Original Article

Contaminated faucets and sinks as a reservoir for antibiotic-resistant bacterial transmission in healthcare settings

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Abstract

Introduction: Multidrug-resistant bacterial infection has emerged as a global hygiene threat in hospitals, and outbreaks cause increased patient morbidity and mortality in the healthcare system. Mounting evidence points to faucets and sinks as the culprits in the outbreaks of multidrug-resistant bacterial infections. However, the mechanism and the route through which faucets and sinks contribute to antibiotic-resistant bacterial transmission are not fully understood.

Methodology: We collected 455 surface samples from faucets and sinks in over 60 areas covering four environmental classes to comprehensively overview the prevalence and distribution of multidrug-resistant bacteria in the hospital.

Results: We detected 32 carbapenem-resistant *Acinetobacter* samples, one methicillin-resistant *staphylococcus aureus* sample, and three carbapenem-resistant *Klebsiella pneumoniae* samples. Thirteen carbapenem-resistant *Acinetobacter spp.* and one *Klebsiella spp.* were identified in the 455 faucet samples.

Conclusions: Some faucets in the hospital were contaminated with antibiotic-resistant *Acinetobacter*, suggesting the possibility that the contaminated faucets and sinks act as a reservoir of antibiotic-resistant bacterial transmission. The current study assessed the prevalence and distribution of multidrug-resistant bacteria on the faucets and sinks in the hospital. It revealed the potential of faucets and sinks as a carrier of antibiotic-resistant bacteria, assisting in spreading them. Improvement of hand hygiene facilities to prevent antibiotic resistance deserves better attention. This study can further instruct us on a surveillance strategy to be used in hospitals.

Key words: Multidrug-resistant (MDR) bacteria; faucet; hospital hygiene; antibiotic resistance; MRSA; CRAB; CR-Kpn.

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Introduction

Multidrug-resistant (MDR) bacteria are those resistant to three or more classes of antibiotics. MDR bacterial infection has been recognized as one of the most important public health threats of the 21st century [1]. In the hospital environment, the transmission of antibiotic resistance can lead to an outbreak of MDR bacterial infection inwards and, even more dangerously, in the intensive care unit (ICU), causing serious morbidity and mortality to patients under vulnerable health conditions. MDR bacteria led to a significant burden on inpatient care hospitals [2]. MDR in Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa, and Acinetobacter baumannii has been causing a significant burden on hospital hygiene [3]. Antibiotic development hysteresis and antibiotic treatment efficiency limitation contributed to infections

with the so-called 'superbug' methicillin-resistant *Staphylococcus aureus* (MRSA), resistant to all antimicrobial categories, that has emerged as a 'cancer that never dies' [4]. Carbapenem-resistant *Klebsiella* species (CRK), particularly carbapenem-resistant *K. pneumoniae* (CR-Kpn), have become a threat to nosocomial infection control [5].

The extensive application of antibiotics, therapies with invasive devices, and long-term hospitalization are factors that exacerbate the prevalence of antibiotic resistance in hospitals, especially in ICUs [6]. Multiple mechanisms contribute to the development of MDR bacteria. These include the acquisition of β -lactamase enzymes, bacterial adaptation of the antibiotic target protein, increased antibiotic efflux by overexpression of transmembrane efflux pumps, transmission of antibiotic resistance by mobile genetic elements such as plasmids, and decreased antibiotic permeability [7]. Antibiotic resistance can be transmitted in the hospital environment by horizontal gene transfer between the bacteria. For example, it has been shown by whole-genome sequencing that MDR bacterial outbreaks can be mediated by transmissible genetic elements between bacterial species in the hospital [8].

Hand hygiene is a crucial strategy to prevent nosocomial infection [9]. The role of sinks in MDR bacterial transmission is being increasingly recognized [10]. Sinks and drains can function asreservoirs for MDR bacteria due to the nutritious waste disposed through them [11-12]. The role of sinks as reservoirs of gram-negative bacteria, including E. coli, Enterobacter species, P. aeruginosa, and Burkholderia cepacian, was documented in various studies [13-15]. Studies found that gram-negative bacteria can exchange drug-resistance genes through horizontal exchange in the sink and faucet niche [16-17]. Extensive environmental surveillance has demonstrated that faucets can be contaminated by MDR bacteria, including multidrug-resistant A. baumannii [18]. It was demonstrated that the rate of ICU-acquired gramnegative bacilli was limited upon removal of the water sinks [10].

To get an overview of MDR bacterial prevalence and distribution in the hospital, we collected 910 faucets and sink surface samples from all four hospital environment classes and identified the antibiotic resistance pattern of the isolated bacteria. By comparing the prevalence between the different areas, we generated an overview of MDR bacterial distribution in the hospital and detected the areas most vulnerable to MDR transmission.

Methodology

Sampling environments

Faucets and matched sinks from four environmental classes in Tongji Hospital, Wuhan, Hubei province, China, were sampled for MDR bacterial contamination surveillance. The samples were collected following the GB15982-2012 hygienic standard for disinfection in hospitals. The Class I environment included regions using the air cleaning technology. The Class II environment was the non-clean operating region that included the delivery room, catheter room, blood disease wards, burn wards, ICU, newborn room, etc. The Class III environment included the mother and baby cohabitation rooms, the packaging and sterilization area, the sterilized supplements' storage area in the central sterile supply department, the hemodialysis center, other ordinary inpatient areas, etc. The Class IV environment consisted of the ordinary outpatient (emergency) clinics, their examination and treatment rooms, and the Infectious Diseases Department outpatient clinic and ward.

Overall, we sampled 16 Class I areas, 21 Class II areas, 12 Class III areas, and 13 Class IV areas. No more than ten water faucets and matched sinks were sampled in any ward. The faucet ratio between those used by the medical staff and the patients was 7:3. The specific sample collecting sites are listed in Table 1.

Sampling locations

The relevant information on the faucet and the matched sink sampling is presented in Table 2.

Detection of microbial contamination on target surfaces

Sampling was conducted by scratching an immersed in physiological salt solution cotton swab five times horizontally and five times vertically on target surfaces no larger than 10×10 cm. The samples were spread on MRSA, *Klebsiella pneumoniae* carbapenemase (KPC)-producing bacteria and carbapenem-resistant *Acinetobacter* mediums. Another similarly treated swab was eluted with 10 mL physiological saline solution and incubated at 1 mL per plate in a regular bacteria culture medium.

Microbiology culturing

The antibiotic testing and regular plates were cultured at 37 °C in a bacteria incubator. After 24–48 hours, a single experienced investigator counted the number of Colony-Forming Units per plate.

Matrix-assisted laser desorption/ionization time-offlight mass spectrometry (MALDI-TOF-MS) of the bacteria

The bacteria colonies cultured from the samples were analyzed by MALDI-TOF-MS.

Statistical analysis

SPSS Statistics for Windows, Version 19.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The MDR counts among the four Classes and various classifications were compared using the chi-squared test [13]. Statistical significance was set at p < 0.05.

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Classification Environment	MRSA	MRSA Ratio (%)	р	CRAb	CRAb Ratio (%)	р	CR- Kpn	CR-Kpn Ratio (%)	р	Normal Without MDR	Normal Ratio (%)	Total	Environment Ratio
Class I	0	0		3	1.316%		0	0		225	98.68%	228	0.330%
Class II	1	0.4464%		9	4.018%		0	0		214	95.53%	224	1.099%
Class III	0	0		18	7.759%		3	1.2931%		211	90.94%	232	2.308%
Class IV	0	0	0.382	2	0.885%	< 0.001	0	0	0.008	224	99.11%	226	0.220%

Table 2. The distribution of MDR in 4 Classifications of Environments.

Results

Identification and distribution of antibiotic-resistant bacteria on the faucets and sinks

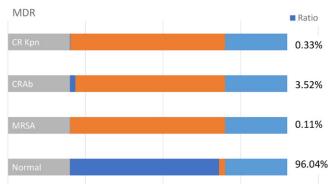
To understand MDR bacterial colonization patterns in the hospital, we sampled 455 sinks and 455 faucets from the outpatient and inpatient areas.

The samples were assessed for the targeted pathogens, including MRSA, carbapenem-resistant *A. baumannii* (CRAb), and CR-Kpn. MALDI-TOF-MS identified the bacteria in the cultured colonies. We detected 556 bacterial colonies on plates without antibiotics, including 28 of *P. aeruginosa*, 11 of *S. haemolyticus*, 17 of *S. epidermidis*, and nine of *Bowman Acinetobacter*. We detected 32 carbapenem-resistant *Acinetobacter spp.*, one MRSA, and three CRK *spp.* on plates containing antibiotics.

The 32 carbapenem-resistant *Acinetobacter spp.* included *A. baumannii* and *A. pittii*. As shown in Figure 1, CRAb contamination was found in 4.4% of the sinks and 2.6% of the faucets. The three CRK *spp.* samples, 0.33% of all samples, included one positive for *K. pneumoniae* and two for *Klebsiella oxytoca*. Carbapenem-resistant *Klebsiella spp.* contamination rate was 0.33%. MRSA isolation showed the lowest rate (0.11% of all samples).

Of the 32 carbapenem-resistant Acinetobacter spp. samples, MALDI-TOF-MS identified 13 as A. baumannii, 12 as A. Pittii, two as A. johnsonii, two as

Figure 1. The overall multidrug-resistant bacterial contamination rates.



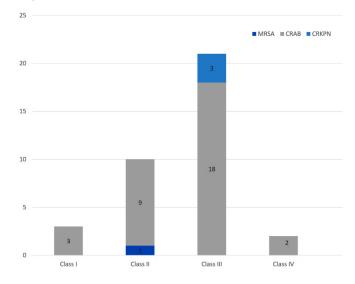
The Carbapenem-resistant *Acinetobacter baumannii*, Carbapenemresistant *Klebsiella pneumoniae*, and Methicillin-resistant *Staphylococcus aureus* contamination rates in the samples are summarized.

A. radioresistens, two as A. ulmoides, and one as A. gilot.

Highest rate of MDR bacteria was isolated in Class III areas

We further analyzed the relationship between the MDR bacterial contamination rate and environment classification. As shown in Table 2, the MDR bacterial contamination rates were 0.3% in Class I. 1.1% in Class II, 2.3% in Class III, and 2.2% in Class IV. Three carbapenem-resistant Acinetobacter SPD. were identified in the Class I environment, one MRSA and nine carbapenem-resistant Acinetobacter spp. in the Class II environment, 18 carbapenem-resistant Acinetobacter spp. and three CRK spp. in the Class III environment. carbapenem-resistant and two Acinetobacter spp. in the Class IV environment. As shown in Figure 2, the highest MDR bacterial contamination rate was in the Class III environment. The distribution of carbapenem-resistant Acinetobacter spp. and CRK spp. differed significantly among the four environmental classes, analyzed by r×c chi-squared test (p < 0.001 and p = 0.008, respectively).

Figure 2. The contamination rates in four environment Classes.



The Carbapenem-resistant *Acinetobacter baumannii*, Carbapenemresistant *Klebsiella pneumoniae*, and Methicillin-resistant *Staphylococcus aureus* contamination rates in the four environment Classes.

Ten of the 32 carbapenem-resistant Acinetobacter spp. and one of the three CRK spp. were isolated from faucets and sinks in the Department of Thoracic Surgery, significantly higher than any other department in the hospital. These data suggested a particular problem specific to this department during the sampling period, warranting the implementation of more stringent preventive strategies against MDR bacterial infection.

Automatic faucets showed a lower contamination rate than traditional faucets

Analysis of MDR bacterial contamination by faucet type found 11 carbapenem-resistant Acinetobacter spp. and one CRK spp. isolated from the 232 traditional faucets in the hospital (4.7%) and two carbapenemresistant Acinetobacter spp. isolated from the 220 automatic faucets (0.9%), no MDR isolated from 1 pedaled tap. We found no correlation between the faucet and sink contamination types, suggesting that their MDR bacterial contamination might not be from the same sources. However, a larger sample is needed for a more reliable assessment of the correlation between faucets and sinks (Table 3).

No significant difference in the MDR bacterial rates between the sinks and faucets

Several studies have shown that sinks in hospitals can preserve multiple bacteria types [19], including P. aeruginosa, Enterobacteriaceae, and E. coli [20-22]. We further investigated whether the MDR bacterial contamination rate differed between faucet and sink

surfaces. As shown in Table 4, 13 carbapenem-resistant Acinetobacter spp. and one CRK spp. were recovered from the 455 faucets in the hospital (2.9%), and one MRSA, 19 carbapenem-resistant Acinetobacter spp., and one CRK spp. were recovered from the 455 sinks in the hospital (4.2%). The difference between the two rates was statistically insignificant.

Non-medical-use faucets had a higher MDR bacterial contamination rate than medical-use faucets

Table 5 shows the MDR bacterial contamination rates in faucets for medical and non-medical use. We recovered 13 carbapenem-resistant Acinetobacter spp. (1.9%) and one CRK spp. (CR-Kpn; 0.1%) samples from the 698 medical-use faucets in the hospital. One MRSA (0.5%), 19 carbapenem-resistant Acinetobacter spp. (9.0%), and two CRK spp. (0.9%) were recovered from the 212 non-medical-use faucets in the hospital. Non-medical-use faucets were colonized by a higher CRAb rate than medical-use faucets.

Distribution of MDR bacteria on plates without antibiotics

We detected 556 bacteria colonies on the plates without antibiotics, including 28 P. aeruginosa, 11 S. haemolyticus, 17 S. epidermidis, nine Bowman Acinetobacter, 36 A. jones, two K. pneumoniae, nine A. lwoffii, 12 P. monteilii, and 43 A. johnsonii.

Carbapenem-resistant Acinetobacter spp. was significantly easier to detect in sites with other bacteria colonies. As shown in Tables 6 and 7, only four carbapenem-resistant Acinetobacter spp. were isolated

Table 3. The distr	ibution of	MDR in mul	tiple type	s of Taps.						
Classification	MRSA	MRSA	CRAb	CRAb Ratio		CD Van	CR-Kpn	Normal without	Normal	Total
Classification	WIK5A	Ratio (%)	CKAD	(%)	р	CR-Kpn	Ratio (%)	MDR	Ratio (%)	Sample
Traditional Tap	0	0	10	4.310%		1	0.431%	221	95.259%	232
Automatic Tap	0	0	2	0.901%		0	0	220	99.09%	222
Pedaled Tap	0	0	0	0	0.076	0	0	1	100%	1

Table 4. The distribution of MDR on Taps and Sinks.

Classification	MRSA	MRSA Ratio (%)	р	CRAb	CRAb Ratio (%)	р	CR- Kpn	CR-Kpn Ratio (%)	р	Normal without MDR	Normal Ratio (%)	Total	Kappa
Taps	0	0		12	2.637%		1	0.220%		442	97.143%	455	
Sinks	1	0.2197%	0.317	20	4.396%	0.150	2	0.4396%	1.0	432	94.945%	455	-0.004

Table 5. The distribution of MDR on medical use or not faucets.

Medical use or not Classification	MRSA	MRSA Ratio (%)	р	CRAb	CRAb Ratio (%)	р	CR-Kpn	CR-Kpn Ratio (%)	р	Normal Without MDR	Normal Ratio (%)	Total
For medical staff	0	0		12	1.719%		1	0.1433%		685	98.138%	698
Not for medical staff	1	0.4716%	0.069	20	9.434%	0.053	2	0.9434%	0.075	189	89.151%	212

Environment	Counts and Ratio	Bacteria 0	Bacteria Not exceeding the limit	Bacteria Out of Specification	Total	р
Ι	Counts	122	34	72	228	
	Ratio in environments	53.5%	14.9%	31.6%	100.0%	
	Ratio in total bacteria	35.1%	29.8%	16.1%	25.1%	
II	Counts	77	18	129	224	
	Ratio in environments	34.4%	8.0%	57.6%	100.0%	
	Ratio in total bacteria	22.1%	15.8%	28.8%	24.6%	
III	Counts	53	30	149	232	
	Ratio in environments	22.8%	12.9%	64.2%	100%	
	Ratio in total bacteria	15.2%	26.3%	33.3%	25.5%	
IV	Counts	96	32	98	226	
	Ratio in environments	42.5%	14.2%	43.4%	100%	
	Ratio in total bacteria	27.6%	28.1%	21.9%	24.8%	< 0.001

Table 6.	The	distribution	of bacteria	pattern on	plates	without	antibiotics

on the faucets and sinks where no other bacteria were detected. In contrast, 22 carbapenem-resistant *Acinetobacter spp.* were isolated on the faucets and sinks with diverse bacterial contamination.

Discussion

The current study analyzed 455 faucets and 455 sink samples from 64 wards and clinics in the hospital. We found that the CRAb contamination rate of faucets and sinks was more common than MRSA and CR-Kpn. One MRSA, 32 carbapenem-resistant *Acinetobacter spp.*, and three CR-Kpn isolates were recovered from the 910 samples. Of these, 13 carbapenem-resistant *Acinetobacter spp.* and one CRK *spp.* were identified in the 455 faucets samples.

CRAb has emerged as one of the most prevalent MDR bacteria in hospitals [23]. Faucets and sinks contaminated by CRAb could serve as reservoirs for transmitting MDR bacteria [15]. Several studies have reported that antibiotic elements, e.g., drug-resistance genes of Gammaproteobacteria, might be spread from colonized sink traps to vulnerable patients in the hospital [24-27]. The mechanisms or transmission routes are not fully understood. An in situ study by Kotay et al. [21] on green fluorescent proteinexpressing E. coli examined the spread of the bacteria from the sink to patients. They found the bacteria biofilm formed in the P-taps spread upwards to the strainer and, from there, dispersed through droplets to the surrounding area. This study provided experimental evidence that organisms can spread by upward mobilization and droplet dispersion rather than spreading directly from the tap to the environment. Zong's group [28] performed a prospective multicenter whole-genome sequencing study of all isolates recovered from sink samples collected in 16 ICUs of 11 hospitals. Through multilocus sequence typing, sequence type analysis, and inter-isolate correlation analysis, they traced the source of a clinical isolate cluster to certain sink isolates.

The possibility that the faucet was the source of the CRAb infection in the ward cannot be excluded. Further studies, including multilocus sequence typing and single nucleotide polymorphism analysis, are needed to determine the association between the faucet/sink and clinic patient isolates.

Antimicrobial stewardship in the hospital has played an increasingly important role in controlling the emergence of antibiotic resistance [29]. In China, tremendous efforts have been made to establish a comprehensive management system of antimicrobial stewardship, including limiting the unnecessary use of antibiotics and normalizing antibiotic treatment procedures; however, little progress has been made [30]. More efforts are required to alleviate antimicrobial resistance. Various bacterial identification methods have been used, including 16s rRNA, reverse transcription real-time polymerase chain reaction, and fluorescence *in situ* hybridization [31]. The MALDI-TOF-MS method allows for more accurate identification of bacterial colonies [31], and its use in

 Table 7. Correlation of CRAb contamination and bacteria amount.

Bacteria 0 / Not exceeding the limit / Out of Specification	Counts and Ratio	CRAb Not detected	CRAb Detected	Total	р
0	Counts	344	4	348	
	Ratio in total bacteria	98.9%	1.1%	100.0%	
	Ratio in CRAb	39.2%	12.5%	38.2%	
Not exceeding the limit	Counts	108	6	114	
-	Ratio in total bacteria	94.7%	5.3%	100.0%	
	Ratio in CRAb	12.3%	18.8%	12.5%	
Out of Specification	Counts	426	22	448	
-	Ratio in total bacteria	95.1%	4.9%	100%	
	Ratio in CRAb	48.5%	68.8%	49.2%	0.009

analyzing clinical and environmental samples has been reported by several groups [32-33]. In our study, we implemented MALDI-TOF-MS to analyze the bacteria isolated from the faucet and sink samples.

Strategies that can improve environmental hygiene include replacing hand-operated faucets with footoperated ones or introducing faucets that are less likely to transmit pathogens into wards. Shaw et al. [34] reported that removing the water sinks from all patient rooms in the ICU and implementing a new water-safe policy helped control the endemic infection-causing gram-negative bacteria, especially MDR Κ. pneumoniae. Jonge et al. suggested that installing disinfecting sink drains in ICUs would reduce the colonization and patient infection rates with MDR P. aeruginosa [35]. Our study supported the potential role faucets and sinks play in the MDR bacterial infection epidemic. Strategies for improving the hand hygiene facilities will improve the control over MDR infection.

Even though sparse MDR bacterial contamination on the faucets represents qualified prevention and control of nosocomial infections, more sampling sites are needed to significantly determine the contamination rate of rare MDR bacteria such as MRSA and CR-Kpn. It is necessary to identify them to ascertain whether the contamination is an accidental occurrence or has a regular pattern. Moreover, other factors might also affect the investigation results, including the frequency at which the faucets are utilized in hand hygiene, their locations, the temperature in the ward or clinic, and whether the clinical waste is dumped in the sinks. These influence the factors could MDR bacterial contamination rate and should be considered.

Conclusions

The current study examined the MDR bacterial contamination rates on faucets and sinks in multiple sites around the hospital, covering the ICU, wards, and clinics. The fewer bacteria recovered from automatic faucets suggests that the MDR bacterial contamination could be reduced by using these faucets to eliminate contact. Non-contact faucet use could contribute to controlling infections originating from sinks and faucets. Further investigation will be performed to unveil the routes and mechanisms of MDR bacterial transmission in patients and the hospital. The molecular-level correlation between the MDR bacteria contaminating the faucets and sinks and those infecting patients will also be examined. Further study is necessary to determine whether faucets and sinks are a reservoir of MDR bacteria that can be transmitted to the patients. Prospective investigations of the transmission routes will contribute to this endeavor.

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Conflict of interests

No conflict of interests is declared.

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