

*Full Length Research Paper*

# Detection of *Legionella pneumophila* as the cause of atypical pneumonia in the water sources of the holy places of Makkah

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***Legionella pneumophila* is an important pathogen and is involved in more than 95% of cases of severe atypical pneumonia. The current study focused on the ablution water in the grand mosque in Makkah as well as water tanks in hotels surrounding the holy mosque. A total of 100 water samples were collected from ablution water in the Haram and the hotels in the central area around the grand mosque and sent to the research laboratory of microbiology, Faculty of Medicine, University of Umm Al Qura. Samples were filtrated and inoculated onto buffer charcoal-yeast extract agar base and incubated at 37°C in a CO<sub>2</sub> incubator. The plates were examined after seven days of incubation. Isolated organisms were confirmed by using the “Microgen Legionella” (latex agglutination test). Out of the 100 water samples tested, 11 samples were positive for *L. pneumophila*. All positive water samples were from hotels water tanks. This indicates that this type of bacteria existing in the water sources. So requires further research to cover all sources of water to avoid an outbreak of this infection among the pilgrims. To avoid the possibility of this, constant maintenance of hotel water tanks regularly and the use of chlorine in specific proportions according to recommended specifications can help reduce the spread of these microbial infections.**

**Key words:** Holy Mosque, *Legionella pneumophila*, water tanks, environmental.

## INTRODUCTION

Legionnaires disease is considered a major form of travel-associated pneumonia. *Legionella pneumophila* is the main causal agent of this disease (Heuner and Steinert, 2003; Miyashita et al., 2020). This infection is transmitted between people through the air polluted with water droplets loaded with this microbe which may be present in water tanks and warm water systems. Transition occurs during the shower through inhalation of this water, which may be contaminated with this type of

pathogenic bacteria. This disease, often in the summer season where the temperature rises which is considered an important environmental factor for the growth and multiplication of this type of bacteria. Symptoms start as high temperature, headache, muscle pain, and coughing. The people with major risk factors for community-acquired Legionnaires are immunocompromised people, chronic diseases, cigarette smokers, and the elderly; they are the most vulnerable to this bacterial infection (Smith

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et al., 2019). Therefore, *Legionella* is responsible for an uncommon type of human respiratory disease called Legionnaires' disease that could be fatal if not treated (Leoni et al., 2005). This disease was first identified in the seventies of the last century in America and was initially believed to be a flu-like disease when a group of people who attended a conference in America died in 1976 (Terranova et al., 1978; Han et al., 2019). According to some studies issued by the center for disease control and prevention (CDC), more than 20,000 patients every year, in addition to nearly four thousand deaths (Chamberlain et al., 2017; Borges et al., 2016). Interestingly, a published study of formerly collected data, investigated both water samples from different sources include water coolers, sprinkles, in addition to clinical samples collected from patients who were admitted to five specialized hospitals in Makkah during Hajj season (Cristovam et al., 2017; Pierre et al., 2017; Ohno et al., 2003; Khodr et al., 2016). Another study revealed that most of the causes of acute pneumonia during the Hajj season are *Haemophilus influenzae* and *Streptococcus pneumoniae*. However, *Legionella* was not mentioned, and this may be due to the lack of tools available for testing for *Legionella*. Another study to identify the etiological agents of severe community-acquired pneumonia during Hajj declared that most of the bacterial infected samples were due to *H. influenzae* and *S. pneumoniae*, but no *Legionella* test was performed (Azhar et al., 2010; Correia et al., 2016; van Heijnsbergen et al., 2015). For a clear understanding of the factors causing the colonization of water pipes and domestic water systems by *L. pneumophila* in Saudi Arabia, this study was designed to detect the presence of *L. pneumophila* in the water sources in the hotels of the accommodation of pilgrims surrounding the Grand Mosque in Makkah city and to obtain data on *L. pneumophila* to help in controlling this type of infection.

## MATERIALS AND METHODS

### Collection of samples

The number of samples collected in this study was 100 water samples, 90 of them from the hotel tanks surrounding the campus and 10 samples from the ablution water in the Haram during three months in the middle of the year 2014. The water samples were collected in 1 L sterile bottles containing 1 ml of 0.1 N sodium thiosulfate to neutralize the chlorine in the water samples so that the bacteria to be isolated will not be affected. The source of water for the ablution water in the Grand Mosque in Makkah city is chlorinated municipal water, while the water of hotel tanks is a mixture of chlorinated municipal water and non-chlorinated well water in the city of Makkah. Water is transferred from wells to hotel tanks by vehicles equipped for this. The sample size in the current study was designed according to the international organization for standardization (ISO) 11731. This study was conducted during the year 2014.

### Transportation of the specimens

After collecting the samples, they were sent to the microbiology

research center in Medicine College, Umm Al-Qura University.

### Water sample preparation and analysis

The samples were filtered by using a filtration vacuum pump device. The polycarbonate membrane filter 0.2  $\mu$ m was used for filtering the water samples. The filter was removed and placed in 10 ml of sterile water inside a sterile tube with a capacity of 50 ml. Then the tube was placed in a centrifuge at 3000 rpm for 1 min. The supernatant was removed and the sedimentation was mixed using the vortex to obtain homogenous suspension. Then 0.1 ml of the final concentration was taken and inoculated on a selective medium Buffered Charcoal Yeast Extract (BCYE). The agar plates were incubated under CO<sub>2</sub> conditions at 37°C.

### Identification and confirmation

After seven days incubation, the plates were examined by traditional methods, then the suspected bacterial colonies expected to be *Legionella* were re-cultured on blood agar plates and BCYE agar plates media and were re-incubated for three days. Blood agar plates that had no growth indicating that this is *Legionella* spp. Confirmation tests were carried out using, latex agglutination technique "Microgen Legionella" (latex agglutination test).

To differentiate between *Legionella* spp., a serotyping test was performed using the Legionella Latex test (Cat. No. DR0800M oxid, UK). This test was done by the direct method. All reagents were placed at room temperature before the assay started. The tube was numbered according to the number of samples and then 0.4 mL of 0.85% saline was added to each test tube. A number of growing colonies of each sample were transferred to 0.4 mL of 0.85% saline in the test tube. This was mixed to obtain a homogeneous mixture. One drop of the three reagents and control reagent was distributed in four circles. One drop of the bacterial suspension was added to each of the four circles, then mixed for exactly 1 min. The agglutination in any circle showed *L. pneumophila* within 1 min. Whereas, other circles that did not show agglutination within 1 min confirmed that it was negative for *L. pneumophila*.

## RESULTS AND DISCUSSION

The time period to collect water samples for the current study was three months, according to the action plan that was designed to conduct this study. Hundreds of water samples were collected, 10 of them taken from the ablution water in Grand Mosque and 90 water samples were collected from water tanks in hotels surrounding the Holy Mosque in Makkah city for detection of *L. pneumophila*. 11 (11%) water samples out of 100 water samples showed positive results of *Legionella* spp. All positive samples were from water samples collected from hotels, while water samples collected from ablution water in Grand Mosque showed negative results. The permits granted to approve the collection of water samples in this study were one-time, so we were unable to repeat the water samples.

These isolates were subjected to confirmatory tests to detection the pathogenic strains that cause atypical pneumonia, and this was done by test, "Microgen Legionella" (latex agglutination test) that confirmed that

**Table 1.** Distribution of water samples and prevalence of *Legionella pneumophila* according to specimen collection locations.

Specimen collection locations	No. of specimens	No. of positive specimens	Positive Specimens (%)	No. of negative specimens	Negative specimens (%)	No. of <i>Legionella pneumophila</i>
Ablution water in Grand Mosque in Makkah	10	0	0	10	100	0
Water tanks in hotels	90	11	12.2	79	87.8	11
Total	100	11	11	89	89	11

11 isolates were related to *L. pneumophila* shown in Table 1. Positive and negative controls were also used during the cultivation of these samples on BCYE and blood agar media to compare them with the water samples results, using a sterile water sample containing a reference strain, the American Type Culture Collection (ATCC) of *Legionella pneumophila* and another sample for sterile water only as a negative control.

Published studies regarding pilgrim's health particularly respiratory tract infections during Hajj showed that pulmonary infections during Hajj are of great burden in Saudi health authorities. The study also showed that 160 admitted patients in Arafat and Mona hospitals during Hajj were diagnosed with respiratory tract infections and were the highest health attributed problem in hospitalized pilgrims during mild weather (Memish et al., 2014; Al-Ghamdi et al., 2003; Madani et al., 2006). Another cross-sectional study in the same year has confirmed that pneumonia was recorded as the highest cause of hospitalization in 808 patients admitted to seven hospitals in Arafat and Mona during the Hajj festival (Khan, 2006). Indeed, these findings were also confirmed by a review article published which studied 689 hospitalized cases belonging to 49 countries in a tertiary hospital in Makah for 5 weeks during Hajj and the findings showed that pneumonia was identified as a major cause of illness and the leading cause of death in 28 patients diagnosed with pneumonia (Alzeer, 2009). Another published review article of respiratory tract infection during Hajj identified these infections as the most common cause of hospital admissions (Mandourah et al., 2012). Interestingly, a published study of formerly collected data, showed both water samples from different sources include water coolers, sprinkles, and storage tanks in Makah areas such as Arafat, Mona, Muzdaliphah, and nearby the Holy Mosque "Haram", and clinical specimens from pneumonia diagnosed patients admitted to five tertiary hospitals in Makkah and the findings showed that pneumonia was the major cause of sickness in pilgrims and recommended with more studies on water sources (Sreenath et al., 2020; Al-Tawfiq et al., 2013, 2014; Spiegelman et al., 2020; Zahran et al., 2018). Absence of documented data on the isolation rates of this type of bacteria cause this disease in Saudi Arabia and in view of the results of previous studies during the Hajj season; the reason for the emergence of this type of bacteria may be due to the fact that these hotels use water from wells which can be

the main source of these pathogenic bacterial strains. This requires more research to cover all water sources. As well as the work for the creation of a database on the prevalence rates of this type of bacteria that causes Legionnaires disease and finding mechanisms to resist and control them to avoid the events of outbreaks of this type of disease during the Hajj and Umrah seasons.

## Conclusion

Based on the results of the current study, the water samples that showed positive results for *L. pneumophila* were collected from hotel water tanks, while no positive samples were recorded for *L. pneumophila* from ablution water sources in the Holy Haram. It is likely that the reason for the presence of these bacterial strains in the water tanks of hotels is due to the use of well water that is not treated with chlorine often during the congestion in the Hajj and Umrah seasons. To avoid the possibility of this, constant maintenance of hotel water tanks regularly and the use of chlorine in specific proportions according to recommended specifications can help reduce the spread of these microbial infections.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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## REFERENCES

- Al-Ghamdi SM, Akbar HO, Qari YA, Fathaldin OA, Al-Rashed RS (2003). Pattern of admission to hospitals during muslim pilgrimage (Hajj). Saudi Medical Journal 24(10):1073-1076.

- Al-Tawfiq JA, Zumla A, Memish ZA (2013). Respiratory tract infections during the annual Hajj: potential risks and mitigation strategies. *Current Opinion in Pulmonary Medicine* 19(3):192-197.
- Al-Tawfiq JA, Memish ZA (2014). Mass gathering medicine: 2014 Hajj and Umra preparation as a leading example. *International Journal of Infectious Diseases* 27:26-31.
- Alzeer AH (2009). Respiratory tract infection during Hajj. *Annals of Thoracic Medicine* 4(2):50-53.
- Azhar El AA, Asghar AH, Bukhari SZ, Zafar TA, Jukdar HA (2010). An Environmental and Clinical study during Hajj season. *Professional Medical Journal* 17(3):479-482.
- Borges V, Nunes A, Sampaio DA, Vieira L, Machado J, Simões MJ, Gonçalves P, Gomes JP (2016). Legionella pneumophila strain associated with the first evidence of person-to-person transmission of Legionnaires' disease: a unique mosaic genetic backbone. *Scientific Reports* 6:26261.
- Chamberlain AT, Lehnert JD, Berkelman RL (2017). The 2015 New York City Legionnaires' Disease Outbreak: A Case Study on a History-Making Outbreak. *Journal of Public Health Management and Practice* 23(4):410-416.
- Correia AM, Ferreira JS, Borges V, Nunes A, Gomes B, Capucho R, Gonçalves J, Antunes DM, Almeida S, Mendes A, Guerreiro M, Sampaio DA, Vieira L, Machado J, Simões MJ, Gonçalves P, Gomes JP (2016). Probable Person-to-Person Transmission of Legionnaires' Disease. *New England Journal of Medicine* 374(5):497-498.
- Cristovam E, Almeida D, Caldeira D, Ferreira JJ, Marques T (2017). Accuracy of diagnostic tests for Legionnaires' disease: a systematic review. *Journal of Medical Microbiology* 66(4):485-489.
- Han XY (2019). Solar and Climate Effects Explain the Wide Variation in Legionellosis Incidence Rates in the United States. *Applied and Environmental Microbiology* 85(22):e01776-19.
- Heuner K, Steinert M (2003). The flagellum of Legionella pneumophila and its link to the expression of the virulent phenotype. *International Journal of Medical Microbiology* 293:133-43.
- Khan NA (2006). Pattern of medical diseases and determinants of prognosis of hospitalization during 2005 Muslim pilgrimage Hajj in a tertiary care hospital. A prospective cohort study. *Saudi Medical Journal* 27(9):1373-1380.
- Khodr A, Kay E, Gomez-Valero L, Ginevra C, Doublet P, Buchrieser C, Jarraud S (2016). Molecular epidemiology, phylogeny and evolution of Legionella. *Infection Genetics and Evolution Journal* 43:108-22.
- Leoni E, De Luca G, Legnani PP, Sacchetti R, Stampi S, Zanetti F (2005). Legionella waterline colonization: detection of Legionella species in domestic, hotel and hospital hot water systems. *Journal of Applied Microbiology* 98(2):373-379.
- Madani TA, Ghabrah TM, Al-Hedaithy MA, Alhazmi MA, Alazraqi TA, Albarrak AM, Ishaq AH (2006). Causes of hospitalization of pilgrims in the Hajj season of the Islamic year 1423 (2003). *Annals of Saudi Medicine* 26(5):346-351.
- Mandourah Y, Al-Radi A, Ocheltree AH, Ocheltree SR, Fowler RA (2012). Clinical and temporal patterns of severe pneumonia causing critical illness during Hajj. *BMC Infectious Diseases* 12(1):1-8.
- Memish ZA, Zumla A, Alhakeem RF, Assiri A, Turkestani A, Al Harby KD, Alyemni M, Dhafar K, Gautret P, Barbeschi M, McCloskey B, Heymann D, Al Rabeeah AA, Al-Tawfiq JA (2014). Hajj: infectious disease surveillance and control. *Lancet* 383(9934):2073-2082.
- Miyashita N, Higa F, Aoki Y, Kikuchi T, Seki M, Tateda K, Maki N, Uchino K, Ogasawara K, Kiyota H, Watanabe A (2020). Distribution of Legionella species and serogroups in patients with culture-confirmed Legionella pneumonia. *Journal of Infection and Chemotherapy* 26(5):411-417.
- Ohno A, Kato N, Yamad K, Yamaguchi K (2003). Factors influencing survival of Legionella pneumophila serotype 1 in hot spring water and tap water. *Applied and Environmental Microbiology* 69(5):2540-2547.
- Pierre DM, Baron J, Yu VL, Stout JE (2017). Diagnostic testing for Legionnaires' disease. *Annals of Clinical Microbiology and Antimicrobials* 16:59.
- Smith AF, Huss A, Dorevitch S, Heijnen L, Arntzen VH, Davies M, Robert-Du Ry, van Beest, Holle M, Fujita Y, Verschoor AM, Raterman B, Oesterholt F, Heederik D, Medema G (2019). Multiple Sources of the Outbreak of Legionnaires' Disease in Genesee County, Michigan, in 2014 and 2015. *Environmental Health Perspectives* 127(12):127001.
- Spiegelman J, Pedutem T, Francisco MJ (2020). Legionnaires' Disease Cases at a Large Community Hospital-Common and Underdiagnosed. *International Journal of Environmental Research and Public Health* 17(1):332.
- Sreenath K, Dey AB, Kabra SK, Thakur B, Guleria R, Chaudhry R (2020). Legionella pneumophila in Patients with Pneumonia at a Referral Hospital, New Delhi, India, 2015-2020. *The American Journal of Tropical Medicine and Hygiene* tpm200653 DOI:10.4269/ajtmh.20-0653.
- Terranova W, Cohen ML, Fraser DW (1978). 1974 outbreak of Legionnaires' Disease diagnosed in 1977. Clinical and epidemiological features. *Lancet* 2(8081):122-4.
- Van Heijnsbergen E, Schalk JA, Euser SM, Brandsema PS, den Boer JW, de Roda Husman AM (2015). Confirmed and Potential Sources of Legionella Reviewed. *Environmental Science and Technology* 49(8):4797-815.
- Zahran S, McElmurry SP, Kilgore PE, Mushinski D, Press J, Love NG, Sadler RC, Swanson MS (2018). Assessment of the Legionnaires' disease outbreak in Flint, Michigan. *Proceedings of the National Academy of Sciences of the United States of America* 115(8):E1730-E1739.