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Occurrence of Airborne Methicillin-Resistant *Staphylococcus aureus* in Different Hospital Wards

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Abstract

Airborne transmission is considered as a route for acquisition of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in hospital environments. Since, control and effective prevention of MRSA infections require a better knowledge of airborne bacteria. This study was conducted to determine the occurrence of airborne MRSA in different wards of four hospitals in Isfahan, Iran. Air samples were taken using an all-glass impinger (AGI) in four locations in each hospital. Detection of oxacillin resistant airborne bacteria was carried out using culture plates with and without antibiotics. Oxacillin resistant isolates were screened for the presence of *mec*A gene, a genetic element found in MRSA. Oxacillin-resistant bacteria with a range of 15-207 cfu/m^3 had a high prevalence and formed about 30%-34% of airborne bacteria. We found that about 63% of the oxacillin resistant isolates carried the *mec*A gene. The frequency of *mec*A gene in isolated oxacillin resistant bacteria from different hospital wards ranged between 9%–26%. Detection of the *mec*A gene in different wards with a relatively high frequency indicated potentially airborne transmission of MRS in hospital environments. The presence of these airborne bacteria could pose a considerable threat to human health especially for vulnerable groups of inpatients such as people with weakened immune systems.

Keywords: MRSA, Hospital, Airborne bacteria, Antibiotic resistance

1. Introduction

Health care associated infections (HAIs) are a major cause of morbidity and mortality worldwide. According to the world health organization and centers for disease control and prevention (CDC), antibiotic-resistant agents of HAIs are on the rise (Septimus and Schweizer, 2016; Ventola, 2015). Although direct contact is the most important and the main transmission route in hospitals, airborne transmission of some HAIs should not be ignored. The most important causes of HAI are those that have the potential to spread by the airborne route (Beggs

et al., 2015; Kowalski, 2006; Mirhoseini, *et al*., 2016a).

There is evidence for airborne transmissions of MRSA, Acinetobacter and Pseudomonas in health care settings 2006; Shamsizadeh *et al*., 2017; Talon *et al*., 2008). Among causative agents of HAIs, MRSA is one of the most important microorganisms and represents a serious concern from the hospital hygiene point of view (Saadoun *et al*., 2015). In healthcare settings, this pathogen has increasingly become an important source of life-threatening blood stream infections, pneumonia, and surgical site infections (Caron, 2015). Airborne transmission of these infections has been reported in different hospital wards including operating theatres, intensive care, burns and orthopedic units (Beggs, 2003; Creamer *et al*., 2014). MRSA strains are endemic in many hospitals in the developed countries and account for 29%–35% of all clinical isolates (Haddadin *et al*., 2002). The impact of MRSA infections can be worse in the developing countries such as Iran due to high consumption of antibiotics. Numerous studies have shown that the hospital environment may play a significant role in the spread of MRSA (Sexton *et al*., 2006; Solomon *et al*., 2017; Talon *et al*., 2008). MRSA can survive prolonged periods in a dry condition such as environmental surfaces and dust (Tolba *et al*., 2007). MRSA bioaerosols can contaminate air and cause airborne infection. Airborne dispersal of MRSA has also occurred in cases where it has colonized the respiratory tract of patients (Andersen *et al*., 2002; Creamer *et al*., 2014). The presence of such nosocomially significant pathogen in the indoor air of hospitals can pose a threat to patients, especially in developing countries.

Therefore, understanding *S. aureus* presence in the hospital environment is vital in preventing of MRSA infection transmission and colonization. There is relatively little information on the airborne dispersal and transmission of MRSA in different hospital wards. The aim of the current study was to assess and compare the occurrence of airborne MRSA in different wards of four educational hospitals and to evaluate the frequency detection of *mec*A gene in isolated resistant bacteria.

2. Material and Methods

2.1 Sampling sites and procedure

The study was conducted in four public hospitals in Isfahan, Iran. Samples of air were collected using all-glass impinger (AGI) with 25ml total volume, containing 10 ml of phosphate buffer solution at each sampling site including: internal medicine wards (IMs), intensive care units (ICUs), operating theatres (OTs) and surgery wards (SWs). At the time of investigation all wards of the hospitals were equipped with central operation HVAC (heating, ventilating and air conditioning systems). Furthermore, high efficiency particulate air (HEPA) filters were within HVAC systems of OT wards.

Approximately 2.5 m^3 of air was drawn using a portable pump at a calibrated flow rate of 12.5 L/min from each site. The impinger fluid was replenished after each 30 min with sterlized phosphate buffer solution to maintain the sampler collection efficiency (Lin *et al*., 1997). Sampling was performed four times in each site and a total of 64 air samples were analyzed for the presence of MRSA. Air sampler was placed 1.5m above the floor to simulate the breathing height. Temperature and relative humidity were monitored throughout the sampling periods and were 26°C ± 2°C and 28 ± 6%, respectively. All samples were stored in cooling box and transported back to the laboratory. At each hospital, air samples from 4 locations were taken on 1 single day from 9 AM to 12 PM after routine cleaning.

2.2 MRSA detection

For detection of MRSA, aliquots of each impinger collection medium were plated onto tryptic soy agar (TSA) supplemented with oxacillin (OX) after a vigorous shaking. In this study 4 mg/L OX were used according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2012). Type culture strains used for quality control were *S. aureus* ATCC 25923 and *E. coli* ATCC 25922. All

culture media were incubated at 35°C for 2-3 d. After an incubation time colonies were counted and the results expressed as colony-forming units per cubic meter (cfu/m^3).

For identification of MRSA, oxacillin resistant isolates were subcultured on Mueller-Hinton agar plates containing oxacillin. All colonies were screened for the presence of *mecA* gene. For DNA amplification, we used the oligonucleotide primer pair *mecA*F 5′-GTA GAA ATG ACT GAA CGT CCG ATAA-3′and *mecA*R 5′-CCA ATT CCA CAT TGT TTC GGT CTAA-3′ in the polymerase chain reaction (Bannoehr *et al*., 2007). DNA was extracted from each isolated colony by boiling for 15 min and centrifugation at 14,000 rpm for 10 min. The supernatant was collected and used as a template DNA for PCR amplification. PCR reaction was performed in a final volume of 25 µL containing 2.5 µL of 10× PCR buffer (2mM $MgCl_2$), 0.2 µM of each primer, 0.2 mM of each of the deoxynucleotides (dNTPs), 2 units of Taq DNA polymerase, and 2 µL template DNA. Amplification was done in a programmable PCR thermal cycler (Corbett Life Science, Australia). After an initial denaturation step for 5 min at 94°C, 35 cycles of amplification were performed as follows: denaturation at 94°C for 45 second, primer annealing at 50°C for 1 min, and DNA extension at 72°C for 45 seconds. The reaction was completed with a final extension at 72°C for 10 min (Mirhoseini *et al*., 2016b). All PCR assays included positive and negative controls. Amplified products were loaded on an agarose gel (2%) and visualized on ultraviolet (UV) transilluminator (UV Tech, France).

2.3 Statistical analysis

Statistical analysis was conducted with SPSS 16.0 (SPSS Inc., Chicago, IL). Kolmogorov-Smirnov normality tests were carried out 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 wards.

3. Results and Discussion

3.1 Levels of resistant airborne bacteria

Total airborne bacteria and oxacillin-resistant airborne bacteria concentrations in the hospitals ranged from 30 to 3250 and 0 to 578 CFU/m^3, respectively. The average concentrations of oxacillin-resistant airborne bacteria were the highest in SW (126 cfu/m^3) of hospitals followed by OT (86 cfu/m^3), ICU (65 cfu/m^3) and IM (52 cfu/m^3) (Table 1). However, there was no significant difference between all wards and hospitals ($p>0.05$). Oxacillin resistant airborne bacteria in the SW ranged between 0 and 492 cfu/m^3, with maximum values in the hospital1. Higher detection of oxacillin resistant airborne bacteria in SW can be caused by different factors such as human activities, number of visitors and the nature of infection of SW patiants. In the study of Li *et al*. the mean concentrations of total airborne *S. aureus* and MRSA were 72 and 32 cfu/m^3 in SW wards of general hospital in China (Li *et al*., 2015).

The mean concentrations of OT airborne bacteria in hospital 4 (693 cfu/m^3) and resistance rate to oxacillin (207 cfu/m^3) were very higher than the limits recommended by some countries for airborne bacteria in OT, despite ventilation

Table1. Mean concentration (cfu/m^3) of Oxacillin resistant airborne bacteria (MRSA)[a] in different hospital wards.

	Hospital1	Hospital2	Hospital3	Hospital4
Hospital wards				
ICU	79(43)	41(7)	64(ND)	76(12)
IM	43(3)	42(9)	83(ND)	40(ND)
SW	174(83)	26(8)	154(ND)	149(3)
OT	85(19)	15(ND)	35(1)	207(ND)

ICU intensive care unit, OT operating theatre, SW surgery ward, IM internal medicine ward, ND not detected

[a]Concentration of MRSA in positive sample.

system with a HEPA filter. Inadequately of filtered incoming air, the number and activity of OT personnel could be the major sources of airborne bacteria. Few countries have set bacterial threshold limits for conventionally ventilated OT, although most reports have recommend an acceptable bacterial limit for a working OT below 180 cfu/m^3 (Shaw *et al*., 2018). The lowest level of airborne OX-resistant bacteria was obtained from Hospital 2. Nevertheless, ICU ward of this hospital had the highest concentration of airborne bacteria (699 cfu/m^3), which can be caused by the type of used antibiotics, low efficiency of the ventilation system and higher occupant density in this ward. Different studies revealed that indoor airborne bacteria may be affected by various factors such as human occupancy and activities, type and efficiency of ventilation, outdoor air source and environmental conditions (Meadow *et al*., 2014; Mirhoseini *et al*., 2016b; Xu *et al*., 2017).

3.2 Distribution of MRSA

The gold standard for identifying MRSA is to detect the *mecA* gene by amplifying it using PCR (Saadoun *et al*., 2015). All resistant isolates were analyzed for presence of *mecA* gene (Figure 1). The results showed that 63 percent of the OX-resistant isolates carried the *mecA* gene. The frequency of *mecA* gene (expressed as percent of *mecA* gene in isolated bacteria with respect to the total number of resistant airborne isolates) in different wards of each hospital is presented in Figure 2. This gene was detected in resistant isolates from all hospital ward samples except isolates from ICU of hospital 3 and OT of hospital 4 (Figure 2). The results showed that the highest frequency of *mecA* positive isolates were detected in SWs (26%) and ICUs (22%) followed by IMs (18%) and OTs (11%), respectively (Figure 2). Frequency of *mecA* positive isolates in all sampling wards of hospital1 was significantly higher than other hospitals ($P<0.001$). According to the obtained results of airborne MRSA isolates from various wards, the ICU of hospital1 had the highest occurrences of airborne MRSA (54%) and therefore patients are at risk to infection with MRSA in this part of the hospital. This hospital is the biggest and most crowded teaching hospital among the hospitals. ICU of this hospital contains 32 beds that are almost fully occupied and crowded with an extensive number of patients and nurse mix. Previous studies concluded that overcrowding, presence of various types of patients and understaffing in nursing, associated significantly to the dissemination of MRSA in ICUs (Andersen *et al*., 2002; Hardy *et al*., 2004). Sexton *et al*. (2006) in the study of environment of the isolation rooms of MRSA patients reported a relationship between the number of patients in a relatively small confined space and the possibility of increased colonization or infection. They reported that about 28% of air samples and over half of the surface samples were positive for MRSA (Sexton *et al*., 2006). On the other hand, higher level of MRSA contamination in ICUs than other wards may in part be related to the additional use of antibiotics and the condition of the patient infection and the critical conditions of the ICU occupants (Saadoun *et al*., 2015). Wilson *et al*. revealed that daily number of MRSA-infected patients in a general ICU were correlated significantly with the daily number of MRSA positive air sample cultures (Wilson *et al*., 2004). Molecular identification of isolated *Staphylococcus aureus* from the air and environment of an ICU at a hospital in Tehran showed that air isolated MRSA strains were typically of the same type as those isolated from patients who are colonized or infected with MRSA (Mirzaii *et al*., 2015). In study by Hardy *et al*., ICUs have the highest incidence rate of MRSA followed by surgical wards and medical wards (Hardy *et al*., 2004). As previously cited, MRSA pneumonia causes a significant degree of mortality (greater than 50%) amongst ICU patients (Hardy *et al*., 2004). The ICUs have been suggested as main sources of the intra- and inter-hospital transition of MRSA. Incidence rates of MRSA in different ICUs are difficult to compare because of differing surveillance methods, lack of uniformity in diagnostic criteria, and lack of adequate systems to compare the severity of illness. In United State hospitals, the over 60% of *S. aureus* isolates in ICUs are methicillin-

resistant and an increasing ratio of these are community-acquired (Haddadin *et al*., 2002). In the study the same frequency of *mecA* gene (15%) obtained from air isolates of ICUs in Hospital 2 and hospital 4, even though the level of airborne bacteria in ICU2 was about three times higher than the ICU 4. In an assessment of dust from the prefilters of stand-alone hospital isolation room air cleaners, the *mecA* gene was detected and concluded that the presence of this gene in all samples indicates that MRSA commonly becomes airborne in hospital isolation rooms (Drudge *et al*., 2012).

High frequencies of *mecA* were also detected in air isolates from SWs wards of hospital 1 (48%) and hospital 2 (30%). It has been shown that *S. aureus* is the common cause of post-surgical infection in most hospitals and it can survive on the environmental surfaces with the ability to resist disinfection (Solomon *et al*., 2017). It is possible that environmental surfaces of surgical sites are contaminated with airborne MRSA, which is the common cause of post-surgical infections. High level of airborne MRSA in these hospital wards is an alarming call because the units are places where strictly sick and post-surgical rehabilitated patients are accepted.

We found that approximately high frequency (22%) of OT resistant airborne isolates in hospital 1 were MRSA positive (Figure 2). However, *mecA* gene was not detected in OT of hospitals 4. This large difference could be attributed to the differences in the location, design of the hospital and cleanness of the OT units. The risk factors that

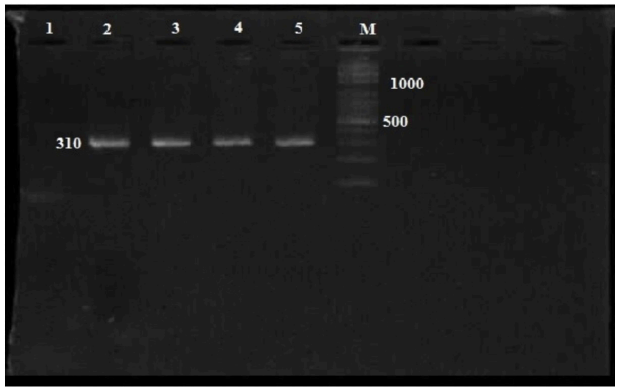

Figure 1. Polymerase chain reaction for *mecA* gene in methicillin-resistant *S. aureus*. M: DNA Maker; lane 1: airborne strains of methicillin-susceptible *S. aureus*, lane 2, 3, 4, 5: airborne strains of methicillin-resistant *S. aureus*.

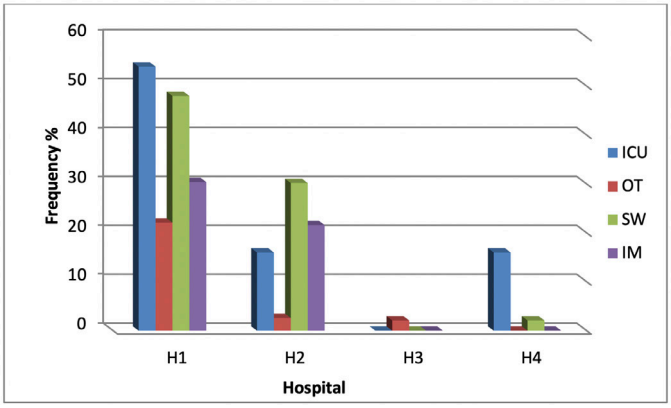

Figure 2. The frequency of *mec*A gene in airborne resistant isolates.

influence the number of bacterial colonies in OT were the type of surgery, site of procedure, number of indoor staff, surgical staging and indoor air temperature. Some reports noted that *S. aureus* was highest in all locations in the OTs and showed resistance to methicillin (Saadoun *et al*., 2015; Shaw *et al*., 2018). The bacterial level in OT air is directly related to the number of people moving about in the ward (Andersen *et al*., 2002). In the present study, the mean number of indoor active persons in the OT ward of hospital 1 was twenty people. The number and activity of OT personnel could be contribute to high percent of MRSA positive in this ward. In recent years, concern has also been expressed about the risks posed by contaminated mechanical ventilation ductwork in hospital buildings.

4. Conclusions

Airborne transmission has been implicated in nosocomial outbreaks of methicillin-resistant *Staphylococcus aureus* (MRSA). Our study showed high prevalence of airborne MRSA in various wards (especially SW and ICU) in educational hospitals in Isfahan, Iran. Despite the presence of HEPA filter in OTs wards, MRSA was also isolated from three hospital OTs.

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Conflict of Interest

The authors have no conflicts of interest to declare for this study.

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