Original Article

Investigation of Hospital Water Systems Contamination to Bacterial Agents of Nosocomial Infections

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Abstract

Aim: Nosocomial infections have become increasingly a major health concern in many hospitals. Gram-negative bacteria (GNB), including *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Legionella* have emerged among the most problematic microorganisms in hospital settings, which can cause a variety of nosocomial infections, especially in susceptible individuals. Biofilm formation allows these waterborne agents to persist in hospital water systems for extended periods. Since the transmission is the initial step in disease occurrence, effective prevention of nosocomial infections requires a better knowledge about waterborne bacteria. The aim of this study was to investigate the frequency of presence of GNB in hospital water systems by a rapid and reliable assay. **Materials and Methods:** A total of 33 water samples were collected from 11 hospitals of Isfahan University of Medical Sciences, Iran and analyzed for the presence of GNB by a polymerase chain reaction (PCR) assay with the application of specific primer sets. **Results:** From the 11 hospitals surveyed, 91% (10 of 11) were positive for at least one of the types of GNB. GNB were detected in 58% (19 of 33) of water samples. 45% (15 of 33) of samples were positive for *legionella. A. baumannii* and *P. aeruginosa* were detected in 18% (6 of 33) of water samples. The mean concentration of heterotrophic bacteria was 36 CFU/ml. **Conclusion:** Detection of GNB in hospital water systems with a relatively high frequency revealed that hospital water may act as an important route for transmission of nosocomial infections. The results emphasize the importance of rapid microbiological monitoring and the implementation of strict control measures in hospital water systems.

Keywords: Gram-negative bacteria, hospital, nosocomial infection, polymerase chain reaction, water

Introduction

Nosocomial infections are among the most important health concerns, with a prevalence of 1.4 million cases worldwide.[1] At the moment, the rate of these infections in developed and developing countries is estimated at about 11% and 25%, respectively.[2] Gram-negative bacteria (GNB), including *Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *Legionella,* are among the most important causes of nosocomial infections.[3] Pneumonia, urinary tract infections, and blood infection are the most prevalent infections caused by *A. baumannii* and *P. aeruginosa,* as well as legionellosis and Pontiac fever by *Legionella*.[4] Immunocompromised patients are the most susceptible individual and at risk of developing these infections.[5] The hospital water system could serve as a potential source for the dissemination of Gram-negative microorganisms, which are the cause of nosocomial infections.[6] The biofilm formation in the water system promotes the growth of these bacteria in the hospital water systems and causes various infections through contaminated water.[6,7]

Exposure to waterborne microorganisms in the hospital environment can occur during bathing, breathing of bio-aerosols and contact with medical equipment contaminated with water containing these microorganisms.[8,9] In several studies, the role of water has been proven as the source of

Access this article online

Quick Response Code:

Website: www.ijehe.org

DOI: 10.4103/ijehe.ijehe_12_19

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How to cite this article: Shamsizadeh Z, Ehrampoush MH, Nikaeen M, Ebrahimi AA, Asghari FB. Investigation of hospital water systems contamination to bacterial agents of nosocomial infections. Int J Env Health Eng 2020;9:10.

Received: 24-12-2019, **Accepted:** 20-06-2020, **Published:** 31-07-2020

A. baumannii infections.[10] The prevalence of amikacin and ciprofloxacin-resistant *A. baumannii* in Tokai University Hospital emergency ward was due to the use of contaminated tap water for oral care.[10] In the case of *legionella*, inhalation of contaminated bioaerosols is the principal route of exposure to the bacterium.[3]

In recent years, due to the increasing number of immunocompromised patients,[11] GNB infections are one of the biggest challenges facing hospitals, especially in developing countries. Therefore, it is necessary to reduce the exposure of patients to infectious agents by rapid monitoring of the source of these bacteria. The culture technique is a common method for the detection of GNB. However, this method is time-consuming and costly. In addition, the culture technique cannot detect bacteria in viable but nonculturable (VBNC) state. Therefore, the use of molecular techniques such as polymerase chain reaction (PCR) as a quick, sensitive, and reliable method for detecting GNB could be useful in the prevention and management of nosocomial infections.[5]

The present study was undertaken to investigate the presence of GNB in hospital water systems using a PCR based method. Furthermore, the relationship of heterotrophic plate count (HPC) with the contamination of hospital water systems with GNB was investigated.

Materials and Methods

Water samples

A total of 33 water samples were collected in sterilized 500-ml glass bottles,[12] from 11 hospitals of Isfahan University of Medical Sciences, Iran. In each hospital, water samples were obtained from different locations, including taps and showers, and were transferred to the laboratory. The amount of residual free chlorine (METERRC) was measured at the time of sample collection. HPC bacteria were determined by culture on R_2A agar and incubation at 35°C for 48 h.

Detection of Gram-negative bacteria

To detection of GNB, water samples were concentrated using membrane filters (0.22 µm, 47 mm diameter; Millipore). Membrane filters were washed in sterile phosphate buffer and shaken for 30 min.[5] Finally, the suspensions were centrifuged, and the resulting sediment was used for DNA extraction. The DNA extraction was performed by several cycles of freezing-thawing and then DNA purified by a Promega DNA Extraction Kit (Promega Wizard® Genomic DNA Purification Kit Madison, WI) according to the manufacturer's instructions. The purified DNA was finally resolved in distilled water and used for PCR assay.

For the detection of *P. aeruginosa* and *legionella*, a nested PCR method was used to increase the sensitivity. In the first PCR step, a portion of the 16s-rRNA gene was amplified using general primers Eubac 27F and 1492R [Table 1]. The use of general primers also makes it possible to identify the problem of DNA extraction or the presence of PCR inhibitors in the extracted sample. In the second PCR step, the specific primer sets of *P. aeruginosa* and *Legionella* were used [Table 1]. For the detection of *A. baumannii*, the OXA-51 primer set was used [Table 1].

PCR amplification was conducted in a final volume of 25 µL, as described by Nikaeen *et al*.[13] The PCR cycling conditions were as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of 45 s at 94°C, primer annealing at varied temperatures (according to the selected primers) for 45 s, primer extension at 72°C for 45 s, and final extension at 72°C for 10 min. PCR products were analyzed by agarose gel electrophoresis using 1.5% agarose gel. Gels were viewed on an ultraviolet transilluminator (UV Tech, France).

Results

In the present study, a total of 33 water samples were taken from different tap water outlets from 11 hospitals and examined for the presence of *A. baumannii. Legionella* and *P. aeruginosa.*

Among the studied bacteria, *legionella* was the most frequently (15/33) detected Gram-negative microorganism in hospital water. Furthermore, the frequency of *A. baumannii* (6/33) and *P. aeruginosa* (6/33) was in the next rate [Table 2]. Figure 1 shows the agarose gel electrophoresis of PCR products.

We found that the percentage of hospital systems that contaminated with *Legionella* was 82% (9/11) and in the case of *A. baumannii* and *P. aeruginosa* 36% (4/11). Furthermore, results of the PCR, revealed that 10 out of 11 hospital systems were contaminated to at least one of the GNB, and so GNB were detected in 91% of hospitals [Figure 2].

The mean concentration of heterotrophic bacteria was 36 CFU/ml and ranged from 4 to 100 CFU/ml. We found that the mean concentration of HPC was no different in GNB-positive samples and in GNB-negative samples. In other words, our study showed no relationship between the presence of GNB and the concentration of HPC bacteria. The mean chlorine residue concentration was 0.06 mg/l and in the range of 0–0.21 mg/l, and therefore, we could not find a relationship between the chlorine residue concentration and the presence of GNB or HPC concentration.

Discussion

Colonization of the hospital water systems with GNB can lead to nosocomial infections.[3] The results of this study indicate the presence of GNB in hospital water [Table 2]. Several studies have confirmed the detection of GNB, including *Acinetobacter*, *Legionella*, *Pseudomonas,* and *Enterobacteriaceae* in hospital water.[10,14-16] However, because of the GNB susceptibility to environmental conditions,[3] they may be found in VBNC form and so on, could not be detected with cultural-based methods. In fact, VBNC bacteria are alive and virulent but are not able to divide the cell and grow on the culture medium, but the

Table 1: Primers used for detection of bacteria

Primer	Sequence (5' → 3')	Amplified fragment (bp)	Annealing temperature (°C)	Reference
Eubac 27F	F: AGAGTTTGATCCTGGCTCAG	1420	55	[5]
1492R	R: AGAGTTTGATCCTGGCTCAG			
OXA51 F	F: TAATGCTTTGATCGGCCTTG	353	57	[17]
OXA R	R: TGGATTGCACTTCATCTTGG			
LEG 448	F: AGGGGTTGATAGGTTAAGAG	386	55	[14]
LEG JRP	R: CCAACAGCTAGTTCACATCG			
P. aeruginosa F	F: GGGGGATCTTCGGACCTCA	956	55	[5]
P. aeruginosa R	R: TCCTTAGAGTGCCCACCCG			

P. aeruginosa: Pseudomonas aeruginosa

Table 2: Detection frequency of gram-negative bacteria in hospital water samples

Hospital number	Positive samples/total samples		
	Legionella	*P. aeruginosa*	*A. baumannii*
Hospital 1	1/3	ND	ND
Hospital 2	2/3	2/3	ND
Hospital 3	1/3	ND	ND
Hospital 4	2/3	2/3	ND
Hospital 5	ND	1/3	1/3
Hospital 6	2/3	ND	2/3
Hospital 7	1/3	ND	ND
Hospital 8	2/3	ND	1/3
Hospital 9	2/3	ND	2/3
Hospital 10	ND	ND	ND
Hospital 11	1/3	1/3	ND

ND: Not detected, *P. aeruginosa: Pseudomonas aeruginosa, A. baumannii: Acinetobacter baumannii*

PCR method can detect these microorganisms. Therefore, it is expected that the high prevalence of GNB in this study is due to the identification of VBNC bacteria by PCR. Shamsizadeh *et al.* have been reported the detection of *A. baumannii* in only one hospital water sample out of 42 samples that they analyzed using the culture method.[17] In the present study, the PCR method was used as a qualitative method for identifying GNB, so it is suggesting to take into account quantitative methods such as real-time PCR to determine the number of bacteria in the hospital environment. However, there are disagreements over the importance of using quantitative methods in the detection of GNB because there is no acceptable level for the presence of GNB bacteria including *Legionella* in hospital environments. Centers for Disease Control and Prevention (CDC) emphasis on the importance of using a quick and accurate monitoring method for the detection of microorganisms in hospital water to reduce the potential exposure of patients to these bacteria.[3]

The presence of *Legionella* in hospital water is recognized as the main reason for Hospital-acquired *legionellosis*.[7] Several studies have been reported the detection of *Legionella* in hospital water.[18,19] Napoli *et al.* detected *Legionella* in 79.1% (102/109) of the health care facilities water samples in Italy.[19] In the present study, 82% of hospitals and 45% of water samples were contaminated with *Legionella* [Figure 2]. Although the *Legionella pneumophila* (*Lpn*) does not form robust biofilms, the ability of this bacterium to join pre-established biofilms by other bacteria such as *Mycobacterium chelonae*, *Acinetobacter lwoffii*, *Pseudomonas putida* leads to stability of *Legionella* in biofilm. In contrast, *P. aeruginosa* and *Pseudomonas fluoresces* biofilms have an antagonistic effect on *Legionella* survive due to the production of antibiotic-like inhibitors.[18]

P. aeruginosa and *A. baumannii* were detected in 18% of the water samples [Figure 2]. Furthermore, 36% of the investigated hospitals were contaminated with *P. aeruginosa* and *A. baumannii* [Table 2]. Several studies have reported the presence of *P. aeruginosa* in water.[18,20] Rogues *et al.* detected *P. aeruginosa* in 11.4% of 484 tap water samples taken from patient rooms.[21] One study in a hospital in Birmingham between 2013 and 2017 also showed that 1%–14% of water samples obtained from the hematology ward were contaminated with *P. aeruginosa*.[20] In another study by Varin *et al.*, *P. aeruginosa* was detected in 46.4% of samples (121 out of 261 samples).[22]

Legionella, *P. aeruginosa,* and *A. baumannii* can also be detectable in hospital air.[23] It is important to consider that GNB presence is probable in aerosols formed from contaminated water and so on airborne transmission.[3] The study of Montagna *et al.* on the hospitals in Italy showed that air and water samples in hospitals (3/10) were *Lpn* positive. Molecular investigation showed that *Lpn* strains, which were detected in the air and water samples, had the same allelic profile.[24]

A. baumannii was also found in 18% of samples. Although *Acinetobacter* has been identified as the relatively low virulence bacterium,[25] it is now recognized as an important opportunistic pathogen that causes hospital infections, especially in immunocompromised patients, and in patients admitted to the intensive care units.[26] Several studies have shown the role of water as a source of *A. baumannii* infection.[27,28] Horii *et al.* Suggested that the shower bath could act as one of the factors causing *Acinetobacter* related hospital infections.[29] The study of Volkow *et al.* also showed that *A. baumannii* isolates from showerhead water, intravenous catheter, and blood cultures of bloodstream-infected patients were related to the same strain.[28]

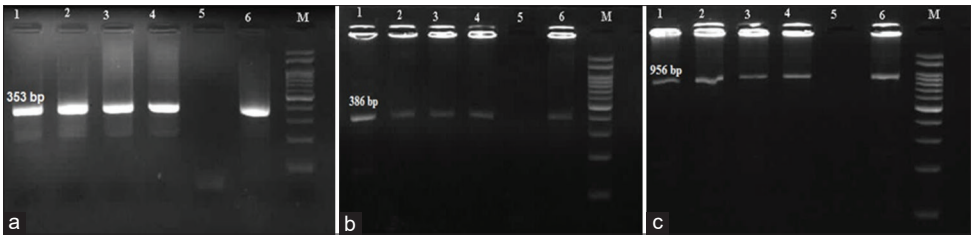

Figure 1: Agarose gel electrophoresis of polymerase chain reaction products: (a) *Acinetobacter baumannii* (b) *Legionella*. (c) *Pseudomonas aeruginosa*. M, DNA Marker (100 bp); 1–4, polymerase chain reaction products; 5, negative control 6, positive control

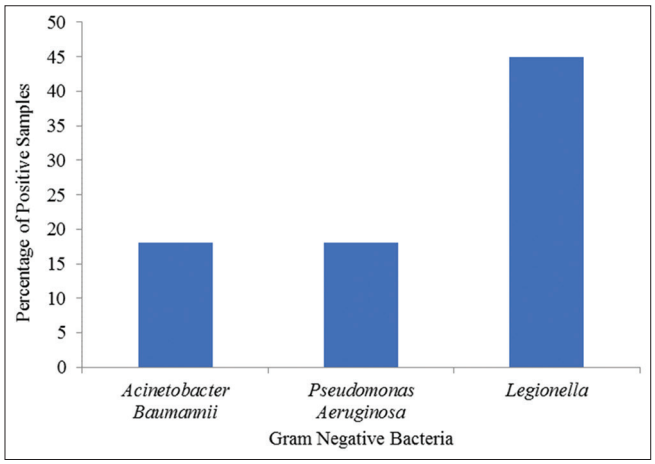

Figure 2: Percentage of positive water samples which contained detected gram-negative bacteria

Several factors affect the survival of GNB in the hospital environment. Pipe size and network design, water systems with low flow, temperature, the concentration of residual chlorine, nutrient level, and hydraulic of water system could affect the biofilm formation and consequently presence and survival of GNB in the hospital water systems.[9,30]

Therefore, the variation of these factors in different hospital water systems may, in part, lead to the different detection rates of GNB in various studies. The mean concentration of heterotrophic bacteria was 36 CFU/ml in a range of 4–100 CFU/ml. However, our study showed no relationship between the HPC and the presence of GNB.

Our results revealed that 91% of the hospital's water system was contaminated at least one type of GNB. Biofilm formation in the water supply system allows these bacteria to survive in the hospital environment for a long time.[10,27] The low concentration of residual chlorine in the hospital's water system could be a reason for the growth of GNB. Although the detection rate of GNB varies in different studies, in accordance with our results, other studies have also shown that the hospital water system is often a proper haven for GNB and therefore requires enhanced monitoring and contaminant control techniques. An effective method for controlling hospital water contamination to inactivate etiological agents of hospital infection is point-of-use disinfection by ultraviolet radiation.[31]

Conclusion

Our results showed that hospital water can act as a potential route for the transmission of GNB. A key factor in the prevention of hospital infections is the rapid identification of the contamination source to use effective control strategies for the proper management of nosocomial infection agents in hospital environments. The method used in this study to identify GNB is a sensitive and reliable method that provides rapid monitoring of GNB in the hospital water system.

Acknowledgments

This work was supported by the Shahid sadoughi University of Medical Sciences, Student Research Committee (grant number 5930). The authors would like to appreciate the all those who have assisted in conducting this study at Isfahan University of Medical Sciences.

Financial support and sponsorship

Shahid sadoughi University of Medical Sciences, Yazd, Iran.

Conflicts of interest

There are no conflicts of interest.

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