

Evaporative Coolers Air Conditioners Devices as a Source of *Legionella Pneumophila* in Khartoum State, Sudan and Recommended Measures of Control

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Abstract: This study was designed to substantiate the role of the evaporative coolers air conditioners in the proliferation of the genus *Legionella*. In addition to sort out, isolate and identify the genus *Legionella* including *Legionella pneumophila*, the causative agent of Legionnaires' disease and Pontiac fever from a new source. This type of air conditioners depends on water in their operation. They cool the air by filtering it through evaporation of water which is widely used in the Sudan. The evaporative air conditioning units considered as reservoirs of the ubiquitous bacteria which it harbored by amoeba. *Legionella* species can proliferate in amoeba and in biofilms that promote the growth of the bacteria even in the bad conditions. The study was done in Khartoum State including different premises from which water samples of evaporative coolers air conditioners were collected. Five hundred and fifty five water samples were collected, treated, cultured, identified and confirmed by gold standard culture technique using Buffered Charcoal Yeast Extract Agar (BCYE - α medium), Glycine Vancomycin Polymyxin-Cycloheximide Agar (G V P C Medium) and biochemical techniques well as Real Time PCR test. The results of *Legionella* species obtained from this study were 42.3% of the total collected samples with a rate of 11% for *Legionella pneumophila*. Recommended measures of control must be applied including this type of air conditioners to prevent *Legionella* out breaks.

Keywords: Evaporative Coolers Air Conditioners Devices, *Legionella pneumophila*, Control Measures

1. Introduction

Evaporative cooler conditioners operation is by the theory of evaporation. The evaporation depends on the transition of liquid water to water vapor. This can cool the air using much less energy than refrigeration [8]. Since the 1976 outbreak in Philadelphia, which led to the detection and description of the bacteria *Legionella pneumophila*, many outbreaks followed and were reported and they frequently involved hospitals. Infections were traced to the colonization parts of air - conditioning plants [1, 5, 21, 24]. But most outbreaks and recurrent single cases in hospitals were associated with contaminated potable water and hot-water systems [13]. *Legionella pneumophila* is the causative agent of Legionnaires' disease which can infect specific species of amoebae like *Acanthamoeba polyphaga* and *Acanthamoeba castellanii* in aquatic environments and can multiply as an

intracellular parasite in human phagocytic cells [2]. *Legionella* are ubiquitous bacteria found in different types of water sources in the environment. Their growth is especially favored in human made warm water systems, including cooling towers, hot tubes, showers and swimming pools [19]. *Legionella* species are able to survive in moist environments for long times and grow well at temperature ranging between 20°C – 42°C [6]. *Legionella pneumophila* serogroup 1 was reported to be responsible for up to eighty percent (80%) of Legionnaires' disease reported cases [11]. *Legionella longbeachae* and other species were identified as the cause of several infections [22]. A previous study in England by Kirby *et al.* [16] showed that the total cases of mortality associated with *Legionella pneumonia* was 19%, but it can be as high as 80% among immunocompromised patients and an early initiation of appropriate therapy will improve better treatment. Temperature is a crucial factor in the colonization

and proliferation of *Legionella* species in the environment and water systems. Therefore, adjustment and maintenance of the temperature of hot- and cold- water systems within the range below 20°C for the cold water and above 60°C for the hot water to prevent or minimize the growth of *Legionella* species is an important control measure to avoid the risk of *Legionella* infection [9]. *Legionella* species are difficult to control in environmental sources due to their resistance to disinfectants, association with biofilms and parasitism of protozoan hosts [15, 20, 23]. Disinfection can help to control the multiplication of *Legionella* species in water and in air - conditioning systems by applying more effective measures including the usage of filters, copper- silver ionization, and chlorine- dioxide or mono- chloramines [18]. Hospitals that care for immunocompromised patients should monitor potable water systems for the presence of *Legionella* species and treat water, if it is found [12]. Decontamination of colonized devices is important to interrupt outbreaks and prevent recurrence of sporadic cases. Two American studies in hospitals confirmed that the frequency with *Legionella pneumophila* isolated from patients with pneumonia was reduced from 16.3% to 0.1% over a 6 – year period and in immunocompromised patients from 76% to 0.8% over a 10 - years period after applying control measures, including decontamination of the plumbing systems, monitoring of *Legionella* species in water, examination of all clinical cases and specimens for the genus *Legionella*, using sterile water for all medical applications [10, 14].

2. Material and Methods

Five hundred and twenty five evaporative coolers air conditioners water samples were collected from different premises including hospitals, universities, schools, offices and houses. Samples were transferred to the research laboratory for bacteriological examination which inoculated onto Buffered Charcoal Yeast Extract Agar (BCYE – α medium) and Glycine Vancomycin Polymyxin Cycloheximide Medium (G V P C medium) (Oxoid Laboratories, London). They were subjected to Gram's stain and biochemical assay according to Barraow and Feltham [3]. Samples were confirmed using Real Time PCR technique (Sacace Biotechnologies, Italy). Samples temperature and pH were measured. All methods of bacterial isolation, identification and confirmation were prescribed by Elsanousi and Elsanousi

[7].

Data Analysis

The data were analyzed using SPSS 21 with reference P-value $\leq (0.05)$. 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.

3. Results

Temperature of all collected samples were ranged between 14°C - 37°C, during sampling and pH ranged between 6.7- 8.6 for most of the samples. All positive samples showed growth on BCYE medium and GVPC medium. (Figure 1) *Legionella* isolates gave negative reaction to Gram's stain technique (figure 2) and positive reactions to: catalase, oxidase and gelatin hydrolysis tests. *Legionella pneumophila* was confirmed using Real Time PCR technique. Collected samples gave 42.3% positive results for *Legionella* species with a rate of 11% for *Legionella pneumophila* (table 1).

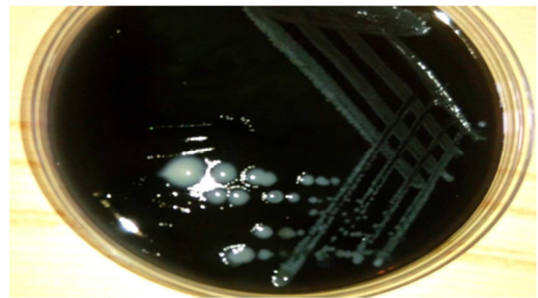


Figure 1. Colonies of *Legionella pneumophila* on CVPC medium.

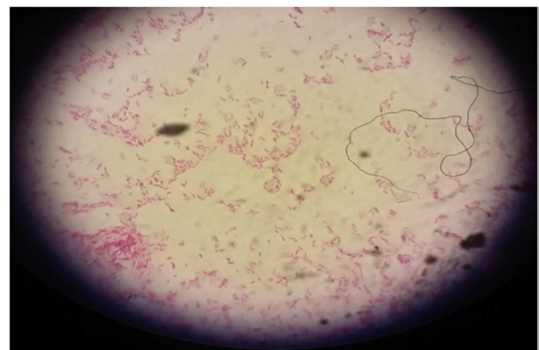


Figure 2. Gram's stain of *Legionella pneumophila*.

Table 1. The ratio of each category calculated from the total of positive and the total of negative samples (P-value of chi-square test=0.002 (Significant)).

Premises category	Number of samples	Positive samples (out of 222)		Negative samples (out of 303)	
		Number	%	Number	%
Hospitals	105	58	26	47	15.5
Offices	105	52	23.4	53	17.5
Schools	105	41	18.5	64	21.1
Houses	105	38	17.1	67	22.1
Universities	105	33	15	72	23.8
Total	525	222	42.3	303	57.7

4. Discussion

Evaporative coolers air conditioners devices are an oldest forms of climate control still found today. They remain good alternatives to traditional air conditioners. They are very effective and efficient when used properly. Due to the evaporative process used by these swamp coolers, these cooling units are best used in hot, dry climates, which prevail worldwide. In the Sudan, it is the most prevalent type of air conditioners. Because of that it requires low energy and inexpensive costs of manufacturing. This study was focusing on the prevalence of *Legionella pneumophila* in evaporative coolers devices as reservoirs of the bacteria, because they depend on water in their operation which create wet, favorable environment for this ubiquities bacteria. There are other factors that can maintain the growth of the bacteria like suitable temperature that was found between 20°C and 37°C in most of our studied samples. Some studies showed that, the isolation of *Legionella pneumophila* was obtained on water at temperatures between 25°C and 45°C with an optimal temperature range of 32°C – 42°C. Also, the conditioners reservoirs contain sediments that can support bacterial growth, such as rust, sludge, protozoa and organic matters, which enhance the biofilm formation [25]. The study paid great attention to the status of the air conditioner whether new or old. We hypothesized that the old devices may contain a lot of organisms than the new devices, but we found that of no value. The most important matter was the cleanliness of their water storages and continuing in water renewal and periods of their operation. The instrument, which operates for long periods, should be looked after for its cleanliness and water renewal. This will give negative results for growth of *Legionella* most of the time. This is in contrast to others that were operated for fewer periods without cleaning and without water changing. The climate and weather play a big role in adjusting the external temperature which affects the bacterial prevalence and affect the conditioners performance. Khartoum State revealed a prevalence rate of 42.3% for *Legionella* species in the evaporative coolers conditioners which may cause a great risk, especially when we notice that the prevalence of *Legionella pneumophila* was 11%. Evaporative coolers air conditioners devices under the study were supplemented with treated water, for this reason we referred to the above measures and we recommend to apply general measures of control like eradication of the microorganisms from water systems using disinfectants [4]. Also we recommend strongly for adding additional doses disinfectant like chlorine and chlorine derivatives to the water reservoirs of the evaporative coolers air conditioning units to avoid the multiplication of the bacteria and amoeba. The American center for disease control and prevention (CDC) recommend with the best practices based on the scientific information that is currently available to disinfect a hot tube contaminated with *Legionella* which it can be apply to disinfect water reservoirs of evaporative coolers air conditioners which modified as: Maintaining water temperatures outside the ideal range of

Legionella growth, ensuring adequate disinfection, maintaining devices to prevent corrosion and biofilm formation and regular monitoring for water management programs [17].

5. Conclusion

Evaporative coolers air conditioners units are remain highly sought after alternatives to traditional air conditioning devices especially in hot developing countries. This study was added and enrolled these devices as a source of *Legionella pneumophila* and other *Legionella* species. Measures of prevention and control must be applied to avoid *Legionella* outbreaks.

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