (Basel)*. (2021) 9:436. doi: 10.3390/vaccines9050436

148. Virk MS, Virk MA, He Y, Tufail T, Gul M, Qayum A, et al. The anti-inflammatory and curative exponent of probiotics: A comprehensive and authentic ingredient for the sustained functioning of major human organs. *Nutrients*. (2024) 16:546. doi: 10.3390/nu16040546

149. Short CS, Quinlan RA, Wang X, Preda VG, Smith A, Marchesi JR, et al. Vaginal microbiota, genital inflammation and extracellular matrix remodelling collagenase: MMP-9 in pregnant women with HIV, a potential preterm birth mechanism warranting further exploration. *Front Cell Infect Microbiol*. (2021) 11:750103. doi: 10.3389/fcimb.2021.750103

150. Vaduva CC, Petrescu AM, Dira LM, Ruican D, Pana RC. Probiotics in the prophylaxis of premature rupture of membranes and cervical incompetence. *Nutrients*. (2024) 16:4230. doi: 10.3390/nu16234230

151. Vanda R, Dastani T, Taghavi SA, Sadeghi H, Lambert N, Bazarganipour F. Pregnancy outcomes in pregnant women taking oral probiotic undergoing cerclage compared to placebo: two blinded randomized controlled trial. *BMC Pregnancy Childbirth*. (2024) 24:311. doi: 10.1186/s12884-024-06496-x

152. Al-Saafin BA, Al-Bakri AG, Abdelrazig S, Dahabiyeh LA. Investigating the effect of the probiotic Lactobacillus plantarum and the prebiotic fructooligosaccharides on Pseudomonas aeruginosa metabolome, virulence factors and biofilm formation as potential quorum sensing inhibitors. *Microb Pathog*. (2023) 177:106057. doi: 10.1016/j.micpath.2023.106057

153. Farr A, Sustr V, Kiss H, Rosicky I, Graf A, Makristathis A, et al. Oral probiotics to reduce vaginal group B streptococcal colonization in late pregnancy. *Sci Rep*. (2020) 10:19745. doi: 10.1038/s41598-020-76896-4

154. Moumne O, Hampe ME, Montoya-Williams D, Carson TL, Neu J, Francois M, et al. Implications of the vaginal microbiome and potential restorative strategies on maternal health: a narrative review. *J Perinat Med*. (2021) 49:402–11. doi: 10.1515/jpm-2020-0367

155. Lev-Sagie A, Goldman-Wohl D, Cohen Y, Dori-Bachash M, Leshem A, Mor U, et al. Vaginal microbiome transplantation in women with intractable bacterial vaginosis. *Nat Med*. (2019) 25:1500–4. doi: 10.1038/s41591-019-0600-6

156. Al-Madboly LA, Aboulmagd A, El-Salam MA, Kushkevych I, El-Morsi RM. Microbial enzymes as powerful natural anti-biofilm candidates. *Microb Cell Fact*. (2024) 23:343. doi: 10.1186/s12934-024-02610-y

157. Liu S, Lu H, Zhang S, Shi Y, Chen Q. Phages against pathogenic bacterial biofilms and biofilm-based infections: A review. *Pharmaceutics*. (2022) 14:427. doi: 10.3390/pharmaceutics14020427

158. Zimmermann ES, Ferreira LM, Denardi LB, Sari MHM, Cervi VF, Nogueira CW, et al. Mucoadhesive gellan gum hydrogel containing diphenyl diselenide-loaded nanocapsules presents improved anti-candida action in a mouse model of vulvovaginal candidiasis. *Eur J Pharm Sci*. (2021) 167:106011. doi: 10.1016/j.ejps.2021.106011

159. Pandey M, Choudhury H, Abdul-Aziz A, Bhattamisra SK, Gorain B, Carine T, et al. Promising drug delivery approaches to treat microbial infections in the vagina: A recent update. *Polymers (Basel)*. (2020) 13:26. doi: 10.3390/polym13010026

160. Tas GG, Sati L. Probiotic Lactobacillus rhamnosus species: considerations for female reproduction and offspring health. *J Assist Reprod Genet*. (2024) 41:2585–605. doi: 10.1007/s10815-024-03230-6

161. Kim SY, Seol D, Jung M, Kwak W, Kim H, Cho S, et al. Assessing the Efficacy of Ligilactobacillus salivarius CLS0420 and Lacticaseibacillus paracasei CLPC0603 on Vaginal Well-Being in Healthy Women: A Pilot, Randomized, Double-Blind, Placebo-Controlled Trial. *Probiotics Antimicrob Proteins*. (2025). doi: 10.1007/s12602-025-10512-7

162. Koirala R, Gargari G, Arioli S, Taverniti V, Fiore W, Grossi E, et al. Effect of oral consumption of capsules containing Lactobacillus paracasei LPC-S01 on the vaginal microbiota of healthy adult women: a randomized, placebo-controlled, double-blind crossover study. *FEMS Microbiol Ecol*. (2020) 96:fiaa084. doi: 10.1093/femsec/fiaa084

163. Ng KYB, Mingels R, Morgan H, Macklon N, Cheong Y. *In vivo* oxygen, temperature and pH dynamics in the female reproductive tract and their importance in human conception: a systematic review. *Hum Reprod Update*. (2018) 24:15–34. doi: 10.1093/humupd/dmx028

frontiers | Frontiers in Immunology

TYPE Review
PUBLISHED 25 September 2025
DOI 10.3389/fimmu.2025.1636876

Check for updates

OPEN ACCESS

EDITED BY
Nar Singh Chauhan,
Maharshi Dayanand University, India

REVIEWED BY
Angela Klain,
University of Campania Luigi Vanvitelli, Italy
Amit Grover,
AstraZeneca, United Kingdom

*CORRESPONDENCE
Zheng Liu
liuzheng1707@163.com

RECEIVED 28 May 2025
ACCEPTED 08 September 2025
PUBLISHED 25 September 2025

CITATION
Wang Z, Yu J, Liu Y, Gong J, Hu Z and Liu Z (2025) Role of the microbiota–gut–lung axis in the pathogenesis of pulmonary disease in children and novel therapeutic strategies. *Front. Immunol.* 16:1636876. doi: 10.3389/fimmu.2025.1636876

COPYRIGHT
© 2025 Wang, Yu, Liu, Gong, Hu and Liu. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.

Role of the microbiota–gut–lung axis in the pathogenesis of pulmonary disease in children and novel therapeutic strategies

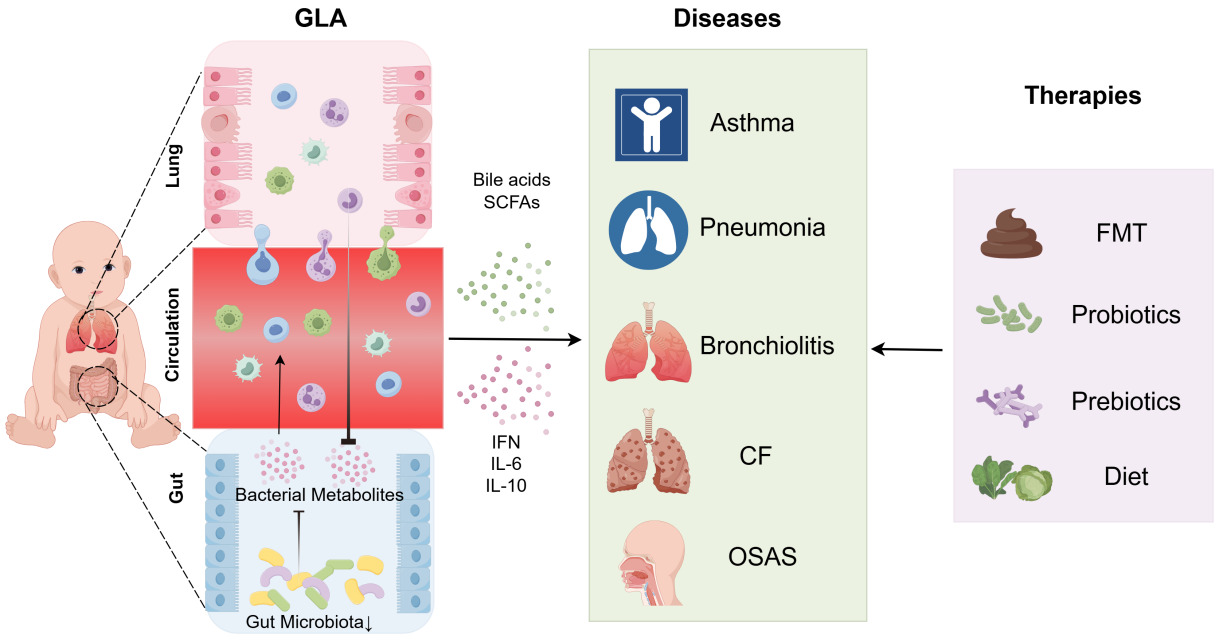
Zhifang Wang[1], Jiayao Yu[2], Yaqi Liu[3], Jiaqi Gong[2], Zepeng Hu[4] and Zheng Liu[5]*

[1]Department of Pediatrics, Shaoxing Keqiao Women and Children's Hospital, Shaoxing, Zhejiang, China, [2]Department of Medical Imaging, School of Medicine, Shaoxing University, Shaoxing, Zhejiang, China, [3]Department of Nursing, School of Medicine, Shaoxing University, Shaoxing, Zhejiang, China, [4]Department of Clinical Medicine, School of Medicine, Shaoxing University, Shaoxing, Zhejiang, China, [5]Department of Pharmacology, School of Medicine, Shaoxing University, Shaoxing, Zhejiang, China

Emerging evidence highlights the microbiota–gut–lung axis (MGLA) as a pivotal regulator of pediatric respiratory health, yet mechanistic insights are lacking and therapeutic applications remain unclear. This review synthesizes cutting-edge findings to delineate how gut microbiota-derived metabolites, particularly short-chain fatty acids (SCFAs), orchestrate pulmonary immunity and disease pathogenesis in children. Leveraging multi-omics integration (metagenomics, metabolomics, transcriptomics), emerging studies have uncovered novel microbe–host interactions driving immune dysregulation in asthma, pneumonia, and cystic fibrosis. A comprehensive map of gut–lung crosstalk has been established across these conditions. Current studies suggest that early-life gut dysbiosis, shaped by delivery mode, antibiotics, and diet, disrupts SCFA-mediated immune homeostasis, amplifying T-helper 2 cell inflammation and impairing alveolar macrophage function. Crucially, we identified disease-specific microbial signatures (e.g., depletion of *Lachnospira* and *Faecalibacterium* in asthma) and demonstrated that fecal microbiota transplantation and probiotic interventions restore microbial balance, attenuating airway inflammation in preclinical models. This work pioneers the translation of MGLA insights into precision medicine strategies, highlighting dietary modulation and microbial therapeutics as viable alternatives to conventional treatments. By bridging microbial ecology and immune dynamics, our findings provide actionable biomarkers for early diagnosis and personalized interventions, addressing critical gaps in pediatric respiratory disease management. The integration of multi-omics frameworks not only advances mechanistic understanding but also positions the MGLA as a transformative target in reducing global childhood morbidity. Future research must prioritize longitudinal studies and clinical trials to validate these innovations, ultimately redefining therapeutic paradigms for GLA-driven pathologies.

KEYWORDS

gut–lung axis, pediatric respiratory diseases, microbial metabolites, multi-omics, precision medicine



GRAPHICAL ABSTRACT
Regulatory Mechanisms and Therapeutic Strategies of the GLA in Pediatric Pulmonary Diseases. The gut microbiota interacts bidirectionally with the pulmonary microenvironment through microbial metabolites (e.g., SCFAs) and immune signaling molecules (e.g., IFN, IL-6, IL-10), forming a central mechanism underlying this axis. Gut dysbiosis disrupts SCFA-mediated immune homeostasis, contributing to the pathogenesis of childhood respiratory diseases such as asthma, pneumonia, and cystic fibrosis. Integrated multi-omics studies have identified disease-specific microbial signatures and propose novel strategies—including FMT, probiotics, prebiotics, and dietary interventions—to restore gut microbiota equilibrium, suppress airway inflammation, and advance precision-based therapeutic approaches for pediatric respiratory disorders. CF, cystic fibrosis; FMT, fecal microbiota transplantation; GLA, gut–lung axis; IFN, interferon; IL, interleukin; OSAS, obstructive sleep apnea syndrome; SCFAs, short-chain fatty acids.

1 Introduction

Acute respiratory infections and influenza-like illnesses (ILIs) account for approximately 10% of annual outpatient visits globally, with pediatric populations bearing a disproportionate burden. Children aged 1–17 years exhibit a fourfold higher incidence of ILIs than adults (1). This vulnerability stems from structurally immature airways and dynamically evolving immune regulation, creating a suitable environment for recurrent infections. Beyond acute morbidity, such episodes disrupt developmental homeostasis and amplify lifelong cardiopulmonary risks (2), necessitating urgent research into early-life susceptibility mechanisms.

The human microbiome associated with the respiratory tract is characterized by its diversity, heterogeneity, and dynamism. The complexity of the microbiome, along with the intricate interactions among microorganisms, host cells, and the host immune system, involves multiple factors. There is often an interaction between gut and respiratory microbiota, with the lymphatic system providing a direct pathway, known as the gut–lung axis (GLA), via which the gut microbiome can influence outcomes related to respiratory diseases and modulate the host's immune response (1). The GLA is a bidirectional communication network mediated by microbiota and metabolites that links intestinal and pulmonary health (3). Emerging evidence indicates that perturbations in one organ (e.g., gut dysbiosis (4)) can propagate systemic immune dysregulation, exacerbating pathologies in the other (e.g., asthma exacerbations (5)). For example, gut-derived microbial components such as lipopolysaccharide (LPS) modulate pulmonary inflammation via lymphatic dissemination (6) whereas respiratory viral infections reciprocally reshape the gut microbial ecology (7). Despite these advances, the spatiotemporal dynamics of microbiome–immune crosstalk in pediatric populations remain poorly mapped.

The gut microbiome critically regulates pediatric health, with its assembly during infancy dictating immune maturation trajectories (8). The composition of the intestinal microbiome influences health from before birth into childhood, with numerous diseases linked to imbalances in this ecosystem. The gut microbiome evolves continuously from infancy to maturity, influenced by various factors that shape its development and composition. Characteristics of the gut microbiota can impact brain development, immune function, lung health, and overall physical growth (9). Gut microbial dysbiosis can have long-term consequences, as supported by data from mouse models in which mice have an increased predisposition to allergic inflammation following early-life antibiotic use (10, 11). Early-life disruptions,

Abbreviations: CF, cystic fibrosis; DC, dendritic cell; FMT, fecal microbiota transplantation; GLA, gut–lung axis; HDAC, histone deacetylase; IFN, interferon; IL, interleukin; ILI, influenza-like illness; LPS, lipopolysaccharide; MGLA, microbiota–gut–lung axis; OSAS, obstructive sleep apnea syndrome; PRRs, pattern recognition receptors; RSV, respiratory syncytial virus; SCFAs, short-chain fatty acids; Th, T helper; TLR, Toll-like receptor; TNF, tumor necrosis factor; Treg, regulatory T cell.

such as antibiotic exposure or dietary insufficiency, induce persistent dysbiosis, elevating the risks of allergic sensitization and recurrent respiratory infections (12).

Microbial metabolites such as short-chain fatty acids (SCFAs) emerge as key orchestrators of mucosal immunity, yet their role in gut–lung interactions remains underexplored in children. Recent studies have integrated multi-omics approaches (metagenomics, metabolomics, transcriptomics) to decode microbiota–immune networks in pediatric respiratory diseases. These efforts have identified disease-specific microbial signatures (e.g., *Lachnospira* depletion in asthma) and have demonstrated that SCFAs enhance alveolar macrophage function not only via epigenetic modulation but also by inducing tolerance-related genetic signatures (13). Such findings highlight the potential of microbiota-targeted therapies (e.g., probiotics, dietary interventions) to recalibrate immune homeostasis.

2 Gut microbiota and children's respiratory health

2.1 Characteristics of the pediatric gut and respiratory microbiota

The pediatric gut microbiome represents a dynamic ecosystem of bacteria, fungi, viruses, and archaea. Indeed, the human gut is inhabited by between 100 thousand and 100 billion bacteria per milliliter of luminal content depending on the region and is therefore the most densely colonized organ (14). Crucially, this microbial consortium not only supports nutrient metabolism but also calibrates systemic immunity via endocrine and metabolic crosstalk (15, 16). This symbiotic relationship is established from birth with the infant gut microbiota and continues to evolve during the critical early years of life. During this period, infants experience rapid growth, with substantial increases in height, weight, and head circumference. Concurrently, their metabolic organs, immune system, digestive system, and neurocognitive functions undergo substantial development and maturation. This phase is also pivotal for formation of the gut microbiota, which is essential for maintaining overall health (17).

In contrast to the gut, the respiratory microbiome exhibits spatiotemporal stratification—nasal cavities favor *Staphylococcus and Corynebacterium*, whereas lower airways are dominated by Prevotella and Streptococcus—with bacterial loads (10^3–10^5 CFU/mg) that are orders of magnitude lower than intestinal levels (18, 19). Our understanding of bacterial component development in the airways is more limited than our knowledge of the gut microbiota. Some evidence (primarily from mouse models) suggests that the respiratory microbiota matures during childhood, a process that is crucial for promoting tolerance to airborne allergens (20). Despite shared dominant phyla (Bacteroidetes/Firmicutes), the lung microbiome displays unique assembly rules; microbial immigration (inhalation/microaspiration), elimination (mucociliary clearance), and local replication dynamically sculpt the community structure (21).

Deciphering the co-evolution of gut and respiratory microbiomes is fundamental to understanding pediatric immune ontogeny. During early life, these microbial communities transcend passive colonization; they actively direct immune cell differentiation via metabolite signaling (e.g., SCFAs), epigenetic modulation, and pathogen exclusion (22). Integrated analysis of human cohort data has revealed that synchronized gut–lung microbiota maturation establishes a systemic immune "set-point," dysregulation of which underlies susceptibility to pneumonia and asthma. This paradigm shifts the focus from cataloging microbial taxa to decoding their functional networks, a cornerstone of our therapeutic discovery platform.

2.2 Gut microbiota affects development of the immune system in children

Upon birth, infants start to develop their initial microbiome, with its composition being shaped by the delivery method, whether through natural, vaginal, or cesarean birth (23). The initial bacterial colonization and various other factors following childbirth strongly influence an infant's early-life microbiome, which subsequently regulates immune system development of the newborn (24). Whereas vaginal birth establishes a maternally derived microbiome enriched in *Lactobacillus* and *Bifidobacterium*, cesarean delivery favors skin-associated *Staphylococcus* and environmental taxa (25). Crucially, this immunomodulatory process exhibits developmental stage-specific sensitivity, as evidenced by murine and human studies demonstrating irreversible immune programming defects when microbial exposure is disrupted during early postnatal windows (26, 27). This foundational microbial assembly orchestrates lymphoid tissue development and immune cell education—processes that are vulnerable to disruption by antibiotics or formula feeding. Such dysbiosis propagates systemic immune misprogramming, elevating the risks for asthma and obesity via GLA signaling (28).

The gut microbiome operates as a microbial tutor during infancy, instructing immune cell differentiation through metabolite- and antigen-driven dialogues. Key taxa (e.g., Clostridia clusters) promote expansion of regulatory T cells (Tregs) via SCFA production, and segmented filamentous bacteria drive T-helper 17 cell (Th17) polarization via interleukin 23 (IL-23)/IL-17 axis activation (29). This trans-organ immunity, mediated by circulating microbial metabolites and trained immune cells, forms the mechanistic bedrock of the GLA (30).

In the intestinal environment, pattern recognition receptors (PRRs), including toll-like receptors (TLRs), C-type lectin receptors, NOD-like receptors, and RIG-I-like receptors, can identify specific molecular patterns in the microbiota, thereby initiating immune responses. The activation of PRRs not only promotes the maturation and activation of immune cells but also modulates their migration and function through the production of cytokines and chemokines (31). Specifically activated PRRs can induce dendritic cells (DCs) to produce IL-12p70, a crucial Th1-polarizing cytokine for anti-infection and anti-tumor immune

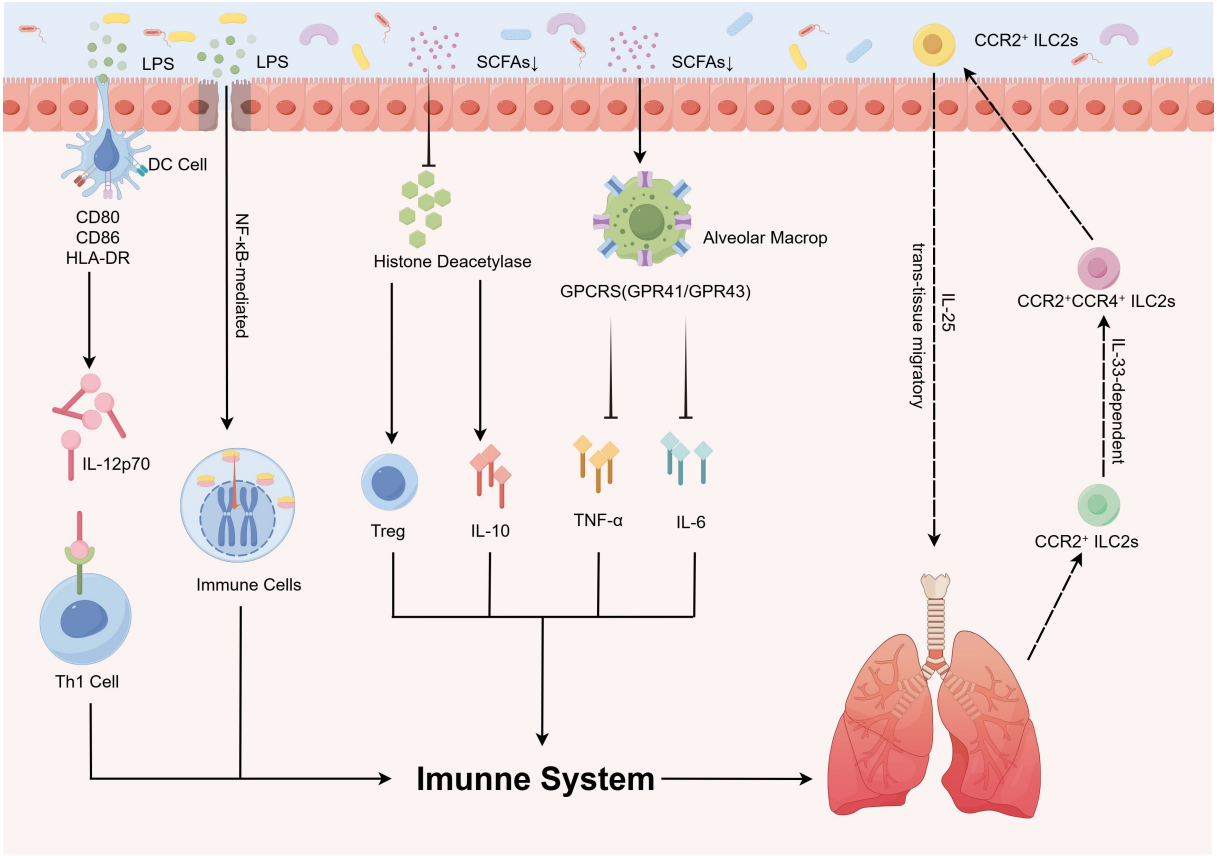


FIGURE 1
Gut microbiota modulates pathophysiological processes in pediatric pulmonary diseases via immune regulatory mechanisms. The gut microbiota orchestrates immune regulatory mechanisms that influence the pathogenesis of childhood pulmonary diseases via microbial metabolites (e.g., SCFAs and LPS) and immune signaling networks, which critically regulate immune system development and disease progression. DCs recognize LPS, leading to upregulated CD80/CD86/HLA-DR expression, enhanced antigen-presenting capacity, and IL-12p70 secretion, thereby driving Th1 polarization to reinforce anti-infective immunity. Gut-derived LPS activates the TLR4/NF-κB pathway, triggering pulmonary inflammation. SCFAs inhibit histone deacetylase activity to promote Treg differentiation and anti-inflammatory IL-10 secretion, while simultaneously activating alveolar macrophages via GPCRs to suppress pro-inflammatory cytokine (TNF-α, IL-6) release. Additionally, trans-tissue migration of $CCR2^+$ ILC2s further modulates pediatric pulmonary disease pathogenesis. CCR2+CCR4+ILC2s, C-C chemokine receptor type 2-positive and C-C chemokine receptor type 4-positive type 2 innate lymphoid cells; CCR2+ILC2s, C-C chemokine receptor type 2-positive type 2 innate lymphoid cells; CD80/CD86, cluster of differentiation 80/86; DC, dendritic cell; GPCRs (GPR41/GPR43), G protein-coupled receptors (G protein-coupled receptor 41/G protein-coupled receptor 43); HLA-DR, human leukocyte antigen – DR isotype; IL, interleukin; LPS, lipopolysaccharide; SCFAs, short-chain fatty acids; Th1, T helper 1 cells; TNF-α, tumor necrosis factor alpha; Treg, regulatory T cells.

responses. Moreover, PRR activation enhances the expression of surface molecules such as CD80, CD86, and human leukocyte antigen-DR (HLA-DR) on DCs, thereby improving their antigen-presenting capacity and T-cell activation ability (32).

A previous study redefined the gut microbiome as a systemic immune rheostat, with microbial metabolites (e.g., SCFAs, indoles) serving as endocrine-like messengers that synchronize lung immunity (33). Via integrated metagenomic–metabolomic analysis, spatial gradients of these molecules have been mapped from gut to bronchoalveolar lavage, correlating their depletion with neutrophilic inflammation in severe asthma (34) (Figure 1). This paradigm-shifting discovery positions fecal metabolite profiling as a noninvasive biomarker for predicting corticosteroid responsiveness—a cornerstone of our precision pulmonology framework. By decoding the gut–lung dialogue, we can pioneer microbiota-centric strategies to reset immune homeostasis in pediatric respiratory diseases.

3 Role of the GLA in children's pulmonary physiological function

3.1 Core immunoregulatory mechanisms of the GLA

The GLA operates as a bidirectional immunoregulatory circuit wherein microbial components and metabolites systemically modulate pulmonary immunity via three primary mechanisms: 1) molecular mimicry: bacterial LPS primes alveolar macrophages via TLR4 (35); 2) metabolite trafficking: gut-derived SCFAs, particularly propionate and butyrate, suppress IL-13-driven eosinophilia by enhancing airway epithelial barrier integrity (36); 3) immune-cell migration: intestinal Th17 cells recruited to the lungs exacerbate neutrophilic inflammation in asthma, a process amplified by dysbiosis-induced IL-23 signaling (37). Disruption of

TABLE 1 Multi-omic insights into gut–lung axis mechanisms in respiratory diseases.

Omics technology	Study participants/disease	Key findings	Mechanism/functional relevance	Ref.
Metagenomics	Children with asthma	Reduced abundance of *Lachnospira*, *Veillonella*, and *Faecalibacterium* in the gut microbiota of 3-month-old infants	Reduced microbial diversity correlates with heightened asthma risk	(52) (53)
	Pneumonia (mouse model)	Gut microbiota dysbiosis (*Bifidobacterium*↓) → impaired phagocytic function of alveolar macrophages	Acetate enhances anti-*Streptococcus pneumoniae* capacity via GPR43 signaling	(64)
	Patients with cystic fibrosis	Depletion of *Bacteroides* and *Faecalibacterium prausnitzii* in the gut	Propionate deficiency impairs neutrophil function; bile acid dysregulation exacerbates airway inflammation	(81) (86) (87)
Metabolomics	Asthma (mouse model)	Decreased fecal SCFA levels (propionate, butyrate)	SCFA depletion promotes Th2 inflammation via HDAC-dependent pathways	(11) (40)
	Patients with COVID-19	Impaired L-isoleucine biosynthesis and reduced acetate levels	Metabolic dysregulation drives immune dysfunction and intestinal barrier disruption	(98)
	OSAS (children)	Reduced butyrate levels → compromised intestinal barrier integrity	SCFA deficiency exacerbates systemic inflammation and hypertension risk	(95)
Transcriptomics	RSV bronchiolitis (mouse model)	Acetate activates GPR43 → NF-κB pathway → enhanced IFN-β production	Augmented antiviral immunity attenuates RSV severity	(77)
	Patients with asthma (alveolar macrophages)	SCFAs (butyrate) inhibit HDAC activity	SCFAs promote Treg differentiation via epigenetic modulation	(42) (44)
	Pneumonia (mouse macrophage model)	Gut microbiota depletion → metabolic reprogramming in alveolar macrophages	Microbial metabolites (e.g., acetate) regulate macrophage function through GPR43 signaling	(65)

GPR43, G protein-coupled receptor 43; HDAC, histone deacetylase; IFN-β, interferon-beta; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; OSAS, obstructive sleep apnea syndrome; RSV, respiratory syncytial virus; SCFA, short-chain fatty acids; Th2, T helper type 2; Treg, regulatory T cells.

gut barrier integrity promotes microbial translocation (e.g., circulating LPS), which triggers nuclear factor-kappa B (NF-κB)-mediated pulmonary hyperinflammation (38). A murine study demonstrated that elevated circulating propionate levels promote hematopoiesis of DC precursors in bone marrow, subsequently affecting DCs in the lungs and draining lymph nodes, thereby resulting in an impaired ability of these DCs to activate Th2 cells within the lung tissue (39). These pathways collectively enhance intestinal mucosal immunity while systemically modulating immune cell differentiation and function. Notably, animal studies substantiate that SCFAs can attenuate pulmonary inflammation by activating Tregs and conditioning DCs to suppress Th2-polarized responses in murine models, thereby recalibrating Th1/Th2 equilibrium (40) (Figure 1, Table 1).

3.2 Systemic immunomodulatory roles of SCFAs

SCFAs—primarily acetate, propionate, and butyrate—serve as keystone metabolites linking the gut microbiota to pulmonary immune homeostasis (11, 41). Emerging evidence suggests that elevated levels of SCFAs in infants are associated with a reduced incidence of asthma, potentially mediated through dual anti-inflammatory mechanisms (42). First, SCFAs activate G protein-coupled receptors (GPR41/GPR43) on alveolar macrophages via receptor-dependent signaling, enhancing pathogen clearance while suppressing pro-inflammatory cytokine release (e.g., tumor necrosis factor alpha [TNF-α], IL-6) (22, 43). Second, SCFAs modulate immune cell function through epigenetic regulation: by inhibiting histone deacetylase (HDAC) activity; these promote anti-inflammatory factor expression (including IL-10) and Treg differentiation while simultaneously restraining Th17 cell overactivation. These coordinated actions maintain pulmonary immune homeostasis and mitigate inflammation (42, 44) (Table 1). Although SCFA concentrations are low in the lungs, gut-derived propionate can induce bone marrow precursors to generate macrophages. These macrophages then migrate to the lungs and attenuate allergic inflammation by promoting IL-10 production (11). These findings establish SCFAs as systemic immunomodulators that exert hormone-like signaling functions (45). Collectively, the above findings underscore SCFAs as pivotal gut-derived mediators that orchestrate pulmonary immune homeostasis via receptor-dependent signaling and epigenetic

mechanisms. The ability of SCFAs to prime anti-inflammatory responses both locally and systemically highlights their therapeutic potential in asthma and other inflammatory lung diseases (Figure 1).

3.3 Trans-organ migration mechanisms of immune cells

Emerging evidence highlights the pivotal role of immune cell trafficking in gut–lung communication wherein innate lymphoid cells (ILCs) and gut-primed Tregs migrate to pulmonary tissues via mesenteric lymphatics under chemokine guidance (46). Extending these observations, one study provided mechanistic insights into the cytokine-regulated bidirectional trafficking of ILC2s using integrated pseudotemporal analysis, lineage tracing, and ectopic transplantation assays. That study delineated a unidirectional maturation trajectory wherein lung-derived C-C chemokine receptor type 2-positive ($CCR2^+$) ILC2s migrate to the intestine via IL-33-dependent pathways, undergoing phenotypic transition characterized by a transient $CCR2^+CCR4^+$ double-positive population and terminal differentiation into $CCR4^+$ gut-resident ILC2s. This migratory axis demonstrates strict tissue tropism under homeostasis, with no detectable reverse migration from intestinal ILC2s to pulmonary niches. Strikingly, IL-25 stimulation subverts this physiological unidirectionality, potentiating intestinal ILC2s to acquire trans-tissue migratory competence toward pulmonary compartments (47). This cellular crosstalk establishes a "mobile immune network" that integrates microbial signals and tissue-specific immunity (Figure 1).

3.4 Translational implications and future directions

The GLA, which is crucial for immune balance and respiratory health, strongly influences pediatric pulmonary diseases. Understanding its role in diseases such as asthma and bronchopulmonary dysplasia could offer new therapeutic strategies. Future research priorities include standardizing methodologies for gut microbiota and metabolite profiling in pediatric cohorts, elucidating age-dependent variations in GLA functionality, and developing targeted interventions (e.g., SCFA prodrugs, microbiota transplantation) to restore immune homeostasis.

4 Role of the GLA in pediatric pulmonary diseases

4.1 Asthma

Asthma remains a leading chronic pediatric condition, accounting for approximately 400,000 annual deaths globally (48, 49). The current global prevalence of asthma exceeds 300 million individuals, with a projected increase to 400 million by 2025 (50). Emerging evidence implicates GLA dysbiosis in asthma pathogenesis, with microbial alterations influenced by perinatal factors including cesarean delivery, neonatal antibiotic exposure, maternal nutritional patterns, formula supplementation, and microbial environmental exposures (51, 52). Longitudinal cohort studies demonstrate that diminished gut microbial diversity during early infancy correlates with subsequent asthma development. Infants who develop asthma exhibit significantly reduced α-diversity metrics as early as 1 month postpartum (52), with temporal microbiota dynamics showing critical developmental windows for asthma predisposition. Specifically, transient depletion of immunomodulatory taxa (*Lachnospira*, *Veillonella*, Faecalibacterium, and *Rothia*) at 3 months postnatally distinguishes high-risk infants (53) (Table 1). Phylum-level analysis reveals Firmicutes dysregulation in pediatric patients with asthma, characterized by decreased anti-inflammatory species (e.g., *Roseburia*) and expansion of pro-inflammatory genera (*Enterococcus*, *Clostridium*) (54) (Figure 2).

This ecological imbalance coincides with impaired mucosal immune programming during critical developmental windows, as evidenced by multi-omics integration of microbial community dynamics, host metabolomic signatures, and nutritional patterns (49, 55, 56). Notably, gut dysbiosis, particularly the depletion of *Akkermansia*, *Bifidobacterium*, and *Faecalibacterium* species during critical developmental windows, is strongly associated with heightened susceptibility to asthma and allergic disorders (26, 27) (Figure 2). Mechanistically, microbial metabolic output—particularly SCFA biosynthesis—mediates dietary microbiota–immune crosstalk, with reduced SCFA production correlating with asthma risk in low-diversity microbiomes (1, 11).

Breastfeeding exerts important modulatory effects on asthma-related microbial metabolites (49). Contrary to historical sterility assumptions, human milk contains dynamic microbial communities and prebiotic compounds that shape the infant gut ecology (57, 58). Multivariate analysis has revealed that exclusive breastfeeding ≥4 months is an independent protective factor against asthma development (49), highlighting nutritional–microbial interactions in early-life asthma prevention (Figure 3).

4.2 Pneumonia

Pneumonia is an infection that inflames the air sacs and other parts of the lungs and is often caused by bacteria, viruses, or other pathogens (59, 60). Pneumonia remains a leading cause of childhood morbidity and mortality, accounting for 14% of deaths in children under 5 years globally, with an estimated 120 million cases annually (61). Research has shown that viral infections, such as influenza, can substantially alter the gut microbiome, even without detectable viral particles in the gastrointestinal tract (62). Compelling evidence links the gut microbiota composition to pneumonia susceptibility and severity (63). Mice with a depleted gut microbiome exhibit increased bacterial spread, heightened inflammation, organ dysfunction, and elevated mortality following *Streptococcus* pneumoniae infection, compared to those

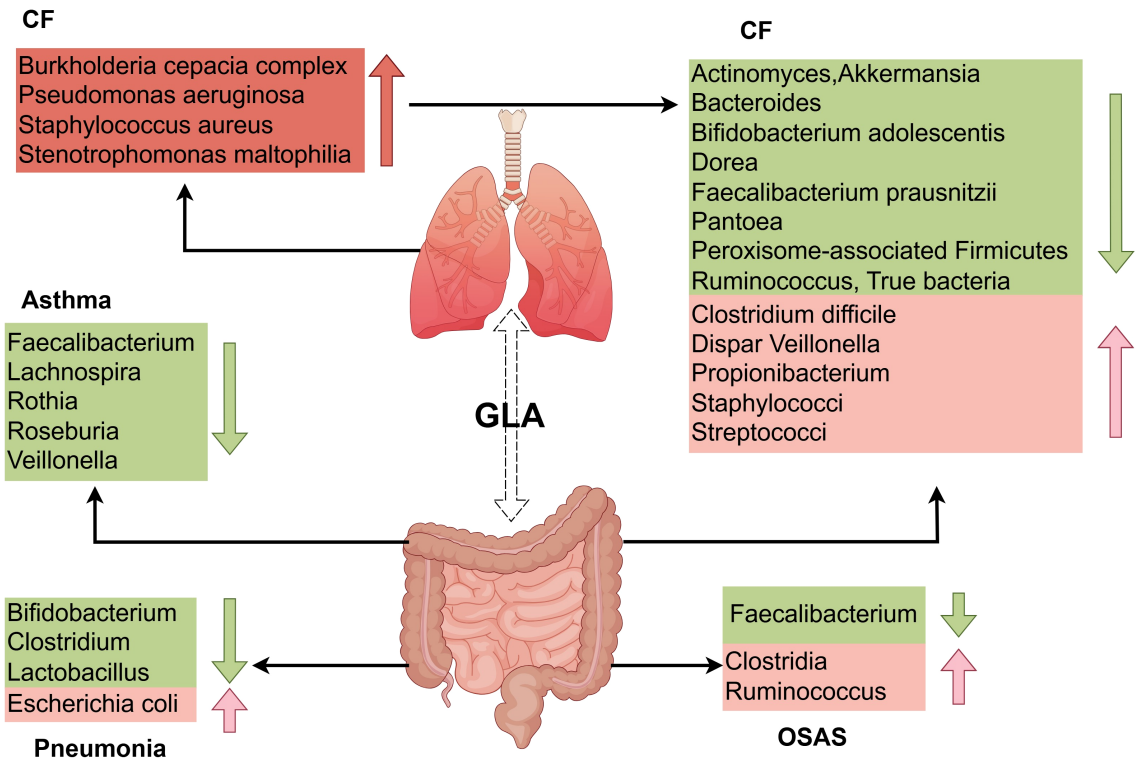


FIGURE 2
Distinct features of gut and respiratory microbiota dysbiosis in pediatric pulmonary diseases. The GLA exhibits dynamic microbiota alterations in childhood asthma, pneumonia, CF, and OSAS. Children with asthma have reduced gut abundance of immunomodulatory taxa (e.g., *Lachnospira, Faecalibacterium, Rothia*) alongside expansion of pro-inflammatory genera (e.g., Enterococcus). Patients with pneumonia display diminished intestinal Bifidobacterium and Lactobacillus abundance with concurrent enrichment of *Escherichia coli*. In CF, the gut microbiota is characterized by substantial enrichment of opportunistic pathogens (e.g., Burkholderia cepacia complex, Pseudomonas aeruginosa) and depletion of beneficial species (e.g., Bacteroides, Faecalibacterium prausnitzii). OSAS is associated with reduced levels of SCFA-producing bacteria (e.g., Clostridia, Ruminococcus), which exacerbates systemic inflammation via the metabolic–immune axis. GLA, gut–lung axis; CF, cystic fibrosis; OSAS, obstructive sleep apnea syndrome; SCFAs, short-chain fatty acids.

with a normal microbiome. Fetal microbiota transplantation (FMT) can restore pulmonary bacterial levels and normalize immune responses in these mice. The gut microbiome modulates metabolic pathways in alveolar macrophages, affecting cellular responsiveness, with macrophages from mice that have a depleted microbiome showing reduced phagocytic activity against *S. pneumoniae* (64) (Table 1). Mechanistic studies reveal two key pathways: 1) macrophage priming: gut-derived acetate enhances alveolar macrophage phagocytic capacity against *S.* pneumoniae via GPR43-dependent metabolic reprogramming (65) (Table 1); antiviral defense: microbiota-derived desaminotyrosine protects the host from influenza by modulating the type I interferon (IFN-I) response (66) (Figure 3).

Pneumonia poses a serious health risk to children worldwide, with high case numbers and mortality rates, emphasizing the need for prevention and better treatment (67). Preterm infants with pneumonia exhibit reduced gut microbiota diversity and disturbances of the gut (68). Children's underdeveloped immune systems make them vulnerable to severe illness and death due to pneumonia. The gut microbiota, particularly early in life, influences lung immunity and the pneumonia risk, highlighting the importance for health of a balanced gut microbiome (23). Pneumonia can affect the composition of the gut microbiota, primarily leading to a decrease in *Bifidobacterium* (10), *Lactobacillus* (69), and *Clostridium* (70), and causing an increase in *Escherichia coli* (59). Infant gut microbiota diversity and functionality increase during the first years of life, influenced by the delivery mode and feeding type (71). In pneumonia, *Pseudomonas*, *Escherichia/Shigella*, *Streptococcus*, and *Akkermansia* are decreased *Bacillota* are increased, at the phylum level, with specific genera being more or less abundant in cases of pneumonia. Preterm infants with pneumonia exhibit reduced gut microbiota diversity and gut disturbances (68) (Figure 2).

The prevalence of pediatric COVID-19 cases ranges from 1% to 13.3% of total reported infections (72). A recent study revealed significantly reduced α-diversity in young patients with COVID-19 (aged <6 months), aligning with prior studies attributing such disparities to the unique infant microbiota, which is shaped by feeding patterns (73). Beyond gut microbiota alterations, intestinal inflammation, microbial translocation, and intestinal barrier dysfunction have also been identified as factors associated with COVID-19 infection and that are potentially attributable to microbiota-driven perturbations (74). The critical role of microbiota has prompted experts to question whether the host

10.3389/fimmu.2025.1636876

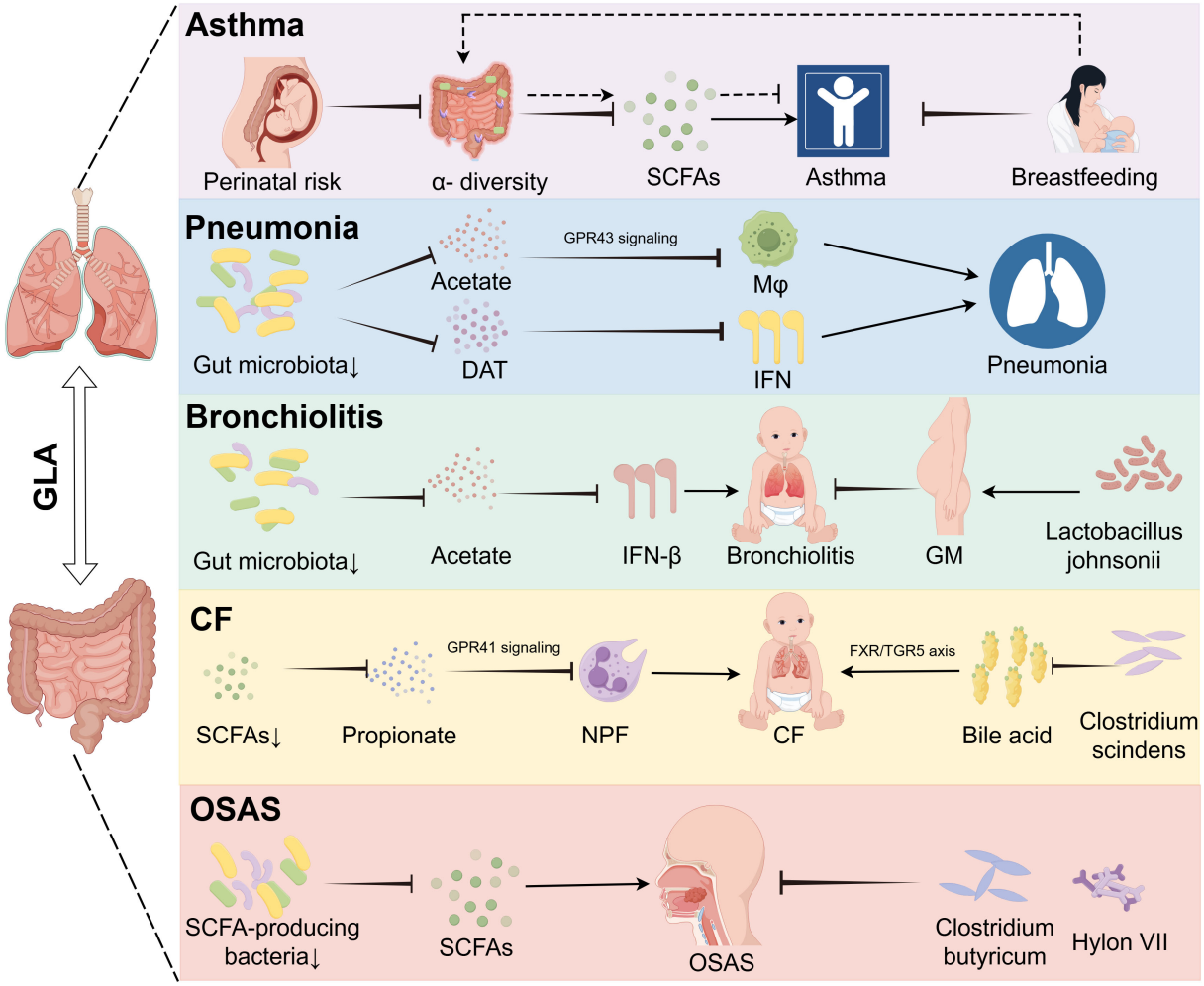


FIGURE 3
Gut dysbiosis drives disease-specific mechanisms in pediatric pulmonary disorders via metabolic and signaling pathways. The GLA orchestrates disease-specific mechanisms underlying childhood asthma, pneumonia, bronchitis, CF, and OSAS. In children with asthma, perinatal microbiota alterations with reduced alpha diversity are modulated by maternal breastfeeding-associated metabolites. In pneumonia, gut dysbiosis reduces acetate levels, impairing the GPR43-mediated phagocytic function of alveolar macrophages, and deaminotyrosine deficiency compromises IFN-I-dependent anti-influenza immunity. Bronchitis is linked to diminished gut-derived acetate, leading to pulmonary IFN-β reduction whereas prenatal supplementation with Lactobacillus jensenii lowers disease incidence. In CF, Bacteroides-derived propionate deficiency disrupts GPR41 signaling, causing neutrophil phagocytic dysfunction, and Clostridioides difficile depletion exacerbates inflammation via dysregulated bile acid metabolism (FXR/TGR5 axis). Reduced SCFA-producing bacteria contribute to OSAS pathogenesis, which is alleviated by C. butyricum and prebiotics. GLA, gut–lung axis; DAT, desaminotyrosine; GM, gestational mother; GPR43, G protein-coupled receptor 43; IFN-I, type I interferon; Mφ, macrophage; NPF, neutrophil phagocytic function; OSAS, obstructive sleep apnea syndrome; SCFAs, short-chain fatty acids.

microbiota status should be considered prior to vaccine development. Furthermore, the development of oral vaccines and maintenance of microbial homeostasis may facilitate early containment of COVID-19 outbreaks or future pandemics (74).

4.3 Bronchiolitis

Respiratory syncytial virus (RSV)-associated bronchiolitis affects 33 million children under age 5 years annually, with 3.2 million requiring hospitalization (75). Emerging data implicate gut microbiota dynamics in disease progression. Cohort studies reveal that infants hospitalized with severe RSV bronchiolitis exhibit gut dysbiosis characterized by *Enterobacteriaceae* overgrowth and *Bifidobacterium* depletion (76). RSV infections are particularly dangerous for infants under age 2 years, leading to high rates of morbidity and mortality. Early infancy is a key period in gut microbiota development that may play a role in bronchiolitis through various mechanisms beyond the Th1/Th2 imbalance theory. Research indicates that acetic acid produced by gut microbes can stimulate lung IFN-β production, enhancing type 1 IFN responses by activating GPR43 and NF-κB (77) (Table 1). This activation can modulate the protective effects of acetic acid against RSV, potentially reducing pneumonia symptoms (Figure 3).

Current research is investigating intervention strategies for respiratory illnesses, with clinical trials demonstrating that *Bifidobacterium* species stimulated by human milk oligosaccharides contribute to the prevention of subsequent

respiratory infections (e.g., bronchitis). This protective mechanism is mediated via *Bifidobacterium*-driven enhancement of acetate production, with elevated acetate levels being mechanistically linked to improved intestinal barrier function (78). Emerging clinical evidence has revealed that maternal supplementation with *Lactobacillus johnsonii* confers transgenerational protection against RSV infection, with offspring exhibiting attenuated airway mucus production and Th2-mediated immune responses. In a murine study, this intervention maintained congruent gut microbiota profiles in both dams and progeny, accompanied by a synchronized reduction in pro-inflammatory metabolites in the maternal plasma, breast milk, and offspring circulation (79). Mechanistically, *Lactobacillus*-driven modulation of maternal microbial ecosystems and associated metabolic reprogramming are positively correlated with enhanced neonatal airway defense mechanisms against RSV pathogenesis (79).

4.4 Cystic fibrosis

CF is an inherited disorder transmitted through an autosomal recessive pattern, resulting from mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. This defect induces the accumulation of abnormally viscous mucus secretions, which primarily compromise pulmonary and gastrointestinal functions (80). CF affects approximately 70,000 people worldwide (81). Lung infections caused by bacteria such as *Pseudomonas aeruginosa*, *S. aureus*, *Burkholderia cepacia* complex, and *Stenotrophomonas maltophilia* worsen respiratory function and contribute to disease morbidity (19). Research supports the existence of a "GLA" in chronic respiratory diseases such as CF, affecting mucosal immunity across these systems (82, 83). Cross-sectional studies have revealed that pediatric patients with CF exhibit marked gut dysbiosis, characterized by a marked depletion of beneficial taxa including *Bacteroides, Bifidobacterium adolescentis, Faecalibacterium prausnitzii, Actinomyces, Eubacterium, Ruminococcus, Dorea, Akkermansia, Peroxisome-associated Firmicutes and Pantoea* (84, 85), alongside concurrent enrichment of opportunistic pathogens such as *Staphylococci, Streptococci, Veillonella dispar, Propionibacterium*, and *Clostridium difficile* (84) (Figure 2).

Mechanistic studies identify two critical pathways linking gut dysbiosis to pulmonary decline. Reduced levels of Bacteroides-derived propionate in SCFA deficiency impair neutrophil phagocytic function via compromised GPR41 signaling (81, 86). Concurrently, diminished *Clostridium scindens* activity disrupts bile acid metabolism by reducing deoxycholic acid production, which exacerbates airway inflammation through dysregulation of the FXR/TGR5 signaling axis (87) (Figure 3, Table 1).

A prospective cross-sectional study elucidated that children with CF exhibit a dietary pattern characterized by high fat and low fiber, which is associated with intestinal dysbiosis, elevated fecal calprotectin, and respiratory microbial disturbance, collectively leading to impaired lung function and increased lung exacerbation (82). Another study highlighted the therapeutic potential of microbiota-targeted interventions (e.g., probiotics, CFTR modulators) in mitigating systemic and respiratory morbidity in children with CF by restoring the gut microbial balance, enhancing immunomodulation, reversing SCFA deficits, and disrupting pathogenic gut–lung crosstalk, thereby attenuating chronic inflammation and improving pulmonary outcomes (88). These findings highlight the gut microbiome as a modifiable determinant of CF progression, paving the way for microbiota-centric adjuvant therapies.

4.5 Obstructive sleep apnea syndrome

OSAS affects 2%–4% of children, with increasing prevalence linked to pediatric obesity and adenotonsillar hypertrophy (89). OSAS is a prevalent chronic respiratory disorder marked by repeated pharyngeal collapse and ventilation disruption during sleep, leading to apnea and sleep disturbances (90). Emerging evidence implicates gut microbiota dysbiosis as both a contributor and consequence of OSAS pathophysiology (91, 92). Cross-sectional studies demonstrate that children with moderate-to-severe OSAS exhibit gut microbiome alterations characterized by an increase in *Clostridia* and *Ruminococcus* species, coupled with depletion of SCFA-producing bacteria such as *Faecalibacterium* and other taxa associated with SCFA synthesis (93, 94) (Figure 2).

In one study, researchers hypothesized that diminished intestinal SCFAs underlie hypertension pathogenesis in OSA. OSA significantly elevated systolic blood pressure after 7 and 14 days, an effect abolished by administration of the probiotic *Clostridium butyricum* or the prebiotic Hylon VII. 16S rRNA sequencing revealed significant enrichment of SCFA-producing bacterial taxa following C. butyricum or Hylon VII treatment. OSA exposure reduced cecal acetate concentrations by 48%, which was prevented by both interventions. Furthermore, C. butyricum and Hylon VII attenuated OSA-induced gut dysbiosis, goblet cell depletion, mucus layer thinning, and cerebral microglial activation (95) (Figure 3, Table 1). A systematic review and meta-analysis indicated that OSA is associated with intestinal barrier dysfunction. Furthermore, the severity of OSA appears to be associated with elevated levels of biomarkers of intestinal barrier dysfunction (96). These preclinical findings suggest that microbiota modulation may serve as a potential adjuvant strategy for OSAS management, although clinical validation in pediatric populations is warranted.

5 Clinical importance of GLA research

5.1 GLA as a biomarker for pediatric pulmonary disease diagnosis

The gut microbiota and its metabolites are increasingly recognized as potential biomarkers for pediatric pulmonary diseases. Omics approaches have been used to identify microbial signatures associated with disease states, including an elevated

Firmicutes/Bacteroidetes ratio indicative of dysbiosis in respiratory conditions (15, 97). In COVID-19, the gut microbiota composition correlates with disease severity and immune dysregulation, characterized by depleted SCFA-producing taxa and impaired L-isoleucine biosynthesis (98, 99) (Table 1). Experimental models have further demonstrated metabolic disturbances, such as increased serum lactate and reduced acetate levels in pulmonary hypertension (97). Although these findings underscore the diagnostic potential of microbiota profiling, current evidence remains fragmented, necessitating large-scale longitudinal studies to validate microbial and metabolic biomarkers across diverse pediatric populations (15).

5.2 Potential application of the GLA in treating pediatric pulmonary diseases

Therapeutic strategies targeting the GLA show promise in modulating immune responses and improving clinical outcomes. Probiotics, such as *Lactobacillus plantarum*, enhance IFN-I production and reduce viral loads in influenza models whereas *Clostridium orbiscindens*-derived desaminotyrosine strengthens antiviral defenses (62, 69). SCFAs exert systemic anti-inflammatory effects by promoting Treg differentiation (via HDAC inhibition) and suppressing Th2/Th17 polarization (through GPR41/43 signaling), with fecal propionate levels inversely correlated with asthma risk in infants (39, 40). Omega-3 polyunsaturated fatty acids (PUFAs) such as DHA (docosahexaenoic acid) and EPA (eicosapentaenoic acid) can regulate immune cell functions, reduce inflammatory responses, and enhance antiviral immune reactions, thereby playing a protective role in pediatric pulmonary diseases. Research has found that prenatal supplementation with omega-3 PUFAs can influence the airway microbiota of infants, reducing the risk of respiratory infections (100). Despite these advances, standardized protocols for strain selection, dosing, and long-term safety monitoring remain critical challenges in clinical translation.

6 Challenges and future directions in GLA research

6.1 Current challenges in GLA research for pediatric respiratory diseases

Despite growing interest in the GLA and its role in pediatric respiratory disorders, critical methodological and translational gaps hinder progress. A predominant reliance on cross-sectional studies limits causal inference because observed associations—such as the reduced gut microbiota diversity in asthma—cannot be used to distinguish whether dysbiosis drives disease or vice versa. Large-scale longitudinal cohorts tracking microbiota dynamics from early life to disease onset are essential to resolve such ambiguity. Furthermore, small sample sizes (<100 participants) and geographical biases (e.g., overrepresentation of European populations) reduce generalizability, and inconsistent sample handling protocols introduce technical variability that obscures biological signals.

The complexity of gut microbiota data presents another layer of difficulty. Integrating taxonomic, metabolic, and functional profiles requires advanced bioinformatics capabilities that are often unavailable to smaller research teams. Compounding this issue, key confounders such as diet are insufficiently addressed. Most studies focus narrowly on the short-term effects of isolated nutrients, overlooking the cumulative impact of dietary patterns and individualized eating behaviors. Genetic predispositions and environmental exposures (e.g., air pollution, antibiotic use) further interact with microbiota in ways that remain poorly characterized, highlighting the need for multifactorial models.

Translating preclinical findings to clinical applications involves additional hurdles. Although animal studies have elucidated mechanisms such as SCFA-mediated immune modulation, they fail to replicate the complexity of human pediatric immune development and environmental exposures. Clinical trials must navigate inherent challenges including stringent participant matching for age, feeding practices, and comorbidities; prolonged follow-up (≥5 years) to assess outcomes in slowly progressing diseases; and unresolved safety concerns regarding probiotics or microbiota-targeted therapies in children.

Finally, the dynamic nature of the gut microbiota during childhood complicates research and intervention design. Most studies capture static snapshots, ignoring developmental trajectories and cumulative microbial effects on disease. Pronounced inter-individual variability, shaped by gene–environment–diet interactions, further complicates the development of personalized approaches. Addressing these limitations requires multinational longitudinal cohorts, multi-omics integration driven by artificial intelligence, and pediatric-specific therapeutic frameworks to advance GLA research from correlation to causation and clinical utility.

6.2 Development and clinical trials of novel treatments

6.2.1 Fecal microbiota transplantation

FMT is a treatment method in which a fecal suspension obtained from a healthy donor is transferred to the patient's digestive tract to restore the normal microbial composition and function of the intestinal tract (101, 102). FMT can be delivered through the upper GI route via a duodenal tube or capsules taken orally (103) or through the lower GI route via colonoscopy or enema (104) (Figure 4). FMT may have applications in the treatment of many diseases, such as asthma, and has been used to treat a variety of gastrointestinal and non-gastrointestinal disorders (105).

6.2.2 Probiotics

Interest in probiotics has grown substantially owing to their complex mechanisms, which are strain- and compound-specific

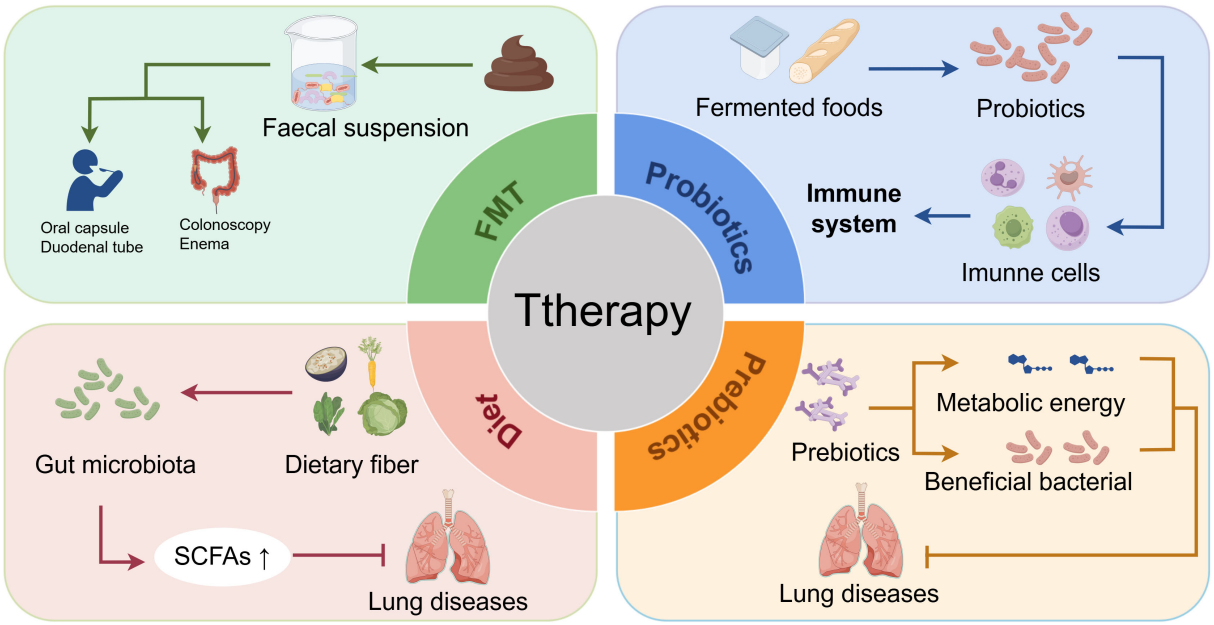


FIGURE 4
Therapeutic strategies targeting the GLA for pediatric pulmonary diseases: mechanisms and clinical interventions. Targeting the GLA offers a novel approach to managing childhood pulmonary diseases through gut microbiota modulation. FMT restores microbial homeostasis by administering healthy donor fecal suspensions via oral capsules, duodenal tubes, or colonoscopy/enema delivery. Probiotics alleviate respiratory symptoms via immunomodulation; prebiotics promote the growth of beneficial bacteria and provide metabolic substrates, thereby reducing pulmonary disease risk. Dietary interventions reshape gut microbiota composition, enhance SCFA production, and mitigate disease progression (e.g., pneumonia). Current evidence indicates that probiotics are more effective against eczema and allergic rhinitis than against asthma, highlighting the need to optimize strain selection, dosage, and treatment duration to improve GLA-targeted therapeutic precision. FMT, fecal microbiota transplantation; GLA, gut–lung axis; SCFAs, short-chain fatty acids.

(106). The mechanisms by which probiotics operate are intricate and varied, typically depending on the specific strain and compound (107). Probiotics can alter the gut microenvironment, compete with harmful bacteria, and suppress pathogens using antimicrobial substances, thereby influencing gut health in nuanced ways (59). Emerging strategies targeting OSA-related gut dysbiosis using prebiotics, probiotics, and SCFAs are being considered for lung disease management and represent a promising therapeutic avenue (91, 108). Probiotics are present in fermented foods and supplements as scientifically validated beneficial strains and exhibit bidirectional immunomodulatory effects on the host, being capable of inducing pro-inflammatory responses while eliciting anti-inflammatory reactions (109). Under immunostimulatory conditions, macrophages, DCs, neutrophils, and natural killer cells in the intestinal mucosa participate in immune responses through enhanced phagocytic activity, inflammatory cytokine release, and Th1/Th17 polarization (15). Concurrently, probiotics activate innate immune responses and cytokines secreted by T cells to stimulate lamina propria-associated immune cells. This immunomodulatory activity is specifically characterized by inducing the production of immunoglobulin A antibody, increased population density, and functional enhancement of macrophages and DCs within the lamina propria, thereby sustaining their functional reinforcement in the small intestine (110) (Figure 4). A placebo-controlled, double-blind, randomized study investigated the effects of a probiotic mixture containing *Bifidobacterium longum BB536*, *Bifidobacterium infantis M-63*, and *Bifidobacterium breve M-16V* in 40 children with seasonal allergic rhinitis and intermittent asthma over a 4-week intervention period. Children receiving the probiotic supplementation demonstrated significant reductions in respiratory symptoms and improved quality of life whereas the placebo group exhibited symptom exacerbation and quality of life deterioration (111). A meta-analysis suggested that Lactobacillus rhamnosus GG (LGG) supplementation, especially postnatally, may prevent asthma (112); however, a database review indicated that probiotics are more effective for eczema and allergic rhinitis than for asthma prevention or treatment (113). Health organizations emphasize the need for further research on probiotic efficacy in asthma prevention because studies may not have used the appropriate strain, dosage, timing, duration, or population (114). Future research will focus on the duration, administration, dosage, and follow-up period for specific probiotic strains (115).

6.2.3 Prebiotics

According to the updated scientific definition established by the International Scientific Association for Probiotics and Prebiotics in 2017, prebiotics are defined as a substrate that is selectively used by host microorganisms to confer a health benefit (116). These compounds exert their effects primarily via two mechanisms: 1) by modulating the gut microbiota composition through the selective promotion of beneficial bacterial growth and provision of metabolic

energy (117) (Figure 4); and 2) by enhancing intestinal barrier function and stimulating the production of beneficial metabolites, thereby inducing multifaceted physiological regulation in the host (118). Given their potential immunomodulatory properties and mechanistic actions, a theoretical basis exists for prebiotics to potentially reduce the risk of COVID-19 infection or mitigate its clinical symptoms (119). However, this hypothesis requires rigorous validation in further experimental and clinical investigations (120). A review of 2,419 pediatric participants analyzed asthma exacerbations, pulmonary function, and immune modulation. Studies that have focused on Lactobacillus/Bifidobacterium (10 randomized controlled trials [RCTs]), bacterial lysates (6 RCTs), and synbiotics (2 RCTs) represent a notable paucity of prebiotic research (121).

6.2.4 Diet

Studies have shown that factors such as diet, genetics, and age affect diversity of the gut microbiota, with diet being a key modifiable factor for treating dysbiosis (27, 122). Clinical and preclinical data highlight how dietary changes can rapidly alter the gut microbiota composition, such as shifts occurring within 24 hours of switching from an animal-based and to a plant-based diet (123, 124). Dietary fiber intake boosts bacterial metabolites, particularly SCFAs (41). Dietary patterns influence the β diversity of the gut microbiota without affecting its α diversity, which varies among individuals consuming an animal-based diet (125, 126). High-calorie diets can worsen LPS-induced pneumonia by disrupting the gut microbiota balance and Th17/Treg cell ratios (127). Future prevention and therapy may involve dietary pattern modifications, such as reducing specific nutrients or adopting lifestyle changes to address physical inactivity and obesity (Figure 4).

Probiotics and dietary changes aim to foster a balanced gut microbiota, which is beneficial for managing respiratory conditions and offering a safer alternative to traditional pharmacological treatments, especially in chronic disease management (3). These strategies collectively bridge mechanistic insights and clinical translation to reshape pediatric respiratory disease management.

6.3 Future research directions

The role of the gut microbiome in pediatric respiratory diseases is an emerging field with implications for new therapeutic strategies. Future research should focus on the GLA, particularly the impact of microbial metabolites on immune modulation. Multi-omics approaches will help deepen understanding regarding the complex interactions between the gut microbiota and children's respiratory health. A key objective is to identify microbial signatures and metabolites that serve as biomarkers for early diagnosis and prognosis of diseases such as asthma and pneumonia, enabling personalized medicine based on individual microbiome profiles. It is also crucial to explore the role of the gut microbiome in responses to treatments such as FMT and probiotics/prebiotics, as well as to design clinical trials assessing their efficacy and safety. Research should also investigate the microbiome's influence on vaccine responses and immunotherapies for respiratory diseases, potentially leading to microbe-based adjuvants that boost vaccine efficacy. The impact of diet on the gut microbiome and lung health in children merits further study, with a focus on dietary interventions to improve respiratory outcomes. Finally, translating preclinical findings into clinical practice is essential. Future research should connect laboratory discoveries with clinical applications, implementing evidence-based interventions that leverage the GLA to prevent and treat pediatric respiratory diseases, with the aim to reduce the impact of these diseases on children's health and quality of life globally.

7 Conclusions

This review synthesizes critical evidence on the MGLA as a central regulator of pediatric respiratory health. Early-life gut microbiota perturbations—driven by birth mode, antibiotics, and diet—disrupt immune programming via metabolite signaling (e.g., SCFAs), epigenetic modulation, and pathogen exclusion, predisposing to asthma, pneumonia, and bronchiolitis. Multi-omics integration has revealed evolutionarily conserved microbiota–immune network architectures across disease phenotypes, with mechanistic prioritization identifying Faecalibacterium-derived SCFA-mediated Treg modulation as therapeutically actionable biological circuitry. To systematically characterize the methodological framework underpinning these findings, we constructed a comprehensive reference table (Table 1) detailing the multi-omics platforms, analytical pipelines, and data integration strategies used, thereby providing explicit technical documentation to enhance methodological reproducibility and cross-study interoperability. Clinically, microbiome-modulating strategies—including precision probiotics, FMT, and dietary SCFA boosters—demonstrate efficacy in respiratory diseases such as asthma and pneumonia. For OSAS, the current evidence is primarily derived from preclinical models, necessitating clinical trials to establish therapeutic efficacy. Future research must prioritize longitudinal birth cohorts to map developmental windows of microbiota–immune crosstalk, coupled with mechanistic studies dissecting microbial vesicles and mobile immune cell trafficking. Standardizing microbiota therapeutics and leveraging AI-driven biomarker panels will accelerate translation from bench to bedside, ultimately enabling the personalized management of childhood respiratory diseases.

Author contributions

ZW: Conceptualization, Supervision, Writing – original draft, Funding acquisition, Writing – review & editing. JY: Conceptualization, Writing – review & editing, Visualization, Writing – original draft. YL: Visualization, Writing – review & editing. JG: Visualization, Writing – review & editing. ZH:

Visualization, Writing – review & editing. ZL: Supervision, Funding acquisition, Writing – review & editing, Conceptualization.

Funding

The author(s) declare financial support was received for the research and/or publication of this article. This work was funded by Shaoxing University Enterprise Important Horizontal Topic (No. 2024USXH287).

Acknowledgments

We thank LetPub (www.letpub.com.cn) for its linguistic assistance during the preparation of this manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that Generative AI was used in the creation of this manuscript. During the preparation of this work the author(s) used DeepSeek and Kimi in order to improve language and readability. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Kloepfer KM, Kennedy JL. Childhood respiratory viral infections and the microbiome. *J Allergy Clin Immunol*. (2023) 152:827–34. doi: 10.1016/j.jaci.2023.08.008

2. Lloyd CM, Saglani S. Early-life respiratory infections and developmental immunity determine lifelong lung health. *Nat Immunol*. (2023) 24:1234–43. doi: 10.1038/s41590-023-01550-w

3. Du B, Fu Y, Han Y, Sun Q, Xu J, Yang Y, et al. The lung-gut crosstalk in respiratory and inflammatory bowel disease. *Front Cell Infect Microbiol*. (2023) 13:1218565. doi: 10.3389/fcimb.2023.1218565

4. Zhu W, Wu Y, Liu H, Jiang C, Huo L. Gut-lung axis: microbial crosstalk in pediatric respiratory tract infections. *Front Immunol*. (2021) 12:741233. doi: 10.3389/fimmu.2021.741233

5. Kahhaleh FG, Barrientos G, Conrad ML. The gut-lung axis and asthma susceptibility in early life. *Acta Physiol (Oxf)*. (2024) 240:e14092. doi: 10.1111/apha.14092

6. Stricker S, Hain T, Chao CM, Rudloff S. Respiratory and intestinal microbiota in pediatric lung diseases-current evidence of the gut-lung axis. *Int J Mol Sci*. (2022) 23):6791–820. doi: 10.3390/ijms23126791

7. Boncheva I, Poudrier J, Falcone EL. Role of the intestinal microbiota in host defense against respiratory viral infections. *Curr Opin Virol*. (2024) 66:101410. doi: 10.1016/j.coviro.2024.101410

8. Nunez H, Nieto PA, Mars RA, Ghavami M, Sew HC, Sukhum K. Early life gut microbiome and its impact on childhood health and chronic conditions. *Gut Microbes*. (2025) 17:2463567. doi: 10.1080/19490976.2025.2463567

9. Ronan V, Yeasin R, Claud EC. Childhood development and the microbiome—The intestinal microbiota in maintenance of health and development of disease during childhood development. *Gastroenterology*. (2021) 160:495–506. doi: 10.1053/j.gastro.2020.08.065

10. Korpela K, Salonen A, Virta LJ, Kekkonen RA, Forslund K, Bork P, et al. Intestinal microbiome is related to lifetime antibiotic use in Finnish pre-school children. *Nat Commun*. (2016) 7:10410. doi: 10.1038/ncomms10410

11. Dang AT, Marsland BJ. Microbes, metabolites, and the gut–lung axis. *Mucosal Immunol*. (2019) 12:843–50. doi: 10.1038/s41385-019-0160-6

12. Kelderer F, Mogren I, Eriksson C, Silfverdal SA, Domellof M, West CE. Associations between pre- and postnatal antibiotic exposures and early allergic outcomes: A population-based birth cohort study. *Pediatr Allergy Immunol*. (2022) 33:e13848. doi: 10.1111/pai.13848

13. Tan JK, Macia L, Mackay CR. Dietary fiber and SCFAs in the regulation of mucosal immunity. *J Allergy Clin Immunol*. (2023) 151:361–70. doi: 10.1016/j.jaci.2022.11.007

14. Barcik W, Boutin RCT, Sokolowska M, Finlay BB. The role of lung and gut microbiota in the pathology of asthma. *Immunity*. (2020) 52:241–55. doi: 10.1016/j.immuni.2020.01.007

15. Ma P, Wang M, Wang Y. Gut microbiota: A new insight into lung diseases. *BioMed Pharmacother*. (2022) 155:113810. doi: 10.1016/j.biopha.2022.113810

16. Budden KF, Gellatly SL, Wood DL, Cooper MA, Morrison M, Hugenholtz P, et al. Emerging pathogenic links between microbiota and the gut-lung axis. *Nat Rev Microbiol*. (2017) 15:55–63. doi: 10.1038/nrmicro.2016.142

17. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. *Cell*. (2014) 157:121–41. doi: 10.1016/j.cell.2014.03.011

18. Kim YJ, Bunyavanich S. Microbial influencers: the airway microbiome's role in asthma. *J Clin Invest*. (2025) 135:e184316–26. doi: 10.1172/JCI184316

19. Stricker S, Hain T, Chao C, Rudloff S. Respiratory and intestinal microbiota in pediatric lung diseases—Current evidence of the gut–lung axis. *Int J Mol Sci*. (2022) 23:6791. doi: 10.3390/ijms23126791

20. Singh N, Vats A, Sharma A, Arora A, Kumar A. The development of lower respiratory tract microbiome in mice. *Microbiome*. (2017) 5:61. doi: 10.1186/s40168-017-0277-3

21. Huffnagle GB, Dickson RP, Lukacs NW. The respiratory tract microbiome and lung inflammation: a two-way street. *Mucosal Immunol*. (2017) 10:299–306. doi: 10.1038/mi.2016.108

22. Ashique S, De Rubis G, Sirohi E, Mishra N, Rihan M, Garg A, et al. Short Chain Fatty Acids: Fundamental mediators of the gut-lung axis and their involvement in pulmonary diseases. *Chem Biol Interact*. (2022) 368:110231. doi: 10.1016/j.cbi.2022.110231

23. Tamburini S, Clemente JC. Gut microbiota: Neonatal gut microbiota induces lung immunity against pneumonia. *Nat Rev Gastroenterol Hepatol*. (2017) 14:263–64. doi: 10.1038/nrgastro.2017.34

24. Tamburini S, Shen N, Wu HC, Clemente JC. The microbiome in early life: implications for health outcomes. *Nat Med*. (2016) 22:713–22. doi: 10.1038/nm.4142

25. Inchingolo F, Inchingolo AD, Palumbo I, Trilli I, Guglielmo M, Mancini A, et al. The impact of cesarean section delivery on intestinal microbiota: mechanisms,

consequences, and perspectives-A systematic review. *Int J Mol Sci*. (2024) 25:1055–75. doi: 10.3390/ijms25021055

26. Fujimura KE, Sitarik AR, Havstad S, Lin DL, Levan S, Fadrosh D, et al. Neonatal gut microbiota associates with childhood multisensitized atopy and T cell differentiation. *Nat Med*. (2016) 22:1187–91. doi: 10.1038/nm.4176

27. Rastogi S, Mohanty S, Sharma S, Tripathi P. Possible role of gut microbes and host's immune response in gut–lung homeostasis. *Front Immunol*. (2022) 13:954339. doi: 10.3389/fimmu.2022.954339

28. Eladham MW, Selvakumar B, Saheb SN, Saheb SF, Ibrahim SM, Halwani R. Unraveling the gut-Lung axis: Exploring complex mechanisms in disease interplay. *Heliyon*. (2024) 10:e24032. doi: 10.1016/j.heliyon.2024.e24032

29. Sun CY, Yang N, Zheng ZL, Liu D, Xu QL. T helper 17 (Th17) cell responses to the gut microbiota in human diseases. *BioMed Pharmacother*. (2023) 161:114483. doi: 10.1016/j.biopha.2023.114483

30. Harris NL, Marsland BJ. The gut-lung axis: Protozoa join the party. *Cell*. (2025) 188:275–77. doi: 10.1016/j.cell.2024.12.027

31. Johnson CC, Ownby DR. The infant gut bacterial microbiota and risk of pediatric asthma and allergic diseases. *Transl Res*. (2017) 179:60–70. doi: 10.1016/j.trsl.2016.06.010

32. Gilmour BC, Corthay A, Oynebraten I. High production of IL-12 by human dendritic cells stimulated with combinations of pattern-recognition receptor agonists. *NPJ Vaccines*. (2024) 9:83. doi: 10.1038/s41541-024-00869-1

33. Wang J, Zhu N, Su X, Gao Y, Yang R. Gut-microbiota-derived metabolites maintain gut and systemic immune homeostasis. *Cells*. (2023) 12:793–819. doi: 10.3390/cells12050793

34. Ozcam M, Lynch SV. The gut-airway microbiome axis in health and respiratory diseases. *Nat Rev Microbiol*. (2024) 22:492–506. doi: 10.1038/s41579-024-01048-8

35. Tang J, Xu L, Zeng Y, Gong F. Effect of gut microbiota on LPS-induced acute lung injury by regulating the TLR4/NF-kB signaling pathway. *Int Immunopharmacol*. (2021) 91:107272. doi: 10.1016/j.intimp.2020.107272

36. Kim CH. Control of lymphocyte functions by gut microbiota-derived short-chain fatty acids. *Cell Mol Immunol*. (2021) 18:1161–71. doi: 10.1038/s41423-020-00625-0

37. Shibuya A, Shibuya K. Exploring the gut fungi-lung allergy axis. *Cell Host Microbe*. (2018) 24:755–57. doi: 10.1016/j.chom.2018.11.012

38. Zhang S, Paul S, Kundu P. NF-kappaB regulation by gut microbiota decides homeostasis or disease outcome during ageing. *Front Cell Dev Biol*. (2022) 10:874940. doi: 10.3389/fcell.2022.874940

39. Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat Med*. (2014) 20:159–66. doi: 10.1038/nm.3444

40. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, DeRoos P, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature*. (2013) 504:451–55. doi: 10.1038/nature12726

41. Koh A, De Vadder F, Kovatcheva-Datchary P, Backhed F. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. *Cell*. (2016) 165:1332–45. doi: 10.1016/j.cell.2016.05.041

42. Mann ER, Lam YK, Uhlig HH. Short-chain fatty acids: linking diet, the microbiome and immunity. *Nat Rev Immunol*. (2024) 24:577–95. doi: 10.1038/s41577-024-01014-8

43. Caetano-Silva ME, Rund L, Hutchinson NT, Woods JA, Steelman AJ, Johnson RW. Inhibition of inflammatory microglia by dietary fiber and short-chain fatty acids. *Sci Rep*. (2023) 13:2819. doi: 10.1038/s41598-022-27086-x

44. Abdelhalim KA. Short-chain fatty acids (SCFAs) from gastrointestinal disorders, metabolism, epigenetics, central nervous system to cancer - A mini-review. *Chem Biol Interact*. (2024) 388:110851. doi: 10.1016/j.cbi.2023.110851

45. Ney LM, Wipplinger M, Grossmann M, Engert N, Wegner VD, Mosig AS. Short chain fatty acids: key regulators of the local and systemic immune response in inflammatory diseases and infections. *Open Biol*. (2023) 13:230014. doi: 10.1098/rsob.230014

46. Shi C, Zhou L, Li H, Shi X, Zhang Y, Lu Y, et al. Intestinal microbiota metabolizing Houttuynia cordata polysaccharides in H1N1 induced pneumonia mice contributed to Th17/Treg rebalance in gut-lung axis. *Int J Biol Macromol*. (2022) 221:288–302. doi: 10.1016/j.ijbiomac.2022.09.015

47. Zhao M, Shao F, Yu D, Zhang J, Liu Z, Ma J, et al. Maturation and specialization of group 2 innate lymphoid cells through the lung-gut axis. *Nat Commun*. (2022) 13:7600. doi: 10.1038/s41467-022-35347-6

48. GBD 2015 Chronic Respiratory Disease Collaborators. Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Respir Med*. (2017) 5:691–706. doi: 10.1016/S2213-2600(17)30293-X

49. Lee-Sarwar KA, Kelly RS, Lasky-Su J, Zeiger RS, O'Connor GT, Sandel MT, et al. Integrative analysis of the intestinal metabolome of childhood asthma. *J Allergy Clin Immunol*. (2019) 144:442–54. doi: 10.1016/j.jaci.2019.02.032

50. Stern J, Pier J, Litonjua AA. Asthma epidemiology and risk factors. *Semin Immunopathol*. (2020) 42:5–15. doi: 10.1007/s00281-020-00785-1

51. Ege MJ. The hygiene hypothesis in the age of the microbiome. *Ann Am Thorac Soc*. (2017) 14:S348–53. doi: 10.1513/AnnalsATS.201702-139AW

52. Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. *Cell Res*. (2020) 30:492–506. doi: 10.1038/s41422-020-0332-7

53. Valverde-Molina J, Garcia-Marcos L. Microbiome and asthma: microbial dysbiosis and the origins, phenotypes, persistence, and severity of asthma. *Nutrients*. (2023) 15:486–511. doi: 10.3390/nu15030486

54. Stokholm J, Blaser MJ, Thorsen J, Rasmussen MA, Waage J, Vinding RK, et al. Maturation of the gut microbiome and risk of asthma in childhood. *Nat Commun*. (2018) 9:141. doi: 10.1038/s41467-017-02573-2

55. Gensollen T, Iyer SS, Kasper DL, Blumberg RS. How colonization by microbiota in early life shapes the immune system. *Science*. (2016) 352:539–44. doi: 10.1126/science.aad9378

56. Oliphant K, Allen-Vercoe E. Macronutrient metabolism by the human gut microbiome: major fermentation by-products and their impact on host health. *Microbiome*. (2019) 7:91. doi: 10.1186/s40168-019-0704-8

57. Charbonneau MR, O'Donnell D, Blanton LV, Totten SM, Davis JC, Barratt MJ, et al. Sialylated milk oligosaccharides promote microbiota-dependent growth in models of infant undernutrition. *Cell*. (2016) 164:859–71. doi: 10.1016/j.cell.2016.01.024

58. Urbaniak C, Angelini M, Gloor GB, Reid G. Human milk microbiota profiles in relation to birthing method, gestation and infant gender. *Microbiome*. (2016) 4:1. doi: 10.1186/s40168-015-0145-y

59. Zhang D, Jian Y, Zhang Y, Li Y, Gu L, Sun H, et al. Short-chain fatty acids in diseases. *Cell Commun Signal*. (2023) 21:212–32. doi: 10.1186/s12964-023-01219-9

60. Dong J. Signaling pathways implicated in carbon nanotube-induced lung inflammation. *Front Immunol*. (2020) 11:552613. doi: 10.3389/fimmu.2020.552613

61. Quach A, Spence H, Nguyen C, Graham SM, von Mollendorf C, Mulholland K, et al. Slow progress towards pneumonia control for children in low-and-middle income countries as measured by pneumonia indicators: A systematic review of the literature. *J Glob Health*. (2022) 12:10006. doi: 10.7189/jogh.12.10006

62. Hanada S, Pirzadeh M, Carver KY, Deng JC. Respiratory viral infection-induced microbiome alterations and secondary bacterial pneumonia. *Front Immunol*. (2018) 9:2640. doi: 10.3389/fimmu.2018.02640

63. Zhang S, Chen DC. Facing a new challenge: the adverse effects of antibiotics on gut microbiota and host immunity. *Chin Med J (Engl)*. (2019) 132:1135–38. doi: 10.1097/CM9.0000000000000245

64. Schuijt TJ, Lankelma JM, Scicluna BP, de Sousa E Melo F, Roelofs JJTH, de Boer JD, et al. The gut microbiota plays a protective role in the host defence against pneumococcal pneumonia. *Gut*. (2016) 65:575–83. doi: 10.1136/gutjnl-2015-309728

65. Xie Q, Li Q, Fang H, Zhang R, Tang H, Chen L. Gut-derived short-chain fatty acids and macrophage modulation: exploring therapeutic potentials in pulmonary fungal infections. *Clin Rev Allergy Immunol*. (2024) 66:316–27. doi: 10.1007/s12016-024-08999-z

66. Wang Q, Fang Z, Xiao Y, Wang H, Zhang P, Lu W, et al. Lactiplantibacillus pentoses CCFM1227 Produces Desaminotyrosine to Protect against Influenza Virus H1N1 Infection through the Type I Interferon in Mice. *Nutrients*. (2023) 15:3659–75. doi: 10.3390/nu15163659

67. Amin R, Hatakeyama Y, Kitazawa T, Matsumoto K, Fujita S, Seto K, et al. Capturing the trends in hospital standardized mortality ratios for pneumonia: a retrospective observational study in Japan (2010 to 2018). *Environ Health Prev Med*. (2020) 25:2. doi: 10.1186/s12199-019-0842-4

68. Ma Y, Peng X, Zhang J, Zhu Y, Huang R, Li G, et al. Gut microbiota in preterm infants with late-onset sepsis and pneumonia: a pilot case-control study. *BMC Microbiol*. (2024) 24:272. doi: 10.1186/s12866-024-03419-w

69. Steed AL, Christophi GP, Kaiko GE, Sun L, Goodwin VM, Jain U, et al. The microbial metabolite desaminotyrosine protects from influenza through type I interferon. *Science*. (2017) 357:498–502. doi: 10.1126/science.aam5336

70. Jiang Y, Bao C, Zhao X, Chen Y, Song Y, Xiao Z. Intestinal bacteria flora changes in patients with Mycoplasma pneumoniae pneumonia with or without wheezing. *Sci Rep*. (2022) 12:5683. doi: 10.1038/s41598-022-09700-0

71. Shao Y, Forster SC, Tsaliki E, Vervier K, Strang A, Simpson N, et al. Stunted microbiota and opportunistic pathogen colonization in caesarean-section birth. *Nature*. (2019) 574:117–21. doi: 10.1038/s41586-019-1560-1

72. Nikolopoulou GB, Maltezou HC. COVID-19 in Children: Where do we Stand? *Arch Med Res*. (2022) 53:1–08. doi: 10.1016/j.arcmed.2021.07.002

73. Franchitti E, Bottino P, Sidoti F, Carpino A, Pruccoli G, Ramenghi U, et al. Investigating the role of gut microbiota in pediatric patients with severe COVID-19 or MIS-C. *Microorganisms*. (2025) 13:83–97. doi: 10.3390/microorganisms13010083

74. Bacorn M, Romero-Soto HN, Levy S, Chen Q, Hourigan SK. The gut microbiome of children during the COVID-19 pandemic. *Microorganisms*. (2022) 10:2460–89. doi: 10.3390/microorganisms10122460

75. Li Y, Wang X, Blau DM, Caballero MT, Feikin DR, Gill CJ, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in children younger than 5 years in 2019: a systematic analysis. *Lancet*. (2022) 399:2047–64. doi: 10.1016/S0140-6736(22)00478-0

76. Alba C, Aparicio M, Gonzalez-Martinez F, Gonzalez-Sanchez MI, Perez-Moreno J, Toledo DCB, et al. Nasal and fecal microbiota and immunoprofiling of infants with

and without RSV bronchiolitis. *Front Microbiol*. (2021) 12:667832. doi: 10.3389/fmicb.2021.667832

77. Antunes KH, Fachi JL, de Paula R, Da SE, Pral LP, Dos SA, et al. Microbiota-derived acetate protects against respiratory syncytial virus infection through a GPR43-type 1 interferon response. *Nat Commun*. (2019) 10:3273. doi: 10.1038/s41467-019-11152-6

78. Dogra SK, Martin FP, Donnicola D, Julita M, Berger B, Sprenger N. Human milk oligosaccharide-stimulated bifidobacterium species contribute to prevent later respiratory tract infections. *Microorganisms*. (2021) 9:1939–53. doi: 10.3390/microorganisms9091939

79. Fonseca W, Malinczak CA, Fujimura K, Li D, McCauley K, Li J, et al. Maternal gut microbiome regulates immunity to RSV infection in offspring. *J Exp Med*. (2021) 218:e20210235–48. doi: 10.1084/jem.20210235

80. Shahrebabak AG, Rezaei M, Shahpar A, Nezhad NZ, Sarasyabi MS, Nakhaie M, et al. The efficacy of COVID-19 vaccination in cystic fibrosis patients: a systematic review. *BMC Infect Dis*. (2025) 25:358. doi: 10.1186/s12879-025-10736-6

81. Ranganathan SC, Hall GL, Sly PD, Stick SM, Douglas TA, Australian REST. Early lung disease in infants and preschool children with cystic fibrosis. What have we learned and what should we do about it? *Am J Respir Crit Care Med*. (2017) 195:1567–75. doi: 10.1164/rccm.201606-1107CI

82. McKay I, van Dorst J, Katz T, Doumit M, Prentice B, Owens L, et al. Diet and the gut-lung axis in cystic fibrosis – direct & indirect links. *Gut Microbes*. (2023) 15:2156254–69. doi: 10.1080/19490976.2022.2156254

83. Enaud R, Prevel R, Ciarlo E, Beaufils F, Wieers G, Guery B, et al. The gut-lung axis in health and respiratory diseases: A place for inter-organ and inter-kingdom crosstalks. *Front Cell Infect Microbiol*. (2020) 10:9. doi: 10.3389/fcimb.2020.00009

84. Enaud R, Hooks KB, Barre A, Barnetche T, Hubert C, Massot M, et al. Intestinal inflammation in children with cystic fibrosis is associated with crohn's-like microbiota disturbances. *J Clin Med*. (2019) 8:645–58. doi: 10.3390/jcm8050645

85. Vernocchi P, Del CF, Russo A, Majo F, Rossitto M, Valerio M, et al. Gut microbiota signatures in cystic fibrosis: Loss of host CFTR function drives the microbiota enterophenotype. *PloS One*. (2018) 13:e208171. doi: 10.1371/journal.pone.0208171

86. Caparros-Martin JA, Saladie M, Agudelo-Romero SP, Reen FJ, Ware RS, Sly PD, et al. Detection of bile acids in bronchoalveolar lavage fluid defines the inflammatory and microbial landscape of the lower airways in infants with cystic fibrosis. *Microbiome*. (2023) 11:132. doi: 10.1186/s40168-023-01543-9

87. Bass R, Brownell JN, Stallings VA. The impact of highly effective CFTR modulators on growth and nutrition status. *Nutrients*. (2021) 13:2907–18. doi: 10.3390/nu13092907

88. Price CE, O'Toole GA. The gut-lung axis in cystic fibrosis. *J Bacteriol*. (2021) 203:e31121. doi: 10.1128/JB.00311-21

89. Bitners AC, Arens R. Evaluation and management of children with obstructive sleep apnea syndrome. *Lung*. (2020) 198:257–70. doi: 10.1007/s00408-020-00342-5

90. Lv R, Liu X, Zhang Y, Dong N, Wang X, He Y, et al. Pathophysiological mechanisms and therapeutic approaches in obstructive sleep apnea syndrome. *Signal Transduct Target Ther*. (2023) 8:218. doi: 10.1038/s41392-023-01496-3

91. Yan W, Jiang M, Hu W, Zhan X, Liu Y, Zhou J, et al. Causality investigation between gut microbiota, derived metabolites, and obstructive sleep apnea: A bidirectional mendelian randomization study. *Nutrients*. (2023) 15:4544–59. doi: 10.3390/nu15214544

92. Liu W, Du Q, Zhang H, Han D. The gut microbiome and obstructive sleep apnea syndrome in children. *Sleep Med*. (2022) 100:462–71. doi: 10.1016/j.sleep.2022.09.022

93. Wang F, Zou J, Xu H, Huang W, Zhang X, Wei Z, et al. Effects of chronic intermittent hypoxia and chronic sleep fragmentation on gut microbiome, serum metabolome, liver and adipose tissue morphology. *Front Endocrinol (Lausanne)*. (2022) 13:820939. doi: 10.3389/fendo.2022.820939

94. Ko CY, Liu QQ, Su HZ, Zhang HP, Fan JM, Yang JH, et al. Gut microbiota in obstructive sleep apnea-hypopnea syndrome: disease-related dysbiosis and metabolic comorbidities. *Clin Sci (Lond)*. (2019) 133:905–17. doi: 10.1042/CS20180891

95. Ganesh BP, Nelson JW, Eskew JR, Ganesan A, Ajami NJ, Petrosino JF, et al. Prebiotics, probiotics, and acetate supplementation prevent hypertension in a model of obstructive sleep apnea. *Hypertension*. (2018) 72:1141–50. doi: 10.1161/HYPERTENSIONAHA.118.11695

96. Mashaqi S, Rangan P, Saleh AA, Abraham I, Gozal D, Quan SF, et al. Biomarkers of gut barrier dysfunction in obstructive sleep apnea: A systematic review and meta-analysis. *Sleep Med Rev*. (2023) 69:101774. doi: 10.1016/j.smrv.2023.101774

97. Callejo M, Mondejar-Parreno G, Barreira B, Izquierdo-Garcia JL, Morales-Cano D, Esquivel-Ruiz S, et al. Pulmonary arterial hypertension affects the rat gut microbiome. *Sci Rep*. (2018) 8:9681. doi: 10.1038/s41598-018-27682-w

98. Yeoh YK, Zuo T, Lui GC, Zhang F, Liu Q, Li AY, et al. Gut microbiota composition reflects disease severity and dysfunctional immune responses in patients with COVID-19. *Gut*. (2021) 70:698–706. doi: 10.1136/gutjnl-2020-323020

99. Zhang F, Wan Y, Zuo T, Yeoh YK, Liu Q, Zhang L, et al. Prolonged impairment of short-chain fatty acid and L-isoleucine biosynthesis in gut microbiome in patients with COVID-19. *Gastroenterology*. (2022) 162:548–61. doi: 10.1053/j.gastro.2021.10.013

100. Hjelmsø MH, Shah SA, Thorsen J, Rasmussen M, Vestergaard G, Mortensen MS, et al. Prenatal dietary supplements influence the infant airway microbiota in a randomized factorial clinical trial. *Nat Commun*. (2020) 11:426. doi: 10.1038/s41467-020-14308-x

101. de Groot P, Scheithauer T, Bakker GJ, Prodan A, Levin E, Khan MT, et al. Donor metabolic characteristics drive effects of faecal microbiota transplantation on recipient insulin sensitivity, energy expenditure and intestinal transit time. *Gut*. (2020) 69:502–12. doi: 10.1136/gutjnl-2019-318320

102. Leonardi I, Paramsothy S, Doron I, Semon A, Kaakoush NO, Clemente JC, et al. Fungal trans-kingdom dynamics linked to responsiveness to fecal microbiota transplantation (FMT) therapy in ulcerative colitis. *Cell Host Microbe*. (2020) 27:823–29. doi: 10.1016/j.chom.2020.03.006

103. Kao D, Roach B, Silva M, Beck P, Rioux K, Kaplan GG, et al. Effect of Oral Capsule- vs Colonoscopy-Delivered Fecal Microbiota Transplantation on Recurrent Clostridium difficile Infection: A Randomized Clinical Trial. *Jama*. (2017) 318:1985–93. doi: 10.1001/jama.2017.17077

104. Shao T, Hsu R, Hacein-Bey C, Zhang W, Gao L, Kurth MJ, et al. The evolving landscape of fecal microbial transplantation. *Clin Rev Allergy Immunol*. (2023) 65:101–20. doi: 10.1007/s12016-023-08958-0

105. Logon K, Swirkosz G, Nowak M, Wrzesniewska M, Szczygiel A, Gomulka K. The role of the microbiome in the pathogenesis and treatment of asthma. *Biomedicines*. (2023) 11:1618–35. doi: 10.3390/biomedicines11061618

106. Salminen S, Collado MC, Endo A, Hill C, Lebeer S, Quigley E, et al. The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. *Nat Rev Gastroenterol Hepatol*. (2021) 18:649–67. doi: 10.1038/s41575-021-00440-6

107. Cunningham M, Azcarate-Peril MA, Barnard A, Benoit V, Grimaldi R, Guyonnet D, et al. Shaping the future of probiotics and prebiotics. *Trends Microbiol*. (2021) 29:667–85. doi: 10.1016/j.tim.2021.01.003

108. Badran M, Mashaqi S, Gozal D. The gut microbiome as a target for adjuvant therapy in obstructive sleep apnea. *Expert Opin Ther Targets*. (2020) 24:1263–82. doi: 10.1080/14728222.2020.1841749

109. D'Ettorre G, Ceccarelli G, Marazzato M, Campagna G, Pinacchio C, Alessandri F, et al. Challenges in the management of SARS-coV2 infection: the role of oral bacteriotherapy as complementary therapeutic strategy to avoid the progression of COVID-19. *Front Med (Lausanne)*. (2020) 7:389. doi: 10.3389/fmed.2020.00389

110. Wang X, Zhang P, Zhang X. Probiotics regulate gut microbiota: an effective method to improve immunity. *Molecules*. (2021) 26:6076–91. doi: 10.3390/molecules26196076

111. Miraglia DGM, Indolfi C, Capasso M, Maiello N, Decimo F, Ciprandi G. Bifidobacterium mixture (B longum BB536, B infantis M-63, B breve M-16V) treatment in children with seasonal allergic rhinitis and intermittent asthma. *Ital J Pediatr*. (2017) 43:25. doi: 10.1186/s13052-017-0340-5

112. Du X, Wang L, Wu S, Yuan L, Tang S, Xiang Y, et al. Efficacy of probiotic supplementary therapy for asthma, allergic rhinitis, and wheeze: a meta-analysis of randomized controlled trials. *Allergy Asthma Proc*. (2019) 40:250–60. doi: 10.2500/aap.2019.40.4227

113. Wang HT, Anvari S, Anagnostou K. The role of probiotics in preventing allergic disease. *Children (Basel)*. (2019) 6:24–33. doi: 10.3390/children6020024

114. Edwards MR, Walton RP, Jackson DJ, Feleszko W, Skevaki C, Jartti T, et al. The potential of anti-infectives and immunomodulators as therapies for asthma and asthma exacerbations. *Allergy*. (2018) 73:50–63. doi: 10.1111/all.13257

115. He Q, Huang J, Zheng T, Lin D, Zhang H, Li J, et al. Treatment with mixed probiotics induced, enhanced and diversified modulation of the gut microbiome ofxhealthy rats. *FEMS Microbiol Ecol*. (2021) 97:fiab151–64. doi: 10.1093/femsec/fiab151

116. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol*. (2017) 14:491–502. doi: 10.1038/nrgastro.2017.75

117. Calatayud M, Van den Abbeele P, Ghyselinck J, Marzorati M, Rohs E, Birkett A. Comparative effect of 22 dietary sources of fiber on gut microbiota of healthy humans *in vitro*. *Front Nutr*. (2021) 8:700571. doi: 10.3389/fnut.2021.700571

118. Davani-Davari D, Negahdaripour M, Karimzadeh I, Seifan M, Mohkam M, Masoumi SJ, et al. Prebiotics: definition, types, sources, mechanisms, and clinical applications. *Foods*. (2019) 8:92–119. doi: 10.3390/foods8030092

119. Batista KS, de Albuquerque JG, Vasconcelos M, Bezerra M, Da SBM, Pinheiro RO, et al. Probiotics and prebiotics: potential prevention and therapeutic target for nutritional management of COVID-19? *Nutr Res Rev*. (2023) 36:181–98. doi: 10.1017/S0954422421000317

120. Xu L, Yang CS, Liu Y, Zhang X. Effective regulation of gut microbiota with probiotics and prebiotics may prevent or alleviate COVID-19 through the gut-lung axis. *Front Pharmacol*. (2022) 13:895193. doi: 10.3389/fphar.2022.895193

121. Fan D, Hu J, Lin N. Effects of probiotics, prebiotics, synbiotics and postbiotics on pediatric asthma: a systematic review. *Front Nutr*. (2025) 12:1586129. doi: 10.3389/fnut.2025.1586129

122. Rinninella E, Cintoni M, Raoul P, Lopetuso LR, Scaldaferri F, Pulcini G, et al. Food components and dietary habits: keys for a healthy gut microbiota composition. *Nutrients*. (2019) 11:2393–416. doi: 10.3390/nu11102393

123. Tanes C, Bittinger K, Gao Y, Friedman ES, Nessel L, Paladhi UR, et al. Role of dietary fiber in the recovery of the human gut microbiome and its metabolome. *Cell Host Microbe*. (2021) 29:394–407. doi: 10.1016/j.chom.2020.12.012

124. Miao Z, Du W, Xiao C, Su C, Gou W, Shen L, et al. Gut microbiota signatures of long-term and short-term plant-based dietary pattern and cardiometabolic health: a prospective cohort study. *BMC Med*. (2022) 20:204. doi: 10.1186/s12916-022-02402-4

125. Roager HM, Vogt JK, Kristensen M, Hansen L, Ibrugger S, Maerkedahl RB, et al. Whole grain-rich diet reduces body weight and systemic low-grade inflammation without inducing major changes of the gut microbiome: a randomised cross-over trial. *Gut*. (2019) 68:83–93. doi: 10.1136/gutjnl-2017-314786

126. Bisanz JE, Upadhyay V, Turnbaugh JA, Ly K, Turnbaugh PJ. Meta-analysis reveals reproducible gut microbiome alterations in response to a high-fat diet. *Cell Host Microbe*. (2019) 26:265–72. doi: 10.1016/j.chom.2019.06.013

127. Liu H, Bai C, Xian F, Liu S, Long C, Hu L, et al. A high-calorie diet aggravates LPS-induced pneumonia by disturbing the gut microbiota and Th17/Treg balance. *J Leukoc Biol*. (2022) 112:127–41. doi: 10.1002/JLB.3MA0322-458RR

Frontiers in Immunology

Explores novel approaches and diagnoses to treat immune disorders.

The official journal of the International Union of Immunological Societies (IUIS) and the most cited in its field, leading the way for research across basic, translational and clinical immunology.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact