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Preliminary assessment of rural airborne microorganisms as emerging environmental hazards: seasonal variability and antimicrobial resistance in South India

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Airborne microorganisms (bioaerosols) represent hazardous environmental contaminants due to their capacity to replicate, survive in diverse matrices, and disseminate antimicrobial resistance. The sites were selected to represent four distinct rural microenvironments (agricultural, tribal, domestic, and commercial) differing in land use, human activity, and ecological characteristics. Despite increasing evidence from urban and hospital environments, rural bioaerosols remain underexplored, particularly under environmentally relevant conditions where exposure is continuous. Using a MicroBio MB1 air sampler, bacterial concentrations were consistently higher than fungal loads, with winter peaks of 880 colony-forming units (CFU) m⁻³ in commercial zones and 635 CFU m⁻³ in tribal sites. In summer, bacterial levels declined overall except in domestic environments (720 CFU m⁻³), while fungal loads increased, reaching 120 CFU m⁻³ in domestic sites. Gram staining confirmed the predominance of Gram-positive bacilli and cocci (*Bacillus cereus*, *Staphylococcus*), while Lactophenol Cotton Blue staining revealed filamentous fungi, predominantly *Aspergillus* and *Penicillium*. Matrix-assisted laser Desorption/Ionization Time-of-flight Mass Spectrometry validated species-level identities, and antimicrobial susceptibility tests showed resistance to erythromycin and amphotericin B, while ciprofloxacin and chloramphenicol remained effective against bacteria, and clotrimazole and fluconazole demonstrated seasonal efficacy against fungi. These results identify rural bioaerosols as critical yet overlooked reservoirs of hazardous microbes and

antimicrobial resistance and highlight an urgent need for rural antimicrobial resistance surveillance to support integrated monitoring and public health interventions.

KEYWORDS

air quality, antimicrobial resistance, bioaerosol, health effects, rural area

1 Introduction

Airborne microorganisms, known as bioaerosols, represent a significant yet often underestimated class of environmental contaminants (Gauhar et al., 2023). Unlike inert particulates, they are viable organisms capable of replication, mutation, and adaptation, thereby posing hazards to human health and the environment (Akbar et al., 2022). Bioaerosols can induce respiratory diseases and allergic reactions, cause infectious outbreaks, and act as vectors for antimicrobial resistance (AMR) (Endale et al., 2023). Their abundance, diversity, and pathogenic potential vary with environmental context, anthropogenic influence, and seasonal variations (Li et al., 2023; Li et al., 2024; Akhtar et al., 2024).

Urban and institutional bioaerosols have been extensively studied due to high population densities, industrial emissions, and human activity. For example, studies in megacities, schools, and hospitals have documented dominant bacterial and fungal taxa, diurnal and seasonal fluctuations, and the presence of opportunistic pathogens such as *Aspergillus* spp., and highlighted occupational, educational, and nosocomial health risks (Geng et al., 2024; Ivanova et al., 2025; de Sousa et al., 2025; Calderón-Ezquerro et al., 2025; Upadhyay et al., 2024). For example, Geng et al. (2024) reported distinct diurnal microbial patterns in a megacity, de Sousa et al. (2025) observed unexpectedly high bacterial loads in outdoor school environments in Sofia, Bulgaria, and Calderón-Ezquerro et al. (2025) identified *Aspergillus fumigatus* as a major hospital-airborne threat in Mexico City. Indoor occupational and residential spaces have shown high microbial loads influenced by human presence, crowding, and ventilation, with implications for effective health management (Upadhyay et al., 2024; Wang et al., 2025; Kumar and Singh, 2025; Diala et al., 2025). Outdoor and extreme environments, including Arctic aerosols and recreational beaches, also harbor diverse microbes, some of which exhibit antibiotic resistance, underscoring the global relevance of airborne AMR (Andreeva et al., 2024; Ambrose et al., 2025; Polus and Mucha, 2025; Park et al., 2025; Shukla et al., 2025).

Despite these advances, systematic assessments of rural bioaerosols, particularly in developing countries, remain limited. Rural environments combine natural ecological diversity with anthropogenic influences such as agriculture, livestock, and biomass burning. At the same time, populations often reside close to these sources, resulting in continuous exposure under environmentally relevant conditions. Knowledge of seasonal dynamics, microbial diversity, and resistance patterns in rural environments is sparse, and most AMR studies focus on clinical isolates rather than environmental air as a reservoir and vector for resistant microbes (Chawla et al., 2023; Zulkifl et al., 2025; Lou et al., 2022; Cocker et al., 2024; Despotovic et al., 2023). For instance, recent studies have shown that environmental air can facilitate the dissemination of tetracycline-resistant bacteria from agricultural

environments (Lou et al., 2022) and contribute to the regional propagation of antibiotic resistance genes under biomass-burning conditions (Despotovic et al., 2023).

Addressing this gap requires seasonally resolved, environmentally contextualized studies that quantify microbial loads, identify taxa using molecular tools, and determine susceptibility to clinically relevant antibiotics and antifungals. In this context, the present study examines the seasonal distribution, diversity, and antimicrobial susceptibility of airborne bacterial and fungal bioaerosols across four rural microenvironments—agricultural, tribal, domestic, and commercial in Coimbatore, Tamil Nadu, India. Sampling was conducted in winter and summer using standardized culture-based methods, combined with Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) identification and Kirby-Bauer susceptibility testing. By linking microbial abundance, diversity, and resistance profiles with seasonal variation, this study provides insights into the combined hazards of microbial exposure and AMR in rural populations.

The study is short-term, based on two seasonal snapshots, and does not represent a complete longitudinal time series. A short-term seasonal field investigation, seasonally comparative study aimed to quantify airborne bacteria and fungi, identify dominant taxa using MALDI-TOF, and evaluate antibiotic/antifungal susceptibility across rural microenvironments during winter and summer. Although this study provides seasonally contrasting datasets, it relies on two representative seasonal snapshots due to logistical and resource constraints. Consequently, these data reflect short-term seasonal differences rather than a time-series investigation of long-term temporal trends. In addition, culture-based methods may not fully capture viable but non-culturable (VBNC) organisms or the entire microbial diversity. Further studies incorporating multi-year datasets, high-throughput sequencing, and antibiotic resistance gene profiling are needed for a more comprehensive understanding of rural airborne microbiomes and their AMR characteristics.

2 Methodology

2.1 Area of study

Sampling of aerial bioaerosols was conducted in four ecologically and anthropogenically distinct rural microresidences within a 10 km radius of Karunya Institute of Technology and Sciences, Coimbatore, Tamil Nadu, India. The sites were also chosen strategically to represent agricultural, tribal, domestic, and commercial environments with diverse land-use characteristics that affect air quality and microbial communities. Angel Garden, Karuny Nagar (10.9386768 N, 76.7530944 E), was selected as the agricultural location. It is a crop-based area with minor structural enhancement and open-field exposure. In the tribal location, the

Trishul Shelters, Boluvampatti, and Sadivayal (10.9391838 N, 76.7299982 E), a low-density tribal settlement on the fringe of the forest, with natural vegetation and minimal anthropogenic activities impacting the microbial air composition.

The domestic site was located near Nandangarai Dam, Boluvampatti (10.9322942°N, 76.7273448°E) and consisted of a small residential cluster with livestock feedstock, providing a microenvironment characterized by household emissions and organic matter. The commercial site was situated along Siruvani Main Road, Karunya Nagar (10.9383783°N, 76.7418690°E), a roadside business zone with continuous vehicular traffic and elevated pedestrian activity, serving as a hub of anthropogenic aerosol generation. Satellite world views were documented on January 17, 2025, and April 6, 2025, to capture seasonal conditions at the study locations visually. These contrasting temporal snapshots provided the environmental framework for assessing seasonal variability in airborne bacterial and fungal bioaerosols.

2.2 Sample collection protocol

Airborne microbial sampling was conducted during two distinct seasons to capture temporal variability in bioaerosol concentrations. The number of observations (duplicate samples per site per season) was selected to match field feasibility, standard bioaerosol sampling protocols, and equipment availability. The design allowed controlled seasonal contrasts despite limited replication. All samples were collected outdoors, 1.5 m above ground. Indoor activities (e.g., cooking, livestock feeding) may contribute to outdoor microbial loads; however, the sampling was conducted strictly outdoors. Climatic variables (temperature and relative humidity) were recorded during sampling for comparison. Sampling followed a systematic, fixed-point approach, with duplicate runs conducted at predetermined coordinates within each microenvironment. The winter sampling was carried out on January 17, 2025, under ambient conditions of 29 °C temperature and 54% relative humidity. The summer sampling was conducted on April 6, 2025, when the temperature was 35 °C and the relative humidity was 49%. These two seasonal points were selected to represent typical cool-dry and hot-dry climatic conditions of Coimbatore's rural belt.

In each of the four study locations, bioaerosol collection was conducted using standardized procedures to ensure the reproducibility and reliability of the data. The air was sampled with a calibrated MicroBio MB1 air sampler that uses impaction to collect airborne microorganisms onto agar media. All samplings were done around the same time of day to reduce variability and at a consistent sampling time, which corresponds to the human breathing zone. At both sites and across seasons, a sample was collected twice to account for site-specific variation. The open Petri dishes were covered with parafilm, placed in insulated containers, and transported to the microbiology laboratory within 2 h of sampling to avoid desiccation or contamination. The protocol ensured that a consistent determination of seasonal effects on airborne microbial load was possible and that the measure was consistent across locations. This process generated similar datasets for winter and summer by controlling volume, height, and sampling

duration, followed by analysis of bacterial and fungal community structure and AMR profiles.

2.3 Instrumentation

The MicroBio MB1 air sampler (Cantium Scientific, United Kingdom), a single-stage impactor specifically designed to quantify airborne microorganisms in the atmosphere and clinical practice, was used to sample airborne organisms. The working principle is that the instrument passes a specified amount of air through a perforated lid and directs the particles onto the surface of an agar plate, enabling enumeration of viable airborne bacteria and fungi. All runs were scaled to a volume of 0.2 m³ (200 L), which is appropriate for recovering culturable microorganisms without overloading the agar surface. The sampler was set at 1.5 m above ground level, which is within the approximate human breathing zone, making it relevant to respiratory exposure measurements. The instrument was placed on a tripod to reduce interference and vibration by the operator during sampling. There was consistency in sampling time across sites and seasons, following the manufacturer's operating protocols, resulting in the best recovery rates. The standard 90 mm Petri dish size used by the MB1 gave a real area of exposure of the sample during a sampling run of ~28.3 cm². Agar plates and sampler heads were sterilised before going to the field to avoid cross-contamination between sites. Precision and reproducibility were achieved by using the MicroBio MB1 at all sampling sites, and calibration of the instrument against known airflow rates provided a reliable method for converting colony counts to airborne microbial concentrations of colony-forming units per cubic meter (CFU m⁻³).

2.4 Sampling procedure

The air sampler was mounted on a tripod at 1.5 m above the ground, in the human breathing zone, at each of the four study sites during both sampling seasons. A total of 24 sampling events were conducted across the four study locations (three events per site). For each sampling event, microbial enumeration was performed using four bacterial plates and four fungal plates, and CFU/m³ values were calculated from the average colony counts obtained from the duplicate plates. Sterile 90 mm Petri dishes, filled with suitable culture media, were fitted onto the MicroBio MB1 air sampler before activation. Enumeration of total culturable bacteria was performed using Nutrient Agar (NA), and selective recovery of fungi was performed using Potato Dextrose Agar (PDA) supplemented with antibacterial agents. For each site, duplicate runs were performed to improve reproducibility and account for micro-scale spatial variability. Each run collected 0.2 m³ of air, yielding a total of 0.4 m³ per site per season. The effective surface area exposed to the impaction head during each run was approximately 28.3 cm², ensuring uniform particle deposition across the agar surface.

Immediately after air exposure, Petri plates were carefully sealed with parafilm to prevent post-sampling contamination and transported to the laboratory in insulated boxes maintained under cool conditions. The transport time from the field to the laboratory was kept to 2 h to preserve microbial viability for accurate

quantification. This standardized procedure enabled direct comparison of bacterial and fungal loads across sites and seasons while ensuring consistency in sampling height, air volume, and plate handling. By maintaining rigorous aseptic conditions and duplicate sampling, the methodology provided reliable data for seasonal and spatial assessment of rural airborne bioaerosols.

2.5 Quantification and isolation of airborne microorganisms

Following sample collection, exposed agar plates were incubated under standardised conditions to facilitate the enumeration of viable airborne bacteria and fungi. For bacterial cultures, plates containing NA were incubated at 37 °C for 24–48 h. Colony growth was monitored, and counts were recorded either manually or using a digital colony counter. For fungal cultures, PDA plates were incubated at 25 °C for 3–7 days. To account for variation in fungal growth rates, colonies were recorded at both Day 3 and Day 7, ensuring accurate quantification of both fast- and slow-growing fungi.

The microbial concentrations were expressed as colony-forming units per cubic meter of air (CFU m⁻³). This was calculated by multiplying the number of colonies per plate by a standard conversion factor of 5, corresponding to a sampled air volume of 0.2 m³ per run. The use of duplicate plates per site and season ensured the reliability and reproducibility of results. CFU/m³ values were calculated using the average colony count from duplicate plates for each sampling event.

In addition to enumeration, morphologically distinct colonies were subcultured onto fresh media to obtain pure isolates for further characterisation. Bacterial colonies were streaked onto NA plates, while fungal colonies were transferred onto PDA plates. The streak-plate method was employed to ensure single colony isolation, enabling subsequent characterisation and identification of the predominant airborne microorganisms.

2.5.1 Rationale for using culture-based enumeration

In the present study, culture-based approaches have been adopted since these techniques remain globally standardized and widely accepted for enumerating viable airborne bacteria and fungi in field-based bioaerosol research. These methods allow for the direct enumeration of CFU m⁻³ as informative, exposure-relevant data for public health assessment under environmentally realistic conditions. Culture-based enumeration is also compatible with downstream morphological characterization, MALDI-TOF MS identification, and antimicrobial susceptibility testing, all of which require viable isolates. High-throughput sequencing approaches, such as 16S rRNA/ITS amplicon sequencing, could not be included due to resource and logistical constraints; however, culturable organisms were emphasized, as they represent the active, biologically relevant fraction most likely to survive, disperse, and contribute to the dissemination of AMR in rural air. Hence, culture-based enumeration has been selected to ensure methodological consistency, feasibility, and compatibility for assessing viable airborne microbial hazards in rural environments.

2.6 Microscopic characterization of isolates

Pure isolates obtained from subculturing were subjected to standard staining techniques for preliminary characterization. For bacterial isolates, Gram staining was performed. Thin smears of bacterial culture were prepared on clean glass slides, heat-fixed, and sequentially stained with crystal violet, Gram's iodine, 95% ethanol, and safranin. The slides were observed under oil immersion at 1,000× magnification using a compound microscope. This procedure allowed the determination of cell morphology (cocci or bacilli) and Gram reaction (positive or negative). For fungal isolates, Lactophenol Cotton Blue (LPCB) staining was employed. Small fragments of fungal mycelium were placed on a glass slide, treated with LPCB stain, and covered with a coverslip. The preparations were examined under light microscopy at 400× magnification to visualise structural features, including hyphae, conidiophores, spore arrangement, and septation patterns. These staining methods provided the morphological basis for further molecular identification of the isolates.

2.7 Molecular identification of isolates

Superior isolates of bacteria and fungi were identified by MALDI-TOF Mass Spectrometry (MS). The study was conducted in Microbiological Laboratories, R.S. Puram, Coimbatore, Tamil Nadu, India, using a FLEX-PC Microflex mass spectrometer. New colonies were then selected and inoculated onto a MALDI target plate. All samples were spotted on a suitable matrix solution to ease ionisation. The ready target was then placed in the MALDI-TOF system. The spectral fingerprints of each isolate were compared with the instrument reference database to identify isolates at the species level, enabling fast, reliable analysis. The reason for choosing this method is that it offers high throughput, culture-based accuracy, and reproducibility, making it appropriate for differentiating airborne microbial isolates from environmental samples.

2.8 Antimicrobial susceptibility testing (AST)

The susceptibility of bacteria and fungi to antimicrobial agents was assessed using the Kirby-Bauer disc diffusion test, according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2025) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). AST was performed only on dominant isolates identified by MALDI-TOF: *Bacillus cereus*, *Bacillus flexus*, *Staphylococcus* spp. (bacteria), and *Aspergillus* spp., *Penicillium* spp. (fungi). For bacterial isolates, overnight cultures were prepared and standardized to 0.5 McFarland turbidity. The bacterial suspension was spread evenly on Mueller-Hinton Agar (MHA) using a sterile cotton swab. The agar surface was inoculated antiseptically with antibiotic discs containing chloramphenicol (30 µg), tetracycline (30 µg), ciprofloxacin (5 µg), and erythromycin (15 µg). The plates were incubated at 37 °C for 24 h, after which the areas of inhibition (ZOI) were measured in millimeters using a digital Vernier scale.

For fungal isolates, spores were picked in sterile saline containing Tween-80 and diluted to a 0.5 McFarland standard.

TABLE 1 CFU of airborne bacteria and fungi during winter (January 2025) and summer (April 2025).

Site type	Bacteria (CFU m ⁻³)		Fungi (CFU m ⁻³)	
	Winter	Summer	Winter	Summer
Agricultural	370	315	70	45
Commercial	880	785	35	10
Tribal	635	515	115	90
Domestic	410	720	80	120

The suspension was uniformly swabbed on both PDA and RPMI agar plates. Antifungal discs that were tested consisted of itraconazole (10 µg), amphotericin B (20 µg), clotrimazole (10 µg), and fluconazole (25 µg). The plates were incubated at 25 °C–30 °C for 48–72 h, and the zones of inhibition were measured in millimeters. This standardized assay allowed the evaluation of susceptibility and resistance patterns of airborne bacterial and fungal isolates to the most commonly used antibiotics and antifungals, yielding reproducible, comparable results across locations and seasons. This study focused on phenotypic resistance patterns; genetic determinants of resistance (e.g., *mecA*, *erm*, β -lactamase genes) could not be assessed. We acknowledge that genetic assays would provide deeper insight into horizontal gene transfer and environmental mechanisms of AMR.

3 Results and discussions

3.1 CFU

Table 1 gives the CFU concentrations of bacteria and fungi recorded across the four microenvironments during the winter season. Bacterial concentrations were consistently higher than fungal levels at all sites, with commercial areas showing the maximum load of 880 CFU m⁻³, followed by tribal with 635 CFU m⁻³, domestic with 410 CFU m⁻³, and agricultural zones with 370 CFU m⁻³. Fungal concentrations were comparatively lower, with the tribal area recording the highest value of 115 CFU m⁻³, followed by domestic (80 CFU m⁻³), agricultural (70 CFU m⁻³), and commercial sites (35 CFU m⁻³). These values indicate that winter air quality in rural Coimbatore is dominated by bacterial aerosols, particularly in locations with greater human activity.

Table 1 also gives the CFU values recorded during the summer season. A general decline in bacterial concentrations was observed compared with winter, except in the domestic environment, where bacterial counts rose sharply to 720 CFU m⁻³, the second-highest among the studied zones. Commercial areas continued to exhibit the highest bacterial concentrations (785 CFU m⁻³), followed by tribal (515 CFU m⁻³) and agricultural sites (315 CFU m⁻³). In contrast, fungal concentrations exhibited more pronounced site-specific increases in summer. Domestic zones recorded the maximum fungal load (120 CFU m⁻³), while tribal areas remained relatively high (90 CFU m⁻³). Agricultural sites showed moderate fungal levels (45 CFU m⁻³), and commercial regions registered the lowest (10 CFU m⁻³).

From both tables, three consistent trends are evident. First, higher bacterial concentrations confirmed their dominance in rural airborne microbial communities. Second, commercial zones emerged as the bacterial hotspots, maintaining the highest loads in both winter and summer. Third, fungal concentrations exhibited seasonal variation, with tribal areas showing winter peaks and domestic areas reaching summer maxima (Bowers et al., 2013). These observations confirm the influence of seasonal and microenvironmental factors on airborne microbial distribution and highlight the significance of commercial and domestic sites in determining overall microbial exposure risks in rural Coimbatore.

3.2 Gram staining

Gram staining of bacterial isolates collected from the four rural microenvironments consistently demonstrated the predominance of Gram-positive organisms, which retained the crystal violet stain and appeared purple under oil immersion (Figure 1). These isolates were identified as either cocci or bacilli, with site-specific and seasonal variations in abundance and arrangement that reflected the environmental characteristics of each location. Bacterial isolates from the tribal site were small Gram-positive bacilli with scattered cocci, which are usually found in clumps and short chains. Their morphology and staining pattern are similar to those of the *Bacillus cereus*, a sporing bacterium that can withstand changing environmental conditions in forest-bordering rural areas. Their ecological plasticity is evidenced by their survival in winter and summer, highlighting their adaptability to both highly fertile soils and low-activity environments. Agricultural isolates showed a higher proportion of long Gram-positive bacilli, singly positioned, and fewer cocci in short chains. These characteristics are common to *Bacillus pumilus* and *B. cereus*, as confirmed by MALDI-TOF. Crop residues, soil organic matter, and agricultural activity largely determine their presence. Comparison of summer and winter revealed a slight increase in staining intensity and bacillary population density in summer, which higher microbial growth rates can explain during warmer, drier seasons (Bekhit et al., 2025).

The commercial location showed the most heterogeneous and densely populated areas when observed under a microscope. Gram-positive cocci, frequently in groups like those of *Staphylococcus*, with some scattered bacilli, dominated the winter. Both cocci and bacilli were found in equal numbers in summer, with an evident increase in concentration. This morphologic heterogeneity is consistent with the increased CFU counts observed at commercial sites. It could be explained by increased human activity, motorised emissions, and the resuspension of dust particles carrying human-related bacteria. A seasonal shift was present in domestic samples. Gram-positive cocci dominated the fields in winter, forming clusters, a pattern typical of *Staphylococcus* and *Micrococcus* species, which are common in the critical human environment and on the skin. Conversely, the summer samples showed a more mixed profile, with cocci persisting, but bacilli of varying lengths were also present. The greater diversity can also be associated with higher humidity and poor ventilation in rural houses, creating an environment where microbes can survive and thrive (Almatawah et al., 2022; Rakshith et al., 2025).

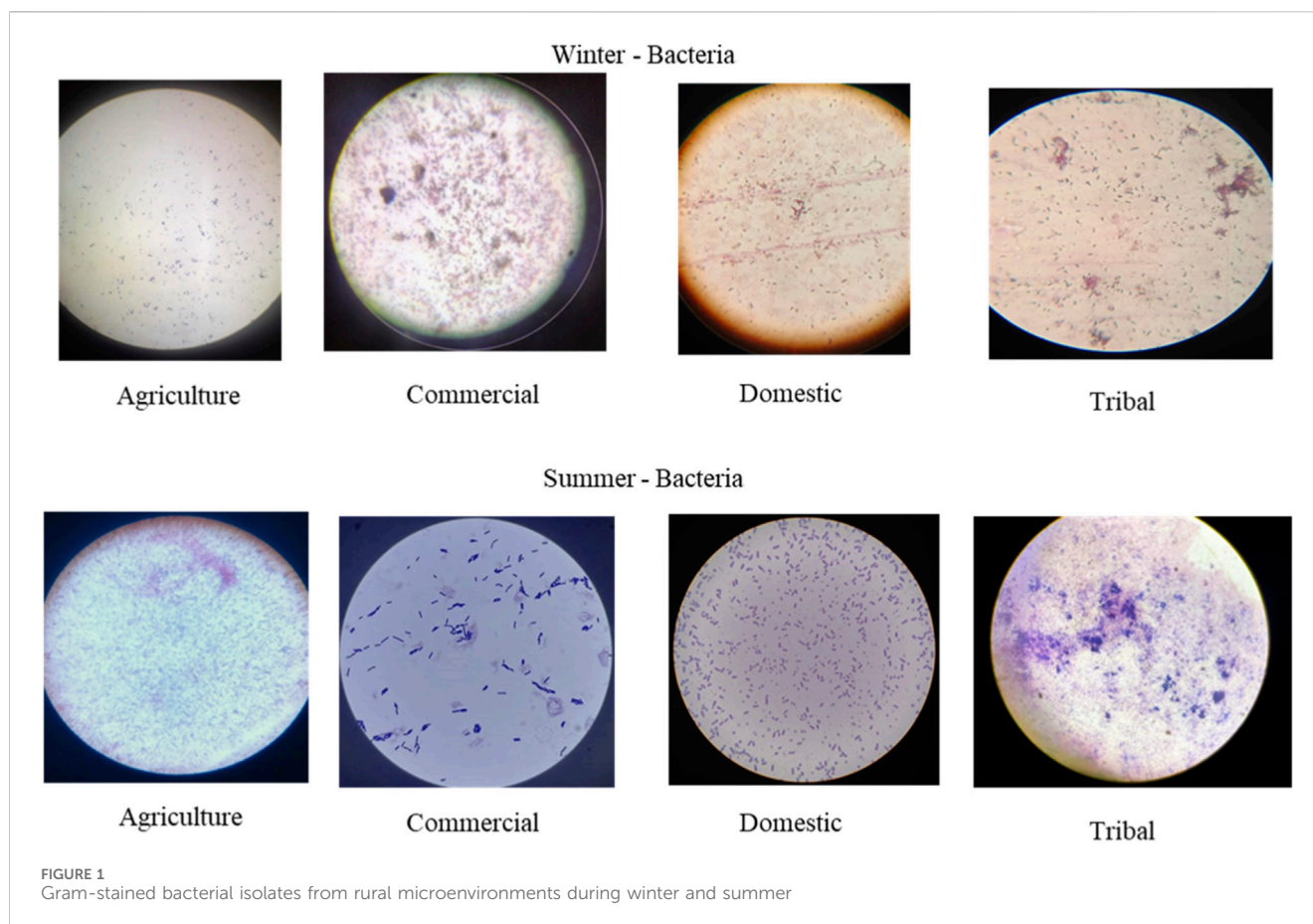


FIGURE 1
Gram-stained bacterial isolates from rural microenvironments during winter and summer

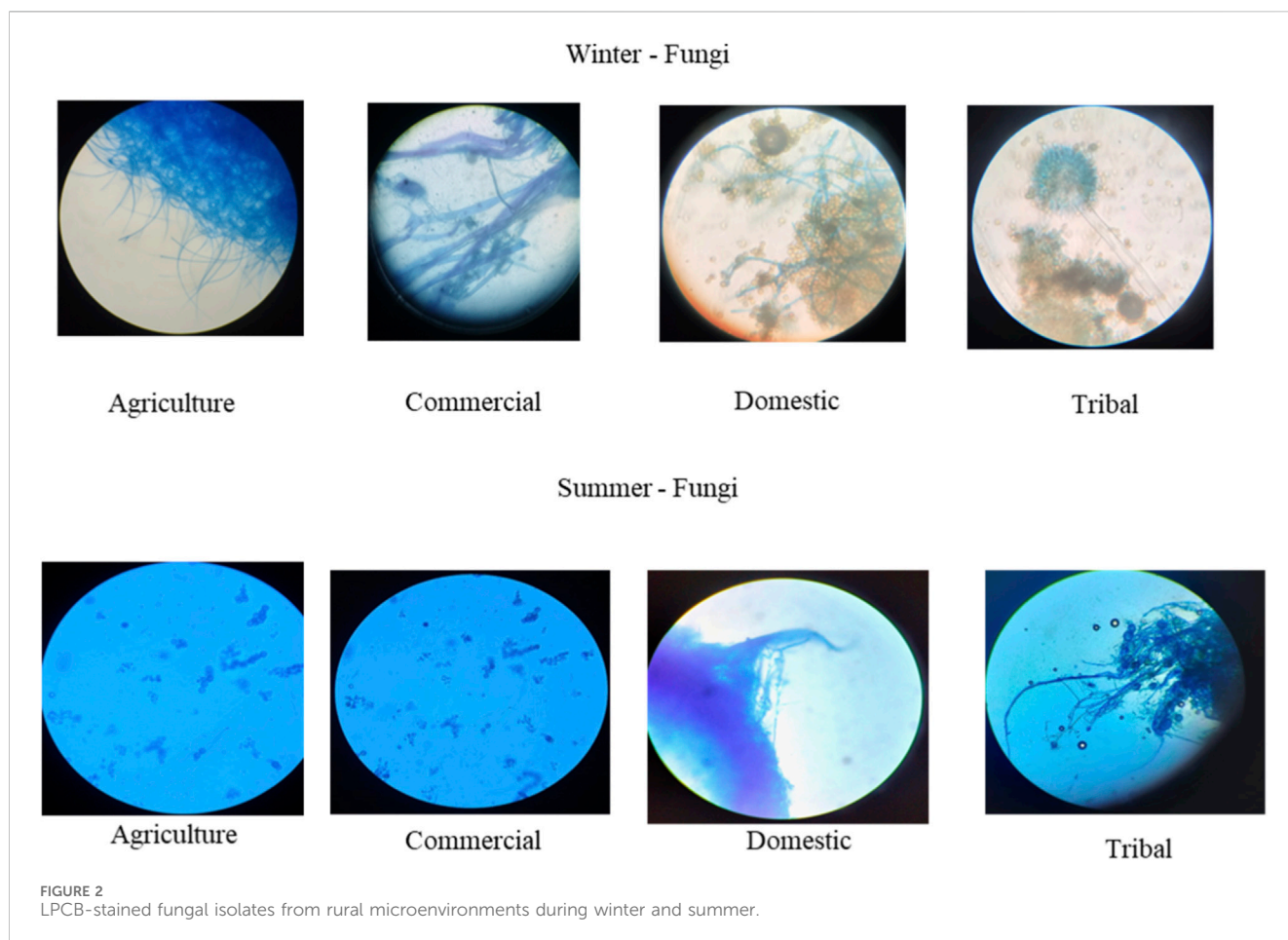
LPCB staining provided clear visualisation of the hyphal and conidial structures of airborne fungi isolated from the rural microenvironments, confirming the predominance of filamentous fungi belonging to the genera *Aspergillus* and *Penicillium*, along with site-specific differences in sporulation intensity and morphology as shown in Figure 2. In the agricultural samples, long, septate hyphae with dense branching and well-developed conidiophores were observed, indicative of *Penicillium oxalicum* and *Aspergillus* species. Their morphology reflects the strong soil–crop association of agricultural bioaerosols, where organic matter supports fungal growth (Lone, 2025). In commercial environments, LPCB staining revealed compact spore heads and rough conidiophores characteristic of *Penicillium citrinum* and *Aspergillus parasiticus*. The dense sporulation observed here is consistent with higher dust and vehicular activity, which favour the dispersal of spores into the atmosphere.

Domestic samples presented a mixed fungal profile. Winter isolates exhibited smaller spore clusters with limited branching, while summer isolates showed more abundant sporulation, with *Aspergillus parasiticus* being dominant (Andersson Aino et al., 2022). The increased hyphal density and sporulation in summer suggest that indoor humidity and ventilation constraints strongly enhance fungal persistence in residential environments. In tribal sites, LPCB staining highlighted conidial heads typical of *Aspergillus terreus*, with roughened conidiophores and spherical conidia (Henß et al., 2022). These structures were more pronounced in summer,

reflecting the suitability of forest margins and humid surroundings for fungal proliferation. Seasonal comparison across all sites showed that winter isolates often had thinner hyphae and weaker sporulation, whereas summer isolates consistently exhibited abundant, mature conidiophores and denser spore arrangements.

3.3 MALDI-TOF analysis

Representative MALDI-TOF spectra of bacterial isolates from the four rural environments are shown in Figures 3a–d. Each spectrum produced reproducible peak clusters primarily within the 3,000–12,000 m/z window, which is characteristic of ribosomal proteins and suitable for species-level microbial identification. Distinct differences in spectral fingerprints were observed between sites, highlighting spatial variation in airborne microbial composition. The domestic environment spectrum, as given in Figure 3a, displayed its dominant peaks at 3,444.1, 4,293.9, 5,252.8, 6,665.2, 7,466.8, 9,950.3, and 11,129.7 m/z. The most intense signal was recorded at 5,252.8 m/z, with an amplitude of 1.08×10^4 a. u. The presence of secondary high-mass peaks at 12,275.6 and 15,405.9 m/z further confirmed the protein complexity of the isolate. This fingerprint matched *Rhodococcus rhodochrous*, a species often associated with soil and dust particles that can infiltrate indoor environments (Singhal et al., 2015).

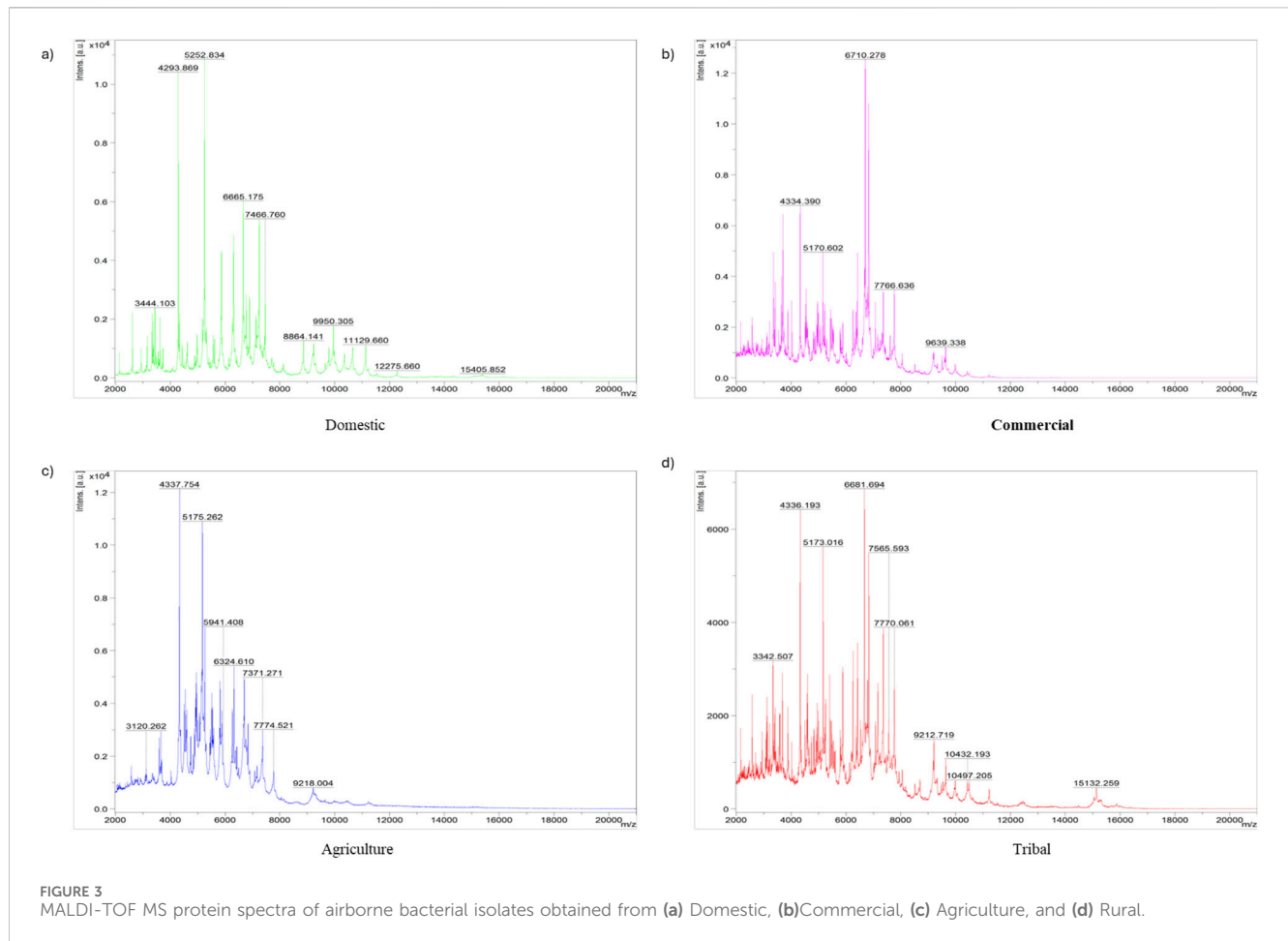


The commercial site spectrum, as shown in [Figure 3b](#), exhibited a distinct profile dominated by peaks at 4,334.4, 5,170.6, 6,710.3, 7,766.6, and 9,639.3 m/z. Among these, the most intense peak occurred at 6,710.3 m/z, with an amplitude of 1.24×10^4 a. u., which is a defining marker of *Bacillus flexus*. The strong dominance of the 6,700 m/z region distinguishes this isolate from others, reflecting its adaptation to outdoor environments with high human and vehicular activity. The relatively narrow cluster of peaks compared to domestic isolates indicates a lower protein diversity, which is typical of spore-forming *Bacillus* species well adapted to survive in fluctuating air conditions of commercial roadsides. The agricultural spectrum, as illustrated in [Figure 3c](#), showed high-intensity peaks at 3,120.3, 4,337.8, 5,175.3, 5,941.4, 6,324.6, 7,371.3, and 7,774.5 m/z, with an additional peak at 9,218.0 m/z. The most prominent signals were observed at 4,337.8 and 5,175.3 m/z, each exceeding 1.0×10^4 a. u. These markers are consistent with *Bacillus cereus*, which has been repeatedly associated with soil, crop residues, and agricultural aerosols. The broad distribution of peaks, particularly those between 5,900 and 7,700 m/z, reflects the ribosomal heterogeneity of this species. Its consistent presence in farming zones can be explained by the constant release of spores during soil-plant interactions, making *B. cereus* a dominant airborne bacterium in agricultural landscapes. The tribal environment spectrum, as depicted in [Figure 3d](#), red showed a different pattern, with significant peaks recorded at 3,342.5, 4,336.2, 5,173.0, 6,681.7,

7,565.6, 7,770.1, and 9,212.7 m/z, along with higher-mass peaks at 10,432.2, 10,497.2, and 15,132.3 m/z. The most prominent peak was observed at 6,681.7 m/z, with an amplitude of $\sim 6.8 \times 10^3$ a. u. The profile again corresponded to *Bacillus cereus*, confirming its ecological resilience across both agricultural and tribal sites. The presence of additional peaks above 10,000 m/z indicates the protein complexity of these isolates, consistent with the influence of the natural forest margins surrounding the tribal settlements.

The MALDI-TOF MS fingerprints obtained in this study are consistent with previously published environmental spectral profiles. Earlier work has shown that airborne and soil-associated *Bacillus* spp. typically display dominant ribosomal protein peaks within the 3,000–12,000 m/z range, matching the spectral window observed in our isolates ([Ha et al., 2019](#)). Similarly, characteristic spectral markers of the *Rhodococcus* spp. reported in environmental aerosols support our species-level assignments ([De Alegria Puig et al., 2017](#)). Among airborne fungi, *Aspergillus* species are known to produce reproducible peptide/protein signatures in this same mass range, consistent with our fungal spectral clusters ([De Carolis et al., 2012](#)). Incorporating these comparisons strengthens the reliability of our MALDI-TOF-based identification and confirms that the protein spectral patterns observed in rural air are consistent with established environmental microbial profiles.

The fungal isolates exhibited reproducible spectral clusters primarily within the 3,000–15,000 m/z region, in agreement with



published fungal MALDI-TOF MS spectral windows (Chalupová et al., 2014).

3.4 Resistivity test

3.4.1 Antibiotic susceptibility of bacterial isolates

The antibiotic susceptibility of bacterial isolates is presented in Figure 4. Distinct seasonal and spatial variations were observed in inhibition profiles across agricultural, commercial, domestic, and tribal environments. In agricultural sites, inhibition zones were relatively low during winter, ranging from 15 mm for chloramphenicol to 30 mm for ciprofloxacin, with complete resistance to erythromycin. However, in summer, the identical isolates displayed greater sensitivity, with zones of 35 mm for chloramphenicol, 42 mm for tetracycline, 34 mm for ciprofloxacin, and 28 mm for erythromycin. This seasonal improvement in susceptibility suggests a dynamic shift in bacterial populations under warmer, drier conditions, leading to reduced resistance traits. Commercial isolates showed broader susceptibility in both seasons but remained stable in their resistance to erythromycin. Winter isolates demonstrated inhibition zones of 33 mm for chloramphenicol, 26 mm for tetracycline, 28 mm for ciprofloxacin, and 25 mm for erythromycin. In comparison, summer values included 30 mm for chloramphenicol, 34 mm for tetracycline, and 30 mm for

ciprofloxacin, with no inhibition for erythromycin. The persistent resistance to erythromycin across seasons emphasizes its limited therapeutic potential in environments with high anthropogenic activity.

Domestic isolates exhibited extreme variability between antibiotics and seasons. During winter, they were susceptible to chloramphenicol (40 mm) and ciprofloxacin (40 mm), but entirely resistant to tetracycline and erythromycin. In summer, the identical isolates demonstrated susceptibility to all antibiotics tested, with inhibition zones of 37 mm for chloramphenicol, 18 mm for tetracycline, 27 mm for ciprofloxacin, and 30 mm for erythromycin. This seasonal transition suggests selective antibiotic pressure within enclosed domestic settings, where microbial communities are more prone to fluctuation. Tribal isolates displayed moderate inhibition during winter (24 mm chloramphenicol, 20 mm tetracycline, 27 mm ciprofloxacin) but complete resistance to erythromycin. By summer, resistance intensified, with no inhibition observed for chloramphenicol and erythromycin, while tetracycline (34 mm) and ciprofloxacin (30 mm) maintained activity. The complete lack of inhibition by two antibiotics underscores the emergence of multidrug resistance in tribal environments, raising concerns about environmental exposure and the potential misuse of antimicrobials (Ghodsi et al., 2025).

These resistance patterns are consistent with environmental AMR trends reported in earlier studies. The erythromycin

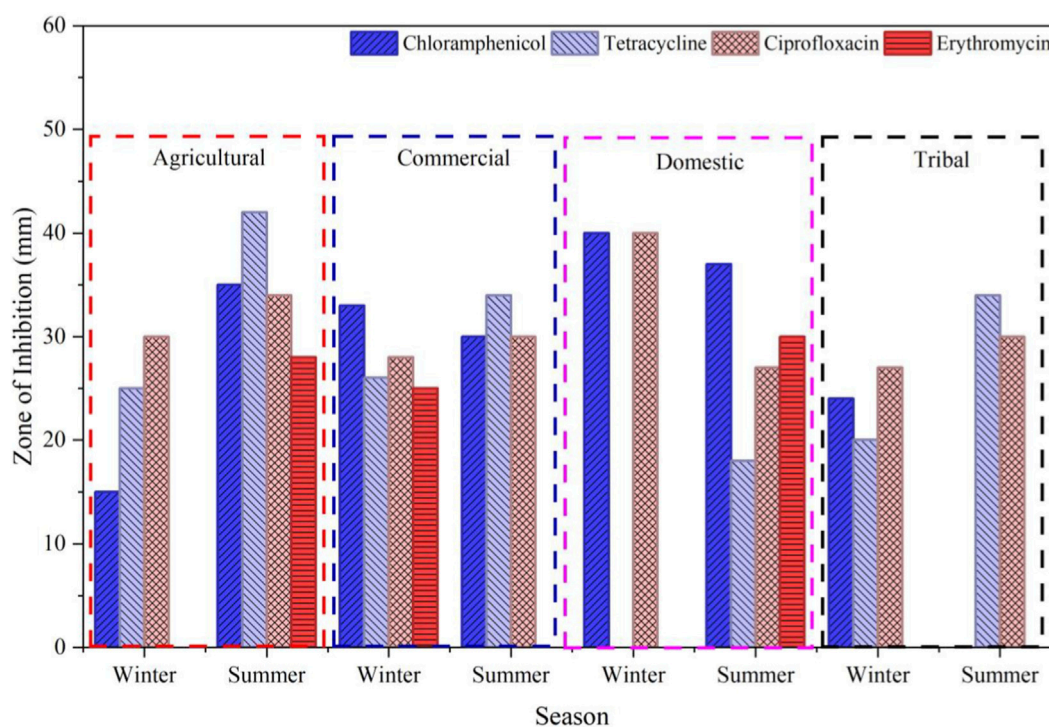


FIGURE 4
Seasonal antibiotic susceptibility of bacterial isolates from agricultural, commercial, domestic, and tribal environments, expressed as inhibition zone diameters (mm).

resistance observed aligns with previous evidence that rural households, livestock waste, and domestic wastewater streams contribute macrolide residues into the environment, which may exert selective pressure on airborne microbes (Polianciuc et al., 2020). Similarly, our finding of universal resistance to amphotericin B aligns with reports showing intrinsic polyene resistance in environmental *Aspergillus* and *Penicillium* isolates (Ghodsi et al., 2025). Fungal resistance patterns observed in our study are consistent with earlier indoor environmental studies, indicating that ecological *Penicillium* and *Aspergillus* species exhibit intrinsic tolerance to certain antifungal classes (Akhtar et al., 2025). These supporting studies reinforce the robustness and ecological relevance of the resistivity profiles reported in this study.

The disc diffusion assays provided direct visual confirmation of the antibiotic resistance and susceptibility patterns, as shown in Figure 5. The plates clearly illustrate the spatial and seasonal variations in bacterial responses to the four tested antibiotics. In agricultural isolates, winter plates showed small or absent halos around erythromycin discs, confirming complete resistance, while moderate zones were observed for chloramphenicol and ciprofloxacin. In summer, however, inhibition halos became more pronounced, particularly around tetracycline (42 mm) and chloramphenicol (35 mm), supporting the quantitative trends.

Commercial isolates consistently exhibited visible inhibition halos for chloramphenicol, tetracycline, and ciprofloxacin in both seasons, whereas erythromycin discs remained surrounded by dense bacterial growth with no zones. Domestic isolates displayed striking dual behavior: during winter, large inhibition zones were observed around chloramphenicol and ciprofloxacin discs (~40 mm), whereas

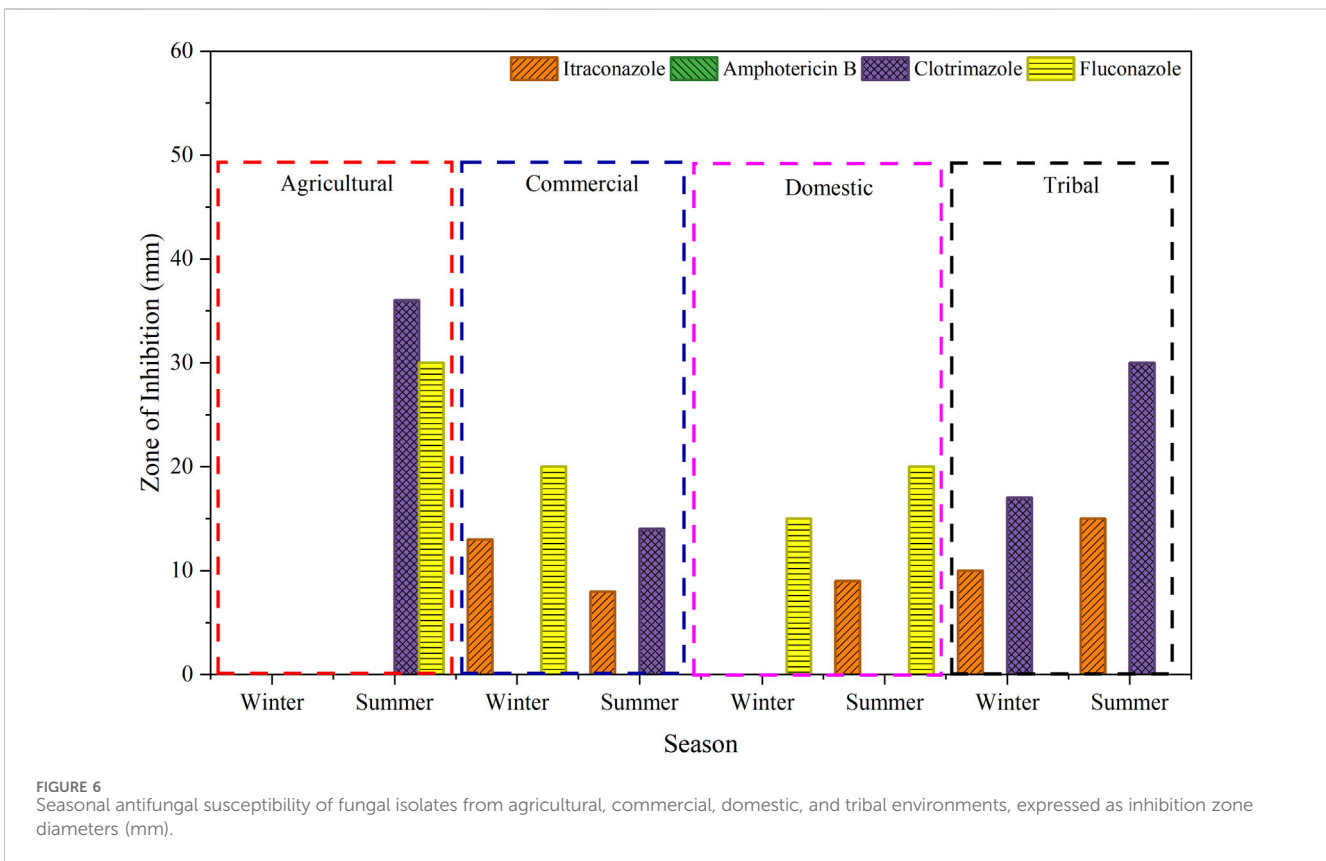
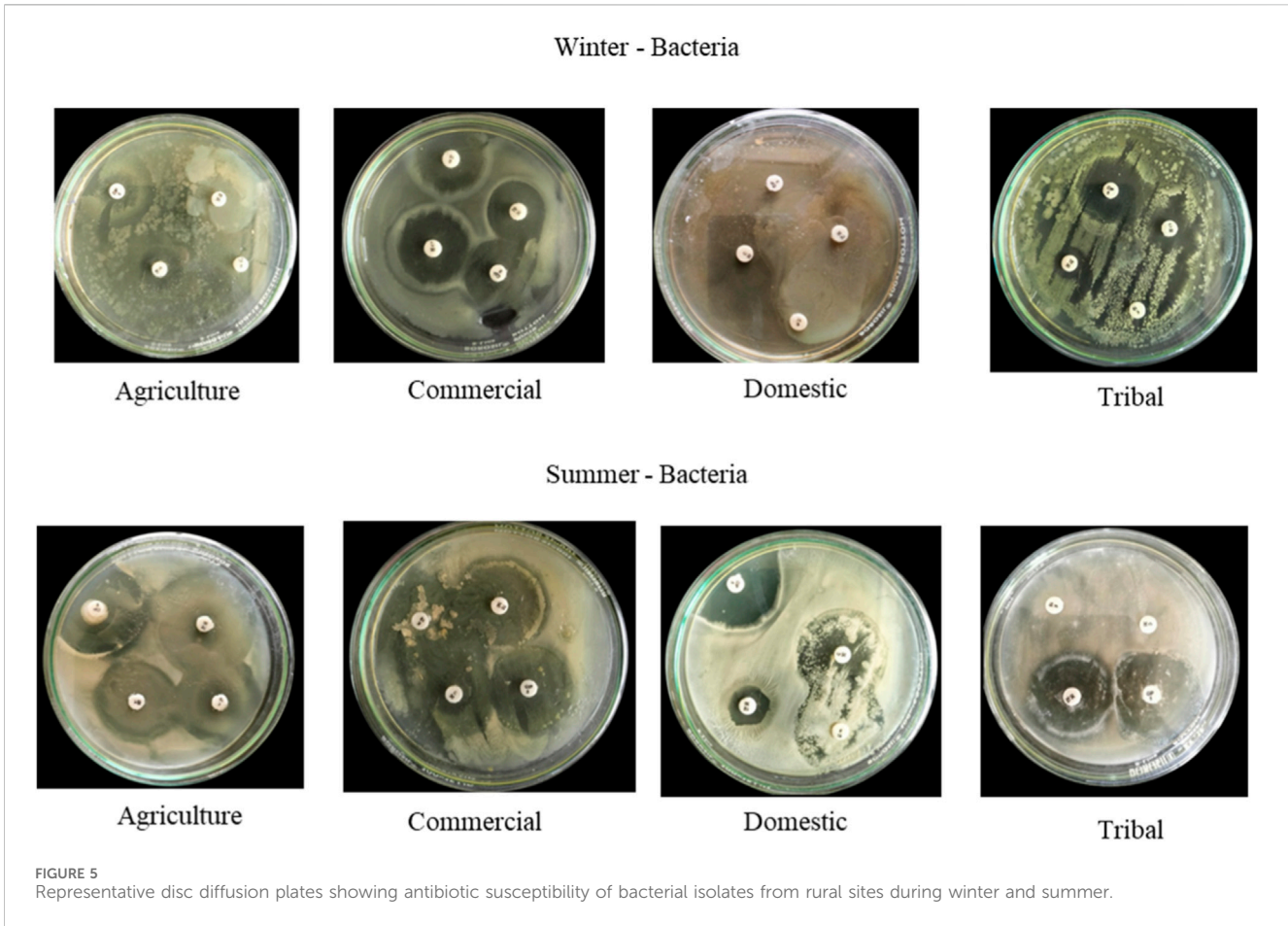
no activity was observed with tetracycline or erythromycin. By summer, however, inhibition halos became visible around all four antibiotics, albeit smaller for tetracycline (18 mm). Tribal isolates demonstrated reduced susceptibility overall, with small inhibition zones in winter (20–27 mm) and complete resistance to chloramphenicol and erythromycin in summer.

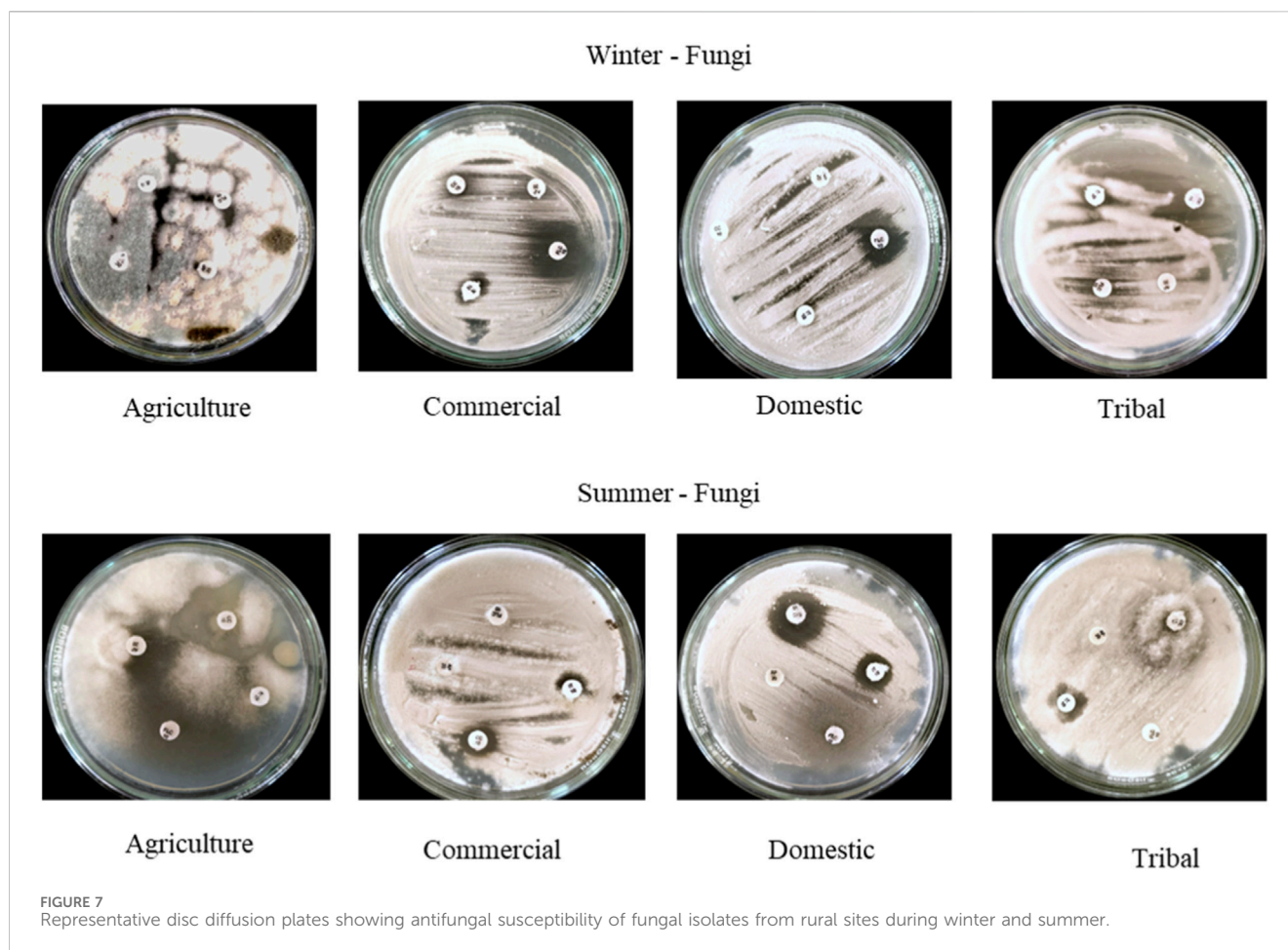
The visual evidence in Figure 4 supports the interpretation that ciprofloxacin and chloramphenicol remain the most effective antibiotics in rural settings, while erythromycin remains persistently ineffective. Seasonal changes, particularly in agricultural and domestic isolates, are also more apparent when comparing winter and summer plates.

3.4.2 Antibiotic susceptibility of fungal bacterial isolates

The antifungal susceptibility patterns are presented in Figure 6, which illustrates the seasonal variations in inhibition zone diameters across agricultural, commercial, domestic, and tribal environments. A consistent observation across all sites and seasons was the complete lack of amphotericin B activity, with inhibition zones remaining at 0 mm, confirming universal resistance to this critical antifungal agent.

In the winter season, fungal isolates exhibited widespread resistance. Agricultural samples showed no inhibition to any of the antifungals tested, reflecting total resistance. Commercial isolates demonstrated only moderate inhibition, with itraconazole at 13 mm and fluconazole at 20 mm, while clotrimazole was ineffective. Domestic isolates displayed minimal susceptibility, with fluconazole producing a 15 mm zone, whereas itraconazole





and clotrimazole showed no activity. Tribal isolates showed partial activity with 10 mm inhibition for itraconazole and 17 mm for clotrimazole, though fluconazole was ineffective. Overall, winter profiles confirmed strong resistance, with only limited site-specific responses.

In summer, a marked improvement in susceptibility was recorded, particularly in agricultural and tribal zones. Agricultural isolates demonstrated high inhibition zones of 36 mm for clotrimazole and 30 mm for fluconazole, contrasting sharply with their total resistance in winter. Tribal isolates also showed significant susceptibility, with 30 mm inhibition for clotrimazole and 15 mm for itraconazole, while fluconazole remained inactive. Domestic isolates displayed moderate sensitivity, with fluconazole producing a 20 mm zone and itraconazole producing a 9 mm zone, although clotrimazole showed no inhibition. Commercial isolates, however, remained largely resistant, with low inhibition values of 8 mm for itraconazole and 14 mm for clotrimazole.

The comparison between winter and summer clearly highlights that susceptibility to clotrimazole and fluconazole increases substantially in agricultural and tribal environments during the warmer season, while resistance remains dominant in commercial areas. The improved summer response may be attributed to changes in fungal community composition or to physiological stress under higher temperatures and lower

humidity, which can increase susceptibility to azole antifungals. The antifungal susceptibility assays are presented in [Figure 7](#), which provides direct visual confirmation of the resistance and sensitivity profiles shown in the bar chart. In winter, agricultural plates displayed complete fungal overgrowth around all discs, confirming resistance to itraconazole, clotrimazole, fluconazole, and amphotericin B. Commercial plates showed only faint halos for itraconazole and fluconazole (≤ 20 mm), while domestic isolates exhibited partial inhibition with fluconazole (~ 15 mm). Tribal isolates were moderately susceptible to clotrimazole (17 mm) and itraconazole (10 mm), though fluconazole remained ineffective.

Summer plates showed contrasting susceptibility profiles, with distinct, broader halos observed in agricultural and tribal isolates. Agricultural plates showed large inhibition zones exceeding 30 mm for clotrimazole and fluconazole, highlighting their effectiveness against summer fungal isolates. Tribal isolates also exhibited potent inhibition for clotrimazole (~ 30 mm) and moderate inhibition for itraconazole (~ 15 mm). Domestic plates revealed visible halos for fluconazole (~ 20 mm), while commercial isolates produced only small inhibition zones for itraconazole (8 mm) and clotrimazole (14 mm), confirming their persistence as resistant environments. Amphotericin B discs, however, consistently showed no inhibition across all plates and seasons, providing explicit visual confirmation of universal resistance. The visual patterns in [Figure 7](#) reinforce the

conclusion that clotrimazole and fluconazole are the most effective antifungals against rural airborne fungi, particularly in summer, while amphotericin B is entirely ineffective. The stark seasonal differences visible in these plates highlight the influence of environmental conditions on fungal susceptibility.

The observed bacterial and fungal loads, combined with resistance to erythromycin and amphotericin B, underscore the emerging public-health implications for rural communities. Continuous occupational exposure from agriculture, livestock handling, indoor biomass combustion, and proximity to forest margins may heighten inhalation risks. Furthermore, local antimicrobial usage patterns in livestock and humans may contribute to the environmental selection of resistant strains. Understanding these drivers within a One Health framework is essential for designing rural AMR surveillance and community-level interventions.

3.5 Study limitations

This study has several limitations. First, sampling was conducted on a single winter and a single summer day, providing seasonal contrasts but not long-term temporal trends. Multi-year or repeated within-season sampling would improve generalizability. Second, culture-based enumeration does not capture VBNC organisms or the full spectrum of environmental microbial diversity. Third, MALDI-TOF identification relies on available database spectra and may miss rare or unculturable ecological taxa. Fourth, the AMR assessment was limited to phenotypic disc diffusion without genetic confirmation of resistance determinants. Fifth, we acknowledge that a larger sample size and more extensive spatial replication would enhance generalizability. However, logistical and resource constraints restricted sampling to two seasonal snapshots. Plate-wise raw colony count data from each sampling round were not available for retrospective inclusion due to the completed nature of the study, and this is acknowledged as a methodological limitation. Finally, contextual variables such as antibiotic usage patterns in livestock, agricultural practices, and wind-driven dispersal were not measured but could influence spatial AMR patterns. These limitations should be addressed in future One Health bioaerosol studies.

4 Conclusion

Despite the study's temporal and methodological limitations, the findings provide an essential first baseline dataset on airborne microorganisms and AMR in rural South India. This study offers one of the first systematic evaluations of airborne bacterial and fungal bioaerosols across rural microenvironments in Coimbatore, Tamil Nadu, under environmentally relevant conditions. By combining seasonal sampling, culture-based enumeration, Gram and LCB staining, MALDI-TOF MS identification, and antimicrobial susceptibility testing, the findings clearly demonstrate that rural air is a significant reservoir of hazardous microorganisms and AMR.

Across both seasons, bacterial concentrations remained higher than fungal levels. These seasonal differences are

mainly driven by climatic factors—temperature, relative humidity, and wind speed, which influence microbial survival and dust resuspension. In commercial areas, higher vehicular load and human movement contribute to increased bacterial emissions, explaining the peak winter value (880 CFU m⁻³). Fungal patterns differed, with tribal areas showing higher winter levels due to cooler and more humid microclimates, while domestic environments recorded maximum summer counts (120 CFU m⁻³) likely from household activities, biomass cooking, and indoor moisture. Overall, both climate and site-specific human/vehicular activities shape the seasonal aerosol variations. Morphological characterization through Gram staining highlighted the predominance of Gram-positive bacilli and cocci, notably *Bacillus cereus*, *Bacillus pumilus*, and *Staphylococcus* spp., which are known for their environmental persistence and pathogenic potential. Fungal isolates identified through LPCB staining and MALDI-TOF analysis were dominated by *Aspergillus* and *Penicillium*, taxa frequently associated with respiratory allergies, mycotoxicosis, and nosocomial infections.

Antimicrobial susceptibility testing revealed concerning resistance patterns. Bacterial isolates displayed complete resistance to erythromycin across most sites and seasons. In contrast, fungal isolates exhibited universal resistance to amphotericin B. Seasonal susceptibility shifts were observed, with ciprofloxacin and chloramphenicol remaining broadly effective against bacteria, and clotrimazole and fluconazole demonstrating higher activity against fungi during summer. These findings highlight the adaptive nature of rural microbial populations and their potential role in disseminating AMR under natural climatic conditions. Taken together, this study establishes that rural bioaerosols, though less studied than their urban or hospital counterparts, pose risks comparable to those of their urban or hospital counterparts. Their dual role as infectious agents and reservoirs of AMR underscores the urgent need for integrated surveillance, especially in developing regions where human populations live in proximity to agricultural, domestic, and tribal environments. Continuous monitoring and incorporation of rural bioaerosols into national AMR mitigation strategies are vital to safeguard environmental and human health.

Data availability statement

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

Author contributions

HS: Conceptualization, Software, Investigation, Writing – review and editing, Funding acquisition, Resources, Writing – original draft, Project administration, Methodology, Visualization, Validation, Supervision, Formal Analysis, Data curation. SG: Project administration, Visualization, Data curation, Supervision, Formal Analysis, Validation, Methodology, Investigation, Conceptualization, Writing – review and editing, Funding acquisition, Software, Writing – original draft,

Resources. JV: Writing – original draft, Investigation, Software, Writing – review and editing, Resources, Funding acquisition, Visualization, Methodology, Formal Analysis, Validation, Conceptualization, Project administration, Data curation, Supervision. BR: Resources, Investigation, Conceptualization, Writing – review and editing, Funding acquisition, Supervision, Writing – original draft, Visualization, Project administration, Validation, Data curation, Software, Methodology, Formal Analysis. LA: Supervision, Methodology, Project administration, Validation, Investigation, Data curation, Conceptualization, Writing – review and editing, Software, Funding acquisition, Formal Analysis, Writing – original draft, Resources, Visualization. ER: Writing – original draft, Conceptualization, Writing – review and editing, Investigation. DS: Conceptualization, Writing – review and editing, Writing – original draft. AB: Writing – original draft, Investigation, Conceptualization, Writing – review and editing. YH: Conceptualization, Writing – original draft, Data curation, Writing – review and editing. C-HH: Methodology, Visualization, Data curation, Validation, Project administration, Conceptualization, Supervision, Software, Investigation, Formal Analysis, Writing – original draft, Resources, Funding acquisition, Writing – review and editing.

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Conflict of interest

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