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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*.

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INTRODUCTION

Legionella pneumophila have emerged as an important pathogen in both community and hospital-acquired pneumonia.[1,2] 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*.[3-5] 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.[6] Numerous outbreaks of Legionnaires' disease have been reported all over the world.[7,8] High isolation rates of *Legionella* spp from aquatic environments such as showers, faucets, springs, etc were reported worldwide.[9-11] 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.[12-15] The mortality rate of legionellosis patients can reach 30% in immunocompromised and elderly patients.[16] 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 36ºC 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 -80ºC.

***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 gene[18] 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.

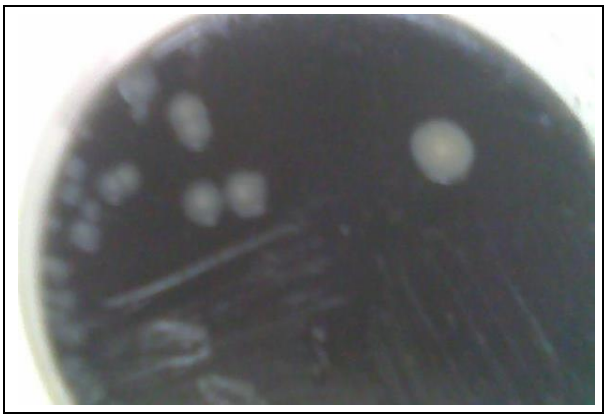

Figure 1: Appearance of *Legionella* spp colonies as white-grey color in BCYE plates containing GVPC supplements.

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.[20-22] Standard culture method was widely used to detect and quantify *Legionella* in water samples.[23] 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.[24] 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.[25] In the current study, one isolate of *L. pneumophila* was detected by PCR but not culture

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

Hospital	No. of water samples / ward or department	No. of positive *Legionella* spp samples / ward or department	No. of positive *L. pneumophila* samples by culture / ward or department	No. of positive *L. pneumophila* samples by PCR / ward or department
H1	5 / Adm., Derma., Ped.,	0	0	0
H2	9 / Adm., A+E., ICU, Onc., Ortho.	1 / Onc.	0	0
H3	8 / Derma., Int., Neuro., Psycho., Surg.	0	0	0
H4	7 / Adm., Cardio., ENT.	0	0	0
H5	7 / A+E, ICU., NICU, Onc.	2 / A+E, ICU	2 / A+E, ICU	2 / A+E, ICU
H6	6 / Adm., Cardio., Int.,	0	0	0
H7	5 / Gyn., Int., Surg., Uro.	2 / Int., Uro.	1 / Int.	1/ Int.
H8	6 / Cardio., NICU, Onc., Psycho.	3 / Cardio.; Psycho.	0	
H9	9 / Chest, ENT, ICU, Int., Neuro., Surg.	0	0	1 / Chest
H10	4 / A+E, Derma., Ped.	0	0	0
Total	**66**	**8**	**3**	**4**

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.

method. It may be either non-cultivable or dead organism. The role of viable but non-culturable *Legionella* in causing human health risk is still uncertain. It is also reported that using both culture and molecular identification methods gives better estimates of *L. pneumophila* 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 Egypt[27] 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 Jordan[28], 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 study[11] 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 Iran[30], to a high rate of 59%, in a study from China[9].

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.

Conflict of Interest

The authors declare that they don't have any conflict of interest.

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