

LEGIONELLA INFECTION; AN ENVIRONMENTAL AND CLINICAL STUDY DURING HAJJ SEASON

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SUMMARY...Respiratory system is most commonly affected during Hajj season. **Objectives:** (1) to determine the prevalence of Legionella in drinking water, cooling sprinklers and other sources of water consumed in pilgrimage area of the holy city, Makkah; (2) to study the prevalence of pneumonia caused by Legionella species from clinically suspected cases; and (3) to determine whether there is any relationship disease incidence and water supply system. **Material & methods:** The clinical samples were received from in-patients of the following five Ministry of Health (MOH) Hospitals; Hera General Hospital, Al-Noor Specialist Hospital, King Faisal Hospital, King Abdul-Aziz Hospital, and Ayyad Hospital during Hajj 2003. The patients included in the study were those who fulfilled the following criteria; clinically diagnosed as pneumonia, negative on routine cultures and found negative for acid fast bacilli (AFB) from sputum/bronchial aspirate (BAL). Serological tests was done by Enzyme linked Immunosorbent assay (ELISA) for Legionella antibodies Total IgG and IgM. **Results:** Out of total 133 patients suspected for Legionella, 83 (62.4%) were male and 50 (37.6%) female. The male versus female ratio was 1:0.6. The major age group affected was between 50-70 years (63.2%). There were 4 (3.0%) cases of Legionella species isolated from Microbiological cultures. The overall seropositive rate among clinically suspected cases was 4.51%. A total of 560 water samples were collected from Arafat, Muzdalifa, Mena, and areas around the Holy Mosque and found negative for Legionella species. **Conclusion:** Pneumonia was one of the most common illnesses among pilgrims. The water supplied to all areas of the pilgrimage was free from the Legionella and no link has been established among the patients with water supply systems. Both clinicians' awareness and availability of specialized laboratory tests for atypical pneumonia such as Legionella is necessary. Although, all water samples found negative for Legionella but it is recommended to screen all water reservoirs for Legionella annually before Hajj season.

INTRODUCTION

Legionellosis is an environment-related acute respiratory infection and later may leads to Pneumonia caused by the Gram-negative Legionella bacteria, of which most pathogenic is Legionella pneumonphila. The infection is usually acquired by the inhalation of aerosols

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contaminated with the pathogenic *Legionella*. Such contaminated aerosols are generated in man-made water systems, such as air-conditioning cooling towers, whirlpool spas, and fountains that have not been properly maintained¹. It was first discovered in 1976 after an outbreak in Philadelphia during a convention of the American Legion. Many studies have related the environmental sources and pathologies of infections with *Legionella*^{2,3}. *Legionella* is transmitted through aerosols produced from environmental sources, and person-to-person transmission does not take place⁴. More than 40 species of *Legionella* have been identified, and more than 20 linked with human diseases. It is difficult to distinguish *Legionella* from other forms of pneumonia, because many cases go unreported. Approximately 1,000 cases of Legionnaires' disease are reported annually to Centers for Disease Control and Prevention (CDC) but is estimated that over 25,000 cases of the illness occur each year and cause more than 4,000 deaths⁵. The most susceptible persons for Legionnaires' disease are elderly, smokers, and immunosuppressed⁶.

Respiratory system is the most commonly affected during Hajj season due to the gathering of pilgrims, mostly elderly subjects from poor countries with a high prevalence of infectious diseases, under crowded conditions and performing physically exhausting religious rites. Since water sprinklers are used to reduce the atmospheric temperature during Hajj, however, pilgrims may acquire pneumonia due to *Legionella* infection^{7,8}.

The study was conducted with the following objectives: (1) to determine the prevalence of *Legionella* in drinking water, cooling sprinklers and other sources of water consumed in pilgrimage area of the holy city, Makkah; (2) to study the prevalence of pneumonia caused by *Legionella* species from clinically suspected cases; and (3) to determine whether there is any relationship disease incidence and water supply system.

MATERIAL & METHOD

The clinical samples were collected from in-patients of following five MOH hospitals; Hera General Hospital (HGH), Al-Noor Specialist Hospital (NSH), King Faisal

Hospital (KFH), King Abdul-Aziz Hospital (KAH), and Ajjad Hospital (AJH) during Hajj period 1423H. The patients included in the study were those who fulfilled the following criteria; patients with diagnosis of pneumonia or bronchopneumonia with sputum specimens negative for both acid fast bacilli and routine microbiological culture. The following specimens were collected; sputum or bronchial aspirate for microbiological culture, and blood for serology. Sputum specimens were inoculated on *Legionella* selective media-Buffer Charcoal Yeast Extract (BCYE) medium (Oxoid, UK) and incubated in 5% CO₂ atmosphere at 35°C. The plates were examined at regular intervals for up to 10 days. Suspicious *Legionella* colonies were further investigated using biochemical tests⁹. Serum samples were examined for total *Legionella* IgG and IgM antibodies using enzyme linked immunosorbent assay (ELISA) Kit (Vircell, Spain) as has been proposed by the manufacturer's instructions.

The water testing was applied to all kinds of water consumed by pilgrims including; drinking water, cooling sprinklers and storage tanks. A 100 ml of water samples were collected in sterile glass bottles from different areas in Makkah including; Arafat, Muzdalifa, Mena and the area around the Holy Mosque. The concentration method was used for identification of *Legionella* organisms. Bacteria including *Legionella*, if present in water sample, were concentrated by membrane filtration. The filtrate was tested for *Legionella* by inoculating on BCYE medium and incubated in 5% CO₂ atmosphere at 35°C. The plates were examined at regular intervals for up to 10 days. Identification of *Legionella* species was made by colony morphology, Gram-stain, and positive reactions with catalase, oxidase and DNase tests.

RESULTS

A total of 133 clinical specimens of patients belonged to 26 nationalities were received from the following five hospitals; HGH 68 (51.1%), NSH 29 (21.8%), KFH 18 (13.5%), KAH 10 (7.5%), and AJH 8 (6.0%). There were 83 (62.4%) male and 50 (37.6%) female. The male versus female ratio was 1:0.6. The age groups of patients included in the study was as follows; age group ≤ 20 years 6 (4.5%), 21-40 years 8 (6.0%), 41-60 years 61

(45.9%), and ≥ 61 years 58 (43.6%) cases.

Legionella species were identified on microbiological culture in 4 (3.0%) specimens. On reviewing the medical records, the patients belonged to the positive isolated had severe pneumonia with difficulty in breathing, and fever $\geq 40^\circ\text{C}$. All positive patients were above 50 years old and 3 were male and 1 female. Legionella antibodies total IgG & IgM, were detected in 6 (4.5%) samples. There were 2 patients serology positive but the culture was

negative.

A total of 560 water samples were collected and tested for Legionella from different areas of Makkah including; Arafat 200 (35.7%), Muzdalifah 90 (16.1%), Mena 150 (26.8%) and around the Holy Mosque 120 (21.4%). Microbiological culture for Legionella were negative for all water samples involved in the study. Table-I shows the detail of positive cases for Legionella and correlation with water supply systems.

Table-I. Positive cases for legionella and correlation with water supply systems

Age	Sex	Nationality	Microbiology Culture	Anti-Legionella (Total igG/IgM)
59 years	Male	Indian	+	+
55 years	Male	Sudanis	+	+
52 years	Male	Indonesian	+	+
65 years	Female	Indonesian	+	+
51 years	Male	Pakistani	-	+
58 years	Female	Bangladesh	-	+

DISCUSSION

Although, pneumonia is the commonest disease during Hajj period^{7,8} and Legionella infection is one of the causes of this illness¹. The occurrence of legionellosis within a facility requires an environmental source of Legionella. An association of pneumonia due to Legionella and environmental samples has been documented in many studies^{9,10,11} but no such studies conducted in Makkah so far.

This study showed that 3% of the suspected cases of Legionella were positive on microbiological culture and 4.5% on serological investigation. This figure is similar to which has been reported in other area of the kingdom. However, these studies were performed on national and international resident^{12,13}. These percentages even are low, should not be ignored because pneumonia disease is one of the most common infections during Hajj and risk factors for acquiring Legionella's disease is present

during this season⁹⁻¹¹.

The elderly patients had a higher risk of developing Legionella infection than do young adults and children as reported in this study. Our results showed that men were affected more than the women. Similar preponderance of the elderly patients and male gender has been reported by Goh et al., 2005¹.

In present study, all environmental samples collected from pilgrimage areas were found negative for Legionella and no link of water distribution system has been established among patients in clinical care units in contrast to other international studies where they established a link between clinical specimens and environmental samples⁹⁻¹¹.

All patients involved in the study had community-acquired legionellosis as all cases belonged to the different hospitals. The source of Legionella in patient's care

facilities of Makkah was unknown and can be explained that it may be from their country of origin of pilgrims. Diagnosis of Legionnaires' disease requires the application of specific diagnostic tests that are not usually available in local health care facilities. The organism can not be differentiated on Gram stain and will not grow on standard microbiological media typically used to process sputum specimens. Selective media BCYE with added antibiotics to inhibit respiratory flora are also not available in routine Microbiology laboratory. Approximately, 80% of unknown etiology of pneumonias cases can be diagnosed by providing appropriate laboratory support⁸.

In conclusion, pneumonia is the one of the most common illness among our pilgrims, which can be justified on the fact the combination of crowdedness and elderly pilgrims. Both clinician's awareness and availability of specialized laboratory tests for atypical pneumonia such as Legionella is necessary. Although authors could not document any positive water sample but it is recommended to screen all water reservoirs for Legionella annually before Hajj season.

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