

Seroprevalence of *Legionella pneumophila* in Pneumonia Patients in Four Major Hospitals in Trinidad and Tobago

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ABSTRACT

Trinidad and Tobago is an island-state in the Caribbean with a size of 5 128 square kilometers and a population of 1.3 million. Pneumonia is a leading cause of death in Trinidad. This project determined the frequency of *Legionella pneumophila* in patients with pneumonia, investigated the relationship between pneumonia and selected risk factors. Serum and demographic data were collected from 123 patients, diagnosed with pneumonia. Sera were tested for *L pneumophila* Ig G/M/A and IgM. All analyses were done using the SPSS statistical package. Of a total of 123 serum samples tested, 39 (31.7%) were positive for *L pneumophila* IgM/G/A while 2 (1.6%) were positive for IgM only. Hospitals, gender and ethnicity did not significantly ($p > 0.05$; χ^2) affect the seroprevalence of *L pneumophila*. Overall, the prevalence of *L pneumophila* assayed was not significantly ($p > 0.05$, χ^2) affected by co-morbidities.

Seroprevalencia de la *Legionella Pneumophila* en Pacientes con Neumonía en los Cuatro Hospitales Principales de Trinidad

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RESUMEN

Trinidad and Tobago es una isla del Caribe que posee una extensión de 4828 kilómetros cuadrados y una población de 1.3 millones. La neumonía es una de las principales causas de muerte en Trinidad. Este proyecto determinó la frecuencia de *Legionella pneumophila* en pacientes con neumonía, e investigó la relación entre la neumonía y los factores de riesgo seleccionados. Se recopilaron datos séricos y demográficos de 123 pacientes, diagnosticados con neumonía. Los sueros fueron sometidos a análisis a fin de determinar la presencia de *L. pneumophila* Ig G/M/A y Ig M. Todos los análisis se realizaron usando el paquete estadístico SPSS (Statistical Package for the Social Sciences). De un total de 123 muestras de suero analizadas, 39 (31.7%) dieron positivas frente a *L. pneumophila* Ig M/G/A, mientras que 2 (1.6%) dieron positivo frente a Ig M solamente. Los hospitales, el género y la etnicidad no afectaron significativamente la seroprevalencia de *L. pneumophila* ($p > 0.05$; χ^2). En general, la prevalencia de *L. pneumophila* ensayada no fue afectada significativamente por comorbidades ($p > 0.05$, χ^2).

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INTRODUCTION

Legionnaire's disease outbreaks have been identified from time to time but sporadic community acquired disease varies from 2.5–31% as reported by Pachon *et al* (1). In Spain, *Legionella* seroprevalence ranged from 6% (2, 3) to 12% (4) using IgG titres 1:128. For current infections, France had a

prevalence of 1% (5) from immunofluorescent assays (IFA) 1:256, which was similar to Japan with 1.6% (6) and Switzerland with 0.6% (7) also with IFA. However, higher values were seen in Thailand with 5.4% (8) and in the United States of America (USA) with 6.7% (9).

Legionella pneumophila is the major cause of Legionnaire's Disease, but other species have also been known to be aetiological agents of pneumonia, such as *L micdadei* in the Pittsburgh outbreak (10, 11). The *Legionella* bacterium is found in warm environments about 32–45°C and so can colonize humans. A potentially large reservoir is man-made water supplies where hot water is stored. These include spas, hot tubs, cooling towers and humidifiers. The bacteria are

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found on the biofilm present along the surface of the container and not freely swimming (12).

In the past few years, nosocomial cases have predominated and this trend is attributed to two factors: new and easy methods of identification *eg* urinary antigen assay, and the presence of the *Legionella* in hospital water supply (11).

To date, only a study reported by Hospedales *et al* (13) in 1996 on Legionnaire's Disease in Antigua exists, otherwise, there is little data available in Trinidad or the region with regards to this pathogen.

This study was done to determine the prevalence of *L pneumophila* in 123 pneumonia cases in four hospitals in Trinidad and Tobago.

MATERIALS AND METHODS

Case definition

The diagnosis of community-acquired pneumonia (CAP) requires the following to be present (8): new pulmonary infiltrate seen on a chest radiograph that was taken within 24 hours of presentation; the confirmatory clinical finding is presence of at least one of the major criteria, which include, cough, sputum production, temperature $>37.8^{\circ}\text{C}$ or at least two of the minor criteria: pleuritic chest pains, dyspnoea, altered mental status, pulmonary consolidation by physical examination, and white blood cell count of > 12000 cells/ μl . These published criteria were used by the clinicians and once they were met, blood samples were taken by the physicians.

Exclusion criteria

Patients of the following categories were excluded from the study: presence of tuberculosis, since this is highly contagious and therefore not allowed in the routine microbiological laboratory; presence of human immunodeficiency virus (HIV) infection, since the condition leaves patients susceptible to infections that members of an otherwise healthy population would not be subjected to (14); children < 5 years of age, since they cannot expel sputum voluntarily developed yet or admission from a nursing home or hospital to avoid possible nosocomial pneumonia.

Source of samples

The study commenced only after relevant authorizations were given by the hospitals involved as well as the Ethics Committee of the Faculty of Medical Sciences of the University of the West Indies, St Augustine. The sample population was 123 cases with pneumonia that presented to the five hospitals: Port-of-Spain General Hospital (POSGH), Eric Williams Medical Sciences Complex (EWMSC), San Fernando General Hospital (SFGH), and Sangre Grande Health Centre (SGHC). The samples were from patients between the ages of 5–70 years. Port-of-Spain General Hospital is located in the northwest region of the island, EWMSC is in the north and SGHC is northeast. The northern part of the country is more urban than the central and southern parts,

thus, a large population is served by these health facilities. San Fernando General Hospital is southwest and normally serves the south and central inhabitants. Sample collection was done between the months of October 2002 and October, 2003.

Administration of questionnaire

A questionnaire together with letter of consent was provided for participants in the study to complete. The questionnaire elicited demographic information (age, gender, ethnicity), history and current or past co-morbidities.

Collection of samples

Samples of serum were used for the serological tests. Blood samples were collected from patients diagnosed as having pneumonia by the attending physician. Approximately 4 ml of blood was collected from each patient and serum used for serological tests. The blood sample was drawn into a red-capped Vacutainer[®] tube, transported to the laboratory within two hours and centrifuged at 2000 rpm for 15 minutes. The serum was dispensed into three 1.5 ml Ependorf[®] vials and stored at -70°C until needed. Grossly haemolyzed, icteric or lipaemic samples were not used for the serological tests. Overall, 123 samples were collected.

Detection of *Legionella pneumophila* immunoglobulins in sera

This procedure was done using the Sigma Diagnostics[®] kit for *Legionella pneumophila* serotypes 1–7 Ig G/Ig M/Ig A (15). The EIA multi-well reader was set to read at 450 nm. Positive samples were found by using a cut-off optical density value. This value was obtained by multiplying the mean of the low positive values, found from the low positive controls provided, by the correction factor that is provided by the manufacturer. Then an optical density (OD) ratio was found for each sample by dividing the OD value by the cut-off OD. Negative samples were those with an OD ratio ≤ 0.90 . This indicated that IgG/M/A antibodies to *L pneumophila* were not detected. A non-reactive result may be equivalent to an immunofluorescent antibody (IFA) titre of $< 1:256$ (15). Positive samples were ≥ 1.10 and may be equivalent to an IFA titre of $\geq 1:256$. Equivocal samples, 0.91–1.09 were treated as negative samples. Specificity was 92.0% and sensitivity, 90.1%.

Detection of current infection for *L pneumophila* serotypes 1–7 through IgM antibodies was done using the diagnostic kit from Virion/Serion[®] (16). The EIA reader was set at 405 nm with reference of 655 nm. Positive samples were found reading the cut-off point from a standard graph provided by the manufacturer. Negative samples were those with an U/ml of < 120 . This indicated that IgM antibodies to *L pneumophila* were not detected. Positive samples were ≥ 140 and equivocal samples, 120–139, were treated as negative samples. Only samples with an OD ratio of more

than 140 U/ml were used, hence all equivocal samples were not considered. Specificity was 97.5% and sensitivity, 59.8%.

RESULTS

Thirty-nine (31.7%) of the 123 samples tested were positive for *L pneumophila* IgM, IgG and IgA. Two (1.6%) were positive for *L pneumophila* IgM only.

Table 1 shows that the differences in seroprevalence across hospitals were not statistically significant ($p = 0.457$;

Table 1: Seroprevalence of *L pneumophila* in pneumonia patients by source of samples and demography

| | No (%) of samples positive for <i>L pneumophila</i> | <i>p</i> -value |
|------------------|--|-----------------|
| Hospital | | |
| SFGH | 21 (32.3) | 0.46 |
| POSGH | 12 (38.7) | |
| SGHC | 6 (25.0) | |
| EWMSC | 0 (0.0) | |
| Gender | | |
| Male | 19 (30.2) | 0.43 |
| Female | 20 (33.3) | |
| Age | | |
| <21 | 5 (35.7) | 0.19 |
| 21–30 | 5 (41.7) | |
| 31–40 | 1 (11.1) | |
| 41–50 | 7 (38.9) | |
| 51–60 | 8 (25.8) | |
| 61–70 | 10 (50.0) | |
| >70 | 3 (15.8) | |
| Ethnicity | | |
| East Indian | 14 (24.1) | 0.13 |
| African | 17 (43.6) | |
| Other | 8 (30.8) | |

χ^2). Overall, for existing infections there was almost an equal distribution between the genders. Ten (50.0%) of 20 tested positive for *L pneumophila* in the 61–70 group. The prevalence of infections by *L pneumophila* among the ethnic groups was not statistically significant ($p = 0.321$; χ^2).

Table 2 shows underlying disease compared with exposure to bacteria. Regardless of the underlying diseases

Table 2: Seroprevalence of *L pneumophila* in pneumonia patients by co-morbidities

| | No (%) of samples positive for <i>L pneumophila</i> | <i>p</i> -value |
|---------------------|--|-----------------|
| Asthma | 3 (21.4) | 0.362 |
| COPD | 2 (100.0) | 0.099 |
| Diabetes mellitus | 9 (34.6) | 0.450 |
| Heart disease | 10 (38.5) | 0.274 |
| Hypertension | 15 (55.6) | 0.053 |
| Liver disease | 2 (100.0) | 0.099 |
| Renal insufficiency | 4 (50.0) | 0.221 |
| Smoking | 9 (25.0) | 0.175 |

Values < 0.05 are statistically significant.

such as asthma, chronic obstructive pulmonary disease (COPD), diabetes mellitus, heart disease, hypertension, liver disease and renal insufficiency, the prevalence of *L pneumophila* in pneumonia patients was not significantly affected ($p > 0.05$).

DISCUSSION

Seroprevalence of *L pneumophila* in the general population of the USA is 5–10% (17). Antibody detection showed exposure to *Legionella pneumophila* in 31.7% (39 of 123) of pneumonia patients in the sample studied. A positive result could have been due to any of these showing past exposure or current infection so there is a possibility of existing infection prior to the pneumonia experience. However, only two (1.6%) had current infections, which is comparable to findings in France of 1% (5) and to Japan with 1.6% (6).

In a study done on lower respiratory tract infections in Spain, 11% of the patients tested using IgG antibody detection had *Legionella* (18). The high seroprevalence of *L pneumophila* found in the present study sample may be explained in part, by the presence of *L pneumophila* in the environment, soil and water. In these situations, the microorganisms can multiply with the help of bacteria and protozoa found naturally in the environment by using them as a vehicle of reproduction (14). Furthermore, those that are able to survive for a long period in the environment tend to be more virulent (14). Thus, infection by these strains of *L pneumophila* leads to severe pneumonia requiring hospitalizations. Within the Caribbean, there were two cases of *Legionella* isolation from English tourists who stayed at a hotel in Antigua (13). After investigation, it was found that despite the fact that the chlorine levels in the cold water were satisfactory, the hotel's main supply and hot and cold-water distribution system contained *L pneumophila*. The concentrations were highest in the hot water system and in samples collected in rooms not used for days.

Although reports state that there are obvious regional differences in *L pneumophila* and, indeed, most bacterial pneumonia (19), there were no significant differences found for the hospitals and any of the pathogens. This was expected since Trinidad is relatively small (5 128 km² with a population of about 1.3 million including Tobago) and the population would not be isolated and thereby would not show differences according to geography. In fact, it is a common practice to send patients from one hospital to another when there are demands on space and equipment.

Although no significant difference was found between the genders, *L pneumophila* immunoglobulin was detected in 48.7% and 51.3% of male and female patients respectively. A study by El-Ebiary *et al* (20) reported finding a higher mortality in *L pneumophila* positive male patients than in females. These differences observed between the two sexes are consistent with animal and human studies (21), however, this study did not assess mortality rates hence cannot draw a comparison with these findings.

There were no significant differences in co-morbidities between patients infected with *L pneumophila* and those who were not. Although most *Legionella* infection is due to *L pneumophila* (11, 12, 14), both kits used quoted specificities of above 90% each (15, 16), which may reduce the probability of a false-positive result due to other species.

Limitations of the study included demographic data retrieval since this depended on a questionnaire, some information may not be accurate and also difficult to verify, for example, ethnicity. Also, the test kits may not detect early infections by *L pneumophila*, as detectable seroconversion may not have taken place yet.

In conclusion, *L pneumophila* infection is highly prevalent in the study sample but may be asymptomatic since only 1.6% of the pneumonia patients actually had current infection with the bacteria. Nevertheless, the high prevalence is an indication that this bacterium has the potential to pose a health hazard in the immunocompromised patients if not monitored.

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