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A metagenomics-based approach to understanding the transmission of healthcare-associated antimicrobial resistance in Pakistan

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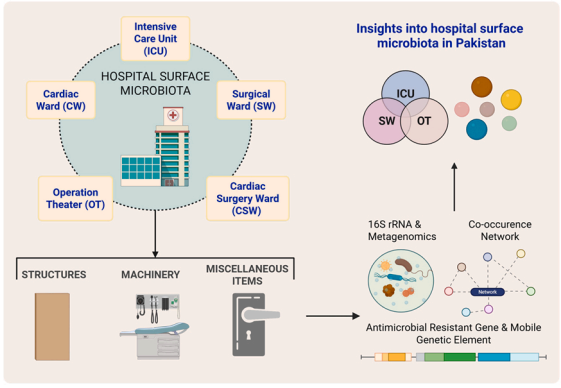
HIGHLIGHTS

- Hospital environments serve as reservoirs for healthcare-associated infections.
- Culture-based techniques limit resistome detection.
- Antibiotic resistance genes are highly prevalent in hospital settings in Pakistan.
- Study reveals potential transmission routes of ARGs across hospital wards.
- Findings show the urgent need for improved AMR detection in low-resource settings.

GRAPHICAL ABSTRACT



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ABSTRACT

Hospital environments are critical yet underexamined reservoirs for hazardous antimicrobial resistance (AMR), particularly in lower-middle-income countries (LMICs) where resource constraints often hinder comprehensive surveillance. In this study, we employed 16S rRNA gene sequencing and shotgun metagenomics to characterize

Abbreviations: AMR, Antimicrobial resistance; ARGs, Antibiotic-resistant genes; ASVs, Amplicon Sequence Variants; CSW, Cardiac surgery ward; CW, Cardiac ward; FEAST, Fast expectation-maximization for microbial source tracking; HAIs, Hospital-associated infections; HGT, Horizontal gene transfer; ICU, Intensive care unit; IS, Insertion Sequence; MGEs, Mobile genetic elements; NCBI, National center for biotechnology information; OT, Operation theatre room; OTUs, Operational Taxonomic Units; PCoA, Principal Coordinates analysis; PDA, Primary data archive; SW, Surgical ward.

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Antimicrobial resistance
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the microbiome, resistome, and potential transmission routes across five clinical environments within a hospital in Pakistan: the intensive care unit (ICU), surgical ward (SW), cardiac surgery ward (CSW), cardiac ward (CW), and operating theater (OT). Microbial community analysis revealed compositional similarities among the ICU, SW, and OT, with the ICU emerging as a primary source of microbial dissemination. Species-level profiling identified hospital-associated pathogens such as *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Enterobacter cloacae,* and metagenome-assembled genome (MAG) analysis enabled the linkage of antimicrobial resistance genes (ARGs) and mobile genetic elements (MGEs) to specific bacterial hosts. ARGs and MGEs displayed setting-specific patterns, with the SW harboring the highest abundance of ARGs, particularly those conferring resistance to β-lactams and biocides. Insertion sequences were the most prevalent MGEs and were commonly linked to ARGs, indicating potential horizontal gene transfer. Co-occurrence network analysis identified *Staphylococcus*, *Enterococcus*, and *Escherichia* as central hub taxa within the microbial communities of the ICU, SW, and OT, indicating their critical roles in potential ARG transmission. These findings provide critical insights into the environmental transmission dynamics of AMR in LMIC healthcare settings and underscore the urgent need for metagenomics-informed infection control strategies.

1. Introduction

Hospital environments, including surfaces, medical equipment, air, waste, and occupants, represent significant reservoirs for healthcare-associated infections (HAIs) and multidrug-resistant pathogens [1–4]. These complex hospital settings offer ideal niches for microbial colonization and persistence [4,5]. Various nosocomial pathogens, including vancomycin-resistant *enterococci* (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), *Acinetobacter baumannii*, *Clostridioides difficile*, and *Pseudomonas aeruginosa*, have been found to persist in hospital environments for extended periods of time [2,3,6,7]. The survival of these pathogens on surfaces and within hospital infrastructure contributes to the environmental dissemination of antimicrobial resistance (AMR), posing a critical public health hazard [5,8].

Proper hygiene and disinfection practices are essential for maintaining low levels of bacterial contamination in hospital environments and preventing HAIs associated AMR [8–10]. Inadequate infection control, poor sanitation infrastructure, and inconsistent disinfection protocols in resource-limited healthcare systems further increase the risk [11]. Despite growing concern over AMR, most surveillance efforts, especially in lower-middle-income countries (LMICs), have focused predominantly on clinical isolates, often neglecting the environmental reservoirs that play a pivotal role in the transmission pathways [10–12]. Recent advancements in molecular techniques and high-throughput sequencing have enabled the rapid characterization of microbial communities and evaluation of the presence of antibiotic resistance genes (ARGs) in environmental samples [13–16]. However, LMICs including Pakistan, for instance, microbiological investigations are still largely reliant on conventional culture-based techniques, with limited integration of high-resolution molecular tools capable of characterizing microbial communities and resistome. Meanwhile, inappropriate antibiotic prescribing remains rampant in many LMICs [17,18]; recent surveys indicate that up to 70.3 % of the patients had at least one inappropriate antimicrobial, accelerating the evolution and spread of ARGs [19,20].

To address these gaps, this study investigates the microbial ecology and resistome of hospital environments in a major cardiac care center in Pakistan. By integrating 16S rRNA gene sequencing, shotgun metagenomics, and microbial source tracking, we aim to profile microbial diversity, identify ARGs, and elucidate the interactions and origins of microbial contaminants in both general wards and the operating theater. These findings provide critical insights into environmental AMR risks and inform future infection control strategies in LMIC healthcare settings.

2. Materials and methods

2.1. Ethics statement

The study was approved by the Institutional Ethical Review Committee of the Faisalabad Institute of Cardiology Hospital (17–2019/DME/FIC/FSD).

2.2. Sampling sites and sample collection

Environmental samples were collected from a tertiary-level autonomous public hospital specializing cardiac care in Punjab province, Pakistan, the country's most populous region, from January to December 2021. The hospital, located in Faisalabad, has a capacity of 202 beds and serves as a major referral center. Samples were primarily collected across five major units: the intensive care unit (ICU), surgical ward (SW), cardiac surgery ward (CSW), cardiac ward (CW), and modern operating theater (OT). While these units share similar layouts, they differ in clinical function, with the OT dedicated exclusively to surgical procedures.

Environmental swab samples were collected from three categories from each ward and the OT: structural components (walls, floors, and switches), machinery and medical equipment (elevators, biometric machines, electrocardiograms, cardiac monitors, infusion pumps, spirometers, pediatric cardiac monitors, anesthesia machines, permanent pacemakers, defibrillators, pediatric infusion pumps, nebulizers, and pacemakers), and miscellaneous items (chairs, lights, doorknobs, nursing counters, pharmacy counters, and pediatric tables). For each unit, three independent environmental swab samples were collected per site by swabbing a 10×10 cm area using sterile cotton swabs (Oxoid, Basingstoke, UK) as previously illustrated [21,22], at the ICU, SW, CSW, CW, and OT. A total of 115 environmental swab samples were collected from five different hospital units (23 samples/unit) and stocked in −80 ℃ freezer before further processing.

2.3. Genomic DNA extraction and sample pooling

Genomic DNA was extracted from the swab samples using the QIAamp DNA extraction kit, according to the manufacturer's guidelines (Qiagen, Germantown, Maryland, USA). DNA concentration and purity were measured using a Nanodrop instrument (Spectrophotometer ND-1000, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Subsequently, genomic DNA from each swab sample was pooled based on their primary categories, namely structural, machinery, and miscellaneous, resulting in three pooled samples from each ward. A total of 15 pooled DNA samples (three environmental sample DNA categories/ward) were prepared for further analysis (Table 1).

2.4. 16S rRNA gene sequencing and microbial community analysis

The DNA library was prepared and sequenced as previously described [23]. Briefly, the V4 region of the 16S rRNA gene was amplified via polymerase chain reaction (PCR) using dual-index PCR primers and Pfx AccuPrime master mix (Invitrogen, Carlsbad, California, USA). The resulting amplicons were purified and normalized to equimolar amounts using the Sequa Prep plate normalization kit (Invitrogen,

Table 1
List of Samples.

Sample ID	Isolation source	sample categories	Accession number	Bio Project ID
16S sequencing				
UM−09	ICU	Structure	SAMN41105533	PRJNA1105564
UM−10	ICU	Miscellaneous	SAMN41105534	
UM−11	ICU	Machines	SAMN41105535	
UM−13	SW	Structure	SAMN41105536	
UM−14	SW	Miscellaneous	SAMN41105537	
UM−15	SW	Machines	SAMN41105538	
UM−17	OT	Structure	SAMN41105539	
UM−18	OT	Miscellaneous	SAMN41105540	
UM−19	OT	Machines	SAMN41105541	
UM−37	CSW	Structure	SAMN41105542	
UM−38	CSW	Miscellaneous	SAMN41105543	
UM−39	CSW	Machines	SAMN41105544	
UM−41	CW	Structure	SAMN41105545	
UM−42	CW	Miscellaneous	SAMN41105546	
UM−43	CW	Machines	SAMN41105547	
Shotgun metagenomic sequencing				
UA−11	ICU	Pooled DNA	SAMN23408116	PRJNA782966
UA−37	OT		SAMN23412800	
UA−38	SW		SAMN23412799	

*ICU (Intensive care unit); SW (Surgical ward); OT (Operation theater room); CSW (Cardiac surgery ward); CW (Cardiac ward).

Carlsbad, California, USA). The barcoded V4 amplicons from each sample were pooled in equal amounts to construct the DNA library. The size and concentration of the DNA library fragment were determined using tape station and Kapa quantitative PCR (qPCR) (Kapa Biosystems, Wilmington, Massachusetts, USA). The final DNA library (600 μL of 6 pmol/L library) was loaded onto a MiSeq v2, 2 × 250 cycle cartridge (Illumina, San Diego, California, USA) and sequenced using the Illumina MiSeq platform at the Interdisciplinary Center for Biotechnology Research (ICBR) at the University of Florida.

Raw sequencing data were analyzed using the QIIME 2 pipeline [24]. Sequence quality control was performed using the Divisive Amplicon Denoising Algorithm 2 (DADA2), integrated within QIIME 2, to generate high-quality amplicon sequence variants (ASVs). The ASVs were taxonomically classified using the SILVA 132 reference database (https://www.arb-silva.de/documentation/release-132/). The resulting ASV table, rooted phylogenetic tree, taxonomy assignments, and sample metadata were imported into R (v. 4.4.2) as phyloseq object for downstream analyses. A total of 286,560 raw reads were generated across all samples. The sequencing depth was normalized to the minimum read count observed (5020 reads per sample), resulting in a final dataset comprising 60,348 reads. Three samples (two from the ICU and one from the CW) were excluded due to insufficient microbial content.

Alpha diversity was assessed to evaluate within-sample richness and diversity using observed ASVs and the Shannon index. Statistical differences among wards were tested using the Kruskal–Wallis test, followed by Dunn's post-hoc test for pairwise comparisons using GraphPad Prism (v. 10.4.1, GraphPad Software, USA). Beta diversity was calculated based on Bray–Curtis dissimilarity to assess differences in microbial community composition between wards and visualized using principal coordinate analysis (PCoA) in R (v. 4.4.2). Statistical differences of beta diversity between the hospital environments were tested by PERMANOVA. Bacterial genera with a relative abundance greater than 1 % and present in more than 20 % of the samples were classified as the main taxa. Differences in the relative abundance of these genera across hospital wards were assessed using the Kruskal–Wallis test and visualized using R (v. 4.4.2).

To estimate the extent of shared microbiome between the wards and OT room samples, the Fast Expectation-maximization Microbial Source Tracking (FEAST) tool was used [25]. FEAST is a machine learning method that employs neural networks and transfer learning to quantify the transmission ability from one site to another [26]. The contribution value of the source tracking task serves as a quantitative index to present the microbial transfer ability from a single 'source' site to a 'sink' site. For this analysis, ASVs presenting fewer than two samples were excluded from the input ASV table. The FEAST tool was executed following the standard tutorial available at https://github.com/cozygene/FEAST.

2.5. Shotgun metagenomic sequencing and resistome analysis

The resistome was investigated in the three facilities where microbial transfer was observed: ICU, SW, and OT. The Illumina TruSeq® Nano DNA Library Prep kit (Illumina, Inc., San Diego, California, USA) was used to construct the shotgun metagenomic library for the three corresponding DNA pools (Table 1), following the manufacturer's protocols. The quality of the library, including its size and quantification, was assessed using a Bioanalyzer and qPCR. Paired-end sequencing was performed using an Illumina PE150-NovaSeq (Novogene Bioinformatics Technology Co., Ltd., Davis, California, USA). Raw reads were filtered for quality using Trimmomatic [27]. Briefly, in the first step, TruSeq3 adapter sequences were removed using 'ILLUMINACLIP.' Adapter clipping occurred once a match score of 30 was reached, with a maximum of two mismatches set up in the initial seed. Both paired-end reads were retained after clipping. Next, a sliding window of 4 nucleotides was used to remove nucleotides from the 3' end when the Phred score within the window was below 20.

The AmrPlus pipeline (http://megares.meglab.org/amrplusplus) was used to identify ARGs and mobile genetic elements (MGEs) in the different wards and the OT room [28]. The sequences were aligned to the reference ARGs included in the Antimicrobial Resistance Database. MEGARes (v. 2.0), and reference MGEs were included in the Mobile Genetic Element database MGEdb using the Resistome Analyzer. Only ARGs and MGEs that were covered by sample reads for more than 80 % of their length and those with resistance not conferred by Single Nucleotide Polymorphisms were considered for analysis.

2.6. Taxonomic classification and microbial diversity analysis

Taxonomic profiling of shotgun metagenomic datasets was performed using Kraken2 (v.2.1.3) following established protocols [29,30]. Briefly, host-derived sequences were removed by aligning raw reads to the human reference genome (GRCh38.p14) using Bowtie2 (v.2.5.4) [31]. Taxonomic classification was then conducted using the k2_standard_20240904 Kraken2 database with the minimum-hit-groups parameter set to 3. Species-level abundance estimation was performed using Bracken (v2.9) [32] with the read length parameter set to 100 bp and a minimum of eight reads required per taxon before estimation. Relative abundance tables were generated from Bracken output. Alpha-diversity (Shannon index) and beta-diversity (Bray-Curtis dissimilarity) were calculated using KrakenTools (v.1.2). All the data visualizations were generated using ggplot2 (v.3.5.1) [33] in R (v.4.4.3).

2.7. Metagenome-assembled genomes (MAGs) analysis and annotations

Metagenomic reads were first cleaned with fastp (v.0.23.4) [34], to retain sequences with Phred quality ≥ Q15 and length > 50. Filtered reads were assembled using MEGAHIT (v.1.2.9) [35] with minimum contig length set to 1000 bp. Paired-end reads were mapped to the assembled contigs using BWA (v0.7.1) [36]. Contig depths and coverages were further calculated using SAMtools (v.1.20) [37] and MetaBAT2 (v.2.13) [38]. Then the MAGs were reconstructed using MetaBAT2 (v.2.13) [38] with a minimum contig length of 2500 bp. Genome quality (estimated completeness and contamination) was evaluated using CheckM2 (v.1.0.2) [39], and only MAGs with > 50 % completeness and < 5 % contamination were included for downstream analysis.

Taxonomic classification of high-quality MAGs was performed with the classify workflow in GTDB-Tk v2.3.2 [40]. For two low-quality

MAGs, species-level taxonomy was inferred via sequence alignment against the PubMLST database [41]. ARGs and plasmids were identified using ABRicate (v.1.0.1) [42] against the CARD databases [43]. MGEs were detected using MobileElementFinder (v.1.0.3) [44] in the Center for Genomic Epidemiology (https://www.genomicepidemiology.org/) and Prokka (v.1.14.6). ARGs were linked to their corresponding MAGs based on contig co-localization and visualized using Proksee (v.1.2.0) [45]. A Sankey diagram was generated using sankeydiagram.net (v.1.6.2) (https://sankeydiagram.net/), to visualize the relationships among sample source, MAG identity, species-level taxonomy, ARGs, and associated MGEs.

2.8. Co-occurrence network analysis

To investigate potential bacterial-bacterial interactions within the hospital microbiome, microbial co-occurrence network analysis was conducted among core bacterial genera, defined as those present in at least 50 % of samples. Pairwise Spearman's rank correlations (ρ) were calculated based on relative abundance data using R, as previously described [46]. A co-occurrence was defined as a statistically significant correlation with ecoefficiency > 0.25 and a false discovery rate (FDR)-adjusted p-value < 0.05. Significant associations were represented as edges connecting bacterial genera in a co-occurrence network, which was visualized using the Force Atlas layout algorithm in Gephi (v.0.10).

3. Results

3.1. Microbial diversity within and among sampling sites

To characterize the microbial communities across distinct hospital environments and explore potential transmission patterns, we performed 16S rRNA gene amplicon sequencing of environmental surface samples collected from four hospital wards and one operating room: the intensive care unit (ICU), surgical ward (SW), cardiac surgery ward (CSW), cardiac ward (CW), and operating theater (OT). Alpha diversity was assessed using observed ASVs to estimate microbial richness and the Shannon index to evaluate both richness and evenness (Fig. 1A–B). Although statistical analysis revealed no significant differences in alpha diversity across the hospital environment ($p = 0.0699$ and $p = 0.2091$, respectively), the SW and OT exhibited relatively higher richness and diversity, suggesting a more complex or heterogeneous microbial community in these locations.

Beta diversity was analyzed using Bray–Curtis dissimilarity to compare the microbial community composition between the wards and OT. Principal coordinate analysis (PCoA) revealed a significant separation among samples by ward ($p = 0.009$, PERMANOVA), with samples from the ICU, SW, and OT clustering closely and those from the CSW and CW forming a distinct group (Fig. 1C). Although pairwise comparisons between individual wards did not reach statistical significance, the observed clustering suggests ecological similarity among the ICU, SW, and OT, which are associated with frequent patient contact and procedural interventions. In contrast, the microbial profiles of CSW and CW, which likely experience fewer intensive interventions, appeared to have distinct microbial community compositions.

These findings suggest that shared functional use, human activity, and environmental conditions may contribute to the convergence of microbial communities within certain clinical spaces. The microbial compositional similarities among the ICU, SW, and OT may reflect potential microbial exchange routes or common selective pressures, such as disinfection practices or antibiotic exposure. Understanding these spatial dynamics is critical for identifying reservoirs of opportunistic pathogens and designing targeted infection control strategies.

3.2. Bacterial population varies across hospital wards

To further characterize the microbial composition across hospital wards and identify potentially shared or disseminated bacterial taxa, we focused on main genera with a relative abundance greater than 1 % and present in more than 20 % of samples. Bacterial genera exhibited clear variation in their relative abundance across wards (Fig. 2), indicating spatial heterogeneity within the hospital microbiota. Notably, *Staphylococcus* was dominant in the ICU, while *Prevotella* and *Enterococcus* were more abundant in the SW and OT (Fig. 2). *Massilia* predominated in the cardiac CSW and CW. *Escherichia–Shigella* showed relatively high abundance in the ICU, SW, and OT but was nearly absent in CSW and CW (Fig. 2). In contrast, *Pseudomonas* was more abundant in CSW and CW but present at lower levels in the ICU, SW, and OT (Fig. 2).

Statistical analysis using the Kruskal–Wallis test identified several genera with significantly different abundances across wards. Specifically, *Massilia* ($p = 0.044$), *Prevotella* ($p = 0.019$), and *Staphylococcus* ($p = 0.013$) exhibited significant variation (Fig. 2), suggesting ward-specific microbial prevalence. These findings demonstrate that while some bacterial genera are commonly shared across wards, a distinct microbial profiling emerges in different clinical environments, suggesting that understanding these differences in microbial composition among wards is essential for informing targeted surveillance and infection control strategies in healthcare settings.

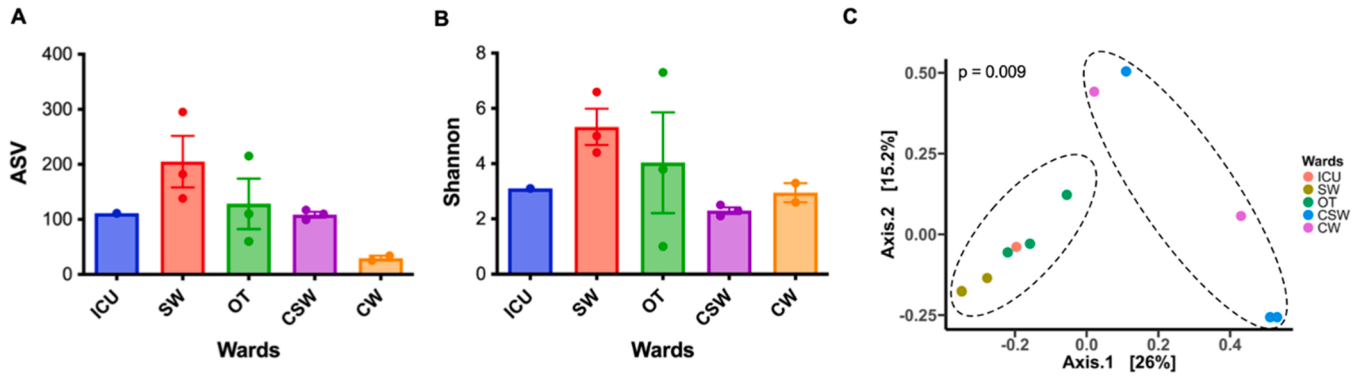


Fig. 1. Microbial richness, diversity, and community structure across hospital environments. (A) Microbial richness, represented by the number of observed amplicon sequence variants (ASVs), was compared across five hospital sites: the intensive care unit (ICU), surgical ward (SW), operating theater (OT), cardiac surgery ward (CSW), and cardiac ward (CW). No statistically significant differences in richness were observed among the groups. (B) Microbial diversity was evaluated using the Shannon diversity index, reflecting both the richness and evenness of the community. Similar to ASV richness, Shannon diversity did not differ significantly among wards. (C) Differences in microbial community composition were assessed using Bray–Curtis dissimilarity, followed by principal coordinate analysis (PCoA). Samples clustered by locations, and PERMANOVA test indicated a statistically significant difference in community composition among the sampled sites ($p = 0.009$).

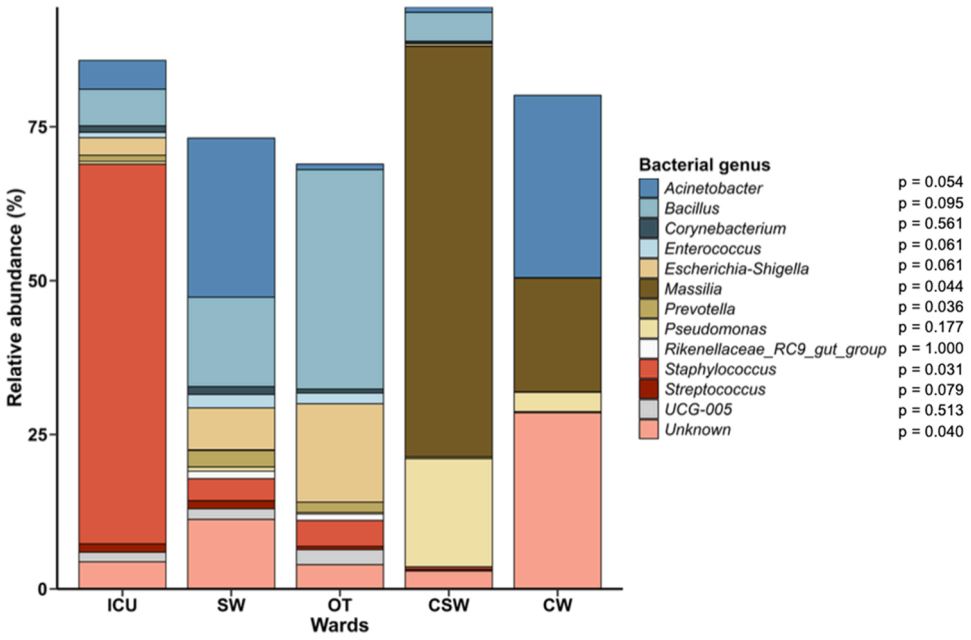


Fig. 2. Differential microbial colonization patterns across clinical settings. Bacterial genera with relative abundance greater than 1 % and detected in more than 20 % of the samples were considered as the major taxa. The stacked bar plot illustrates the relative abundance (%) of these genera across five hospital environments: intensive care unit (ICU), surgical ward (SW), operating theater (OT), cardiac surgery ward (CSW), and cardiac ward (CW).Differences in the distribution of major genera across wards were assessed using the Kruskal–Wallis test. Significant variation was observed for *Massilia* ($p = 0.044$), *Prevotella* ($p = 0.036$), and *Staphylococcus* ($p = 0.031$), suggesting differential colonization or contamination patterns across clinical settings.

3.3. ICU serves as a primary source of microbial transmission to SW and OT

To investigate potential routes and directions of microbial transmission across hospital wards, we performed microbial source tracking using the fast expectation-maximization for microbial source tracking (FEAST) algorithm. Each ward was designated as a "sink" in separate models to identify the relative contributions of microbial sources from other wards. When the SW was analyzed as the sink, the majority of the microbial community was traced back to the ICU (40 %), followed by OT (30 %), with the remaining 30 % attributed to unknown sources (Fig. 3A). Similarly, when the OT was treated as the sink, 40 % of the microbial input originated from the ICU, 20 % from SW, and 40 % from unknown sources (Fig. 3B). In contrast, CSW and CW did not exhibit any clear source-sink relationships, suggesting limited microbial exchange between SW, ICU, and OT. These results highlight the ICU as a major microbial source for both SW and OT, suggesting bidirectional transmission between SW and OT (Fig. 3C). Notably, the ICU in this facility functions as an intermediary between SW and OT in terms of patient transfer, which may facilitate microbial exchange through both human-associated and surface-based routes.

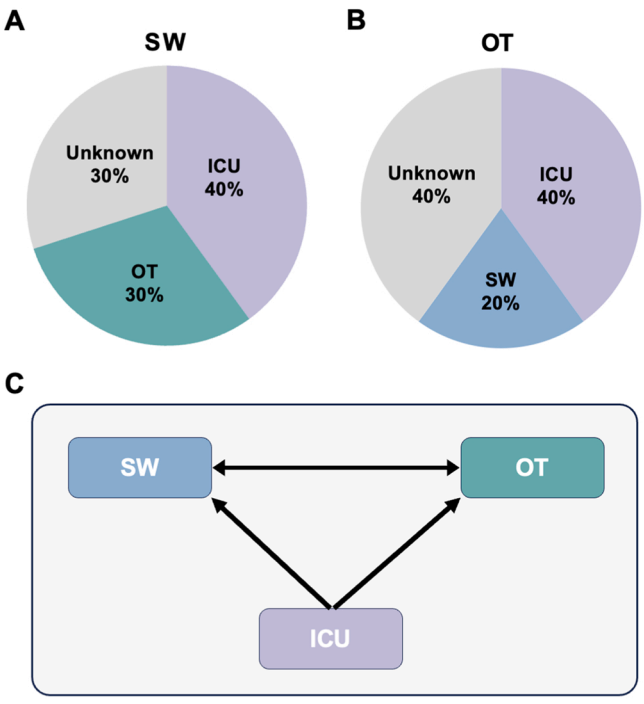


Fig. 3. Microbial transmission was observed between ICU, SW and OT, with ICU acting as a major source**.** Microbial source tracking was analyzed among hospital wards. (A) When the surgical ward (SW) was designated as the sink, the primary microbial sources were the intensive care unit (ICU, 40 %) and the operating theater (OT, 30 %), with the remaining 30 % attributed to unknown sources. (B) When the operating theater (OT) was treated as the sink, microbial contributions were evenly split between the ICU (40 %) and unknown sources (40 %), with a smaller contribution from the surgical ward (SW, 20 %). (C) Inferred microbial transmission dynamics among ICU, SW, and OT. The ICU served as a major source of microbial dissemination to both SW and OT, while bidirectional transmission was observed between SW and OT.

3.4. Microbial and resistome profiling revealed ward-specific distributions

Given the presence of microbial transmission among the ICU, SW, and OT, we hypothesized that these hospital environments may harbor critical pathogens and act as reservoirs of antimicrobial resistance genes (ARGs). To investigate this, we performed shotgun metagenomic sequencing on pooled samples from each environment (Table 1) and assessed microbial diversity, community structure, and the distribution of ARGs and mobile genetic elements (MGEs). The ICU exhibited the highest microbial diversity based on Shannon index, followed by the SW and OT (Fig. 4A). Beta diversity analysis using Bray-Curtis dissimilarity revealed substantial differences in microbial community composition across the three environments (Fig. 4B). Species-level taxonomic profiling further supported environment-specific microbial structures

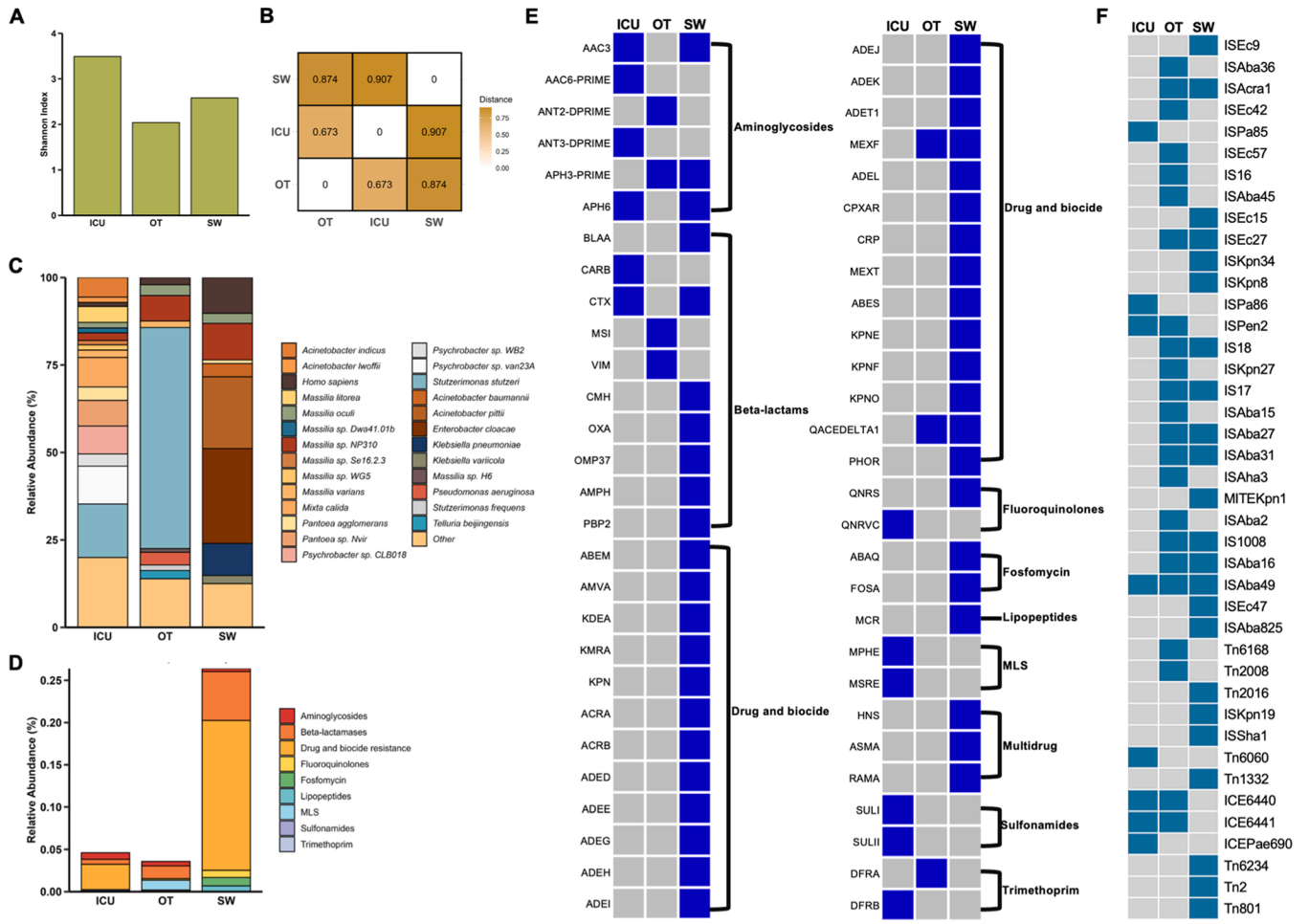


Fig. 4. Microbial and resistome profiles across hospital environments. (A) Alpha diversity (Shannon index) of microbial communities in the intensive care unit (ICU), operating theater (OT), and surgical ward (SW), based on shotgun metagenomic sequencing data. The ICU showed the highest microbial diversity among the three environments. (B) Heatmap of beta diversity (Bray–Curtis distances) showing dissimilarities in microbial composition. Distinct microbial community structures were observed across hospital environments. (C) Stacked bar graph showing the relative abundance of bacterial species in each hospital environment. Several hospital-associated pathogens were identified. Species with less than 1 % relative abundance were grouped as other. (D) The relative abundance of ARGs detected in the hospital environments. The SW exhibited the highest overall ARG abundance, followed by the ICU, with the highest abundance of ARGs related to drug and biocide resistance. (E) Heatmap showing the distribution of specific ARGs across the three locations. Multiple classes of resistance genes were identified. The observed site-specific patterns suggest that ARGs may not be uniformly transmitted between locations but could be independently acquired from the local environment. (F) Distribution of mobile genetic elements (MGEs) associated with β-lactam resistance. A location-dependent pattern was also observed. *ISAba49* was detected in all locations, highlighting its potential role in the dissemination of β-lactam resistance across different hospital environments.

(Fig. 4C). Several hospital-associated pathogens were identified across the samples, including *Acinetobacter baumannii*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Notably, *Stutzerimonas stutzeri*, an opportunistic pathogen with environmental persistence, dominated the microbial community in the OT. *Enterobacter cloacae*, a common nosocomial pathogen associated with a wide range of hospital-acquired infections, was particularly abundant in the SW. The ICU, by contrast, harbored a more diverse microbial community.

This distinct microbial community structure across hospital environments also corresponded with variations in the resistome. The relative abundance of ARGs was greater in SW, accounting for 0.26 % of the total metagenomic content, compared to 0.04 % in the ICU and 0.03 % in the OT (Fig. 4D). In total, 56 unique ARGs were identified, which were associated with a wide range of resistance mechanisms (Fig. 4E). The majority of ARGs conferred resistance to drugs and biocides (46.4 %, $n = 26$), followed by genes related to β-lactam resistance (17.8 %, $n = 10$), aminoglycosides (10.7 %, $n = 6$), fluoroquinolones (3.5 %, $n = 2$), fosfomycin (3.5 %, $n = 2$), macrolides (3.5 %, $n = 2$), sulfonamides (3.5 %, $n = 2$), trimethoprim (3.5 %, $n = 2$), and lipopeptides (1.7 %, $n = 1$). A small subset (5.3 %, $n = 3$) was associated with multidrug resistance. SW harbored the greatest diversity and abundance of ARGs (76.7 %, $n = 43$), dominated by genes related to drug and biocide resistance and β-lactam resistance. In contrast, the OT room contained the fewest ARGs (12.5 %, $n = 7$), including only two genes associated with β-lactam resistance (Fig. 4E). As MGEs are major drivers of horizontal gene transfer (HGT), especially for β-lactam resistance, we further analyzed the prevalence and distribution of MGEs in the ICU, SW, and OT samples. The identified MGEs included integrative and conjugative elements (ICEs), insertion sequences (*ISs*), and transposons (Fig. 4F). ICEs accounted for 8.3 % of the total MGEs and were not detected in the SW. Insertion sequences were the most abundant class of MGEs, with a similar high prevalence in the SW (60 %) and the OT room (56 %) and significantly lower abundance in the ICU (13.3 %). Notably, *ISAba49* was detected in all three hospital units, suggesting that it may play a central role in facilitating ARG transmission across clinical environments. These findings indicate that ARGs, particularly those conferring β-lactam resistance, are unevenly distributed across wards and are potentially mobilized via MGEs. The higher abundance of ARGs and MGEs in the SW highlights its importance as a reservoir of resistance genes within the hospital microbiota.

3.5. Bacterial co-occurrence network revealed hub taxa in clinical settings

To explore potential microbe-microbe interactions and ecological relationships among bacterial taxa in clinical environments, we performed a co-occurrence network analysis using data from the ICU, SW, and OT, where microbial transmission was previously observed (Fig. 3). A total of 46 bacterial genera were included in the analysis, resulting in 227 statistically significant interactions (edges), the majority of them were positively correlated (Fig. 5). Only a few negative associations were identified, among them *Massilia* exhibited the highest number of negative correlations. This may explain its low abundance across these wards (Fig. 2), potentially due to competitive exclusion or antagonistic interactions with coexisting taxa.

Notably, three major HAI genera, *Staphylococcus*, *Enterococcus*, and *Escherichia*, emerged as central hubs in the network, as indicated by their larger node size and greater connectivity with other taxa (Fig. 5). These genera showed extensive co-occurrence with both clinically and nosocomially important bacteria. Their central positioning in the network suggests that they may play a key role in shaping the microbial community structure and facilitating the transmission of MGEs, including antimicrobial resistance genes. The strong co-occurrence patterns observed among HAI-associated genera and other taxa within these high-contact clinical settings underscore the potential for HGT and microbial persistence. These findings highlight the need for targeted surveillance of microbial interactions, not only at the pathogen level but also across the broader hospital microbiota, to better understand and mitigate the risk of the dissemination of antimicrobial resistance.

3.6. Metagenome-assembled genomes and bacterial host-ARG-MGE associations

To improve resolution beyond community-level bacterial distribution and further investigate transmission patterns and host–ARG–MGE relationships, we performed MAGs analysis. A Sankey diagram (Fig. 6A) illustrates the connections among hospital environment, bacterial hosts, ARGs, and associated *ISs*. A total of 11 MAGs were recovered from the three hospital environments, including four from the ICU, five from the SW, and two from the OT. Of these, nine were considered high quality (> 50 % completeness and < 5 % contamination, Supplementary Table 1). All recovered MAGs belonged to Gram-negative bacteria, including several hospital-associated bacteria such as *Stutzerimonas*, *Enterobacter*, *Acinetobacter*, *Klebsiella*, and *Psychrobacter* (Fig. 6A). Several MAGs harbored multiple ARGs, with those from the SW exhibiting the highest resistance gene density (Fig. 6A, B). Notably, *Enterobacter cloacae* contained 21 ARGs, and *Acinetobacter pittii* carried 12 ARGs (Fig. 6B). Mover, the extended-spectrum beta-lactamase gene, CTX-M-15, was identified in *Pantoea sp018842675* from the SW, carbapenemase encoding genes, including MSI-1 and MSI-OXA were detected in *Telluria* sp. from the OT, and OXA-417 was identified in *A. pittii* from the SW (Fig. 6B).

To investigate potential HGT, we analyzed the genomic context of ARGs and mobile genetic elements. The sulfonamide resistance gene *sul1* was found co-localized with the insertion sequence *IS6100* on contigs from both *Pantoea alvi* (ICU) and *Stutzerimonas stutzeri* (OT), suggesting a conserved genetic structure or transfer event across spatial and taxonomic boundaries (Fig. 6C). Similarly, CTX-M-15 was located adjacent to *ISEc9* on a contig from *P. sp018842675* in the SW, further supporting its HGT potential (Fig. 6C). Together, these findings

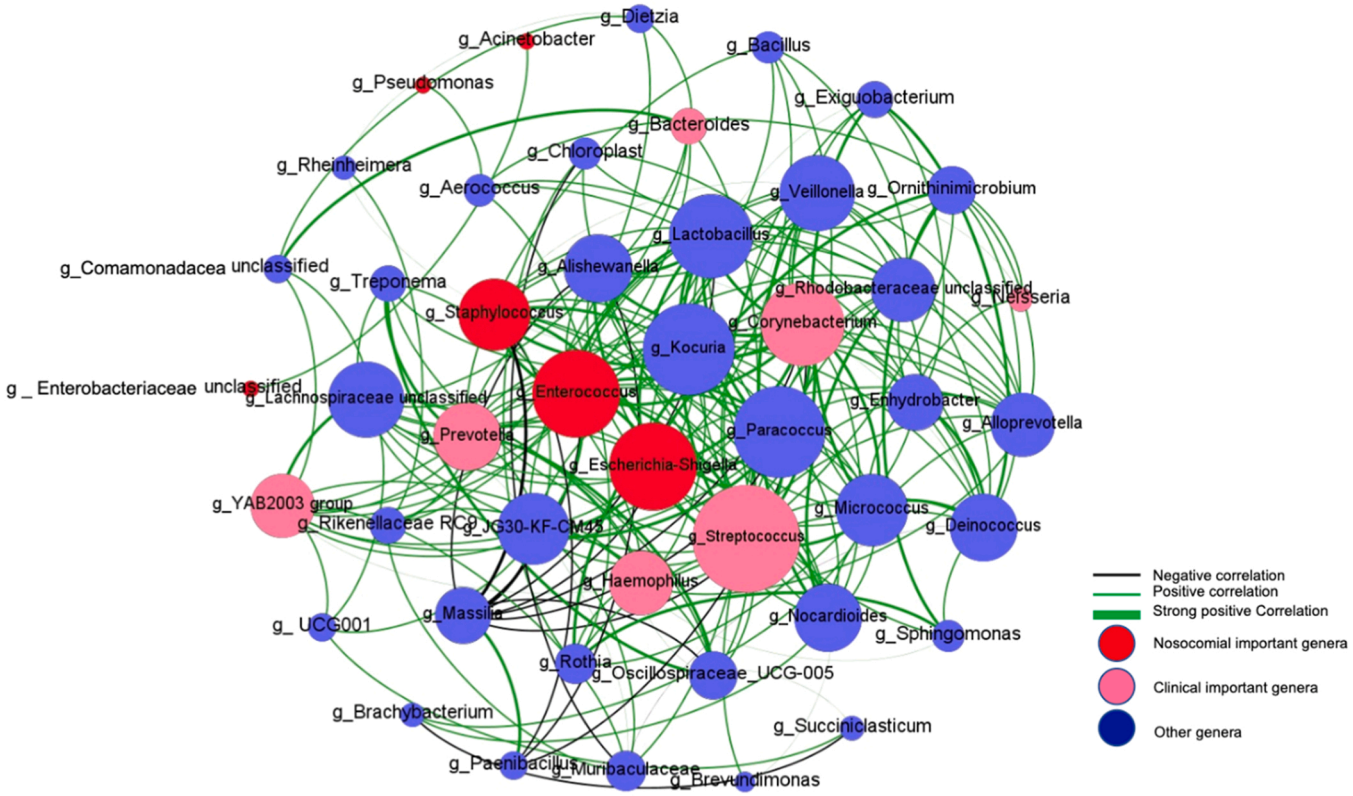
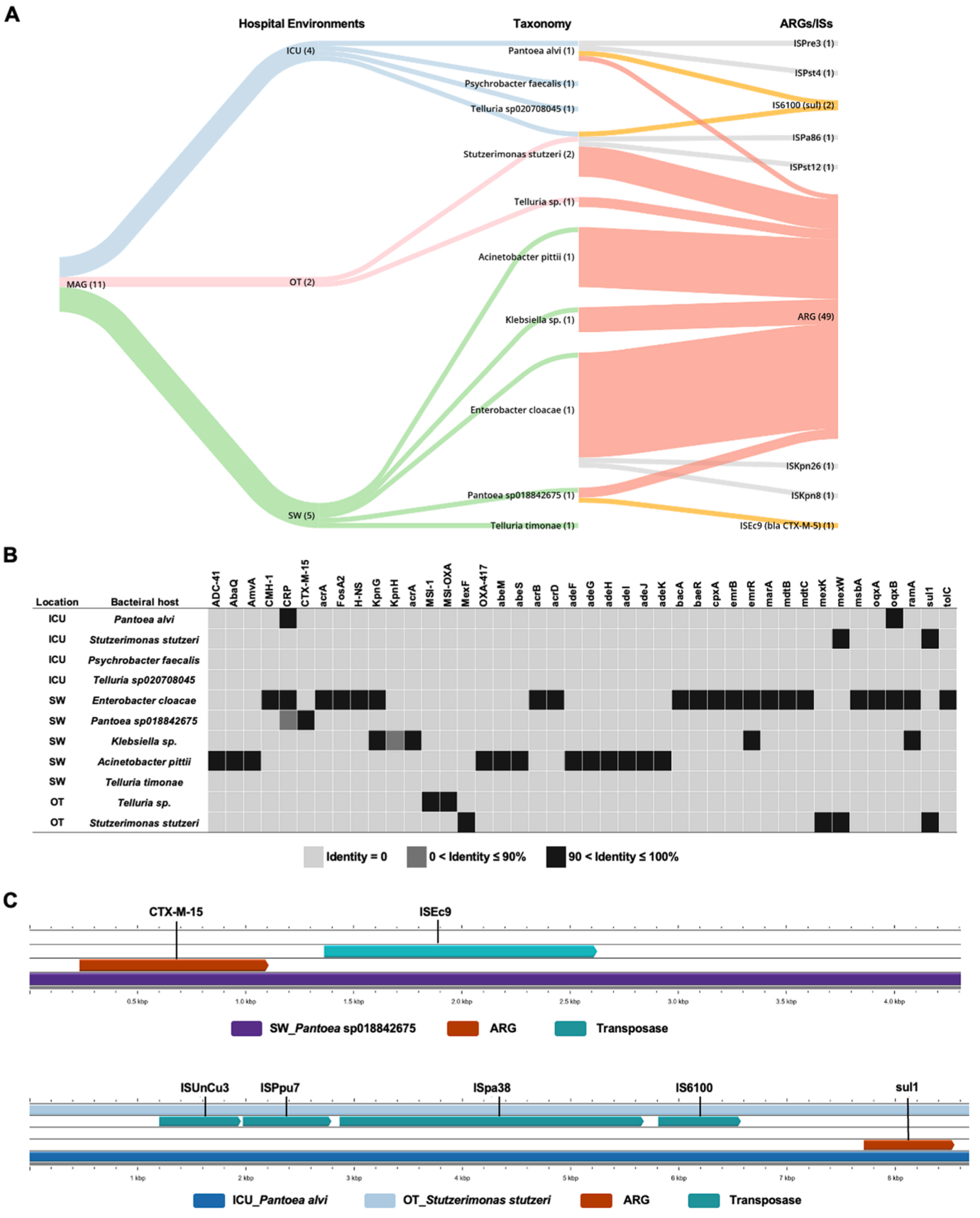


Fig. 5. Co-occurrence network of microbial genera in hospital environments. The network was constructed based on significant pairwise Spearman correlations (FDR-adjusted $p < 0.05$, $|\rho| > 0.25$) among 46 bacterial genera identified in samples from the intensive care unit (ICU), surgical ward (SW), and operating theater (OT) of the hospital. The nodes represent bacterial genera, with node size proportional to the number of connections. Node color indicates clinical relevance: red for nosocomial important genera, pink for clinically important genera, and blue for other genera. A total of 227 edges were identified: green for positive, bold green for strong positive, and black for negative correlations.



(caption on next page)

Fig. 6. Metagenome-assembled genomes (MAGs) across hospital environments. (A) Sankey diagram of MAGs across three hospital environments. A total of 11 MAGs were constructed from shotgun metagenomic sequencing data, including four from the intensive care unit (ICU), two from the operating theater (OT), and five from the surgical ward (SW). Bacterial species were identified and linked to the presence of ARGs and insertion sequences (*ISs*). A contig encoding both *IS6100* and the sulfonamide resistance gene *sul1* was detected in *Pantoea alvi* from the ICU and *Stutzerimonas stutzeri* from the OT. (B) Distribution of ARGs identified in MAGs from different bacterial hosts. Multiple resistance mechanisms were observed, including carbapenem resistance genes (MSI-1, MSI-OXA, and OXA-417) and the extended-spectrum beta-lactamase gene CTX-M-15. MAGs from SW harbored the highest number of ARGs. (C) Genetic context of ARG and MGE co-localization. The beta-lactam resistance gene CTX-M-15 was identified in *Pantoea sp018842675* from the SW, co-localized with the insertion sequence *ISEc9*. A homologous genomic region carrying multiple *ISs* elements and *sul1* was shared between *P. alvi* from the ICU and *S. stutzeri* from the OT, indicating potential horizontal gene transfer.

underscore the risk of ARG dissemination across different hospital environments and bacterial hosts and highlight the importance of genome-resolved metagenomics in tracing ARG-MGE linkages in healthcare environments.

4. Discussion

In this study, we present a comprehensive characterization of the hospital surface microbiome, ARGs, and MGEs across multiple clinical wards and operating theater in a tertiary care hospital in Pakistan. Using both 16S rRNA gene sequencing and shotgun metagenomics, we revealed both ecological and functional aspects of hospital microbiota that may contribute to the persistence and dissemination of AMR in clinical settings. Notably, by reconstructing MAGs, we were able to assign several ARGs to specific bacterial hosts, including globally significant hospital-associated pathogens. These host-ARG-MGE linkages provide strong evidence for the mobility of ARGs and existence of potential environmental reservoirs or persistent colonization within hospital environments. These findings are specially critical in LMIC settings, where routine AMR surveillance remains limited.

Consistent with previous studies, our results demonstrate that microbial diversity and composition vary across hospital environments, likely influenced by factors such as patient traffic, disinfection practices, ward function, and environmental conditions [23,47–51]. While no statistically significant differences were observed in microbial richness or alpha diversity between wards, beta diversity analysis revealed clear microbial community composition clustering. Notably, microbial communities in the ICU, SW, and OT were more similar to each other than to those in the CSW and CW, which may reflect differences in patient demographics, contact frequency, or operational protocols, such as ventilation and surgical activity.

The ICU, OT, and SW are closely interconnected, as patients are frequently transferred between these wards as part of pre-operative, surgical, and post-operative care. The higher microbial richness and diversity observed in the SW and OT by 16S sequencing may be attributed to increased patient turnover, greater procedural complexity, and shared equipment use, all of which can introduce microbial heterogeneity. The OT and SW commonly receive patients directly from the ICU, facilitating microbial spillover and the exchange of taxa. These clinical areas also involve invasive procedures and frequent patient movement, which likely contribute to increased environmental heterogeneity and promote microbial exchange. Additionally, we observed overlapping ARG classes, particularly β-lactam resistance genes, and MGEs such as *ISAba49*, across these wards (Fig. 4). These findings indicate not only microbiome overlap but also potential ARG transmission routes, likely driven by both clinical connectivity and gaps in infection control practices. These findings align with studies showing either localized microbial variability [47,51] or broader homogeneity [52,53] depending on hospital architecture and patient turnover. FEAST-based microbial source tracking further suggested that the ICU may serve as a key source of microbial transfer to SW and OT (Fig. 3). The bidirectional flow between the SW and OT and the central role of the ICU in this network likely reflect the high frequency of patient and staff movement among these spaces, as well as shared surface contact points. Although CSW and CW did not show clear microbial transmission patterns, they exhibited distinct community profiles, possibly due to the presence of more stable or immunocompromised cardiac patients [54].

Resistome profiling revealed a ward-specific distribution of ARGs in the hospital. SW harbored the greatest relative abundance and diversity of ARGs, particularly those conferring resistance to biocides and β-lactams (Fig. 4D). This may be due to the frequent use of disinfectants, elevated microbial loads, and more complex patient interventions in this area. While previous studies have largely focused on ARGs in hospital wastewater or high-touch areas such as sinks [55–57], our findings highlight the potential of general ward surfaces to act as significant reservoirs of ARGs. MGEs, particularly insertion sequences (ISs), were widespread and unevenly distributed across wards. ISs elements accounted for over 80 % of the total MGEs identified, with the highest prevalence in the SW and OT (Fig. 4F). Several of these IS families, including IS5 and IS3, are strongly associated with carbapenemase genes, such as bla_{NDM} and bla_{OXA} [58–60], underscoring their potential role in the horizontal transfer of β-lactam resistance. Transposons, particularly Tn3 and Tn2, were linked to bla_{TEM} in *E. coli*, a relationship previously observed in commensal ampicillin-resistant strains [61]. The absence of ICEs in the SW, despite high ARG abundance, suggests alternative mechanisms of resistance dissemination in this ward.

Overall, these findings indicate that the hospital microbiome is shaped by spatially distinct ecological and functional dynamics, with certain wards acting as hotspots for microbial exchange and the dissemination of ARGs. Furthermore, this study identified the abundance of ARGs and MGEs, enabling deeper investigation into their species level associations with bacterial hosts. By leveraging shotgun metagenomic sequencing and MAGs, we gained critical insight into the mechanisms underlying resistome and AMR transmission within hospital environments. Importantly, these results emphasize the need to focus not only on known pathogens but also on broader microbial networks and environmental reservoirs when designing AMR surveillance and infection control strategies. While this study focused on AMR surveillance within hospital environments, it did not include wastewater sampling, which may serve as an important route for AMR dissemination beyond the clinical setting. Future studies incorporating hospital wastewater surveillance will be essential to better characterize the environmental transmission pathways of AMR. To the best of our knowledge, this study is the first metagenomic analysis of the hospital surface microbiota in Pakistan and offers valuable baseline data for understanding microbial ecology and resistance risks in clinical environments in LMICs. By identifying high-risk wards and key bacterial taxa associated with ARGs and MGEs, our findings have the potential to guide infection prevention policies and support the development of more effective AMR monitoring frameworks.

Environmental implication

This study reveals that hospital environments in low-resource settings, such as those in Pakistan, act as significant and under-recognized reservoirs of antimicrobial resistance genes (ARGs) and mobile genetic elements. The presence and transmission of these genetic determinants across hospital wards not only pose risks to patient health but also represent a broader environmental hazard through potential dissemination into wastewater and surrounding communities. By identifying specific ARG hotspots and transmission pathways, our findings underscore the urgent need for environmentally integrated AMR surveillance strategies. This work highlights how inadequate infection control and waste management practices can facilitate environmental spread of

healthcare-associated resistance, with potential consequences for public and ecological health.

CRediT authorship contribution statement

Muhammad Tariq: Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Conceptualization. **Jeong KwangCheol:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Raul C. Mainar-Jaime:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Data curation. **Yuting Zhai:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Muhammad Umer Asghar:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Peixin Fan:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Data curation, Conceptualization. **Ting Liu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Data curation, Conceptualization. **Arsalan Haseeb Zaidi:** Writing – review & editing, Writing – original draft, Project administration, Funding acquisition, Conceptualization. **Noor Ul Ain:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2025.139384.

Data Availability

Data will be made available on request.

References

[1] Chemaly, R.F., et al., 2014. The role of the healthcare environment in the spread of multidrug-resistant organisms: update on current best practices for containment. Ther Adv Infect Dis 2 (3-4), 79–90.
[2] Li, W., et al., 2022. Evaluation of culturable 'last-resort' antibiotic resistant pathogens in hospital wastewater and implications on the risks of nosocomial antimicrobial resistance prevalence. J Hazard Mater 438, 129477.
[3] Yuan, Q., et al., 2024. Genetic traits and transmission of antimicrobial resistance characteristics of cephalosporin resistant Escherichia coli in tropical aquatic environments. J Hazard Mater 479, 135707.
[4] Sukhum, K.V., et al., 2022. Antibiotic-resistant organisms establish reservoirs in new hospital built environments and are related to patient blood infection isolates. Commun Med 2 (1), 62.
[5] Cocker, D., et al., 2024. Healthcare as a driver, reservoir and amplifier of antimicrobial resistance: opportunities for interventions. Nat Rev Microbiol 22 (10), 636–649.
[6] Cohen, B., et al., 2017. Transmission of health care-associated infections from roommates and prior room occupants: a systematic review. Clin Epidemiol 9, 297.
[7] Mulani, M.S., et al., 2019. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. Front Microbiol 10, 539.
[8] Suleyman, G., Alangaden, G., Bardossy, A.C., 2018. The role of environmental contamination in the transmission of nosocomial pathogens and healthcare-associated infections. Curremt Infect Dis Rep 20, 1–11.
[9] Zhang, M., et al., 2023. Cascade capture, oxidization and inactivation for removing multi-species pollutants, antimicrobial resistance and pathogenicity from hospital wastewater. J Hazard Mater 457, 131730.
[10] Ogunsola, F.T., Mehtar, S., 2020. Challenges regarding the control of environmental sources of contamination in healthcare settings in low-and middle-income countries - a narrative review. Antimicrob Resist Infect Control 9 (1), 81.
[11] Lewnard, J.A., et al., 2024. Burden of bacterial antimicrobial resistance in low-income and middle-income countries avertible by existing interventions: an evidence review and modelling analysis. Lancet 403 (10442), 2439–2454.
[12] Sulis, G., Sena, S., Gandra, S., 2022. Antimicrobial resistance in low- and middle-income countries: current status and future directions. Expert Rev AntiInfect Ther 20 (2), 147–160.
[13] Padmanabhan, R., et al., 2013. Genomics and metagenomics in medical microbiology. J Hazard Mater 95 (3), 415–424.
[14] Goldberg, B., et al., 2015. Making the leap from research laboratory to clinic: challenges and opportunities for next-generation sequencing in infectious disease diagnostics. MBio 6 (6), e01888-15.
[15] Michán, C., Blasco, J., Alhama, J., 2021. High-throughput molecular analyses of microbiomes as a tool to monitor the wellbeing of aquatic environments. Microb Biotechnol 14 (3), 870–885.
[16] Wang, C., et al., 2025. The quest for environmental analytical microbiology: absolute quantitative microbiome using cellular internal standards. Microbiome 13 (1), 26.
[17] Do, N.T.T., et al., 2021. Community-based antibiotic access and use in six low-income and middle-income countries: a mixed-method approach. Lancet Glob Health 9 (5), e610–e619.
[18] Klein, E.Y., et al., 2024. Global trends in antibiotic consumption during 2016–2023 and future projections through 2030. Proc Natl Acad Sci 121 (49), e2411919121.
[19] Atif, M., et al., 2017. Investigation of antimicrobial use at a tertiary care hospital in Southern Punjab, Pakistan using WHO methodology. Antimicrob Resist Infect Control 6 (1), 41.
[20] Saleem, Z., et al., 2019. Pattern of inappropriate antibiotic use among hospitalized patients in Pakistan: a longitudinal surveillance and implications. Antimicrob Resist Infect Control 8, 188.
[21] Griffith, C., 2016. Chapter 44 - Surface Sampling and the Detection of Contamination. In: Lelieveld, H., Holah, J., Gabrić, D. (Eds.), in Handbook of Hygiene Control in the Food Industry (Second Edition). Woodhead Publishing, San Diego, pp. 673–696.
[22] Asghar, M.U., et al., 2023. Molecular distribution of biocide resistance genes and susceptibility to biocides among vancomycin resistant Staphylococcus aureus (VRSA) isolates from intensive care unit (ICU) of cardiac hospital- A first report from Pakistan. Heliyon 9 (12), e22120.
[23] ElRakaiby, M.T., et al., 2019. Hospital Microbiome Variations As Analyzed by High-Throughput Sequencing. OMICS 23 (9), 426–438.
[24] Bolyen, E., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol 37 (8), 852–857.
[25] Shenhav, L., et al., 2019. FEAST: fast expectation-maximization for microbial source tracking. Nat Methods 16 (7), 627–632.
[26] Klassert, T.E., et al., 2021. Bacterial colonization dynamics and antibiotic resistance gene dissemination in the hospital environment after first patient occupancy: a longitudinal metagenetic study. Microbiome 9, 1–17.
[27] Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30 (15), 2114–2120.
[28] Chng, K.R., et al., 2020. Cartography of opportunistic pathogens and antibiotic resistance genes in a tertiary hospital environment. Nat Med 26 (6), 941–951.
[29] Lu, J., et al., 2022. Metagenome analysis using the Kraken software suite. Nat Protoc 17 (12), 2815–2839.
[30] Wood, D.E., Lu, J., Langmead, B., 2019. Improved metagenomic analysis with Kraken 2. Genome Biol 20 (1), 257.
[31] Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods 9 (4), p. 357-U54.
[32] Lu, J., et al., 2017. Bracken: estimating species abundance in metagenomics data. Peerj Comput Sci.
[33] H, W., 2016. ggplot2: elegant graphics for data analysis. Springer-Verlag, New York.
[34] Chen, S.F., 2023. Ultrafast one-pass FASTQ data preprocessing, quality control, and deduplication using fastp. Imeta 2 (2).
[35] Li, D., et al., 2015. MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics 31 (10), 1674–1676.
[36] Li, H., Durbin, R., 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25 (14), 1754–1760.
[37] Danecek, P., et al., 2021. Twelve years of SAMtools and BCFtools. Gigascience 10 (2).
[38] Kang, D.W.D., et al., 2015. MetaBAT, an efficient tool for accurately reconstructing single genomes from complex microbial communities. Peerj 3.
[39] Chklovski, A., et al., 2023. CheckM2: a rapid, scalable and accurate tool for assessing microbial genome quality using machine learning. Nat Methods 20 (8), 1203.
[40] Chaumeil, P.A., et al., 2020. GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. Bioinformatics 36 (6), 1925–1927.

[41] Jolley, K.A., Bray, J.E., Maiden, M.C.J., 2018. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. Wellcome Open Res 3, 124.
[42] Seemann, T., ABRicate: mass screening of contigs for antiobiotic resistance genes; 2016.
[43] Alcock, B.P., et al., 2023. CARD 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. Nucleic Acids Res 51 (D1), D690–D699.
[44] Johansson, M.H.K., et al., 2021. Detection of mobile genetic elements associated with antibiotic resistance in Salmonella enterica using a newly developed web tool: MobileElementFinder. J Antimicrob Chemother 76 (1), 101–109.
[45] Grant, J.R., et al., 2023. Proksee: in-depth characterization and visualization of bacterial genomes. Nucleic Acids Res 51 (W1), W484–W492.
[46] Cai, L., et al., 2021. Antimicrobial resistance bacteria and genes detected in hospital sewage provide valuable information in predicting clinical antimicrobial resistance. Sci Total Environ 795, 148815.
[47] Allerton, F., Blacklock, K.L.B., 2023. Infection control in small animal clinical practice. CABI.
[48] Chen, C.-H., et al., 2017. Bacterial diversity among four healthcare-associated institutes in Taiwan. Sci Rep 7 (1), 8230.
[49] Chen, J., et al., 2023. The distinct microbial community patterns and pathogen transmission routes in intensive care units. J Hazard Mater 441, 129964.
[50] Kembel, S.W., et al., 2012. Architectural design influences the diversity and structure of the built environment microbiome. ISME J 6 (8), 1469–1479.
[51] Rampelotto, P.H., et al., 2019. Exploring the hospital microbiome by high-resolution 16S rRNA profiling. Int Joutnal Mol Sci 20 (12), 3099.
[52] He, P., et al., 2020. Characteristics of and variation in airborne ARGs among urban hospitals and adjacent urban and suburban communities: a metagenomic approach. Environ Int 139, 105625.
[53] Lomtadze, M., et al., 2010. Incidence and risk factors of nosocomial infections after cardiac surgery in Georgian population with congenital heart diseases. Georgian Medival N 178, 7–11.
[54] Hudson, C.M., et al., 2014. Resistance determinants and mobile genetic elements of an NDM-1-encoding Klebsiella pneumoniae strain. PloS One 9 (6), e99209.
[55] Campos, J.C., et al., 2015. Characterization of Tn 3000, a transposon responsible for bla NDM-1 dissemination among Enterobacteriaceae in Brazil, Nepal, Morocco, and India. Antimicrob Agents Chemother 59 (12), 7387–7395.
[56] Poirel, L., et al., 2012. Tn 125-related acquisition of bla NDM-like genes in Acinetobacter baumannii. Antimicrob Agents Chemother 56 (2), 1087–1089.
[57] Mahillon, J., Chandler, M., 1998. Insertion sequences. Microbiol Mol Biol Rev 62 (3), 725–774.
[58] Zhai, Y., et al., 2021. Dissemination mechanisms of NDM genes in hospitalized patients. JACAntimicrob Resist 3 (1).
[59] Acman, M., et al., 2022. Role of mobile genetic elements in the global dissemination of the carbapenem resistance gene blaNDM. Nat Commun 13 (1), 1131.
[60] Shankar, C., et al., 2019. Rapidly disseminating blaOXA-232 carrying Klebsiella pneumoniae belonging to ST231 in India: multiple and varied mobile genetic elements. BMC Microbiol 19 (1), 137.
[61] Partridge, S.R., Hall, R.M., 2005. Evolution of transposons containing bla TEM genes. Antimicrob Agents Chemother 49 (3), 1267–1268.