سرویس های تخصصی
سرویس ترجمه
کارگاه های آموزشی
مرکز اطلاعات علمی
سامانه ویراستاری STES
فیلم های آموزشی

کارگاه های آموزشی مرکز اطلاعات علمی

مقاله نویسی علوم انسانی
اصول تنظیم قراردادها
آموزش مهارت های کاربردی در تدوین و چاپ مقاله
Detection of Legionella Contamination in Tabriz Hospitals by PCR Assay

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ARTICLE INFO

Article Type: Research Article

Article History:
Received: 18 September 2012
Revised: 7 November 2012
Accepted: 7 November 2012
ePublished: 7 February 2013

Keywords:
Hospital
Legionella spp.
Water system

ABSTRACT

Purpose: The present study was designed to evaluate the occurrence of Legionella contamination in the tap water of Tabriz hospitals, Azerbaijan, Iran. Methods: One hundred and forty water samples from diverse water supply systems of 17 hospitals were collected and analyzed for the presence of Legionella spp. by PCR assay. Results: In this study, 10 of 140 (7.1%) samples were positive for Legionella which L. pneumophila was detected in 4 (2.85%) water samples. Conclusion: In conclusion, hospital potable systems are the primary reservoirs for Legionnaires’ disease. This study concludes that Legionella spp. are present in aquatic hospitals environment of Tabriz. Due to the serious risk of infections, it is better to make efforts to eliminate Legionella spp. in water supplies.

Introduction

Legionella spp. are gram-negative, fastidious and ubiquitous bacteria responsible for mild upper respiratory tract infections or pneumonia following inhalation of contaminated water droplets from a variety of water sources.1 Probably 3 to 8% of all community-acquired pneumonias are caused by Legionella spp., and 85% of those pneumonias are caused by Legionella pneumophila.2 Non- L. pneumophila species have also been reported to be infectious.1 The case-fatality rate for patients with legionellosis is 5 to 30%, with the elderly and immunocompromised patients being at greater risk.1 Legionella infections can be the cause of extrapulmonary inflammatory diseases too.2 Although hospital cases are not infrequent, most cases occur sporadically.1 Numerous reports have shown an obvious association between the presence of legionellae in hot water supplies and the occurrence of legionellosis.1,3 Therefore, for risk assessment of nosocomial Legionella infections, examination of hospital water systems is required.4 Legionella are detected by various methods. Culture is considered the gold standard for the laboratory detection of Legionella infections. It has a sensitivity of 50 to 90%; however, colonies appear after 3 to 4 days.2 Legionellae are slow-growing bacteria, and successful culture needs selective media.3 Nucleic acid amplification tests have been shown to be helpful for the detection of Legionella.6 Recently, novel methods have been developed for finding of Legionella in water samples by PCR methods to overcome the limitations of culture. PCR techniques have the advantages of quickness, detection of nonculturable legionellae, and easier handling of large samples.4 This study presents the results of an environmental investigation on Legionella spp. that was carried out on the water distribution systems of 17 healthcare facilities in Tabriz, Iran.

Materials and Methods

Sample collection

A total of 140 tap water samples were collected from diverse water supply systems of 17 hospitals. The size of hospitals ranged from under 200 beds to 500 beds. All hospitals were supplied with chlorinated domestic drinking water. All samples were transported to the laboratory of microbiology department and kept at 4 to 8°C until analysis, and water samples were concentrated by filtration through 0.45μm pore size nitrocellulose membranes (HTTP, Millipore, Ireland). The membranes were aseptically removed, put into sterilized 50mL tubes and resuspended in 10mL of the original water samples. Each concentrated water sample was shaken for 30 min to get out bacterial cells from the membranes.7 For acidic treatment, the chloride acid was used as the acidic buffer with a pH of 2.2 over a period of 15 minutes.8 The overgrowth of faster growing bacteria and fungi was decreased with the heat treatment of samples.
Microbiological analysis
Aliquots of 100μL of prepared samples were inoculated on plates of buffered charcoal yeast extract agar (BCYE) with supplements (Difco Laboratories, Detroit, Mich., USA), and the plates were incubated at 37 °C in a humidified atmosphere with 2.5% CO₂ for 6-14 days. Colonies with the typical ground glass form of Legionella were Gram stained and subcultured on selective BCYE [containing polymyxin B (80U/ml), vancomycin (0.5μg/ml) anisomycin (80μg/ml), cefamandole (4μg/ml)] and non-selective media, such as sheep-blood agar and MacConky agar. Colonies that grew on selective BCYE agar but not on non-selective media were considered supposed Legionella. The identification of Legionella spp. were done by biochemical tests.79

DNA extraction and PCR assay
DNA extraction was performed as described previously.10 The PCR test was carried out in a 25μl of reaction mixture with the following compositions: DNA extract (5μl), 0.1 units Taq DNA polymerase (Fermentas), 1.5mM magnesium chloride, 0.5mM of deoxy-nucleotriphosphate, 2.5 mM buffer, and 0.5μM of each primer (Table 1). For amplification, an initial denaturation for 4min at 95 °C was followed by 30 cycles of 95 °C for 30 s, 61 °C for 30 s and 72 °C for 30 s. In this study, DNA of Legionella pneumophila ATCC 33152 was used as positive control and DNA of a laboratory isolate strain of E. coli as negative control. The PCR products were analyzed by agarose gel electrophoresis using 1.5% agarose gel containing ethidium bromide, and gels were viewed on a UV transilluminator.

| Table 1. Primers for PCR amplification of Legionella spp. |
|---|---|---|
| Target | Sequence | Product size (bp) | Ref |
| 16S rRNA | GCTTTGCCATCAAATCTTTCTGAA | 212 bp | 11,12,13 |
| 16S rRNA | CTTGCGTCGAGTTGAGCG | 212 bp | 11,12,13 |
| Mip | GCATTGGGCACGATTGAGC | 168 bp | 9,12 |
| Mip | GCATTGGGCACGATTGAGC | 168 bp | 9,12 |
| Mip | GCATTGGGCACGATTGAGC | 168 bp | 9,12 |

Results
In this research, various water supplies of hospitals were investigated for the presence of Legionella spp. by culture and PCR methods. Overall, 140 samples from various areas of hospitals were cultured, and Legionella spp. were isolated from 8(5.7%) of samples. Table 2 shows the number of Legionella spp. and the source of samples and the detecting methods. Two strains isolated from the water samples were identified as L. pneumophila by culture. In order to detect Legionella spp., at first 16Sr RNA gene was amplified using PCR test (Figure 1). Of the total of 140 samples, 10 (7.1%) were PCR positive for 16Sr RNA. L. pneumophila was detected in 4 (2.85 %) samples of hospital water systems by Mip gene PCR. In this study, the culture method showed excellent correlation with PCR results (P< 0.001).

Table 2. The detection of Legionella spp. according to the source and the methods.

<table>
<thead>
<tr>
<th>Source</th>
<th>Culture</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entrance water system</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Heating system</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Bathroom</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Kitchen</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Patient room</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ventilator</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>10</td>
</tr>
</tbody>
</table>

Discussion
The levels of Legionella contamination in hospital water system have been reported to correlate with the occurrence of nosocomial Legionella diseases.14 For risk evaluation of nosocomial Legionella infections; surveillance of hospital water systems is needed. A sampling program may be designed to perform an overall evaluation of the level of contamination of the hospital, or to detect the presence of Legionella during epidemiologic studies of clinical cases. In the absence of ascertained cases of legionellosis, the value of routine environmental sampling aimed at primary prevention is controversial.15 Although surveys of Legionella colonization in hospitals have been conducted in the UK, Canada, USA, and Spain16, but in Azerbaijan region, the health care facilities don’t obtain routine environmental cultures. The results of this study showed that Legionellae was detected in Tabriz hospitals. To the best of our knowledge, this is the first
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report of Legionella spp. isolation in this area. Many techniques have been reported to detect Legionella, including culture,7,13,17 Direct Florescence,7,17,18 specific PCR,7,17,19,20 and real-time PCR.4,6,11,13 Isolation of Legionellae spp. from water samples by culture technique is generally preferred. Culture is the gold standard and very important for antibiotic susceptibility tests, but it has some limitations such as the growth requirements of the organisms, long incubation periods, overgrowth of other bacteria, and nonculturable Legionellae in some environmental samples.4 PCR method has some advantages and some limitations. Non viable and non cultivable Legionella are detected by PCR. Our genus-specific 16S rRNA PCR test was detected all Legionella species. The 16S rRNA gene is exceptionally suited as a target gene since it exists in several copies per genome and thus allows a high sensitivity of the PCR. Another method using mip or 16S rRNA gene sequencing was also considered promising because it offer sequence-based identification for L. pneumophila and non-L. pneumophila species, but it is complex and time-consuming.3 We found 2 samples were positive for PCR but culture negative; it may be due to dead or non-cultivable Legionella spp. Dusserre et al. showed that L. pneumophila DNA detected by PCR in water samples treated with chlorine. Indeed, culture became negative after the addition of 0.5ppm chlorine for 24 h, whereas the PCR was preserved.12 In this study, 2 and 4 isolated strains were identified as L. pneumophila by culture and mip gene, respectively. There are nearly 40 specious Legionella spp. and mip target is specific for L. pneumophila.

In a previous study have shown that hospital water system may be contaminated by Legionella spp., and these strains can persist for long periods in water.14 According to an international study, 12-75% of all hospital samples are contaminated with Legionella in hospitals.22 Samples of hot water and biofilms were collected from 41 hospitals in Italy’s Piemonte region between June 1999 and March 2008, and Legionella spp. were isolated from 32% of them.13 In some studies conducted in Iran, prevalence of Legionella isolation from hospitals was 36.6% and 22.7% (8, 14). In a USA national surveillance study of 20 hospitals in 13 states, 6 hospitals were colonized with Legionella.23 In another study from Spain, the rate of Legionella contamination in 12 hospitals was reported 30%.24 There was any information on the prevalence of the Legionella spp. and the value of this bacterium as a nosocomial pathogen is not clear in the Tabriz. Our study confirms that a large portion of water systems in hospitals are contaminated with the bacterium and this may play a key role as a risk factor in the safety of patients in hospitals.

Legionella species have been isolated from a wide variety of water types.7 In this research, the numbers of Legionella positive samples from the kitchens, bathrooms, internal distribution system, and cooling tower were 3, 3, 3, and 1, respectively. Moreover, several authors have described the isolation of Legionella spp from showers, cooling towers and boilers,4,5 which is in agreement with the findings of our study. The results of the present study show that temperature has a key role in the positive samples. In this study, 13.72% of hot water samples were Legionella positive by PCR. The control of hot water supplies in hospitals is crucial, and several reports have shown a clear association between the presence of Legionella in hot water systems and occurrence of legionellosis.10,23

Conclusion
In conclusion, hospital potable water systems are the primary reservoirs for Legionnaires’ disease. Therefore, surveillance of water supplies of hospital is important, and the infection can be prevented by disinfecting hospital water. This study shows that both culture and PCR methods could detect Legionella spp. in water supplies. However, PCR is more sensitive and faster than culture.

Acknowledgments
This project was financially supported by Tropical and Infectious Diseases Research Center, Tabriz University of Medical Sciences. This article was written based in a dataset of M. Sc thesis, registered in Tabriz University of Medical Sciences. We also appreciate Dr. Mosavian from Ahvaz, and Dr. Feizabadi and Dr. Yaslianifar from Tehran who kindly donated some standard samples of Legionella pneumophila.

Conflict of Interest
There is no conflict of interest in this study.

References

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Advanced Pharmaceutical Bulletin, 2013, 3(1), 131-134 | 133

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