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$PM_{2.5}$-Associated Variation in Airborne Antimicrobial Resistance Gene Abundance During Winter Smog Episodes in Urban Pakistan (2022–2023)

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Research Article

Keywords: $PM_{2.5}$, antimicrobial resistance genes, smog, quantitative real-time PCR, airborne resistome, Pakistan

Posted Date: November 6th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11398870/v1

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Additional Declarations: No competing interests reported.

Abstract

Airborne particulate matter ($PM_{2.5}$) is a major component of winter smog and has been increasingly recognized as a potential carrier of microbial contaminants, including antimicrobial resistance genes (ARGs). However, limited data are available on the distribution of airborne ARGs associated with $PM_{2.5}$ in urban environments of Pakistan.

Objective

This study aimed to quantify the relative abundance of clinically relevant ARGs in $PM_{2.5}$-associated bacterial communities across three cities of Pakistan and to evaluate their association with air pollution and meteorological conditions. **Methodology**

$PM_{2.5}$ samples were collected from Lahore, Islamabad, and Okara during the winter months (November–December 2022 and 2023). External DNA (eDNA) associated with particulate matter was extracted and analysed using quantitative real-time PCR (qPCR). The relative abundance of selected ARGs (*sul1, blaTEM, blaCTX-M-32, blaNDM-1, vanA, vanRA, tnpA, intI1*, and *blaTEM-F*) was determined using the 2^(-ΔCt) method and normalized against the 16S rRNA gene. Environmental parameters, including $PM_{2.5}$ concentration, Air Quality Index (AQI), temperature, and humidity, were also assessed.

Results

Lahore exhibited the highest pollution levels, with $PM_{2.5}$ concentrations reaching 276.6 ± 27.4 μg/m³ and AQI values up to 289.3 ± 42.7. $PM_{2.5}$ showed strong positive correlations with AQI (r = 0.92) and relative humidity (r = 0.91) and a negative correlation with temperature (r = − 0.38). Distinct spatial variation in ARG relative abundance was observed across the study sites. The *blaNDM-1* gene demonstrated the highest relative abundance, particularly in Lahore (0.077) and Islamabad (0.056), while *vanA* showed a comparatively higher abundance in Okara. Other ARGs, including *blaTEM-F, sul1*, and *intI1*, exhibited variable distribution patterns across cities, indicating heterogeneous environmental resistome profiles.

Conclusion

The findings reveal significant spatial variability in airborne ARG relative abundance associated with $PM_{2.5}$ and highlight the influence of environmental conditions on resistome distribution. These results underscore the importance of monitoring airborne antimicrobial resistance in polluted urban environments and provide baseline data for future environmental and public health assessments.

1 Introduction

Air pollution remains one of the most significant environmental threats to human health worldwide, with ambient particulate matter playing a central role in disease burden (Chen and Kan 2008). Fine particulate matter ($PM_{2.5}$), characterized by an aerodynamic diameter of ≤ 2.5 μm, is a major component of urban smog and is strongly associated with respiratory and cardiovascular morbidity and mortality (Henning 2024; Organization 2024). Global estimates indicate that exposure to ambient air pollution contributes to

approximately 4.2 million premature deaths annually, highlighting its substantial public health impact (Organization 2024). Rapid urbanization, industrialization, and increased energy consumption have further intensified air pollution levels, particularly in developing regions (Zhang et al. 2022).

In recent years, airborne particulate matter has also been recognized as a potential carrier of microbial communities, collectively referred to as the air microbiome (Cao et al. 2014). These microbial populations include bacteria, fungi, archaea, and viruses that can attach to particulate matter and remain suspended in the atmosphere. Importantly, particulate-associated microorganisms may harbor antimicrobial resistance genes (ARGs), which are genetic determinants that enable bacteria to survive antibiotic exposure (Vassallo et al. 2022). The presence of ARGs in airborne environments has raised concerns regarding their potential role in the environmental dissemination of antimicrobial resistance.

Several studies have reported the occurrence of ARGs in $PM_{2.5}$-associated microbial communities, suggesting that airborne particles may act as reservoirs or carriers of resistance determinants (Xie et al. 2018). However, the extent to which ARGs are distributed across different environmental settings and pollution levels remains insufficiently characterized, particularly in regions experiencing severe smog conditions. Previous metagenomic analyses have demonstrated that urban air microbiomes can contain diverse resistance genes, including β-lactamases, aminoglycoside resistance genes, and vancomycin resistance determinants, reflecting complex environmental resistome profiles (Khan et al. 2025).

South Asia is among the most polluted regions globally, with Pakistan consistently ranking among the countries with the highest $PM_{2.5}$ levels (WHO 2024; Zdero 2025). Urban centers such as Lahore frequently experience hazardous air quality conditions, with AQI values exceeding 200 during winter smog episodes (IQAir 2025). Despite these conditions, there remains a lack of systematic data on airborne ARGs associated with $PM_{2.5}$ in this region. In particular, limited information is available regarding the relative abundance and distribution of clinically relevant ARGs under real-world smog conditions.

Therefore, the present study aims to quantify the relative abundance of selected antimicrobial resistance genes in $PM_{2.5}$-associated external DNA across three cities in Pakistan (Lahore, Islamabad, and Okara) during winter smog periods (2022–2023). In addition, the study evaluates the relationship between environmental factors, including $PM_{2.5}$ concentration, air quality index (AQI), temperature, and humidity, and the distribution of airborne ARGs. By focusing on DNA-based quantification, this study provides insight into the environmental presence and spatial variability of ARGs in polluted urban atmospheres, contributing to a better understanding of airborne resistome dynamics.

2 Materials and methods

2.1 Study area and sample collection

$PM_{2.5}$ sampling was conducted during two consecutive winter seasons (November–December 2022 and 2023) across three cities of Pakistan: Islamabad, Lahore, and Okara. These locations represent urban and semiurban environments with varying air pollution levels. Ambient $PM_{2.5}$ was collected using an air

sampling device equipped with quartz microfibre filters under controlled and standardized sampling conditions with consistent operational settings across all sites to ensure comparability of collected particulate matter. At each site, one 24-hour $PM_{2.5}$ sample was collected daily throughout the sampling period (November–December of each year), resulting in approximately 60 individual samples per city per sampling season. Following collection, the filters were carefully handled and stored at − 20°C until further processing. Sampling was conducted at approximately the breathing height (~ 1.5–2 m above ground level) to represent typical ambient human exposure conditions.

Due to the low biomass and limited external DNA (eDNA) yield from individual $PM_{2.5}$ filters, samples collected within each month were pooled prior to DNA extraction. Specifically, daily filter samples corresponding to each month (November and December of 2022 and 2023) were combined to obtain a representative composite sample for each city and time point. This pooling approach enabled sufficient DNA recovery for downstream molecular analysis. Consequently, each pooled sample represents aggregated monthly environmental conditions rather than individual daily variability.

2.2 Environmental Data Acquisition and Analysis

To characterize environmental conditions during the sampling period, air quality and meteorological parameters, including **the air quality index** (AQI), $PM_{2.5}$ concentration, ambient temperature (°C), and relative humidity (%), were obtained from publicly available air quality monitoring databases corresponding to each study site and sampling period. These platforms compile standardized measurements from ground-based monitoring stations and provide validated datasets for urban air quality assessment. Daily values corresponding to each sampling day (November–December 2022 and 2023) were retrieved and aggregated to calculate monthly mean ± standard deviation (SD) values for each parameter. These aggregated values were used to represent environmental exposure conditions during each sampling period.

To evaluate spatial and temporal variability, comparative analyses across cities (Lahore, Islamabad, and Okara) and sampling periods were performed. Differences among cities were assessed using one-way analysis of variance (ANOVA), and a p value of < 0.05 was considered to indicate statistical significance. In addition, relationships among environmental variables were evaluated using Pearson correlation analysis, where correlation coefficients (r) were calculated between AQI, $PM_{2.5}$, temperature, and relative humidity.

2.3 Extraction and quality assessment of external DNA (eDNA)

The external DNA (eDNA) associated with particulate matter was extracted according to a modified protocol. In this study, eDNA refers specifically to extracellular or surface-associated DNA present on particulate matter, excluding intracellular genomic DNA contained within intact bacterial cells (Nielsen et al. 2007; Torti et al. 2015). To preferentially recover external DNA, a mild extraction approach was employed that facilitates the desorption of DNA from particle surfaces while minimizing the disruption of intact microbial cells (Alawi et al. 2014). Prior to DNA extraction, pooled $PM_{2.5}$ filter samples corresponding to each month and sampling site were used as input material, as described in section 2.1.

Briefly, 1 g of PM collected from dust filters was suspended in 9 mL of 0.1 M sodium phosphate buffer at pH 8.0 in a sterile Falcon tube, to which 0.5 g of acid-washed polyvinylpyrrolidone (PVP) was added. The use of phosphate buffer and gentle agitation promotes the release of loosely bound extracellular DNA from particulate surfaces without inducing extensive cell lysis (Alawi et al. 2014; Corinaldesi et al. 2005). The sample was horizontally shaken at 150 rpm for 1 min three times with 1 min intervals on ice. Next, SDS was added to a final concentration of 0.1% and shaken for 10 s. The low concentration and short exposure to SDS were applied to enhance DNA recovery while limiting disruption of intact microbial cells (Torti et al. 2015).

The suspension was subsequently centrifuged at 500 × g for 10 min at 4°C, after which the supernatant containing extracellular and particle-associated DNA was carefully collected (Thomas et al. 2018). This low-speed centrifugation step enables separation of intact cells and larger particles into the pellet, while extracellular and particle-associated DNA remains in the supernatant fraction (Alawi et al. 2014). The pellet was washed twice, and all the supernatants were combined and centrifuged at 10,000 × g for 20 min at 4°C. One volume of cetyltrimethylammonium bromide solution was added to the supernatant, which was subsequently incubated at 65°C for 30 min, followed by centrifugation at 5000 × g for 10 min at 4°C. The pellet was dissolved in TE buffer containing 10 mM Tris-HCl, 0.1 mM EDTA, and 1 M NaCl, pH 8.0.

DNA was precipitated by adding 0.6 volumes of cold isopropanol, incubating on ice for 1 h and centrifuging at 10,000 × g for 15 min at 4°C. The resulting pellet was redissolved in TE buffer (10 mM Tris-HCl, 0.1 mM EDTA; pH 8.0) and cleaned with an equal volume of phenol—chloroform-isoamyl alcohol (25:24:1, v/v) and chloroform-isoamyl alcohol (24:1, v/v), followed by centrifugation at 10,000 × g for 5 min. The final supernatant was treated with 70% ethanol containing 0.2 M NaCl and incubated for 1 h at -20°C (Fauchery et al. 2022). Following centrifugation (10,000 × g, 15 min, 4°C), the DNA pellet was washed with ethanol, vacuum-dried and redissolved in TE buffer. The extracted DNA was stored at 4°C until subsequent use (FitzGerald et al. 2024).

It is important to note that this protocol is designed to recover extracellular and particle-associated DNA and does not include intensive mechanical or enzymatic lysis steps typically required for total genomic DNA extraction; therefore, the recovered DNA predominantly represents noncellular or surface-associated genetic material (Nielsen et al. 2007; Torti et al. 2015). The integrity of the extracted eDNA was confirmed via 1% agarose gel electrophoresis using ethidium bromide (8 µL per 100 mL gel) for visualization under a UV transilluminator. The DNA purity and concentration were further measured spectrophotometrically (NanoDrop), and an A_{260}/A_{280} ratio of 1.8–2.0 was considered acceptable for downstream analysis (Westermeier 2016).

2.4 Target Genes, Primer Selection and PCR Optimization

Nine clinically significant antimicrobial resistance genes (ARGs) were targeted for relative abundance analysis, including sul1 (sulfonamide), blaTEM, blaCTX-M-32, blaNDM-1 (β-lactamases), vanA, vanRA (vancomycin), tnpA (transposase), intI1 (class-1 integrase), and blaTEM-F, along with the 16S rRNA gene used as an internal reference for normalization. These genes were selected based on their clinical relevance, widespread environmental occurrence, and association with mobile genetic elements

contributing to antimicrobial resistance dissemination (Berendonk et al. 2015; Huang et al. 2021). Sul1 is commonly used as a marker of anthropogenic pollution, β-lactamase genes (blaTEM, blaCTX-M-32, blaNDM-1) represent clinically critical resistance mechanisms, vanA and vanRA are associated with vancomycin resistance of high clinical importance, while tnpA and intI1 serve as indicators of horizontal gene transfer potential in the environmental resistome (Gillings et al. 2015; Manaia et al. 2018).

In addition, blaTEM-F was included as a variant/fragment-specific target of the blaTEM gene to assess the consistency and stability of β-lactam resistance detection across different primer systems and to account for potential sequence-level variation within environmental samples. The inclusion of blaTEM-F alongside blaTEM enables more robust detection and comparison of β-lactamase-associated resistance patterns in $PM_{2.5}$-associated DNA. The primer sequences and melting temperatures used are summarized in Table 1. All primers were selected from previously validated studies to ensure the specificity and reproducibility of ARG detection (Ja'afar et al. 2021). PCR optimization was performed with eDNA (isolated from $PM_{2.5}$ filters) in a Galaxy XP Thermal Cycler (BIOER, PCR). Each reaction mixture contained a total volume of 10 μL, which included Taq polymerase, $MgCl_2$, dNTPs, 10 × buffer, 10 μM forward/reverse primers, PCR high-quality water and 1 μL of eDNA template.

Table 1
Primer details for antibiotic resistance and 16S rRNA genes

Sr#	Gene Name	Forward Primer	Reverse Primer	Tm
1	*sul1*	CGCACCGGAAACATCGCTGCAC	TGAAGTTCCGCCGCAAGGCTCG	F-67.7°C, R-67.7°C
2	*blaTEM*	TTCCTGTTTTTGCTCACCCAG	CTCAAGGATCTTACCGCTGTTG	F-67.7°C, R-67.7°C
3	*blaCTX-M-32*	CGTCACGCTGTTGTTAGGAA	CGCTCATCAGCACGATAAAG	F-59.4°C, R-62.1°C
4	*blaNDM-1*	TTGGCCTTGCTGTCCTTG	ACACCAGTGACAATATCACCG	F-56.1°C, R-59.4°C
5	*vanA*	AAAAGGCTCTGAAAACGCAGTTAT	CGGCCGTTATCTTGTAAAAACAT	F-60.1°C, R-59.3°C
6	*vanRA*	CCCTTACTCCCACCGAGTTTT	TTCGTCGCCCCATATCTCAT	F-60.3°C, R-58.4°C
7	*tnpA*	AATTGATGCGGACGGCTTAA	TCACCAAACTGTTTATGGAGTCGTT	F-56.4°C, R-62.5°C
8	*intI1*	CGAACGAGTGGCGGAGGGTG	TACCCGAGAGCTTGGCACCCA	F-66.6°C, R-65.2°C
9	*blaTEM-F*	ATCTCAACAGCGGTAAGATC	GAGAATAGTGTATGCGGCG	F-56.4°C, R-57.3°C
10	*16S rRNA*	GGGTTGCGCTCGTTGC	ATGGYTGTCGTCAGCTCGTG	F-55.8°C,

Sr#	Gene Name	Forward Primer	Reverse Primer	Tm
				R-61.5°C

The cycling conditions were as follows: 95°C for 10 min; then, 40 cycles of 95°C/1 min, 55–69°C/1 min (gradient), 72°C/1 min; and a final extension at 72°C/10 min. Optimal annealing temperatures and template dilutions (1:10 or 1:100) were determined through gradient PCR to ensure specific amplification, confirmed by the presence of single, distinct bands without nonspecific amplification on 1.5% agarose gels (Ja'afar et al. 2021). These optimization steps ensured the high specificity and reliability of downstream qPCR-based quantification.

2.5 Quantitative real-time PCR (qPCR)

To measure the relative abundance of specific ARGs in eDNA harvested from $PM_{2.5}$ filters, quantitative real-time PCR (qPCR) was performed using the Mic Real-Time PCR System (BioMolecular Systems, Australia) with SYBR Green detection chemistry. The 10 µL reaction mixture contained 5 µL of 2× SYBR Green Master Mix, 0.2 µL of both forward and reverse primers (10 µM), 3.6 µL of nuclease-free water, and 1 µL of diluted (1:10) eDNA template. The cycling conditions included initial denaturation at 95°C for 12 min, followed by 40 cycles of amplification at 95°C for 15 s, 60 ± 2°C for 20 s, and 72°C for 20 s, followed by melt curve analysis to verify amplification specificity and **the** absence of **nonspecific** products.

Each gene was run in triplicate as technical replicates for each pooled sample to ensure reproducibility, and the relative abundance of each ARG was calculated using the 2^(-ΔCt) method and normalized to the 16S rRNA reference gene. The use of the ΔCt method is appropriate for DNA-based qPCR analysis, as it enables comparison of gene abundance across samples without requiring a defined experimental calibrator. It should be noted that these triplicates represent technical replication only, while biological replication was limited due to the pooling of samples described in Section 2.1. The efficiency and specificity of amplification were confirmed through real-time amplification plots and melt curves showing a single sharp peak for each gene (Fig. 1 and Fig. 2).

2.6 Statistical analysis

Triplicate quantitative PCR assays were performed (technical replicates for each pooled sample to ensure reproducibility). Relative abundance data are reported as 2^(-ΔCt) values (normalized to the 16S rRNA reference gene) and are presented as the mean values for each pooled sample. All statistical analyses and data visualizations were performed using RStudio (version 4.3.2) with the ggplot2, dplyr, and ggpubr packages. The 2^(-ΔΔCt) method was not applied, as no single experimental calibrator condition was defined; therefore, relative abundance values based on the ΔCt method were used for comparison across samples.

Due to the use of pooled monthly samples, statistical comparisons of ARG relative abundance among sampling locations were primarily descriptive. Variations in ARG abundance across cities were evaluated

through graphical visualization and comparison of mean values rather than inferential statistical testing. The use of pooled samples limited statistical power and precluded robust inferential analysis of ARG data. Associations between environmental variables ($PM_{2.5}$, AQI, temperature, and humidity) were evaluated using Pearson's correlation coefficient (r). In addition, differences in environmental parameters among cities were assessed using one-way analysis of variance (ANOVA), with a p value of < 0.05 considered statistically significant.

3 Results

3.1 Variation in Air Quality across Study Sites

Air quality parameters showed clear spatial and temporal variation across the study sites during the sampling period (Fig. 3; Table 2). Lahore consistently exhibited the highest pollution levels among the three cities. AQI values in Lahore ranged from 189.1 ± 35.4 in November 2022 to 289.3 ± 42.7 in December 2023. Similarly, $PM_{2.5}$ concentrations were elevated, reaching a maximum of 276.6 ± 27.4 μg/m³ during December 2023. In comparison, Islamabad showed lower pollution levels, with AQI-US values ranging between 143.8 ± 18.6 and 185.1 ± 22.1 and $PM_{2.5}$ concentrations between 71.5 ± 14.2 and 110.9 ± 12.8 μg/m³. Okara demonstrated intermediate values, with AQI-US ranging from 172.1 ± 26.4 to 214.5 ± 27.8 and $PM_{2.5}$ concentrations between 67.5 ± 18.2 and 99.5 ± 23.1 μg/m³.

Table 2
Summary of one-way ANOVA results for environmental parameters

Parameter	F value	p value	Interpretation
AQI-US	8.77	0.0077	Significant difference among cities
$PM_{2.5}$	17.29	< 0.001	Significant difference among cities
Temperature	1.74	0.229	No significant difference
Humidity	55.14	< 0.001	Significant difference among cities

Across all sites, higher pollution levels were generally observed during December compared to November in both years. One-way ANOVA indicated significant differences among cities for AQI-US ($F = 8.77$, $p = 0.0077$) and $PM_{2.5}$ ($F = 17.29$, $p < 0.001$) (Table 2). Post hoc Tukey analysis (Table 3) showed that AQI-US levels in Lahore were significantly higher than those in Islamabad ($p = 0.0066$), whereas differences involving Okara were not statistically significant. For $PM_{2.5}$, Lahore exhibited significantly higher concentrations compared to both Islamabad ($p = 0.0017$) and Okara ($p = 0.0017$), while no significant difference was observed between Islamabad and Okara ($p > 0.05$).

Table 3
Tukey post hoc comparison between cities

Parameter	Comparison	Mean Difference	p value	Interpretation
AQI-US	Lahore vs Islamabad	89.93	0.0066	Significant
	Okara vs Islamabad	30.23	0.3888	Not significant
	Okara vs Lahore	-59.70	0.0549	Not significant
$PM_{2.5}$	Lahore vs Islamabad	150.65	0.0017	Significant
	Okara vs Islamabad	0.98	0.9994	Not significant
	Okara vs Lahore	-149.68	0.0017	Significant
Temperature	Lahore vs Islamabad	0.65	0.9542	Not significant
	Okara vs Islamabad	3.88	0.2426	Not significant
	Okara vs Lahore	3.23	0.3578	Not significant
Humidity	Lahore vs Islamabad	32.98	< 0.001	Significant
	Okara vs Islamabad	-2.23	0.8274	Not significant
	Okara vs Lahore	-35.20	< 0.001	Significant

3.2 Variation in Meteorological Conditions

Meteorological parameters varied across the study sites and sampling periods (Fig. 4; Table 2). The temperature ranged from 12.7 ± 1.1°C to 20.0 ± 2.6°C in Lahore, from 13.6 ± 1.9°C to 18.9 ± 3.1°C in Islamabad, and from 17.4 ± 3.2°C to 24.2 ± 3.1°C in Okara. Relative humidity showed notable variation, with higher values in Lahore (62.7 ± 5.1% to 77.8 ± 6.3%) than in Islamabad (27.8 ± 6.8% to 40.9 ± 6.7%) and Okara (29.8 ± 7.3% to 35.1 ± 6.4%). Statistical analysis indicated that temperature did not differ significantly among cities ($F = 1.74$, $p = 0.229$), whereas relative humidity showed significant variation ($F = 55.14$, $p < 0.001$) (Table 3). Tukey post hoc analysis (Table 3) confirmed that humidity levels in Lahore were significantly higher than those in both Islamabad ($p < 0.001$) and Okara ($p < 0.001$), while no significant difference was observed between Islamabad and Okara ($p > 0.05$).

3.3 Relationship Among Environmental Parameters

Correlation analysis based on monthly aggregated data showed relationships among environmental variables (Fig. 5). A strong positive correlation was observed between $PM_{2.5}$ and AQI-US ($r = 0.92$). Relative humidity showed a strong positive correlation with $PM_{2.5}$ ($r = 0.91$) and a moderate positive correlation with AQI-US ($r = 0.74$). In contrast, temperature exhibited negative correlations with $PM_{2.5}$ ($r = -0.40$), AQI-US ($r = -0.26$), and relative humidity ($r = -0.36$). These results indicate distinct associations among air quality and meteorological parameters across the study sites.

3.4 Airborne Antimicrobial Resistance Gene (ARG) Relative Abundance Across Study Sites

3.4.1 Relative abundance of airborne antimicrobial resistance genes across cities

The relative abundance of nine clinically relevant antimicrobial resistance genes (ARGs) associated with $PM_{2.5}$ was assessed across Lahore, Islamabad, and Okara using qPCR-based quantification normalized to 16S rRNA (2^−ΔCt). The distribution of ARGs across the study sites is presented in Fig. 4, illustrating gene-specific variability in abundance patterns. Among the analysed genes, blaNDM-1 exhibited the highest relative abundance across all cities, with mean values of approximately 0.077 in Lahore, 0.056 in Islamabad, and 0.009 in Okara (Figs. 6 and 7). Similarly, blaCTX-M-32 showed higher abundance in Islamabad (0.026) compared to Lahore (0.008) and minimal levels in Okara (0.001), indicating spatial variability in β-lactamase gene distribution.

The blaTEM gene displayed consistently low abundance across all sites (≈ 0.001–0.002). In contrast, blaTEM-F exhibited comparatively higher levels, particularly in Lahore (0.031) and Islamabad (0.025), with a substantially lower abundance in Okara (0.005). Genes associated with mobile genetic elements also demonstrated notable variation. The class 1 integrase gene (intI1) showed moderate abundance across all cities, ranging from 0.013 in Okara to 0.028 in Islamabad, while the transposase gene (tnpA) exhibited higher levels in Islamabad (0.021) than in Lahore (0.010) and Okara (0.007). Among the vancomycin resistance genes, vanA and vanRA displayed distinct spatial patterns. The vanA gene showed its highest abundance in Okara (0.002), whereas it remained lower in Lahore (0.0005) and nearly absent in Islamabad. Conversely, vanRA exhibited higher abundance in Islamabad (0.016) and Lahore (0.012) than in Okara (0.008). The sulfonamide resistance gene (sul1) also demonstrated marked variation, with the highest abundance observed in Islamabad (0.028), followed by Okara (0.005) and minimal levels in Lahore (0.001).

Overall, the results indicate substantial spatial variability in ARG relative abundance across the studied cities, with several clinically relevant genes showing higher levels in urban environments. It should be noted that these findings represent relative abundance patterns derived from pooled monthly samples and therefore reflect aggregated environmental signals rather than individual sample-level variability.

3.4.2 Distribution patterns of ARGs across cities

To further evaluate the spatial distribution of ARGs, mean relative abundance values were visualized using a heatmap (Fig. 6), providing an integrated comparison of gene prevalence across the three study sites. The heatmap highlights distinct patterns of gene enrichment, with several ARGs showing clear variation among cities. Consistent with the distribution trends observed in Figs. 7 and 8, blaNDM-1 exhibited the highest relative abundance overall, with markedly elevated levels in Lahore (0.077) and Islamabad (0.056) compared to Okara (0.009). Similarly, blaTEM-F showed higher abundance in Lahore (0.031) and Islamabad (0.025) but remained substantially lower in Okara (0.005).

In contrast, sul1 displayed a distinct pattern, with the highest abundance in Islamabad (0.028), followed by lower levels in Okara (0.005) and minimal abundance in Lahore (0.001). The intI1 gene showed a relatively consistent presence across all cities, with moderate abundance ranging from 0.013 to 0.028, suggesting its widespread distribution. Genes associated with mobile genetic elements and resistance variability also exhibited site-specific differences. The tnpA gene showed a higher abundance in Islamabad (0.021) than in Lahore (0.010) and Okara (0.007), while blaCTX-M-32 was enriched in Islamabad (0.026) relative to Lahore (0.008) and Okara (0.001).

Among vancomycin resistance genes, vanA showed its highest abundance in Okara (0.002), whereas vanRA was more abundant in Islamabad (0.016) and Lahore (0.012). The blaTEM gene remained consistently low across all cities (≈ 0.001–0.002), indicating minimal variation. Overall, the heatmap analysis confirms a heterogeneous distribution of ARGs across the study sites, with several genes showing preferential enrichment in specific cities. These patterns are consistent with the genewise distributions presented in the preceding figures and provide a consolidated overview of ARG prevalence across different environmental settings.

3.4.3 Correlation among antimicrobial resistance genes

To investigate potential relationships among the detected ARGs, a Spearman correlation analysis was performed based on relative abundance values (Fig. 9). The analysis revealed both positive and negative associations among genes, indicating variable co-occurrence patterns within the airborne resistome. Several ARGs demonstrated moderate to strong positive correlations. Notably, blaNDM-1 showed positive correlations with blaTEM-F ($r \approx 0.72$) and intI1 ($r \approx 0.68$), suggesting potential co-occurrence under similar environmental conditions. Similarly, tnpA exhibited a positive correlation with intI1 ($r \approx 0.64$), indicating a possible association with mobile genetic elements.

In contrast, weaker or negative correlations were observed for certain genes. For example, vanA showed weak to negative associations with β-lactamase genes such as blaNDM-1 ($r \approx -0.30$), suggesting distinct distribution patterns across sampling sites. Overall, the correlation analysis indicates that while some ARGs tend to cooccur, others exhibit independent or contrasting distribution patterns, reflecting the heterogeneous nature of ARG presence in airborne particulate matter.

4 Discussion

The present study reveals a clear spatial gradient in air pollution across the selected cities, with Lahore consistently exhibiting the highest burden of particulate pollution during the winter smog seasons. $PM_{2.5}$ concentrations in Lahore reached 276.6 ± 27.4 μg m^{-3} alongside AQI-US values approaching 289.3 ± 42.7, substantially exceeding those observed in Islamabad and Okara. These findings are consistent with previous reports identifying Lahore as a major hotspot of winter smog in South Asia, driven by dense traffic emissions, industrial activity, and regional biomass burning (Ashraf et al. 2022; Guttikunda and Gurjar 2012; Gurjar et al. 2008; Naureen et al. 2022). The persistence of such elevated concentrations highlights the cumulative effect of local emissions and transboundary pollution, which has been widely documented across the Indo-Gangetic Plain (Lelieveld et al. 2015).

The significant differences observed in this study further support the existence of distinct urban and semiurban pollution profiles. Significant variation in $PM_{2.5}$ and AQI-US across cities, particularly between Lahore and Islamabad, reflects the influence of urbanization intensity and emission density. Similar spatial contrasts have been reported in multicity assessments, where megacities consistently exhibit higher particulate loads than smaller or less industrialized regions (Gurjar et al. 2008; Zeb et al. 2025). In contrast, the lack of a significant difference between Islamabad and Okara suggests that moderate urban environments and semiurban regions may share comparable pollution characteristics under certain meteorological conditions, despite differences in emission sources.

Meteorological factors emerged as critical determinants of pollution variability, particularly the role of humidity and temperature. The strong positive correlations observed between $PM_{2.5}$ and relative humidity ($r = 0.91$), as well as between AQI-US and humidity ($r = 0.74$), indicate that moisture-rich atmospheric conditions favour particulate accumulation. This agrees with previous studies demonstrating that high humidity enhances the hygroscopic growth of aerosol particles, increases light scattering, and promotes secondary aerosol formation through heterogeneous chemical reactions (Naureen et al. 2022; Seinfeld and Pandis 2016).

Conversely, the negative relationship between temperature and $PM_{2.5}$ ($r = -0.40$) reflects the role of thermal dynamics in pollutant dispersion. Lower temperatures are typically associated with reduced planetary boundary layer height, which limits vertical mixing and traps pollutants near the surface (Ashraf et al. 2022; Jacob and Winner 2009). The absence of significant temperature differences among cities suggests that while temperature influences pollution temporally, it may not be the primary driver of spatial variability compared to humidity and emission sources.

The strong correlation between $PM_{2.5}$ and AQI-US ($r = 0.92$) confirms that fine particulate matter is the dominant contributor to air quality deterioration in the studied regions. This finding aligns with global observations that $PM_{2.5}$ is a key determinant of AQI in heavily polluted urban environments (Lelieveld et al. 2015; Organization 2021). In addition to physicochemical characteristics, $PM_{2.5}$-associated environments may also influence microbial and genetic components. Previous studies have suggested that particulate matter is associated with the presence and transport of microorganisms, providing protection against environmental stressors such as ultraviolet radiation and desiccation (Smets et al. 2016).

Collectively, the findings of this study highlight the multifactorial nature of winter smog formation, driven by the interaction between anthropogenic emissions and meteorological conditions. The observed pollution–climate relationships provide a critical environmental context for understanding airborne particulate dynamics in Pakistan. The present study provides a comprehensive assessment of the relative abundance and distribution of airborne antimicrobial resistance genes (ARGs) associated with $PM_{2.5}$ across urban and semiurban environments in Pakistan. The results revealed pronounced spatial variability in ARG profiles, with several clinically relevant genes exhibiting elevated relative abundance in more urbanized settings, particularly Lahore and Islamabad. These findings are consistent with the influence of environmental conditions and anthropogenic activities on the airborne resistome.

Among the analysed genes, blaNDM-1 exhibited the highest relative abundance across all study sites, with mean values of approximately 0.077 in Lahore, 0.056 in Islamabad, and 0.009 in Okara. This marked gradient may be associated with urbanization intensity and the presence of carbapenem resistance determinants in airborne particulate matter. Similar findings have been reported in previous studies, where blaNDM-1 has been detected in environmental matrices, including wastewater, aerosols, and urban air (Li et al. 2018).

In contrast, blaCTX-M-32 demonstrated a distinct spatial pattern, with the highest relative abundance observed in Islamabad (0.026) compared to Lahore (0.008) and Okara (0.001). This variability may reflect that different β-lactamase genes are influenced by localized emission sources and environmental conditions. The blaTEM gene showed consistently low abundance across all sites (≈ 0.001–0.002), whereas blaTEM-F exhibited comparatively higher levels, particularly in Lahore (0.031) and Islamabad (0.025). This difference between closely related gene variants highlights the complexity of ARG distribution in environmental samples.

Genes associated with mobile genetic elements (MGEs), including intI1 and tnpA, also showed notable patterns. The intI1 gene demonstrated moderate and relatively consistent abundance across all cities (0.013–0.028), suggesting its widespread presence. Similarly, tnpA exhibited a higher abundance in Islamabad (0.021) than in Lahore (0.010) and Okara (0.007). The sulfonamide resistance gene sul1 displayed a distinct distribution pattern, with the highest abundance in Islamabad (0.028). This observation may be associated with differences in antibiotic usage patterns and environmental contamination sources.

The correlation analysis further provided insights into potential co-occurrence patterns among ARGs. Positive correlations observed between blaNDM-1, blaTEM-F, and intI1 indicate that these genes may be associated with similar environmental sources or carriers. However, it is important to note that correlation does not imply causation, and these relationships should be interpreted cautiously. The observed patterns indicate that environments with higher $PM_{2.5}$ levels were associated with greater ARG relative abundance.

It is important to emphasize that the present study is based on DNA-derived relative abundance, which reflects the presence of ARGs rather than their transcriptional activity or expression. Therefore, conclusions regarding gene activity or microbial viability cannot be drawn from these data. Additionally, the use of pooled monthly samples represents a methodological limitation. While pooling was necessary to obtain sufficient eDNA from low-biomass $PM_{2.5}$ samples, it reduces the ability to assess temporal variability and limits statistical inference.

Despite these limitations, the study provides valuable insights into the distribution of ARGs in airborne particulate matter under real-world environmental conditions. The observed spatial variability, combined with the presence of clinically relevant resistance genes and mobile genetic elements, underscores the potential role of air pollution in the environmental presence and distribution of antimicrobial resistance.

5 Conclusions

This study provides a comprehensive assessment of airborne antimicrobial resistance genes (ARGs) associated with $PM_{2.5}$ across major urban and semiurban environments in Pakistan during winter smog conditions. The findings demonstrate clear spatial variability in both air pollution and ARG relative abundance, with highly urbanized regions such as Lahore exhibiting elevated $PM_{2.5}$ levels alongside increased abundance of clinically relevant resistance genes.

Among the detected ARGs, blaNDM-1 emerged as the most dominant gene, particularly in Lahore (0.077) and Islamabad (0.056), indicating a strong association between urban pollution intensity and the environmental presence of carbapenem resistance determinants. Other genes, including blaTEM-F, intI1, and sul1, also showed variable distributions across cities, reflecting the influence of localized environmental conditions and potential anthropogenic sources. The observed co-occurrence patterns between ARGs and mobile genetic elements further suggest that airborne particulate matter may contribute to the environmental persistence and distribution of resistance determinants.

Importantly, the study demonstrates that $PM_{2.5}$-associated DNA can act as an environmental reservoir of ARGs; however, these findings reflect relative abundance rather than gene expression or microbial activity. While the use of pooled monthly samples enabled sufficient DNA recovery from low-biomass $PM_{2.5}$ filters, it limits the assessment of temporal variability and restricts statistical inference at the individual sample level.

Overall, these findings highlight the potential role of air pollution as a contributor to the environmental dissemination of antimicrobial resistance and emphasize the need to incorporate airborne pathways into Antimicrobial resistance (AMR) surveillance frameworks. Future studies employing higher-resolution sampling, biological replication, and RNA-based approaches are necessary to better understand the functional dynamics and public health implications of airborne resistomes.

Declarations

Ethics approval and consent to participate

This study involved environmental air sampling and molecular analysis of particulate matter-associated environmental DNA. No human participants, human tissues, personal data, vertebrate animals, or regulated biological materials were involved. Therefore, ethical approval and informed consent were not required for this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contribution

M.I.K., A.A. and I.A. conceived and designed the study. M.I.K. performed the experimental work, analysed the data and prepared the initial manuscript draft. N.K. contributed to writing, review and editing. A.A. and I.A. supervised the study and contributed to review and editing. All authors reviewed and approved the final manuscript.

Acknowledgements

Not applicable.

Data Availability

All the data generated or analysed during this study are included in this published article files.

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Figures

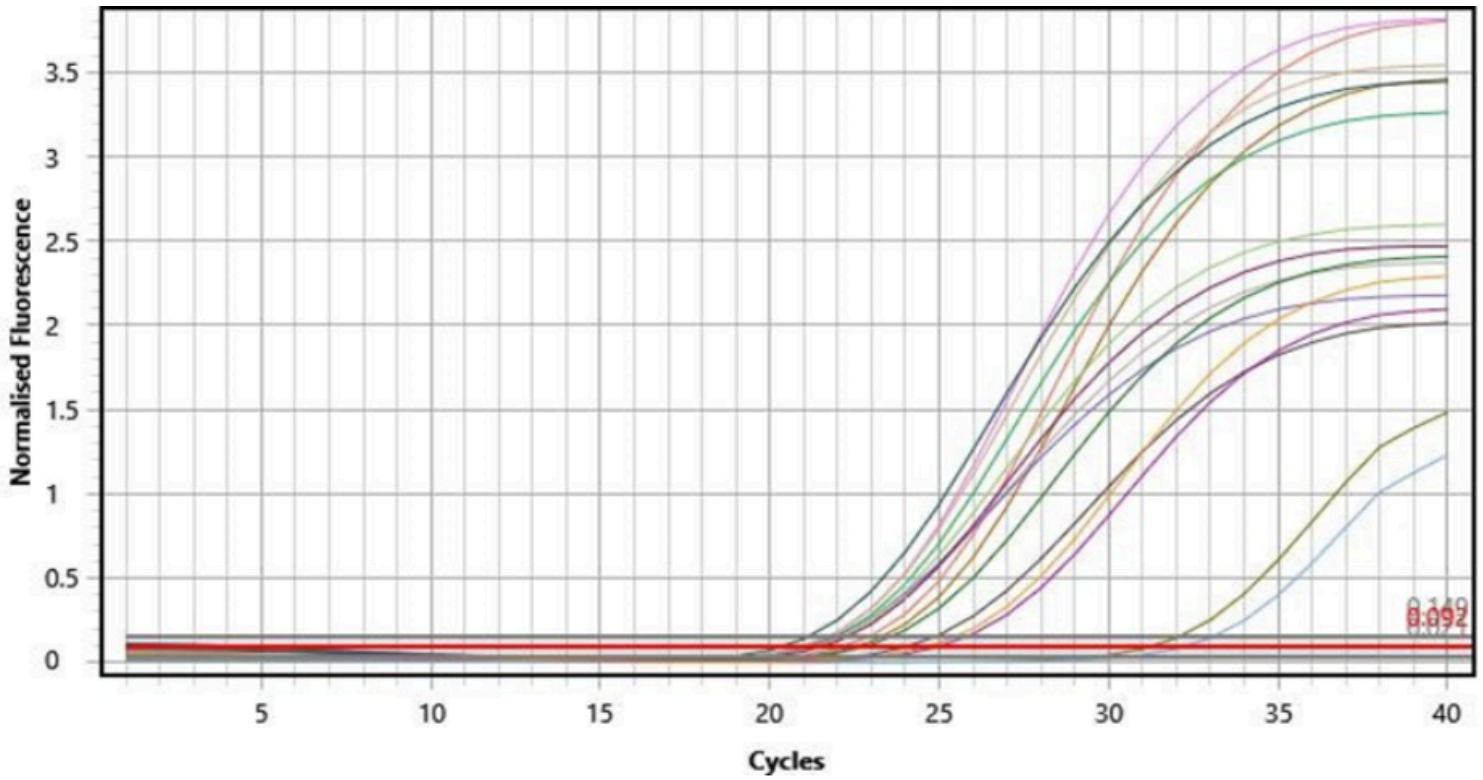


Figure 1

Image showing the cycling graph of real-time PCR.

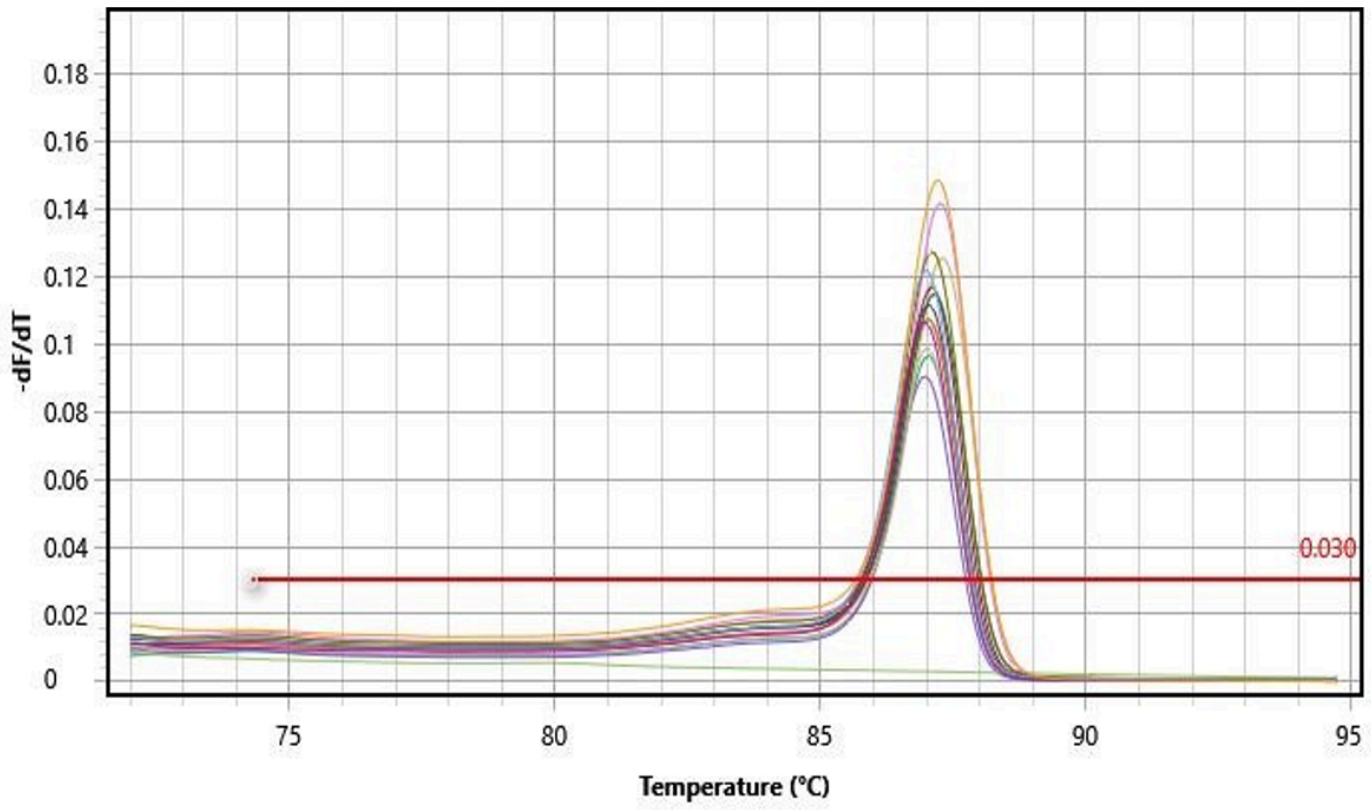


Figure 2

Real-time PCR melt curve

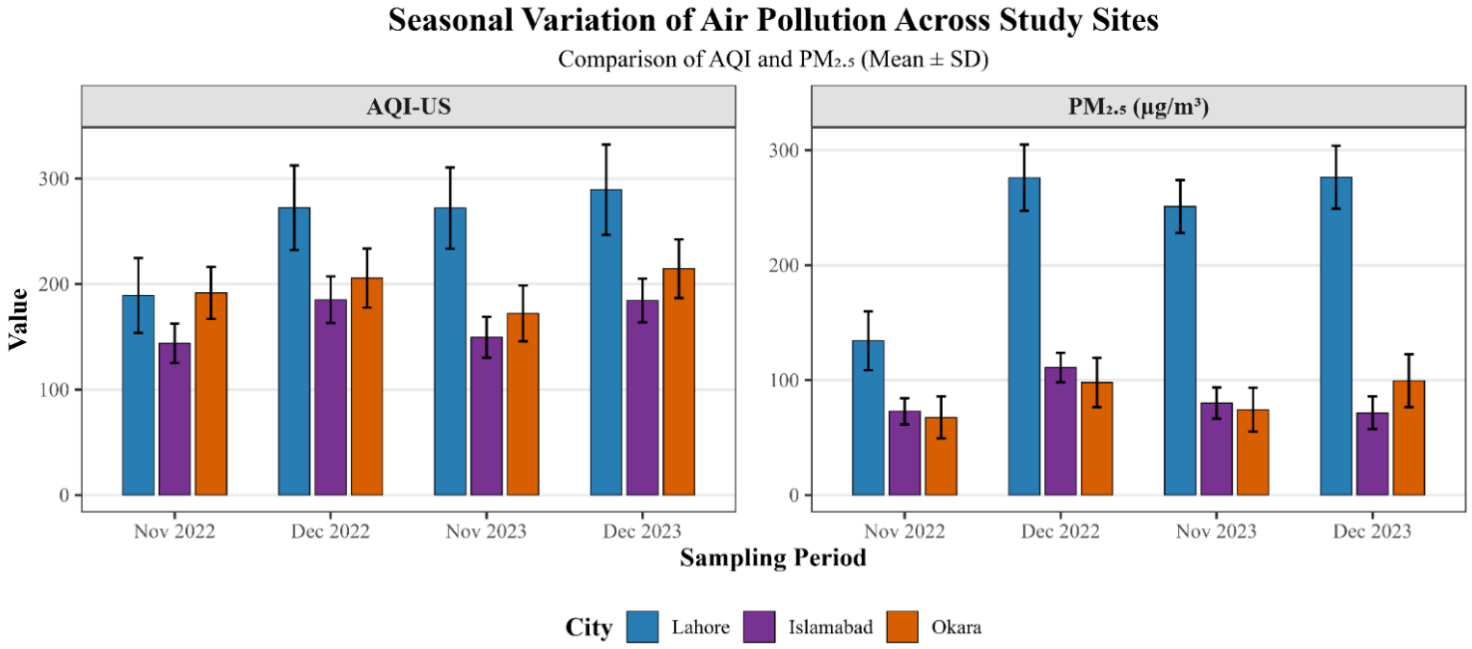


Figure 3

Variation in AQI-US and $PM_{2.5}$ across Lahore, Islamabad, and Okara during November–December 2022 and 2023

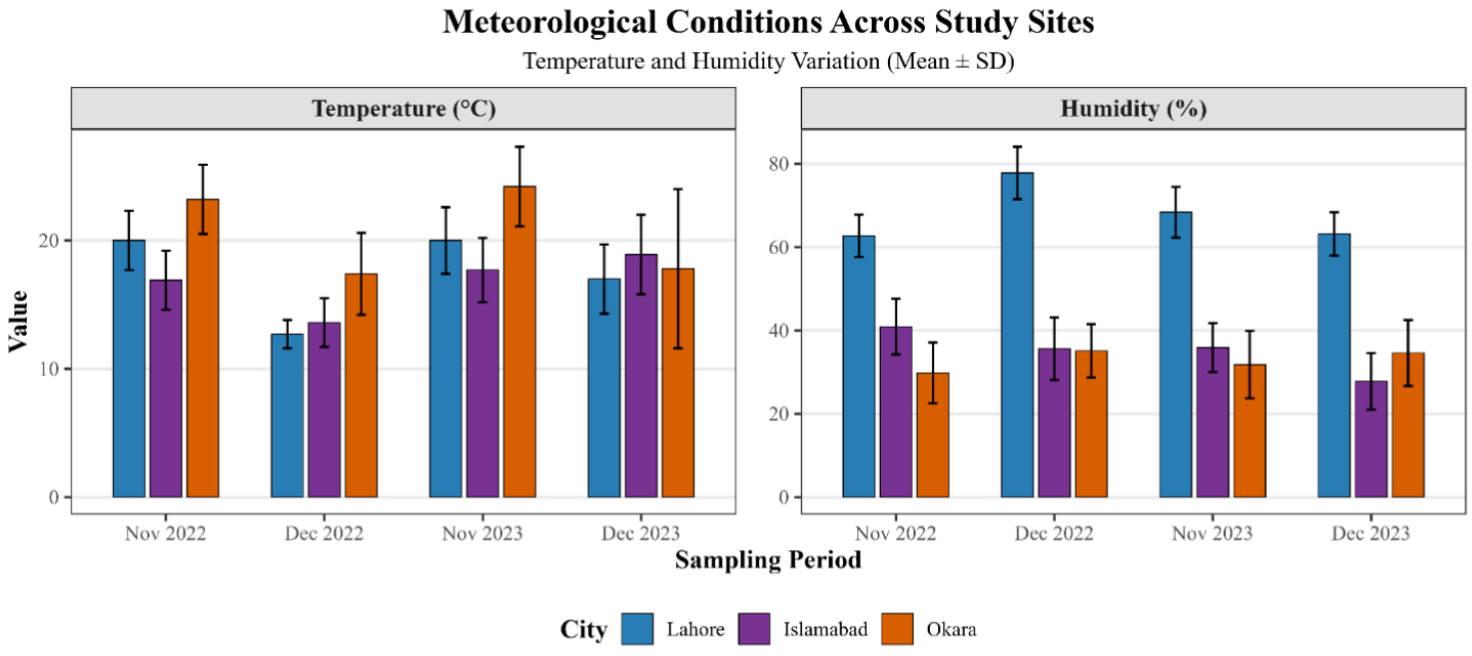


Figure 4

Variation in temperature and relative humidity across the study sites during the sampling period.

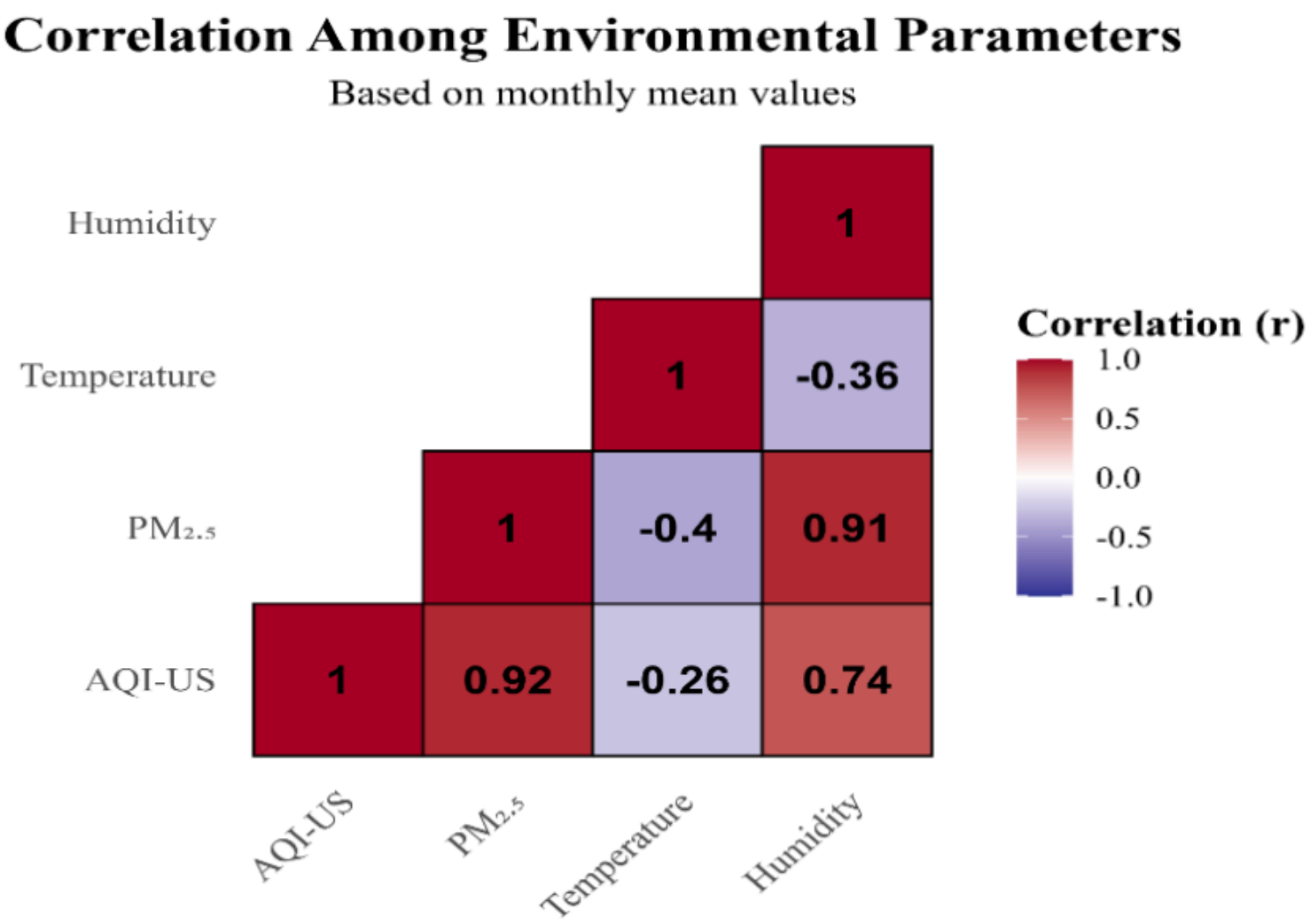


Figure 5

Correlation heatmap showing relationships among AQI-US, $PM_{2.5}$, temperature, and humidity.

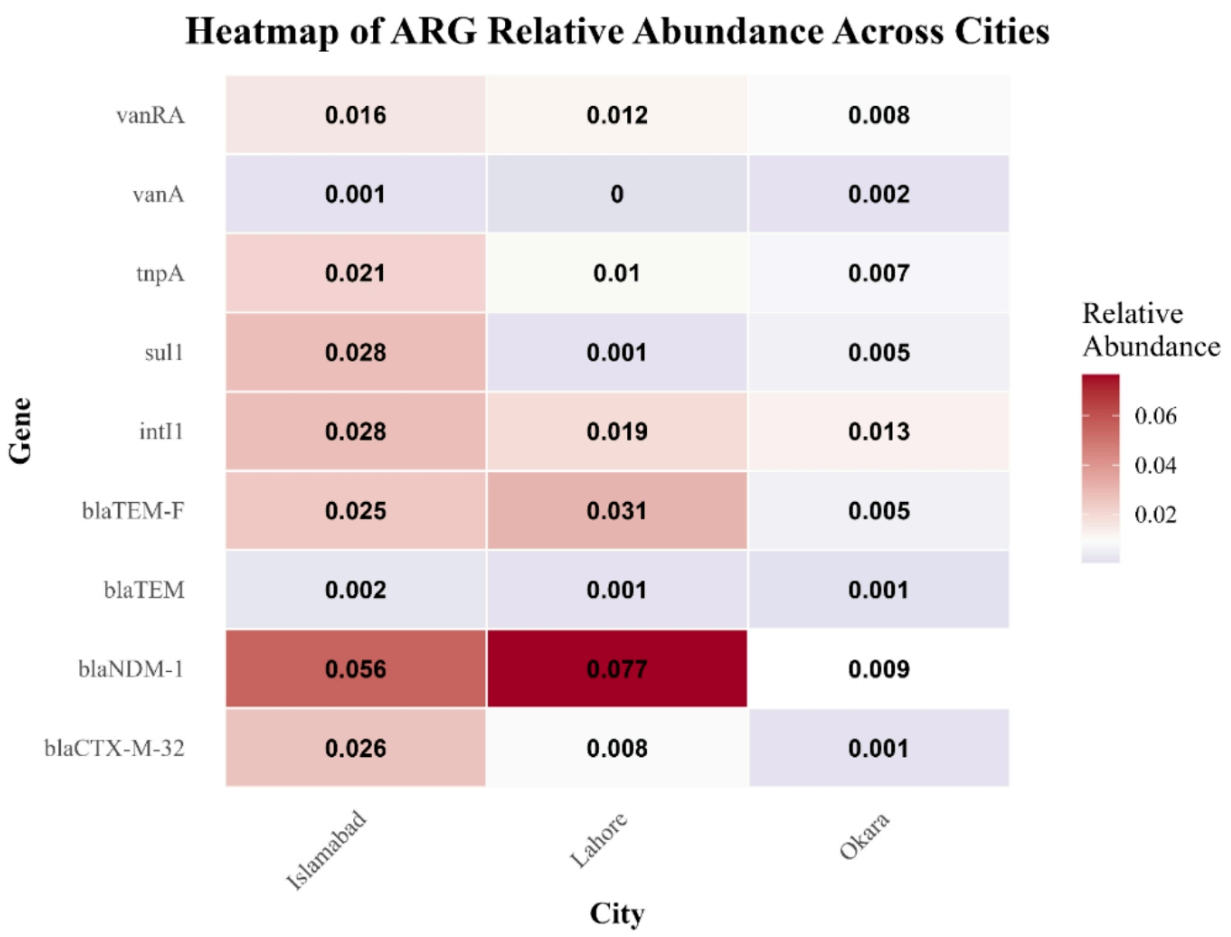


Figure 6

Heatmap showing the mean relative abundance of ARGs across Lahore, Islamabad, and Okara. Color intensity represents the magnitude of relative abundance for each gene.

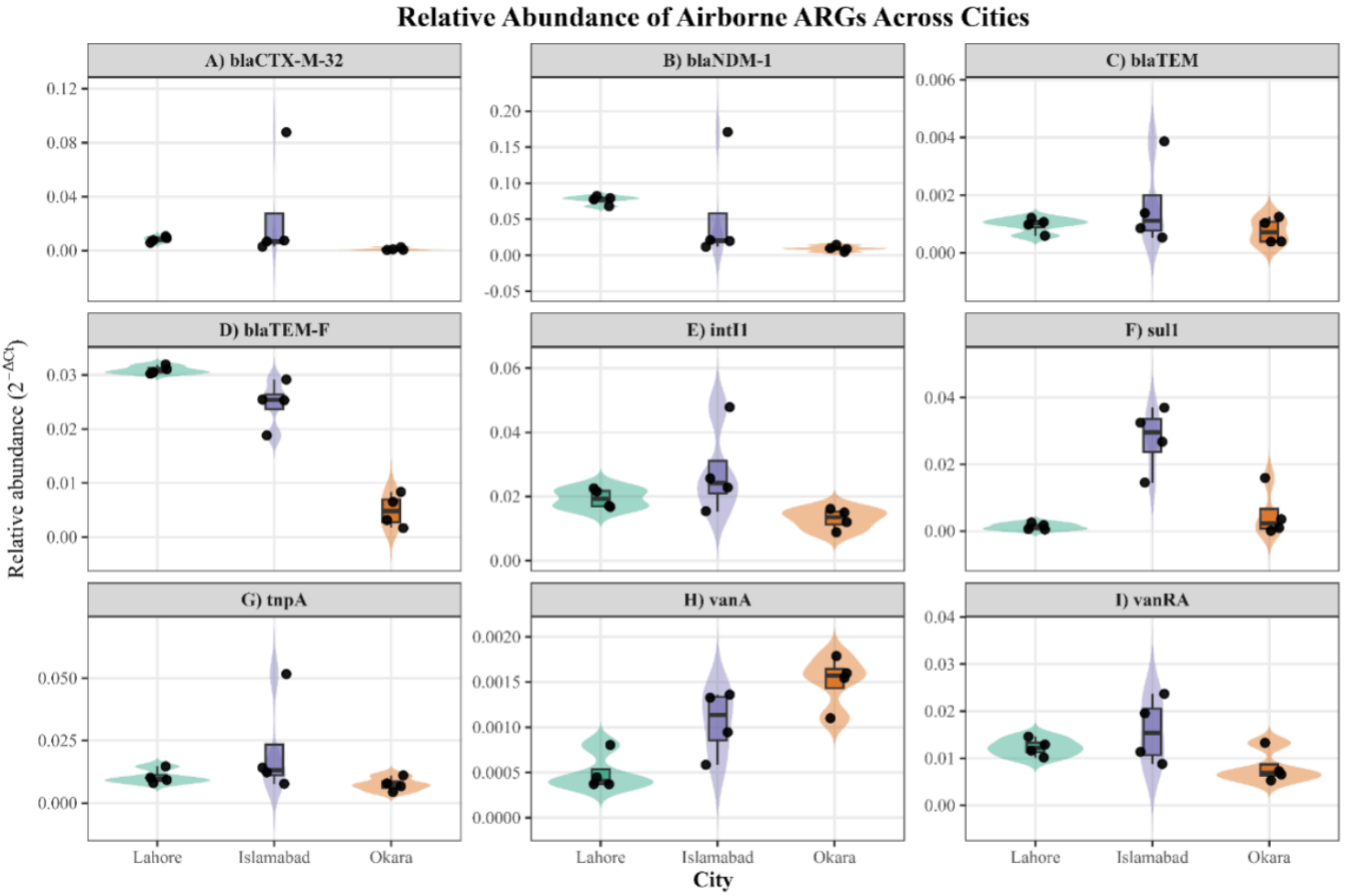


Figure 7

Relative abundance of airborne antimicrobial resistance genes (ARGs) across Lahore, Islamabad, and Okara, determined by qPCR and expressed as 2^−ΔCt. Violin plots with overlaid boxplots and individual data points illustrate the distribution of ARGs across the study sites.

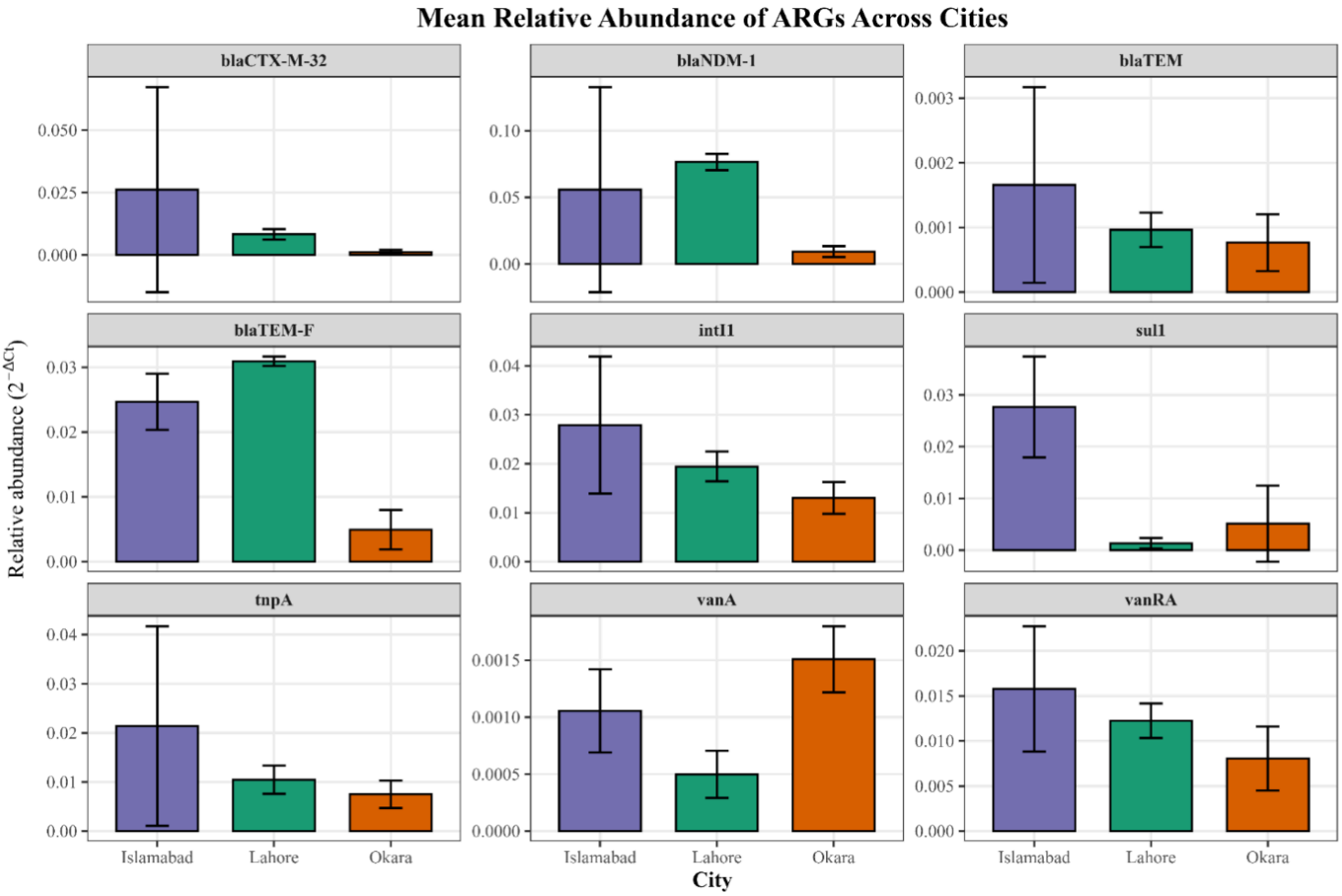


Figure 8

Mean relative abundance of ARGs across cities, presented as the mean ± standard deviation (SD) based on qPCR quantification (2^−ΔCt).

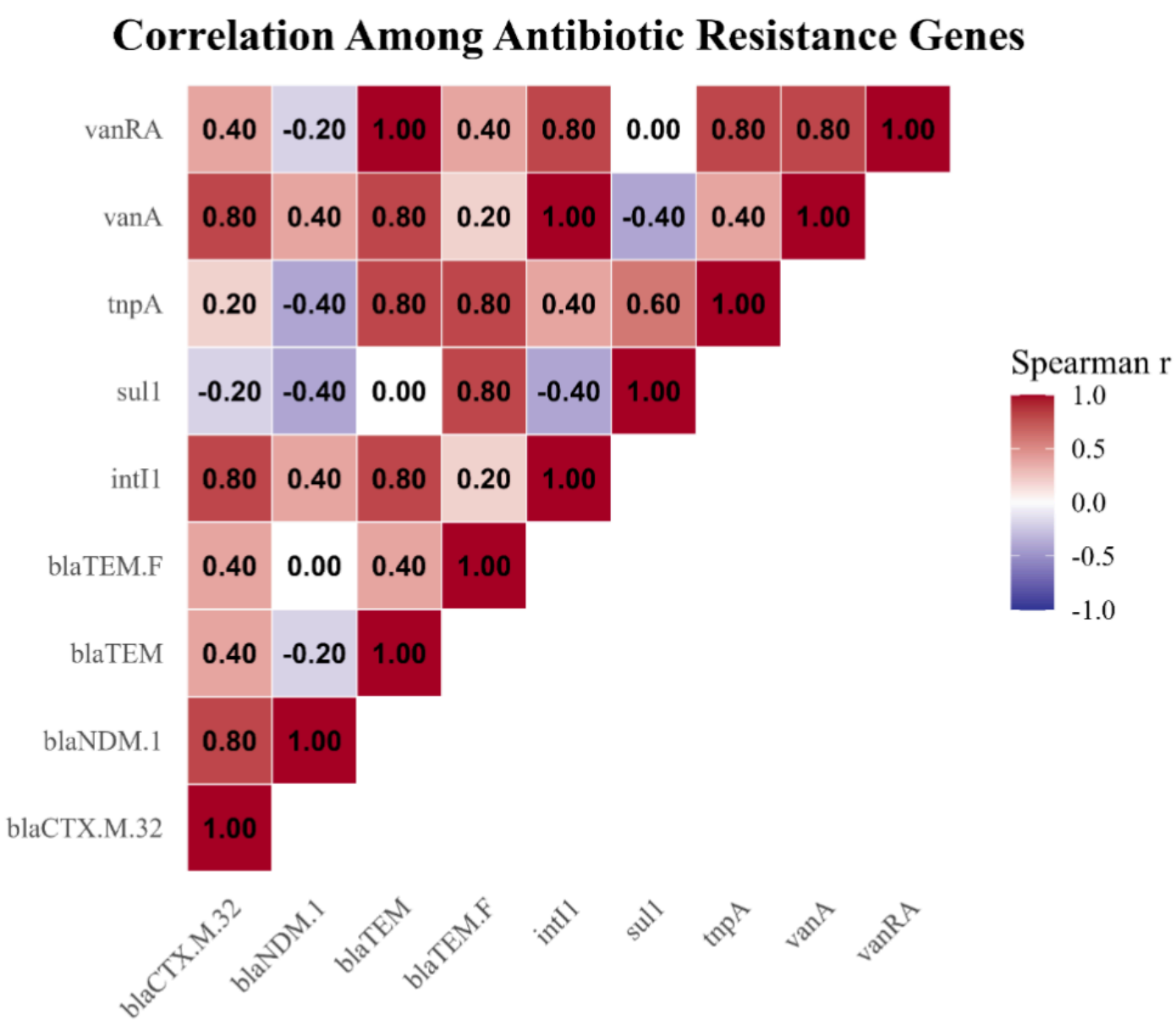


Figure 9

Spearman correlation heatmap illustrating relationships among ARGs based on relative abundance values. Correlation coefficients (r) are displayed within each cell.

Supplementary Files

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- GraphicalAbstract.docx