Direct monitoring of gram-negative agents of nosocomial infections in hospital air by a PCR-based approach

Mahnaz Nikaeen[1*], Zahra Shamsizadeh[1], Seyed Hamed Mirhoseini[2]

[1]Department of Environmental Health Engineering, School of Health, Isfahan University of Medical Sciences, Isfahan, Iran.

[2]Department of Environmental Health Engineering, School of Health, Arak University of Medical Sciences, Arak, Iran.

*Corresponding author, Tel.: 98-313-792-3278; Fax: 98-313-668-2509

E-mail address: nikaeen@hlth.mui.ac.ir

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. This study was designed to investigate the presence of GNB in hospital air as a potential source for spread and transmission of these bacteria, by a rapid detection method. A total of 51 air samples were taken from different wards of 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 of surgery wards. Intensive care units and operating teaters were the high-risk areas due to the more presence of GNB in these wards. The results of this study revealed the presence of GNB in various hospital wards. The results highlight the usefulness of PCR-monitoring of hospital air as a rapid and reliable tool for identification of GNB, to prevent and control the nosocomial infections especially for protection of vulnerable patients.

Key words: 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 be 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 (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 mean 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 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 throught 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 (Table 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 non culturabe (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 industrial cooling towers showed no cultivable *Legionella* bacteria using the standard culture method, even when the concentrations of ~1×10^3 *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) (Table 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 truma ICU, 23% (12/50) of patient air zones contained *A. baumannii* (Munoz-Price et al., 2013). Shamsizadeh et al. (2017) have been 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*, particullarly 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 theaters and locations surrounding operating theaters 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 French (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 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 be 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 bee transmited through contaminated hospital air.

Conclusion

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

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List of tables:

Table 1:

Hospital	Location				Total
	ICU	OT	SW	IM	
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:

Hospital	Location				Total
	ICU	OT	SW	IM	
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:

Hospital	Location				Total
	ICU	OT	SW	IM	
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

Figure captions:

Figure 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

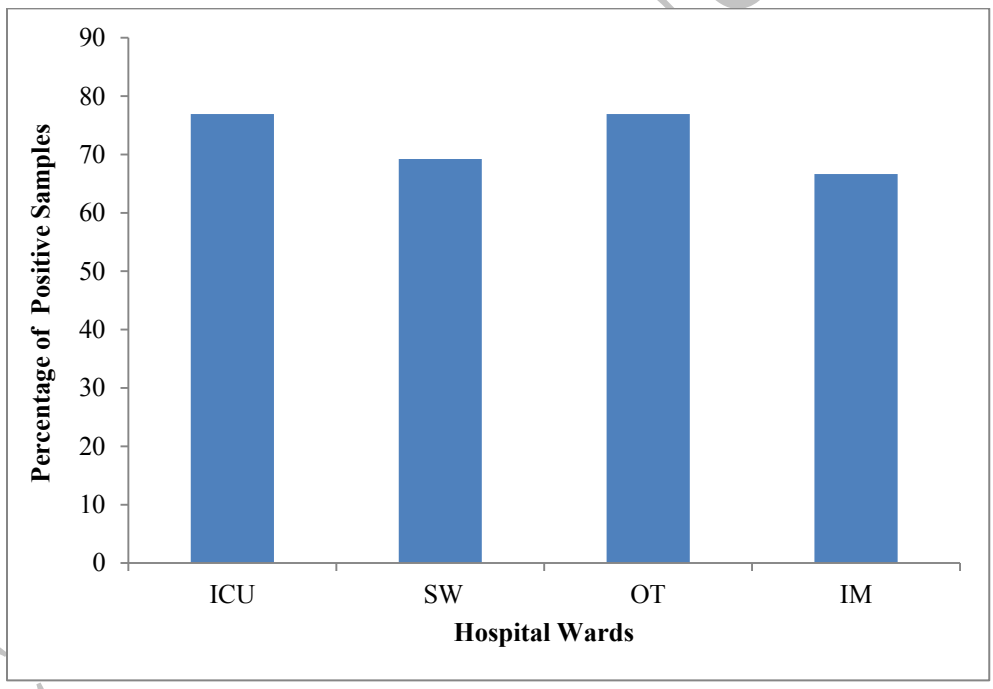

Fig. 1.