Original Article

Occurrence of airborne vancomycin- and gentamicin-resistant bacteria in various hospital wards in Isfahan, Iran

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Abstract

Background: Airborne transmission of pathogenic resistant bacteria is well recognized as an important route for the acquisition of a wide range of nosocomial infections in hospitals. The aim of this study was to determine the prevalence of airborne vancomycin and gentamicin (VM and GM) resistant bacteria in different wards of four educational hospitals.

Materials and Methods: A total of 64 air samples were collected from operating theater (OT), Intensive Care Unit (ICU), surgery ward, and internal medicine ward of four educational hospitals in Isfahan, Iran. Airborne culturable bacteria were collected using all glass impingers. Samples were analyzed for the detection of VM- and GM-resistant bacteria.

Results: The average level of bacteria ranged from 99 to 1079 CFU/m^3. The highest level of airborne bacteria was observed in hospital 4 (628 CFU/m^3) and the highest average concentration of GM- and VM-resistant airborne bacteria were found in hospital 3 (22 CFU/m^3). The mean concentration of airborne bacteria was the lowest in OT wards and GM- and VM-resistant airborne bacteria were not detected in this ward of hospitals. The highest prevalence of antibiotic-resistant airborne bacteria was observed in ICU ward. There was a statistically significant difference for the prevalence of VM-resistant bacteria between hospital wards ($P = 0.012$).

Conclusion: Our finding showed that the relatively high prevalence of VM- and GM-resistant airborne bacteria in ICUs could be a great concern from the point of view of patients' health. These results confirm the necessity of application of effective control measures which significantly decrease the exposure of high-risk patients to potentially airborne nosocomial infections.

Key Words: Airborne transmission, gentamicin-resistant bacteria, nosocomial infection, vancomycin-resistant

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INTRODUCTION

Hospital-acquired infections (HAIs) are the major causes of death and increased morbidity among hospitalized patients.[1] However, antibiotic-resistant bacteria are involved in many of these infections (about 70%).[1,2] Antibiotic-resistant bacteria have been a significant health concern during recent decades[3,4] and have been described as "nightmare bacteria" that "pose a catastrophic

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threat" to people worldwide.[4] Vancomycin-resistant enterococci (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA) are the most prevalent resistant Gram-positive bacteria that are responsible for HAI. Among nosocomial Gram-negative pathogens, extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, multidrug-resistant *Pseudomonas aeruginosa*, and multidrug-resistant *Acinetobacter baumannii* are the most common.[1] There is evidence that the hospital environment may play a significant role in the spread of bacteria, including those that are resistant to antibiotics. Most of the bacteria released from patients can disperse in hospital environment by air currents and finally can be inhaled by susceptible individuals.[5] Today, airborne transmission of pathogenic bacteria is well recognized as an important route for the acquisition of a wide range of nosocomial infections.[6] Various studies estimate that 10% of nosocomial infections are airborne, and 16% of Intensive Care Unit (ICU) infection results from airborne pathogen transmission.[7] Airborne bacteria could result from a range of ward activities. Previous studies have been shown that bed making and mechanical floor cleaning release large numbers of bacteria into the air.[6]

Furthermore, airborne transmissions of some pathogens such as MRSA lead to an increase in the spread of antibiotic-resistant genes.[4] Therefore, identification of airborne bacteria potentially harmful to patients is a key parameter in control and effective prevention of HAI.

Vancomycin and gentamicin (VM and GM) are two important antibiotics used commonly in the treatment of some infections. VM is the standard treatment for MRSA infections and GM as an aminoglycoside most often used because of its low cost and dependable activity against Gram-negative bacteria.[8] According to the results of study by Askari *et al*., the number of vancomycin-resistant *S. aureus* (VRSA) reported in Iran is extremely high.[9] Several studies have shown the prevalence of MRSA and VRSA in Iran, and also, some results indicated to the existence of vancomycin-intermediate *S. aureus*, MRSA and even VRSA in our hospitals.[9-11]

However, the emergence of resistant bacteria against these antibiotics has led to a global concern about the treatment of these infections.[12] There has been a tenfold increase in the prevalence of GM resistance enterococci during 2003–2008 as reported by Norwegian surveillance system for antimicrobial resistance.[13] There are several studies which describe the role of VM- and GM-resistant bacteria in HAI. However, these investigations have been mostly directed toward clinical isolates, and there are very few studies for airborne strains of these resistant bacteria.[14,15]

Therefore, this study was carried out to determine the prevalence of airborne VM- and GM- resistant bacteria in different wards of four educational hospitals (including Kashani, Amin, Alzahra and Noor hospitals) of Isfahan, Iran.

MATERIALS AND METHODS

Sampling sites and sample collection

The study was performed from March to December 2014 in four educational hospitals of Isfahan University of Medical Sciences, Isfahan, Iran. Airborne cultureable bacteria were collected using all glass impinger (AGI) in four locations in each hospital including operating theater (OT), ICU, surgery ward, and internal medicine ward. Approximately, 3000 L of air at a flow rate of 12.5 L/min was drawn into AGI, which contained 10 ml of phosphate buffer solution. Sampling was conducted at a height of 1.5 m above the ground level to simulate the breathing zone. A total of 64 samples were analyzed for the presence of airborne resistant bacteria. During sampling, temperature, and relative humidity were also recorded by use of a portable weather station (Kimo) at each sampling site and were about 26 ± 2.3°C and %28 ± 5.7, respectively. All samples were transferred to the laboratory in an insulated box with cooling packs and were processed immediately after arrival at the laboratory.

Detection of vancomycin- and gentamicin-resistant airborne bacteria

For detection of VM- and GM-resistant bacteria, aliquots of each impinger collection medium after a vigorous shaking were plated onto Tryptic Soy Agar (TSA) containing VM (16 mg/l) and GM (10 mg/l) according to Clinical and Laboratory Standards Institute recommendations.[16] Simultaneously, aliquots of each sample were plated onto TSA without any antibiotic to evaluate the total counts of airborne bacteria. All plates were incubated at 35°C for 2–3 days. For quality control, standard strains of *Escherichia coli* (ATCC 25922), and *S. aureus* (ATCC 25923) were used. Colonies growing on TSA with and without antibiotics were enumerated and calculated as colony-forming units per cubic meter (CFU/m^3). Prevalence of antibiotic resistance airborne bacteria (ARAB) was calculated as the counts of bacteria growing on TSA along with antibiotics divided by the numbers of bacteria growing on TSA without antibiotics.

Statistical analysis

Statistical analysis was performed with SPSS 20.0 for windows. Kolmogorov–Smirnov normality tests were performed for recognition the use of parametric or nonparametric tests. The Kruskal–Wallis test was used to compare the concentration differences of airborne bacteria among the sampling sites. Differences were considered significant when P values were smaller than 0.05.

RESULTS

The average concentration of airborne bacteria and ARAB in various wards of hospitals is shown in Table 1. The total airborne bacteria count in hospital 4 was higher than in other hospitals and the highest average concentration of GM- and VM-resistant airborne bacteria were found in hospital 3. Nevertheless, statistically, significant differences were not found among the four hospitals ($P > 0.05$). The mean concentration of airborne bacteria was the lowest in OT wards and GM- and VM-resistant airborne bacteria were not detected in this ward of hospitals [Table 1]. The prevalence (percentage of ARAB with respect to total airborne bacteria) of GM- and VM-resistant airborne bacteria in different hospitals and hospital wards are presented in Figures 1 and 2, respectively. As results show, the prevalence of VM-resistant bacteria was higher than GM-resistant bacteria in investigated hospitals [Figure 1]. The highest prevalence of ARAB was observed in ICU ward of hospitals [Figure 2]. There was a statistically significant difference in the prevalence of VM-resistant bacteria between hospital wards ($P = 0.012$).

DISCUSSION

Airborne-resistant bacteria pose a considerable threat to human health, and this is, especially important for vulnerable groups of inpatients such as people with weakened immune systems. The hospital environment could act as a vast reservoir for resistant microorganisms and their resistance genes.[11] This study showed that there were fluctuations in airborne bacterial concentrations with hospitals and different wards from 99 to 1079 CFU/m^3 [Table 1] which were comparable with the results obtained by others.[17,18] However, the average concentration of airborne bacteria (464 CFU/m^3) was higher compared to those by others.[14,19] The various environment factors such as hospital design, the type of ventilation system, temperature, relative humidity, population density, and disinfection methods can influence the levels of airborne bacteria in different wards.[6,17]

The concentration of airborne bacteria in OT wards was low when compared to other hospital wards. This

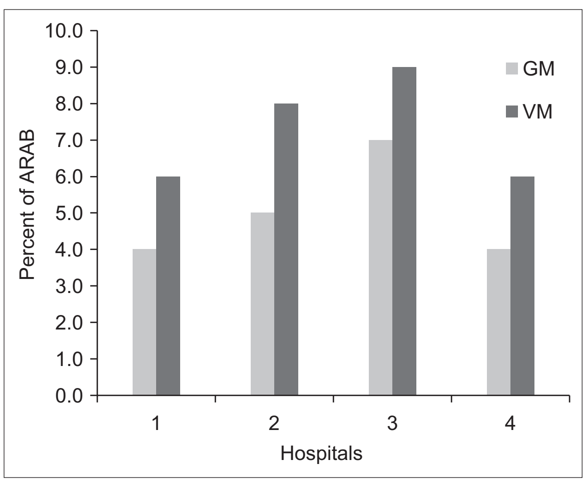

Figure 1: Prevalence of antibiotic-resistant airborne bacteria isolated from investigated hospitals. GM: Gentamicin, VM: Vancomycin, ARAB: Antibiotic resistant airborne bacteria

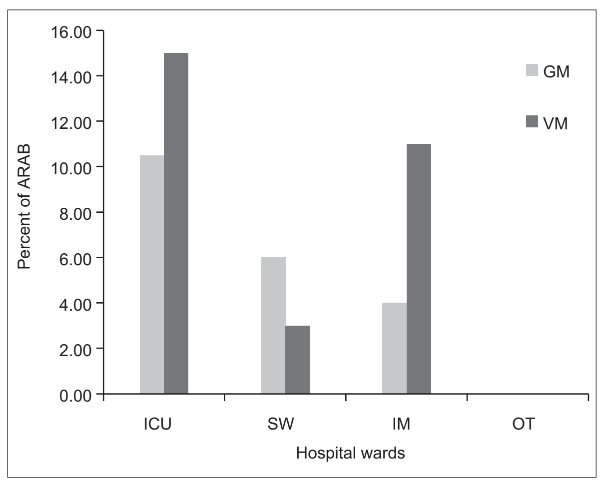

Figure 2: Prevalence of antibiotic-resistant airborne bacteria isolated from air samples of hospital wards. ICU: Intensive Care Unit, OT: Operating theater, SW: Surgery ward, IM: Internal medicine, GM: Gentamicin, VM: Vancomycin, ARAB: Antibiotic-resistant airborne bacteria

Table 1: Average concentration of airborne bacteria and antibiotic-resistant airborne bacteria (CFU/m^3) in different wards of hospitals

Location	Hospital 1				Hospital 2				Hospital 3				Hospital 4			
	ICU	OT	SW	IM	ICU	OT	SW	IM	ICU	OT	SW	IM	ICU	OT	SW	IM
Airborne bacteria	335	351	337	518	699	284	149	99	354	119	919	740	208	693	532	1079
VM	5	0	0	17	0	0	0	10	21	0	6	9	10	0	19	5
GM	14	0	7	6	18	0	0	18	14	0	32	4	14	0	28	0

ICU: Intensive Care Unit, OT: Operating theater, SW: Surgery ward, IM: Internal medicine, VM: Vancomycin, GM: Gentamicin can be because of the high level of health standards, as well as proper disinfection and air purification systems such as ultraviolet light, in this ward.[20] The average concentration of airborne bacteria in hospital 4 was higher than in other hospitals. However, the concentration of ARAB was the highest in hospital 3. Some studies have showed that various factors such as architectural design, in particular, the source of ventilation air, density of patients, and patient activity does influence the diversity and composition of airborne bacteria in hospital.[17,20] This study also showed that about 13% of airborne bacteria isolated from ICU wards were found resistant to both VM and GM [Figure 2]. On the other hand, the highest concentration of ARAB was observed in ICUs. ICUs are an important hospital ward for the emergence of antimicrobial resistance due to the frequent use of a wide range of antibiotics; high density of patients within relatively small specialized areas; the presence of more chronically and acutely ill patients who require prolonged hospitalization, and often harbor antibiotic-resistant bacteria.[21] Other factors that affect the level of ARAB in ICUs might be weakly maintained or operated ventilation systems, insufficiently disinfection program, and lack of trained nursing staff and other support staff due to economic pressures.

Ventilator-associated pneumonia which caused by Gram- or Gram-negative bacteria, and line-associated bacteremia (principally by Gram-positive) are the two most common nosocomial infections of ICUs.[22] There is an increased mortality rate in patients with ventilator-associated pneumonia caused by multidrug-resistant organisms.[22]

In this study, the higher level of VM-resistant airborne bacteria may be due to selective pressure caused by continuous use of VM in hospitals for any *S. aureus* infection.[23] Aligholi *et al*. showed that of the 356 *S. aureus* clinical isolates from the Imam Khomeini Hospital in Tehran, 149 *S. aureus* strains were resistant to methicillin and two strains of MRSA were VRSA strains.[11]

Xu *et al*.[24] also detected 32 VRE strains from a Tertiary Care Hospital of Beijing, China. For many years, VM has been considered as the alternative for MRSA infections but after a report of transfer of the *vanA* gene from VRE to MRSA and creating MRSA with high-level resistance to VM, concerns about this transfer were increased.[25] Rossi *et al*.[23] reported the case of a bloodstream infection caused by MRSA that was susceptible to VM but during treatment with antibiotic became resistant to VM and *vanA* gene was detected in it.

Although VRE strains are commonly transmitted by direct contact with infected persons or indirect contact with contaminated environments,[26] evidence suggests that airborne dispersal and transmission may also be important.[27] Muzslay *et al*.[15] have showed that VRE may be inhaled in hospital wards. Therefore, the prevalence of VRE in hospital environments may create a reservoir of mobile resistance genes for other nosocomial pathogens.[28]

High level of resistant bacteria to aminoglycosides, in particular to GM, has been reported worldwide.[29] In our study, the average prevalence of GM-resistant airborne bacteria in four hospitals ranged from 4% to 7%. By contrast, a study in Iran has reported a high level (about 52%) prevalence of GM-resistant strains in clinical samples.[30] Some studies have been reported the concurrent resistance of bacteria to GM and VM.[29,31] The incidence of high-level GM-/VM- resistance has been reported in many *Enterococcus* species.[32] Study of Tomita *et al*.[29] demonstrated that GM plasmid might co transfer VM-resistance plasmids.

CONCLUSION

ARAB can survive in the various hospital environments and remain suspended in the air for long periods of time. The results of this study also showed that the relatively high prevalence of VM- and GM-resistant airborne bacteria in ICUs could be a great concern from the point of view of patients' health.

These results confirm the necessity of application of effective control measures which significantly decrease the exposure of high-risk patients to potentially airborne nosocomial infections.

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Conflicts of interest
There are no conflicts of interest.

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