

# Detection of *Legionella pneumophila* from Evaporative Coolers Air Conditioners in Khartoum State

Elsanousi R.M.A.<sup>1,\*</sup>, Elsanousi S.M.<sup>2</sup>

<sup>1</sup>Department of Microbiology, University of Bahri, Sudan

<sup>2</sup>Department of Microbiology, University of Khartoum, Sudan

\*Corresponding author: sanousireem@gmail.com

**Abstract** This study was designed to detect the prevalence of *Legionella pneumophila* in evaporative coolers air conditioners in Khartoum State. A total of 525 water samples were collected from water reservoirs of air conditioning systems in the three main cities of Khartoum State (Khartoum, Omdurman and Khartoum North) in the period between March – October, 2012, 2013, 2014, 2015 and 2016. The premises from which the samples were collected were divided into five categories: universities, schools, hospitals, offices and houses (35 samples for each category in the three cities, with a total of 175 samples for each city). *Legionella* species were isolated from 222 (42.3%) out of the total samples taken from Khartoum State, which were divided as follows: 75 (33.8%) from Omdurman, 74 (33.3%) from Khartoum North and 73 (32.9%) from Khartoum. Regarding the premises categories, 58 (55.2%) were from hospitals, 52 (49.5%) from offices, 41 (39%) from schools, 38 (36.2%) from houses and 33 (31.4%) from universities were positive for *Legionella* spp. The highest prevalence rate of *Legionella* spp was found in hospitals (55.2%), followed by offices (49.5%), schools (39%), houses (36.2%) and universities (31.4%).

**Keywords:** *Legionella pneumophila*, evaporative coolers air conditioners, water samples

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## 1. Introduction

An evaporative cooler is a device that cools air through the evaporation of water. The temperature of dry air can be dropped significantly through the phase transition of liquid water to water vapor (evaporation) [16].

Legionnaires' disease acquired its name in July 1976 when an outbreak of pneumonia occurred among people attending a convention of the American Legion at the Bellevue – Stratford Hotel in Philadelphia. On January 1977 the causative agent was isolated from the cooling towers of the hotel's air conditioning system, identified and named *Legionella* [19].

*Legionella* are Gram negative bacilli, non capsulated and non - spore forming, most species exhibit motility and have non fermentative metabolism [38]. The bacteria are ubiquitous, which found in environmental water sources such as rivers, lakes and soil. The organism passes into sites that constitute an artificial reservoir like piped water in towns and cities, water net works, water systems in individual buildings and cooling towers [33]. The organisms do not multiply below 20°C and are killed within a few minutes at temperatures above 60°C [31]. Environmental surveillance of *Legionella* is the standard culture technique. Recently Real - time - PCR method for rapid detection and quantification of *Legionella* in water samples was

developed [22,37]. All *Legionella* organisms require L- cysteine and iron for primary isolation and subcultures. Buffered Charcoal Yeast Extract (BCYE) agar containing L- cysteine and iron as well as  $\alpha$  - ketoglutarate. It can be made more selective by the addition of antibiotics for *Legionella* spp selection [14,32]. *Legionella* spp prefer 80 – 90% humidity with 2 - 5% CO<sub>2</sub>. They are catalase positive and do not ferment or oxidize sugars. *Legionella pneumophila* is oxidase positive but other *Legionella* species are variable in oxidase activity, Most *Legionella* spp produce gelatinase and  $\beta$  – lactamase enzymes. [3]. *Legionella* bacteria multiply within amoebae, specially, when environmental conditions are unfavorable [15]. Also it can grow and multiply intracellularly within human alveolar macrophages. [2]. The term Legionellosis, is collectively refers to Legionnaires' disease and Pontiac fever [18]. Inhalation of contaminated aerosols is the most commonly accepted mechanism of infection [9]. The potential health risk of *Legionella* to humans is theoretically associated with cells densities in a range between 10<sup>4</sup> - 10<sup>5</sup> CFU per liter of water [4,23]. Most community and nosocomial cases of Legionnaires' disease are typically associated with cooling towers [27]. *Legionella* spp. are responsible for 1 to 5% of cases of community - acquired pneumonia (CAP) [5,35]. Immuno suppression, chronic lung disease, cigarette smoking and old in age are risk factors for the disease, signs and symptoms are non specific and do not distinguish

*Legionella* infection from other common causes of pneumonia. [10].

## 2. Material and Methods

### 2.1. Samples Collection

A total of 525 water samples were collected from evaporative air conditioning systems from Khartoum State, which distributed according to the statistic data with a rate of 175 samples for each city; Khartoum, Omdurman and Khartoum North, including five categories for each city: universities, schools, hospitals, offices and houses which divided on 35 samples for each category in the three cities of the Khartoum State, the samples were taken under aseptic conditions, using sterile syringes and sterile containers. The specimens were labeled, put into thermos containing ice and transferred to the research laboratory for bacteriological examination. Samples were tested for pH and temperature during sampling. Also the status and type of the air conditioners were investigated. Samples were taken according to geographical distribution of the cities including the four directions and following the microbiological quality control measures. Also water distribution in each area was considered. Samples that were collected from offices included public offices like commercial offices, markets, pharmacies and others according to the geographic distribution of the areas. Firstly, for proper evaluation we took samples in different volumes; 1000 ml, 500 ml, 100 ml, 50 ml and 20 ml, but most samples were taken as 20 ml of evaporative cooling conditioners water reservoirs.

### 2.2. Bacterial Isolation

Five ml of each water sample were centrifuged at 4000 rpm for 10 minutes using a cold centrifuge. The sediment was inoculated onto Buffered Charcoal Yeast Extract Agar BCYE –  $\alpha$  medium and Glycine Vancomycin Polymyxin Cycloheximide Medium (G V P C Medium) (Oxoid Laboratories, London). Inoculated media were incubated at 37°C, in an atmosphere of 5 -10% CO<sub>2</sub> (using the candle jar) and humidity using wet cotton swamp on water in a Petri Dish, for 3 - 7 days.

### 2.3. Bacterial Identification

Cultures were examined by Gram stain technique. And sub cultured on Blood agar to ensure its negative growth. Primary identification was done by light microscopy using Gram stain technique, Secondary identification was done by biochemical tests like catalase, oxidase and gelatin hydrolysis tests, which were performed according to Barro and Feltham [3]. Purified isolates were confirmed using Real time PCR.

### 2.4. Real Time PCR

This procedure was done following manufacture instructions– (Sacace Biotechnologies, Italy) - Real Time PCR Kit for quantitative detection of *Legionella pneumophila*

– Complete Real Time PCR test with DNA purification kit (TB50-50FRT). Pure colonies suspected for *Legionella pneumophila* were emulsified in 0.5 ml saline solution. 50  $\mu$ l of the suspension was used for DNA extraction. Required quantity of PCR - mix-1 reaction tubes were prepared for samples and controls. Seven (7  $\mu$ l) of PCR – mix - 2 was added on the wax surface in addition to ten (10  $\mu$ l) of extracted DNA to appropriate tube. Ten (10  $\mu$ l) of DNA - buffer was added to the tube labeled to amplification negative control. Also ten (10 $\mu$ l) of QS1, QS2 and QS3, were added to the tubes labeled to amplification positive controls. All of the mentioned tubes were inserted to the thermo cycler for amplification.

### 2.5. Data Analysis

The results were subjected to statistical analysis in the form of frequencies and percentages. The significance of these frequencies was tested using chi- square test. The data were analyzed using SPSS 21 with reference P-value  $\leq 0.05$ .

## 3. Results

Khartoum State gave results as follows:

A total of 222(42.3%) positive samples, including, (33.8%) for Omdurman, (33.3%) for Khartoum North and (32.9%) for Khartoum, which calculated out of 222 positive samples obtained from 525 collected samples. (Table 2) All samples were between 14°C - 37 °C, during sampling and showed pH between 6.7- 8.6 for most of the samples. Also all positive samples showed growth on BCYE -  $\alpha$  medium and GVPC medium and gave negative growth on Blood Agar medium. All *Legionella* spp isolates revealed negative reaction to Gram stain technique and positive reactions to: catalase, oxidase and gelatin hydrolysis tests. 63% from 38 randomly selected *Legionella* spp isolates showed positive results for *Legionella pneumophila* using Real Time PCR technique (Table 1). Khartoum State positive results (42.3%) were divided as follows: Omdurman city gave the highest prevalence rate of *Legionella* spp, which was 14.3%, followed by Khartoum North (14.1%) and Khartoum (13.9%) (Table3). Hospitals category showed: 23.8% for Omdurman hospitals, 16.2% for Khartoum hospitals and 15.2% for Khartoum North hospitals, with a total of 55.2% positive samples. The second higher prevalence rate of *Legionella* spp was obtained from the offices category, which revealed a rate of 49.5% for the three cities, with 22% for Khartoum, 17 % for Khartoum North and 10.5% for Omdurman. The third category that gave high level of *Legionella* spp was the schools, which showed 39%, for the three cities, with a rate of: 14.3% for Khartoum North, 13.3% for Khartoum, and 11.4% for Omdurman. The fourth category was the houses which revealed 36.2% of *Legionella* spp, with a rate of 12.4% for both Khartoum North and Omdurman and 11.4% for Khartoum. The lowest rate of *Legionella* spp was reported in universities as: 31.4% for the three cities with a rate of 13.3% for Omdurman, 11.4% for Khartoum North and 6.7% for Khartoum (Table 4).

**Table 1. Quantitative Real Time PCR results of *Legionella pneumophila* isolated from four water samples obtained from evaporative air conditioning systems in Khartoum State**

Sample Name	Standard Concentration	Calculated Concentration	Ct
QS1	6.23×10 <sup>6</sup>	6.23×10 <sup>6</sup>	5.98
QS2	1.70×10 <sup>5</sup>	1.70×10 <sup>5</sup>	22.12
QS3	4.00×10 <sup>3</sup>	4.00×10 <sup>3</sup>	35.03
NTC	-	-	-
9		3.56×10 <sup>6</sup>	15.67
13		1.09×10 <sup>5</sup>	25.46
21		1.60×10 <sup>5</sup>	22.29
43		5.29×10 <sup>6</sup>	13.12

QS: Positive standard control, NTC: Negative Standard control, Ct: Cycle threshold.

**Table 2. Prevalence of *L. pneumophila* in water samples collected from evaporative air conditioning systems with their rate in each city of Khartoum State**

City	Positive samples (out of 222)		Negative samples (out of 303)	
	Number	%	Number	%
Omdurman	75	33.8	100	33
Khartoum north	74	33.3	101	33.3
Khartoum	73	32.9	102	33.7

**Table 3. Percentages and calculation of the total of positive and negative samples in the three cities of Khartoum State from the total number of collected samples**

City	Positive samples (out of 525)		Negative samples (out of 525)	
	Number	%	Number	%
Omdurman	75	14.3	100	19.1
Khartoum North	74	14.1	101	19.2
Khartoum	73	13.9	102	19.4
Total	222	42.3	303	57.7

**Table 4. Prevalence of *L. pneumophila* in water samples collected from evaporative air conditioning systems including their rate in each group under study in Khartoum State**

Categories (out of 105)	Khartoum	Khartoum north	Omdurman	Total % of positive samples
Universities	6.7%	11.4%	13.3%	31.4%
Schools	13.3%	14.3%	11.4%	39%
Hospitals	16.2%	15.2%	23.8%	55.2%
Offices	22%	17%	10.5%	49.5%
Houses	11.4%	12.4%	12.4%	36.2%

## 4. Discussion

In general view of this study, it was found that hospitals' evaporative cooling systems reported the highest prevalence rate (55.2%) of *Legionella* species among all categories under study, which agrees with many studies on hospitals that reported evidence to *Legionella* species in hospitals environments and demonstrated their role in hospital acquired pneumonia, like a previous study done in India by Anbumani, Gururajkumar and Chaudhury [1] for the isolation of *Legionella pneumophila* from clinical and environmental sources in a tertiary care hospital. They found that *Legionella pneumophila* was present in the hospital environment and was the aetiological agent of lower respiratory tract infection in 2.55% of the patients.

Findings of our study substantiate the study of Leoni *et al.* [24], who detected *Legionella* species in 40% of domestic, hotel and hospital hot water systems in Italy. *Legionella pneumophila* isolated from 33.3% of the samples and the highest colonization was found in the hot water systems of hospitals. Another study in Iran carried by Ghotaslou *et al.* [20], detected *Legionella* contamination in Tabriz hospitals using PCR assay where they reported that 10 of 140 (7.1%) were positive for *Legionella*, with *Legionella pneumophila* detection in 4 (2.85%) of the water samples. This study concluded that hospitals potable system were the primary reservoirs for Legionnaires' disease and *Legionella* species, were presented in aquatic hospitals environment. Such hospital systems are also the main cause of nosocomial infection through aspiration [25]. Offices group revealed the second highest rate of *Legionella* prevalence. This result is important because it covered different types of premises under this category, especially when we are considering the proportion of community acquired pneumonia (CAP) that results in severe pneumonia is higher for Legionnaires' disease than for other causes; with a consequently higher mortality rate [17]. Community acquired pneumonia has a high rate of hospital admission, with less than 1% being managed at home. Legionnaires' disease can account for up to 30% of community acquired pneumonia requiring admission to intensive care units [26,39]. Infection with *Legionella* requires both proliferation and exposure. Potable water systems containing *Legionella* are a significant cause of sporadic cases of legionellosis acquired in the community [4,34,36,40]. Legionnaires' disease is thought to be rare in children but one study identified 76 cases of *Legionella* infection in children [21]. Schools group demonstrate a rate of 39% of *Legionella* spp prevalence in air conditioners, which may become a threat if not controlled. Results of this study revealed 36.2% *Legionella* positive growth for the houses, which is a higher result when compared with the results obtained by Dimitriadi and Velonakis [13], whom detected *Legionella* species from houses domestic water in Greece in only 17% from a total of 100 collected samples. Our results was almost similar to that of a previous study done by Burak and Zeybek [6], who collected 122 samples from hot water and swabs from the showers in 61 houses in Istanbul, who isolated *Legionella pneumophila* from 13 (21.3%) of the houses. The lowest prevalence rate of *Legionella* spp. in this study was found in the universities category, with 31.4% *Legionella* positive growth. This seems to be similar to the results obtained by Chin *et al.* [8] who isolated *Legionella* spp from cooling towers and potable water systems in a university campus in Kuala Lumpur with prevalence rate of 23.5% for *Legionella pneumophila*. 63% from a total of 38 selected isolates gave positive results for *Legionella pneumophila* using Real-Time PCR technique, which is considered a rapid diagnostic method in comparison with different PCR assays [11].

## 5. Conclusion

In conclusion, the rate of the prevalence of *Legionella* spp. in evaporative cooler air conditioning systems in Khartoum State, found in this study indicates a

considerable prevalence of this pathogenic organism in the environment which may result in a great biological burden leading to serious outbreaks if measures of prevention and control were not taken.

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