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Co-selection of antibiotic and disinfectant resistance in environmental bacteria: Health implications and mitigation strategies

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A R T I C L E I N F O

Keywords:
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A B S T R A C T

Background: The rapid emergence of co-selection between antimicrobials, including antibiotics and disinfectants, presents a significant challenge to healthcare systems. This phenomenon exacerbates contamination risks and limits the effectiveness of strategies to combat antibiotic resistance in clinical settings. This study aimed to investigate the prevalence and characteristics of bacteria in hospital environments that exhibit co-selection mechanisms and their potential implications for patient health, framed within the One Health perspective.
Methods: Air and surface samples were collected from seven large hospitals and analyzed to detect antibiotic-resistant bacteria (ARB). The resistance profiles of isolated ARB to various disinfectants were determined. Bacterial species were identified using 16S rRNA gene sequencing, and the presence of antibiotic resistance genes (ARGs) and class 1 integrons (*intI*1) was investigated.
Results: A high percentage (85%) of samples contained ARB, with β-lactam resistance being the most frequently observed. Alarmingly, 94% of isolated ARB exhibited resistance to at least one disinfectant, and 91% demonstrated resistance to three or more disinfectants. *Staphylococcus* and *Bacillus* emerged as the dominant genera displaying co-selection. The presence of ARGs, including *mec*A (associated with methicillin resistance) and *qac*B (associated with disinfectant resistance), along with *intI*1, provided further evidence supporting co-selection mechanisms.
Conclusion: These findings underscore the critical need for robust antimicrobial resistance surveillance and the prudent use of disinfectants in healthcare settings. Further research into co-selection mechanisms is essential to inform the development of effective infection control strategies and minimize the spread of resistant bacteria.

Abbreviations

ARB	Antibiotic Resistant Bacteria
AMR	Antimicrobial Resistance
ARGs	Antibiotic Resistance Genes
CLSI	Clinical And Laboratory Standards Institute
HAIs	Healthcare-associated Infections
HP	Hydrogen Peroxide
ICUs	Intensive Care Units
*intI*1	Class 1 integrons
MAR	Multi-Antimicrobial Resistant
*mec*A	Associated With Methicillin Resistance
PBS	Phosphate Buffer Saline
*qac*B	Associated With Disinfectant Resistance
QACs	Quaternary Ammonium Compounds
SH	Sodium Hypochlorite
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
WHO	World Health Organization

1. Introduction

Antibiotic resistance has emerged as a global health crisis, significantly hindering the treatment of infections, particularly in hospitals (Maki and Zervos, 2021; United Nations Environment Programme, 2023). In the European Union alone, antibiotic-resistant bacteria (ARB) caused 671,689 infections in 2015, with 63% being nosocomial, resulting in an estimated 33,110 deaths and 874,541 disability-adjusted

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life years (DALYs) (Cassini et al., 2019). The rise of ARB has drastically complicated the management of life-threatening infections, increasing morbidity, mortality, and economic burden due to prolonged hospital stays (Maki and Zervos, 2021; United Nations Environment Programme, 2023). Without effective intervention, projections estimate that drug-resistant infections will cause 10 million deaths annually by 2050 (Haenni et al., 2022) and inflict over $100 trillion in economic losses on low- and middle-income countries over the next three decades (Basiry et al., 2022). Consequently, limiting the spread of ARB and associated infections, particularly in healthcare settings, has become a paramount public health priority (Haenni et al., 2022; United Nations Environment Programme, 2023).

Among the critical factors in curbing ARB is addressing the infectious agents and transmission routes responsible for healthcare-associated infections (HAIs). Eliminating microbial agents and restricting the transmission of organisms within hospital environments are vital for breaking the chain of infection (World Health Organization, 2002). Disinfectants, widely regarded as essential tools in infection control, play a central role in reducing the microbial load in healthcare settings. However, an emerging concern is the increasing prevalence of bacteria resistant to both disinfectants and antibiotics, referred to as multi-antimicrobial resistant (MAR) bacteria (van Dijk and Verbrugh, 2022; Krewing et al., 2024). The emergence of cross- and co-resistance among antimicrobials poses a severe threat to healthcare systems, potentially exacerbating contamination and limiting available strategies to combat ARB in clinical settings (United Nations Environment Programme, 2023; Krewing et al., 2024). Cross-resistance arises when bacterial genes conferring antibiotic resistance also mediate resistance to disinfectants, such as through efflux pump mechanisms. However, co-resistance involves the co-localization of genes conferring resistance to distinct antimicrobial agents, such as β-lactams and quaternary ammonium compounds, within the same genetic element (Basiry et al., 2022; Murray et al., 2024). A review of outbreaks linked to HAIs found that, in cases where the susceptibility of infectious agents to disinfectants was assessed, the pathogens were frequently highly resistant to the disinfectants (van Dijk and Verbrugh, 2022). This underscores the significant challenge posed by pathogenic or opportunistic bacteria demonstrating simultaneous resistance to both antibiotics and disinfectants, which complicates the treatment and management of HAIs (United Nations Environment Programme, 2023).

Sublethal exposure to disinfectants, as well as the misuse or overuse of antimicrobial agents, has been identified as a key driver of bacterial stress responses, promoting antimicrobial resistance (AMR) (Basiry et al., 2022; van Dijk and Verbrugh, 2022, United Nations Environment Programme, 2023; Boyce, 2023). It is essential to recognize that the intensive and indiscriminate use of disinfectants, particularly in healthcare settings during the current COVID-19 pandemic, has raised concerns about the emergence of MAR bacteria (Chen et al., 2021; van Dijk and Verbrugh, 2022; Boyce, 2023). In response, national and international authorities have developed action plans under the One Health approach to mitigate AMR (Haenni et al., 2022; United Nations Environment Programme, 2023). Within this framework, prevention plays a central role, with environmental monitoring in healthcare settings serving as a crucial component of integrated surveillance for antimicrobial resistance. Such monitoring enables the identification and control of potential sources of antimicrobial resistant infections, helping to mitigate their spread (Haenni et al., 2022; United Nations Environment Programme, 2023; Murray et al., 2024). However, addressing antibiotic resistance alone may fall short of achieving the desired outcomes without a deeper understanding of co-selective agents, particularly in hospital environments (United Nations Environment Programme, 2023). While antibiotic resistance is widely recognized as a major health threat, resistance to disinfectants has received comparatively little attention, especially in human healthcare settings (van Dijk and Verbrugh, 2022). Recent studies have explored the co-selection of resistance to biocides and antibiotics in the food and veterinary sectors (Romero et al., 2017; Al-Mustapha et al., 2022; Enany et al., 2023; Wu-Chen et al., 2023), but data on this phenomenon in hospital environments remain scarce. Additionally, existing hospital-based studies on co-selection have primarily focused on specific causative agents of HAIs, such as *Staphylococcus* and *Acinetobacter* (Martro et al., 2003; Sekiguchi et al., 2004; Reichel et al., 2014; Tarashi et al., 2022; Pallós et al., 2024). Consequently, critical knowledge gaps remain regarding the co-selection of resistance to antimicrobial agents among hospital bacteria. This gap underscores the urgent need for research to clarify the role of hospital environments in the transmission and dissemination of antimicrobial-resistant pathogens responsible for HAIs. Our study aims to address these gaps by providing novel insights into the co-selection of resistance to clinically relevant antibiotics and commonly used disinfectants in bacterial strains isolated from hospital environments. To the best of our knowledge, this is the first study to comprehensively evaluate the efficacy of disinfectants against ARB isolated from hospital settings and to explore the potential role of MAR bacteria in the transmission and persistence of HAIs.

2. Methods

2.1. Sampling

The study was conducted in seven large hospitals. Table S1 (supplementary file) provides details about the characteristics of these hospitals. A total of 61 samples were collected from the air and surfaces within the intensive care units (ICUs) of these hospitals. Air sampling was conducted twice in each ICU using an all-glass impinger containing phosphate-buffered saline (PBS). To simulate the respiratory zone, sampling was conducted at a height of 1.5 m above from the ground level and approximately 2400 L of air were collected using a portable pump during a 4- hour period.

For surface sampling, a single sample was collected from each ICU by swabbing frequently touched surfaces, such as patient beds and tray tables. Sterile Dacron swabs pre-wetted with PBS were used to ensure effective collection. Each swab covered a surface area of up to 400 cm^2 and was placed in tubes containing 3 mL of PBS. Samples were stored in a cold box and immediately transferred to the laboratory for microbial analysis. To detach the collected cells from the swabs, the samples were subjected to vigorous vortexing for 15 s, followed by sonication for 180 s. Afterward, the swab was removed, and the resulting PBS suspensions were utilized for microbial analysis.

2.2. Detection of ARB

To detect ARB, aliquots from each impinger and surface collection tube medium were thoroughly shaken before being plated onto tryptic soy agar (TSA) supplemented with antibiotics. Four different antibiotics were utilized in this study: ampicillin and ceftazidime from the β-lactam class, as well as tetracycline and gentamicin representing the tetracycline and aminoglycoside classes, respectively. Antibiotic concentrations were 32, 16, 32 and 16 mg/L, respectively, following the recommendations of the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2021). In parallel, aliquots of each sample were plated onto TSA without any antibiotic. All plates were incubated at 35 °C for 2–3 days. The quality of the media and antibiotics was evaluated using specific bacterial strains, including *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923), following the recommendations of the CLSI (CLSI, 2021). All experiments were conducted in duplicate. Colonies that grew on antibiotic-supplemented culture media were subjected to isolation and resistance confirmation by re-culturing them on TSA containing the respective antibiotics.

2.3. Susceptibility/resistance to disinfectants

This study evaluated the resistance of isolated ARB to commercially available disinfectants commonly used in hospital environments. These disinfectants contained active agents such as quaternary ammonium compounds (QACs), alcohol, biguanides, chlorine, and hydrogen peroxide. The Kirby-Bauer disk diffusion method was utilized to assess the inhibitory effects of nine hospital disinfectants, including sodium hypochlorite (SH) at 1% and 2% concentrations, hydrogen peroxide (HP), and various commercial products labeled as SS, ST, IS, VS, SHI, and SHP, against isolated ARB. Detailed characteristics of these disinfectants are provided in Table S2. To conduct the experiment, a concentration equivalent to 0.5 McFarland standard (approximately 1×10^6 CFU/mL) were prepared from ARB isolates in tryptic soy broth (TSB). Then, a homogenous bacterial suspension (approximately 200 μL) was streaked onto Mueller-Hinton Agar using a sterile swab. Next, sterile paper disks (6 mm in diameter) were impregnated with each of the selected disinfectants and placed onto culture agar plates that had been pre-adsorbed with bacterial cells. The plates were then incubated at 35 °C for 18–24 h. As controls, sterile distilled water and phenol were used as negative and positive controls, respectively. The experiments were conducted in duplicate, and the average zone diameters of growth inhibition were determined for each ARB isolate. The results were interpreted as follows: Resistant (≤14 mm), Intermediate (15–19 mm), and Susceptible (≥20 mm) (Khan et al., 2016).

2.4. Molecular identification of antimicrobial resistant bacteria

Genomic DNA was extracted from disinfectant-resistant ARB isolates that demonstrated resistance to at least three disinfectants. In the initial step, the presence of *Staphylococcus* spp., *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* among the disinfectant-resistant ARB isolates was confirmed via polymerase chain reaction (PCR) using specific primer sets, as previously described (Gholipour et al., 2024). For isolates that were not identified, as well as a subset of identified isolates, the extracted DNA was subjected to PCR amplification using Eubac 27F and 1492R primers (Farhadkhani et al., 2014). These primers target an approximately 1420-bp fragment of the 16S rRNA gene, a widely used marker for bacterial species identification. Following amplification, the gene sequencing was performed and DNA sequences were analyzed using BLAST algorithms and databases from the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences of the identified bacteria were deposited in GenBank under accession numbers PP922288-PP922324.

2.5. Detection of antibiotic resistance genes in the isolated ARB

Disinfectant-resistant ARB isolates were screened for the presence of seven common antibiotic resistance genes (*bla*$_{TEM}$, *bla*$_{CTX-M}$, *mec*A, *tet*W, *aac*(6')-Ib-cr, and *sul*1) and one disinfectant-resistance encoding gene (*qac*B) using PCR, as presented in Table S3 (supplementary file). Additionally, the isolates were analyzed for the presence of the class 1 integrons (*intI*1), a genetic element that can harbor many ARGs.

3. Results and discussion

There is a growing concern globally about the increasing prevalence of MAR bacteria especially in environments which overuse or misuse of disinfectants could confer co-selection of resistance to both disinfectants and antibiotics (van Dijk and Verbrugh, 2022; (United Nations Environment Programme, 2023)).

3.1. Detection of ARB in ICUs environment

Samples collected from the air and surfaces of seven hospital ICUs, revealed that 85% (52 out of 61) of the samples tested positive for the presence of ARB (Fig. 1). The frequency of ARB-positive samples among the hospitals ranged from 67% to 100%, with air samples exhibiting a higher detection rate compared to surface samples. Hospitals No. 3, 5, and 6 demonstrated the highest frequency of ARB-positive samples, whereas Hospital No. 7 had the lowest detection rate (Fig. 1). These findings align with other research that has reported the presence of ARB in hospital environments, particularly in bioaerosols (Gwenzi et al., 2022; Mirhoseini et al., 2016). Bacteria can become airborne in hospitals through various means, such as sneezing, coughing, speaking, linen shaking, and water shower sprays. Due to their size, bioaerosols can remain suspended in the air for extended periods or travel short distances when attached to droplets or dust particles (particles larger than 5 μm), eventually settling on surfaces (Beggs, 2003).

The highest resistance was observed against ampicillin (74%), followed by ceftazidime (67%), gentamicin (30%), and tetracycline (25%) (Fig. 1). The findings indicate a relatively high prevalence of β-lactam resistance across all ICU samples. Notably, no tetracycline resistance was detected in samples from three hospitals, and gentamicin resistance was absent in samples collected from Hospital No. 7. Bacteria present in hospital air, especially those resistant to antibiotics, play a crucial role in the transmission of HAIs, either through airborne transmission or

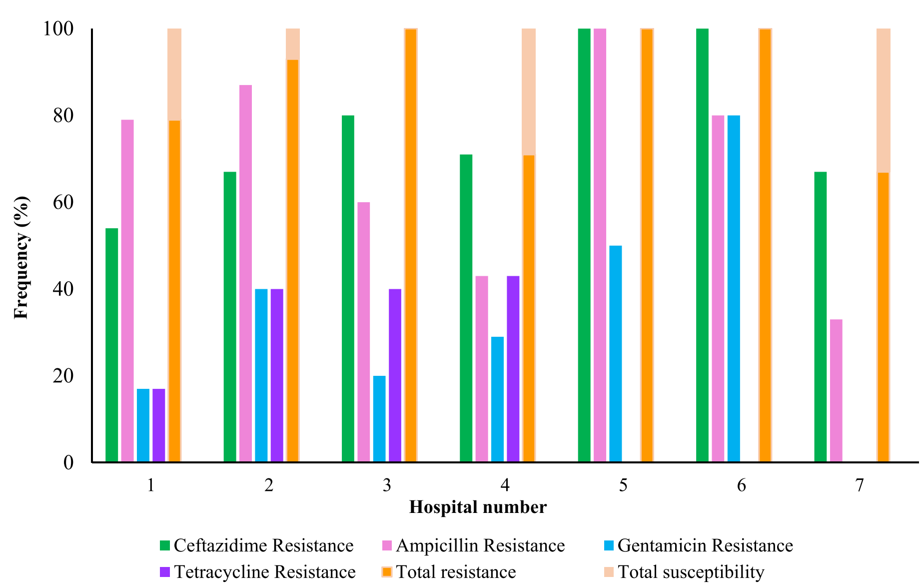

Fig. 1. Frequency of positive samples for the presence of resistant bacteria in hospitals.

indirectly when they settle on surfaces. Contaminated surfaces in hospital environments serve as significant sources for the transmission of HAIs. Therefore, limiting the transmission of organisms through the hospital environment is a critical factor in breaking the infection chain (World Health Organization, 2002; Blot et al., 2022). It is noteworthy that a substantial portion of the samples exhibited resistance to more than one antibiotic. Alarmingly, 26% of the samples were resistant to three different classes of antibiotics. The high frequency of ARB-positive samples, particularly those resistant to β-lactam antibiotics, underscores the potential role of hospital environments in the dissemination of ARB. The incidence of HAIs in ICU patients is reported to be up to 30%. These patients, with underlying diseases and weakened immune levels due to specific therapeutic interventions, are at an increased risk of acquiring hospital infections, especially those caused by resistant bacteria (Blot et al., 2022). Additionally, it's important to note that although ARB may not opportunistic, they have the ability to transfer resistance genes to pathogenic bacteria, especially on surfaces where biofilms may form (Gwenzi et al., 2022).

Out of the 61 environmental samples collected from ICUs, 174 colonies of ARB were isolated. Among these, 105 colonies were from the air and 69 colonies were from surfaces. The highest frequency of resistance was observed in β-lactams-resistant bacteria, including ceftazidime-resistant (38%) and ampicillin-resistant (35%), followed by gentamicin-resistant bacteria (16%) (Fig. 2). Tetracycline-resistant bacteria were the least frequently detected, with a prevalence of 11%, and were entirely absent in samples from three hospitals (Fig. 2). These findings are consistent with previous studies reporting a high prevalence of β-lactams-resistant bacteria in hospital environments (Aali et al., 2014; Mirhoseini et al., 2016; Chaoui et al., 2019; Gwenzi et al., 2022; Lee and Yoo, 2022; Mehdipour et al., 2023; Gholipour et al., 2024). The frequency of detection of specific ARB is likely influenced by the se-lective pressure exerted by antimicrobials commonly used in hospitals. β-lactam antibiotics, which account for approximately 50%–70% of total antibiotic use in many countries, create significant selective pressure on bacterial populations. This widespread usage contributes to the emer-gence of extended-spectrum β-lactamase (ESBL)-producing and multidrug-resistant strains. Notably, resistance to β-lactam antibiotics in pathogenic or opportunistic bacteria often correlates with resistance to other antibiotic classes, further complicating treatment options (Tiwari et al., 2022; World Health Organization, 2018). β-lactam antibiotics are often regarded as the last line of defense for treating severe bacterial infections, including urinary tract infections, bloodstream infections, wound infections, and pneumonia. Consequently, resistance to this critical class of antibiotics represents a serious threat to clinical patient outcomes (Tooke et al., 2019). Our results also showed the detection of gentamicin-resistant bacteria (16%) in hospital environments, consistent with other studies (Gwenzi et al., 2022; Mehdipour et al., 2023). Although the use of aminoglycosides, such as gentamicin, is relatively limited in the general population, their application in hospital settings and veterinary medicine, combined with the presence of aminoglycoside resistance genes in environmental matrices, may account for the detection of these ARB in hospital environments.

3.2. Resistance of ARB to disinfectants

Disinfectants are antimicrobial agents designed for application on non-living surfaces to eliminate microorganisms effectively. Unlike antibiotics, they typically do not target specific microbes, making them suitable for general-purpose applications but less effective against resistant microorganisms (Krewing et al., 2024). Several studies have reported resistance of certain microorganisms to various types of disinfectants (Martro et al., 2003; Sekiguchi et al., 2004; Reichel et al., 2014; Tarashi et al., 2022; Pallós et al., 2024). In our study, out of 174 ARB isolates, 94% showed resistance to at least one disinfectant, and 91% were resistant to three or more disinfectants (Fig. 3a). Furthermore, 14% of the isolates were resistant to all types of disinfectants tested. As depicted in Fig. 3b, the highest resistance among the isolates was to sodium hypochlorite 1% (78%), followed by SH 2% (73%) and SS (70%). The highest sensitivity was to hydrogen peroxide (66%), fol-lowed by VS (57%) and IS (32%). Although SS, VS, and IS share similar compositions, primarily consisting of alcohol and QACs, the high resis-tance of ARB to SS may be attributed to differences in the concentrations of active agents within the disinfectant formulation. Research has demonstrated that bacterial exposure to subinhibitory concentrations of QACs can lead to increased minimum inhibitory concentrations, often driven by the upregulation of QAC efflux pumps (United Nations Envi-ronment Programme, 2023; Boyce, 2023). A study by Awodele et al. (2007) found that 38% of ARB exhibited resistance to quaternary ammonium compounds.

On the other hand, the substantial increase in the use of sodium hypochlorite in healthcare settings and the community during the COVID-19 pandemic raises concerns about the potential development of resistance to this disinfectant due to overuse (van Dijk and Verbrugh, 2022; United Nations Environment Programme, 2023). As shown in Table 1, the most resistant ARB to disinfectants were spore-forming bacteria, such as *Bacillus* species. It is well-documented that spore-forming bacteria and mycobacteria exhibit intrinsic resistance to disinfectants and antiseptics, primarily due to their complex cell wall structures. Additionally, Gram-negative bacteria are generally less sus-ceptible than Gram-positive bacteria to

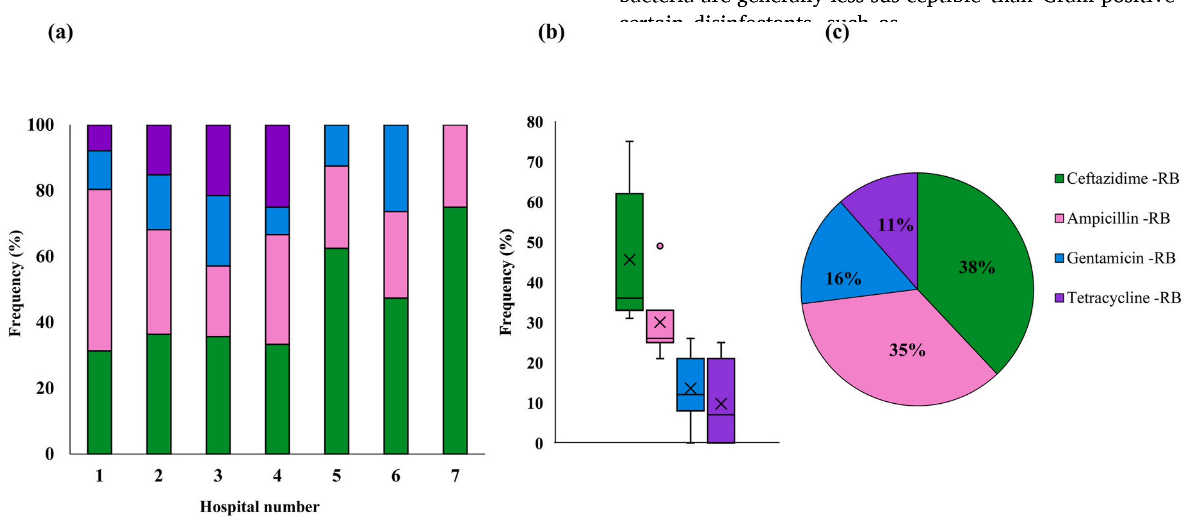

Fig. 2. Prevalence of various antibiotic-resistant isolates (%) in hospital environment samples (RB: Resistant Bacteria).

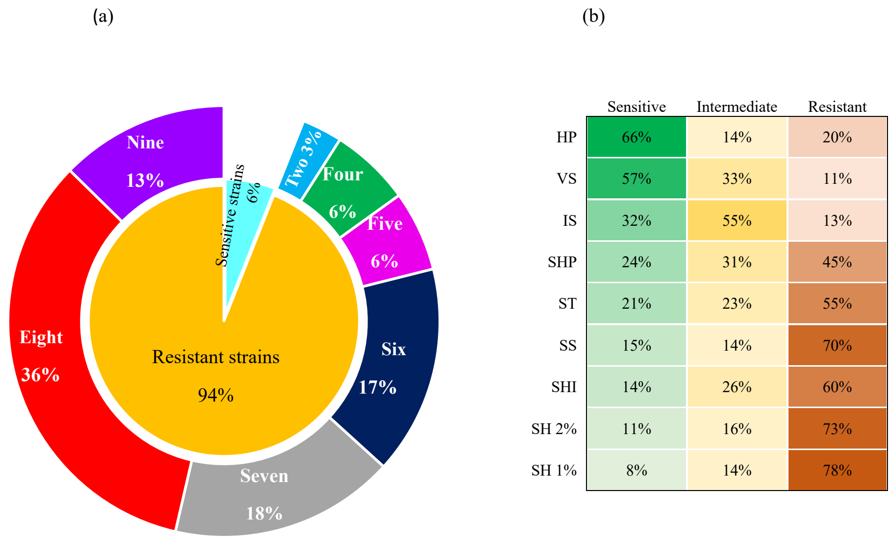

Fig. 3. Resistance of antibiotic-resistant bacteria to disinfectants (a) Frequency distribution of ARB exhibiting multi-disinfectant resistance (in the accompanying pie chart, alphabetic numbers indicate the count of disinfectants rendered ineffective). (b) Frequency distribution of ARB categorized as resistant, intermediate, or sensitive for each disinfectant.

Table 1
Characteristics of antimicrobial-resistant bacteria isolated from hospitals environments.

Identified species	Class	Characteristics	Clinical Significance
Brevundimonas spp.	Alphaproteobacteria	Gram-negative, non-fermenting bacilli	Some strains can cause opportunistic infections
Roseomonas cervicalis	Alphaproteobacteria	Gram-negative, non-fermenting coccobacilli	pathogenic for humans and can cause urogenital or eye infections
Acinetobacter junii	Gammaproteobacteria	Gram-negative, non-fermenting bacilli	Can cause hospital-acquired infections including catheter-related blood stream infections and cellulitis
Acinetobacter baumannii	Gammaproteobacteria	Gram-negative, non-fermenting bacilli	Multidrug-resistant pathogen causing difficult-to-treat hospital infections
Acinetobacter lwoffii	Gammaproteobacteria	Gram-negative, non-fermenting bacilli	Can cause opportunistic infections in immunocompromised individuals
Pseudomonas spp.	Gammaproteobacteria	Gram-negative, non-fermenting bacilli	Except for *Pseudomonas aeruginosa*, are generally not considered opportunistic pathogens
Pseudomonas aeruginosa	Gammaproteobacteria	Gram-negative, non-fermenting bacilli	Can cause hospital-acquired infections like pneumonia and urinary tract infections
Chryseobacterium taklimakanense	Flavobacteriia	Gram-negative bacilli	some strains can cause opportunistic infections in immunocompromised individuals
Staphylococcus spp.	Bacilli	Gram-positive, non-spore forming cocci	Some species can cause opportunistic infections
Staphylococcus aureus	Bacilli	Gram-positive, non-spore forming cocci	Common skin and nasal flora, can cause a variety of infections including skin infections, pneumonia, and food poisoning
Bacillus licheniformis	Bacilli	Gram-positive, spore forming bacilli	can cause infection in immunocompromised individuals
Bacillus paralicheniformis	Bacilli	Gram-positive, spore forming bacilli	Not typically associated with human disease
Paenibacillus lactis	Bacilli	Gram-positive, spore forming bacilli	Not typically associated with human disease
Brevibacillus borstelensis	Bacilli	Gram-positive, spore forming bacilli	Not typically associated with human disease
Priestia megaterium	Bacilli	Gram-positive, spore forming bacilli	Not known to cause human disease
Siminovitchia fordi	Bacilli	Gram-positive, spore forming bacilli	Not typically associated with human disease
Planomicrobium spp.	Bacilli	Gram-positive cocci	Not typically associated with human disease
Aeromicrobium spp.	Actinomycetia	Gram-positive, non-spore forming	Typically not considered pathogenic
Brachybacterium spp.	Actinomycetia	Gram-positive, non-spore forming	Rarely causes human disease
Microbacterium petrolearium	Actinomycetia	Gram-positive, non-spore forming	Not known to cause human disease

QACs, due to their outer membrane, which can impede biocides from reaching their target sites. *Pseudomonas aeruginosa* serves as a notable example of Gram-negative bacteria with intrinsic resistance to QACs (Boyce, 2023; Chen et al., 2021). On the other hand, the presence of bacteria on surfaces, such as walls, floors, or medical devices, often in the form of biofilms, significantly reduces the effectiveness of disinfectants, even when applied at high concentrations (Haenni et al., 2022; Boyce, 2023). However, co-selection may contribute as an important factor in the simultaneous resistance to antibiotics and disinfectants in hospital environment bacteria.

3.3. Co-selection of resistance

Co-selection refers to the simultaneous selection for resistance to multiple agents, such as antibiotics and disinfectants. It can occur through two main mechanisms: 1) cross-resistance which happens when a single mechanism provides resistance to two or more dissimilar agents. For instance, drug efflux pumps can remove multiple agents from bac-terial cells, or changes in cell membrane structure can affect suscepti-bility to both antibiotics and disinfectants. 2) co-resistance which occurs when multiple resistance genes are genetically linked. The presence of integron genes, such as *intI*1, exemplifies co-resistance (Zheng et al., 2020; Shamsizadeh et al., 2021; Boyce, 2023; Murray et al., 2024; Mpongwana et al., 2024). Our study revealed that 94% of the isolated ARB exhibited resistance to disinfectants, underscoring the significant risk associated with co-selection mechanisms in hospital environments. These mechanisms often function by either reducing the effective con-centration of microbicides that the organism encounters or enabling the bacteria to evade the action of the compound entirely (Boyce, 2023). As shown in Fig. 4 simultaneous resistance to antibiotics and disinfectants was observed for all groups of ARB. However, because of more fre-quency detection of β-lactams-resistant bacteria, resistance of this group of ARB to disinfectants is a concern for healthcare settings. It has been reported that QACs and the biguanide chlorhexidine can promote cross-resistance by inducing the expression of non-specific efflux pumps, potentially explaining the resistance of ARB to the disinfectants analyzed in this study (Basiry et al., 2022; Krewing et al., 2024). A re-view of pathogens isolated from dairy farms also demonstrated cross-resistance between QACs and antibiotics such as penicillin and tetracycline in bacterial species including *Staphylococcus*, *Pseudomonas*, and *Klebsiella* (Mpongwana et al., 2024). Additionally, disinfectants based on oxidative agents, such as hydrogen peroxide and hypochlorite, have been reported to induce non-specific oxidative stress, which may lead to cross-resistance with antibiotics (Krewing et al., 2024). While our results indicated a high resistance of ARB to hypochlorite, low resistance to hydrogen peroxide was observed. This finding suggests that the hypothesis of oxidative stress-induced cross-resistance may not fully apply to the ARB isolates analyzed in our study. However, high resistance of β-lactams-resistant bacteria to chlorine may be attributed to selective pressures resulting from the widespread use of this antibiotic class and chlorine-based disinfectants in hospital settings, particularly during the COVID-19 pandemic (United Nations Environment Programme, 2023; Boyce, 2023). Additionally, sublethal concentrations of disinfectants such as chlorine and chloramine have been reported to facilitate the exchange of ARGs between bacteria (UN Environment Programme, 2023). The results of a study on biofilms of water distribution systems in healthcare settings showed co-selection to antibiotics and chlorine especially to β-lactam antibiotics. The authors suggested that the exposure of biofilm-associated bacteria to sublethal concentrations of free chlorine may have contributed to this co-selection within hospital water systems (Mehdipour et al., 2023). However, the rela-tionship between antibiotic resistance and disinfectant resistance re-mains highly complex, underscoring the need for further research to elucidate the underlying mechanisms (United Nations Environment Programme, 2023).

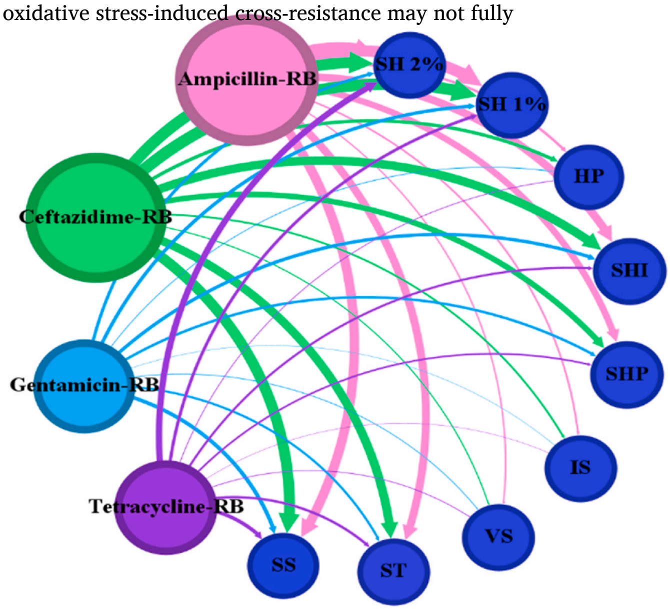

Fig. 4. Network analysis of resistance of ARB against different disinfectants (The size of nodes and the width of edges is related to the relative abundance of bacteria) (RB: Resistant Bacteria).

3.4. Characterization of species with co-selection

The presence of MAR bacteria in environment, particularly those that are opportunistic or pathogenic, poses a significant concern. Our molecular analysis of ARB that exhibited resistance to at least three disinfectant agents revealed the presence of Gram-positive bacteria, including Bacilli and Actinomycetia, as well as gamma- and alpha-proteobacteria (Table 1). Among the bacteria exhibiting co-selection, the dominant genera were *Staphylococcus*, followed by *Bacillus* (Fig. 5). While certain species of *Bacillus*, such as *B. pumilus* and *B. thuringiensis*, have been associated with human illness, *Staphylococcus* remains one of the most significant causative agents of HAIs (Blot et al., 2022). The high resistance of *Staphylococcus* to both antibiotics and disinfectants (Fig. 5a and b) may present an alarming situation for ICUs, posing a threat to vulnerable patients. Similarly, the relatively frequent detection of *Acinetobacter baumannii* exhibiting co-selection also is a concern for patient health. Additionally, certain species of non-fermenting, Gram-negative bacteria such as *Brevundimonas*, *Roseomonas*, and *Pseudomonas* are potential opportunistic pathogens. *Brevundimonas* spp. have been identified as causative agents of bacteremia in hospitalized patients (Fig. 5a and b) (Ryan and Pembroke, 2018).

The detection of these opportunistic bacterial pathogens in hospitals, along with their simultaneous resistance to antibiotics and disinfectants, raises health concerns, particularly for vulnerable populations. As illustrated in Fig. 5a and b, *Staphylococcus* exhibited resistance to all tested disinfectants and antibiotics, underscoring the significant chal-lenge in managing *Staphylococcus*-related infections in hospital envi-ronments. However, VS demonstrated relatively higher efficacy as a disinfectant against *Staphylococcus*. In contrast, VS was less effective against *Acinetobacter baumannii*. These findings emphasize the critical importance of antimicrobial surveillance for key causative agents of hospital infections, particularly when a specific bacterium is responsible for a high incidence of HAIs.

3.5. Detection of antibiotic resistance genes

In this study, we analyzed isolated ARB for a variety of ARGs, as well as the *intI*1 gene, a key indicator of resistance genes presence (Zheng et al., 2020; Nguyen et al., 2021). The results of this analysis are presented in Fig. S1 (Supplementary file). The ARGs analysis revealed that 9% of the isolates carried the *mec*A gene, all of which were *Staphylococcus* species, including *S. aureus*. The *intI*1 gene was observed in 12% of the isolates, with 9% of these also testing positive for the *sul*1 gene. Additionally, the *aac(6′-Ib-cr* gene, which confers resistance to aminoglycosides, was identified in 4% of the isolates, with 3% of these isolates harboring both the *aac(6′-Ib-cr* and *intI*1 genes. In three isolates confirmed as *Pseudomonas* and *Acinetobacter*, the *intI*1 gene was detected

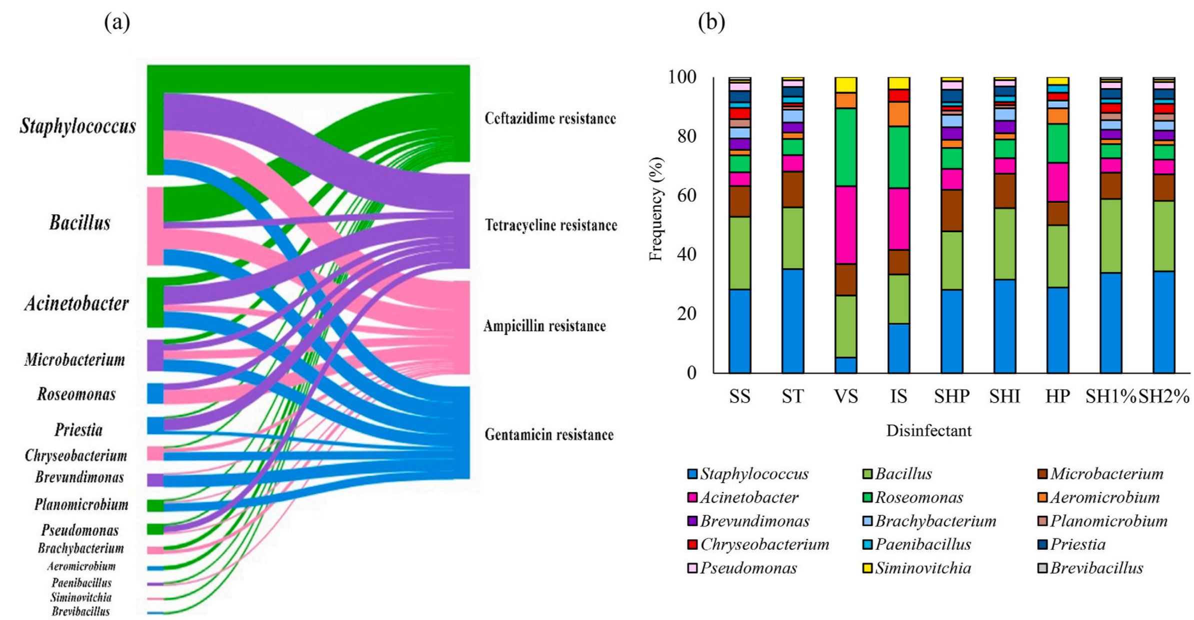

Fig. 5. Community structure of antimicrobial-resistant bacteria (genus level) (a) Antibiotic resistance profile of identified antimicrobial-resistant isolates (b) Relative abundance of identified antimicrobial-resistant isolates against different disinfectants (The size of curved lines is related to the relative abundance of bacteria) (RB: Resistant Bacteria).

alongside *aac(6')-Ib-cr* and *sul*1, indicating the potential presence of multidrug-resistant causative agents of HAIs in hospital environments. The *intI1* gene, known for its ability to accumulate diverse resistance mechanisms, may also contribute to ARB resistance to disinfectants through co-resistance (Zheng et al., 2020). Despite a high fraction of ARB showing resistance against β-lactam antibiotics, we did not identify the β-lactamase genes $bla_{CTX\text{-}M}$ and bla_{TEM}. Furthermore, *tet*W, associated with tetracycline resistance, was absent in all tetracycline-resistant isolates, suggesting other ARGs may play a role. Notably, the *qac*B gene, which confers resistance to disinfectants, was detected in half of the resistant *S. aureus* isolates, confirming the co-selectivity of these bacteria for both antibiotics and disinfectants. Furthermore, methicillin-resistant *S. aureus* (MRSA) strains were identified to harbor the *qac*B gene in combination with *intI1* and *sul*1, suggesting the presence of co-resistance mechanism in these bacteria (Basiry et al., 2022). However, in *S. aureus* isolates lacking the *qac*B gene, cross-resistance may explain their simultaneous resistance. These findings underscore the complex interplay of resistance genes and highlight the urgent need for further research to guide infection control strategies and antimicrobial management in healthcare settings.

3.6. Mitigating the risk of antimicrobial resistant bacteria in hospital environments

Eliminating microbial agents, particularly antibiotic-resistant bacteria, is a fundamental aspect of infection control, with the environment playing a critical role in this effort (Haenni et al., 2022; United Nations Environment Programme, 2023). Consequently, the detection of ARB with resistance to disinfectants in hospital environments underscores the urgent need to ensure hygienic conditions for patients by mitigating antimicrobial-resistant bacteria in these settings.

- The role of disinfectant resistance: The development of resistance to disinfectants is often associated with improper usage, particularly regarding the type and concentration of the disinfectant. Hospital environments host a diverse bacterial community with varying levels of susceptibility to these agents. Disinfection protocols must prioritize the inactivation of key causative agents of HAIs, especially in cases where a specific infection is prevalent.
- Factors influencing disinfectant effectiveness: Several factors can undermine the efficacy of disinfectants (van Dijk and Verbrugh, 2022), including:

Physical barriers: Microorganisms can persist in dirt, nooks, and crevices, where they may be exposed to suboptimal disinfectant concentrations. The WHO highlights that thorough cleaning of hospital environments, including the removal of dust, is a critical component in controlling HAIs (World Health Organization, 2002).

Organic matter: The presence of organic matter can significantly reduce disinfectant effectiveness by interfering with their action or causing oxidation.

Dissipation: Volatile disinfectants may dissipate rapidly, leading to reduced exposure time and diminished efficacy.

- Alternative strategies for microbial control: In scenarios where, disinfectant resistance poses a challenge, alternative microbial control methods should be considered. sterilization and UV radiation are highly effective against resistant bacteria, including spore-forming bacteria, and should be employed where feasible (World Health Organization, 2002).
- Improved Hygiene Practices: The WHO emphasizes the critical role of thorough cleaning in removing dust and potential sources of microbial contamination (World Health Organization, 2002).
- Addressing the challenges of AMR: The effective control and prevention of AMR necessitate a comprehensive, multi-faceted approach as follows:

Standardized resistance testing: Establishing standardized methods is essential for accurately identifying and quantifying resistance, multidrug resistance, and co-selection.

Evidence-based disinfection protocols: Disinfectant use should follow evidence-based guidelines, ensuring appropriate selection and concentration to maximize efficacy.

Transparency in biocide production: Manufacturers of biocides should be encouraged to provide accurate and detailed information on formulations and efficacy data to ensure responsible use.

- Addressing resource disparities: Low-income countries are disproportionately more impacted by AMR due to economic challenges, inadequate sanitation, and limited access to healthcare resources. To mitigate these disparities, the development of low-cost, accessible methods for monitoring and preventing AMR is essential (United Nations Environment Programme, 2023).
- Surveillance networks: Establishing local surveillance networks integrated with national and regional systems is critical for monitoring and comparing AMR prevalence. These networks enable the collection of actionable data to guide targeted interventions and evaluate the effectiveness of existing strategies.
- The impact of emerging infections: The emergence of infections like COVID-19 has underscored the importance of proper disinfectant use. During pandemics, the widespread application of disinfectants can drive co-selection mechanisms, further contributing to AMR. This highlights the necessity of careful management, monitoring, and evidence-based guidelines for disinfectant use to minimize unintended consequences.

4. Conclusions

This study identified a concerningly high frequency of ARB, including pathogenic strains, particularly those resistant to β-lactam antibiotics, within ICUs. This finding underscores the challenges of infection control in healthcare settings. Moreover, the results demonstrated significant resistance of ARB to a broad spectrum of disinfectants, raising major health concerns. The co-selection mechanism is especially troubling for opportunistic pathogens detected in hospital environments like *Staphylococcus* and *Acinetobacter*, both of which are well-known causative agents of HAIs. A considerable proportion of the identified ARB comprised *Staphylococcus* spp., which exhibited high resistance to various disinfectants. Notably, nearly half of these *Staphylococcus* isolates carried both the *mec*A and *qac*B genes simultaneously. While the number of isolates harboring the tested ARGs was relatively low, the presence of *intI*1, a well-known marker of horizontal gene transfer, alongside ARGs suggests that both cross- and co-resistance mechanisms may contribute to simultaneous resistance to antibiotics and disinfectants. These findings emphasize the critical importance of robust antimicrobial resistance surveillance as a cornerstone for controlling and preventing HAIs. Such surveillance not only guides the selection of the most effective antimicrobials for patient care but also informs strategies to inactivate resistant microorganisms in healthcare environments. However, further research is essential to unravel the precise mechanisms of co-selection and the genetic factors involved. In parallel, careful management and monitoring of disinfectant use are imperative to mitigate the risks associated with antimicrobial resistance.

CRediT authorship contribution statement

Mina Sheikh: Writing – original draft, Investigation, Data curation. **Sahar Gholipour:** Writing – original draft, Visualization, Software, Formal analysis. **Soudabeh Ghodsi:** Validation, Methodology. **Mahnaz Nikaeen:** Writing – review & editing, Project administration, Funding acquisition, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2024.120708.

Data availability

Data will be made available on request.

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