Research Square

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Airborne bacterial diversity and antimicrobial resistance in restaurant environments in southwestern Nigeria

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Abstract

Background

Food service environments may favour the airborne spread of antimicrobial-resistant bacteria. This study characterised airborne bacteria and their antimicrobial resistance in restaurants in the Ile-Ife–Ipetumodu area, southwestern Nigeria.

Methods

Air was sampled in 58 restaurants using settle plates (Trypticase Soy, MacConkey and Mannitol Salt Agar) exposed for 15 minutes during business hours. Isolates were identified biochemically and with Microbact™ kits. Susceptibility was tested by disc diffusion (CLSI) and by broth microdilution for vancomycin and teicoplanin. Phenotypic MRSA and ESBL producers were characterised by PCR, and factors associated with multidrug-resistant (MDR) bacteria were assessed by bivariate analysis.

Results

A total of 382 bacterial isolates were recovered, comprising 249 (65.2%) Gram-positive and 133 (34.8%) Gram-negative isolates. Bacterial loads ranged from 1.0×10^2 to 1.96×10^2 CFU/plate/15 min. *Staphylococcus aureus* predominated among Gram-positive isolates (152; 61%), while *Escherichia coli* was the most frequent Gram-negative pathogen (58; 43.6%). MRSA was confirmed in 23.7% of *S. aureus* isolates. Among Gram-negative isolates, 22.6% (30/133) were phenotypically ESBL-positive, while 20.3% (27/133) carried at least one of the ESBL-associated genes tested. MDR bacteria were recovered from 36/58 (62.1%) restaurants. High occupancy density, proximity to restrooms, inadequate ventilation, and high peak activity were significantly associated with MDR bacterial recovery ($p < 0.05$).

Conclusion

Restaurant air harbours diverse antimicrobial-resistant bacteria, including MDR pathogens. Improved ventilation and routine microbiological monitoring are warranted.

Introduction

The global rise of antimicrobial resistance (AMR) is no longer a distant prospect; it is a present-day public health emergency. Resistant microorganisms are rapidly spreading across clinical and community settings alike, and projections suggest that, if current trends remain unchecked, AMR-attributable deaths could reach 10 million annually by 2050 [1, 2]. Although much early scientific and policy attention centred mostly on healthcare-associated transmission, accumulating evidence now implicates the broader

environment as a niche and transmission route for antimicrobial-resistant bacteria and the resistance genes they carry [3, 4].

Among environmental matrices, indoor air represents one of the least studied yet most consequential [3]. Bacterial bioaerosols can be generated from diverse sources including human respiratory activity, contaminated surfaces, and aerosolised organic material. These bioaerosols may carry pathogenic and antimicrobial-resistant bacteria capable of spreading across enclosed spaces through inhalation and gravitational deposition [5]. This concern is particularly salient in urban settings, where individuals are estimated to spend approximately 90–95% of their daily time indoors [6]. Routine activities like breathing, speaking, coughing, and even walking are seen as continuous drivers of bacterial aerosol generation in confined environments [7].

Food service environments, particularly busy restaurants, represent a distinct and underappreciated category of indoor setting with elevated potential for bacterial bioaerosol accumulation. The convergence of high patron density, uninterrupted food preparation, and heterogeneous ventilation arrangements renders these spaces particularly susceptible to airborne microbial build-up [8]. Cooking activities release particulate matter and organic compounds [9], and airborne particulate matter can carry viable bacteria [10]. Consequently, restaurant indoor air may act as a potential niche for antimicrobial resistance for diverse bacterial populations, including organisms of direct public health significance [11].

These concerns are especially pertinent in Nigeria, where potentially pathogenic bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp. and *Pseudomonas aeruginosa*, have been recovered from the air of high-traffic urban settings [12], and where antimicrobials are routinely sold over the counter [13]. The presence of enteric organisms such as *Escherichia coli* in indoor air environments is particularly alarming, as it signals faecal contamination and heightened risk of foodborne disease transmission [14].

Despite a growing literature on environmental AMR, studies specifically examining bacterial bioaerosols in Nigerian food service environments as potential AMR reservoirs remain scarce. This study therefore aimed to characterise the diversity and antimicrobial resistance profiles of airborne bacteria recovered from food service environments in the Ile-Ife–Ipetumodu area of Osun State, southwestern Nigeria.

Materials and Methods

Study Design

This was an analytical cross-sectional study. Ethical approval was obtained from the Health Research Ethics Committee of Obafemi Awolowo University Teaching Hospital Complex (IPHOAU/12/1467). Written permission was obtained from the management of each participating restaurant before sampling commenced. Written informed consent was obtained from the management of each participating restaurant prior to sample collection.

Study Area

The study was conducted in the Ile-Ife–Ipetumodu area of Osun State, southwestern Nigeria. The study area is a major educational and commercial hub, accommodating tertiary institutions including Obafemi Awolowo University and Oduduwa University, whose substantial student and staff populations contribute to a large and active restaurant sector. The diversity of food service establishments in the area, which includes fully enclosed air-conditioned dining spaces, fan-assisted semi-enclosed premises, and open-fronted roadside kitchens, made it a representative and ecologically varied setting for this investigation (Fig. 1).

Selection of Sampling Sites

Fifty-eight restaurants were selected using a purposive sampling approach, on the basis of three pre-established criteria: (i) regular customer patronage (typically > 10 persons/hour during business hours); (ii) uninterrupted food preparation and service operations throughout the working day; and (iii) willingness of management to grant unrestricted access for sampling and administration of questionnaire. Selected restaurants were sampled across the study area to provide spatial representation of the restaurant environments included in the study. The establishments varied in physical size, seating capacity, and ventilation arrangement, encompassing naturally ventilated open spaces, fan-assisted environments, and enclosed premises with limited mechanical ventilation. Sampling was conducted in both the dining and kitchen areas of each establishment, as these represent the primary breathing environments for patrons, food handlers, and kitchen staff.

Air Sampling and Sample Processing

Air sampling was performed between December 2023 and April 2024 using the validated passive gravitational settle plate method [15, 16]. At each restaurant, sterile Petri dishes containing Trypticase Soy Agar (TSA), MacConkey Agar (MA), and Mannitol Salt Agar (MSA) (Oxoid Ltd., Basingstoke, UK) were simultaneously positioned at a height of approximately one metre above floor level [17]. Plates were exposed for 15 minutes during business hours to maximise bioaerosol capture. An unexposed set of plates was maintained as negative controls throughout sampling and transport to detect potential laboratory contamination. Following exposure, plates were immediately sealed, placed in insulated containers maintained at 4°C, and transported to the Department of Biological Sciences Laboratory, Oduduwa University Ipetumodu where they were processed within one hour of collection to preserve microbial viability.

Enumeration and Identification of Bacterial Isolates

Exposed plates were incubated aerobically at 37°C for 24 hours. Colonies were enumerated using a digital colony counter, and bacterial loads were recorded as colony-forming units per plate per 15-minute

exposure time (CFU/plate/15 min). Morphologically distinct colonies were sub-cultured on Nutrient Agar for pure culture isolation and subsequently identified using standard microbiological methods [18] and Microbact™ 12S/24E identification kits.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) was performed using the Kirby–Bauer disc diffusion method in accordance with CLSI [19] guidelines. The isolates were evaluated using two distinct panels: a Gram-negative susceptibility panel comprising ampicillin (10µg), amoxicillin–clavulanate (30µg), cefotaxime (30µg), ceftazidime (30µg), cefoxitin (30µg), meropenem (10µg), and trimethoprim (5µg), alongside the broad-spectrum overlap agents ciprofloxacin (5µg), nalidixic acid (30µg), tetracycline (30µg), chloramphenicol (30µg), streptomycin (30µg), and trimethoprim–sulfamethoxazole (25µg); and a Gram-positive panel consisting of cefoxitin (30µg), oxacillin (1µg), ceftaroline (30µg), erythromycin (15µg), azithromycin (15µg), clindamycin (2µg), linezolid (30µg), and trimethoprim–sulfamethoxazole (25µg), supplemented with the broad-spectrum agents (ciprofloxacin, gentamicin, streptomycin, tetracycline, and chloramphenicol) (Oxoid Ltd., Basingstoke, UK). Vancomycin and teicoplanin susceptibility profiles were determined via minimum inhibitory concentration (MIC) determination using the broth microdilution method in accordance with EUCAST breakpoints [20]. Methicillin resistance and ESBL production were assessed phenotypically using cefoxitin discs and the double-disc synergy test respectively.

Genomic DNA Extraction

Genomic DNA was extracted from presumptive staphylococcal isolates for amplification of the 16S rRNA, *nuc* and *mecA* genes. DNA was also extracted from phenotypically ESBL-positive Gram-negative isolates for detection of *bla*TEM, *bla*SHV, and *bla*CTX-M. DNA extraction was done by using a genomic DNA extraction kit (Promega Corporation, Madison, USA) according to the manufacturers' instructions under aseptic conditions. The concentration and purity of the extracted DNA were assessed spectrophotometrically (NanoDrop; DeNovix US). The samples were stored at -20°C for further molecular analysis.

Molecular Confirmation and Resistance Gene Detection

Isolates with an ESBL phenotype were screened by PCR for bla_{TEM}, bla_{SHV}, and bla_{CTX-M} genes using specific primers as shown in Table 1 [21, 22]. Each 25µL reaction contained 12.5µL of 2× PCR master mix (dNTPs, PCR buffer and Taq polymerase), 0.5µL of each primer, 3µL of template DNA and 8.5µL of nuclease-free water. The β-lactamase gene PCRs were run using a standard cycling protocol: initial denaturation at 94°C for 3 min, then 30 cycles of denaturation (94°C, 30 s), annealing (60°C, 30 s; the annealing temperature validated for the TEM and SHV primers [21]), and extension (72°C, 1 min), followed by a final extension at 72°C for 7 min. PCR products were checked by running them on a 1.5% agarose gel in TBE buffer at 100 V for 45 min. Gels were stained in ethidium bromide for 15 min,

destained for 30 min, then examined under UV light with a transilluminator and photographed [22]. All presumptive *Staphylococcus aureus* isolates were confirmed by PCR amplification of the thermonuclease (*nuc*) gene using established primers [23], as previously described [24]. Methicillin resistance was genotypically confirmed by the detection of the *mecA* gene [25]. Presumptive staphylococcal isolates were confirmed to the genus *Staphylococcus* by PCR amplification of the 16S rRNA gene using the primers listed in Table 1 [26]. The 16S rRNA, *nuc* and *mecA* PCRs were run as previously described [24], with initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min; and a final extension at 72°C for 10 min.

Table 1
Oligonucleotide primers and amplicon sizes for molecular confirmation and resistance-gene detection.

Target Gene	Primer	Sequence (5'→3')	Amplicon (bp)
16S rRNA	Forward	ACGGTCTTGCTGTCACTTATA	257
	Reverse	TACACATATGTTCTTCCCTAATAA	
nuc	Forward	GCGATTGATGGTGATACGGTT	270
	Reverse	AGCCAAGCCTTGACGAACTAAAGC	
mecA	Forward	AAAATCGATGGTAAAGGTTGGC	533
	Reverse	AGTTCTGCAGTACCGGATTTGC	
blaTEM	Forward	CATTTCCGTGTCGCCCTTATTC	800
	Reverse	CGTTCATCCATAGTTGCCTGAC	
blaSHV	Forward	AGCCGCTTGAGCAAATTAAAC	713
	Reverse	ATCCCGCAGATAAATCACCAC	
blaCTX-M	Forward	CGCTGTTGTTAGGAAGTGTG	754
	Reverse	GGCTGGGTGAAGTAAGTGAC	

Statistical Analysis

All data were analysed using Epi Info™ version 7.2 (CDC, Atlanta, USA). Bacterial isolate frequencies and resistance profiles were expressed as proportions and percentages. Statistical significance was set at $p < 0.05$. Proportions are reported with 95% confidence intervals calculated using the Wilson score method. Isolates were classified as multidrug-resistant (MDR) when they exhibited acquired non-susceptibility to at least one agent in three or more antimicrobial classes [27]. A restaurant was classified as MDR-positive when at least one MDR isolate was recovered from its air samples.

Environmental characteristics of each restaurant were recorded at the time of sampling using a structured questionnaire (supplementary file 1). The variables assessed were occupancy density (the

number of persons present in the sampled area during plate exposure, > 10 versus ≤ 10; distinct from the hourly patronage used as an inclusion criterion), proximity of the sampling zone to a restroom (< 5 m versus ≥ 5 m), ventilation type (natural or inadequate versus mechanical, i.e. air conditioning or fans), activity level during sampling (high, with cleaning or food service in progress, versus low), kitchen layout (open-plan versus partitioned), waste management (open bins present versus lidded or no bins), food category served (cooked or hot meals versus pastries or cold service) and time of sampling (afternoon peak versus morning or evening). The association between each variable and restaurant-level MDR status was assessed by bivariate analysis of 2×2 tables. Odds ratios (ORs) with 95% CIs were calculated by the Taylor series (Woolf) method, and p-values were obtained with the Pearson chi-square test, as all expected cell counts were ≥ 5.

Results

Bacterial Loads and Overall Distribution of Isolates

Bacterial loads ranged from 1.0×10^2 to 1.96×10^2 CFU/plate/15 min. A total of 382 bacterial isolates were recovered from the 58 restaurants sampled. Of these, 249 (65.2%) were Gram-positive and 133 (34.8%) were Gram-negative. Among the Gram-positive isolates, *Staphylococcus aureus* was the most prevalent species (39.8%; 152/382), followed by coagulase-negative staphylococci (16.5%; 63/382), *Bacillus* spp. (5.5%; 21/382) and *Micrococcus* spp. (3.4%; 13/382). Among the Gram-negative isolates, *Escherichia coli* predominated (15.2%; 58/382), followed by *Pseudomonas aeruginosa* (10.2%; 39/382), *Klebsiella pneumoniae* (4.2%; 16/382), *Enterobacter* spp. (2.9%; 11/382), and *Acinetobacter baumannii* (2.4%; 9/382) (Table 2).

Table 2
Distribution of bacterial isolates recovered from the indoor air of restaurants in the Ile-Ife–Ipetumodu area, Osun State, Nigeria.

Bacterial Species / Group	Number of Isolates (n)	Percentage (%)
GRAM-POSITIVE (n = 249)		
Staphylococcus aureus	152	39.8
Coagulase-negative staphylococci	63	16.5
Bacillus spp.	21	5.5
Micrococcus spp.	13	3.4
Sub-total	**249**	**65.2**
GRAM-NEGATIVE (n = 133)		
Escherichia coli	58	15.2
Pseudomonas aeruginosa	39	10.2
Klebsiella pneumoniae	16	4.2
Enterobacter spp.	11	2.9
Acinetobacter baumannii	9	2.4
Sub-total	**133**	**34.8**
TOTAL	**382**	**100.0**

Antimicrobial Susceptibility of Gram-Positive Isolates

Among the 249 Gram-positive isolates, the highest resistance was observed to co-trimoxazole (40.6%) and tetracycline (37.3%), followed by erythromycin (26.9%), azithromycin (23.7%), oxacillin (21.7%) and cefoxitin (20.5%). No resistance was observed to vancomycin, teicoplanin, ceftaroline or linezolid (Table 3).

Table 3
Antimicrobial resistance profiles of Gram-positive isolates recovered from restaurant indoor air in the Ile-Ife–Ipetumodu area, southwestern Nigeria.

Antibiotic	*S. aureus* (n = 152)	CoNS (n = 63)	*Bacillus spp.* (n = 21)	*Micrococcus spp.* (n = 13)	Total Resistant (N = 249)
Cefoxitin	36 (23.7%)	15 (23.8%)	0 (0.0%)	0 (0.0%)	51 (20.5%)
Oxacillin	36 (23.7%)	18 (28.6%)	0 (0.0%)	0 (0.0%)	54 (21.7%)
Ceftaroline	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Erythromycin	42 (27.6%)	22 (34.9%)	2 (9.5%)	1 (7.7%)	67 (26.9%)
Azithromycin	38 (25.0%)	19 (30.2%)	1 (4.8%)	1 (7.7%)	59 (23.7%)
Clindamycin	14 (9.2%)	8 (12.7%)	0 (0.0%)	0 (0.0%)	22 (8.8%)
Linezolid	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Ciprofloxacin	5 (3.3%)	2 (3.2%)	1 (4.8%)	0 (0.0%)	8 (3.2%)
Gentamicin	8 (5.3%)	4 (6.3%)	0 (0.0%)	0 (0.0%)	12 (4.8%)
Streptomycin	19 (12.5%)	9 (14.3%)	3 (14.3%)	2 (15.4%)	33 (13.3%)
Tetracycline	58 (38.2%)	24 (38.1%)	7 (33.3%)	4 (30.8%)	93 (37.3%)
Co-trimoxazole	64 (42.1%)	29 (46.0%)	5 (23.8%)	3 (23.1%)	101 (40.6%)
Chloramphenicol	3 (2.0%)	1 (1.6%)	0 (0.0%)	0 (0.0%)	4 (1.6%)

Note: CoNS = Coagulase-Negative Staphylococci. Vancomycin and teicoplanin are not shown because they were tested by broth microdilution (MIC) rather than disc diffusion; all Gram-positive isolates were susceptible to both agents.

Antimicrobial Susceptibility of Gram-Negative Isolates

Excluding intrinsic resistance, resistance among the Gram-negative isolates was highest to co-trimoxazole (47.1%), ampicillin (41.4%), trimethoprim (35.3%) and tetracycline (26.6%) (Table 4). Resistance to cefotaxime, ceftazidime and cefoxitin was 7.4%, 6.0% and 9.0%, respectively. All isolates were susceptible to ciprofloxacin and meropenem. Phenotypic ESBL production was detected in 30/133 (22.6%) Gram-negative isolates, while 27/133 (20.3%) carried at least one of the tested ESBL-associated genes (*bla*TEM, *bla*SHV or *bla*CTX-M). MDR bacteria were recovered from 36/58 (62.1%) restaurants.

Table 4
Antimicrobial resistance profiles (% resistant) of Gram-negative isolates recovered from restaurant indoor air in the Ile-Ife–Ipetumodu area, southwestern Nigeria.

Antibiotic	***E. coli* (n = 58)**	***P. aeruginosa* (n = 39)**	***A. baumannii* (n = 9)**	***Enterobacter spp.* (n = 11)**	***K. pneumoniae* (n = 16)**	**Total, n/N (%)**
Amoxicillin–clavulanate	10 (17.2%)	–	–	–	6 (37.5%)	16/74 (21.6%)
Cefotaxime	6 (10.3%)	–	0 (0.0%)	1 (9.1%)	0 (0.0%)	7/94 (7.4%)
Ampicillin	24 (41.4%)	–	–	–	–	24/58 (41.4%)
Nalidixic acid	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (9.1%)	0 (0.0%)	1/133 (0.8%)
Ceftazidime	5 (8.6%)	3 (7.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	8/133 (6.0%)
Cefoxitin	5 (8.6%)	3 (7.7%)	3 (33.3%)	–	0 (0.0%)	11/122 (9.0%)
Tetracycline	20 (34.5%)	–	0 (0.0%)	5 (45.5%)	0 (0.0%)	25/94 (26.6%)
Chloramphenicol	1 (1.7%)	–	–	6 (54.5%)	0 (0.0%)	7/85 (8.2%)
Streptomycin	5 (8.6%)	6 (15.4%)	0 (0.0%)	3 (27.3%)	0 (0.0%)	14/133 (10.5%)
Ciprofloxacin	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0/133 (0.0%)
Trimethoprim	22 (37.9%)	–	–	8 (72.7%)	0 (0.0%)	30/85 (35.3%)
Meropenem	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0/133 (0.0%)
Trimethoprim–sulfamethoxazole	32 (55.2%)	–	0 (0.0%)	8 (72.7%)	0 (0.0%)	40/85 (47.1%)

Note: – indicates intrinsic resistance of the organism to the agent (CLSI M100, 2024 [19]); Totals (n/N) include only organism–agent combinations without intrinsic resistance.

ESBL Characterisation and MRSA Detection

Among the 133 Gram-negative isolates, 22.6% (*n* = 30; 95% CI: 16.3–30.4) exhibited a phenotypically ESBL-positive profile by double-disc synergy testing, and 27 of these 30 isolates (90.0%; 95% CI: 74.4–96.5) were found to harbour at least one of the targeted β-lactamase genes (*blaTEM, blaSHV*, or *blaCTX-M*) by PCR, corresponding to 20.3% (27/133; 95% CI: 14.3–27.9) of all Gram-negative isolates. Similarly,

the species-specific thermonuclease (nuc) gene was confirmed across all 152 isolates (100.0%). Subsequent phenotypic screening with cefoxitin disks, followed by confirmatory genotypic profiling for the *mecA* determinant, revealed a methicillin-resistant *S. aureus* (MRSA) prevalence of 23.7% (*n* = 36; 95% CI: 17.6–31.0) within this population, with all 36 cefoxitin-resistant isolates carrying the *mecA* gene.

Multidrug Resistance Profile

Resistance patterns by organism are shown in Tables 3 and 4. Among Gram-positive isolates, coagulase-negative staphylococci showed resistance to co-trimoxazole (46.0%), tetracycline (38.1%), erythromycin (34.9%) and oxacillin (28.6%), and *S. aureus* showed resistance to co-trimoxazole (42.1%), tetracycline (38.2%), erythromycin (27.6%) and oxacillin (23.7%) (Table 3). Methicillin resistance among coagulase-negative staphylococci (cefoxitin 23.8%; oxacillin 28.6%) was determined phenotypically only, as *mecA* detection was performed on *S. aureus* isolates. Among Gram-negative isolates, *Enterobacter* spp. exhibited the broadest resistance profile, with resistance to trimethoprim–sulfamethoxazole (72.7%), trimethoprim (72.7%), chloramphenicol (54.5%) and tetracycline (45.5%). *E. coli* showed resistance to trimethoprim–sulfamethoxazole (55.2%), ampicillin (41.4%) and tetracycline (34.5%) (Table 4).

Analysis of environmental risk factors associated with MDR bacterial recovery is presented in Table 5. High occupancy density (> 10 persons present during sampling) (OR 5.25, 95% CI 1.66–16.59; p = 0.004), restroom proximity within five metres of the sampling zone (OR 5.07, 95% CI 1.27–20.22; p = 0.015), reliance on natural or inadequate ventilation, compared with mechanical ventilation systems (OR 3.76, 95% CI 1.23–11.51; p = 0.018), and high peak activity level during sampling (OR 3.37, 95% CI 1.10–10.32; p = 0.030) showed statistically significant associations with MDR bacterial recovery. In contrast, kitchen layout configuration (open-plan versus partitioned; OR 1.68, p = 0.340), waste management practices (OR 0.76, p = 0.622), food category served (OR 0.76, p = 0.670), and time of sampling (OR 0.93, p = 0.896) did not reach statistical significance. It should be noted that unadjusted bivariate associations were used, and confounding between environmental variables cannot be excluded.

Table 5
Bivariate analysis of environmental risk factors associated with the recovery of MDR bacteria (N = 58)

Risk Factor	MDR Positive (n = 36)	MDR Negative (n = 22)	Odds Ratio (95% CI)	p-value
Occupancy Density				
High (> 10 persons present)	27 (75.0%)	8 (36.4%)	5.25 (1.66–16.59)	0.004
Low (≤ 10 persons present)	9 (25.0%)	14 (63.6%)	1.00 (Ref)	--
Restroom Proximity				
Near (< 5 metres)	16 (44.4%)	3 (13.6%)	5.07 (1.27–20.22)	0.015
Far (≥ 5 metres)	20 (55.6%)	19 (86.4%)	1.00 (Ref)	--
Ventilation Type				
Natural/Inadequate	26 (72.2%)	9 (40.9%)	3.76 (1.23–11.51)	0.018
Mechanical (AC/Fans)	10 (27.8%)	13 (59.1%)	1.00 (Ref)	--
Peak Activity Level				
High (Cleaning/Serving)	22 (61.1%)	7 (31.8%)	3.37 (1.10–10.32)	0.030
Low (Static)	14 (38.9%)	15 (68.2%)	1.00 (Ref)	--
Kitchen Layout				
Open-plan	21 (58.3%)	10 (45.5%)	1.68 (0.58–4.89)	0.340
Partitioned	15 (41.7%)	12 (54.5%)	1.00 (Ref)	--
Waste Management				
Open Bins Present	14 (38.9%)	10 (45.5%)	0.76 (0.26–2.24)	0.622
Lidded/No Bins	22 (61.1%)	12 (54.5%)	1.00 (Ref)	--
Food Category				
Cooked/Hot Meals	26 (72.2%)	17 (77.3%)	0.76 (0.22–2.63)	0.670
Pastries/Cold Service	10 (27.8%)	5 (22.7%)	1.00 (Ref)	--
Time of Sampling				
Afternoon (Peak)	19 (52.8%)	12 (54.5%)	0.93 (0.32–2.70)	0.896

Risk Factor	MDR Positive (n = 36)	MDR Negative (n = 22)	Odds Ratio (95% CI)	p-value
Occupancy Density				
Morning/Evening	17 (47.2%)	10 (45.5%)	1.00 (Ref)	--

Discussion

This study demonstrates the presence of appreciable bacterial loads in the indoor air of restaurant environments within the Ile-Ife–Ipetumodu area, southwestern Nigeria. The recovery of 382 bacterial isolates from 58 restaurants reveals a diverse airborne microbial community that includes clinically relevant and antimicrobial-resistant pathogens. Elevated airborne bacterial counts have likewise been reported from restaurants elsewhere in Nigeria [16], and indoor air in a university cafeteria in Ethiopia has been identified as a potential vehicle for bacterial contamination [28]. These parallel observations underscore the need for attention to hygiene and ventilation in food service establishments in the region.

The predominance of Gram-positive organisms, particularly *S. aureus*, is consistent with findings from comparable indoor air investigations. Studies of indoor air in restaurants in Yola, Nigeria, and in a hospital in Hawassa, Ethiopia, have likewise identified staphylococci as the dominant airborne bacteria [29, 30], a pattern attributable to their continuous human shedding from skin, nasal passages, and respiratory secretions. The prevalence of *S. aureus* in the present study substantially exceeds the 19% reported by Gulumbe et al. [16] in Nigerian restaurants in Kano, suggesting that the particular occupancy dynamics and ventilation characteristics of the local food service sector may be associated with elevated settled bacterial contamination; whether this corresponds to elevated airborne exposure cannot be determined from settle-plate data alone.

The high prevalence of *S. aureus* carries direct public health implications. Beyond its established role as a principal cause of foodborne illness and healthcare-associated infection, this organism is highly adaptable to airborne dispersal and is capable of depositing on food, preparation surfaces, and utensils, thereby creating downstream risks for food safety and consumer health [31, 32]. The confirmation of MRSA isolates is especially concerning, as it documents the presence of a priority-tier resistant phenotype well beyond the boundaries of the clinical environment. MRSA has previously been recovered from raw meat intended for human consumption in Nigeria [33], and the present data contribute further evidence that restaurant environments represent a plausible setting for community-level MRSA contamination. However, this study's cross-sectional design cannot demonstrate active transmission. Longitudinal investigations incorporating molecular strain typing would be required to confirm whether environmentally detected organisms colonise restaurant patrons or staff.

The recovery of *Escherichia coli*, a recognised faecal indicator organism, from restaurant indoor air is a finding of considerable sanitary significance. Under clean environmental conditions, *E. coli* is not a typical airborne commensal. Its presence in sampled air therefore suggests possible secondary

aerosolisation from contaminated surfaces, resuspension of settled particulate, or toilet plume effects during flushing in adjacent restroom facilities. This finding is consistent with the recovery of *E. coli* and other Gram-negative bacteria from air in high-traffic settings in Osun State [12] and with the poor hygiene practices documented in Nigerian restaurants with high airborne microbial counts [16]. Beyond *E. coli*, several of the other Gram-negative isolates were recovered: *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. These organisms belong to the ESKAPE group of pathogens that are among the leading causes of difficult-to-treat, frequently multidrug-resistant infections worldwide. Their detection in restaurant indoor air is of added concern, as these organisms combine intrinsic and acquired resistance mechanisms with a strong capacity for environmental persistence and horizontal gene transfer [2].

The antimicrobial resistance profiles documented in this study are consistent with the substantial burden of AMR reported for Nigeria [13]. Erythromycin resistance in *S. aureus* (27.6%) and coagulase-negative staphylococci (34.9%), along with tetracycline resistance across multiple species (Tables 3 and 4), is consistent with the documented over-the-counter availability and widespread unsupervised use of these agents in Nigeria [1, 13]. The environmental dissemination of such resistant isolates within restaurant settings presents a meaningful public health concern, as settled resistant bacteria on surfaces and in the food-handling environment could plausibly contribute to community-level resistance gene transfer [3, 4].

Of particular reassurance is the complete preservation of susceptibility to vancomycin, teicoplanin, and linezolid across all Gram-positive isolates, and to meropenem and ciprofloxacin among Gram-negative species. The intact activity of these critically important agents in this environmental context is a positive finding, though it should not engender complacency: the global trajectory toward glycopeptide and carbapenem resistance in community settings demands continued vigilance and proactive surveillance.

ESBL-producing organisms present a formidable clinical challenge, as they confer broad-spectrum beta-lactam resistance and are frequently co-resistant to multiple other antibiotic classes [34]. Their detection in a community food service setting supports the premise that non-clinical environments constitute meaningful epidemiological intersections in the ecology of AMR dissemination. This finding is in keeping with the detection of antibiotic-resistant airborne bacteria in restaurant environments in Riyadh, Saudi Arabia, by Aldosary et al. [11].

Phenotypic ESBL production was detected in 30 isolates, whereas comparatively few isolates were resistant to cefotaxime (7/94) or ceftazidime (8/133) by disc diffusion (Table 4). Several phenotypic and genotypic factors may explain this discordance. First, the double-disc synergy test detects clavulanate-inhibitable β-lactamase activity, and ESBL-producing isolates, particularly those with low-level enzyme expression, can test susceptible to third-generation cephalosporins in vitro [34]. Second, the *bla*TEM and *bla*SHV primers used in this study amplify both ESBL variants and non-ESBL broad-spectrum variants such as TEM-1, TEM-2 and SHV-1, which can be distinguished only by sequencing the amplicons [21]; some of the 27 PCR-positive isolates may therefore carry non-ESBL TEM or SHV enzymes. Third, co-

production of AmpC β-lactamases can mask or mimic synergy in phenotypic ESBL tests [22], and the double-disc synergy test is not standardised for non-fermenters, so some positive results in *P. aeruginosa* and *A. baumannii* may not reflect true ESBL production. The three phenotypically positive isolates that carried none of the targeted genes may harbour ESBL families not covered by the PCR panel. Sequencing of the amplified genes, together with confirmatory combination-disc testing, would help resolve these discrepancies.

The MDR profile documented in this study carries substantial public health significance that extends well beyond the immediate food service setting. Overall, 62.1% of sampled establishments yielded at least one MDR isolate, defined at isolate level as non-susceptibility to at least one agent in three or more antimicrobial classes, a prevalence that underscores how widely antimicrobial resistance has permeated community-level environments in the study area. The resistance observed among coagulase-negative staphylococci is consistent with their growing recognition as important MDR vectors in both healthcare and community settings, where their capacity for biofilm formation and horizontal resistance gene acquisition enables rapid phenotypic diversification [35]. The MDR resistance pattern observed in *Enterobacter* spp. spanning folate pathway inhibitors, tetracyclines and phenicols simultaneously is of particular clinical concern given this organism's demonstrated capacity for plasmid-mediated resistance gene acquisition and its association with difficult-to-treat nosocomial infections [2, 34].

The risk factor data provide mechanistic context for understanding how these MDR organisms accumulate and persist in restaurant indoor air. The strong association between high patron occupancy density and MDR recovery is biologically coherent. This is because crowded enclosed environments accelerate bioaerosol generation from respiratory and dermal sources, increasing the probability that sampled air contains resistant organisms shed by colonised individuals [6, 8]. The particularly elevated odds ratio associated with restroom proximity is consistent with the previously described toilet plume mechanism, whereby flush-generated aerosol droplets can carry viable faecal bacteria into adjacent spaces; however, this association does not establish aerosolisation as the confirmed source of the MDR *E. coli* and *Enterobacter* spp. recovery patterns observed here [36]. The significant protective effect of mechanical ventilation is consistent with an extensive body of evidence linking adequate air exchange rates to reduced settled bacterial contamination in indoor environments; this settle-plate based association does not, by itself, quantify bioaerosol concentration or airborne infection risk [8]. Notably, kitchen layout and waste management practices did not emerge as significant predictors of MDR recovery, suggesting that the spatial configuration of individual workstations matters less than the holistic ventilation quality and occupancy dynamics of the broader establishment. Taken together, these findings reinforce the growing scientific consensus that food service environments represent underappreciated but consequential environmental niche for antimicrobial resistance, particularly in settings where ventilation, sanitation, and infection control infrastructure are inadequate. Limited regulatory oversight of indoor air quality in Nigerian food service establishments may contribute to these patterns, although this was not assessed in the present study.

Conclusion

This study reveals that restaurant environments within the Ile-Ife–Ipetumodu area harbour a diverse airborne bacterial community with considerable antimicrobial resistance, including methicillin-resistant *Staphylococcus aureus* (MRSA) and ESBL-producing Gram-negative bacteria. The recovery of antimicrobial-resistant bacteria from indoor restaurant air highlights a potential public health concern associated with environmental hygiene and ventilation practices in food service environments. These findings suggest that restaurant indoor air may represent an underappreciated environmental reservoir of antimicrobial-resistant bacteria in Nigeria and support the need for coordinated, multi-stakeholder interventions, including improved ventilation, strengthened hygiene practices, appropriate antimicrobial stewardship, and routine microbiological monitoring of food service environments.

Limitations

This study employed the passive settle plate method, which while widely validated and well-suited for field-based environmental surveillance captures only organisms of sufficient mass to sediment gravitationally and does not permit volumetric quantification of airborne concentration in CFU/m³. Additionally, the cross-sectional design precludes assessment of temporal trends in bacterial burden or resistance profiles across seasons. Restaurants were selected purposively and each was sampled on a single occasion, and outdoor air was not sampled for comparison. Because the *bla*TEM and *bla*SHV primers amplify all variants of these gene families, including the non-ESBL TEM-1 and SHV-1, and the amplicons were not sequenced, a positive PCR result could not confirm that the gene detected encoded an ESBL. Future longitudinal investigations incorporating molecular epidemiological tools would further elucidate the dynamics of AMR gene dissemination in these settings.

Abbreviations

AmpC Ambler class Cβ
lactamase

Declarations

Ethics Approval and Consent to Participate

Ethical approval for this study was obtained from the Health Research Ethics Committee of Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife (Approval No. IPHOAU/12/1467). Written informed consent was obtained from the management of each participating restaurant prior to sample collection. All procedures were conducted in accordance with the ethical standards of the institutional research committee and the Declaration of Helsinki as revised in 2013. Confidentiality of all participant data was strictly maintained throughout the study.

Consent for publication

Not applicable

Competing Interests

The authors declare no competing interests.

Clinical trial number:

Not applicable.

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Author Contribution

A.A.O. conceived the study, the adapted methodology, and drafted the manuscript. P.A.I. carried out the sample collection and performed the experiments. A.A.O. and O.S.A. carried out the data analysis. O.S.A. prepared all the tables and figures with the assistance of A.A.O. and P.A.I. All authors reviewed and approved the final version of the manuscript.

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Data Availability

The datasets generated and analysed during this study are available from the corresponding author upon reasonable request.

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Figures

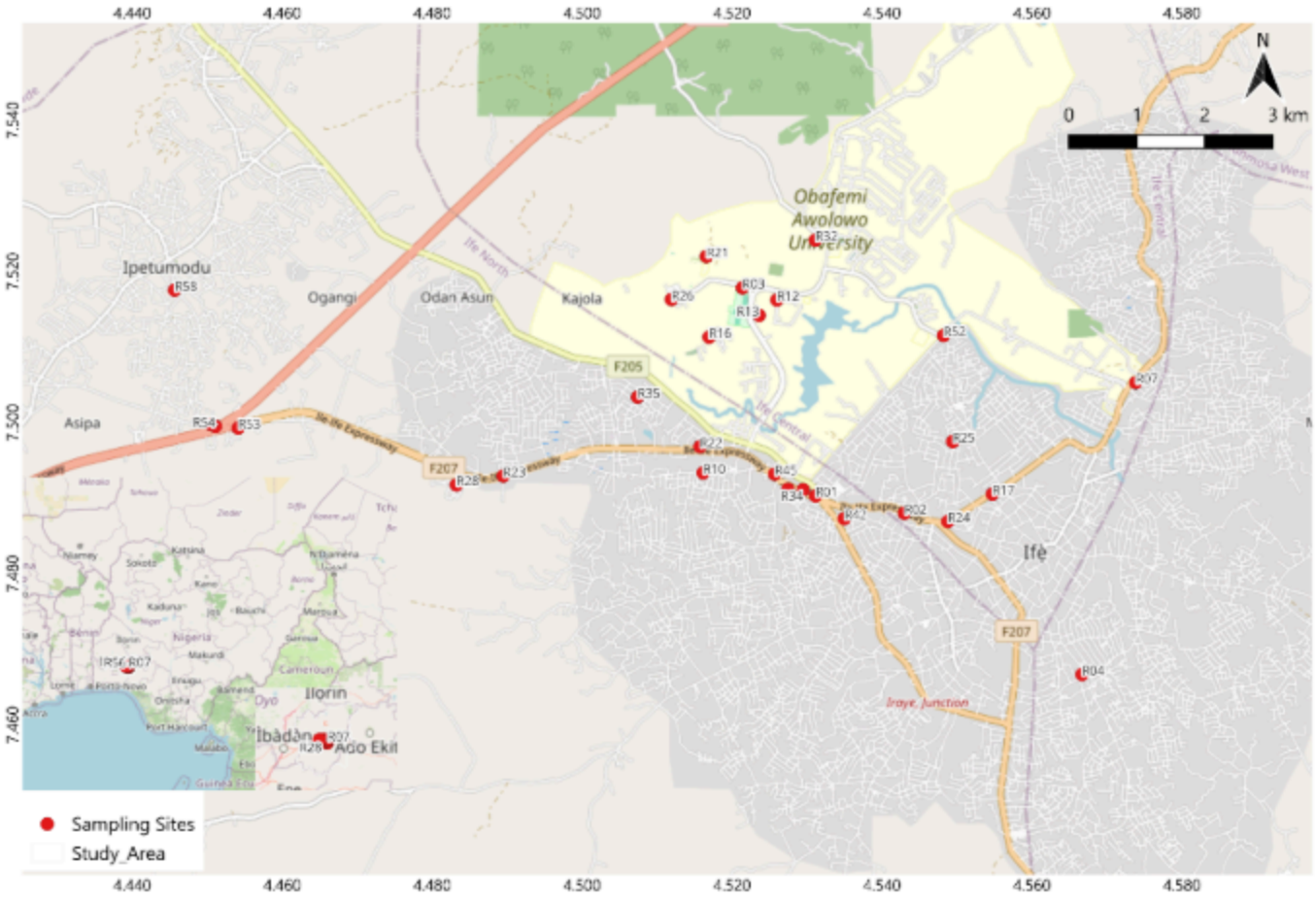


Figure 1

Map of the study area showing selected restaurant sampling sites in the Ile-Ife–Ipetumodu area, Osun State, Nigeria. Base map data © OpenStreetMap contributors, available under the Open Database Licence (https://www.openstreetmap.org/copyright).

Supplementary Files

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- SupplementaryMaterial1Questionnaire.docx