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Original Research Article

Hospital air: A potential route for transmission of infections caused by β-lactam–resistant bacteria

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Background: The emergence of bacterial resistance to β-lactam antibiotics seriously challenges the treatment of various nosocomial infections. This study was designed to investigate the presence of β-lactam–resistant bacteria (BLRB) in hospital air.

Methods: A total of 64 air samples were collected in 4 hospital wards. Detection of airborne bacteria was carried out using culture plates with and without β-lactams. BLRB isolates were screened for the presence of 5 common β-lactamase–encoding genes. Sequence analysis of predominant BLRB was also performed.

Results: The prevalence of BLRB ranged between 3% and 34%. Oxacillin-resistant bacteria had the highest prevalence, followed by ceftazidime- and cefazolin-resistant bacteria. The frequency of β-lactamase–encoding genes in isolated BLRB ranged between 0% and 47%, with the highest and lowest detection for OXA-23 and CTX-m-32, respectively. *Mec*A had a relatively high frequency in surgery wards and operating theaters, whereas the frequency of *bla*TEM was higher in intensive care units and internal medicine wards. OXA-51 was detected in 4 wards. *Acinetobacter* spp, *Acinetobacter baumannii*, and *Staphylococcus* spp were the most predominant BLRB.

Conclusions: The results revealed that hospital air is a potential route of transmission of BLRB, such as *Acinetobacter* and *Staphylococcus*, 2 important causative agents of nosocomial infections. Therefore, improvement of control measures against the spreading of airborne bacteria in hospital environments is warranted.

Nosocomial infections represent a significant health concern, with approximately 1.4 million people affected worldwide.[1] Approximately 60% of some these infections involve antimicrobial-resistant bacteria[2] and account for about 99,000 deaths per year in the United States.[3] Resistance to antibiotics has been a particular problem over the last decades, increasingly hampering the treatment of hospital-acquired infections.[4] Vulnerable groups of inpatients are especially at high risk of developing antibiotic-resistant infections. Such infections pose a serious threat to immunocompromised patients, causing increased morbidity, mortality, and medical costs.[2,3,5] Because of evolution and emergence of bacterial resistance to antibiotics and an increase in the number of immunosuppressed individuals worldwide because of HIV infection, chemotherapy, drug therapies, and genetic disorders, hospitals are now more often facing the problem of antibiotic-resistant nosocomial infections.[6] Several bacterial pathogens involved in epidemics of human disease have evolved into multidrug-resistant strains after antibiotic use.[5] Multidrug-resistant bacteria, specifically *Mycobacterium tuberculosis*, *Enterococcus faecium*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*, are becoming common in hospitals.[5,7] Although contact spread is the main route of transmission for most infections, there is increasing evidence that *P aeruginosa*, methicillin-resistant *S aureus* (MRSA), *M tuberculosis*, and *A baumannii* could be transmitted via the airborne route.[4,8] Airborne microorganisms are spread from numerous sources, including air conditioning systems and respiratory droplets produced by patient coughing or sneezing. Ward activities, such as those generated by

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bed making and mechanical floor cleaning, have been shown to release large numbers of bacteria into the air.[4,8] Therefore, control and effective prevention of antibiotic-resistant nosocomial infections require a better identification of airborne bacteria that are potentially harmful to patients. This information is critical to implementing more appropriate control measures against the spread of airborne hospital-acquired infections. It is clear that the potential hazards posed by airborne bacteria depend on the pathogenicity of a specific strain, environmental factors, and bacterial gene pool, including antibiotic resistance genes. Beta-lactam antibiotics are generally used to treat inpatients, accounting for approximately 50%-70% of the total antibiotic use; of these, subgroups of penicillins, cephalosporins, and carbapenems comprise the largest share of antibiotics used for human use in most countries.[9] However, the emergence of bacterial resistance to β-lactam antibiotics seriously challenges the control and treatment of some nosocomial infections. The main nosocomial pathogens in the group for which hospital air has been implicated in the transmission include MRSA and *A baumannii*.[10] There are several studies describing the presence and transmission routes of these pathogens in hospital environments, especially in intensive care units (ICU) and burn units.[2,10] However, to our knowledge, no studies have described the levels of β-lactamase–producing bacteria and their resistance genes in hospital environments.

Therefore, this study was carried out (1) to determine the prevalence of airborne β-lactam–resistant bacteria (BLRB) in different wards of 4 educational hospitals, (2) to evaluate the frequency of 5 common β-lactamase–encoding genes in isolated resistant bacteria, and (3) to identify the most predominant BLRB by 16s rRNA gene sequencing.

MATERIALS AND METHODS

Sampling sites and strategies

The study was carried out from March to December 2014 in 4 educational hospitals of Isfahan University of Medical Sciences, Isfahan, Iran. Sampling locations in each hospital included operating theatres (OTs), ICUs, surgery wards (SWs), and internal medicine wards (IMs). For detection of airborne culturable bacteria, each hospital was visited 4 times, and a total of 64 samples were collected using an all-glass impinger, containing 10 mL of phosphate buffer solution. Approximately 2,500 L of air were collected using portable pumps at a flow rate of 12.5 L/min from each site. Air sampling was performed at a height of 1.5 m above the ground level to simulate the breathing zone. At each hospital, air samples from 4 locations were taken on 1 single day from 9 AM to 12 PM after routine cleaning. During the study period, patients, staff, and patient attendants were present, but visitors were limited. Windows were kept closed during sampling, and air exchange between indoor and outdoor environments was restricted. Furthermore, a similar disinfection procedure was used for all hospitals. The characteristics of hospitals and wards are presented in Table 1.

Temperature and relative humidity were recorded by use of a portable weather station (KIMO, France) throughout the sampling periods and were approximately 26°C ± 2.3°C and 28% ± 5.6%, respectively.

All samples were transferred to the laboratory in an insulated box with cooling packs and processed immediately on arrival in the laboratory.

Table 1
Characteristics of investigated hospitals and wards

Type of ward	Construction or renovation age (y)	No. of rooms*	No. of Beds	No. of People†	Area (m^2)	Ventilation system	Sampling location
Hospital A	22						
ICU		4	8	10-15	120	Central operation HVAC‡	Inside ICU, near staff counter
OT		4	4	20-30	240	Central operation HVAC with HEPA filter	Corridor of ward
SW		12	28	40-50	360	Central operation HVAC	Corridor of ward
IM		12	28	40-50	360	Central operation HVAC	Corridor of ward
Hospital B	24						
ICU		1	6	5-10	90	Central operation HVAC	Inside ICU, between patient beds
OT		2	2	4-8	180	Central operation HVAC with HEPA filter	Corridor of ward
SW		9	20	15-30	225	Central operation HVAC	Corridor of ward
IM		6	14	15-30	150	Central operation HVAC	Corridor of ward
Hospital C	15						
ICU		1	4	6-8	70	Central operation HVAC	Inside ICU, near staff counter
OT		3	3	5-8	150	Central operation HVAC with HEPA filter	Corridor of ward
SW		11	20	8-12	220	Central operation HVAC	Corridor of ward
IM		8	18	10-15	160	Central operation HVAC	Corridor of ward
Hospital D	50						
ICU		1	8	6-10	80	Central operation HVAC	Inside ICU, near staff counter
OT		2	2	4-8	120	Central operation HVAC with HEPA filter	Corridor of ward
SW		8	22	20-35	180	Central operation HVAC	Corridor of ward
IM		8	24	20-35	180	Central operation HVAC	Corridor of ward

HEPA, high efficiency particulate air; *HVAC*, heating, ventilating, and air conditioning systems; *ICU*, intensive care unit; *IM*, internal medicine ward; *OT*, operating theatre; *SW*, surgery ward.
*Number of active rooms in the operating theater during the sampling period.
†Including patients, staff, and patient attendants.
‡Heating, ventilating, and air conditioning systems contain 65% efficiency filters.

Detection of BLRB

For detection of BLRB, aliquots of each impinger collection medium were plated onto tryptic soy agar (TSA) supplemented with antibiotics after a vigorous shaking. In this study, 3 different β-lactam antibiotics were selected from the penicillin and cephalosporin groups, including oxacillin (OX), cefazolin (CFZ), and ceftazidime (CAZ) with concentrations of 4, 32, and 30 mg/l, respectively, according to the recommendations of the Clinical and Laboratory Standards Institute.[11] For quality control, standard strains of *Escherichia coli* (ATCC 25922) and *S aureus* (ATCC 25923) were used. In parallel, aliquots of each sample were plated onto TSA without any antibiotic to determine the total number of airborne bacteria. All plates were incubated at 35°C for 2-3 days. Colonies growing on TSA with and without antibiotics were enumerated and calculated as colony-forming units (cfu) per cubic meter. All experiments were carried out in duplicates, and the mean values were analyzed. The prevalence of BLRB was calculated as the number of bacteria growing on TSA along with antibiotics divided by the number of bacteria growing on TSA without antibiotics.

Beta-lactamase–encoding genes

For detection of β-lactamase encoding genes, BLRB were isolated and subcultured on Mueller-Hinton agar plates containing specific antibiotics. All isolates were screened for the presence of 5 common β-lactamase (*bla*) encoding genes, including bla_{TEM}, $bla_{CTX\text{-}m\text{-}32}$, $bla_{OXA\text{-}23}$, $bla_{OXA\text{-}51}$, and *mec*A. *bla* genes were amplified using the following oligonucleotide primers: *mecA* (ATAGAAATGACTG AACGTCCGATA and CCAATTCCACATTGTTTCGGTCTAA),[12] bla_{TEM} (ATAAAATTCTTGAAGACGAAA and GACAGTTACCAATGCTTAATCA),[13] $bla_{OXA\text{-}23}$ (GATCGGATTGGAGAACCAGA and ATTTCTGACCGCATTTCCAT), $bla_{OXA\text{-}51}$ (TAATGCTTTGATCGGCCTTG and TGGATTGCACTTCA TCTTGG),[14] and $bla_{CTX\text{-}m\text{-}32}$ (CGTCACGCTGTTGTTAGGAA and CGCTCATCAGCAGCATAAAG).[15]

Genomic DNA was extracted from isolated colonies by boiling for 15 minutes. Ethanol precipitation was performed on aqueous phase, and then DNA was suspended in 50 μL of distilled water. Polymerase chain reaction (PCR) amplification was conducted in a final volume of 25 μL containing 2.5 μL of 10× PCR buffer (2 mM $MgCl_2$), 0.2 μM of each primer, 0.2 mM of each of the deoxynucleotides (dNTPs), 2 U of Taq DNA polymerase, and 2 μL template DNA. The PCR cycling conditions were as follows: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 45 seconds at 94°C, primer annealing at varied temperatures (according to the selected primers) for 45 seconds, primer extension at 72°C for 45 seconds, and final extension at 72°C for 10 minutes. All PCR assays included positive and negative controls. PCR products were analyzed by electrophoresis using 1.5% (w/v) agarose gel. Gels were analyzed using an ultraviolet (UV) transilluminator (UVITEC, Cambridge, United Kingdom).

Molecular identification of predominant BLRB

Extracted genomic DNA of resistant bacteria was used for PCR amplification with Eubac 27F and 1492R primers (BIONEER, Daejeon, South Korea), which amplify an approximately 1,420-bp fragment of the 16S ribosomal RNA (rRNA) gene. DNA sequencing of the amplified gene 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).

Statistical analysis

Statistical analysis was performed with SPSS 15.0 (SPSS Inc., Chicago, IL). Kolmogorov-Smirnov normality tests were performed for evaluation of the applicability of parametric or nonparametric tests. The Kruskal-Wallis test was used to compare concentration differences of airborne bacteria among the sampling sites. Differences were considered significant when probability (P) values were <.05.

RESULTS

The concentration of detected airborne bacteria and BLRB in each hospital are presented in Table 2. The highest average concentration of airborne bacteria and BLRB were obtained from hospital D. However, there was no significant difference between concentrations of bacteria among the 4 hospitals. Figure 1 shows the prevalence of specified groups of BLRB (percentage of bacteria in each specified antibiotic group with respect to total airborne bacteria) in different hospital wards. OX-resistant bacteria had the highest prevalence in all 4 hospitals (Fig 1a) and investigated hospital wards (Fig 1b). Statistical analysis showed no significant differences for BLRB between similar wards of the 4 hospitals.

The frequency of resistance genes (expressed as percent of detected genes in isolated bacteria with respect to the total number of BLRB isolates) in different hospitals and hospital wards are shown in Figure 2. A high frequency of the OXA-23 gene (28%) was observed in BLRB isolates. However, this gene was not detected in the OT of hospital D. Screening for the OXA-51 gene revealed that 5.2% of BLRB of all hospital ward samples were positive for this gene, and the highest frequency of the OXA-51 gene was detected in ICU wards (10%). This gene was not identified in isolated BLRB from hospital B. *Mec*A was detected with the highest frequency in hospital A. However, in hospital C, it was detected only in BLRB from the OT. CTX-m-32 was not detected in hospitals A and B or in the OTs and SWs of any of the hospitals. The TEM gene showed various frequencies in hospitals and hospital wards.

Sequencing results of predominant bacteria revealed the presence of 15 species among the BLRB isolated in the hospitals (Table 3).

DISCUSSION

People take to hospitals to receive treatment for diseases, including infections. However, the air quality in hospitals is a great concern because of the presence of airborne bacteria that may cause nosocomial infections.[4,8] In the present study, the concentration of airborne bacteria in hospitals ranged from 99-1,079 cfu/m^3 (Table 2), with an average of 464 cfu/m^3, which is in accordance with data reported by some authors,[4,16] but higher than that found by other authors.[7,17,18] Augustowska and Dutkiewicz showed that the mean monthly airborne bacterial level measured in a hospital ward in a pneumonologic department in Poland ranged between 257 and 436 cfu/m^3.[16] However, the concentration of airborne bacteria in hospital environments may be affected by several factors, such as ward activities, population density, ventilation efficiency, and environmental factors.[4,8]

Although there was no significant difference between concentrations of airborne bacteria among the 4 hospitals, the mean total concentration of airborne bacteria and BLRB was highest in hospital D (Table 2). Elevated levels of bacteria in the hospital may result from poor ventilation as a result of building design and construction age. Nevertheless, the ICU of hospital D had the lowest contamination among the studied ICUs, which might be related to ventilation efficiency because of the recent installation of new filters in the ventilation system. However, the mean concentration of

Table 2
Mean concentration of airborne bacteria and β-lactam–resistant bacteria (colony forming units/m^3) in different hospital wards

Type of bacteria	Hospital A				Hospital B				Hospital C				Hospital D			
	ICU	OT	SW	IM	ICU	OT	SW	IM	ICU	OT	SW	IM	ICU	OT	SW	IM
Airborne bacteria ± SD	335 ± 312	351 ± 205	337 ± 118	518 ± 360	699 ± 337	284 ±84	149 ±88	99 ± 56	354 ± 287	119 ±34	919 ± 1,154	740 ± 973	208 ±92	693 ± 305	532 ± 112	1,079 ± 485
OX	79	85	174	43	41	15	26	42	64	35	154	83	76	207	149	40
CFZ	0	0	4	0	0	5	8	0	14	9	0	0	20	0	10	9
CAZ	35	19	32	32	35	61	37	53	28	15	50	27	0	28	361	42

CAZ, ceftazidime; *CFZ*, cefazolin; *ICU*, intensive care unit; *IM*, internal medicine ward; *OT*, operating theatre; *OX*, oxacillin; *SW*, surgery ward.

airborne bacteria in ICUs, especially in the ICU of hospital B, was higher than that reported by Kim et al in 5 general hospitals in Seoul (202 cfu/m^3).[19] A possible explanation for the highest concentration of bacteria in the ICU of hospital B could be sampling location (Table 1). Our results also showed a relatively high bacterial concentration in OTs compared with other studies.[17,18] Ortiz et al reported a mean bacterial concentration of 25.6 cfu/m^3 for OT samples in a hospital located in Murcia, Spain, which was lower than the concentration of bacteria in class 100,000 cleanrooms (<100 cfu/m^3).[17] Although variable threshold limits for bacterial contamination in OT have been proposed, our results revealed bacterial concentrations exceeding the values reported by Ortiz et al for class 100,000 cleanrooms.[17] Various factors influence the concentration of airborne bacteria in OT, including efficiency of filtration systems, occupant density, and type of surgical procedures. As shown in Table 1, the relatively high number of people in the OT of hospital A may have contributed to a high concentration of airborne bacteria. The activity of OT personnel could be the main source of airborne bacteria. Stocks et al found a relationship between the number of persons present in the operating room and the CFU per cubic meter at the surgery site.[18] Hospital A is the largest public hospital among the hospitals studied, and the high concentrations of airborne bacteria observed in the OT and also in the IM and SW of this hospital may be the result of relatively crowded wards and a wide range in patient types. Hence, the type of surgical procedure and hospitalized patients might influence the concentration of airborne bacteria. Lower concentrations of bacteria in the OT of hospital C (mostly kidney diseases) and in the SW and IM of hospital B (neurologic disorders) could be related to the limited types of surgical procedures and particular type of patients in these wards. However, comparison of means and SDs of bacterial levels in the SW and IM of hospital C were higher than those in hospitals A and B. Renovation of some parts of hospital C adjacent to these wards during 1 sampling event might have contributed to these high levels.

Our study was not performed at a single type of ward or a single hospital; therefore, it may not represent variables that significantly influence the levels of airborne microorganisms. However, it appears that the concentration of airborne bacteria could be reduced through proper maintenance of air ventilation systems and improved control of human activity, especially in the ICUs and OTs of the hospitals.

The spreading of antibiotic-resistant bacteria in hospital environments poses a serious threat to human health. Our results showed a relatively high prevalence (approximately 30%-40%) of BLRB for a specific group of β-lactam antibiotics (oxacillin; Figure 1). In a study by Yadav et al, approximately 17% (52/300) of airborne bacteria isolated from a fair ground and residential area in Gwalior, Central India were found to be resistant to oxacillin.[20]

Among the BLRB, CFZ resistance was least common in airborne bacteria isolated from the 4 hospitals monitored in the present study. However, the prevalence of CFZ-resistant airborne bacteria in ICU wards (6%) was higher than in other investigated wards (Fig 1). Variation in the prevalence of BLRB in different wards and hospitals could be affected by selective pressure of antimicrobials used in hospitals. Beta-lactam antibiotics represent the largest share of antibiotics used for human use in most countries, accounting for approximately 50%-70% of the total antibiotic use.[9]

In the isolated BLRB in the present study, a low frequency of the CTX-m-32 gene was detected (Fig 2). This gene was neither detected in hospitals A and B nor in any of the OTs and SWs. Some studies showed that CTX-m-32 is the most prevalent gene in extended-spectrum β-lactamase–producing Enterobacteriaceae, such as *K pneumoniae*.[5,13] However, these studies were carried out using different types of clinical specimens, and the difference in detec-

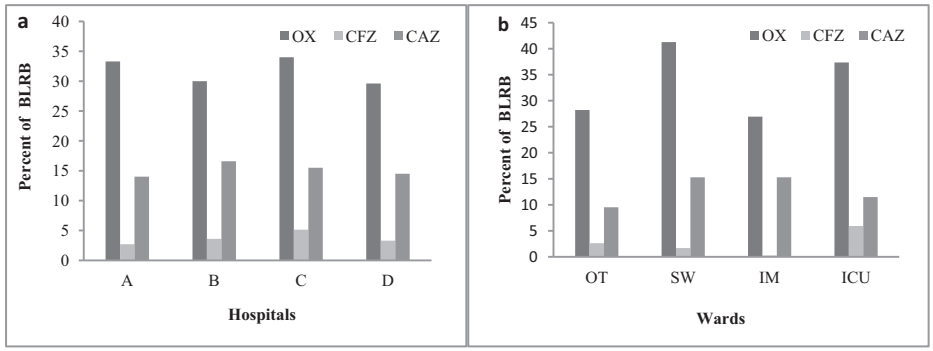

Fig 1. Prevalence of β-lactam–resistant bacteria (BLRB) isolated from (a) different hospitals and (b) different hospital wards. *CAZ*, ceftazidime; *CFZ*, cefazolin; *ICU*, intensive care unit; *IM*, internal medicine ward; *OT*, operating theatre; *OX*, oxacillin; *SW*, surgery ward.

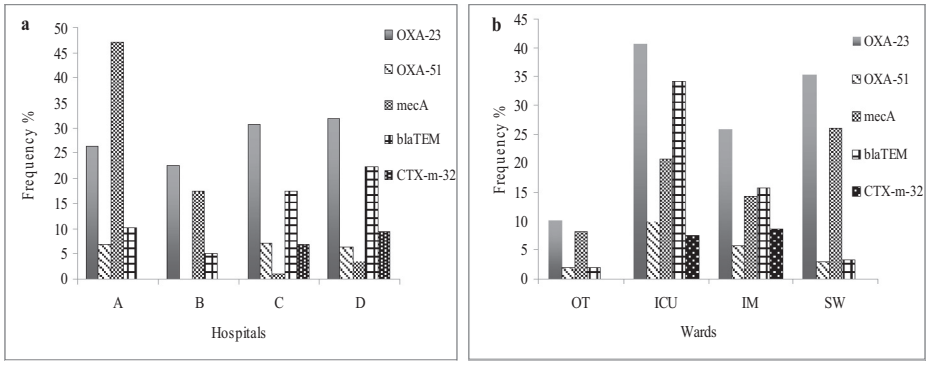

Fig 2. Frequency of detection of different groups of *bla* genes in (a) different hospitals and (b) different hospital wards. *ICU*, intensive care unit; *IM*, internal medicine ward; *OT*, operating theatre; *SW*, surgery ward.

Table 3
Predominant airborne β-lactam–resistant bacteria as identified by 16S rDNA sequence analysis

Genus	Species	GenBank accession number
Bacillus	*Bacillus* spp	KU514027
Paenibacillus	*Paenibacillus* spp	KU514036
Brevundimonas	*Brevundimonas* spp	KU514041
Staphylococcus	*Staphylococcus epidermidis*	KU514040-KU514043
	Staphylococcus hominis	KU514037
	Staphylococcus saprophyticus	KU514038-KU514050
	Staphylococcus haemolyticus	KU514028-KU514048
Acinetobacter	*Acinetobacter radioresistens*	KU514029
	Acinetobacter baumannii	KU514033-KU514039
		KU514042
		KU514031-KU514034
	Acinetobacter spp	KU514046-KU514049
Corynebacterium	*Corynebacterium mucifaciens*	KU514030
	Corynebacterium spp	KU514032-KU514044
Delftia	*Delftia lacustris*	KU514045
Stenotrophomonas	*Stenotrophomonas maltophilia*	KU514047
Pseudomonas	*Pseudomonas stutzeri*	KU514035

tion could be related to the short survival periods of these gram-negative bacteria in the airborne state.[4,8]

*bla*TEM is another common extended-spectrum β-lactamase produced by Enterobacteriaceae. However, it is also found in *P aeruginosa*.[21] The *bla*TEM gene appeared most frequently in air samples from ICU wards (34%) (Fig 2).

The results of this study indicated that among the β-lactamase–encoding genes, OXA-23 was the most frequent gene found in airborne BLRB (Fig 2). OXA-23 is commonly found in *Acinetobacter* spp.[14] OXA-51 is a chromosomally located intrinsic gene in *A baumannii*[14]; therefore, identification of OXA-23 along with OXA-51 demonstrated that the species of *Acinetobacter* was *A baumannii*. The OXA-51 gene was detected in all hospital wards with the highest frequency in ICUs (Fig 2). Previous studies have found that *Acinetobacter* spp have emerged as a particularly important organisms in ICUs.[22]

Detection of the *mec*A gene, a genetic element found in methicillin-resistant *Staphylococcus* (MRS) spp, in all hospitals and different wards with a relatively high frequency indicated potentially airborne transmission of MRS in hospital environments. We found that approximately 17% of the BLRB isolates carried the *mec*A gene. The frequency of *mec*A in different hospital wards showed that hospital SWs had the highest occurrence (26%). Other investigators have also reported airborne transmission of MRS, such as MRSA, in different hospital wards, especially in ICUs.[2,10] It has been reported that approximately 40% of nosocomial *S aureus* infections in the United States are methicillin-resistant. Drudge et al detected the *mec*A gene in dust from the prefilters of stand-alone hospital isolation room air cleaners and concluded that the identification of this gene in all samples indicates that MRSA commonly becomes airborne in hospital isolation rooms.[23] However, in a study by Gilbert et al, no antibiotic resistance genes were detected in any airborne antibiotic-resistant bacteria.[7]

Analysis of DNA sequences of predominant BLRB showed that 8 of 25 sequenced isolates could be identified as *Acinetobacter* spp, with 3 isolates being *A baumannii*.

Antibiotic resistant *A baumannii* has increasingly been recognized as an important causal agent of a variety of nosocomial infections, including bacteremia, urinary tract infection, and secondary meningitis. *A baumannii* also plays an important role in the etiology of nosocomial pneumonia, especially in ICUs.[22]

Staphylococcus spp were the second most predominant resistant bacteria, and *mec*A was detected in all of the isolates except for one isolate and identified as MRS. MRS spp are of particular importance in hospital environments because they have been responsible for many nosocomial infections.[24] *S aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, and *Staphylococcus haemolyticus* are recognized as the major human pathogens in hospital environments.[24,25] *S saprophyticus* has frequently been isolated from young women with uncomplicated urinary tract infections.[24] Other studies also reported *Staphylococcus* as the main bacteria isolated from air samples in hospital rooms.[6,26]

The highly frequent detection of *Acinetobacter* and *Staphylococcus* could be related to persistence of these microorganisms in hospital environment for many days. Furthermore, these bacteria spread easily in the environment of infected or colonized patients.[4,8]

Corynebacterium as an example of aerobic gram-positive bacteria was also found among isolated BLRB. Sarica et al. reported *Staphylococcus*, *Corynebacterium*, and *Micrococcus* as the predominant bacteria in air samples from various hospital areas.[26] A number of studies reported on the etiological role of *Corynebacterium* spp in infections in groups of people experiencing symptoms of immunodeficiency. Different species of *Corynebacterium* resistant to antibiotics have been isolated from clinical samples.[27] *Corynebacterium diphtheriae* may be transmitted via the airborne route.[3]

Sequence analysis of BLRB enabled recognition of 2 gram-positive isolates affiliated with *Bacillus* and *Paenibacillus*. There is a possibility that *Paenibacillus* spp may cause human infection.[28]

Brevundimonas spp, *Stenotrophomonas maltophilia*, *Delftia lacustris*, and *Pseudomonas stutzeri* were other gram-negative BLRB recovered from air samples. *Brevundimonas* spp and *S maltophilia* were described as opportunistic bacteria that may exhibit antibiotic resistance.[22,29,30] *S maltophilia* could cause various serious infections in humans and has most commonly been associated with respiratory infections.[30] However, the role of air in transmission of these bacteria has not been determined.

The relatively high concentration of BLRB and the high frequency of β-lactamase–encoding genes among isolated BLRB in hospital environments revealed the potential role of airborne bacteria in transmission of nosocomial infections. Therefore, it is required to properly eliminate and control the spreading of antibiotic-resistant bacteria in hospital environments. Implementation of enhanced cleaning and environmental disinfection, isolation procedures of infected patients, application of properly designed and working air ventilation systems, and control of antibiotic usage are measures that could efficiently reduce nosocomial infection rates.[4,8] Air quality monitoring and identification of the dissemination sources of pathogenic or opportunistic airborne bacteria are also key factors that would help to inform efforts taken against the spreading of infectious agents in hospital environments. In addition, knowledge about the prevalence of potentially pathogenic airborne bacteria in hospital environments and their antimicrobial susceptibility patterns could help reduce and possibly eliminate the exposure of high-risk patients through effective control measures.

CONCLUSIONS

The results of the present study showed a relatively high prevalence of 3 groups of airborne BLRB in various wards in 4 hospitals in Isfahan, Iran. Five β-lactamase–encoding genes were detected with various frequencies in isolated BLRB. Isolation of β-lactam–resistant *Staphylococcus* spp and *A baumannii* as the most predominant BLRB indicated the potential role of airborne bacteria in dissemination of nosocomial infections. The results confirm the necessity for application of effective control measures that significantly decrease the exposure of high-risk patients to potentially airborne nosocomial infections. However, further studies are needed to identify the sources of airborne bacteria and interventions that could reduce the levels of such bacteria in hospital environments.

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