Isolation of *Legionella pneumophila* From Hospitals Water Supply in Egypt

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ABSTRACT

Objectives: The study was conducted to screen the water samples isolated from hospitals in Egypt for contamination with *Legionella* spp. Methods: A total of 66 water samples were collected from different departments in 10 governmental hospitals. Identification of *Legionella* spp and *Legionella pneumophila* was performed by standard culture method and by PCR assay. Serogrouping of *L. pneumophila* was carried out utilizing latex agglutination test. Results: The current study revealed that 8 of 66 (12%) water samples were positive for *Legionella* spp by culture method. However, only 4 (6%) samples were contaminated with *L. pneumophila* identified by PCR, 3 of them were also identified by culture method. *L. pneumophila* were found in different hospital departments; accident and emergency department, intensive care unit, internal medicine department and chest department. All identified *L. pneumophila* were of serogroup 1. Conclusions: *Legionella* spp is ubiquitously present in water samples. Potable hospital water supply is a primary source of Legionnaires’ disease if contaminated with *L. pneumophila*. Routine screening of *Legionella* spp in water supply to hospitals is required periodically to remove the pathogen and enhance health care during hospital stay.

Keywords: Egypt; Hospital; Legionella pneumophila; Legionnaires’ disease; Water.

INTRODUCTION

*Legionella pneumophila* have emerged as an important pathogen in both community and hospital-acquired pneumonia. The bacterium is gram negative rods and fastidious. Approximately 10% of community-acquired pneumonias were due to infection with *Legionella* spp and 90% of them were caused by *L. pneumophila*. Other commonly identified pathogens causing community-acquired pneumonia are *Streptococcus pneumoniae, Haemophilus influenzae, Chlamydia pneumoniae, Mycoplasma pneumonia* and viruses. Inhalation of water droplets containing *Legionella* can cause legionellosis disease which includes Legionnaires’ disease, a type of atypical pneumonia, and Pontiac fever, an acute febrile self-limited illness. Numerous outbreaks of Legionnaires’ disease have been reported all over the world. High isolation rates of *Legionella* spp from aquatic environments such as showers, faucets, springs, etc were reported worldwide. Consequently, routine screening of *Legionella* in water samples is important in avoidance of human infection. *L. pneumophila* contaminated water systems in hospital supply is a potential source of Legionnaires’ disease transfer especially to hospitalized patients. The mortality rate of legionellosis patients can reach 30% in immunocompromised and elderly patients. The present study aimed to detect the prevalence ratios of *Legionella* spp and *L. pneumophila* in hospital water supply from 10 governmental hospitals in Egypt.

MATERIALS AND METHODS

Samples collection

A total of 66 samples were collected from faucets and showers in various departments and wards in 10 tertiary care governmental hospitals in Egypt. All water supplies in those hospitals were potable water treated with chlorine. More than one water sample might be taken from the same hospital ward/department.
Sterile cotton swabs were used to swab the interior walls of faucets and showers several times after removing their filters. The swabs were utilized to inoculate BCYE (buffered charcoal yeast extract) plates containing GVPC supplements (glycine, vancomycin, polymyxin B and cycloheximide) (bioMérieux, Marcy l’Etoile, France) by streaking. Cultured plated were incubated at 36°C for 48 hours.

**Morphological identification of L. pneumophila**

_Legionella_ spp colonies appeared white-grey color and smooth with regular margin (Figure 1). _L. pneumophila_ colonies were yellowish green under UV light. Confirmation of presumed colonies was carried out by absence of growth in BCYE medium without L-cysteine (bioMérieux, Marcy l’Etoile, France). _L. pneumophila_ confirmed isolates were preserved in glycerol/nutrient broth; 1:1 at -80°C.

**Molecular identification of Legionella spp and L. pneumophila by PCR**

DNA extraction of suspended water sample swabs in 1 ml sterile ultrapure water were performed as previously described.17 PCR amplification was carried out using two primer pairs; for _Legionella_-specific 16S rRNA gene18 and for _mip_19 gene specific to _L. pneumophila_. PCR reaction condition was performed following previously reported.19 Positive and negative controls were run in every PCR cycle. PCR products were analyzed by 1.5% agarose gel electrophoresis. Gel was stained by ethidium bromide and photographed under UV lamp.

**Serogrouping of L. pneumophila water isolates**

Latex agglutination test, Slidex® Legionella kit (bioMérieux, Marcy l’Etoile, France), was used to identify _L. pneumophila_ serogroup SG1 and serogroup SG2-15 according to the manufacturer’s protocol. The kit utilizes latex particles coated with antibodies. _L. pneumophila_ antigens bond to kit’s antibodies causing visible agglutination in 30 seconds to few minutes.

**RESULTS**

In the current study, 66 water samples from various departments and wards in 10 hospitals in Egypt were screened for the presence of _Legionella_ spp and _L. pneumophila_. Table 1 shows the distribution of water samples among different departments in the hospitals and the detected _Legionella / L. pneumophila_ isolates. Eight _Legionella_ spp were isolated from water samples of 4 of 10 hospitals examined. Three water samples of 66 samples (4.5%) from 2 hospitals were detected to carry _L. pneumophila_ isolates by culture; whereas four samples of 66 (6%) in 3 hospitals were found to have the organism by PCR molecular identification. Hospital 5 was detected to harbor _L. pneumophila_ in two water samples isolated from Intensive Care Unit (ICU) and Accident & Emergency department. However, hospital 8 had 3 _Legionella_ spp from its Cardiology and Psychology wards. One _L. pneumophila_ isolate from chest department in hospital 9 was identified as _L. pneumophila_ by PCR but not by culture identification. Overall, the correlation between isolates identification by culture method and PCR was excellent. All the 4 _L. pneumophila_ isolates detected by PCR were characterized to be of serogroup SG1 using latex agglutination test.

**DISCUSSION**

Water contamination with _Legionella_ spp is considered a potential risk for transfer of Pontiac fever and Legionnaires’ disease. Surveillance of hospitals water supply for the routine screening of the pathogen is of great importance to avoid _L. pneumophila_ outbreaks specifically in hospitalized and immunocompromised patients. The correlation between detection of _L. pneumophila_ in water samples and human health risk of infection is still an issue of debate. Some studies linked the detection of the organism in potable water samples with outbreaks of Legionnaires' disease in the supplied areas;12-15 whereas other studies failed to prove the same linkage.16-20 Standard culture method was widely used to detect and quantify _Legionella_ in water samples.21 However, the method has several limitations; it is time-consuming, viable but non-culturable _Legionella_ will fail to grow and false positives/false negatives are frequently occur.22 Alternatively, molecular identification of _Legionella_ with PCR or quantitative (real-time) PCR (qPCR) is increasingly applied and sometimes replacing the culture method although it may overestimate the bacterial count as it detects both living and dead organisms.23 In the current study, one isolate of _L. pneumophila_ was detected by PCR but not culture
method. It may be either non-cultivable or dead organism. The role of viable but non-culturables in causing human health risk is still uncertain. It is also reported that using both culture and molecular identification methods gives better estimates of Legionella count and hence improves the human health care.26

In Egypt, screening of L. pneumophila in water samples has not been drawn a sufficient care. A recent study from Egypt27 screened the presence of Legionella in 25 water samples from chest department, emergency ICU and surgery ICU in one hospital over two-year study period. They detected high ratio of the organism 10 of 25 (40%) in water samples, mostly (7 samples) from chest department by real-time PCR method. To compare with our study findings, only 8 of 66 (12%) water samples were found to have Legionella spp by culture method. This difference in rates of Legionella spp between our study and theirs may be also due to different techniques of pathogen identification used in both studies. Moreover, they examined Legionella in 100 patients with pneumonia (50 patients had community-acquired pneumonia and the rest were diagnosed as having hospital-acquired pneumonia) in the same hospital. They found that 18 of 100 were infected with Legionella, 10 of them acquired Legionella from hospital stay.

Another study from Jordan28, a Middle East country in vicinity of Egypt, determined the prevalence of Legionella spp in domestic hot water systems of private apartments. They identified Legionella spp in 8.5% (17 of 200 water samples) by culture method, 15 of those were L. pneumophila and 10 of them belonged to serogroup 1. Similarly, in the present study, L. pneumophila serogroup 1 is prevalent and detected in all our 4 L. pneumophila isolates. Approximately 80% of L. pneumophila infections were caused by serogroup 1.29 An international study31 reported a wide range (12-75%) of Legionella spp contamination in hospital water samples. Additionally, the isolation rates of L. pneumophila from different public facilities including hospitals ranged from as low as 3%, in a study from Iran30, to a high rate of 59%, in a study from China9.

CONCLUSION

Environmental surveillance system is highly needed to obtain incidence data of Legionella spp and L. pneumophila in water samples isolated from public facility sources specifically hospital supplies. Despite the sporadic occurrence of legionellosis (Pontiac fever and Legionnaires' disease), hospital public health boards must include isolation of L. pneumophila from hospital water supply as a potential risk factor of L. pneumophila outbreak incidence. Further studies and routine screening of water samples for the detection of Legionella spp is urgently needed in our region to compensate the shortage of prevalence data.

Table 1. Water samples distribution in hospitals and detected Legionella spp / L. pneumophila samples

<table>
<thead>
<tr>
<th>Hospital</th>
<th>No. of water samples / ward or department</th>
<th>No. of positive Legionella spp samples / ward or department</th>
<th>No. of positive L. pneumophila samples by culture / ward or department</th>
<th>No. of positive L. pneumophila samples by PCR / ward or department</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>5 / Adm., Derma., Ped.,</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H2</td>
<td>9 / Adm., A+E, ICU, Onc., Ortho.</td>
<td>1 / Onc.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H3</td>
<td>8 / Derma., Int., Neuro., Psychos., Surg.</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H4</td>
<td>7 / Adm., Cardio., ENT.</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H5</td>
<td>7 / A+E, ICU, NICU, Onc.</td>
<td>2 / A+E, ICU</td>
<td>2 / A+E, ICU</td>
<td>2 / A+E, ICU</td>
</tr>
<tr>
<td>H6</td>
<td>6 / Adm., Cardio., Int.,</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H8</td>
<td>6 / Cardio., NICU, Onc., Psycho.</td>
<td>3 / Cardio., Psycho.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>H9</td>
<td>9 / Chest, ENT, ICU, Int., Neuro., Surg.</td>
<td>0</td>
<td>0</td>
<td>1 / Chest</td>
</tr>
<tr>
<td>H10</td>
<td>4 / A+E, Derma., Ped.</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>66</td>
<td>8</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

Hospital departments’ abbreviations:
Adm.: Administration; A+E: Accident and Emergency; Cardio.: Cardiology; Derma.: Dermatology; ENT: Ear, Nose and Throat; Gyn.: Gynecology; ICU: Intensive Care Unit; Int.: Internal Medicine; Neuro.: Neurology; NICU: Neonatal Intensive Care Unit; Onc.: Oncology; Ortho.: Orthopedics; Ped.: Pediatric; Psycho: Psychology; Surg.: Surgical; Uro.: Urology.
Conflict of Interest
The authors declare that they don’t have any conflict of interest.

REFERENCES


