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Original Article

Prevalence of *Legionella pneumophila* in a Variety of Environmental Water Systems

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ABSTRACT

The purpose of the current study is to isolate and identify *Legionella pneumophila* by bacteriological and molecular methods from water and swab samples collected from a variety of water systems in Fallujah City, Iraq. A total of 227 samples were collected, including 146 swab samples and 81 of 1 L water samples. Bacteriological and molecular assays were performed compromised cultural, gram stain, a set of biochemical tests, and serological tests. The phenotypically validated isolates underwent a 16s rRNA gene by conventional PCR assays. The results showed 28 (12.33%) were positive with the presence of *legionella pneumophila* isolates. including 5 (17.86%) positive isolates from water samples and 23 (82.14%) positive isolates from swabs. The current study showed that the majority of the water and swab samples were detected to be negative, but there is an appropriate exposure to this pathogen in the community. The diversity of the presence of these bacteria in several water systems, as well as the diversity in the use of multiple sources of water and exposure to them, leads to an increase in the potential risks of infection by *L. pneumophila*.

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INTRODUCTION

Legionella pneumophila is a fastidious bacterium, Gram-negative, non-spore-forming, rod-shaped, and aerobic.[1] The *Legionella* genus contains about 60 species. While new species are being discovered, *L. pneumophila* is still the most common pathogen in humans, accounting for 70-90% of infections.[2] *L. pneumophila* is common in both natural and man-made aquatic habitats, including potable water systems, cooling towers, and different water plumbing parts. *Legionella* can be spread to people by inhaling contaminated water aerosols from a variety of water sources.[3]

Legionella pneumophila (Lp) causes Legionnaires' disease (LD), a severe pneumonia. This infection accounts for 1.9% of the total community-acquired pneumonia infections, 4.0% of hospitalized cases, and 7.9% of patients requiring intensive care unit admission.[4] LD was initially diagnosed in 1976, following an epidemic caused by a polluted air conditioning system during an American Legion convention in Philadelphia. Through that outbreak, 182 people were infected, with 29 (16%) dying.[5] L. *pneumophila* serogroup 1 is the most frequently detected in the environment, which is also frequently linked to the illness.[6] In the United States and Europe, serogroup 1 is responsible for 70%-80% of LD cases.[7] The rate of fatality of legionnaires disease was determined to be around 10%. The rate of mortality varies greatly, varying from 1% to 80%. The Mortality rates are mostly determined by the following determinants: the patient's therapy, the speed with which the medication is administered, and whether the disease is considered sporadic, nosocomial, or associated with a big outbreak.[8] Even though *Legionella* outbreaks are common worldwide, they aren't reported in developing countries since it is difficult to isolate this bacterium and there aren't any straightforward diagnostic procedures.[9]

Legionella may survive in a variety of physical and chemical environments. The bacteria move from the natural reservoirs to city water supply systems, where they are incorporated into domestic water buildings or additional facilities that rely on water to function.[2]

Generally, the proportion of *Legionella spp.* in the water is often very small (less than 1% of the total bacterial population in residential environments).[9] The bacteria may colonize a variety of man-made water systems and are widely spread in diverse habitats, including several protozoa species.[10] L. pneumophila thrives and replicates inside free-living amoebae in aquatic conditions. As a result, when these host cells are present in a water system, they are regarded as a risk factor for LD.[11] In addition to surviving in the planktonic phase, L. pneumophila can survive and persist inside multi-species biofilms covering the surfaces of water systems.[10,12] Biofilms are a huge public health concern because they provide a protective habitat that permits bacteria to continuously leak into the water supply. Furthermore, biofilms improve bacterial life by improving resistance to temperature changes and antimicrobial agents.[13] These bacteria are found in the highest densities in biofilms found in hot water systems and water outlet openings. Warm stagnant water and water temperatures between 20 and 45 °C give an optimal environment for this bacterium's enormous growth.[14] Previous investigative studies found significant rates of *Legionella spp.* isolation regarding building and housing sites around the world, with 43.6% in Kuwait(15),[15] 32.7% in Germany,[16] 31.5% in Iran,[17] 8.3% in South Korea,[18] 84.1% in Italy,[19] and 34.1% in Japan.[20] In Iraq, there lack of rules governing environmental *Legionella* surveillance, and the majority of buildings are used without being aware of or taking precautions against *Legionella* risk.

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MATERIALS AND METHODS

A total of 227 samples, including 81 of 1 L water samples and 146 swabs, were collected from three distinct sorts of water sources; decorative fountains, freshwater sterilization and distribution plants, and potable water from homes and public places. The sterile 1L polyethylene bottles used for collecting the water samples were sterilized by Ultraviolet rays in the College of Veterinary Medicine at Fallujah University. Some physical and chemical parameters of water were examined for total dissolved solids (TDS), pH, $CL_{2,}$ and temperature. at the point of collection. The samples were taken with the authorized permission of the Al-Anbar Health Directorate and the Al-Anbar Water Directorate. Water samples were collected, analyzed, and evaluated in accordance with international operational standards for the quality of water; *Legionella* identification, and standard procedures for water and wastewater examination developed and published cooperatively by the American Public Health Association, American Water Work Association, and Water Environment Federation were applied.

Sample processing: Concerning water samples, they were put into a sterilized 47mm filter funnel assembled with a 0.22 m polycarbonate Millipore filter (Fisher Scientific, 3970 Johns Creek Ct., Suite 500, Suwanee, GA 30024) and a side-arm flask and a vacuum pump, the samples were filtered and concentrated in a cabinet with biological safety features. After the 1 L of water was completely filtered, the filter paper was in an aseptic manner separated from the funnel by using sterilized filter forceps, pleated to the external side, and then inserted inside a sterilized 50 mL centrifuge tube that already had 10 mL of the sterile water. The centrifuge tube was next vortexed for a minute to remove bacteria and organic substances from the filter before being centrifuged at 3000 rpm for 30 min. All except 1mL of the supernatant were delicately taken out using a sterilized pipette. Vortex mixing was conducted for 30 seconds to resuspend the deposit's contents.

Culture method: All samples were grown on buffered yeast extract charcoal agar BCYE (M809, Himedia) with two supplements, first is the *legionella* growth supplement which contains essentially L-Cysteine hydrochloride and Ferric pyrophosphate (FD016A, Himedia); the most important requirements for *legionella* nutrition. The second supplement is an antibiotic supplement recommended for the selective isolation of *Legionella* species, consisting of four types of antibiotics, they are Colistin sulphate 7500Units Vancomycin 2.500mg Trimethoprim 1.250mg Amphotericin B (FD017, Himedia). After the process of spreading the samples on BCYE media plates and according to previous instructions and studies, the incubation period ranged from 7 to 10 days, the culture began to be observed from the third day of the incubation period. The incubation process was carried out in an incubator set at 37°C, under aerobic conditions. As a negative control, a Confirmation culture was accomplished by subculturing the suspected colonies of *Legionella spp.* using blood agar (which lacks L-cysteine), colonies resembling *legionellae spp.* can be presumedly recognized based on their need for this amino acid. *Legionella spp.* are thought to be present in subcultured colonies that develop on BCYE agar but not on blood agar or BCYE lacking L-cysteine.

Gram stains` and Biochemical test: A colony of the suspected *legionella spp.* was put on a glass slide and stained with a gram stain procedure. The cells were then examined under the oil-immersion lens of the compound light microscope to determine their morphology and clustering. Subsequently, the negative gram stain isolates have conducted a series of biochemical tests, including oxidase, catalase, gelatin qualification, Motility, urease, and Hippurate hydrolysis tests.

The serological test: The L. pneumophila isolates were serogrouped using a commercial Latex agglutination test kit (LK04– HiLegionella Latex Test Kit, Himedia) in accordance with the instructions provided by the manufacturer.

DNA extraction: From bacterial growth overnight cultures, genomic DNA was extracted. Utilizing ABIO pure Extraction's (USA) method. One millilitre of the sample was placed in a 1.5-millilitre microcentrifuge tube together with 20 millilitres of proteinase K solution (20 mg/ml) and 200 millilitres of buffer BL. The tube was then violently shaken using a vortex and left to sit at 56 C for 30 minutes. The extraction was then carried out in accordance with the company's instructions. After that, the extracted DNA was examined using a Quantus fluorometer before being placed in a refrigerator at -20C in order to conduct standard PCR experiments.

PCR amplification of the 16S rRNA gene: A conventional PCR technique was performed for the detection of 16s rRNA genes.[21] To design primers of selected genes, selected primers were blasted by BLast N to compare the sequence of primers with existing GenBank records. The specific L. pneumophila 16s rRNA gene primers sequences were as follows; F-16srRNA: 5'AGGGTTGATAGGTTAAGAGC-3', R-16srRNA: 5'-CCAACAGCTAGTTGACATCG-3'. DNA amplification was performed in a thermocycler (Thermo Fisher Scientific, USA) under conditions of the initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 1 minute and a final step of extension at 72°C for 7 minutes. The PCR product was electrophoresed on 1.5% agarose gel (Promega, USA) in 1 X TAE buffer (Promega, USA) at 100v/mAmp for 60min. The DNA was stained with the Ethidium bromide stain (Promega, USA).

RESULTS

The findings of this investigation revealed the presence of shiny, convex, white-grey colonies on the BCYE agar (Figure 1A). The Gram-negative isolates were retained for further analysis, but the Gram-positive isolates were excluded from the study. specifically, the selected isolates that did not demonstrate any growth on blood cultures, which were classified as presumptive *Legionella* isolates. The results of the biochemical assays indicated that the isolates had positive reactions for oxidase, catalase, a liquefication gelatin test, motility, and Hippurate hydrolysis while yielding negative results for the urease test. Subsequently, all the isolates that exhibited compatible cultural and biochemical characteristics performed molecular testing using the conventional polymerase chain reaction (PCR) method, which confirmed the presence of the 16s rRNA gene amplicons (Figure 1B).

From a total of 227 samples, 28 (12.33%) were positive with the presence of *L. pneumophila* isolates. including 5 (17.86 %) positive isolates from water samples and 23 (82.14%) positive isolates from biofilm swabs (Table 1). The distribution of the detected *L. pneumophila* isolates from the five distinct sorts of water sources was as follows:

Regarding the four parameters that were measured when sampling, the concentrations of TDS ranged from 309 to 7960, while its concentration in the positive samples ranged from 387 ppm to 848 ppm. As for the concentration of chlorine, its concentration ranged from 0.1 ppm to 11.5 ppm in all samples, but its concentrations in the water of positive samples ranged from 0.1 to 5.6. As for the temperature, it ranged in the water samples from 14.4 to 37 Celsius, while the temperatures of the positive samples ranged from 19 to 34.9 Celsius. Finally, the pH of all samples ranged from 6.6 to 7.6. Its concentrations in the positive samples ranged from 6.7 to 7.4.

Table 1: Distribution of *legionella pneumophila* isolates in a variety of environmental sites.

Places` categories	Negative	Positive	Total
Decorative fountains	21	3	24
Freshwater sterilization and distribution plants	26	4	30
potable water from homes and public places	152	21	173
Total	199	28	227

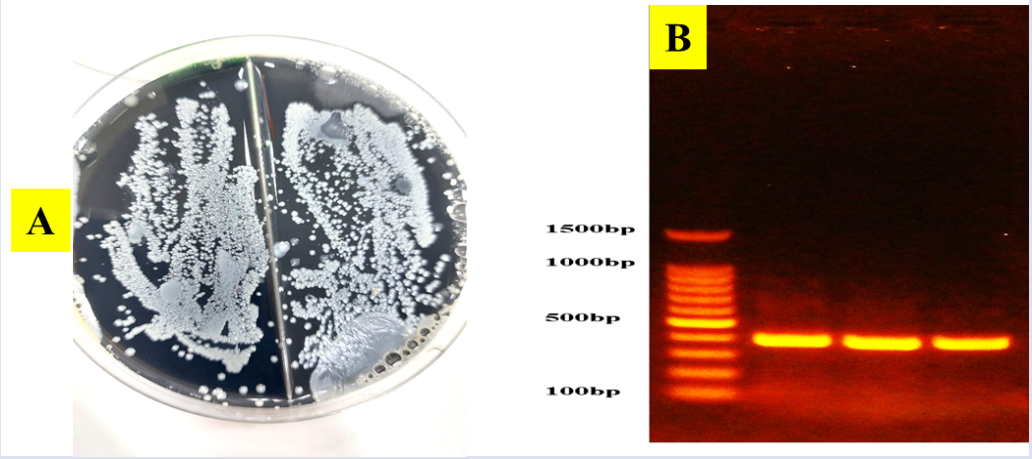

Figure 1: (A) The positive isolates of *legionella pneumophila* (B) Gel electrophoresis of the PCR product of the 16s rRNA gene.

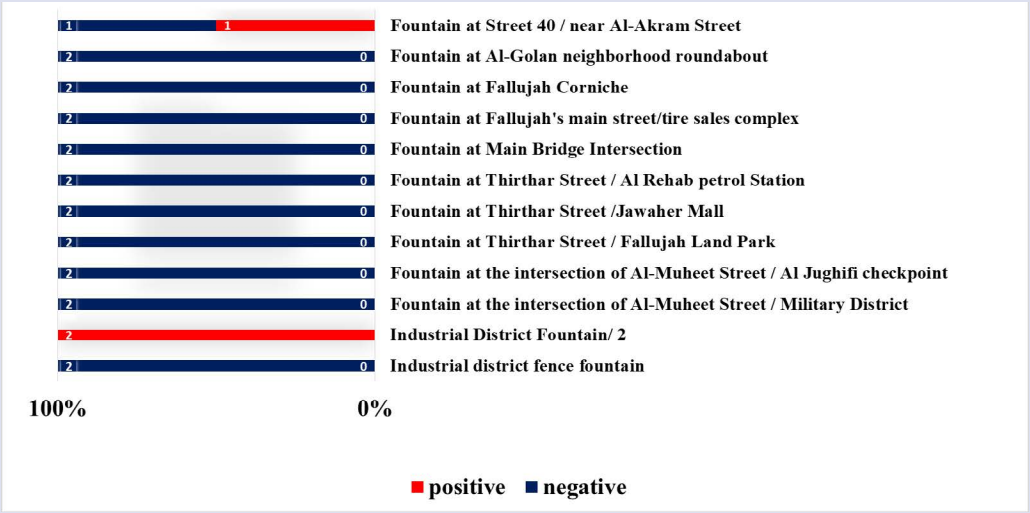

Figure 2: The presence of *legionella pneumophila* isolates in two fountains located in Fallujah city.

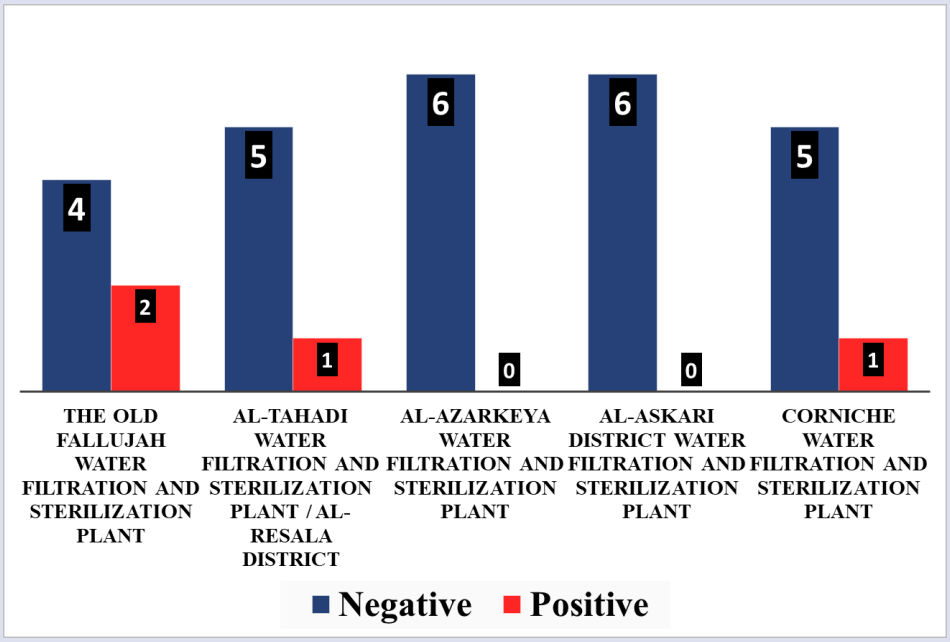

Figure 3: The presence of *legionella pneumophila* isolates in five Freshwater Sterilization and Distribution Plants located in Fallujah city.

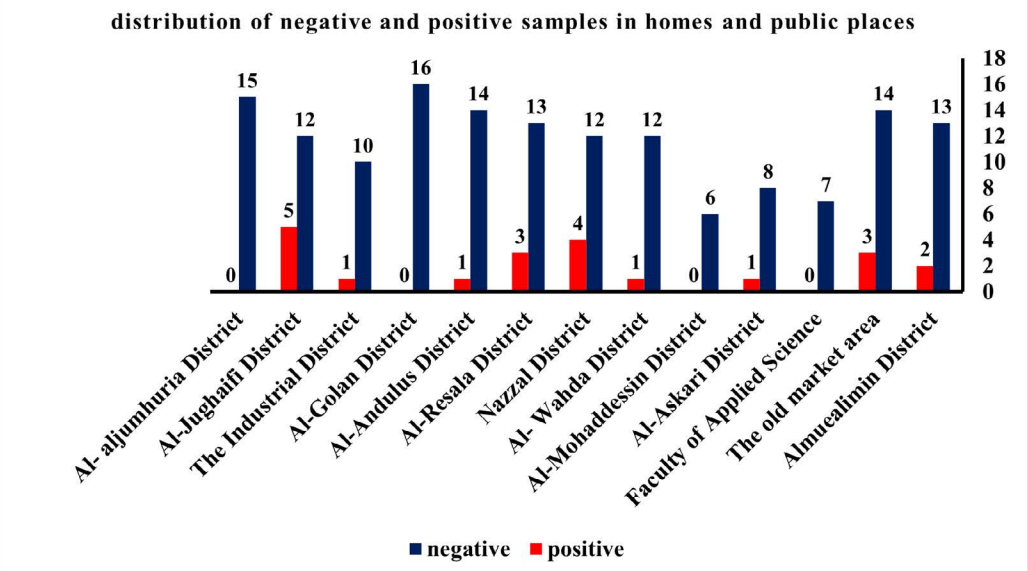

Figure 4: The presence of *legionella pneumophila* isolates from 13 different districts in Fallujah city.

The results of the investigation of *Legionella pneumophila* in the three water systems categories in this study were as follows: (Table 1)

Decorative fountains: 24 samples were collected from 12 sites of decorative fountains on different roadsides and public places in Fallujah City (Figure 2). All these fountains are equipped with potable water, which is supplied by water filtration and sterilization plants within a network of pipes to reach all areas of this city. One of these decorative fountains showed diagnosed isolates of *Legionella pneumophila* by both sample collection methods (swabbing and filtration technique), and the other one was positive only by swabbing method, while the rest of the decorative fountains were negative results and did not show the presence of *Legionella pneumophila*. Serologically, all three positive samples have belonged to serogroup 1 (SG1). Statistically, there were no significant differences observed between the positive results of the *L. pneumophila* isolates and the Sites of the sample *(P* value = 0.054 ns). regarding the type of sampling via both swabbing and filtration techniques, Statistically, there were no significant differences observed between the positive result of the L. pneumophila and the Type of sampling *(P* value = 0.537 NS).

Freshwater Sterilization and Distribution Plants: In this study, samples were taken from five plants for the purification and sterilization of drinking water (Figure 3). These stations cover all districts of Fallujah City through a network of water pipes that supply all homes and public places with potable water. The number of samples taken from these stations was 30 samples, 6 samples from each site. The results of all morphological, cultural, biochemical and molecular tests showed that 4 (13.33%) of them were positive and 26 (86.67%) were negative, as shown in the table below. three of the positive isolates were shown agglutination for serogroup 1 (SG1), while one was shown as (SG 2-15). Statistically, there were no significant differences observed between the positive result of the L. pneumophila and the Sites of the sample (*P* value = 0.401 Ns).

In each of the five water purification and sterilization stations, 6 samples were taken from three sources inside each plant; Two samples were taken from each source, the first source was the raw water that was drawn from the Euphrates River. Samples of the second source were taken from the precipitation reservoir, and the third was inside the Plant after the addition of chlorine to the water. From the raw water sources, it was found that 4 out of 10 samples were positive for L. pneumophila isolates, while it was not any positive results found in the precipitation reservoirs and the sources after chlorine addition. Statistically, there were very highly significant differences observed between the positive result of the L. pneumophila and the Source of sample *(P* value = 0.010**).

Houses and public places: In the current study, 173 samples were collected from 13 different districts in Fallujah city. These samples, like all other samples in this study. The results of the sequential tests for these bacteria showed that 152 (87.86%) samples were negative, while 21 (12.14%) positive samples contained the isolate of this bacteria. The positive isolates serologically categorized into 16 of SG1 (76.19%), and 5 (23.81%) of them belonged to (SG 2-15). Out of a total of 173 samples collected from houses and public places, 119 (68.79%) of them were collected by the swabbing technique, and its results showed that there were 18 (15.13%) positive samples containing L. pneumophila and 101(84.87%) negative samples. while by the other method (filtration technique), 54) 31.21%) samples were collected, and its result showed that there were 3 (5.56%) positive samples and 51(94.44%) negative. Statistically, there were no significant differences observed between the positive result of the L. pneumophila and the type of sampling (*P* value > 0.05) (Figure 4).

Regarding the water sources, a total of 173 samples were collected from 8 types of different water sources. Most of the samples were from water-supplying equipment, which is as follows: out of 88(50.87%) water tap samples, 4 (4.55%) were positive isolates and 84 (95.45%) were negative. out of 13(7.51%) Kitchen sink water mixers, all of their results were negative. Of 8 (4.62%) Bathwater mixer taps, 2 (25%) of them were positive and 6) 75%) were negative. Out of 4 (2.31%) closed-ended water tubes, 3(75%) were positive isolates and 1(25%) was negative. Of 4 (2.31%) Pre-valve seats of water tap samples, 2 (50%) were positive and 2(50%) were negative. Out of 15 (8.67%) first-stage filters of the osmosis filtration system for household use, 8 (53.33%) of them were positive and 7 (46.67%) were negative. 15 (8.67%) samples of air cooler water tanks, all of their results were negative. Out of 26 (15.03%) wash basin water mixer taps, two (7.69%) of them were positive and 24 (92.31%) samples were negative. Statistically, there were very highly significant differences observed between the positive result of the L. pneumophila and the Sources of samples *(P* value = 0.000***).

DISCUSSION

Legionellosis, a disease caused by *Legionella* bacteria, is not frequently documented in Iraq. This could potentially be attributed to the load on healthcare systems due to the prevalence of other more prevalent diseases, limited access to affordable diagnostic techniques, and a general lack of awareness regarding this particular disease among people in the country. According to the findings of the Centers for Disease Control (CDC), those who are 50 years of age or older, smokers, individuals with chronic lung illness, and those with a compromised immune system are most susceptible to the development of Legionnaires' disease.[22] According to available data, there has been a notable rise in the prevalence of Legionnaires' disease in the United States, with a fourfold increase observed since the year 2000. Similarly, in Europe, the incidence of this disease has shown an approximately threefold increase since 1995.[23] The presence of stagnant water at the locations of water usage, specifically at the terminal points, contributes to an elevated colonization rate of *Legionella spp.* bacteria. Numerous studies have demonstrated a correlation between nosocomial infections and the lack of movement or stagnation observed in many settings, including sensor-operated toilet faucets, lavatory drainage pipes, ice machines, and decorative fountains.[24] Hence, the primary objective of this research was to investigate the occurrence of *L. pneumophila* in water samples collected from various sources in Fallujah City, Iraq. Specifically, the study targeted decorative fountains, freshwater sterilization and distribution plants, and potable water sources in residential and public areas, all of which are potential reservoirs for the proliferation of this pathogenic bacterium. A total of 227 water samples obtained from various locations throughout Fallujah city were subjected to screening, which led to the identification of 28 (12.33%) instances of *L. pneumophila*. To the best of our current understanding, this study represents the first documentation of the identification of *L. pneumophila* in Anbar Province, Iraq.

It is thought The actual presence of L. pneumophila is higher than what has been discovered for several reasons, including The force of water flow in the installed water networks in Iraqi buildings and homes is slow due to most of the buildings and single-family homes being horizontal and depend on water tanks on the roofs of their buildings that is characterized by its low height, which makes the water pressure coming down to the points of use have a low flow, so the nature of this slow flow is not strong enough to tear the biofilm formed inside the pipes.[25] Also, the isolation of *Legionella spp*. by microbiological techniques poses challenges due to the presence of a viable but non-culturable (VBNC) state. This VBNC condition can lead to potential misdiagnosis of *legionella spp*.[26] Additionally, this fastidious bacterium grows slowly and is easily overgrown by other microorganisms, making it challenging to cultivate.[27]

In terms of the serotype of the positive isolates that were obtained in this study, it was found that the majority of them (78.57) were from Serogroup 1, and this corresponds with many previous studies, including the study that was conducted in Barah Governorate in Iraq. which was shown from 258 isolates, 77.1% belonged to serogroup 1 and 22.9% of isolates were serogroups 2–15.[28]

It was found that the positive isolates of *Legionella pneumophila* were growing in low concentrations of TDS, which are very close to the normal levels of drinking water, which are less than 500, and it wasn't found in the samples that were taken from fountains whose water had high concentrations of TDS. This was also evident in the water purification plants and in samples taken from the drinking water of homes and public places. Concerning temperatures, it was found that the positive isolates were taken from sources with a temperature ranging from 19 to 34.9°C. These are suitable temperatures for the growth and reproduction of *Legionella*, as indicated by previous studies.

It is known that *Legionella spp.* tolerates somewhat higher concentrations of chlorine in drinking water. During this study, it was found that the positive isolates were in water with chlorine concentrations ranging from 0.1 to 5.6. The highest concentrations of *Legionella* were found in the fountains. Although *Legionella* has been proven in previous studies it tolerates a wide range of changes in pH concentrations. However, in the current study, *Legionella* pneumophila isolates were found in water with moderate concentrations ranging between 6.7 and 7.4.

Fountains, particularly those that operate within a closed water system, have the potential to create ideal conditions for the growth of *Legionella* bacteria.[29] The aerosolization of water from public fountains in Mediterranean cities has the potential to present a substantial public health concern.[8] In the current study, 2 (16.67 %) of a total of 12 decorative fountains were detected with positive *L. pneumophila* isolates. The current study was closely compatible with a study that took place in South Korea,[18] where its results showed that the number of positive isolates was 3 out of a total of 14 fountains. This study was renewed after several years in the same place, as the results showed that there was only one positive isolate out of 80 samples collected from these fountains. A similar study was carried out in Mangalore, Karnataka, India. to explore *Legionella spp.* from several water systems, including fountains. Only one of the 20 samples taken by swab and water from decorative fountains tested positive for *legionella pneumophila*.[30]

This study was contradicting a study carried out in Spain,[31] where its results showed 2 positive isolates out of the total of 4 fountains from which samples were taken. Also, the current study was inconsistent with an investigation study on *Legionella spp*. carried out at the University of Patras, Greece.[8] Its results showed that all 54 samples taken from the decorative fountains were positive isolates. Epidemiology investigation of the LD outbreak in Bresso, Italy revealed that only the environmental isolates from one of the city fountains matched the patient strains.[4] According to a study in South Dakota, USA, the water *Legionella* colony count in the decorative fountain was greater than the remaining *Legionella*-positive water sources.[32] This fountain was in a restaurant, after the tracking investigation, it was proven the fountain was the source of many cases of Legionnaires disease in this city.

Concerning the second category investigated, the Freshwater purification and Distribution Plants, the current study showed that 4 (13.33%) of 30 samples (6 samples from each plant) were positive and 26 (86.67%) were negative. All the positive isolates were detected in raw water. a potential explanation for this phenomenon could be the absence of chlorine in the raw water. Chlorine serves as an inhibitor of biofilm formation and also acts as an agent for preventing protozoa growth, which is known to be favourable hosts for these bacteria. It is worth noting that chlorine is primarily concentrated in the precipitation reservoir where it is added, and subsequently, the water that undergoes distribution relatively retains these chlorine concentrations.

This study agreed with a previous study conducted in the Iraqi city of Basra on 13 water purification plants, its results showed the presence of *Legionella pneumophila* isolates in all plants except one in raw water and precipitation reservoir. While the post-treatment water showed positive isolates in only (5/12) plants.[27] But this research exclusively utilized phenotypic tests, without molecular approaches which considered the definitive diagnosis approaches to identification of *legionella spp*. It has been shown that numerous bacteria exhibit phenotypic similarities to *Legionella*; however, by genotypic analysis, it has been demonstrated that they belong to different bacterial species.[33] Another previous study on *Legionella spp.* and free-living amoebae showed the observed reduction in *Legionella spp.* concentration, as determined by polymerase chain reaction (PCR), was found to be 27.5% in raw water and 3.4% in finished water.[34] A further study investigation relied on the utilization of the Legiolert assay revealed

that L. pneumophila was detected in three out of five raw water samples obtained from a single water purification plant, whereas the remaining 48 samples gathered from 11 other water treatment facilities tested negative for the presence of the bacteria.[35] The results of the current study were somewhat inconsistent with what was stated in a study conducted in the United States,[36] where the results showed the presence of *Legionella pneumophila* was observed in 25% of the source water samples, but it was found in just 4% of the treated water samples. In this study, the identification of *legionella* was performed by qPCR assay which is considered a more sensitive assay to the detection of *legionella spp*. A study carried out in China discovered the presence of *Legionella spp*. had a higher number of gene copies within biofilms than in water samples.[37]

It was clear from the results that *Legionella pneumophila* was isolated from the majority of districts in the city, despite the fact of the low detected isolates in these districts. Further, It has been shown through the results of the water source in homes and public places that these bacteria are prevalent in various equipment, but most of them have appeared where the water stagnates, which means the slow or stagnant water flow in the pipes encourages the formation of biofilm in which these bacteria exist, whether they are inside protozoa or within the microbial community of this biofilm.

Multiple investigations have demonstrated that water samples obtained from electronic faucets had a significantly higher prevalence of *Legionella* contamination as compared to faucets operated by handles. Electronic faucets have a higher propensity for colonization due to reduced water flow, which creates an environment conducive to the proliferation of waterborne pathogens. This is further exacerbated by the presence of favourable conditions, such as a column temperature of approximately 35°C. Additionally, the lower flushing capacity of electronic faucets contributes to the increased likelihood of colonization.[38]

Concerning the prevalence of *Legionella pneumophila* in house water systems, Multiple studies have conducted investigations on L. pneumophilia infections among patients receiving hospital care, and have identified a correlation between these diseases and the presence of bacteria in their domestic shower facilities. This suggests that the patients' exposure to these microbes in their homes may have been the origin of their infections.[39] The aforementioned data suggest that tap and shower water, particularly the biofilms present in pipes, have the potential to serve as sources of everyday pathogen exposure.

As well as, in what is consistent with the current study, through a study conducted on the investigation of *Legionella spp*. in water sources of residential homes,[40] it was found that the presence of *Legionella* was seen with greater frequency in water samples compared to biofilm samples, with a prevalence of 29.8% and 16.9% respectively. It is worth noting that in only one instance was the pathogen exclusively detected in the biofilm. The pathogen L. pneumophila sg 1 was isolated more frequently, accounting for 65.8% of cases.

Nevertheless, the present investigation closely corresponded with a prior study conducted in the United States, which demonstrated that *Legionella pneumophila* was present within residential areas at a rate of 21%.[41] In another investigation, it was found that a total of eight out of 370 house samples collected in June 2016, six out of 28 residence samples, representing 21% of the samples, were found to be positive for culturable L. pneumophila. Notably, these positive results originated from multiple taps inside the same residence.[42] A study conducted in Jordan revealed that *Legionella pneumophila* was identified in 47.4% of the samples collected from the water distribution systems of three hotels under investigation. The point-of-use area with the highest documented positive *legionella* contamination is the cold washbasin, accounting for 33% of cases. This is followed by the showerhead, which accounts for 22.2% of cases.[43] This study provide a further contribution to focus on environmental protection against all the biohazards in our locality in Iraq.[44]

This study provides evidence that various water systems, including decorative fountains, Freshwater Sterilization and Distribution plants, and potable water in homes and public places, are inhabited by L. pneumophila in Fallujah City. This colonization poses a potential risk to the population, highlighting the importance of consistent monitoring and decontamination of water supplies in these locations. The study's observations serve as a foundation for doing further extensive research on the prevalence of L. pneumophila in several probable sources within Iraq.

CONCLUSION

Based on the findings of this study, it can be inferred that there is a potential prevalence of *Legionella pneumophila* in various water systems in Iraq, where many factors were found, including the warm temperature of the water, suitable for the spread and growth of these bacteria during most seasons of the year. However, additional research is required to further investigate these bacteria.

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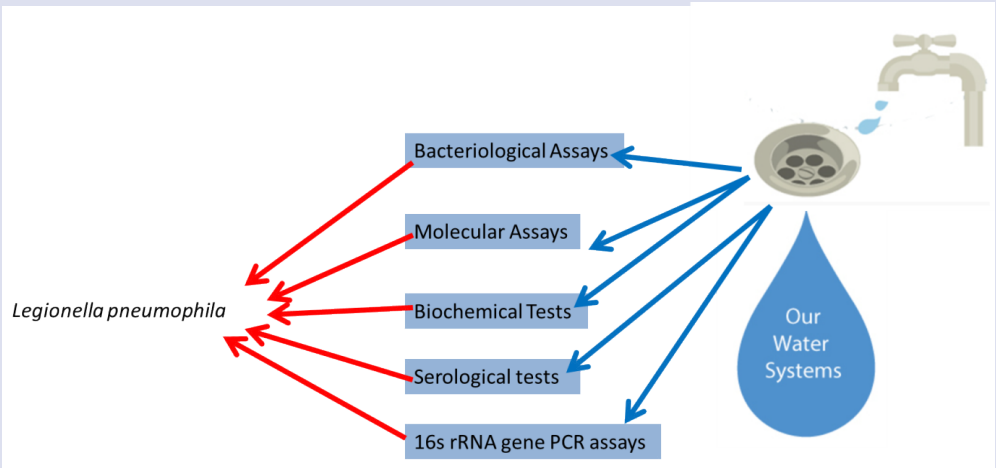
GRAPHICAL ABSTRACT



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