



## **Prevalence of Nosocomial *Legionella Pneumophila* in a Liver Transplant Unit: Clinical and Environmental Study**

**Dalia Moemen<sup>1\*</sup>, Weam Shakra<sup>2</sup>, Ashraf Elshawadfy<sup>2</sup>, Abdou El-Moughith<sup>2</sup> and Mohamed Elsaadany<sup>3</sup>**

<sup>1</sup>Department of Microbiology and Immunology, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

<sup>2</sup>Department of Botany, Faculty of Science, Zagazig University, Zagazig, Egypt.

<sup>3</sup>Department of Gastroenterology Surgery, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

### **Authors' contributions**

*This work was carried out in collaboration between all authors. Author DM designed the study, select the cases and managed the experimental process. Author WS wrote the protocol, identified the bacterial species, managed the literature searches and wrote the first draft of the manuscript. Authors AE and AEM managed the analyses of the study and revised the final draft of manuscript. All authors read and approved the final manuscript.*

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### **ABSTRACT**

**Aims:** *Legionella pneumophila* (*L. pneumophila*) is a human pathogenic bacteria associated with aquatic habitat. It is a causative agent of sever pneumonia known as Legionnaires' disease (LD). LD among liver transplant recipients (LTRs) is difficult to diagnose with routine methods.

**Study Design:** Thus the current study was designed to detect *Legionella* in clinical samples as well as environmental samples in the liver transplant unit of Gastroenterology Surgical Center (GEC), from November, 2014 till June, 2016.

\*Corresponding author: E-mail: [dr\\_daliamoemen@yahoo.com](mailto:dr_daliamoemen@yahoo.com);

**Methods:** Respiratory, urine and blood samples were collected from 30 LTRs who were hospitalized with signs and symptoms of lower respiratory tract infections. The specimens were screened for *Legionella* by plate culture method, urinary antigen detection and serological investigation. A total of 40 environmental samples from hospital water and air ventilation system of the same unit were collected and analyzed for the occurrence of *Legionella*. The minimum inhibitory concentration (MICs) of *L. pneumophila* isolates against 8 antimicrobials were determined.

**Results:** The prevalence of nosocomial *Legionella* pneumonia was 20%. Six patients were positive for *L. pneumophila* by culture, among those, urine antigen was detected in five patients and serum investigation yielded positive in three patients. *L. pneumophila* was recovered from five water samples (12.5%). Azithromycin was found to be the most active against *Legionella* isolates in vitro. Chlorine (2 mg/L) and superheating (70°C) of hospital water successfully eradicated *L. pneumophila*.

**Conclusion:** For hospital with transplant units, periodic monitoring of *Legionella* in hospital water supply and introducing diagnostic tests for LD for patients with nosocomial pneumonia is necessary.

**Keywords:** Nosocomial pneumonia; *Legionella pneumophila*; Legionnaires' disease; liver transplant recipients; hospital water contamination.

## 1. INTRODUCTION

*Legionella pneumophila* (*L. pneumophila*) is a Gram negative, fastidious, aerobic, opportunistic intracellular human pathogen, that is ubiquitous in many water system and responsible for severe pneumonia called Legionnaires' disease (LD). *L. pneumophila* serogroup 1 is associated with almost 85–90% of the cases worldwide [1]. LD occurs sporadically or as outbreak and the case fatality rate associated with outbreaks increased for hospital-acquired infections and transplant patients [2]. LD is acquired by inhalation or aspiration of *Legionella* from contaminated environmental sources. Potable water is an important source of both nosocomial and community acquired *Legionella* infection [1]. Hospital-acquired *Legionella* infections is a serious problem in some hospitals. It was estimated that 20–30% of legionellosis were nosocomial and associated with contamination of hospital's water supply system [3]. Diagnosis of LD is difficult as the pneumonia caused by *Legionella* does not show a unique presentation, so for confirmation of LD the laboratory tests are necessary. Culture is considered the gold standard for the laboratory diagnosis of LD but *Legionella* are slow-growing bacteria, and need selective media; buffered charcoal yeast extract (BCYE) agar supplemented with  $\alpha$ -ketoglutarate, with or without antimicrobial agents [4]. ELISA is a commonly used antibody detection method, nevertheless the reported sensitivities of serological assays vary from 41% to 94 [5]. However, the rapid diagnosis of LD by detection of the soluble *L. pneumophila* serogroup 1

antigen in urine samples is effective in early treatment decisions. *Legionella* antigen in urine is detectable one day after onset of the disease, and persists for days to weeks [6]. Various disinfectants (chlorine, monochloramine) and physical treatments (heat, UV) are used in water systems to control *Legionella* growth and several disinfection studies have been performed on *Legionella* [7]. In case of treatment failure, *L. pneumophila* might be able to recolonize water systems because of protection in the biofilm or in amoebae [8,9]. In Egypt, *L. pneumophila* is misdiagnosed and the information about its involvement in nosocomial infections is limited. Thus, the current study aimed to identify hospital-acquired LD among all cases of nosocomial pneumonias in a liver transplant unit. Also, environmental samples from the same unit were examined to detect *Legionella* within water sources and air ventilation system to control infection.

## 2. MATERIALS AND METHODS

This study was carried out in the liver transplant unit of Gastroenterology Surgical Center (GEC), Mansoura University, Egypt. The liver transplant is 8 bed unit distributed over 4 ICUs (1 recipient and 3 donor) and 2 wards, 5 beds each. Clinical and environmental samples were received in the medical diagnostics and infection control unit (MDICU), Microbiology Department over a period from November, 2014 till June, 2016. This study was conducted with approval from the Medical Research Ethics Committee, Mansoura University.

## 2.1 Clinical Samples

A total of 120 clinical samples were collected from 30 liver transplant recipients (LTRs), who were hospitalized with signs and symptoms of lower respiratory tract infections. The samples included sputum (n=26), transtracheal aspirate (n=4), blood (n=30) and urine (30). All patients were screened for *Legionella* infection by culture of respiratory specimens and antigen detection in urine and serum. Hospital-acquired LD was defined in accordance with CDC criteria; laboratory confirmed legionellosis that occurs in a patient who has been hospitalized continuously for greater than or equal to 10 days before the onset of illness is considered a definite case of nosocomial LD, and laboratory-confirmed infection that occurs 2-9 days after hospital admission is a possible case of the disease [10].

## 2.2 Data Collection

The immune suppression regimen for LTRs consisted of induction with Basiliximab (Simulic) IV (20 mg) + methylprednisolone (Solumedrol) 250 mg IV + Mycophenolate mofetil (Cellcept) 1000 mg (in Nasogastric tube) during the operation and then maintenance with Mycophenolate Mofetil (Cellcept) 1000 mg per day and Tacrolimus (Prograf) according to the trough level. All patients received the usual scheme of antibiotic prophylaxis in accordance with the sensitivity of bacteria in our region. For all patients, the following data were collected: age, sex, induction of immunosuppression, steroid boluses, number of days on ventilator, duration of intensive care unit (ICU) stay, need for retransplantation, underlying pulmonary disease, associated comorbidity e.g. diabetes, malignancy, hepatic and renal impairment.

## 2.3 Environmental Samples

Starting in January 2016, a total of 40 samples were collected from different water sources of the liver transplant unit including tanks, hot and cold faucets and air ventilation system. There was no hospital water treatment other than the routine methods (through the local water chlorination). One liter of water from tanks was collected in a sterile container. Swab samples were collected from faucets and filters of the air ventilation system with sterile swabs. Faucets swab samples were obtained by introducing a sterile cotton swab into the opening of the faucet and rotating it along the inner sides of the nozzles. Then, these swabs were placed in sterile plastic

centrifuge tubes containing 3-5 mL of water obtained from the same point to prevent drying during transport [11]. Water tank samples was concentrated by filtration through a 0.22 µm pore-size polycarbonate filter. The membrane was then immersed in 5 mL of sterile deionized water, vortexed for 1 minute, and shaken vigorously 50 times until the deposit on the membrane was resuspended. The suspension was heated in a water bath at 50°C for 30 minutes and used for culture [12]. Swab samples were centrifuged at 3000 rpm for 30 minutes. After centrifugation, the supernatant was removed from each sample and 0.5mL of the pellet was transferred to 4.5 mL HCl-KCl acidic buffer (pH 2.2) then mixed gently and shaken for five minutes [13].

## 2.4 Microbiologic Methods

Only sputum samples of Murray–Washington classification degrees IV or V were processed for culture (degree IV, 10–25 epithelial cells and >25 leucocytes per field; degree V, ≤ 10 epithelial cells and >25 leucocytes per field using a low magnification lens (×100)). For *Legionella* spp. culture: The samples were plated on BCYE agar. All plates were incubated aerobically at 37°C for 3-10 day. Isolates were identified by standard procedures, using Gram stain, colonial morphology on BCYE agar, lack of growth on blood agar and biochemical tests including catalase, oxidase, urease, nitrate, gelatinase and hippurate hydrolysis tests [13].

## 2.5 Identification

Typical colonies of *Legionella* spp. were grey to white glistening convex colonies, appearing in 3-4 days time, but may take up to 10 days. Thin Gram-negative bacilli, catalase and oxidase positive, urease negative colonies growing only on BCYE and not on blood agar were presumptively identified as *Legionella* spp. (L-cysteine is essential for growth) [14]. To identify *L. pneumophila*, hippurate hydrolysis test was done by standard method [15]. Serotype identification was confirmed by species-specific antisera (Microscreen *Legionella* latex confirmation assay, UK).

## 2.6 Antibiotic Susceptibility Test

### 2.6.1 β- lactamase detection

β- Lactamase produced by *legionella* spp. was tested using nitrocefin β- lactamase disc (Carr-Scarborough microbiological) [16].

**2.6.2 Antibiotic classes susceptibility**

Antibiotic susceptibility test was performed on environmental and clinical isolated bacteria using agar dilution method to determine the minimal inhibitory concentration (MIC) of the following antibacterial agents; erythromycin (ERY), clarithromycin (CLA), ciprofloxacin (CIP), azithromycin (AZI), cefotaxime (CEF), doxycycline (DOX), levofloxacin (LEV), and moxifloxacin (MOX) [17].

**2.7 Antigen Detection Test**

A total of 60 clinical samples including blood (30) and urine (30) specimens were collected from LTRs to detect the soluble antigen of *L.pneumophila* (LP Ag) using ELISA kit provided from Sunlong Biotech Co., LTD.

**2.8 Effect of Chlorine and Heat on *L. pneumophila***

**2.8.1 Free chlorine disinfection treatments**

A chlorine stock solution of 100 mg/ L was prepared using bleach (a commercial 5% sodium hypochlorite solution) and diluted in sterile, distilled, deionized water. Several chlorine concentrations were tested: 0.5 mg/L, 1 mg/L and 2 mg /L [18]. In a flask, 100 mL of water was added to each chlorine solution and stirred. Then, 100 µL of *L. pneumophila* suspension was inoculated into the flask. After each of the disinfection treatments, ten-fold serial dilutions were made in distilled water for each sample and transferred 0.1 ml of a test system to BCYE plates for enumeration of *L. pneumophila* colony-forming units (CFUs). The plates were incubated at 37°C for up to 10 days [19]. The chosen isolates for this experiment were C1, C4, C5, W1

and W5 because their MICs were relatively high. *E. coli* was used as a control and both bacteria were exposed to identical chlorine concentrations under the same environmental conditions.

**2.8.2 Thermal disinfection treatments**

To study the inactivation of microorganisms by thermal treatment, 2 mL of *L. pneumophila* suspension were prepared and transferred to tubes. The tubes were placed in water baths and subjected to various temperature treatments; 55, 60 and 70°C for various exposure times. After that ten-fold serial dilutions were made in distilled water for each sample and transferred 0.1 ml of a test system to BCYE plates for enumeration of *L. pneumophila* CFUs. The plates were incubated at 37°C for up to 10 days [20].

**3. RESULTS**

**3.1 Patients**

Out of 121 patients received liver transplantation at the GEC liver transplant unit from November, 2014 till June, 2016, 30 patients were suspected to have hospital acquired pneumonia and were included in this study. *Legionella* pneumonia was diagnosed in six patients. During that period, the prevalence of *Legionella* infection was 5% (6/112) among liver transplant patients, and 30% (6/30) among liver transplant patients with pneumonia. The characteristics and outcomes of these patients are given in Table 1.

In the 6 patients, *Legionella* infection was hospital acquired as respiratory symptoms developed more than 10 days after admission. They were 4 males and 2 females with age ranged between 38-54 y. All patients presented

**Table 1. Characteristics of patients with Legionnaires' disease**

No	Age (years)	Sex	Underlying disease and predisposing factor	Onset*	Steroid doses	Diagnosis by		Outcome
						Sputum culture	Urine Ag	
1	38	M	Chronic active hepatitis (HCV)	4 w	Yes	<i>L. pneumophila</i> (C1)	+	Recovered
2	48	F	Chronic active hepatitis (HCV)*- IDDM	4 w	Yes	<i>L. pneumophila</i> (C2)	+	Died
3	52	M	Chronic active hepatitis (HCV)- Hepatoma	5 w	No	<i>L. pneumophila</i> (C3)	-	Recovered
4	49	M	Chronic active hepatitis (HCV)- IDDM*	8 w	yes	<i>L. pneumophila</i> (C4)	+	Recovered
5	41	F	Chronic active hepatitis (HCV)- IDDM	6 w	No	<i>L. pneumophila</i> (C5)	+	Recovered
6	54	M	Chronic active hepatitis (HCV)- emphysema	3 w	No	<i>L. pneumophila</i> (C6)	+	Died

\*HCV, hepatitis C virus; IDDM, insulin-dependent diabetes mellitus; Onset, post transplantation

with fever, dyspnea and cough and either unilateral or bilateral infiltrates on chest radiograph. The time of onset of pneumonia after transplantation ranged from 3-8 weeks.

### 3.2 Microbiologic Results

The sputum culture of the 6 cases on selective BCYE agar medium were positive for *L. pneumophila* sero group 1 (Fig. 1). Among those, five patients tested positive by urine *Legionella* antigen detection test while 3 cases were positive by serum test. No cross reaction was present among other patients. The culture method was found to be the most efficient diagnostic test which can screen most number of cases followed by urinary antigen detection test.

### 3.3 Environmental Sampling

A total of 40 environmental samples were collected from hospital tanks, faucets (hot and cold) and air ventilation system and inoculated onto BCYE agar medium. As shown in table 2, five (12.5%) samples were positive for *Legionella* serogroup 1 from hot faucets, cold faucets and air ventilation unit.

### 3.4 Antibiotic Susceptibility Testing Results

All 11 *L. pneumophila* isolates (clinical and environmental) produced beta lactamase enzyme when examined by the chromogenic cephalosporin provided by nitrocefin discs. The susceptibilities of *L. pneumophila* isolates to eight antibiotics were determined by the agar dilution method as tabulated in table 3,

azithromycin was the most active drug, followed by clarithromycin. Doxycycline was the least active drug. All isolates of *L. pneumophila* were sensitive to levofloxacin.

### 3.5 Effect of Chlorine and Heat on *L. pneumophila*

As recorded in Table 5, high concentration of chlorine enhanced the elimination of *L. pneumophila*, taking into account the time of exposure. At a concentration of 2 mg/L, the bactericidal effect was obvious at the first 5-10 minutes resulting in a 99.9% kill of bacteria while the least bacterial growth was observed after 40 minutes exposure to chlorine. In contrast, at 0.5 mg/L, large number of *L. pneumophila* can survive and resist the treatment till contact time between 30 and 60 minutes had elapsed when 99% kill of bacteria occur during this period.

Temperature also exerted a large influence on *L. pneumophila*. A thermal treatment at different temperatures, 55°C, 60°C and 70°C, was applied to *L. pneumophila* isolates during various times and results were tabulated in table 5. *E. coli* was observed to be unable to survive as it was not detected in the samples within 5 minutes of treatment with chlorine. *L. pneumophila* was able to survive and grow at 55°C for long time while 90% of *L. pneumophila* was inhibited after one hour of heating at 60°C. Also, the time required for inhibiting 99% of *L. pneumophila* decreased from more than one hour at 60°C to 10 minutes at higher temperature of 70°C. Comparison of results of different temperatures indicated that thermal disinfection was affective at temperature more than 60°C.



Fig. 1. Buffered charcoal-yeast extract agar plate culture of *Legionella* species, showing smooth, circular, glistening, convex and grayish-white colonies

**Table 2. Environmental screening for *Legionella* in GEC**

Source of sample	No. of samples	No. of positive samples	Isolate code	Positive rate (%)
Tanks	4	0	-	0
Swabs from ICU* faucet (hot)	4	0	-	0
Swabs from ICU faucet (cold)	4	0	-	0
Swabs from wards faucets (hot)	8	3	W1 W2 W3	7.5
Swabs from wards faucets (cold)	8	1	W4	2.5
Swabs from air ventilation system	12	1	W5	2.5
Total	40	5	5	12.5

\*ICU: intensive care units

**Table 3. MIC values of *L. pneumophila* strains against different antibiotics**

<i>L. pneumophila</i> isolates	Antibiotics							
	CIP	AZI	CLA	ERY	CEF	DOX	LEV	MOX
C1	1	0.015	0.125	0.25	2	4	0.031	0.5
C2	0.062	0.015	0.125	0.5	1	0.75	0.125	0.25
C3	1	0.25	0.031	0.125	0.5	1	0.75	0.125
C4	0.125	0.015	0.062	1	2	0.75	0.25	3
C5	0.125	0.75	0.5	3	0.125	2	0.031	0.125
C6	0.25	0.062	0.031	0.125	1	2	0.5	0.25
C7	0.5	0.015	0.031	0.125	0.5	1	0.062	0.75
W1	1	0.125	0.031	2	0.75	3	0.031	0.125
W2	0.25	0.015	0.031	0.125	0.062	1	0.125	3
W3	2	0.125	0.5	0.125	1	3	0.031	0.5
W4	0.062	0.031	0.125	0.25	0.125	2	0.75	0.25
W5	0.25	0.015	0.5	0.125	2	0.75	0.25	1

**Table 4. Bactericidal effect of different concentrations of chlorine on water with *L. pneumophila***

Exposure time (min)	Concentration of chlorine					
	0.5 mg / L		1.0 mg / L		2.0 mg / L	
	count CFU/mL	Percent Survival %	count CFU/mL	Percent survival %	count CFU/mL	Percent survival %
1	495×10 <sup>3</sup>	99	430×10 <sup>3</sup>	86	65×10 <sup>3</sup>	13
5	170×10 <sup>3</sup>	34	140×10 <sup>3</sup>	28	15×10 <sup>3</sup>	3
10	110×10 <sup>3</sup>	22	85×10 <sup>3</sup>	17	500	0.1
20	45×10 <sup>3</sup>	9	34×10 <sup>3</sup>	6.8	200	0.04
30	25×10 <sup>3</sup>	5	10×10 <sup>3</sup>	2	40	0.008
40	8500	1.7	4×10 <sup>3</sup>	0.8	5	0.001
50	4×10 <sup>3</sup>	0.8	1500	0.3	5	0.001
60	1×10 <sup>3</sup>	0.2	500	0.01	5	0.001

**Table 5. Thermal effect of different temperature on water with *L. pneumophila***

Exposure time (min)	Temperature treatment					
	55°C		60°C		70°C	
	Count CFU/mL	Percent Survival %	Count CFU/mL	Percent survival %	Count CFU/mL	Percent survival %
1	499×10 <sup>3</sup>	99.8	433×10 <sup>3</sup>	86.6	300×10 <sup>3</sup>	60
5	496×10 <sup>3</sup>	99.2	350×10 <sup>3</sup>	70	144×10 <sup>3</sup>	28.8
10	450×10 <sup>3</sup>	90	329×10 <sup>3</sup>	65.8	5×10 <sup>3</sup>	1
20	420×10 <sup>3</sup>	84	260×10 <sup>3</sup>	50.2	1×10 <sup>3</sup>	0.2
30	335×10 <sup>3</sup>	67	205×10 <sup>3</sup>	41	15	0.003
40	275×10 <sup>3</sup>	55	175×10 <sup>3</sup>	35	6	0.0012
50	215×10 <sup>3</sup>	43	115×10 <sup>3</sup>	23	4	0.0008
60	180×10 <sup>3</sup>	36	50×10 <sup>3</sup>	10	0	0

### 3.6 Outcome

Patients with pneumonia due to *Legionella* infection were given intravenous azithromycin (zithromax) for 21 days as recommended for immunosuppressed hosts. Four patients improved. Two patients continued to deteriorate and died because of multiple causes. Hospital acquired pneumonias of uncertain etiology were empirically treated with quinolones [21]. After recognition of water contamination, drinking and using tap water was prohibited. Instead, transplant recipients boiled their water and stored it for drinking. Water treatment with hyper chlorination and keeping hot water at temperature above 60°C. Filters were replaced for the air ventilation system. These measures were carried out till eradication of *Legionella* from water was achieved, no other cases acquired *Legionella* pneumonia.

### 4. DISCUSSION

In present study, the prevalence of *L. pneumophila* infection was 20% among bacterial pneumonias. Compatible with Singh et al. who reported that 27% of the bacterial pneumonias were due to *Legionella* [22]. Also, Blanquer et al. study showed a higher prevalence of 33.6% [23]. However, Neumann et al. detected a prevalence of 12.4% among pneumonias in LTRs [24]. The reported high incidence of nosocomial *legionella* pneumonia in our study is directly related to the ready availability of specialized diagnostic tests such as culture using specific medium, urinary antigen assay and serologic screening. Taking into consideration, precautions and specimens pre-treatment prior to inoculation onto culture plates. Moreover the presence of *L. pneumophila* in the liver transplant unit water supply and ventilation system. The symptoms of six cases infected with *L. pneumophila* constituted fever, and other nonspecific symptoms including malaise, myalgia, headache, abdominal pain and cough. The radiological findings showed pulmonary infiltrates developing 3 wk to 12 wk after transplantation. Besides, patients lacked the response to  $\beta$ -lactam antibiotics (penicillin, amino glycosides, cephalosporin). Therefore, while the radiological, clinical and hematological manifestations of LD overlap with those of other typical and atypical causes of pneumonia, a diagnosis based just on these findings are impossible. Thus specific investigation for *Legionella* should be initiated when risk factors for nosocomial LD are identified in a case where pneumonia is suspected, or when there is

insufficient response to empiric antibiotic therapy [25]. The current study showed high sensitivity of urinary antigen detection (86%) as it was compatible with culture results in 5 cases and missed diagnosis in one case. Similarly, Muñoz et al. detected antigen in 74.3% of urine samples [26]. The sensitivity and specificity of techniques for detection of the urinary antigen of *L. pneumophila* serogroup1 have reported variable between 70% - 100% (for sensitivity), and 100% (for specificity) [27]. Therefore, we join others in recommending urinary antigen detection as main technique for laboratory diagnosis of LD, owing to its rapid results, relatively low cost, early diagnosis of *Legionella* infections and most notably its high specificity. The occurrence of cases infected with *Legionella* spp. prompted an epidemiologic investigation include culturing and examination of water distribution system and air ventilation system as potential sources of *Legionella* infection in the intensive care units (ICU) and wards of GEC. Environmental screening resulted in detection of *L. pneumophila* in the water supply system of the liver transplant unit with prevalence rate 12.5%. The levels of *Legionella* contamination in hospital water system have been reported to correlate with the occurrence of nosocomial LD. Isolation of *Legionella* spp. from water samples by culture technique is generally preferred. *Legionella* are generally present at very low or undetectable concentrations in water so it is usually necessary to use a concentration technique (centrifugation and filtration). Also it is necessary to eliminate competitive flora during primary culture. To reduce the growth of unwanted bacteria, the samples can be subjected to a heat treatment (50°C for 30 min) or acid (pH 2.2 for 5 min) [12]. In current research, three *L. pneumophila* positive samples were isolated from the hot faucets representing 7.5%. One from cold faucets and one from air ventilation system with rate 2.1%. Temperature had a key role in the positive samples. This finding was supported by previous literature, in which water temperature was prime factor affecting *L. pneumophila* incidence, that have a predilection for the warm water encountered in man-made systems [28,29]. Susceptibility testing of *L. pneumophila* is not usually performed, since it is a cumbersome procedure and this organism remains susceptible to antibiotic drugs commonly used for treatment. However, susceptibility trends of these pathogens should be monitored periodically in both clinical and environmental isolates [30,31]. Results in this study confirmed that our *L. pneumophila* isolates were inhibited

by low concentrations of macrolides and fluoroquinolones. Among the macrolides, azithromycin followed by clarithromycin were the most active drugs. All isolates of *L. pneumophila* were sensitive to levofloxacin at MIC 0.75 mg/L. Activities of clarithromycin and levofloxacin were almost the same as the MIC range were 0.031–0.5 and 0.031–0.75 mg/L, respectively. In our study, levofloxacin was the most active quinolone as reported by others [32,33]. In the treatment of lower respiratory tract infections, fluoroquinolones have become the most widely used agents because of their broad-spectrum coverage, their ease of administration, and their comparatively fewer adverse effects [34]. The recommended treatment for LD in an immunocompetent host is a macrolide or quinolone for 10–14 days. However, a 21-day treatment duration is recommended for immunocompromised patients to avoid relapses [35,36]. Four of our patients recovered completely, but two died in spite of being treated with azithromycin, which was most probably due to multiple causes. Macrolide antibiotics have a 14-membered lactone ring which inhibit tacrolimus metabolism by affecting hepatic and small intestinal cytochrome P450 enzymes. Increased tacrolimus levels with co-administration of erythromycin and clarithromycin have been reported in the clinical setting [37]. Azithromycin differs from erythromycin and clarithromycin in having a 15-membered ring and had no effect on cytochrome P450 or NADPH-cytochrome c reductase. Therefore, there was no interaction of azithromycin with tacrolimus [38], however there are case reports of tacrolimus-azithromycin interaction that transplant physicians should be aware of [39]. Healthcare centers for immunocompromised and transplant patients are advised to routinely screen for the presence of *Legionella* organisms in their water supply system [40]. Different disinfectants methods are widely used to eradicate *Legionella* from manmade water systems which in turn result in reduction of number of cases and prevent outbreaks of legionellosis worldwide. Chlorination, ozone treatment, superheating and the application of ultraviolet light have been tested [41,42]. The current study involved chlorination and superheating for water treatment to evaluate their effect on the control of *L. pneumophila*. The treatment doses were chosen to be realistic and representative of actual practices. As recommended by the WHO drinking water quality guidelines, the minimum target chlorine concentration at the point of delivery should be 0.2 mg/L in normal states and

0.5 mg/L in high-risk states. Moreover, temperatures above 50°C are also recommended to avoid colonization and regrowth of *Legionella* in the water systems. Our study showed that reduction of *E. coli* bacteria was achieved within a very short period of time (less than 5 minutes) and all tested *L. pneumophila* isolates can survive for periods of longer than 1 hour under the same conditions. The results described in the current study agreed with those reported by Cervero-Aragó et al. who explained that *Legionella* was more resistant to chlorine exposure than other bacteria such as coliform bacteria that were used as indicator organisms to monitor potable water quality. Also they reported significant differences in the inactivation pattern between *L. pneumophila* strains [19]. Our results clearly indicate that high concentration (2 mg/L) of chlorine was more efficient on inactivation of *L. pneumophila* after less than 10 minutes of exposure. Similarly McCall et al. study reported more than 4-log reduction of naturally grown *Legionella* bacteria in less than 1 hour at 2 mg/L chloride concentration in a model plumbing system [43]. Thermal treatments are applied in hot water systems to control and prevent *Legionella* colonization [44]. In the current study, *L. pneumophila* isolates were exposed to different temperatures ranging from 55°C to 70°C in water bath system. The effectiveness of thermal treatments applied increased as the temperatures and exposure times increased, especially for temperatures higher than 55°C. At 70°C, *L. pneumophila* eradication reached 99% within 10 minutes. Similarly, Rogers et al. reported that *L. pneumophila* was not recovered at 60°C [45]. Therefore we associate with others in recommending that the minimum temperature for thermal disinfection is 60°C.

## 5. CONCLUSION

In conclusion, for hospitals with transplant programs, periodic monitoring of *Legionella* spp. in hospital water supply system is recommended as well as including legionellosis in the differential diagnosis of hospital acquired pneumonias and order appropriate diagnostic tests for *Legionella*. (i.e., urine antigen assay, culture, fluorescent antibody serology, and antibiotic sensitivity testing).

## CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this paper.



## ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the author(s).

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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