



The role of the intensive care unit environment and health-care workers in the transmission of bacteria associated with hospital acquired infections

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Summary The goal of this study was to attempt to determine the rate of contamination of health-care workers' (HCWs) hands and environmental surfaces in intensive care units (ICU) by the main bacteria associated with hospital acquired infections (HAIs) in Tehran, Iran. A total of 605 and 762 swab samples were obtained from six ICU environments and HCWs' hands. Identification of the bacterial isolates was performed according to standard biochemical methods, and their antimicrobial susceptibility was determined based on the guidelines recommended by clinical and laboratory standards institute (CLSI). The homology of the resistance patterns was assessed by the NTSYSsp software. The most frequent

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bacteria on the HCWs' hands and in the environmental samples were *Acinetobacter baumannii* (1.4% and 16.5%, respectively), *Staphylococcus aureus* (5.9% and 8.1%, respectively), *S. epidermidis* (20.9% and 18.7%, respectively), and *Enterococcus* spp. (1% and 1.3%, respectively). Patients' oxygen masks, ventilators, and bed linens were the most contaminated sites. Nurses' aides and housekeepers were the most contaminated staff. Imipenem resistant *A. baumannii* (94% and 54.5%), methicillin-resistant *S. aureus* (MRSAs, 59.6% and 67.3%), and vancomycin resistant Enterococci (VREs, 0% and 25%) were detected on the hands of ICU staff and the environmental samples, respectively. Different isolates of *S. aureus* and *Enterococcus* spp. showed significant homology in these samples. These results showed contamination of the ICU environments and HCWs with important bacterial pathogens that are the main risk factors for HAIs in the studied hospitals.

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Introduction

HAIs, such as bacteremia, pneumonia, urinary tract and skin or soft tissue infections, are among the most frequent complications that occur in hospitalized patients in intensive care units (ICUs) [1,2]. Patients in ICUs are at the highest risk for HAIs because of invasive medical procedures during their hospitalizations. The ICU staff and physicians can serve as vehicles for the spread of resident pathogens from different hospital wards to ICUs [2]. Accordingly, the hands of HCWs and ICU personnel require the greatest hygiene standards. Contamination of the ICU environment also plays an important role in the acquisition of nosocomial pathogens by both patients and HCWs. Investigation of the rate of bacterial contamination of the hands of HCWs and the ICU environmental surfaces could provide recommendations for preventing transmission of pathogenic bacteria to patients and personnel in health-care settings [3].

Bacterial strains from patients, the hands of HCWs, and the ICU environment have been demonstrated to be associated with hospital-acquired outbreaks by several studies [4–7]. Although most enteric Gram-negative bacilli cannot remain viable on the dry surfaces of medical equipment or in the ICU environment and are sensitive to common disinfectants, biofilm-forming bacteria, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, are highly resistant to such harsh conditions and are strongly associated with HAIs through contaminated medical devices and other environmental equipment in hospitals [4]. HCWs and ICU staff can serve as major reservoirs of common bacterial pathogens, such as vancomycin resistant *Enterococci* (VRE) [5] and other members of the *Enterobacteriaceae* and multi drug resistant (MDR) Gram-positive and Gram-negative bacteria, which

are responsible for HAIs [6]. Colonization and transmission of these hyper resistant bacterial pathogens are generally considered to be a major problem in infection control programs in ICUs. The role of the ICU staff and environment in HAIs should be considered when devising strategies to prevent or reduce the occurrence of these infections among the highly sensitive patients. There are some reports that showed poor hand hygiene compliance among different hospital staff in Iran [7–9]. However, few data exist on the microbial contamination of HCWs' hands and the hospital environment in the studied hospitals. This study was designed to investigate the frequency and resistance patterns of the main bacterial agents responsible for HAIs on the hands of HCWs and the ICU environments in six ICUs in Tehran, Iran.

Methods

Design and setting

The study was conducted in medical hospitals of Shahid Beheshti University of Medical Sciences in Tehran, Iran, from August 2010 through September 2012. Samples of ICU HCWs aged 25–40 years old, including nurses (79, 426 samples), physicians (6, 26 samples), housekeepers (23, 155 samples), secretaries (4, 32 samples), and nurses' aides (16, 123 samples), were taken randomly after hand hygiene and before any contact with patients, respectively. The ICU environmental surfaces and devices, which were in contact with the hospitalized patients, were also randomly selected for sampling during routine daily patient care in six ICUs in different hospitals (Table 1). All of the HCWs and equipment were sampled several times during the study period. Accordingly, based on the

Table 1 Bacterial isolates from the ICU HCWs hands.

HCWs (N)	<i>S. aureus</i>	<i>S. epidermidis</i>	<i>A. baumannii</i>	<i>S. saprophyticus</i>	<i>Streptococci</i>	<i>Bacillus</i> spp.	<i>Enterococcus</i> spp.	Total
Nurse (426)	13 (3.05)	107 (25.11)	6 (1.41)	0	8 (1.88)	4 (0.94)	6 (1.41)	144 (33.8)
Physicians (26)	4 (15.38)	3 (11.54)	0	0	0	0	0	7 (26.92)
Housekeepers (155)	16 (10.32)	25 (16.13)	1 (0.65)	1 (0.65)	2 (1.29)	3 (1.93)	1 (0.65)	49 (31.61)
Secretary (32)	3 (9.37)	6 (18.75)	1 (3.12)	0	2 (6.25)	2 (6.25)	2 (3.12)	15 (46.87)
Nurse's aide (123)	11 (8.94)	25 (20.31)	3 (2.44)	0	4 (3.25)	5 (4.06)	0	48 (39.02)

types of surfaces that had the greatest and lowest contact with patients, defined numbers of samples were randomly selected for microbial examination. In this study, the procedures were performed without informing the HCWs of the ICUs in advance of the timing and sites of sampling. The environmental samples included bed linens, beds, nurses' stations,ambu bags, patients' tables, oxygen masks, ventilators, telephone handsets, patient files, and other devices that were in contact with the patients.

Bacterial isolation and identification

The standard swab culture method was used to recover bacteria from the surfaces [10]. Surfaces of the ICU environment, medical devices and HCWs' hands were swiped by sterile cotton swabs. The swabs were immediately streaked onto Blood agar and MacConkey agar media for the initial screening of suspected pathogens and an estimation of the total colony counts. The inoculated plates were transported to the laboratory at ambient temperatures and were incubated at 37°C for 24 h under aerobic conditions. The grown colonies were identified by Gram staining and standard biochemical tests. For the biochemical identification of the Gram-positive isolates, catalase, oxidase, nitrate reduction, hydrolysis of esculin, fermentation of mannitol and glycerol, and coagulase tests were used. As a confirmatory test, resistance of Gram-positive cocci to novobiocin was tested to differentiate *S. epidermidis* from *S. saprophyticus*. Members of the *Streptococcus* genus were examined by tests assessing growth in bile esculin medium, catalase and hemolytic reactions. In addition, the common characteristic biochemical tests for Gram-negative bacteria, including oxidase and IMVIC reactions, citrate utilization, decarboxylation of lysine, and urease activity, were carried out [11]. *Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *Klebsiella pneumonia* ATCC 700603, *Enterococcus faecalis* ATCC 51559 and *Staphylococcus aureus* ATCC 25923 were used as the control strains.

Antimicrobial susceptibility testing

Antibiotic susceptibility testing was based on the disk diffusion method and was performed on Mueller-Hinton agar (MHA) plates according to the latest CLSI guidelines [12]. The antibiotic discs were purchased from Padtan Teb (Iran). The tested antibiotics included imipenem (10 mcg), vancomycin (30 mcg), oxacillin (1 mcg), ciprofloxacin (5 mcg), co-amoxiclav (30/10 mcg), gentamicin (10 mcg), norfloxacin (10 mcg), ceftioxin (30 mcg),

lomefloxacin (30 mcg), cefotaxim (30 mcg), amikacin (30 mcg), piperacillin (100 mcg), linezolid (30 mcg) and sulfamethoxazole (25 mcg). *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as quality control strains.

Data analysis

Statistical analyses were performed using SPSS (version 17.0). A *p* value <0.05 was considered statistically significant. NTSYSsp software was used to analyze the possible association between the bacterial isolates based on the diversity of their resistance profiles.

Results

The prevalence of bacterial contamination among HCWs and ICU environments

A total of 51% and 34.5% of the environmental (313/605) and HCWs' (263/762) samples were contaminated with different bacterial species in the studied ICUs. The isolated bacteria were both Gram-positive (60.7%) and Gram-negative bacteria (39.3%). Multi-bacterial contamination of the environmental samples was estimated to be 11% and was mainly found on ventilators (40.4%, *K. pneumoniae*, *S. aureus*, and *S. epidermidis*), beds (14.8%, *S. aureus*, *P. aeruginosa*, *E. coli*, *A. baumannii*, *S. epidermidis*, *K. pneumoniae*, and *Streptococci*) and patient files (10.6%, *S. aureus*, *A. baumannii*, *S. epidermidis*, and *S. saprophyticus*). The coexistence of *A. baumannii* plus *S. epidermidis* (51%), and *A. baumannii* plus *S. aureus* (25.5%) was determined to be the most common multi-bacterial contamination among the studied environmental samples. In total, the frequency of isolated microorganisms was similar among the ICU environments and HCWs' hands (Table 2). The estimated rates of contamination among different environmental samples varied from 21.4% to 82.91%. The most frequently contaminated samples came from ventilators (82.91%), patient oxygen masks (81.81%) and bed linens (67.65%). *K. pneumoniae*, which mainly colonized ventilators 6/11 (54.4%), was the most frequent coliform bacteria among these samples (1.8%). *E. coli* and *Enterococci*, markers of fecal contamination, were observed in approximately 0.5% and 1.3% of the samples, respectively (Table 3). Between 26.92% and 46.87% of the HCWs' hands were contaminated. The rate of contamination varied among ICU secretaries (46.87%), nurses' aides (39.02%), housekeepers (31.61%), nurses (33.80%),

Table 2 Frequency of bacterial species detected in the ICU environments and hands of the ICUs' staff among six hospitals.

Bacterial isolates, N (%)	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>S. saprophyticus</i>	<i>S. epidermidis</i>	<i>A. baumannii</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>Enterobacter</i>	<i>Streptococci</i>	<i>Bacillus</i> spp.	<i>Proteus</i> spp.	<i>Enterococcus</i> spp.
ICU environment	49 (8.1)	11 (1.8)	2 (0.3)	113 (18.7)	100 (16.5)	8 (1.3)	3 (0.5)	2 (0.3)	4 (0.7)	11 (1.8)	2 (0.3)	8 (1.3)
HCWs' hands	47 (5.9)	0 (0)	1 (0.1)	166 (20.9)	11 (1.4)	0 (0)	0 (0)	0 (0)	16 (2.01)	14 (1.8)	0 (0)	8 (1)

N: number of samples with positive culture results for each bacterial genus. Frequency of the bacterial isolates among total samples from ICU environments and HCWs' hands was presented in the parenthesis.

Table 3 Types and percentages of the bacterial isolates in different environmental samples in the studied ICUs.

Site of isolation, N (%)	<i>S. aureus</i>	<i>K. pneumoniae</i>	<i>S. saprophyticus</i>	<i>S. epidermidis</i>	<i>A. baumannii</i>	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>Enterobacter</i>	<i>Streptococci</i>	<i>Bacillus</i> spp.	<i>Proteus</i> spp.	<i>Enterococcus</i> spp.	Total
Ambu bag (14)	0	1 (7.14)	0	1 (7.14)	0	0	0	0	0	1 (7.14)	0	0	3 (21.43)
Nurse's station (16)	0	0	0	1 (6.25)	3 (18.75)	0	0	0	0	0	0	0	4 (25)
Oxygen mask (11)	1 (9.09)	0	0	1 (9.09)	5 (45.45)	0	1 (9.09)	0	0	0	0	1 (9.09)	9 (81.81)
Ventilator (117)	16 (13.68)	6 (5.13)	0	26 (22.22)	39 (33.33)	1 (0.85)	1 (0.85)	0	0	4 (3.42)	2 (1.71)	2 (1.71)	97 (82.91)
Bed (128)	14 (10.94)	1 (0.78)	0	25 (19.53)	16 (12.5)	3 (2.34)	1 (0.78)	0	3 (2.34)	1 (0.78)	0	1 (0.78)	65 (50.78)
Patients' table (124)	7 (5.65)	2 (1.62)	0	28 (22.58)	13 (10.48)	2 (1.61)	0	2 (1.61)	0	2 (1.61)	0	0	56 (45.16)
Bed linen (34)	5 (14.71)	0	0	4 (11.76)	12 (35.3)	0	0	0	0	0	0	2 (5.88)	23 (67.65)
Patient files (97)	4 (4.12)	0	1 (1.03)	15 (15.46)	8 (8.24)	0	0	0	1 (1.03)	2 (2.07)	0	0	31 (31.96)
Phone handset (8)	1 (12.5)	0	0	1 (12.5)	0	0	0	0	0	0	0	0	2 (25)
Others (56)	1 (1.79)	1 (1.79)	1 (1.79)	11 (19.64)	4 (7.14)	2 (3.57)	0	0	0	1 (1.79)	0	2 (3.57)	23 (41.07)

and physicians (26.92%) (Table 1). Multi-bacterial contamination was detected in 0.3% of the HCW samples. All of these isolates, except for *A. baumannii*, were related to Gram-positive bacteria. The most frequent bacteria belonged to *S. epidermidis* (20.9%), *S. aureus* (5.9%), *Streptococci* (2.01%), *Bacillus* spp. (1.8%), *A. baumannii* (1.4%), *Enterococci* (1%), and *S. saprophyticus* (0.1%) genera. *S. aureus* and *S. epidermidis* were the common bacterial isolates found in both the environmental (8.1% and 18.7%) and HCWs' (5.9% and 20.9%) samples, respectively (Table 2).

Antimicrobial resistance patterns

The results of the in vitro susceptibility testing are shown in Tables 4 and 5. Among the *S. aureus* samples, methicillin-resistant (MRSA) isolates were observed among 59.6% and 67.3% of the ICU staffs' hands and environmental samples, respectively. However, all of the isolates were susceptible to vancomycin and linezolid. VREs were found among 25% of the *Enterococcus* spp. isolates that were obtained from the ICU environments, but not among the isolates from staff hands (Tables 4 and 5). VREs were frequently detected in ventilators, and MRSA were mainly observed among the hands of housekeepers (50%) and ventilators (33.36%). In the hands of HCWs, resistance to extended spectrum cephalosporins, including cefepime, ceftazidime, and ceftotaxim, was only found in *A. baumannii* (51.5%), while this resistance in the ICU environmental samples was observed in *A. baumannii* (95.5%), *E. coli* (77.8%), *P. aeruginosa* (62.5%), *Enterobacter* spp. (50%), and *K. pneumoniae* (51.5%). Imipenem resistance phenotypes were found among 67% of the environmental isolates, of which *A. baumannii* had the highest rates of resistance (94/100, 94%) (Table 6). Ventilators were considered to be the main reservoir of imipenem resistant bacteria among the environmental samples (Tables 4 and 5). While the *Streptococcus* and *S. saprophyticus* isolates were among the most sensitive isolates to the studied antibiotics, resistance to gentamicin was observed among all of them (100%). Nearly 50% and 25% of the *Enterococci* isolates from the environmental and HCWs' hands samples were resistant to this antibiotic, respectively (Tables 4 and 5). Multidrug resistance (MDR) phenotypes were detected among 79.38% (127/160) of the environmental and 35.89% (14/39) of the HCWs' samples. In the case of samples from the ICU environments, the MDR phenotype was most common in *A. baumannii* (94/100, 94%), followed by MRSA (22/33, 66.6%), *K. pneumoniae* (6/11, 54.5%), *Enterococcus* spp. (4/8 50%), and *P. aeruginosa* (1/8, 12.5%). On the other hands,

Table 4 Susceptibility testing to antimicrobial agents of isolated species from environment.

Antibiotics ^a (% R) ^b	IMP	V	OX	CP	AMC	GM	TE	NOR	FOX	LOM	CTX	AM	PIP	FEP	Lz	SXT	C	E
<i>E. coli</i>	66.8	—	66.7	0	100	66.7	100	—	100	—	66.7	100	—	66.7	0	0	—	—
<i>Streptococci</i>	0	0	—	0	—	100	—	—	—	—	—	0	—	0	0	50	0	—
<i>A. baumannii</i>	94	—	—	93	100	71	39	—	—	81	97	—	92	94	0	89	—	—
<i>P. aeruginosa</i>	25	—	—	25	100	37.5	—	37.5	—	37.5	—	—	25	62.5	0	12.5	—	—
<i>Staphylococcus saprophyticus</i>	0	0	0	0	—	0	0	—	—	—	—	—	—	—	0	—	—	0
<i>Staphylococcus aureus</i>	32.7	0	67.3	28.6	—	32.7	44.9	—	—	—	—	—	—	—	0	4.1	—	37.5
<i>Enterobacter</i> spp.	50	—	—	0	50	0	50	—	50	—	—	50	—	50	0	100	—	—
<i>K. pneumoniae</i>	45.5	25	—	45.5	81.8	63.6	54.5	—	27.3	—	63.6	88.9	—	63.6	0	81.8	—	—
<i>Enterococcus</i> spp.	—	25	—	25	—	50	37.5	—	—	—	—	37.5	—	—	0	100	0	75
Total (%)	67	3.2	64.8	63.41	97.6	57.2	42.3	37.5	43.8	75	93	57.7	87	85.8	0	60.4	0	41.4

^a IMP, imipenem; V, vancomycin; OX, oxacillin; CP, ciprofloxacin; AMC, amoxi-clav; GM, gentamicin; NOR, norfloxacin; FOX, ceftioxin; LOM, lomefloxacin; CTX, cefotaxim; AM, Amikacin; PIP, piperacillin; Lz, linezolid; SXT, sulfamethoxazole; FEP, cefepime.

^b R: Percentage of resistance to each antibiotic. The results were interpreted according to the CLSI guideline.

Table 5 Susceptibility testing to antimicrobial agents of isolated species from HCWs hands.

Antibiotics ^a (% R) ^b	IMP	V	OX	CP	AMC	GM	TE	FOX	LOM	CTX	AM	PIP	FEP	Lz	SXT	E	C
<i>S. aureus</i>	0	0	59.6	12.8	—	4.3	17	—	—	—	—	—	—	0	4.3	25.5	—
<i>S. saprophyticus</i>	0	0	0	0	—	0	0	—	—	—	—	—	—	0	—	0	—
<i>A. baumannii</i>	54.5	—	—	63.6	100	27.3	36.4	0	63.6	100	—	54.5	54.5	—	36	—	—
<i>Streptococci</i>	0	0	—	—	—	100	—	—	0	—	0	—	0	0	62.5	—	0
<i>Enterococcus spp.</i>	—	0	—	0	—	25	0	—	—	—	0	—	—	0	100	50	0
Total	8	0	58.3	21	100	27.7	18.2	0	25.9	100	0	54.5	22.2	0	21.6	29.1	0

^a IMP, imipenem; V, vancomycin; OX, oxacillin; CP, ciprofloxacin; AMC, amoxi-clav; GM, gentamicin; NOR, norfloxacin FOX, cefoxitin; LOM, lomefloxacin; CTX, cefotaxim; AM, Amikacin; PIP, piperacillin; Lz, linezolid; SXT, sulfamethoxazole; FEP, cefepime.

^b R: percentage of resistance to each antibiotic. The results were interpreted according to the CLSI guideline.

MDR phenotypes in the HCWs' samples were frequently detected in *A. baumannii* (7/11, 63.6%) and *S. aureus* (6/53, 11.3%) (Table 7).

Biotyping and cluster analysis

Biotyping of the strains subjected to antibiotic susceptibility testing was performed according to their resistance profiles based on a distance cut-off point of 100%. While the results showed phenotypic associations between the isolates from the environmental and the HCWs' samples, different clusters of the resistance patterns were detected in all hospitals. No single dominant resistance clone of the studied bacteria was characterized in these ICUs (Supplementary data Fig. 1).

Discussion

Normal human skin is colonized by bacteria, with total aerobic bacterial counts ranging from more than 4×10^4 CFU/cm² to 1×10^6 CFU/cm². The total bacterial counts on the hands of HCWs ranged from 3.9×10^4 to 4.6×10^6 CFU/cm² [13]. HCWs are often contaminated with microbial agents in the hospital environment. Contact between the contaminated HCWs and hospitalized patients in ICUs might cause serious infections. Because nearly 10^6 skin squamous contains viable microorganisms that are shed daily from normal skin, it is not surprising that patient gowns, bed linens, bedsides and other objects in the ICU environment become contaminated. In this study, the swabbing method was used instead of the direct plating method to determine the diversity of bacterial species colonizing the ICU environments and HCWs' hands.

The results of our study showed contamination of the inanimate environments by diverse groups of bacteria, including both Gram-positive (60.7%) and Gram-negative (39.3%) types. Similar rates of contamination with both Gram-positive and Gram-negative bacteria (50.4% and 49.5%, respectively) were observed in the studied respiratory devices (ventilators and oxygen masks). In contrast to our results, in a study in India, it was reported that Gram-negative bacteria constituted the dominant colonized bacteria compared with Gram-positive cocci among the bacteria isolated from respiratory devices (68.85% versus 31.14%) [14]. Ventilators, patients' oxygen masks and bed linens were among the most frequently contaminated environmental samples in our study. This contamination was particularly by *S. aureus* and *A. baumannii*, which are more resistant to desiccation and disinfectants.

Table 6 Frequency of main associated bacterial isolates with HAIs and their antimicrobial resistance rates.

Bacteria and antibiotics	Environment		HCWs		Total	
	Frequency (%)	Resistance (%)	Frequency (%)	Resistance (%)	Frequency (%)	Resistance (%)
<i>E. coli</i>	0.93		0		0.46	
Imipenem		66.8		—		66.8
<i>P. aeruginosa</i>	2.49		0		1.25	
Cefepime		62.5		—		62.5
Piperacillin		25		—		25
Imipenem		25		—		25
<i>K. pneumoniae</i>	3.43		0		1.7	
Imipenem		45.5		—		45.5
<i>Enterococcus</i> spp.	2.49		3.04		2.77	
Vancomycin		25		0		12.5
<i>S. aureus</i>	15.2		17.8		16.5	
Oxacillin		67.3		59.6		63.5
<i>A. baumannii</i>	31.15		4.1		17.63	
Imipenem		94		54.5		74.3

Contamination of the patients' files was observed in 31.95% of the cases, among which coagulase negative *Staphylococci* (CNS) (16.5%), *Acinetobacter* spp. (8.25%), and *S. aureus* (4.1%) were among the more frequent isolates. In a study in Saudi Arabia, the total contamination of patient files in ICUs was estimated to be 85.2% and was higher than our rates. They reported *P. aeruginosa* to be the most commonly isolated bacteria (32.3%), but MRSA was similarly isolated from 6.8% of their ICU patient files [15]. Bacterial contamination was found in 25% of phone handsets (*S. aureus* and *S. epidermidis*), which was lower than what was reported by Messina et al. in Italy (89.2%, including *Staphylococci*, *Enterococci*, and *E. coli*) and Brady et al. in the UK (95.7%, including *S. aureus*, CNS, *Enterococcus* spp., *Bacillus* spp., and *E. coli*) [16,17]. Bacterial contamination of beds and bed linen samples for at least one bacterial species was 45.3% and 61.8%, respectively. The common bacterial species in these samples included *S. aureus* (10.9% and 14.7%), CNS (19.5% and 3.2%), *Acinetobacter* spp. (12.5% and 35.3%), and *Enterococcus* spp. (1.5% and 3.2%). The estimated contamination rate in the case of bed linen was considerably lower than that reported in France in 2013 (mainly including *Staphylococci* 93%; *Pseudomonas* spp. 23%, and *Micrococci* 89%) [18]. Most of these isolates were among the bacteria commonly found in the ICU environment or on the HCWs' skin. This finding suggests the occurrence of cross contamination.

HCW hands are major sources of transmission of nosocomial pathogens [19]. This bacterial contamination is often acquired due to direct contact with patients, body fluid secretions, or touching contaminated environmental surfaces in the ICUs. The

results of the present study show that most of the isolated bacteria from the hands of HCWs are skin microbiota. However, 10.41% of the samples were contaminated with pathogens that are known to be associated with HAIs, including *Enterococci* spp., *S. aureus* and *Acinetobacter* spp. Secretaries had the highest contamination rate among the ICU staff (46.87%). Larson et al. and Waters et al. reported that 21% and 30% of hospital employees' hands were persistently colonized by Gram-negative bacilli, respectively, including *Acinetobacter*, *Klebsiella*, *Enterobacter*, *S. marcescens*, and *E. coli* [19]. In a study by Khodavaisy et al. higher rates of colonization of *Klebsiella* spp. (7.9%), *Enterobacter* spp. (4.7%), *E. coli* (3.9%), *Acinetobacter* spp. (3.1%), and *Pseudomonas* spp. (2.3%) were reported among HCWs' hands [20]. Colonization of the HCWs' hands with Gram-positive bacteria was also reported by some studies. Sepehri et al. showed that nearly 40% of HCWs' hands had bacterial contamination, mostly with *S. epidermidis*, while contamination with nosocomial pathogens was observed among 6% of HCWs' hands [21].

Our results showed alarming rates of drug resistance in the bacteria colonizing different surfaces of the studied ICUs. It seems that these places are possible sources of common nosocomial bacterial pathogens that are resistant to antibiotics used for patients in these hospitals. More than 50% of the *S. aureus* isolates in this study had an in vitro oxacillin resistance phenotype. Ulger et al. found a prevalence of 37.7% of MRSA strains among the hands of HCWs [22]. This rate is far higher than those reported in Libyan hospitals (19%) [23] and in previous reports from Iran (5.5–11.38%) [24,25]. Fortunately, no vancomycin resistant *S. aureus* was

Table 7 Distribution of resistance patterns to more than three antimicrobial categories (MDR) in the ICU environments and hands of the ICU staff among six hospitals.

ICUs environment sites		HCWs' hands samples	
MDR phenotype	Frequency, N (%)	MDR phenotype	Frequency, N (%)
<i>S. aureus</i> (MRSA)			
SXT, CP, GM, OX, E	1/33 (3.03)	CP, GM, TE, OX, E	3/28 (10.71)
SXT, TE, OX, E	1/33 (3.03)	TE, OX, E	3/28 (10.71)
CP, GM, OX, E	3 (9.09)	CP, OX, E	4/28 (14.28)
CP, GM, TE, OX, E	8 (24.24)	Total	7/28 (25)
CP, GM, TE, OX	1/33 (3.03)		
CP, GM, OX, E	1/33 (3.03)		
CP, TE, OX, E	1/33 (3.03)		
GM, TE, OX, E	2 (6.06)		
GM, TE, OX	1/33 (3.03)		
TE, OX, E	3 (9.09)		
Total	22/33 (66.66)		
<i>Enterococcus spp.</i>			
CP, GM, TE, V, AM	1/8 (12.5)		
GM, TE, V, AM	2/8 (25)		
GM, TM, AM	1/8 (12.5)		
Total	4/8 (50)		
<i>P. aeruginosa</i>			
IPM, CP, FEP, GM,	1/8 (12.5)		
<i>Acinetobacter spp.</i>			
IPM, SXT, CP, FEP, GM, TE	27/100 (27)	IPM, SXT, CP, FEP, GM, TE	1/11 (9.09)
IPM, SXT, CP, FEP, GM,	37 (37)	IPM, SXT, CP, FEP,	1/11 (9.09)
IPM, SXT, CP, FEP	15 (15)	IPM, SXT, CP, GM, TE	2 (18.18)
IPM, SXT, CP, FEP, TE	11 (11)	IPM, SXT, CP, FEP, GM	1 (9.09)
IPM, CP, FEP, SXT, GM	2 (2)	IPM, SXT, CP, FEP, TE	1 (9.09)
IPM, CP, FEP	1 (1)	Total	7 (63.63)
SXT, CP, FEP, GM,	1 (1)		
Total	94/100 (94)		
<i>Klebsiella spp</i>			
IPM, CP, FEP, GM, TE, AMC, FOX	2/11 (18.18)		
IPM, CP, FEP, GM, AMC, FOX	2 (18.18)		
CP, FEP, GM, TE, AMC,	1 (9.09)		
FEP, GM, TE, AMC,	1 (9.09)		
Total	6/11 (54.54)		

isolated from our samples. VREs are generally considered important nosocomial pathogens because of their roles in the development of different types of HAIs [26]. The transmission of VREs to patients can occur through both contaminated HCWs and medical devices [27]. In the present study, VREs were found in 25% of the samples obtained from the environmental surfaces, mainly from ventilators. Bonten et al. found that 12% of samples from environmental surfaces in an ICU, most commonly from bedrails and sheets, are contaminated with VREs [28]. They reported that 85% of ventilated patients who acquired VRE were colonized with this pathogen by cross-colonization.

A. baumannii is an opportunistic pathogen that is frequently involved in outbreaks of infections that most frequently occur in ICUs. The spread of a single *A. baumannii* isolate as a source of a nosocomial outbreak is often linked to contaminated respiratory equipment and transmission via the hands of HCWs [29]. The estimated contamination rate of *A. baumannii* in our samples found medical equipment, especially masks and ventilators, as the main possible sources of the bacterium in the studied ICUs. The results of the antimicrobial susceptibility testing also supported this finding, and the highest resistance rate to imipenem was found among isolates from the environmental

samples (94% compared with 54.5% in the HCW samples), which revealed their importance in HAIs in these ICUs. Markogiannakis et al. obtained similar results in a study that found lower frequencies of imipenem resistant *A. baumannii*, which were obtained from HCW samples (30%) relative to the environmental samples (72.5%) [30].

The main bacterial pathogens responsible for HAIs in health care environments present MDR phenotypes [6]. Nearly 79.38% and 35.89% of the bacterial isolates from the environmental and HCW samples in this study had a MDR phenotype. *Acinetobacter* spp. was encountered as the most frequently isolated MDR species among the HCWs' hands and the environmental samples, which is consistent with other reports from Iran [31]. Despite the importance of MDR *Acinetobacter* spp. as a nosocomial pathogen, insufficient attention has been paid to its control in Asian countries. The MDR phenotype was also detected in other bacterial species, including *S. aureus*, *Klebsiella*, *Enterococci*, and *Pseudomonas* spp., that were mostly isolated from the ICU environment. In our study, a direct relationship was found between the rates of bacterial contamination (*S. aureus*, *S. epidermidis*, *Acinetobacter*, *Bacillus*, and *Enterococci*) in HCWs and the types of exposure with the environmental samples so that increased contamination of the medical devices was greatly associated with increased levels of contamination in nurses and nurses' aides, while increased rates of contamination in non-medical devices were correlated with increased rates in ICU housekeepers. These results suggest that more emphasis needs to be placed on the cleaning and disinfection of the ICU environment and education of both environmental services and HCWs. Improved cleaning of frequently contaminated areas, and routine screening of cleaning conditions, could be helpful for controlling infections in these ICUs.

Conclusion

These results identify the ICU staff as well as environmental surfaces as probable sources of bacterial agents involved in HAIs in the studied ICUs in Tehran. Although *S. aureus* and *S. epidermidis* were determined to be the dominant bacteria in both sample types, *A. baumannii* had the highest frequency on the environmental surfaces. The coexistence of different bacterial species, including both Gram-positive and Gram-negative genera, in the medical devices and the environmental surfaces highlight their importance in the production of HAIs in a cooperative manner. Homology of the

resistance patterns between the bacterial isolates from the ICU environment and HCWs suggest the occurrence of cross-contamination between them in the studied hospitals. The presence of multiple clones rather than one dominant clone was proposed in these hospitals based on the observed diversity of the resistance patterns. While HCWs had lower contamination rates compared with environmental samples, their role as vehicles of pathogenic bacteria is suspected. Molecular typing of these isolates in comparison to the isolates from clinical samples will help us to better understand these correlations. Compliance with contact precautions and more aggressive environmental cleaning may decrease such transmission in these ICUs.

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Competing interests

None declared.

Ethical approval

Not required.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jiph.2015.05.010>.

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