Direct Monitoring of Gram-negative Agents of Nosocomial Infections in Hospital Air by a PCR-based Approach

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ABSTRACT

Gram-negative bacteria (GNB), including *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Legionella*, have emerged as causative agents of many problematic infections in healthcare settings worldwide. Using a rapid detection method, this study was designed to investigate the presence of GNB in hospital air as a potential source for spreading and transmitting these bacteria. A total of 51 air samples were taken from different wards in four hospitals over a period of eight months. Air samples were collected using an all-glass impinger and analyzed for the presence of GNB. Detection of *P. aeruginosa* and *Legionella* spp. was performed with a nested PCR assay using specific primer sets of the 16S rRNA gene region of the bacteria. For detection of *A. baumannii*, PCR assay with the specific primers of the inherent blaOXA-51 gene was performed. *A. baumannii* was the most frequently (29/51) detected gram-negative microorganism in hospital air, followed by *P. aeruginosa* (15/51). The lowest detection frequency was related to *Legionella* (9/51), which was not found in air samples from surgical wards. Intensive care units and operating theatres were high-risk areas due to the greater presence of GNB. The results of this study revealed the presence of GNB in various hospital wards and highlight the usefulness of PCR monitoring of hospital air as a rapid and reliable tool for identifying GNB in order to prevent and control nosocomial infections, especially for the protection of vulnerable patients.

Keywords: Gram-negative bacteria; Nosocomial infection; Hospital; Air; PCR.

INTRODUCTION

Nosocomial infections are a significant health concern, globally. These infections lead to increased morbidity and mortality and can extend hospitalization and treatment period which is associated with additional costs (Ducel *et al.*, 2002; Sehulster *et al.*, 2003; De Francesco *et al.*, 2013).

Gram-negative bacteria (GNB) including *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Legionella* spp. are among the most important etiological agents of hospital acquired infections worldwide (Ducel *et al.*, 2002; Sehulster *et al.*, 2003; Rebmann and Rosenbaum, 2011; De Francesco *et al.*, 2013). These ubiquitous bacteria which have been strongly implicated in nosocomial infections, account for a significant proportion of morbidity and mortality in health-care settings. *A. baumannii* and *P. aeruginosa* can cause a variety of infections including pneumonia, urinary tract, wound and blood stream infections (Sehulster *et al.*, 2003; Rebmann and Rosenbaum, 2011; De Francesco *et al.*, 2013). *Legionella* causes Legionnaires disease, a potentially fatal form of pneumonia or an acute self-limiting, influenza-like illness (Pontiac fever) (Ducel *et al.*, 2002; Sehulster *et al.*, 2003). Hospital air has been indicated as a potential source for dispersion and transmission of *Acinetobacter* infections (Sehulster *et al.*, 2003). Exposure to *Legionella* and *P. aeruginosa* occurs through inhalation of aerosols generated from contaminated water sources and faucets (Sehulster *et al.*, 2003). Therefore, exposure to clinically significant gram-negative bacteria released into the air could lead to a variety of infections especially in susceptible patients. In a survey among pediatric patients, identical *Acinetobacter* spp. were cultured from the patients, air, and room air conditioners (Sehulster *et al.*, 2003). A same profile of macrorestriction of genomic DNA has been reported in airborne *L. pneumophila* from an aerated basin and the epidemic strain detected in patients (Nhu Nguyen *et al.*, 2006).

Considering the increased number of susceptible patients due to age, illness, immunosuppression and other risk factors

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(Ducel *et al.*, 2002), and the importance of eliminating exposure of high-risk patients to potential routes of acquisition of gram-negative infections, air quality monitoring is a critical part of the hospital management protocol (Wu *et al.*, 2011; Tang and Wan, 2013). Rapid monitoring of microbial quality of air in health-care settings promotes early awareness of the possible infection sources. In other words, implementation of appropriate risk reduction and control programs of nosocomial infections could be achievable through complete microbiological information about the hospital environments especially high-risk areas.

Detection of GNB in hospital environment samples by conventional culture methods is labour-intensive, time consuming and costly due to the need to several different types of media. Polymerase chain reaction (PCR)-based methods provide a rapid and beneficial means of detection of GNB in environmental samples with high specificity and sensitivity (Asghari *et al.*, 2013; Baghal Asghari *et al.*, 2013).

Based on these premises, the present study was carried out to present a rapid and sensitive detection method for gram-negative agents of nosocomial infections in hospital air and also provide more information on the distribution of these bacteria in air of various parts of hospitals.

MATERIAL AND METHODS

A total of 51 air samples were analyzed for the presence of gram-negative bacteria from four educational hospitals in Isfahan, Iran. Samples were collected from four points in each hospital included operating theatre (OT), intensive care unit (ICU), surgery ward (SW), and internal medicine ward (IM) over a period of 8 months. Air samples were collected using an all-glass impinger, containing 10 mL of phosphate buffer solution at a flow rate of 12 L min^{-1} and a sampling duration of 3 hours after routine cleaning in the morning. The air sampling points were about 1–1.5 m above ground level to simulate the breathing zone. Temperature and relative humidity were recorded throughout the sampling periods and were approximately 26°C ± 2°C and 28% ± 5%, respectively.

For detection of GNB, air samples were concentrated by centrifugation at 4500 g for 15 min. To extract DNA, the resuspended pellets were frozen and heated in liquid nitrogen and boiling water, respectively for five times. The DNA was further extracted and purified using Promega DNA Extraction Kit (Promega Wizard_ Genomic DNA Purification Kit, Madison, WI) according to manufacturer's instruction.

For the detection of *P. aeruginosa* and *Legionella* spp. a nested PCR assay was applied to increase the sensitivity as well as to check the nucleic acid extraction. To check the quality of extracted DNA, the first step of PCR was carried out using universal primer set Eubac 27F and 1492R that target a fragment of the 16S rRNA gene region which is conserved across a broad spectrum of bacteria (Lane, 1991). In the second PCR step, specific primers of the 16S rRNA gene region of *P. aeruginosa* and *Legionella* spp. were used as described previously (Asghari *et al.*, 2013; Baghal Asghari *et al.*, 2013). For the detection of *A. baumannii*, PCR assay with the specific primers of the inherent blaOXA-51 gene was performed (Shamsizadeh *et al.*, 2017).

PCR reactions were run in a final volume of 25 μL as described previously (Asghari *et al.*, 2013; Baghal Asghari *et al.*, 2013; Shamsizadeh *et al.*, 2017) and positive and negative controls were included in all runs. PCR products were visualized by agarose gel electrophoresis using 1.5% gel and the size of amplicons were compared with the 100-bp DNA ladder.

RESULTS AND DISCUSSION

Gram-negative bacteria in hospital air can cause a variety of infections in vulnerable patients via direct inhalation, inhalation of bioaerosols or indirectly through deposition of airborne microorganisms on inanimate surfaces.

The results of this study revealed the presence of GNB in various hospital environments (Tables 1–3). Although detection of some GNB such as *Acinetobacter, Pseudomonas, Klebsiella* and *E. coli* were reported in hospital air (Wu *et al.*, 2011; Maujean *et al.*, 2012; Huang *et al.*, 2013; Tang and Wan, 2013; Luksamijarulkul *et al.*, 2014), gram-positive bacteria are the most commonly found bacteria in the air of hospitals as well as indoor environments (Maujean *et al.*, 2012; Tang and Wan, 2013; Luksamijarulkul *et al.*, 2014; Mihoseini *et al.*, 2016). GNB have less resistance and cannot withstand the inactivating effects of drying (Sehulster *et al.*, 2003) and therefore may be found in viable but nonculturable (VBNC) state that are alive and may still cause infection. High detection rate of GNB in the present study may be related in part to the VBNC bacteria which couldn't be detected by culture method but could be detected by PCR assay. Analysis of aerosols produced by

Table 1. Detection frequency (positive samples/total samples) of *Acinetobacter baumannii* in air of different hospital wards.

Hospital	Location				
	ICU	OT	SW	IM	Total
A	67% (2/3)	67% (2/3)	100% (3/3)	100% (2/2)	82% (9/11)
B	50% (2/4)	25% (1/4)	25% (1/4)	25% (1/4)	31% (5/16)
C	67% (2/3)	100% (3/3)	67% (2/3)	33% (1/3)	67% (8/12)
D	33% (1/3)	67% (2/3)	67% (2/3)	67% (2/3)	58% (7/12)
Total	54% (7/13)	62% (8/13)	62% (8/13)	50% (6/12)	57% (29/51)

ICU: Intensive Care Unit; OT: Operating Theatre; SW: Surgery Ward; IM: Internal Medicine Ward.

Table 2. Detection frequency (positive samples/total samples) of *Pseudomonas aeruginos*a in air of different hospital wards.

Hospital	Location				
	ICU	OT	SW	IM	Total
A	33% (1/3)	67% (2/3)	33% (1/3)	ND	36% (4/11)
B	25% (1/4)	50% (2/4)	50% (2/4)	25% (1/4)	37.5% (6/16)
C	33% (1/3)	67% (2/3)	67% (2/3)	ND	42% (5/12)
D	ND	ND	ND	ND	ND
Total	23% (3/13)	46% (6/13)	38% (5/13)	8% (1/12)	29% (15/51)

ICU: Intensive Care Unit; OT: Operating Theatre; SW: Surgery Ward; IM: Internal Medicine Ward; ND: Not Detected.

Table 3. Detection frequency (positive samples/total samples) of *Legionella* in air of different hospital wards.

Hospital	Location				
	ICU	OT	SW	IM	Total
A	ND	33% (1/3)	ND	50% (1/2)	18% (2/11)
B	75% (3/4)	ND	ND	ND	19% (3/16)
C	33% (1/3)	33% (1/3)	ND	33% (1/3)	25% (3/12)
D	ND	33% (1/3)	ND	ND	8% (1/12)
Total	31% (4/13)	23% (3/13)	ND	17% (2/12)	18% (9/51)

ICU: Intensive Care Unit; OT: Operating Theatre; SW: Surgery Ward; IM: Internal Medicine Ward; ND: Not Detected.

industrial cooling towers showed no cultivable *Legionella* bacteria using the standard culture method, even when the concentrations of $\sim 1 \times 10^3$ *Legionella* cells m^{-3} were detected by use of fluorescent in situ hybridization (FISH) (Mathieu *et al.*, 2006). We used PCR assay as a qualitative method that only detects the presence of GNB in hospital air. However, application of quantitative techniques such as real-time PCR should be taken into account in order to further understand the prevalence of bacteria in hospital environments.

The results showed that the highest detection rate of GNB was related to *A. baumannii* (29/51) followed by *P. aeruginosa* (15/51) (Tables 1–2). *A. baumannii* was detected in various hospital wards with a frequency of 25–100%. *A. baumannii* has become increasingly common cause of nosocomial infections with about 50% mortality which has the highest rate in patients admitted to ICU (Rebmann and Rosenbaum, 2011). In the study, *A. baumannii* was detected in 54% of ICU air samples (Table 1). Detection of *A. baumannii* in ICU air samples has also been reported in other studies (Wang *et al.*, 2003; Huang *et al.*, 2013; Munoz-Price *et al.*, 2013; Shamsizadeh *et al.*, 2017). In air samples of a trauma ICU, 23% (12/50) of patient air zones contained *A. baumannii* (Munoz-Price *et al.*, 2013). Shamsizadeh *et al.* (2017) have reported the detection of *A. baumannii* in air samples of ICU but they couldn't detect it in OT (Shamsizadeh *et al.*, 2017). However, in the present study, *A. baumannii* was detected by PCR in the OT and SW with a higher rate than ICU which indicates that other parts of hospitals than ICU may be high-risk areas for *A. baumannii* infections. This difference may be related to the VBNC state of microorganism or higher detection sensitivity of PCR assay than culture method. Furthermore, we used the liquid impingement sampling as an efficient method for collecting GNB bioaerosols. Previous studies have reported a great capacity of the liquid impingement technique for

collecting airborne *Legionella*, particularly in combination with molecular or FISH detection methods (Deloge-Abarkan *et al.*, 2007; Montagna *et al.*, 2017). Study of Deloge-Abarkan *et al.* (2007) showed that the culturable fraction of airborne *L. pneumophila* recovered with the liquid impingement was 4 and 700 times higher compared to the impaction and filtration techniques, respectively.

P. aeruginosa was also found in various hospital environments with the highest and lowest frequency in OT and IM wards, respectively. No detection of *P. aeruginosa* was reported for hospital D (Table 2). In agreement with our study, Tang and Wan (2013) detected *Acinetobacter* spp. in post-operative recovery room and operating theatres and locations surrounding operating theatres with a higher rate than *P. aeruginosa* in a medical center in Taiwan. However, *P. aeruginosa* was the most abundant and frequently detected species (37.5–42.5%) in two intensive care units of a medical center in central Taiwan. Whereas, detection rates of 7.5–13% were reported for *A. baumannii* (Huang *et al.*, 2013). One study in a hospital in Bangkok also showed that the predominant isolates on the outside of the 230 used surgical masks of hospital personnel were *Staphylococcus aureus* (41%) and *Pseudomonas spp.* (38%) (Luksamijarulkul *et al.*, 2014). *Pseudomonas* spp. (4.8%) was isolated from air samples of a university-hospital autopsy room in France (Maujean *et al.*, 2012). Study of Dettori *et al.* (2014) on environmental investigation in an intensive care unit of a tertiary hospital in northern Sardinia, Italy, showed the detection of *Pseudomonas* spp. in air samples, but they couldn't detect any *A. baumannii* in air samples (Dettori *et al.*, 2014). Since the density of airborne bacteria in hospital environments is affected by several factors such as the number and type of occupants, people activities and ventilation conditions (Wu *et al.*, 2011; Huang *et al.*, 2013), the difference in detection frequency of GNB might be caused in part by the different
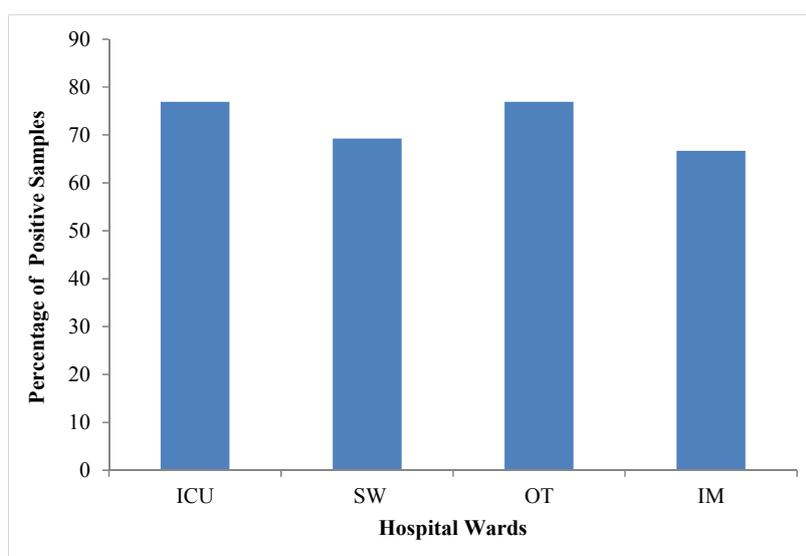
Fig. 1. Percentage of positive air samples which contained at least one type of the detected gram-negative bacteria in each hospital ward. ICU: Intensive Care Unit; OT: Operating Theatre; SW: Surgery Ward; IM: Internal Medicine Ward.

types of infected patients that act as dispersion sources of microorganisms in hospital air. Study of Shimose *et al.* (2015) showed that the ambient air of *Acinetobacter*-positive patients was *Acinetobacter* positive for an average of 21% of the days in samples of up to 10 consecutive days. Their results showed that 4 pairs of 6 air/clinical isolate pairs were closely related according to rep-PCR (Shimose *et al.*, 2015). On the other hand, as air sampling methods are not standardized (Sehulster *et al.*, 2003), comparison of bioaerosol results between different studies may be ambiguous (Pasquarella *et al.*, 2012).

P. *aeruginosa* and *Legionella* can also be frequently found in water systems because of the biofilm formation. Analysis of hospital water samples showed a high rate detection of *Legionella* and *P. aeruginosa* (Asghari *et al.*, 2013; Baghal Asghari *et al.*, 2013). Tap water contamination to these bacteria could lead to hospital air contamination through bioaerosols released to the air by water systems (Sehulster *et al.*, 2003; Deloge-Abarkan, 2007). Montagna *et al.* (2016) detected *Legionella pneumophila* (*Lpn*) in air and water samples of three health-care facilities in Italy. Molecular investigation of *Lpn* isolates showed the same allelic profile for strains in the water and air samples. In an investigation in two health-care centres in northern France, *Legionella* spp. were detected in 72.2% of air samples of hot water shower aerosols (Deloge-Abarkan, 2007). *Legionella* detected with the lowest frequency in hospital air (9/51) and analysis of SW air samples showed no *Legionella* detection (Table 3). Pasquarella *et al.* (2012) reported more frequently detection of *Legionella* spp. in water samples when *P. aeruginosa* was absent, probably due to the growth limitation of *Legionella* in the presence of *Pseudomonas*. Although, contaminated water systems have been considered as the sources of nosocomial legionellosis, Mathieu *et al.* (2006) believe that detection of airborne *Legionella* offers better information than water analysis. However, to minimize

the exposure of patients to contaminated bioaerosols, growth of microorganisms in hospital water systems could be controlled using disinfection methods such as UV irradiation (Nourmoradi *et al.*, 2012). The results of study also showed higher positive samples of GNB in ICU and OT (Fig. 1). Due to the importance of these units in transmission of nosocomial infections, improvement of control measures is needed in order to protect high-risk patients from GNB which may be transmitted through contaminated hospital air.

CONCLUSION

Gram-negative bacteria were frequently detected in the air of various hospital wards, especially the ICU and OT. The introduced rapid and sensitive approach of directly detecting bacteria leads to more information about human exposure to these nosocomial infectious agents. Rapid monitoring of hospital environments provides insights into the proper management of nosocomial infections, especially in high-risk areas. Since humidity is an important factor that affects the survival of GNB, more studies in humid regions are needed.

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