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ENVIRONMENTAL BIOTECHNOLOGY

Department-specific patterns of bacterial communities and antibiotic resistance in hospital indoor environments

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Abstract

The hospital indoor environment has a crucial impact on the microbial exposures that humans encounter. Resistance to antibiotics is a mechanism used by bacteria to develop resilience in indoor environments, and the widespread use of antibiotics has led to changes in the ecological function of resistance genes and their acquisition by pathogens. By integrating the *16S rRNA* Illumina sequencing and high-throughput-quantitative PCR approaches with water and air dust samples across seven departments in Peking University Shenzhen Hospital, China, this study yields intriguing findings regarding the department-specific variations, correlations and source tracing of bacteria, antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) within the hospital indoor environment. A notable observation was the pivotal role played by seasonal variations in shaping the bacterial composition across the entire hospital indoor environment. Another department-specific finding was the correlation between ARGs and MGEs abundance, which was evident in the overall hospital indoor environment, but not found in the blood test room, ophthalmology, and gynecology departments. Notably, as an important source of bacteria and ARGs/MGEs for the blood test room, the gynecology department also presented a close link between bacterial communities and the presence of ARGs/MGEs. Additionally, the results reiterate the importance of surveillance and monitoring of antibiotic resistance, specifically in *Legionella* spp. in man-made water systems, and highlight the significance of understanding genetic elements like *Tp614* involved in gene transfer and recombination, and their impact on antimicrobial treatment efficacy.

Key points

- *The department-specific variations, correlations and source tracing of bacteria, ARGs, and MGEs were uncovered in the hospital's indoor environment.*
- *Although each department exhibited consistent seasonal impacts on bacterial compositions, the co-occurrence between the presence of ARGs and MGEs was exclusively evident in the emergency, surgery, pneumology and otolaryngology departments.*
- *The gynecology department emerged as a crucial source of bacteria, ARGs and MGEs within the hospital. Additionally, it was found to exhibit a significant correlation between bacterial communities and the presence of ARGs and MGEs.*

Keywords Antibiotic resistance genes · Mobile genetic elements · Microbial communities · Hospital indoor environment · High-throughput-quantitative PCR · Department-specific

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Introduction

Hospital indoor environments are characterized by a high-infective risk; firstly, a cause of the compromised immunologic conditions of the patients that make them vulnerable to bacterial, viral, parasitological and fungal opportunistic infections (D'Alessandro and Fara 2017). It has been estimated that around two million patients per year in the USA acquire a nosocomial infection, and tragically at least 90,000 of them succumb to these infections (Pereira da Fonseca et al. 2016). Research has demonstrated that bacteria can persist and accumulate in various locations within the hospital's indoor environment, including white coats (Treakle et al. 2009), stethoscopes (Tang et al. 2011), air conditioners (Li et al. 2021), water faucets (Franco et al. 2020), and water p-traps (Kotay et al. 2020), far longer than previously believed (Kramer et al. 2006). Influenced by the bacterial cell viability and bacterial load (Boyce 2007), the pathogen can be transmitted through contaminated hands or gloves of healthcare workers (Boyce 2007), direct contact with contaminated surfaces (Boyce 2007), splashing of pathogen-contaminated water on sterile goods (Kelsey 2013), and droplets for respiratory pathogens (Hota 2004). The presence of these reservoirs in the hospital environment may heighten the risk of acquiring a nosocomial infection.

Indeed, with extensive usage of antibiotic drugs on patients and routine application of antimicrobial chemicals for sanitation in hospitals, bacteria isolated from hospital environments are frequently resistant to antibiotics. Specifically, in a study conducted by Moges et al. (2014), it was found that a staggering 81.5% of the bacterial isolates from the hospital environment exhibited resistance to multiple antibiotics. Similarly, Phoon et al. (2018) noted that 62.7% of the identified species such as *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* in the tertiary hospital environment were multidrug-resistant (MDR). These pathogens possess the ability to withstand the effects of multiple antibiotics, greatly limiting the available treatment options and significantly increasing the risk of healthcare-associated infection. Of particular concern about the presence of antibiotic resistance is the dissemination of antibiotic resistance genes (ARGs) in the hospital environment. Many of these genes are found on transposons, integrons, or plasmids, which can be mobilized and transferred to other bacteria, belonging to the same or different species (Allen et al. 2010). These findings highlight the alarming reality of a "pre-antibiotic era."

Currently, there is a growing focus on studying the diversity, interaction, and transmission of microbes and ARGs in various components of the hospital environment, including surfaces (Klassert et al. 2021), air dust (Li et al. 2021), water (Sukhum et al. 2022), and during different seasons (Cassone et al. 2021). These studies have shed light on the influence of several factors on the composition of microbes and ARGs in the hospital indoor environment. Factors such as humidity, temperature (Choe et al. 2019), air filtration (Li et al. 2021), chemical residues (e.g., antibiotics) (Ben Maamar et al. 2020), and patient/room occupancy (Ramos et al. 2015; ElRakaiby et al. 2019) have been identified as important contributors to microbial and ARG compositions in hospitals. However, despite the wealth of research in this area, there is still a limited number of studies that have thoroughly investigated the comprehensive patterns of the microbiome and antibiotic resistance in a department-specific manner. While numerous studies have examined microbial and antibiotic-resistance profiles in intensive care units (ICUs) (Oberauner et al. 2013; Bokulich et al. 2013), only a few studies have directed their attention to different ward room sites (Ramos et al. 2015; ElRakaiby et al. 2019) and various hospital departments (Li et al. 2021). Indeed, the diverse ecological interactions and conditions within different hospital sites have significant clinical implications; yet, they have not been extensively explored.

Conventional methods for studying bacteria and antibiotic resistance can be time-consuming and labor-intensive. However, molecular techniques provide rapid and sensitive alternatives for these investigations. For example, *16S rRNA* amplicon sequencing uses highly conserved bacterial regions for detecting diverse bacteria, while high-throughput-quantitative PCR (HT-qPCR) is a relatively rapid and convenient method for simultaneously evaluating a large number of ARGs (up to 384 ARGs) with low-quantity DNA samples (few microliters). The objectives of this study were threefold (workflow in Fig. 1). Firstly, it aimed to identify the factors shaping the composition of bacterial communities, ARGs, and mobile genetic elements (MGEs) in the hospital indoor environment from a department-specific respect. Secondly, it aimed to delve into the mobility of ARGs, and to ascertain whether the proportions of ARGs and MGEs sources could be identified when aggregating towards specific sinks within a particular department. Lastly, the study aimed to investigate department-specific correlations between bacterial communities and ARGs/MGEs, and to investigate the potential bacteria associated with the spread of antibiotic resistance through the horizontal transfer within the hospital. By addressing these objectives, the study will provide a high-throughput exploration of bacterial communities, ARGs, and MGEs within the hospital indoor environment from a department-specific perspective. The results delivered may help to establish priorities to control the spread of nosocomial pathogens and the dissemination of antibiotic resistance determinants.

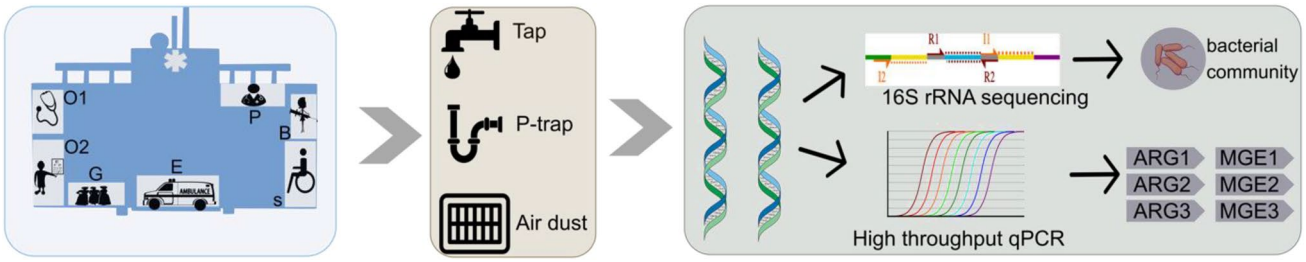

Fig. 1 Overall workflow of the study. O1 is otolaryngology department. O2 is ophthalmology department. G is gynecology department. E is emergency department. S is surgery department. B is blood-test room. P is pneumology department

Materials and methods

Sampling locations, collections, and DNA extraction

We collected 217 samples in the Peking University Shenzhen Hospital, Shenzhen, China (22.55 N, 114.10 E) every month from July 2020 to January 2021. Among 217 samples, 64 samples yielded positive Ct values (Ct < 31) in the HT-qPCR experiments. For the subsequent hospital indoor study, we utilized 60 out of the 64 positive samples (Table S1). Four samples collected from wastewater plants located outside the hospital were excluded from the analysis. The 60 samples constituted water and air dust samples in seven departments (ophthalmology, otolaryngology, emergency, blood test room, surgery, pneumology, and gynecology) from early summer (July, August), late summer (September and October), and to winter (November, December, January).

The water samples contained tap water and p-trap water (a *U*-shaped bend that is connected to the sink and filters water as it enters a plumbing system). Before collecting the p-trap water, the *U*-shaped bend of the p-trap was unplugged to allow the stored water in the bend to be discharged. Then, 500 ml of each type water was collected in a sterile bottle. Next, bacterial cells were captured and collected by pouring the water through the filtration unit containing the sterile mixed cellulose esters (MCE) membrane with a pore size of 0.22 μm and a diameter of 47 mm. The filtration unit consisted of a funnel, a locking ring, a filter flask, and several rubber tubes connected to a water-circulation vacuum pump (− 0.098 Mpa). The captured membrane was promptly stored at 4 °C for subsequent DNA extraction using Dneasy PowerWater Kit (QIAGEN, Germany), which was conducted within 1 day. As for the air dust samples, two filters (30 cm × 30 cm) of the air conditioner (AC) were collected from each studied department. The filters were washed several times with 1 L of sterile reverse osmosis (RO) water, which was filtered through a 0.22-μm MCE membrane to collect microbe cells for subsequent DNA extraction using Dneasy Powerwater Kit (QIAGEN). All collected samples were subject to DNA extraction within 1 day; otherwise, the temporary preservation of samples at − 20 °C was applied. The extracted DNA samples were stored in the EB buffer at − 20 °C for future use.

HT-qPCR

ARGs and MGEs were quantified by HT-qPCR using the Takara (previously WaferGen) SmartChip Real-Time PCR System (#Cat:64,022). Referring to the previous references using customed primers for detecting ARGs and MGEs in the hospital (Stedtfeld et al. 2018; Zhu et al. 2020), we selected 109 gene primer sets (Table S2) for 11 major classes of antibiotics, six transposase genes, five integrase genes, five plasmid genes, and one *16S rRNA* gene primer set. The 11 major classes of antibiotics are aminoglycoside, amphenicol, beta-lactam, fluoroquinolone, multidrug, macrolide/lincosamide/streptogramin B (MLSB), sulfonamide, tetracycline, trimethoprim, vancomycin, and others (peptide, phosphonic acid, and rifamycin). A non-template negative control was used for each primer, and all qPCRs were performed in triplicate. The thermal cycle process amplification was (1) initial denaturation at 95 °C for 10 min, (2) 40 cycles of denaturation at 95 °C for 30 s, and (3) annealing at 60 °C for 30 s (data collection). In the end, melting curve analyses were then automatically generated by the SmartChip qPCR analysis software 2.8.0.65. Only data meeting the following standard were kept for subsequent analysis: (1) amplification efficiencies within the range of 90–110%; (2) amplicons without multiple melting curves; (3) triplicates all within the detection limit of threshold cycle Ct of 31. ARGs and MGEs relative copies represented the gene copies to *16S rRNA* copies ratio.

We used the following formulas (Zhu et al. 2020):

(1) $\text{Gene copies} = 10^{\frac{31-Ct}{10/3}}$ and (2) $\text{Relative copies} = \frac{\text{Gene copies}}{\text{16S rRNA copies}}$

Bacterial 16S rRNA sequencing of 64 samples

Bacteria community structures were determined by a *16S rRNA* gene amplicon sequencing on a HiSeq platform

(Illumina, USA) with PE250 strategy. The V4 to V5 region of the bacterial *16S rRNA* gene was amplified with the universal primer set 515F and 907R (Turner et al. 1999), and labelled with unique 6-nucleotide barcodes for each sample (Table S1). The components of the PCR solution mix and PCR program are shown in Table S3 and Table S4. Following PCR, the *16S rRNA* amplicon samples were grouped into three pools and were sent for Illumina pair-end sequencing, each of which contained 2000 ng DNA individually. To ensure quality control of the raw reads obtained from Illumina sequencing, Fastp software (Chen et al. 2018) was utilized. The post-quality control (QC) reads, specifically those related to the *16S rRNA* gene amplification, were then imported into the Quantitative Insights in Microbiology (QIIME 1) pipeline (Caporaso et al. 2010). Within this pipeline, several steps were performed, including merging pair-end sequences, extracting barcodes, splitting samples, and removing amplification primers. For obtaining operational taxonomic units (OTUs), USEARCH V11 (Edgar 2010) was employed with a 97% similarity threshold. Subsequently, taxonomic assignments were achieved by referencing the obtained OTUs against the Silva 138.1 database (Quast et al. 2013).

Statistical analysis

To analyze the changes and similarities in genus complexity among samples, several statistical methods and packages were employed. Principal component analysis (PCA) and analysis of similarities (ANOSIM) were conducted by the R3.5.3 VEGAN package (Dixon 2003) to reveal patterns and differences. The random forest test, implemented with the RandomForest package (Genuer and Poggi 2020), was utilized to identify the most discriminative variables between two sample categories. Moreover, to visualize the sharing phenomenon of genus and ARGs, Venn graphs were generated by the VennDiagram package (Chen and Boutros 2011). For the investigation of the correlation between ARGs and MGEs, the Mantel test was performed with "mantel" function in the Vegan package. Additionally, a pairwise correlation between the abundances of targeting genes and OTU was calculated using the "corr.test" function, and it was considered statistically robust if the Spearman's correlation coefficient (ρ) was > 0.6 and the p-value was < 0.05. With the pairwise correlation values, the Gephi platform was used to generate networks (Bastian et al. 2009). Further, to perform the source-tracking analysis, Sourcetracker2 (Knights et al. 2011) was utilized. Notably, all graphs during the statistical analysis were generated by Rstudio3.5.3 with the ggplot2 package (Wickham 2016).

Results

Microbial profile

Overall sketch of microbial profile in the hospital indoor environments

From the *16S rRNA* gene amplicon sequencing, the raw data consisted of 598,1256 reads (2.99 Gbps) for all samples, and the quality-filtered (Q30) data consisted of 591,4227 reads with an average value of 92,410 reads per sample, corresponding to 1439 different bacteria types after filtering and quality control. The mapped level of each taxonomic level, namely phyla, class, order, family, genus, and species, against the Silva 138.1 database (Quast et al. 2013) was 100%, 99.9%, 99.7%, 98.8%, 82.8%, and 9.98%, respectively. The limited percentage of identified bacterial species could be attributed to the short reads length that may not provide sufficient resolution for species identification. The rarefaction analysis conducted at the genus level demonstrates that the data obtained was adequate for taxonomic analysis. Despite the limitations of short reads, the analysis is able to provide reliable insights into the composition and diversity of bacterial genera within the sample (Figure S1).

At the genus level, there were 684 genera detected in total. Out of the 684 genera, a subset of 215 genera were identified as being particularly abundant, accounting for over 0.01% of the total *16S rRNA* gene sequences at the genus level. These 215 genera were considered representative of the bacterial community and were selected for further analysis in subsequent steps. In terms of the dominating genera, *Dechloromonas*, *Pseudomonas* and *Flavobacterium*, *Limnohabitans*, *Ralstonia*, and *Acineotobactor* took the leading places (Figure S2a), taking 11.0%, 7.95%, 7.56%, 6.41%, 5.53%, and 4.17% of the community respectively. As *Pseudomonas* is the most concerned waterborne pathogen in healthcare facilities responsible for a wide spectrum of infections (such as pneumonia and urinary tract infections) in humans that can be associated with significant morbidity and mortality (Bonadonna et al. 2017), *Dechloromonas* occurs frequently in the soil and wastewater treatment systems linked with nitrogen cycling roles (Petriglieri et al. 2021) and *Flavobacterium* exists more in soil and freshwater that may cause disease in freshwater fish (Bernardet et al. 1996). Moreover, the pathogenic *Acineotobactor* is particularly noteworthy due to the potential implications of its species (e.g., *A. baumannii, Acinetobacter nosocomialis, Acinetobacter pittii*, and *Acinetobacter seifertii*) as a source of infections (such as pneumonia, meningitis, and bacteremia) in debilitated patients within the hospital (Harding et al. 2018). Apart

from the dominating concerned genera, several other genera of interest were observed in the lower abundance in the studied environment. These included *Enterobacter* (0.806%), *Stenotrophomonas* (0.743%), *Bacillus* (0.418%), *Staphylococcus* (0.263%), *Klebsiella* (0.217%), *Mycobacterium* (0.561%), *Streptococcus* (0.116%), *Escherichia-Shigella* (0.0981%), and *Neisseria* (0.0116%). Among them, *Staphylococcus*, *Klebsiella*, *Acinetobacter*, and *Enterobacter* corresponded to the genus types of *ESKAPE* pathogens (*Enterococcus faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.). However, it is important to note that not all *ESKAPE* pathogens were detected in the hospital environment under study. For instance, *Enterococcus* genus was not classified. Although *Enterococci* are commonly associated with hospital-acquired infections and are notorious for their resistance to vancomycin (Brinkwirth et al. 2021; Hammerum et al. 2024), the limitations of this study, such as DNA extraction efficiency and the sensitivity of V4–V5 *16S rRNA* amplicon sequencing, may have hindered the identification of *Enterococcus* in the samples.

From species-level respect, the results of predominating species (Figure S2b) were concerning, as they indicated the presence of certain pathogens in the studied environments. Among the major species, *Pseudomonas* sp. was found to be the most prevalent, accounting for 45.4% of the samples. This pathogen showed a particularly significant emergence in the emergency and otolaryngology departments. Another alarming pathogen was the presence of *Stenotrophomonas maltophilia*, which accounted for 5.12%. This pathogen was most frequently detected in the pneumology department, indicating a potential association with respiratory-related conditions. Furthermore, the study identified the presence of *K. pneumoniae* in 1.80% of the samples. Although the proportion might be relatively lower compared to other pathogens, the detection of *K. pneumoniae* was still significant due to its potential to cause serious infections and its association with antibiotic resistance (Madebo et al. 2022). In the study conducted by Madebo et al. (2022), *P. aeruginosa* and *K. pneumoniae* were reported as the leading contaminants in the hospital as well. However, another dominating pathogen of *S. aureus* did not appear in our study which could potentially be attributed to a low mapping rate at the species level during the analysis.

Seasonal changes shaped the bacterial composition in each department

When examining microbial alpha diversity in the indoor hospital environments, a significant fluctuation was observed across different departments, as depicted in Fig. 2f. The otolaryngology and surgery departments displayed the highest microbial diversity, with Shannon index values of 2.80 and 2.66, respectively. In contrast, the pneumology department exhibited the lower microbial diversity, recording the lowest Shannon index value of 1.99. To further explain such variation of alpha diversity, the Shannon index values between air dust and water within specific department were compared. Interestingly, in the otolaryngology, surgery, and pneumology departments, air dust consistently displayed higher bacterial alpha diversity. The Shannon index values for air dust were 3.23, 3.43, and 2.50, respectively. The water medium exhibited slightly lower Shannon index values, measuring 2.70, 2.51, and 1.61, respectively. This finding aligns with the understanding that aerosols released within the hospital environment can serve as a reservoir for a diverse range of microorganisms, contributing to the observed higher alpha diversity in air dust samples. Previous studies, such as the one by Bonadonna et al. (2017) have also highlighted the presence of microorganisms in aerosols released through various routes within healthcare facilities. Apparently, the notable increase in alpha diversity within the respiratory-related department, specifically the pneumology department, gives rise to apprehension. This heightened diversity had the potential to introduce a wide range of bacteria, including pathogens, into the respiratory system, thereby elevating the risk of respiratory infections and exacerbating pre-existing respiratory conditions. Notably, aerosol contact has been established as a significant pathway for respiratory infections, such as tuberculosis or Legionnaires' disease (Tang et al. 2006), wherein inhaled bacteria colonize the respiratory tract. Adding to the concern, the formation of bacterial biofilms within the respiratory tract poses a considerable threat. Aerosols can facilitate the dissemination of these biofilms, thereby promoting the transmission of antibiotic-resistant bacteria among individuals (Ding et al. 2023).

In order to assess the primary environmental factor influencing the microbial composition in the hospital indoor environments, a PCA was conducted, considering variations in seasons, mediums, and departments. The PCA results reveal the presence of three distinct clusters along the PC1 and PC2 axes, primarily separated by seasonal differences, rather than variations in departments and mediums (Fig. 2a, d, e). This suggests that the microbial structure within the studied indoor hospital environment significantly differed between summer and winter. The influence of seasons was further supported by the ANOSIM result (Table S5). To gain a deeper understanding of the clusters within the summer season, the summer samples were divided into two subgroups: early summer and late summer. Therefore, another round of PCA analysis was conducted specifically for these subgroups (Fig. 2a). The results uncover that the two summer clusters were composed of different genera components, with early summer and late summer exhibiting distinct microbial compositions. This differentiation within the summer season was strongly supported by the ANOSIM result with a *p*-value

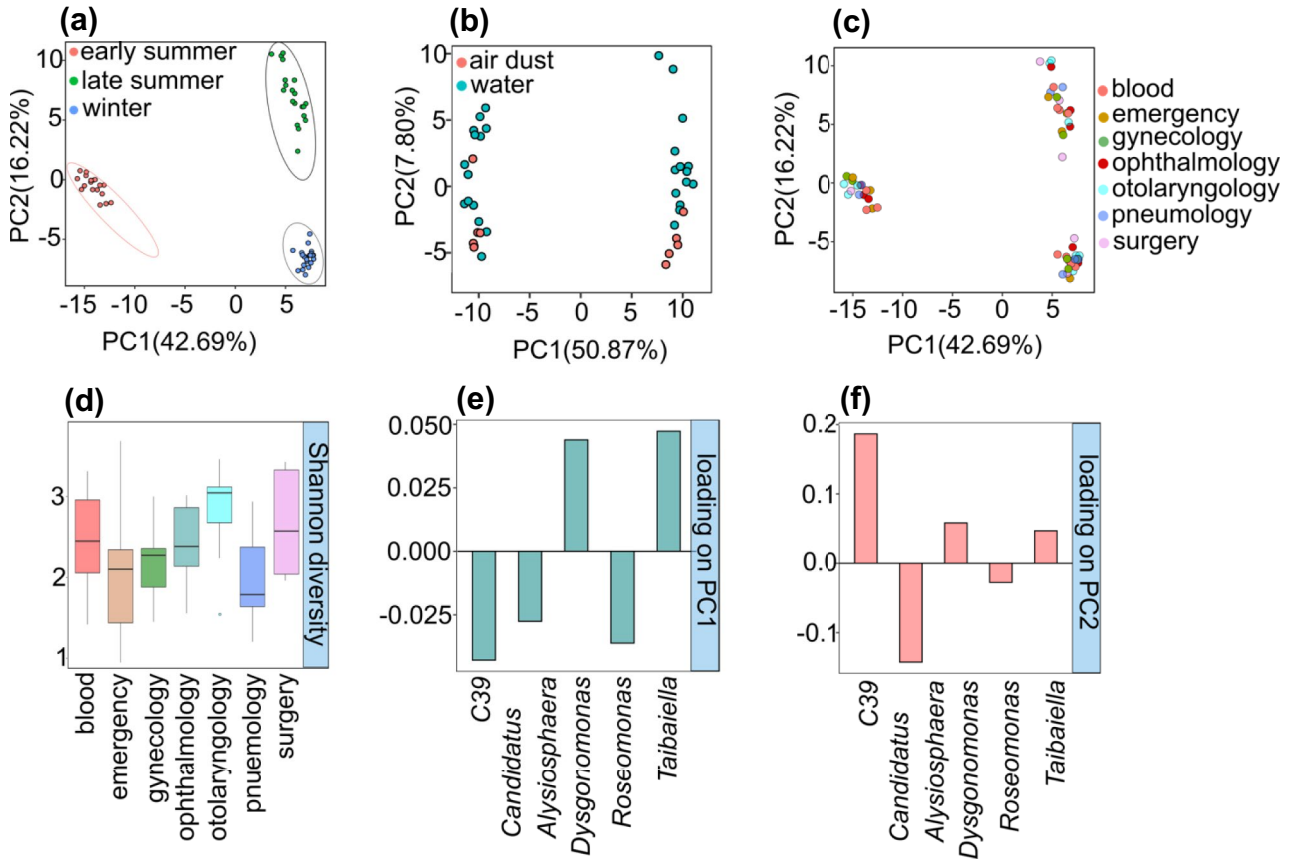

Fig. 2 **a** PCA clusters in the studied hospital indoor environment separated by early summer, late summer and winter, of which the ellipses were drawn in 95% confidence level. **b** PCA characteristics of two mediums. Due to the absence of air dust samples in winter, the analysis in water and air dust was conducted without including the water samples from winter. **c** PCA characteristics of seven departments. **d** Bacterial (genus-level) Shannon diversity in seven departments, where the inside line in the each box represents the median value. **e**, **f** Five key genera that contributed significantly to the observed community compositional differentiation between early summer and late summer in the overall hospital indoor environment

of 1E-04 and an *R*-value of 0.7346 (Table S5). To pinpoint the specific genera responsible for driving this separation between early summer and late summer, a RandomForest analysis was performed. The analysis reveals several key genera that contributed significantly to the observed differentiation (Fig. 2g, h). These genera included *C39*, *Candidatus Alysiosphaera*, *Dysgonomonas, Roseomonas*, and *Taibaiella*. The variation of these genera likely played a crucial role in shaping the distinct microbial compositions observed between the early summer and late summer clusters. Among these genera, except for *Roseomonas*, the other four were found to be more predominant in the late summer samples. It is worth noting that *Taibaiella* exhibited the most pronounced discrimination, showing a 23-fold higher relative abundance in the late summer samples. Furthermore, it is noteworthy that certain species within the *Roseomonas* genus, e.g., *Roseomonas mucosa* have been known to be opportunistic pathogens for humans (Dé et al. 2004). Their increased relative abundance in early summer samples might suggest a potential health risk during that time. Additionally, *Dysgonomonas* bacteria have been recognized as causative agents of gastroenteritis, particularly in immunocompromised individuals (Ryan and Sherris n.d.).

Upon examining each of the three clusters in the PCA plot in Fig. 2c, it is apparent that each cluster encompassed all samples from the seven departments, reflecting the seasonal impact witnessed in each department. However, the ANOSIM results in Table S5 presented contrasting findings, indicating that seasonal variation significantly influenced the bacterial compositions solely in the blood test room and emergency department. Conversely, for the remaining five departments, the ANOSIM outcomes suggest that bacterial compositions varied irrespective of seasonal changes. Upon further investigation into the reason behind these contradictory results, it appears that the inadequate sample size for the ANOSIM test may have played a role.

Overall, the observed alpha- and beta-diversity patterns underscore that the bacterial community structure in the

hospital indoor environments was significantly influenced by seasonal variations. However, while diversity is generally considered beneficial for ecosystem health, it can also introduce challenges, particularly in healthcare settings where cross-infection is a concern for immunocompromised patients. As a result, understanding diversity dynamics can provide valuable insights into the ecological processes and environmental factors that shape microbial communities (Brown et al. 2004), contributing to a broader understanding of microbial ecology and ecosystem functioning.

Tracing the sources of *bacteria* in the hospital indoor environments

Regarding the common genus types present, the Venn analysis reveals several noteworthy findings in the hospital indoor environments. Across all seven departments, there were a total of 197 common genus types, indicating the presence of a core bacterial community within the hospital environment (Figure S3a). To illuminate such phenomenon, the activities of healthcare occupants had a significant influence on it within healthcare facilities (Hospodsky et al. 2012). Through their interactions with patients and contact with various surfaces, the occupants could unintentionally facilitate the transfer of bacteria from one patient to another, thereby influencing the spread of healthcare-associated infections. In addition to the common community types among the department, the common bacteria between water and air dust samples was also observed, with 201 common genus types being observed between these two mediums. The intimation is that bacteria sharing occurred frequently in water and air mediums. Specifically, bacteria present in water sources could become aerosolized and subsequently released into the air. This was particularly notable during the summer season when air conditioners were in operation and were cooled using water from the hospital water, which enormously increased the likelihood of bacterial exposure and potential transmission to individuals within the healthcare facility (Leung et al. 2019).

Assuredly, there is a well-established fact that in the outpatient setting, the blood test room functions as a focal point for accommodating patients from diverse origins. As a consequence, to further seek the sources of the genera present in the blood test room, a source-tracking analysis was undertaken. Indeed, the validation that demonstrates the reliability of selecting the "real" sink as the designated sink role in the SourceTracker2 analysis has been conducted by Wu et al. (2022a, b). Based on this evaluation, during the analysis, the blood test room was considered the "sink" for the communities (genus level), while the other six departments were considered the "sources." The results of the analysis (Figure S3b) reveal that 46.8% of the genera in the blood test room originated from the other six departments, of which the gynecology and emergency departments contributed the most with 21.6% and 8.81% respectively. However, there were still 53.2% of the sources that could not be identified, indicating a substantial portion of the genera in the blood test room were outdoor origins.

Broad-spectrum profile of ARGs and MGEs

MDR and MGE genes were the most abundant in the hospital indoor environments

The analysis of ARGs and MGEs reveals the detection of 107 target ARGs and MGEs across all samples. The total relative copy number per *16S rRNA* gene copy of these ARGs and MGE genes was 74.1, with an average relative copy number of 0.0290. Figure S4a illustrates the relative abundance of the 107 ARGs and MGEs belonging to 14 different types in all the samples. It is witnessed that the largest proportion of detected genes were dominated by MDR and MGE genes, with a total relative copy number of 24.8 and 20.6, respectively. The MDR genes were further classified into the mobile genes and the genes that were not featured with the mobility with a relative copy number of 5.55 and 19.3, respectively. The MGEs category included integrases, transposases, and plasmids. Integrases had a relative copy number of 11.4, transposases had a relative copy number of 9.14, and plasmids that were represented by *tra* genes had a relative copy number of 0.0600. This indicates a high likelihood of horizontal gene transfer (HGT) risk within the hospital, as MGEs play a significant role in facilitating the transfer of ARGs. Following the MGE genes, ARGs delivering resistance to sulfonamide, MLSB, and aminoglycoside were detected, with 9.28 relative copies, 6.90 relative copies, and 4.13 relative copies, respectively. Noticeably, among beta-lactam-resistant genes, there were 57.0% (1.88 relative copies) extended-spectrum beta-lactamases (ESBLs), which has been defined as transmissible β-lactamases that can be exchanged between bacteria (Shaikh et al. 2015). These findings align with a study conducted by Li et al. (2019) on hospital samples collected from air-conditioning units, which also identified similar patterns of dominating drug resistance.

Heterogenous ARGs/MGEs abundance and diversity in the hospital indoor environments

The different departments in the hospital indoor environments presented a heterogeneous distribution regarding the richness of ARGs and MGE genes (Fig. 3a). The blood test room had the highest abundance of ARGs and MGEs, with 12.9 relative copies, while the otolaryngology department had the lowest gene copies, with 6.18 relative copies. The distribution of MGE genes exhibited a different pattern

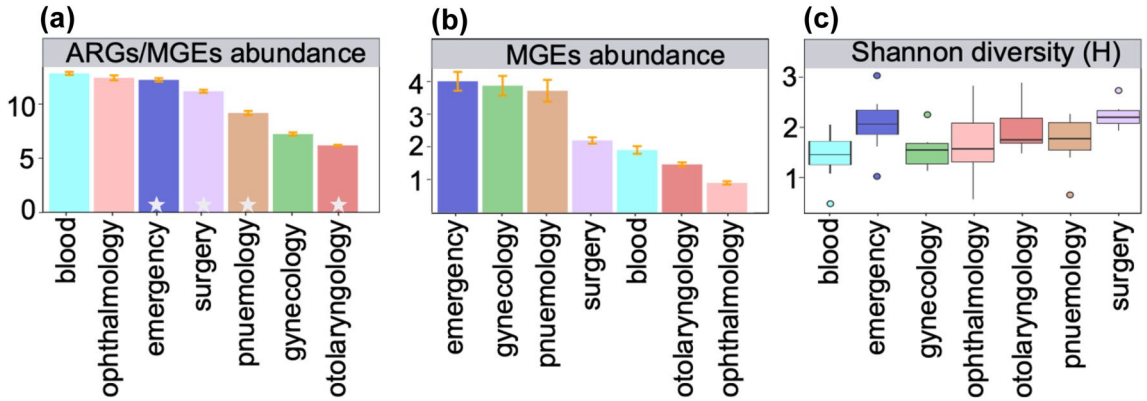

Fig. 3 **a** Abundance of ARGs and MGEs genes in each department, where the abundance represents the relative copy number per *16S rRNA* gene copy. The upper edge of the error bar represents the abundance value plus the standard deviation (SD), and the lower edge of the error bar represents the abundance value minus the SD. The five-star symbols in the bottom of the bar in the emergency, surgery, pneumology, and otolaryngology departments represent a significant correlation between ARGs and MGEs in each of those department. **b** Abundance of MGEs genes in each department, where the abundance represents the relative copy number per *16S rRNA* gene copy. **c** Boxplot of ARGs/MGEs Shannon diversity in each department, where the inside line in the each bar represents the median value, and the outside circles stand for outliers

(Fig. 3b), with the emergency department showing the highest abundance of MGE genes at 4.00 relative copies, indicating a higher frequency of gene transfer events in that department. In contrast to the relatively moderate level of heterogeneity in terms of ARGs and MGEs abundance, the diversity of ARGs and MGEs displayed pronounced fluctuations across departments. The surgery samples exhibited a notable increase in diversity, with an average Shannon index of 2.26 (Fig. 3c). This could be attributed to factors such as the use of antimicrobial chemicals to treat wounds and injuries (Hartmann et al. 2016), and the contribution of human sources from the high occupancy rates (Prussin and Marr 2015).

Given the intricate and diverse distribution of ARGs and MGEs in hospital indoor settings, the primary factors shaping their compositions were expected to be complex. In detail, despite seasonal conditions like temperature and humidity exerting a significant influence on communities' composition, both PCA and ANOSIM analyses indicate that seasonal variations were not the primary drivers altering the composition of ARGs and MGE genes (Figure S5a, Table S6), even though a clear seasonal pattern emerged with higher relative gene copies (48.4) and Shannon diversity (1.97) of ARGs and MGE genes during the summer months (Figure S6a-b). Actually, while a study by Lu and Lu (2020) stated that in the estuarine sediment environment, seasonal changes had a great impact on the abundance of ARGs, and there remains a dearth of research demonstrating the seasonal influence on shaping ARG compositions in hospital environments. Similarly, neither medium nor department variations significantly affected the components of ARGs and MGEs as well (Figure S5b-c, Table S6). While there may be differences in the richness, diversity, and specific types of ARGs/MGEs between different environmental factors (Figure S6), these differences may not be strong enough to be statistically significant. Other factors, such as microbial community dynamics, host factors, or specific environmental conditions, could also contribute to the observed patterns.

Department-specific correlation between ARGs and MGEs

It has been stated that MGEs can contribute to the HGT of ARGs among different microbes, and the host range of the MGEs carrying an ARG is important for determining how far it will spread (Ben Maamar et al. 2020). Consequently, to further prove the mobility of ARGs, the Mantel test between ARGs and MGEs was conducted. The results reveal a moderate correlation between the abundance of ARGs and MGE genes ($p = 0.001$, $r = 0.2100$) in the overall hospital indoor environment. This suggests that the abundance of ARGs was likely to be associated with the presence of MGEs, further supporting the notion of horizontal transfer of ARGs among bacteria in the hospital. Nevertheless, when the Mantel test was performed in each department individually, an intriguing finding emerged. The significant correlation between ARGs and MGEs was observed simply in the emergency, surgery, pneumology, and otolaryngology departments (Fig. 3a), while no significant correlation was found in the other three departments (Table S7). In the emergency, surgery, pneumology, and otolaryngology departments, there were specifically 44, 68, 59, and 45 pairs of ARGs and MGE genes, respectively, showing the robust correlation ($p < 0.5$, $\rho > 0.6$) based on the co-occurrence analysis. Consequently,

to assess whether those ARGs correlating with MGEs exhibited greater similarity between seasons compared to those not correlated with MGEs within these departments, more ANOSIM tests were conducted for correlated ARGs between seasons and non-correlated ARGs between seasons. The results of these ANOSIM tests (shown in the Table S8) imply that there were no significant differences in ARGs composition between seasons within each department. This suggests that the similarity in ARGs composition between seasons was not primarily influenced by the transfer of ARGs carried by MGEs. Other elements, such as microbial structure (He et al. 2020), antibiotic usage patterns (Perry et al. 2021), persistent sources of ARGs, and indoor conditions (Perry et al. 2021), may have contributed to the observed similarity in ARGs composition between seasons in the hospital indoor environment.

Based on the ARGs' mobility indicated by the significant correlation between ARGs and MGEs, the phenomenon of common ARGs and MGEs in the hospital indoor environments is manifested in Fig. 4a. Specifically, the indoor hospital exhibited a core set of 38 ARGs and 8 MGE genes across the studied departments. Significantly, akin to the unique trends observed in representative ARGs, the singular presence of specific ARGs was apparent. For example, the ophthalmology department had vancomycin-resistant genes *vanSB* and *vanWB*. The pneumology department had peptide-resistant gene *mcr-2* and MLSB-resistant gene *ermY*. Additionally, the otolaryngology department had the aminoglycoside-resistant gene *armA*. Among those unique ARGs, *vanSB*, *vanWB*, and *ermY* have been acknowledged as chromosome-mediated ARGs (Leigh et al. 2022); consequently, their unique presence in specific departments could be attributed to their inherent mobility. In terms of *mcr-2* and *armA* gens, they have been known as mobilized resistance genes mediated by plasmids (Liassine et al. 2016; Saadatian Farivar et al. 2018). Hence, the presence of their unique existence in specific departments might result from their limited transferability mediated by MGEs.

Once more, a source-tracking analysis was performed to probe the origins' proportions of ARGs and MGEs found in the blood test room. To the same token, the analysis maintained the postulation that the blood test room functioned as a "sink" for the accumulation of ARGs/MGEs, with the other six departments acting as the "sources." According to the source tracking results (Fig. 4b), it was found that 50.8% of ARGs and MGEs present in the blood test room originated from the other six departments. Among these, the gynecology department was identified as a significant contributor, accounting for 31.5% of the ARGs and MGEs, while the pneumology and surgery departments contributed 7.90% and 5.45% respectively. Considering the gynecology department as one of the sources that contributed 21.6% of the genera to the blood test room, it suggests that the gynecology department played a non-negligible role in the dissemination of bacteria, ARGs, and MGEs. However, it is crucial to note that a substantial portion (49.2%) of the source of ARGs/MGEs could not be identified, implying the possibility of outdoor sources. A similar source-tracking study was also conducted by Li et al. (2021) in the same hospital, which pointed out that in the air dust, the outpatient hall was one of the main ARG transmission sources to the ophthalmology and pediatrics departments.

Co-occurrence between bacterial communities and ARGs/MGEs in the gynecology department

In this study, ARGs were able to be found in all studied hospital indoor environments. Indeed, the use of antibiotics by humans in the hospital has been found to stimulate the acquisition of ARGs by pathogenic bacteria (Martinez et al. 2009). To investigate whether the bacterial community was correlated with ARGs and MGEs composition, we used the

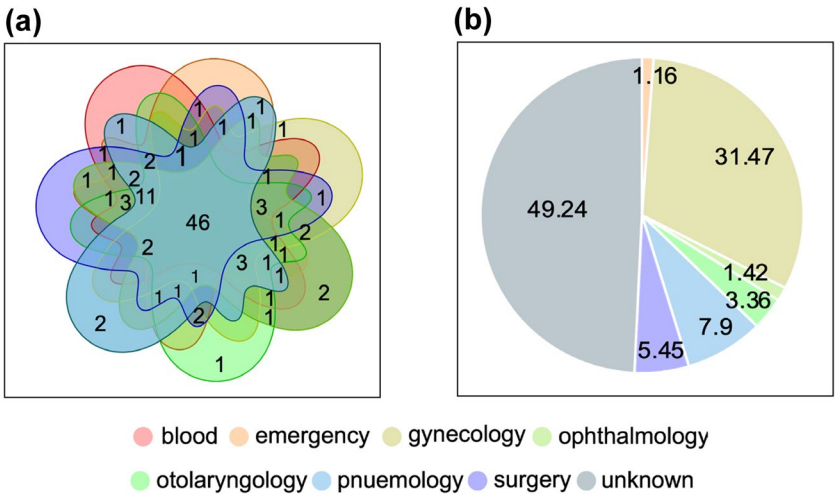

Fig. 4 **a** Venn diagram of common ARGs and MGE genes in seven departments. **b** Source-tracking analysis of ARGs and MGEs across seven departments, where the blood department acted as "sink", and other six departments served as "sources"

Procrustes analysis and the Mantel test to correlate profiles. The Procrustes test shows that there was not a goodness-of-fit test (Table S9, $M^2 = 0.6583$, $p = 1E\text{-}06$, 10,000 permutations) on the basis of Bray – Curtis dissimilarity metrics, reflecting the potential inconsistency between the bacterial community composition and the composition of ARGs and MGEs. The mantel test proved this as well (Table S9, $r = 0.03715$, $p = 0.113$). However, in contrast to the insignificant relationship between all detected genera and ARGs/MGEs, the pairwise Spearman's rank correlation (23,221 pairs in total) displayed the intriguing result that tetracycline-resistant gene *tet(36)* was significantly connected with nine genera (p-value < 0.01, $\rho > 0.6$). It has been previously reported that *tet(36)* was first discovered in *Bacteroides* sp. strain and HGT of *tet(36)* was claimed to occur frequently between divergent phylogenetic groups in the farm environment (Whittle et al. 2003a, b). Further, to assess the relationship between genus types of *ESKAPE* pathogens and ARGs/MGEs, another co-occurrence analysis was conducted. Specifically, the analysis focused on the genera of *Pseudomonas*, *Enterobacter*, *Staphylococcus*, *Klebsiella*, and *Acinetobacter*, in relation to 107 ARGs/MGE genes. The results of the Procrustes test indicate a lack of significant fit (Table S9, $M^2 = 0.7946$, $p = 1E\text{-}04$, 10,000 permutations). This inconsistency was further supported by pairwise Spearman's rank correlation analysis. Among the total of 540 pairs examined, none exhibited a p-value less than 0.05 and ρ greater than 0.6, reinforcing the absence of a strong correlation between the two factors.

Absorbingly, although there was no correlation observed between the community composition (even *ESKAPE*) and ARGs/MGEs profile in the overall studied environment, a strong connection between them was discovered specifically in the gynecology department which was supported by the Procrustes'residuals value. In general, the residuals value represents the closeness between the ARGs/MGEs and genus composition. A lower residuals value indicates a closer relationship. In the gynecology department, we found that the average residuals value of samples was significantly low, suggesting a strong association between ARGs/MGEs and bacterial composition in that specific department. To further evaluate this strong correlation, Procrustes and Mantel analyses were conducted exclusively targeting the ARGs/MGEs and bacterial composition in the gynecology department. Both analyses gave compatible results, showing a strong correlation between ARGs/MGEs and bacterial composition (Table S9, Procrustes with M^2 of 0.1007 and p of 0.0025; Mantel test with r of 0.7571 and p of 0.018).

Based on these results, we proceeded to perform pairwise Spearman's correlation analysis between nine gynecology samples containing 43 ARGs/MGEs, and 181 genera. The results show that all 43 target genes and 181 genera had a significant relationship, with p values below 0.05 and ρ values above 0.6. Moreover, 32 ARGs and 117 genera exhibited ρ values above 0.8, with p values below 0.01. In the study conducted by Li et al. (2015), it has been hypothesized that the non-random co-occurrence patterns between ARGs and microbial taxa could indicate the possible host information of AGRs if the ARGs and the co-existed microbial taxa possessed significantly similar abundance trends among the different environments (Spearman's $\rho > 0.8$, p-value < 0.01). In line with this hypothesis, those matched correlation values of 32 ARGs/MGEs and 117 genera were sent for the undirected network analysis.

The created network of the gynecology department consisted of 185 nodes (each node represented a subtype of ARGs, MGEs, or bacterial taxa) and 366 edges (Fig. 5). The modularity index was 0.642 (value > 0.4), suggesting that the network had a modular structure. Based on the modularity class, the entire network was separated into three major modules. Compared with a random association, clusters of nodes in the same module contained more interactions among themselves than with other nodes. Module I was the largest module comprising 82 nodes, followed by modules II and III including 23 and 20 nodes, respectively. From the network, it was witnessed that transposase *Tp614* and aminoglycoside-resistance *sat4* in module I had the most positive connections with 151 types of genera individually. Besides *Tp614*, MGE genes including integrase *int1-a-marko* and transposase *tnpA 201* in module III had more positive connections with 18 and 17 types of genera as well. Consequently, the substantial number of connections involving MGEs in the network indicate the dissemination of ARGs and the potential acquisition of these ARGs by other microbes within the gynecology department. This phenomenon underscores the impact of a high-antibiotic-selective pressure in the hospital environment. Other ARGs, such as *bacA*, *ampC* in module III, and *tetX* in module V, which conferred resistance to bacitracin, beta-lactamase, and tetracycline, respectively, were associated with multiple candidate genera (20, 15, and 12 genera, respectively), suggesting a higher likelihood of these ARGs being carried by a diverse range of microbial hosts. However, it is important to note that while many ARGs were associated with multiple genera, only a few ARGs were found to be potentially carried by a single host. For example, the MLSB-resistant gene *msr(E)* was exclusively correlated with *Chelatococcus*, and *Paludibacter* was the only connection with MLSB-resistant gene *lnuA* as well. These observations indicate that certain ARGs may have a more restricted distribution and are less likely to be transferred between different microbial taxa.

Furthermore, the network analysis unveiled significant correlations between certain clinically relevant genera and specific ARGs/MGEs. Notably, *Legionella* exhibited a significant correlation with two ARGs associated with antibiotic resistance. Specifically, it was found to be linked to

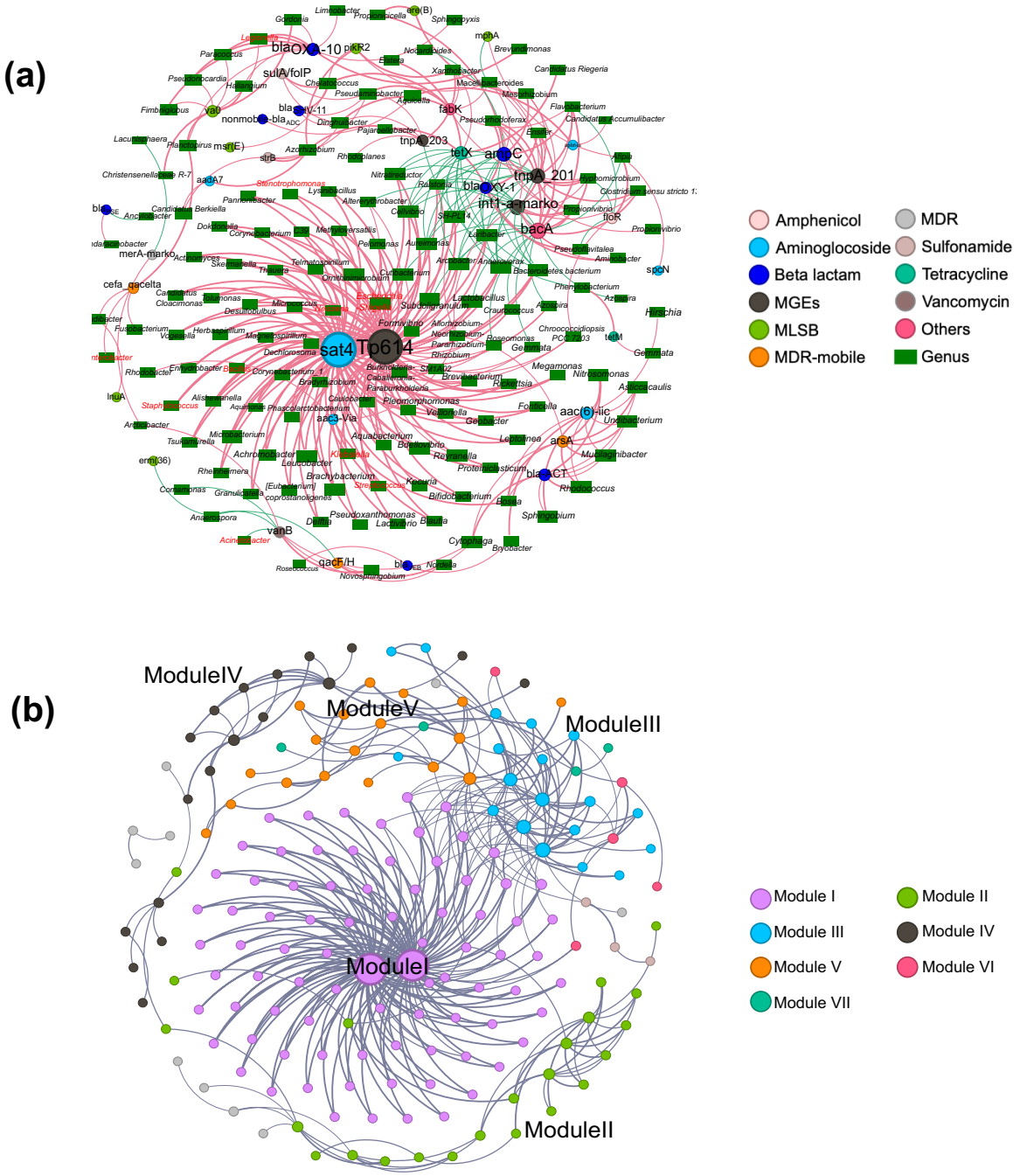

Fig. 5 **a** Co-occurrence analysis between bacterial communities (genus-level) and ARGs/MGEs in gynecology department with nine samples. Circle colors represent ARGs and MGEs types, and dark-green boxes represent the genera. Line colors mean positive (red) or negative (blue) spearman correlation. Font/node size represents the degree of connections. Font color with red represents the concerned genera. Line width represents the absolute value of the correlation index, i.e. the wider the line shows, the bigger the absolute value is. **b** the same network as Fig.(a), while the node colors here represent the Module classes

the beta-lactam-resistance gene *blaOXA*$_{10}$ and the MLSB-resistance gene *va0*. Another notable discovery affirmed a correlation between *Enterobacter* (*ESKAPE*-related) and the MDR *cefa_qacelta*, pinpointing the association between this genus and multidrug resistance. What is more, *Acinetobacter* (*ESKAPE*-related) was acknowledged to be co-occurring with vancomycin-resistant *vanB*, of which the presence has been known to signify a heightened level of resistance to vancomycin (Luqman et al. 2024). Of particular concern were the correlations observed between several genera, including *Bacillus*, *Escherichia-Shigella*, *Klebsiella, Neisseria*, *Staphylococcus*, *Stenotrophomonas*, *Streptococcus*, and

sat4/Tp614. Noticeably, the network presents that *Sandaracinobacter* and *Azorhizobium* had a close loop cycle with bla_{PSE}. This implicates that bla_{PSE} was the low-risk ARG that might not transfer in the gynecology department.

In summary, the network analysis reveals the modular structure of the gynecology department's bacterial community, with specific ARGs, MGE genes, and bacterial taxa–forming distinct modules. MGEs played a prominent role in facilitating the dissemination of ARGs, indicating the potential for HGT among different microbial organisms. While the majority of ARGs showed correlations with a wide range of microbes, others such as bla_{PSE} were exclusively associated with specific bacterial taxa, highlighting the immobility and conservation of certain ARGs.

Discussion

The department-specific dynamic pattern within the hospital indoor environment was initially identified through the seasonal impact on the bacterial composition. The PCA clusters separated by early summer, late summer, and winter suggest that the seasonal change significantly influenced the bacterial structure within the overall indoor hospital environment. The previous research has reported that temperature and humidity can positively influence microbial diversity (Perencevich et al. 2008; Zhou et al. 2016). The higher temperatures and increased humidity experienced during the summer months create favourable conditions for microbial growth and activity. According to the meteorological information of China Meteorological Administration, in the specific case of the studied hospital in Shenzhen city, the temperature at the collection points during the summer season, when the air conditioners are in operation, was approximately 32 °C. However, it is worth noting that the early summer period may experience even higher temperatures, reaching over 34 °C. During this time, the relative humidity level exceeds 50%. As the summer progresses into the late season, the temperature and relative humidity levels tend to moderate. The temperature during this period ranges around 28 to 30 °C, and the relative humidity is over 40%. In contrast, during the winter season when air conditioners are typically not in use at the collection points, the temperature remains approximately 15 °C. The relative humidity during this time is above 20%. Certainly, the consistent observation of such seasonal impact in each department is noteworthy. Despite the fact that bacteria exhibit varying mechanisms of colonization, interaction, and evolution across different departments due to the presence of diverse ecological niches (e.g., air, water, human bodies, and medical equipment), as well as their distinct responses to different stressors like antibiotics (Ben Maamar et al. 2020), along with other factors in the hospital indoor environment such as high occupancy rates and frequent patient movement, the substantial impact of seasonal fluctuations on shaping the bacterial composition remains prominent. Moreover, by tracking the contamination source of communities, it was uncovered that a substantial fraction of bacterial flow among the seven departments could be traced back to the gynecology department, accounting for 21.6% of the bacterial communities identified in the "patient sink" area of the blood test room.

The department-specific dynamic pattern was also evident in the correlation between the abundance of ARGs and MGEs genes. Throughout the entire indoor environment of the hospital, the widespread mobility of ARGs was demonstrated by a moderate correlation between the abundance of ARGs and MGEs. As indicated by the presence of common ARGs and MGE genes, on the one hand, this may suggest the presence of a common pool of genes circulating within the hospital. Alternatively, this may be caused by the common communities across the departments. During the circulation between water and air dust mediums across seven departments, the largest proportions of ARGs and MGE genes were assumed to originate from the gynecology department and outdoor sources, eventually accumulating in the blood test room. Similar correlation between ARGs and MGEs has also been observed in studies focusing on hospital aerosols and wastewater (Wu et al. 2022a, b; Markkanen et al. 2023; Jiao et al. 2023). However, the ARGs' mobility was not witnessed in the blood test room, ophthalmology, and gynecology departments as reflected by the insignificant results of the Mantel test. Therefore, it is estimated that the association between ARGs and MGEs varied across different departments within the hospital. This was presumably caused by different mechanisms of horizontal transfer and the driving forces (e.g., antibiotic stresses, bacteria loads, and the occupant's rate) behind the circulation of ARGs among departments.

The last department-specific pattern was revealed in the correlation between bacterial communities and ARGs/MGEs. Intriguingly, a significant correlation was exclusively found in the gynecology department, of which the network analysis raises concerns about the presence and potential dissemination of pathogens correlated with certain ARGs, such as *Legionella* spp. linked with beta-lactam-resistance $blaOXA_{10}$ and MLSB-resistance *va0*, which other studies have not reported. Currently, beta-lactam, fluoroquinolones, macrolides, and rifampicin are reported as the active antibiotics to which *Legionella* spp. are susceptible (Nimmo and Bull 1995; Sharaby et al. 2019; Pappa et al. 2020). Implicitly, the resistance against beta-lactam in our study might prompt apprehension for the inactive therapy of antimicrobials such as amoxicillin that belongs to beta-lactam to legionellosis patients. Additionally, as the $blaOXA_{10}$ is a frequently encountered ARG capable of HGT in hospitals (Golshani and Sharifzadeh 2013), the presence of beta-lactam antibiotics in

the environment may stimulate the evolution of microbial resistance mechanisms (D'Costa et al. 2006). Therefore, the potential inefficacy of beta-lactam pharmacotherapy is particularly significant and highlights the importance of considering *Legionella* spp. which colonize various man-made water systems and may be exposed to antimicrobial agents from different sources, including those produced by other microorganisms (Almahmoud et al. 2009). Besides, the remarkable co-occurrence between *Acinetobacter* and *vanB* posed alarming challenges. As suggested by Chang et al. (2003), this resistance may have been acquired through the acquisition of *vanB* from vancomycin-resistant *enterococci*. Worse, while vancomycin-containing regimens have been reported to offer therapeutic benefits against infections caused by colistin (peptide)-resistant *A. baumannii*, the emergence of this co-occurrence between *Acinetobacter* and *vanB* implies hidden treatment failures in corresponding infections in the future. Another concerned correlation was between multiple genera (*Bacillus*, *Escherichia-Shigella*, *Klebsiella*, *Neisseria*, *Staphylococcus*, *Stenotrophomonas*, and *Streptococcus*) and *sat4/Tp614*. *Tp614* represents the signatures of various genetic elements involved in gene transfer and recombination. As a result, it is reasonable to estimate that *Tp614* would carry *sat4* transferred in these pathogenesis-related genera, which potentially impeded the antimicrobial treatment to the related infections. A similar pattern was shown by Enany and Alexander (2017) that transposase *Tn5404* carried genes of *sat4* in the *S. aureus* that were spread in the bacterial populations. Actually, *sat4* coding for aminoglycoside resistance is frequently found in clinical and urban wastewater carried by clinical strains (Zaheer et al. 2020), and the risk always arises when *sat4* forms the cluster of *aadE/ant(6)-la-sat4-aph(3')-IIIa*, which is commonly associated with insertion elements from *Tn5405* transposons (Zaheer et al. 2020). Taken together, the network profile underscores the need for careful surveillance and monitoring of antibiotic (e.g., vancomycin) resistance, particularly in *Acinetobacter* and *Legionella* spp. in man-made water systems. The findings also show the importance of monitoring and understanding the genetic elements such as *Tp614* involved in gene transfer and recombination, as well as their impact on antimicrobial treatment efficacy.

In conclusion, hospital indoor environments are indeed dynamic environments that harbor a diverse range of bacteria derived from both the surrounding environment and the individuals present. These factors play a critical role in determining the infectious risk faced by patients within these facilities. Considering that the diverse ecological interactions and conditions within different hospital sites have significant clinical implications, this study yields noteworthy findings by exploring the department-specific variations, correlations and source tracing of bacterial community, ARGs, and MGEs within the hospital indoor environment. These department-specific dynamics are crucial for implementing targeted interventions and infection control measures. By recognizing the unique characteristics and contributions of each department, strategies can be developed to minimize the dissemination of antibiotic resistance and the transmission of bacteria within the hospital, leading to improved patient safety and reduced healthcare-associated infections.

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Data availability The *16S rRNA* amplicon sequencing data has been submitted to China National GeneBank (CNGB). The project ID is CNP0005378.

Declarations

Ethics approval and consent to participate This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication All authors consent to the publication of this manuscript.

Competing interests The authors declare no competing interests.

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