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Occurrence of chlorine-resistant *Pseudomonas aeruginosa* in hospital water systems: threat of waterborne infections for patients

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Abstract

Background Several healthcare-associated infection outbreaks have been caused by waterborne *Pseudomonas aeruginosa* exhibiting its ability to colonize water systems and resist conventional chlorine treatment. This study aims to investigate the occurrence of *Pseudomonas aeruginosa* in hospital drinking water systems and the antimicrobial resistance profiles (antibiotic and chlorine resistance) of isolated strains.

Methods We investigated the presence of *Pseudomonas aeruginosa* in water and biofilms developed in nine hospital water systems ($n = 192$) using culture-based and molecular methods. We further assessed the survival of isolated strains after exposure to 0.5 and 1.5 ppm concentrations of chlorine. The profile of antibiotic resistance and presence of antibiotic resistance genes in isolated strains were also investigated.

Results Using direct PCR method, *Pseudomonas aeruginosa* was detected in 22% (21/96) of water and 28% (27/96) of biofilm samples. However, culturable *Pseudomonas aeruginosa* was isolated from 14 samples. Most of *P. aeruginosa* isolates (86%) were resistant to at least one antibiotic (mainly β-lactams), with 50% demonstrating multidrug resistance. Moreover, three isolates harbored *intI*1 gene and two isolates contained $bla_{OXA-24,}$ $bla_{OXA-48,}$ and bla_{OXA-58} genes. Experiments with chlorine disinfection revealed that all tested *Pseudomonas aeruginosa* strains were resistant to a 0.5 ppm concentration. However, when exposed to a 1.5 ppm concentration of chlorine for 30 min, 60% of the strains were eliminated. Interestingly, all chlorine-resistant bacteria that survived at 30-minute exposure to 1.5 ppm chlorine were found to harbor the *intI*1 gene.

Conclusions The detection of antimicrobial resistant *Pseudomonas aeruginosa* in hospital water systems raises concerns about the potential for infections among hospitalized patients. The implementation of advanced mitigation measures and targeted disinfection methods should be considered to tackle the evolving challenges within hospital water systems.

Keywords Healthcare-associated infections, Antibiotic resistance, Chlorine resistance, Water, Biofilm

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Introduction

Despite improvements in medical care and disease prevention, healthcare-associated infections (HAIs) remain a significant global health threat, leading to increased morbidity, mortality, and economic burdens [1]. Antimicrobial-resistant (AMR) microorganisms play a key role in this challenge, complicating treatment and elevating risks. The environment serves as a reservoir for AMR and facilitating their dissemination through complex pathways [2]. Combating this growing challenge requires acting within the One-Health framework emphasizing collaborative efforts across diverse healthcare disciplines, encompassing human, animal, and environmental sectors [3]. Water systems, as a critical environmental component, play a significant role in influencing the propagation of antimicrobial resistance within the One-Health framework.

Evidence suggests that waterborne transmission significantly contributes to a substantial portion of documented HAIs, estimated at approximately 21.6% [4]. Among waterborne microorganisms, opportunistic premise plumbing pathogens (OPPPs) require particular attention due to their unique ability to persist in water distribution systems (WDSs) [5]. Their ability to survive in low-nutrient environments, resist common disinfectants, and form biofilm makes them particularly worrisome for HAIs [5]. Biofilms within WDSs represent complex microbial communities that adhere to solid-liquid interfaces. These communities are embedded within a matrix of extracellular polymeric substances (EPS) [6]. Bacteria within biofilm are closely clustered and can acquire antibiotic resistance genes (ARGs) from each other thorough horizontal gene transfer (HGT) [7]. Therefore, biofilm detachment from various WDSs components can then disseminate antibiotic resistant bacteria into the hospital environment, posing a risk to exposed patients.

Pseudomonas aeruginosa is a gram-negative gammaproteobacterium with intrinsic and acquired resistance to many antibiotics, which makes infections difficult to treat and contributes to a major healthcare challenge. World Health Organization (WHO) classified *P. aeruginosa* as a "high-priority pathogen" due to its global threat, particularly affecting healthcare settings [8]. Additionally, its classification as one of the "ESKAPE" pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *P. aeruginosa* and *Enterobacter* spp.) emphasizes its significant contribution on HAIs and its ability to "escape" the effects of antimicrobial agents [9]. It has been estimated that *P. aeruginosa* was responsible for over 250,000 deaths associated with AMR in 2019, with the highest burden in Sub-Saharan Africa and the lowest in Australia [10, 11].

P. aeruginosa is a ubiquitous bacterium found in various environments and is a prominent example of an OPPP identified within both water and biofilms of WDSs, often exhibiting high levels of resistance to antimicrobial agents [4]. Globally, several waterborne outbreaks of HAIs caused by AMR *P. aeruginosa* have been documented [12–14]. It can cause various HAIs, including hospital-acquired pneumonia, urinary tract infections, surgical site infections, and bloodstream infections. Hospitalized patients can acquire waterborne *P. aeruginosa* infections through various routes, such as inhaling droplets from contaminated water in showers or faucets, exposure of open wounds to contaminated water during surgery or bathing, and rinsing of medical devices like nebulizers or catheters with contaminated water [13, 15].

P. aeruginosa has developed resistance to a wide range of antibiotics through the acquisition of various resistance genes. This resistance is driven by both chromosomal mutations and the increasing prevalence of transferable resistance mechanisms. These mechanisms primarily involve carbapenemases and extended-spectrum β-lactamases (ESBLs), often accompanied by aminoglycoside-modifying enzymes encoded by associated genes [16, 17]. OXA genes encode for a class of enzymes known as OXA-type β-lactamases confer resistance to β-lactam antibiotics and have been identified in ESKAPE pathogens, including *P. aeruginosa*, *Acinetobacter baumannii*, and *Klebsiella pneumoniae* [18]. As reviewed by del Barrio-Tofiño et al. (2020), OXA genes, along with VIM, IMP, CTX, and TEM, are among the most frequently detected horizontally acquired β-lactamases in *P. aeruginosa* epidemic high-risk clones [16]. Encode aminoglycoside-modifying enzymes, *aac* genes, inactivate aminoglycoside antibiotics which is crucial for treating severe *P. aeruginosa* infections [19]. The presence of these ARGs in *P. aeruginosa* isolated from hospital environments is concerning due to the potential exposure of patients.

Studies on antibiotic and chlorine resistance have primarily been conducted within municipal drinking water distribution systems [20–23]. However, research specifically focusing on both antibiotic and disinfectant resistance of waterborne microorganisms in hospital water systems remains limited [24]. Our understanding of *P. aeruginosa* occurrence and resistance within these systems primarily relies on post-outbreak reports, and to the best of our knowledge, no prior studies have investigated the specific chlorine resistance characteristics of *P. aeruginosa* strains isolated from these settings. Acknowledging the crucial role of chlorine disinfection in preventing the spread of waterborne bacteria in hospitals, this study aims to: (1) determine the occurrence of *P. aeruginosa* in both water and biofilm samples collected from hospital WDSs. (2) evaluate the antimicrobial resistance (antibiotic and chlorine resistance) of isolated *P. aeruginosa* strains. (3) determine the frequency of a number

of common ARGs, encoding resistance to β-lactams and aminoglycosides in isolated *P. aeruginosa*.

Methods

Sample collection and preparation

The occurrence and resistance of *P. aeruginosa* in the WDSs of nine large hospitals of Isfahan province, Iran were investigated. A total of 192 samples were obtained from hand-washing faucets and shower hoses situated in high-risk areas, such as intensive care units and operating rooms. This sample set included 96 water samples and 96 biofilm samples. Water samples were collected in 100 mL sterile glass bottles containing 0.1 g/L of sodium thiosulfate to neutralize the chlorine. Biofilm samples were gathered using Dacron swabs following the U.S. Center for Disease Control and Prevention (CDC) protocol [25]. Water samples were analyzed on-site for free chlorine residual and pH using an AB 142 portable pH/chlorine meter (Behin Ab, Iran). Additionally, turbidity and electrical conductivity measurements were obtained using a 2100Q Portable Turbidimeter and an Eutech EC meter, respectively.

To prepare samples for microbial testing, water samples (50 mL) were concentrated tenfold by centrifugation (6000 rpm, 20 min). After discarding 45 mL of supernatant, the concentrated pellet in the remaining 5 mL was used for subsequent analyses. Biofilm samples were subjected to ultrasonic vibration and vortexing to detach cells. The resulting PBS solution containing detached biofilm cells was used for further testing. Heterotrophic plate count (HPC) analysis of the water samples was conducted by spreading 200 µL of the concentrated water sample on R2A agar (Merck, Darmstadt, Germany), followed by incubation at 25 °C for 72–120 h [26].

Pseudomonas aeruginosa detection

The presence of *P. aeruginosa* in water and biofilm samples was investigated using both molecular and culture-based methods. Direct PCR was used to identify the presence of both culturable and non-culturable *P. aeruginosa* cells potentially present in the samples. Culture-based methods were employed to detect viable *P. aeruginosa* and to assess antimicrobial resistance profiles.

P. aeruginosa detection using PCR

DNA extraction from water and biofilm samples was carried out directly using a combination of proteinase K, sodium dodecyl sulfate (SDS), and repeated freezing-thawing cycles [27]. The extracted DNA was further purified by RIBO-prep nucleic acid extraction kit (AmpliSens®) following the manufacturer's instructions. Water and biofilm samples were subjected to conventional PCR using a species-specific primer set to detect the presence of *P. aeruginosa*, as previously described [27].

Additionally, a StepOne real-time PCR system was employed to quantify *P. aeruginosa* in water samples. For the quantitative PCR (qPCR) assay, a reaction mixture was prepared containing 7.5 µL of 2x Power SYBR Green Master Mix, 0.2 µM of each primer [27], 3 µL of template DNA, and double-distilled water. The thermal cycling protocol consisted of an initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 94 °C for 15 s and annealing at 58 °C for 45 s. Melting curve analysis was performed from 65 °C to 95 °C to verify amplicon specificity. A standard curve for qPCR was generated using a serial dilution of *P. aeruginosa* genomic DNA, ranging from 10^6 to 1 cell-equivalent, quantified with a Qubit fluorometer (Eq. S1). Quantification of *P. aeruginosa* in biofilm samples from swabbed faucets was not feasible due to the inability to accurately measure the inner surface area. Therefore, qPCR analysis was performed on water samples only.

P. aeruginosa identification and isolation using culture-based method

To identify viable *P. aeruginosa*, 200 µL of each biofilm and concentrated water samples were plated on blood agar (Merck, Darmstadt, Germany) and incubated for 24–48 h at 37 °C. Suspected colonies from both blood agar and R2A agar were isolated and subcultured on selective Cetrimide agar plates (Difco, Detroit, USA). After 18–24 h of incubation at 37 °C, colonies from Cetrimide agar were subjected to DNA extraction using the boiling method and confirmed as *P. aeruginosa* by conventional PCR using a species-specific primer set [27]. Confirmed *P. aeruginosa* isolates were preserved in 1-mL Tryptic Soy Broth (Merck, Darmstadt, Germany) supplemented with glycerol (15%) at -20 °C for subsequent antibiotic and chlorine resistance testing.

Antibiotic resistance tests

The confirmed *P. aeruginosa* isolates underwent antibiotic susceptibility testing using the Kirby-Bauer disk diffusion method, as outlined in Clinical and Laboratory Standards Institute (CLSI) document M100 [28]. Antibiotic disks used in this study and the breakpoints are presented in Table 1. The antibiotics were selected from group A and B antibiotics recommended by CLSI for routine and occasional antibiotic susceptibility testing of *P. aeruginosa*. All *P. aeruginosa* isolated colonies were also screened for the presence of class 1 integron gene (*intI*1) as well as genes conferring resistance against aminoglycoside-fluoroquinolone (*aac*(6')-Ib-cr) and β-lactams (bla_{CTX-M}, bla_{TEM}, bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-58}, bla_{OXA-48}, bla_{KPC}) using PCR as previously described [24, 29]. Primer sets used for ARG detection are presented in Table S1.

Table 1 Antibiotic panel used for the isolated *P. Aeruginosa* from water and biofilm samples

Antibiotic group	Antibiotic	Disk content (µg) [28]	Zone diameter breakpoints (mm) [28]		
			Susceptible	Intermediate	Resistant
β-lactam (cepham)	Ceftazidime [A]	30	≥ 18	15–17	≤ 14
β-lactam (cepham)	Cefepime [B]	30	≥ 18	15–17	≤ 14
β-lactam (monobactam)	Aztreonam [B]	30	≥ 22	16–21	≤ 15
β-lactam (carbapenem)	Imipenem [B]	10	≥ 19	16–18	≤ 15
Aminoglycoside	Gentamicin [A]	10	≥ 15	13–14	≤ 12
Aminoglycoside	Amikacin [B]	30	≥ 17	15–16	≤ 14
Fluoroquinolone	Ciprofloxacin [B]	5	≥ 25	19–24	≤ 18

[a] Antibiotics listed as Group A should be routinely tested, [B] antibiotics listed as Group B should be tested occasionally [28]

Table 2 Physicochemical characteristics of water samples and HPC

Parameter	Mean ± STD	Min	Max	WHO recommended values
pH	-	6.2	7.7	6.5–8.5
Free chlorine residual (mg/L)	0.24 ± 0.13	0	0.5	0.2
Turbidity (NTU)	0.68 ± 0.47	0.14	3.4	5
EC [a] (µmho/cm)	975 ± 795	345	3000	-
HPC [b] (CFU/mL)	653 ± 841	0	3240	500

[a] Electrical conductivity

[b] Heterotrophic plate count

Chlorine resistance experiment

P. aeruginosa isolates were tested for chlorine resistance as described [24]. Briefly, *P. aeruginosa* colonies were cultured in Tryptic Soy Broth (Merck, Darmstadt, Germany) and adjusted to a 0.5 McFarland standard (OD600). Chlorine resistance testing involved exposing bacterial suspensions to two concentrations of free chlorine (0.5 and 1.5 ppm) for durations of 5 and 30 min. Post-exposure chlorine residuals were inactivated via sodium thiosulfate treatment. Subsequently, serial dilutions of the treated samples were plated onto Tryptic Soy Agar (Merck, Darmstadt, Germany) and incubated at 37 °C for 24 h. The survival rate was determined by comparing the colony counts of the exposed samples to those of unexposed control samples.

Statistical analysis

Data visualization was achieved using Microsoft Excel 2016, while statistical analyses were conducted with SPSS version 24. To assess the relationship between physicochemical parameters, HPC, and *P. aeruginosa* concentration in water samples, Spearman's correlation was employed. Correlations exhibiting a P-value below 0.05 were deemed statistically significant.

Results and discussion

Water quality

Biofilm provides an ideal environment for the prolonged survival of waterborne opportunistic bacteria such as *P. aeruginosa*, and facilitates HGT between bacterial cells, potentially leading to antibiotic resistance in hospital water systems. Moreover, long-term exposure to sub-optimal disinfectants in WDSs may result in chlorine resistance of opportunistic bacteria and even co-resistance to antimicrobial agents [29].

Heterotrophic bacteria count and physicochemical characteristics of water samples are provided in Table 2. HPC in water samples ranged between 0 and 3240 CFU/mL. Notably, the average HPC exceeded the potable water standard of 500 CFU/mL [30] in six of the nine hospital WDSs. Negative correlations were found between chlorine residual levels and both turbidity and HPC in the water samples, indicating that lower chlorine concentrations were associated with higher turbidity and bacterial counts. These results indicate a potential association between insufficient chlorine residual levels and an increased risk of microbial proliferation and biofilm development, emphasizing critical importance of maintaining adequate chlorine concentrations within WDSs [24].

Presence of *P. aeruginosa* in water and biofilm samples

The presence and concentration of *P. aeruginosa* detected by PCR in the samples are illustrated in Fig. 1A-C. *P. aeruginosa* was identified in 28% of biofilm samples (27/96) and 22% of water samples (21/96) (Fig. 1A), with nine samples exhibiting its presence in both water and biofilm samples (Fig. 1B). In most hospitals, *P. aeruginosa* was more prevalent in biofilm samples compared to water samples, except for hospitals No. 2, 8, and 9 where water samples showed a higher prevalence of *P. aeruginosa* than biofilms (Fig. 1A). Based on the results obtained by qPCR, quantities of *P. aeruginosa* in positive water samples ranged from 0.25 to 50 No./mL (Fig. 1C). However, the culture-based method yielded lower detection rates for *P. aeruginosa* (Fig. 2). Culturable *P. aeruginosa* was found in 14 samples (eight biofilm and six

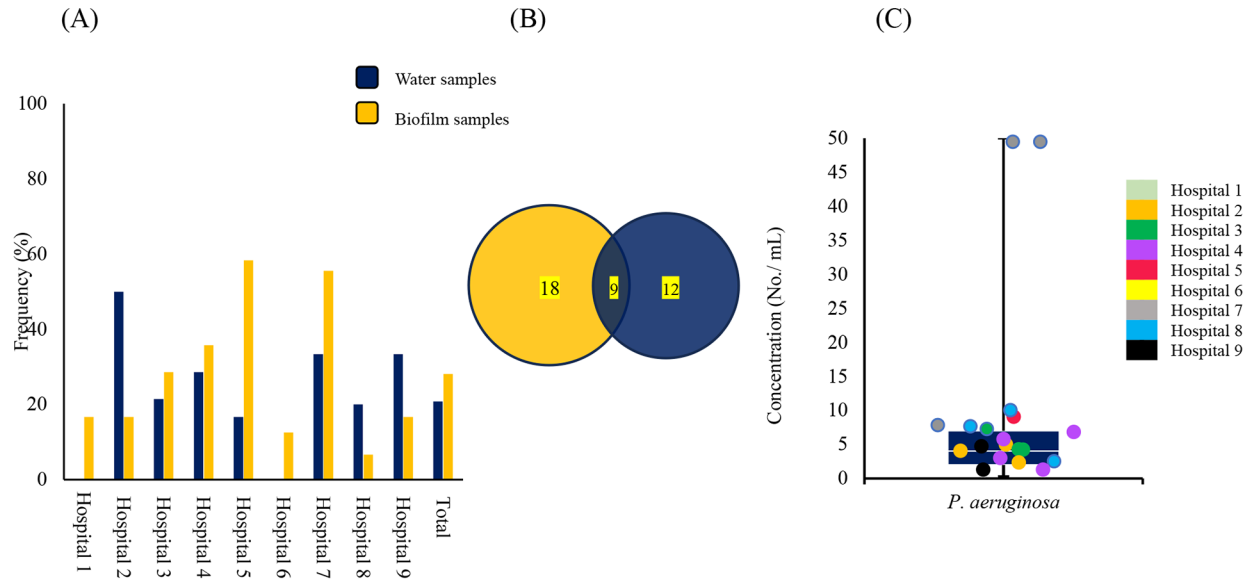

Fig. 1 Frequency of detection and concentration of *P. aeruginosa* in water and biofilm samples using PCR. **(A)** Frequency of detection of *P. aeruginosa* in hospital water systems, **(B)** The numbers of shared and unique *P. aeruginosa* detected in water and biofilm samples, **(C)** Concentration of *P. aeruginosa* in water samples

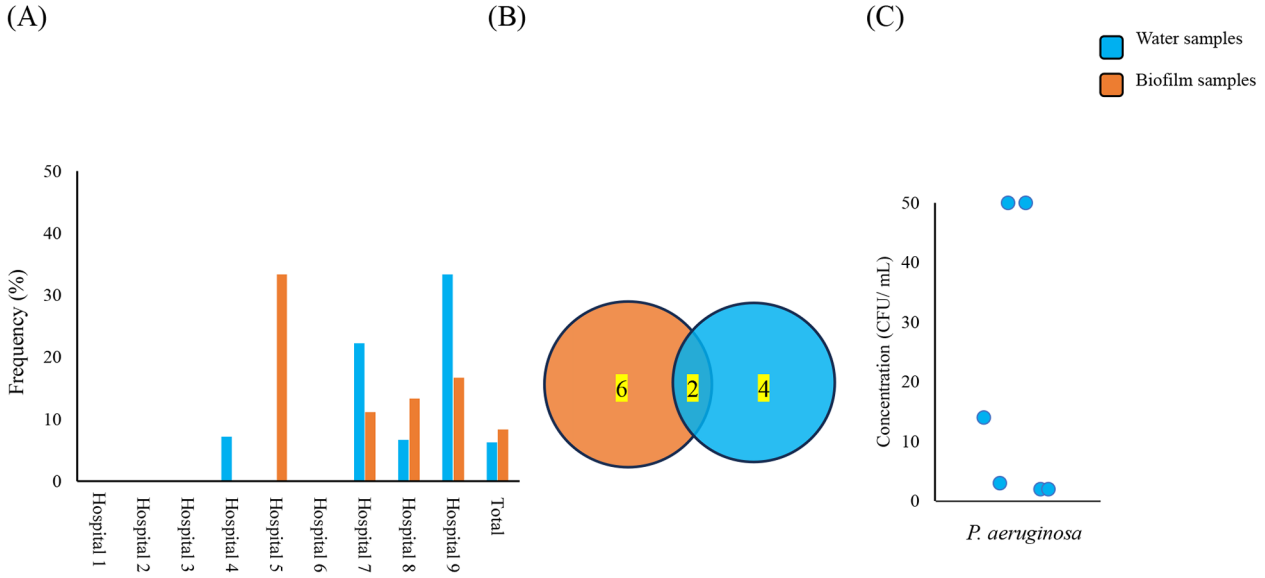

Fig. 2 Frequency of culturable *P. aeruginosa* detected in water and biofilm samples **(A)** Frequency of detection of *P. aeruginosa* in hospital water systems, **(B)** The numbers of shared and unique *P. aeruginosa* detected in water and biofilm samples, **(C)** Concentration of *P. aeruginosa* in water samples

water samples) (Fig. 2A), with simultaneous occurrence in two samples (Fig. 2B). The numbers of *P. aeruginosa* in water samples using the culture-based method ranged from 2 to 50 CFU/mL. Statistical analysis showed a significant positive correlation between the concentrations of culturable *P. aeruginosa* and HPC. No correlation was observed between the physicochemical characteristics of water and *P. aeruginosa* concentrations.

Our findings indicate that *P. aeruginosa* was detected in hospital water systems in both planktonic and biofilm forms, suggesting that these systems represent a hotspot for the dissemination of *P. aeruginosa* into the hospital environment and potentially pose health risks for patients. Previous studies have also reported the occurrence of *P. aeruginosa* in hospital water systems. For instance, a nine-year study in a French hospital using

culture-based methods reported a detection frequency of 17% for *P. aeruginosa* in water samples [31]. Similarly, a study in Taiwanese hospitals identified 14 isolates of *P. aeruginosa* among 162 faucet aerators located in ICUs [32]. In contrast, direct PCR analysis revealed a 50% positivity rate for *P. aeruginosa* occurrence in the water, compared to only 7% recovered by culture methods in a pediatric university hospital in Montreal, Canada [33]. Furthermore, a previous investigation in Isfahan also detected *P. aeruginosa* in 32% of hospital water samples using direct PCR assays [34].

The increased frequency of detecting *P. aeruginosa* through direct PCR assays compared to culture-based methods suggest the potential presence of this bacterium in a viable but nonculturable (VBNC) state. It has been documented that a significant portion of the bacterial population in water system biofilms exists in the VBNC state [35]. When bacteria transit to the VBNC state, they retain metabolic activity and pathogenicity, thereby presenting a potential threat to water quality [36].

Antibiotic resistance analysis

Antimicrobial susceptibility testing using the disk diffusion method revealed that 86% of *P. aeruginosa* isolates were resistant to at least one antibiotic, with 50% demonstrating multidrug resistance (Fig. 3A). Notably, the majority (83%) of antibiotic-resistant *P. aeruginosa* were isolated from biofilm samples, highlighting the increased resistance of biofilm bacteria compared to planktonic ones.

As shown in Fig. 3B, the highest resistance was observed against β-lactam antibiotics. Among the isolated *P. aeruginosa*, 71% were resistant to aztreonam, and the remaining isolates demonstrated intermediate susceptibility. Ceftazidime and cefepime resistance rates were 43% and 29%, respectively, whereas all isolates were susceptible to imipenem. β-lactams are often considered the last resort for treating severe bacterial infections such as urinary tract, bloodstream, wound, and pneumonia infections. Therefore, resistance to this group of antibiotics poses a significant threat to patients [37].

In contrast to other studies reporting high aminoglycoside resistance in waterborne *P. aeruginosa* [38–40], our findings indicated lower resistance levels. Only 14% of our isolates were resistant to amikacin, and 29% showed intermediate susceptibility to gentamicin. This lower resistance might be attributed to the restricted use of these antibiotics in Iran during recent years. Moreover, while we did not find any imipenem-resistant *P. aeruginosa*, a recent study on shower water from a hospital in London, UK, reported approximately 50% of isolated *P. aeruginosa* as imipenem-resistant [41]. *P. aeruginosa* isolated from bathroom water in Indonesian hospitals exhibited resistance to ceftazidime (20%), piperacillin/tazobactam (4%), ciprofloxacin (20%), and gentamicin (20%) [39]. All of *Pseudomonas* spp. isolates from Italian hospital water systems demonstrated resistance to aminoglycosides. Fewer isolates were resistant to meropenem (5.89%) and ceftazidime (17.64%), and no strains were resistant to high levels of ciprofloxacin, aztreonam, or cefepime [40].

Among analyzed genes, bla_{OXA-24}, bla_{OXA-48}, and bla_{OXA-58} were simultaneously detected in one water and one biofilm sample, both obtained from the same faucet. OXA-type β-lactamases have been reported to have profound effect on hydrolysis of cephalosporines, penicillin, piperacillin, aztreonam and carbapenems in *Acinetobacter* spp., Enterobacteriaceae, and *P. aeruginosa* [18]. bla_{OXA-24} and bla_{OXA-58} are typically associated with carbapenem resistance in *Acinetobacter baumannii* [42]. Interestingly, in our study these genes were found in isolates which have shown resistance to ceftazidime,

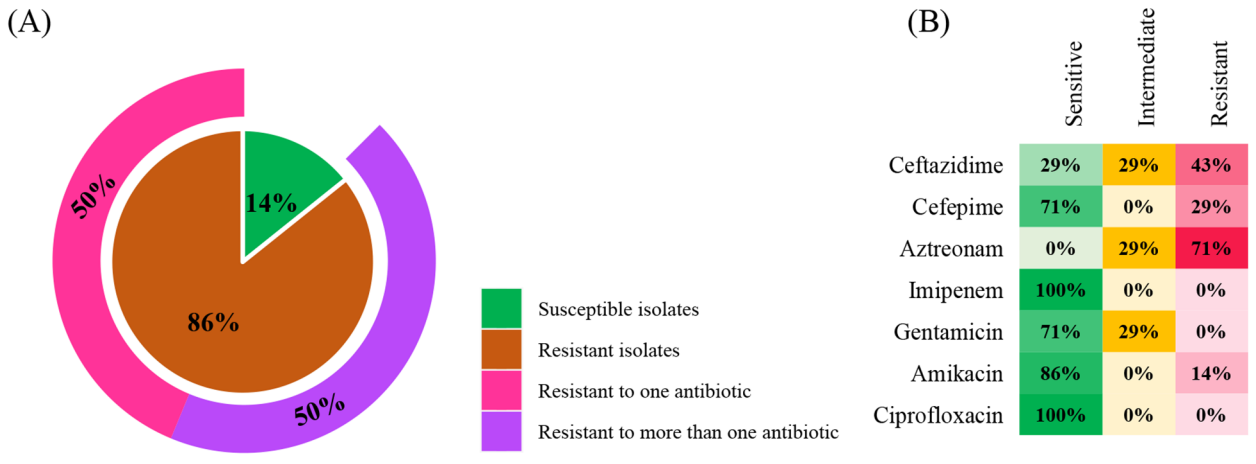

Fig. 3 Resistance of *P. aeruginosa* to antibiotics **(A)** Frequency of resistant isolates to one and more than one antibiotic, **(B)** Frequency of resistant/ intermediate/ susceptible isolates to each antibiotic

aztreonam, and cefepime. However, none of the *P. aeruginosa* in our study identified as carbapenem resistant. The emergence of these types of genes in clinical isolates of *P. aeruginosa* has been recently reported [43, 44]. The presence of these genes in our samples suggests that hospital water systems could serve as a reservoir for the dissemination of β-lactam -resistant *P. aeruginosa*, underscoring the role of hospital WDSs in the transmission of antibiotic-resistant healthcare-associated infections. However, other β-lactam resistance genes (bla_{CTX-M}, bla_{TEM}, $bla_{KPC,}bla_{OXA-23}$), and *aac*(6')-Ib-cr were not detected in *P. aeruginosa* isolates.

Class 1 integron gene (*intI*1) was detected in 21% of the isolates from water and biofilm samples. The *intI*1 gene has the ability to accumulate a diverse range of resistance genes, which facilitates the emergence of bacterial strains resistant to multiple antimicrobial agents [45]. All isolates carrying the *intI*1 gene were exclusively found in samples from one of the hospitals, which specialized in pediatrics. This finding raises serious concerns about the potential for pediatric patients to acquire infections from waterborne, multi-drug-resistant *P. aeruginosa*. Earlier studies have reported outbreaks of multi-drug-resistant *P. aeruginosa* linked to faucet biofilms in neonatal ICUs in Turkey and Ireland. These outbreaks led to fatalities, as well as cases of pneumonia and bloodstream infections among neonates [13, 46].

Chlorine resistance of *P. aeruginosa*

The frequency of resistant *P. aeruginosa* in different concentrations of applied chlorine is depicted in Fig. 4. As recommended by the WHO, effective disinfection of drinking WDSs can be achieved by applying a free chlorine concentration of 0.5 mg/l for a duration of 30 min [47]. As a result, bacteria that tolerate this disinfection dosage are defined as chlorine-resistant bacteria [48]. In our study, all tested *P. aeruginosa* isolates exhibited resistance to a chlorine concentration of 0.5 ppm for both 5- and 30-minute exposure durations. However, when exposed to a higher chlorine concentration (1.5 ppm), 80% of the isolates were able to survive after a 5-minute exposure, with 40% remaining viable even after a 30-minute exposure. A recent study has shown that certain bacteria, such as *Bacillus* and *Staphylococcus*, present in biofilms of hospital water systems can tolerate chlorine concentrations of up to 4 ppm [24]. Jathar et al. (2021) also reported the high resistance of bacteria isolated from water reservoirs, with the highest resistance observed for *Acinetobacter* and *Serratia* [49]. *Pseudomonas peli* was isolated from an urban water supply network in northern China and exhibited a high resistance to chlorine [22]. It has been suggested that bacterial resistance to various antibiotics and disinfectants often relies on shared mechanisms, including expression of efflux pumps and drug resistance operons, as well as inducible mutations in certain genes [50, 51]. Hou et al. (2019) reported that exposure of *P. aeruginosa* to low concentrations of chlorine can lead to the overexpression of drug efflux pumps, resulting in increased antibiotic resistance [52]. Interestingly, in our study, all chlorine-resistant bacteria surviving a 30-minute exposure to 1.5 ppm chlorine were found to harbor the *intI*1 gene. Similarly, a study by Chen et al.

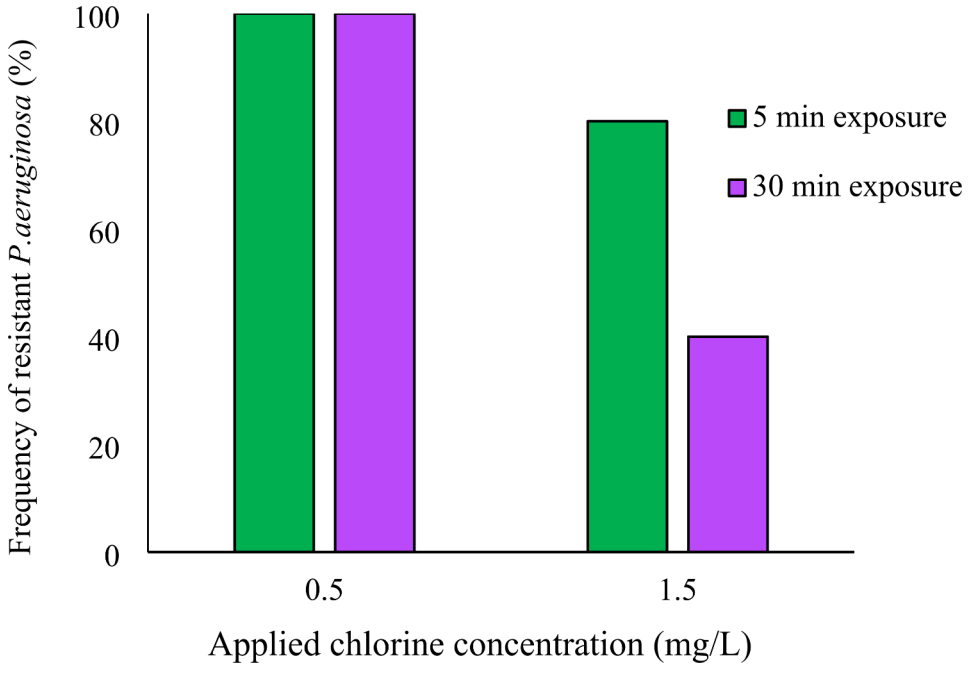

Fig. 4 Frequency of resistant *P. aeruginosa* in different concentrations of applied chlorine

(2023) observed a strong association between resistance to disinfectants and presence of *intI*1 gene in *Salmonella* [53]. Class 1 integrons in environmental bacteria typically contain a 5′ conserved segment (5′CS) with the *intI*1 gene and a 3′ conserved sequence (3′CS) containing efflux pump genes (particularly *sul*1, *qac*E, and *qac*EΔ1) that confer resistance to disinfectants [45]. Therefore, *intI*1 may serve as an indicator for the presence of disinfectants resistance genes.

Conclusions

This study reported the occurrence of *Pseudomonas aeruginosa*, a major causative agent of HAIs, in water and biofilm samples collected from nine different hospital water systems. While the number of *P. aeruginosa* isolates harboring ARGs was low, with OXA-type β-lactamase genes and *intI1* detected in only two and three isolates, respectively, phenotypic antimicrobial susceptibility testing revealed high resistance to β-lactam antibiotics, which are widely used in HAIs treatment. This highlights the potential role of water systems as hotspots for disseminating β-lactam-resistant *P. aeruginosa* in hospitals and raises concerns about the potential risk of infections among hospitalized patients, particularly those who are immunocompromised or have open wounds. Notably, the resistance of these isolates to conventional chlorine disinfection further emphasizes the urgent need for a multifaceted approach to addressing waterborne pathogens in healthcare settings.

To address the growing challenge of antimicrobial-resistant HAIs, collaboration between healthcare professionals, facility managers, and policymakers for developing a comprehensive infection prevention and control (IPC) strategy is required. This should be included not only routine monitoring of hospital water systems but also the implementation of advanced mitigation measures such as innovative faucet technologies and the development of alternative disinfection methods. By prioritizing IPC and adopting a One-Health perspective, healthcare facilities can effectively combat the dissemination of antibiotic-resistant bacteria within the hospital environment.

Supplementary Information

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Supplementary Material 1

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Author contributions
Sahar Gholipour: Methodology, Investigation, Writing-original draft, Mahnaz Nikaeen: Conceptualization, Supervision, Writing- reviewing & editing, Mohammadmehdi Mehdipour: Investigation, Farzaneh Mohammadi: Software, Formal analysis, Davarkhah Rabbani: Conceptualization, Supervision, Writing- reviewing & editing.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethical approval
Not applicable.

Competing interests
The authors declare no competing interests.

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