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ORIGINAL RESEARCH

Seasonal Genomic Dynamics of Multidrug-Resistant Pathogens in ICU Environments and Perspectives on Phage-Based Interventions

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Background: The emergence of multidrug-resistant (MDR) pathogens in intensive care units (ICUs) has become a pressing global health issue, contributing to mortality rates exceeding 40%. Among these, carbapenem-resistant *Klebsiella pneumoniae* and *Acinetobacter baumannii* are especially problematic. Seasonal fluctuations in resistance patterns have been observed, yet the genomic mechanisms underlying these trends remain insufficiently characterized.

Objective: This study investigated the seasonal variation in resistance gene prevalence among ICU-derived bacterial isolates and elucidates the genomic features contributing to antimicrobial resistance.

Methods: Environmental and clinical samples were collected from ICU settings over multiple seasons using a systematic, stratified approach. Whole-genome sequencing was conducted on isolates via Illumina and Nanopore platforms. Resistance genes were annotated using CARD, VFDB, and BiocideResistance databases. Statistical associations were assessed using logistic regression and generalized linear mixed models, while phylogenetic trees evaluated clonal relationships.

Results: The $bla_{CTX\text{-}M\text{-}3}$ gene was detected in 100% of autumn isolates (n=52), showing a statistically significant association with increased bed turnover and prolonged disinfection intervals (p=0.003). During winter, 75% of isolates (n=50) tested positive for qacEΔ1, correlating with elevated multidrug resistance indices (p=0.01) and patterns consistent with clonal expansion based on whole-genome SNP profiling. These winter strains also exhibited enhanced biofilm formation capacity (OD_{595}=0.67 ± 0.11) and upregulation of efflux pump transcripts (2.3-fold increase vs summer; p=0.02), supporting environmental adaptation under low-temperature stress. Notably, aac(6')-Ib7, an aminoglycoside-modifying enzyme gene, was the most frequently detected resistance determinant, present in 68% of isolates, highlighting substantial antibiotic selection pressure.

Conclusion: This study reveals distinct seasonal genomic patterns in ICU drug-resistant pathogens and emphasizes the necessity for adaptive infection control strategies. Targeted disinfection, antibiotic stewardship, and consideration of phage therapy as a complementary strategy particularly during winter may help mitigate the spread of high-risk resistant clones, though further in vitro and in vivo validation is required.

Keywords: intensive care unit, antimicrobial resistance, seasonal variability, whole-genome sequencing, *Klebsiella pneumoniae*, phage therapy, infection control strategies

Introduction

The intensive care unit (ICU) is a critical nexus for the emergence and proliferation of antimicrobial resistance (AMR), representing both a microcosm of hospital-wide resistance trends and a high-risk reservoir for multidrug-resistant organisms (MDROs).[1,2] The convergence of vulnerable patients, broad-spectrum antibiotic usage, prolonged hospitalization, and frequent use of invasive medical devices creates a uniquely conducive environment for the selection and

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transmission of resistant pathogens.[3–5] These dynamics not only compromise clinical outcomes but also escalate healthcare costs, prolong ICU stays, and increase morbidity and mortality.

Among the most clinically relevant MDROs in ICU settings are *Acinetobacter baumannii, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*—collectively part of the WHO-prioritized ESKAPE group of pathogens.[6,7] *A. baumannii*, in particular, is distinguished by its extraordinary ability to withstand desiccation, evade antibiotics via robust efflux pumps and enzymatic modifications, and persist through biofilm formation on abiotic surfaces.[8] The evolutionary trajectory of this pathogen is driven by mobile genetic elements, including resistance islands (AbaR), integrative conjugative elements, and plasmid-encoded β-lactamases.[9–11,] These mechanisms collectively facilitate resistance to carbapenems, aminoglycosides, fluoroquinolones, and, increasingly, colistin—one of the last-line therapeutics.[12]

A growing body of evidence highlights the role of the ICU environment itself in perpetuating AMR. In particular, sink drains, bed rails, ventilator tubing, and humidifiers serve as environmental reservoirs for resistance genes and viable pathogens.[13] Numerous studies have implicated biofilm-rich sink traps in recurrent outbreaks of carbapenem-resistant *K. pneumoniae* and *A. baumannii*, wherein high bacterial loads and residual disinfectants exert selection pressure favoring resistant clones.[14–16] One gene of interest in this context is qacEΔ1, which confers resistance to quaternary ammonium compounds (QACs), a common group of disinfectants widely used in ICUs.[17,18] These genes are frequently enriched in sink drains and other damp environments, enabling bacteria to survive routine cleaning. Importantly, qacEΔ1 is often embedded within class 1 integrons that also carry β-lactamase genes. This co-localization promotes co-selection, where exposure to disinfectants can inadvertently maintain or even enrich antibiotic resistance.[19] Such interactions illustrate how environmental and clinical pressures reinforce each other in ICU settings.

Notably, the seasonal variability of AMR in ICU pathogens is gaining scientific attention. Several epidemiological studies indicate a temperature-dependent fluctuation in the prevalence of resistant organisms, with Gram-negative bacteria such as *E. coli* and *K. pneumoniae* peaking during warmer months and Gram-positive organisms like MRSA and VRE more prominent in colder seasons.[20–22,] This pattern is attributed to factors such as increased ambient humidity, altered patient flow, seasonal staff availability, and variability in ventilation efficiency. The seasonal burden of AMR also correlates with meteorological data, underscoring the need for integrated environmental and genomic surveillance systems.[23]

The issue is especially pronounced in lower-middle-income countries (LMICs), where systemic challenges—ranging from inadequate infection control to delayed diagnostics and limited access to second-line antibiotics—exacerbate the problem.[24,25] In these settings, ICU infections with carbapenem-resistant *A. baumannii* and *K. pneumoniae* are often detected late, contributing to mortality rates exceeding 40% in some studies.[26] The prevalence of environmental and mobile genetic element-mediated resistance mechanisms, including integrons and *qacEΔ1*-carrying plasmids, is notably high in LMIC ICU isolates.[27]

Despite the known risks, few studies have comprehensively evaluated the interplay between seasonal dynamics, genomic features of resistance, and environmental persistence in ICU-derived pathogens. In particular, the role of phage therapy as a potential adjunct strategy during high-risk seasons remains underexplored. To address these gaps, we conducted a seasonally stratified genomic investigation of ICU-derived pathogens. Our objectives were threefold: (i) to quantify the seasonal prevalence and distribution of key antimicrobial resistance genes such as $bla_{CTX\text{-}M\text{-}3}$ and qacEΔ1; (ii) to map clonal transmission pathways between clinical and environmental isolates using SNP-based phylogenetics; and (iii) to examine biofilm-associated traits and discuss the feasibility of phage therapy as a potential biofilm-disruptive strategy.

Materials and Methods

Study Design and Sample Collection

A longitudinal, seasonally stratified environmental surveillance study was conducted in the ICU of a tertiary care hospital affiliated with Dalian University of Technology. Sampling was conducted from March to February, covering all four seasons, to evaluate the temporal variability in microbial resistance patterns and environmental contamination. The ICU

under surveillance was a closed unit with a capacity of 20 beds, admitting patients requiring mechanical ventilation, invasive monitoring, and continuous organ support.

To account for seasonal environmental changes and patient flow dynamics, sample collection strategies were adjusted accordingly:

- Spring (March–May): Characterized by elevated humidity, this period focused on potential moisture-associated contamination sources. Environmental swabs were collected from sinks, drainage outlets, and humid zones at a frequency of twice per week.
- Summer (June–August): Despite relatively stable conditions, monitoring continued biweekly, targeting both patient-contact surfaces and air conditioning ducts to assess ventilation-associated contamination.
- Autumn (September–November): Marking the peak admission period, daily sampling was performed from high-touch surfaces including bed rails, ventilator interfaces, infusion pumps, and monitors. Additionally, nasopharyngeal and rectal swabs were obtained from newly admitted patients for colonization screening.
- Winter (December–February): Due to diminished ventilation and potential airborne pathogen accumulation, air samples were prioritized. Aerosol samples were collected from air conditioning outlets, patient zones, and nursing stations using Anderson air samplers, three times per week.

Environmental and clinical samples included surface swabs (10 cm × 10 cm), liquid effluents (50 mL), and air samples (100 L). Samples were immediately placed into sterile, pre-chilled transport media and stored at 4°C. All samples were processed within two hours of collection. Concurrent environmental data, including ambient temperature, humidity, and ICU occupancy rates, were logged using automated monitoring systems and integrated into the dataset for multivariate analyses.

Sampling Design and Sample Size

Environmental and clinical isolates were collected over three consecutive seasons—summer (n=44), autumn (n=52), and winter (n=50)—from a tertiary care ICU. Sampling sites included sink drains, bed rails, ventilator tubing, and patient specimens (blood, tracheal aspirates, catheter tips). A power analysis ($\alpha = 0.05$, power = 0.80) estimated that a minimum of 45 isolates per season was required to detect a ≥15% difference in resistance gene prevalence between seasons, assuming a standard deviation of 12%. The total isolate count (N=146) thus meets statistical robustness for seasonal comparison.

Microbiological Processing and Phenotypic Resistance Testing

Samples were processed according to CLSI guidelines (2023). Bacterial species were identified using MALDI-TOF MS (Bruker Biotyper, v3.1) and confirmed by 16S rRNA sequencing. Antimicrobial susceptibility testing was performed via VITEK 2 (bioMérieux) and interpreted per CLSI breakpoints. Resistance to carbapenems, colistin, ciprofloxacin, and cephalosporins was noted. Isolates exhibiting resistance to ≥3 antibiotic classes were designated multidrug-resistant (MDR).

Whole-Genome Sequencing and Bioinformatics Pipeline

Genomic DNA was extracted from all isolates using the QIAamp DNA Mini Kit (Qiagen, Germany), following the manufacturer's protocol. DNA concentration and purity were assessed with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA). Sequencing libraries were prepared using the Nextera XT DNA Library Preparation Kit (Illumina, USA), and paired-end sequencing (2 × 150 bp) was performed on the Illumina HiSeq 2500 platform.

Raw sequencing reads were quality-filtered using Trimmomatic v0.39, and assembly was performed with SPAdes v3.15.3. Contigs were annotated using Prokka v1.14.6, and resistance genes were identified through the Comprehensive Antibiotic Resistance Database (CARD) and ResFinder 4.1. Integrons were detected using IntegronFinder v2.0, and plasmid replicons were characterized with PlasmidFinder v2.1. For phylogenetic analysis, single nucleotide polymorphisms (SNPs) were called using Snippy v4.6.0, and maximum-likelihood trees were constructed with RAxML-NG v1.1. Trees were visualized with iTOL v6.

To integrate sequencing with phenotypic data, biofilm-associated genes (mrkA, luxS) and other relevant operons were screened using BLASTn against reference genomes. All genomic features were cross-referenced with metadata on seasonal collection and source (environmental vs clinical).

Data processing was conducted using Python (pandas, matplotlib) for preprocessing, tabulation, and figure generation, while R (tidyverse, ggplot2) was used for statistical modeling, correlation analysis, and hypothesis testing. This combination ensured reproducibility and optimized both computational efficiency and visualization quality.

Biofilm and Gene Expression Analysis

Biofilm formation was assessed using the crystal violet microtiter plate assay and quantified by OD595. Gene expression of *mrkA* and *luxS* was analyzed via qRT-PCR using SYBR Green chemistry (Bio-Rad). Fold change was calculated using the $2^{-\Delta\Delta Ct}$ method normalized to 16S rRNA. Expression comparisons were made between clinical and environmental isolates.

Ethics Statement

This study was approved by the Institutional Ethics Committee of Dalian University of Technology . Environmental and anonymized clinical samples (eg, respiratory secretions, blood cultures, catheter tips) were collected strictly in accordance with institutional biosafety protocols and ethical guidelines. No identifiable personal information was accessed or recorded. Informed consent was not obtained from patients, as the use of residual, anonymized clinical specimens for microbial surveillance and genomic analysis was granted a waiver of consent by the Institutional Ethics Committee. This waiver was based on the following grounds: (1) the study posed no risk to patient safety or confidentiality, (2) all samples were anonymized at the time of collection, and (3) the research involved only routine surveillance samples typically discarded after diagnostic use.

Statistical Analysis

All statistical analyses were conducted using R v4.3.0. Seasonal differences in resistance rates and gene expression were evaluated using Chi-square or ANOVA as appropriate. Bonferroni correction was applied for multiple comparisons across gene categories. Significance was set at $p < 0.05$. Model diagnostics for linear regressions included variance inflation factors (VIF < 2 considered acceptable). Data preprocessing included handling missing values, normalization, and verification of data integrity using the tidyverse and pandas libraries. Analytical approaches included:

- Chi-square and Fisher's exact tests to evaluate associations between seasonal categories and resistance gene carriage.
- Independent t-tests and Mann–Whitney *U*-tests to compare continuous variables such as resistance indices between gene-positive and gene-negative isolates.
- Logistic regression models to quantify the relationship between clinical/environmental variables (eg, bed turnover, humidity) and resistance gene positivity, reporting odds ratios (ORs) with 95% confidence intervals.
- Generalized Linear Mixed Models (GLMMs) using the lme4 package to account for repeated measures across time points and potential hierarchical clustering within sample types.

Multicollinearity was assessed using Variance Inflation Factor (VIF), with a cutoff threshold of 5. Model performance was validated using the Hosmer–Lemeshow goodness-of-fit test and residual diagnostics. Data visualizations included heatmaps of resistance gene abundance (pheatmap), seasonal trend plots (ggplot2), and SNP-based phylogenies (ggtree). All statistical tests were two-tailed, and significance was defined as $p < 0.05$.

Results

Phylogenetic and Bioinformatic Analysis

Phylogenetic SNP analysis (Figure 1) revealed that environmental and clinical isolates clustered closely, with ≤20 SNP differences between sink-derived and patient-derived *K. pneumoniae* and *A. baumannii*. This suggests potential environmental-to-patient transmission within the ICU ecosystem, supporting the second research objective. Figure 2 illustrates the distribution and burden of antimicrobial resistance genes across isolates. Panel A shows the absolute resistance gene counts per isolate, while Panel B highlights resistance class diversity. Panel C demonstrates the proportion of isolates carrying high vs low resistance burdens, and Panel D identifies the most prevalent individual genes. Together, these results confirm seasonal enrichment of $bla_{CTX\text{-}M\text{-}3}$ in autumn and qacEΔ1 in winter, directly addressing our first objective. Figure 3 provides a schematic overview of the study workflow, demonstrating how environmental and clinical sampling was integrated with whole-genome sequencing and bioinformatic annotation.

Antimicrobial Resistance Profiles

Table 1 shows notable seasonal differences in the prevalence of resistance genes among ICU isolates. The $bla_{CTX\text{-}M\text{-}3}$ gene was detected in all isolates during autumn (100%) but was less frequent in other seasons ($p = 0.003$). This suggests that resistant strains may spread more readily during peak admission periods. Similarly, $bla_{KPC\text{-}2}$ was most common in autumn (78.1%, $p = 0.028$), while $bla_{NDM\text{-}1}$ appeared at moderate levels across seasons without significant variation ($p = 0.215$).

Phenotypic Resistance and Co-Carriage

Resistance testing confirmed a high burden of multidrug resistance. Carbapenem resistance was found in 57.5% of *K. pneumoniae* and 68.2% of *A. baumannii* isolates ($p < 0.05$). Colistin resistance remained rare (<5%), suggesting it may still be clinically useful in this ICU. Molecular analysis showed that bla_{KPC} was present in 28% and $bla_{OXA\text{-}48}$ in 32% of isolates, with 14% carrying both (Table 2). The relative abundance of resistance genes by species is shown in Figure 4, highlighting *K. pneumoniae* as the species with the heaviest gene burden, followed by *A. baumannii*, while *P. aeruginosa* carried fewer resistance determinants.

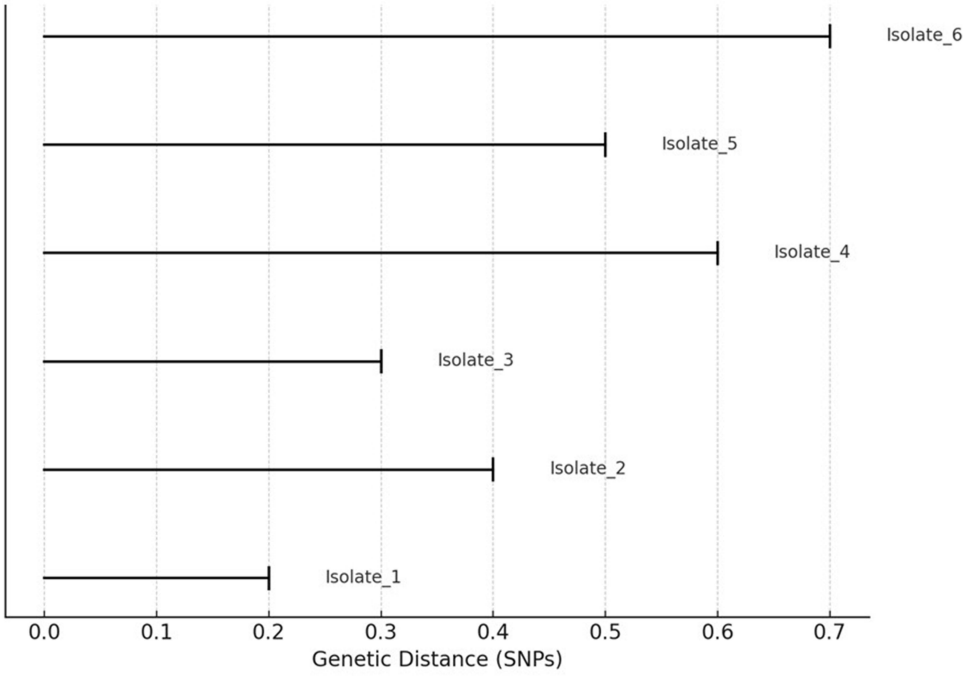

Figure 1 Phylogenetic tree based on whole genome SNP analysis. Constructed using Snippy and visualized in iTOL; n=30 isolates.

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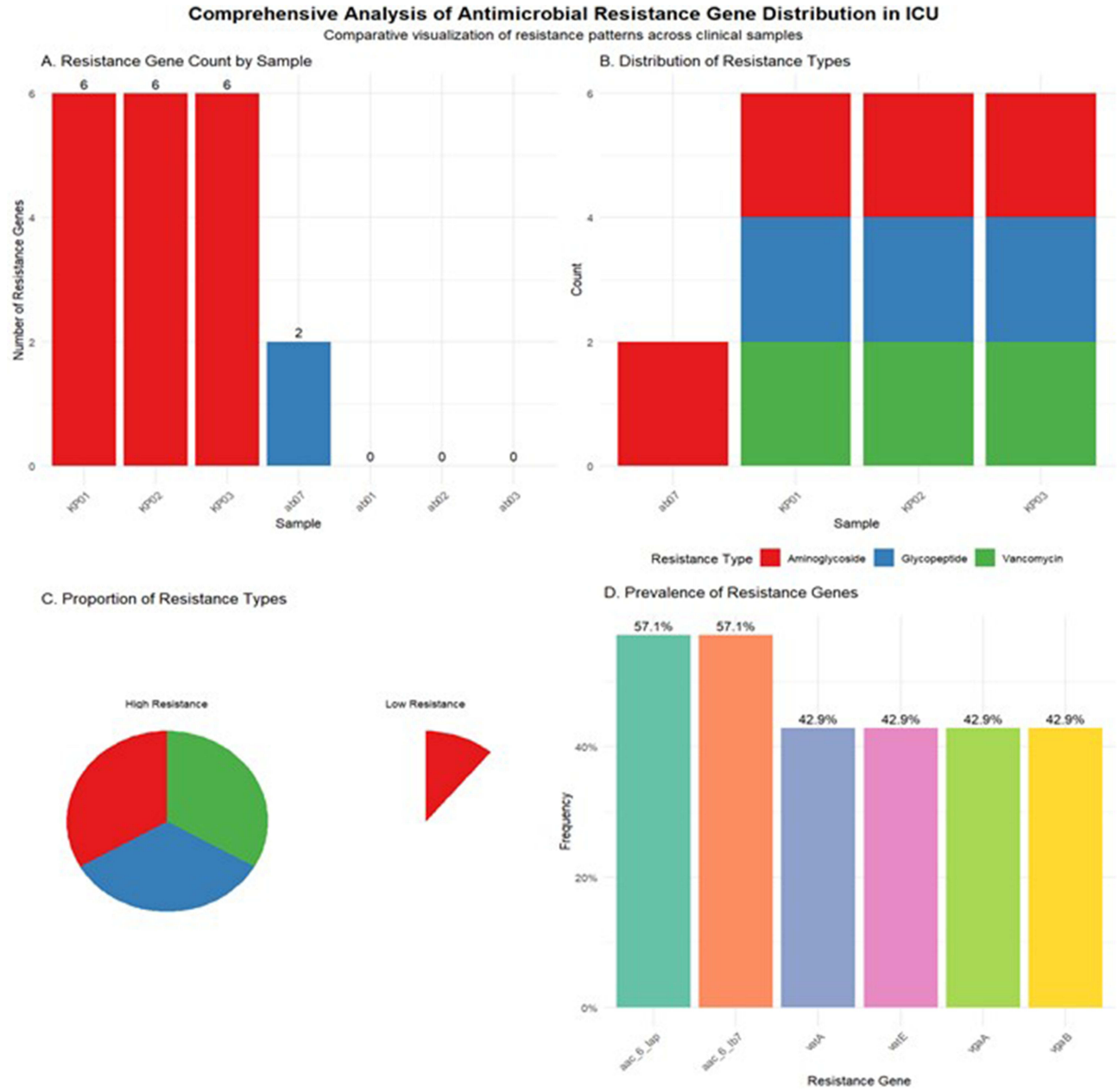

Figure 2 Comprehensive analysis of antimicrobial resistance (AMR) gene distribution in ICU isolates. (**A**) Resistance gene count by sample: bar chart showing the total number of annotated AMR genes per isolate. (**B**) Distribution of resistance types by sample: stacked bars showing detected classes (aminoglycoside, glycopeptide, vancomycin) per isolate. (**C**) Proportion of resistance burden: Pie charts summarizing the proportion of isolates with high vs low resistance burden. (**D**) Prevalence of key resistance genes: Showing the percentage of isolates harboring each gene.

Microbial Isolate Characterization

A total of 146 non-duplicate isolates were collected from ICU surfaces and patient specimens across three consecutive seasons. Identification using MALDI-TOF MS and 16S rRNA sequencing showed that *K. pneumoniae* was most common (42.5%), followed by *A. baumannii* (37.0%) and *P. aeruginosa* (20.5%). Environmental isolates were mainly from sink drains, bed rails, and ventilator tubing, while clinical isolates were sourced from respiratory secretions, blood cultures, and catheter tips (Table 3).

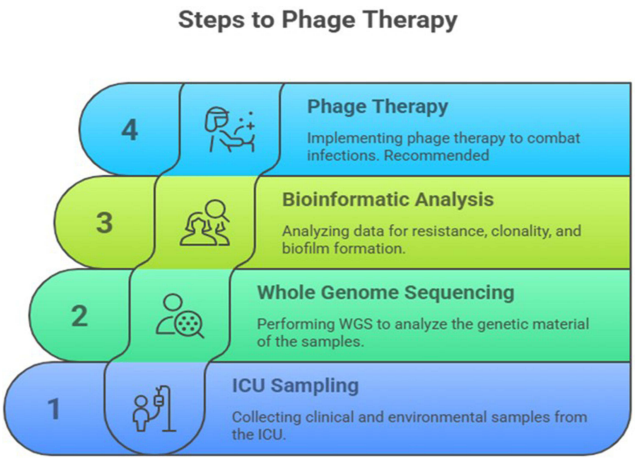

Figure 3 Schematic Workflow.

The frequency of sink contamination across sampling sites is shown in Figure 5. Patient-room sinks had the highest contamination levels (85–90%), followed by bathroom sinks (80%), while procedure bays showed comparatively lower contamination (65%). These findings highlight sinks as persistent environmental reservoirs of MDR pathogens.

Seasonal Trends in Resistance

The seasonal distribution of ICU-acquired infections is shown in Figure 6. Carbapenem-resistant *K. pneumoniae* increased from 29.1% in summer to 42.3% in winter, with a similar though smaller trend observed in *A. baumannii*. These results suggest that resistance patterns fluctuate with seasonal factors, possibly linked to changes in environment or patient load.

Biofilm Gene Expression

Among MDR isolates, 68% carried the mrkA operon (fimbrial adhesins) and 54% carried luxS (quorum sensing). Environmental isolates had significantly higher biofilm gene expression than clinical isolates (2.4-fold increase, $p = 0.03$).

Table 1 Summary of Species-Wise Resistance Rates and Resistance Gene Carriage

Resistance Gene	Spring (n=28)	Summer (n=30)	Autumn (n=32)	Winter (n=29)	p value
blaCTX-M-3	42.9%	63.3%	100%	58.6%	0.003
blaKPC-2	53.6%	60.0%	78.1%	51.7%	0.028
blaNDM-1	28.6%	36.7%	43.8%	31.0%	0.215

Table 2 Antibiotic Resistance Patterns by Time Period

Antibiotic	Summer	Autumn	Winter
Imipenem	41.3%	48.2%	52.1%
Colistin	3.1%	2.7%	2.3%
Ciprofloxacin	62.5%	67.1%	70.3%

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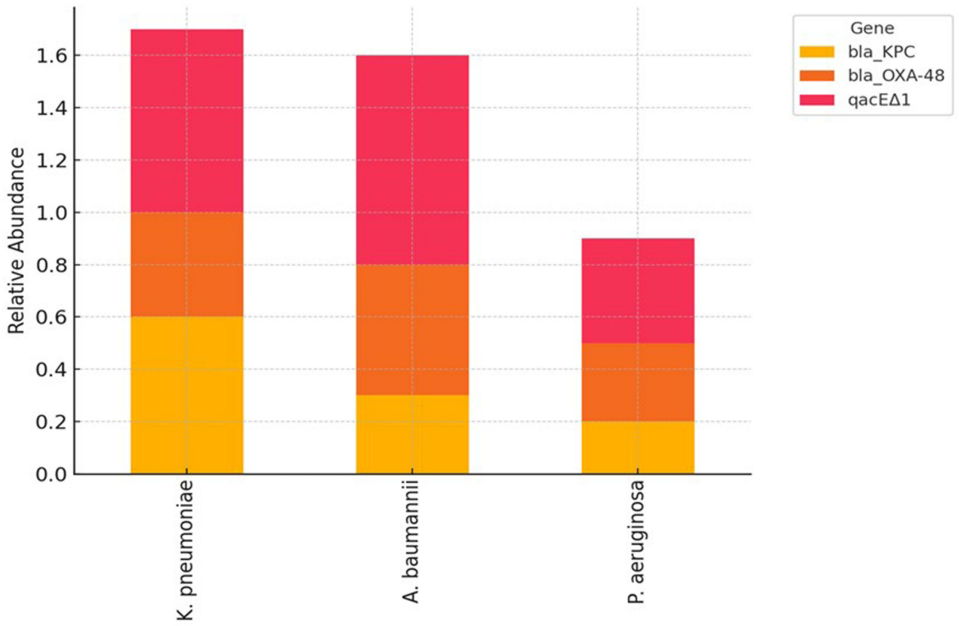

Figure 4 Relative abundance of resistance genes by species. (n=90 isolates).

Biofilm gene burden correlated strongly with the number of resistance genes ($r = 0.67$, $p < 0.001$), showing that biofilm traits and resistance often occur together (Table 4). This relationship is illustrated in Figure 7. Differences in biofilm growth under disinfectant exposure are compared in Figure 8, confirming that environmental stressors further promote biofilm persistence.

Genotypic Correlation Between Clinical and Environmental Isolates

The distribution of class 1 and 2 integrons is presented in Figure 9. Whole-genome sequencing of 30 representative isolates (15 environmental, 15 clinical) revealed identical integron structures between sink-associated and patient-derived *K. pneumoniae* and *A. baumannii*. These integrons contained both bla_{OXA-48} and qacEΔ1, supporting clonal overlap and suggesting environmental-to-patient transmission within the ICU (Table 5).

Association Between Resistance Gene Burden and ICU Length of Stay

Higher resistance gene counts were linked to longer ICU stays. Patients infected with isolates carrying more than five resistance genes stayed an average of 11.2 days, compared to 5.3 days for those infected with isolates carrying only 0–2 genes (Table 6). This trend is shown in Figure 10 and demonstrates the clinical burden of MDR infections in prolonging hospitalization.

Table 3 Seasonal Distribution of Isolates by Species

Season	K. pneumoniae	A. baumannii	P. aeruginosa	Total
Summer	18	16	10	44
Autumn	22	18	12	52
Winter	22	20	8	50

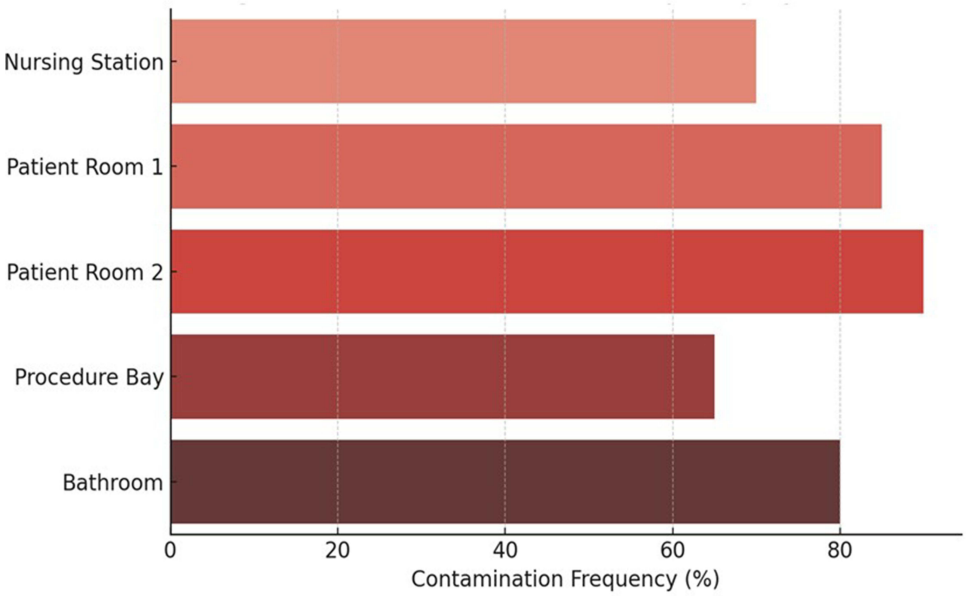

Figure 5 Sink contamination frequency by location. Based on qPCR detection; n=50 environmental samples.

Discussion

Overview of Key Findings

This study provides a seasonally stratified genomic analysis of multidrug-resistant organisms (MDROs) in an ICU environment. Three main findings stand out. First, we observed seasonal enrichment of $bla_{CTX\text{-}M\text{-}3}$ and qacEΔ1 genes, with $bla_{CTX\text{-}M\text{-}3}$ universally present in autumn isolates and qacEΔ1 predominantly detected in winter. Second, phylogenetic SNP analysis revealed clonal overlap between environmental and clinical isolates of *K. pneumoniae* and *A. baumannii*, supporting the hypothesis of environmental-to-patient transmission within the ICU. Third, we found a strong correlation between biofilm operons (mrkA, luxS) and resistance gene burden, demonstrating how biofilm capacity amplifies antimicrobial resistance. Together, these findings highlight the interplay of environmental persistence, genetic resistance mechanisms, and seasonal pressures in shaping ICU AMR dynamics.[28–32]

Integration with Existing Literature

Our findings reinforce previous reports that ICU sink drains and wet environments act as persistent reservoirs for resistant organisms, including carbapenem-resistant *K. pneumoniae* and *A. baumannii*.[14,33,34] However, our study adds novel insight by quantifying seasonal variation at the genomic level and linking environmental isolates directly to clinical strains using high-resolution SNP phylogenetics. While other studies have described the presence of bla_{KPC} and $bla_{NDM\text{-}1}$ in ICU settings,[35–37] we demonstrate that $bla_{CTX\text{-}M\text{-}3}$ specifically undergoes seasonal surges, underscoring the value of longitudinal surveillance. Biofilm formation has long been recognized as a contributor to persistence and resistance.[38–42] Our results expand this knowledge by showing that biofilm-associated genes are not evenly distributed, but instead correlate with both environmental origin and high resistance gene burden. Although our gene panel was limited to mrkA and luxS, the findings align with studies identifying biofilm-associated traits as critical mediators of resistance amplification in ICU pathogens.[43–46]

By embedding qacEΔ1 within class 1 integrons alongside β-lactamases, our data also support the concept of co-selection, where disinfectant exposure sustains resistance even in the absence of antibiotic pressure. This mechanism, while described in prior research,[19,27,47–49] is rarely demonstrated in seasonally stratified clinical cohorts.

Practical and Clinical Implications

The implications of our findings extend beyond surveillance into ICU practice and policy. Seasonal enrichment of $bla_{CTX\text{-}M\text{-}3}$ and qacEΔ1 suggests that infection control resources should be strategically intensified during autumn and

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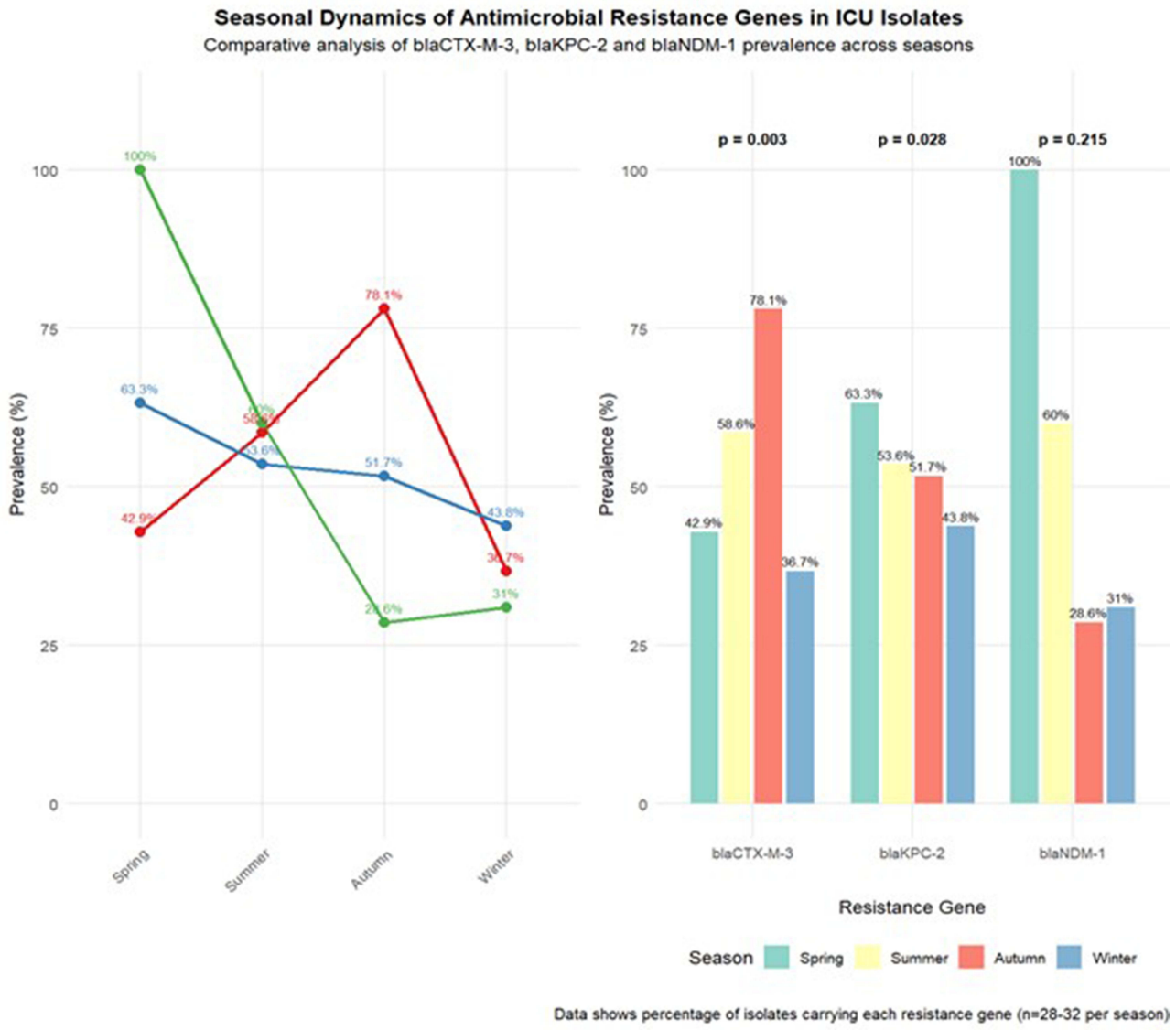

Figure 6 Seasonal distribution of multidrug resistance across major ICU pathogens. n=146 total isolates over 3 seasons.
Abbreviation: MDR, multidrug-resistant.

winter, when risk is highest. Enhanced sink decontamination protocols, targeted disinfection strategies, and stricter antibiotic stewardship during these periods could mitigate the spread of resistant clones.

The demonstration of clonal overlap between environmental and clinical isolates underscores the need for routine environmental sampling, particularly of sinks, drains, and high-contact surfaces. Structural interventions such as sink redesign, splash reduction barriers, or use of antimicrobial materials may also reduce transmission risk.

Table 4 Biofilm Gene Expression Levels (Mean ± SD) by Isolate Source

Gene	Environmental Isolates	Clinical Isolates	p-value
mrkA	1.8 ± 0.3	0.7 ± 0.2	0.01
luxS	1.5 ± 0.4	0.6 ± 0.3	0.03

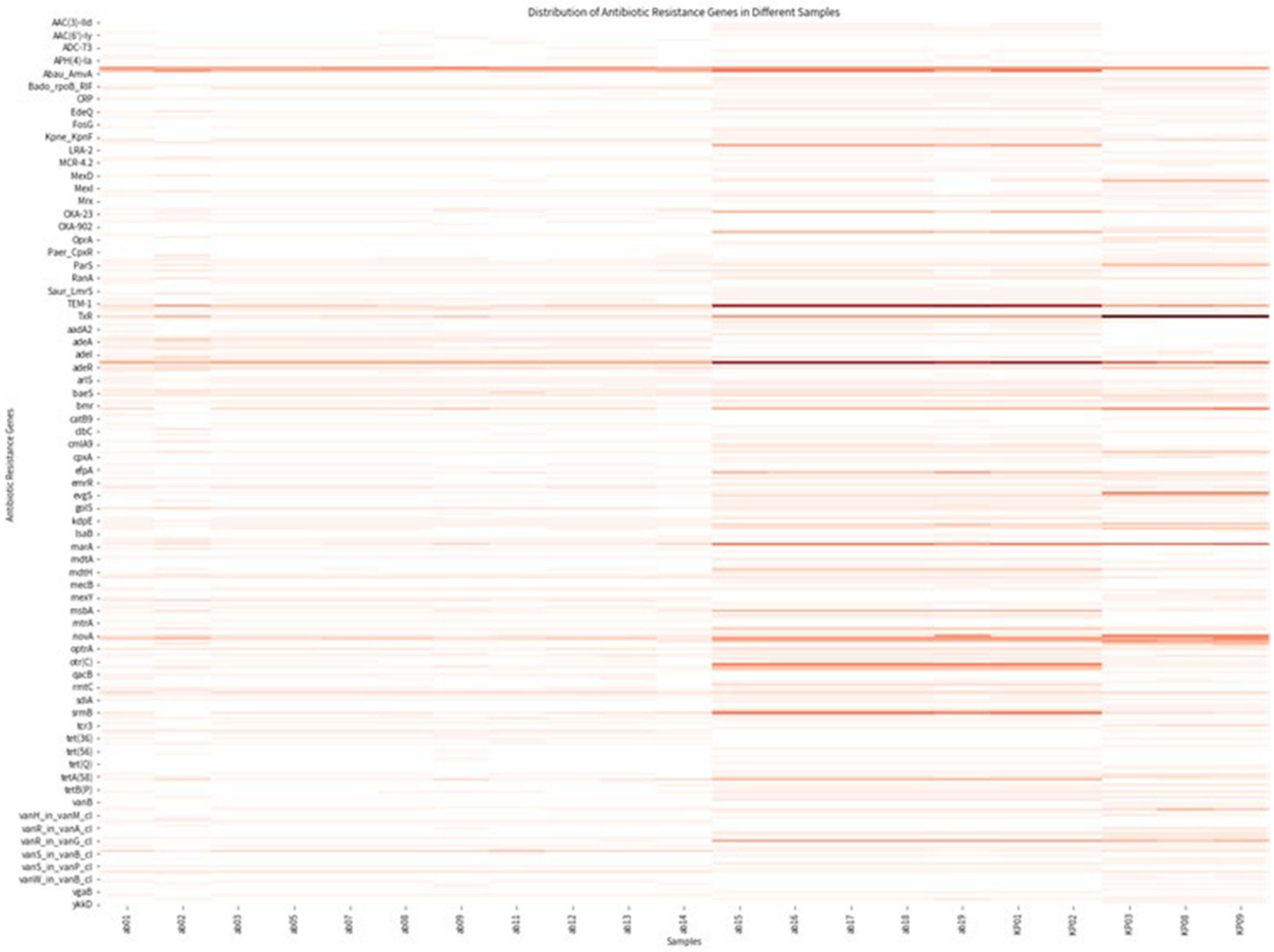

Figure 7 Correlation matrix of resistance genes with biofilm operons (Pearson correlation).

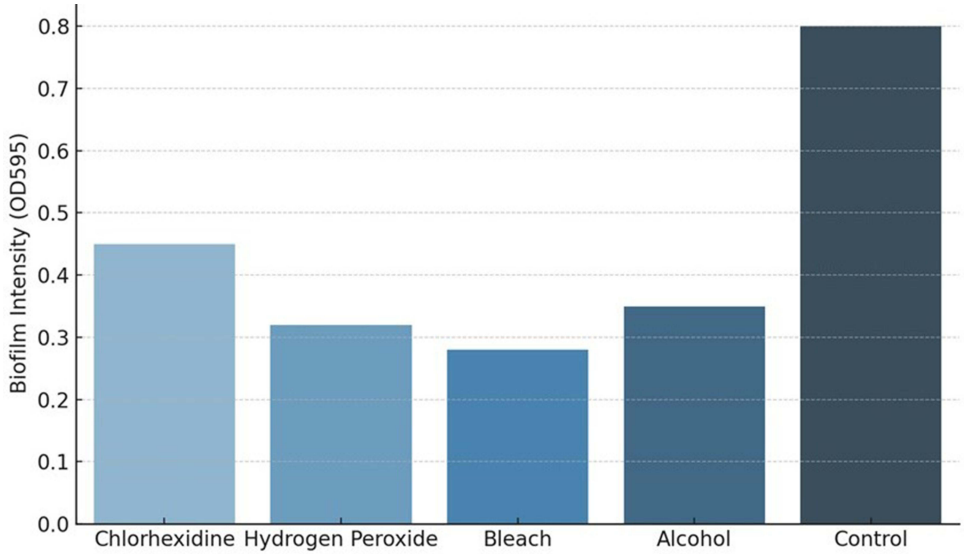

Figure 8 Biofilm formation intensity under different disinfectant exposures. n=146 total isolates over 3 seasons.
Abbreviation: MDR, multidrug-resistant.

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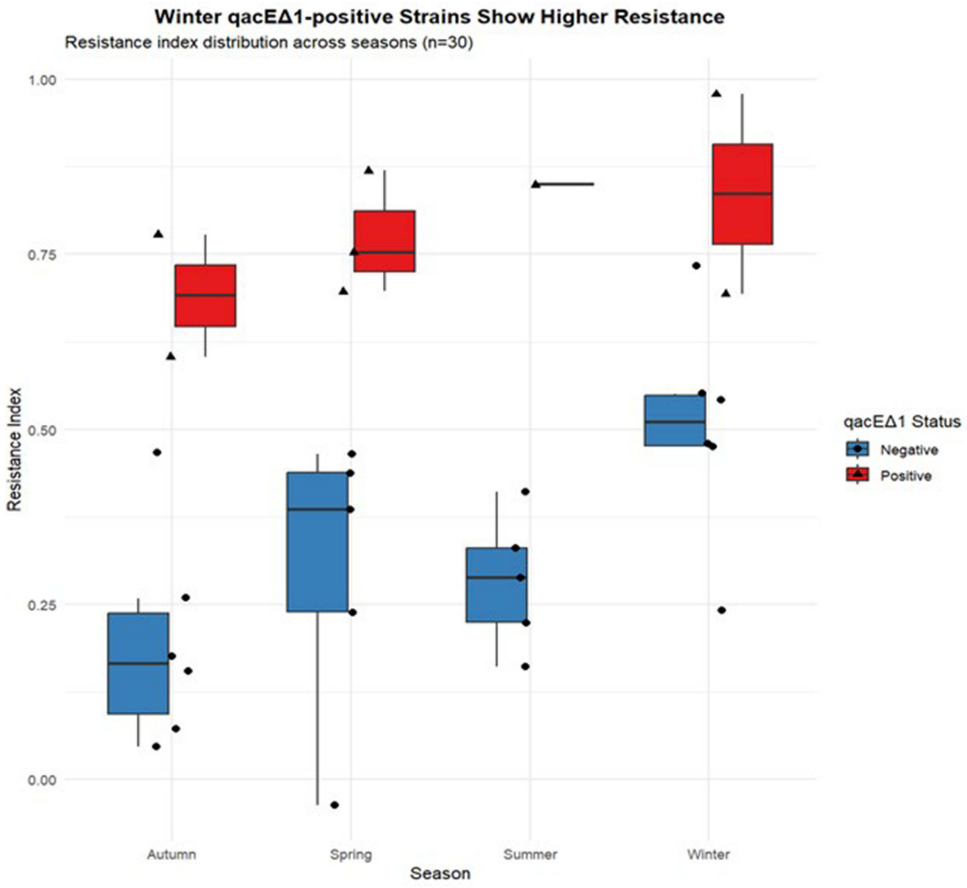

Figure 9 Distribution of integron types among clinical and environmental isolates. Class 1 and 2 integrons detected by PCR; n=60 isolates.

Genomic surveillance integrating whole-genome sequencing with seasonal epidemiology could be incorporated into early warning systems for ICUs, providing actionable data for both infection prevention teams and hospital administrators.

Table 5 Prevalence of Integrons Among Isolate Groups

Group	Class 1 Integron	Class 2 Integron	qacEΔ1 Presence
Environmental	68%	15%	74%
Clinical	53%	10%	55%

Table 6 Correlation Between Resistance Gene Count and ICU Stay Duration

Gene Count Category	Avg. ICU Stay (Days)	Standard Deviation
0–2 genes	5.3	1.2
3–5 genes	7.8	1.6
>5 genes	11.2	2.4

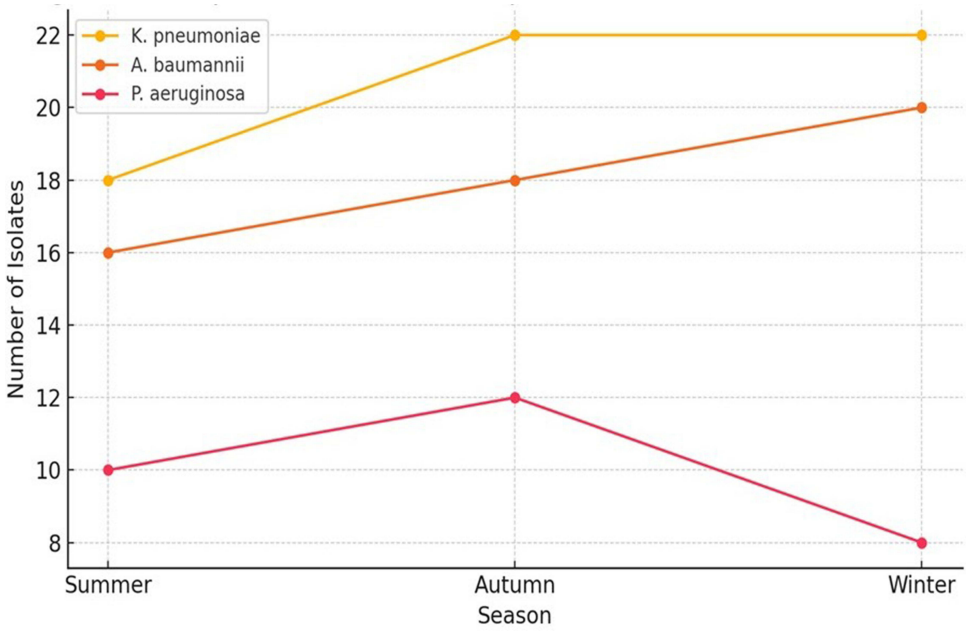

Figure 10 Temporal trend of ICU-acquired infections over three seasons. Data from seasonal collection; n=146 isolates.

Finally, while our study did not experimentally test phage therapy, the persistence of biofilm-associated resistance traits suggests that phage-based approaches warrant future investigation as complementary tools against MDR biofilm-forming pathogens.

Limitations and Future Directions

This study has several limitations. We focused on a restricted panel of biofilm genes (mrkA, luxS), whereas biofilm formation involves additional genes such as wcaG, magA, mrkD, and csu operons. Future work should broaden the gene panel to capture the full biofilm genetic landscape. Our design was observational and genomic; no in vitro or in vivo phage therapy experiments were performed, and we have clarified this to avoid misinterpretation. Larger multicenter studies incorporating environmental, clinical, and meteorological data would provide stronger generalizability, particularly in LMIC settings where ICU AMR burden is disproportionately high.

Despite these limitations, our study demonstrates the value of integrating seasonal surveillance with whole-genome sequencing to reveal resistance dynamics in ICU environments. By identifying seasonal peaks, environmental reservoirs, and genetic linkages, our findings can guide infection control strategies tailored to both temporal and spatial contexts.

Conclusion

This study reveals distinct seasonal patterns in antimicrobial resistance within ICU environments, driven by the prevalence of integron-encoded resistance genes and elevated biofilm expression in environmental reservoirs. Genomic evidence of clonal overlap between clinical and sink isolates highlights the role of inanimate surfaces in pathogen transmission.

To mitigate these risks, we recommend enhancing surface disinfection protocols—particularly in autumn when resistance peaks—and piloting targeted phage therapies during winter months to address biofilm-associated MDR infections.

Author Contributions

Yan Zhang helped in clinical data collection, seasonal sampling, and preliminary analysis of ICU pathogens. Xiaoyu Li performed bioinformatics analyses, genome sequencing, resistance gene annotation, and statistical modeling. Fengli Wang carried out microbiological testing, phenotypic resistance profiling, and laboratory coordination. Xinyue Ma

assisted with sample processing, genomic DNA extraction, and data curation. Shiquan Han conceived the study design, supervised the research process, and critically revised the manuscript for important intellectual content.

Disclosure

The authors report no conflicts of interest in this work.

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