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## International Journal of Biological & Medical Research

Journal homepage: [www.biomedscidirect.com](http://www.biomedscidirect.com)



### Original Article

## Clinical Prevalence of *Legionella*, Associated Risk & Clinical Features

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#### ARTICLE INFO

##### Keywords:

*Legionella* culture - legionellosis - pneumonia - water supply, BAL (Bronchoalveolar lavage) LRTI (Lower respiratory tract infections).

#### ABSTRACT

**Background & objectives:** *Legionella pneumophila* and other members of this genus are important respiratory pathogens but legionellosis often remains a neglected and under reported condition. Hence, this study was done to find out the presence of this organism in patients suffering with lower respiratory tract infection. **Methods:** A total of 2000 lower respiratory tract samples (sputum, BAL, Pleural fluid) were examined. Culture was done on buffered charcoal yeast extract agar with supplements and identification of the isolates was done by microscopy and biochemical tests. **Results:** *L.pneumophila* could be isolated from 32 (1.6%) patients suffering from lower respiratory tract infection, unassociated with other aetiological agents of bacterial pneumonia. associated risk factors co morbid illness also observed and *Legionella* can be effectively treated with macrolids. **Interpretation & conclusion:** Our study has shown that *Legionella* was the aetiological agent of lower respiratory tract infection in 1.6 per cent of patients. Co morbid illness and other clinical findings used to diagnose this disease. A larger study and reports from other parts of the country may help in determining the true significance of legionellosis in India.

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### 1. Introduction

*Legionella pneumophila* is the most frequent species isolated from adult and pediatric patients with either community or Hospital-acquired legionellosis. The severity of legionellosis varies from mild febrile illness (Pontiac fever) to a potentially fatal form of pneumonia (Legionnaires' disease) that can affect any one, but principally affects those who are susceptible due to age, underlying illness, immunosuppression and other risk factors such as sex, smoking, alcoholism etc. (1). Legionellosis is a well recognized problem in developing countries but since risk environments and susceptible populations are found world wide it is likely that the problem of *Legionella* infection is under- appreciated in developing countries like India, Srilanka, Taiwan etc.,(2).

True prevalence of *Legionella pneumophila* remains unclear because it remains unrecognized and empirical treatment for respiratory tract infection leading to recovery. Death rates are therefore difficult to assess. It's estimated that about 10% to 15% of patients with *Legionella pneumonia* die, with the higher mortality occurring in untreated nosocomial cases (3).

#### A Problem Pathogen?

*Legionella* can resist higher temperature and are able to multiply at temperatures between 25 and 43°C and survive in

temperatures of up to 55-60°C therefore, they thrive easily in natural and artificial hot water systems. It intracellularly multiplies in protozoa and thus cannot be affected by the water environment like temperature, water flora, water nutrients, water chemicals and water chlorination and its air born transmission is of at least one to as many as six kilometers. Normal chlorination methods cannot remove *Legionella* in water as it requires hyper-chlorination. This pathogen cannot grow on ordinary media and requires special medium (BCYE) which need to be supplemented with L-Cysteine. *Legionella* outbreaks are inevitably linked to neglected A/C cooling towers(4).

#### MATERIALS AND METHODS

##### Exclusion criteria

Patients of the following categories were excluded from the study: Presence of tuberculosis, presence of Human Immunodeficiency Virus Infection, Children below 5 years of age since they cannot expel sputum voluntarily, samples which have grown other respiratory pathogen on routine laboratory testing.

##### Inclusion criteria:

Patients who were not having (any underlying) infections such as tuberculosis, HIV,HBsAg and samples which did not grow any respiratory pathogen on routine laboratory culture, patients of 16-75 year of age group were included.

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## Collection of samples

### Clinical samples

Sputum and appropriate lower respiratory tract specimens (Pleural fluid and BAL) which were submitted to the laboratory for routine microbiological testing and which did not yield any bacterial respiratory pathogen on routine culture and which were negative for acid fast bacilli were processed to find out the etiologic role of *Legionella* spp. Overall, 2000 samples were collected and processed from inpatients and outpatients at Sri Venkateswara Institute of Medical Sciences hospital, Tirupati, Andhra Pradesh from March, 2006 to May, 2010.

### Preparation of specimens for the isolation of *Legionella*

Recovery in the presence of other bacterial species present in the sample can be improved by heating, usually at 50°C for 30 minutes, and by treating with acid. If using an acid treatment, an acid buffer of 0.2N HCl-KCl (pH 2.2) buffer should be used for five minutes and samples were neutralized with 1N KOH, although this may also inhibit the growth of legionellae (5).

Clinical samples were also treated with heat treatment by maintaining 2 ml of sample at 50°C for 30 minutes and then the samples were brought to the room temperature and then streaked on the GVPC selective BCYE medium and incubated at 37°C with higher humidity. Humidity was maintained by keeping a beaker of water inside the incubator. All cultures (incubated plates) were examined at 72 and 96 hrs (3rd and 4th day). If no colonies were observed, the plates were incubated for further 7 days. Microscopic examination was done with a dissecting microscope using the scanner objective (4X) to detect bacterial colonies resembling *Legionella*. Suspected colonies were aseptically picked and subcultured on blood agar plate and on selective BCYE agar plate and incubated for 24 hrs. If no growth was observed on blood agar after 48 hrs the blood agar plates were discarded only the BCYE streaked plates were further incubated (6).

### Processing the suspected colonies

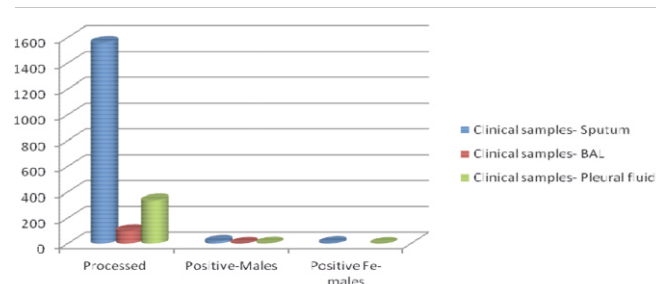
The suspected colonies were further processed by Gram staining and modified Gram staining. Modified Gram staining was done by using a carbol-fuchsin as counter-stain. If the bacteria were Gram-negative it appeared pink under the microscope. Oxidase test, Catalase test, & Hippurate hydrolysis tests also done to the suspected colonies to identify *legionella* from other contaminants. Medical records were referred to find out the patients' clinical profile (6).

## RESULTS & DISCUSSION

### Samples processed

Respiratory specimens from clinically suspected cases of LRTI which did not yield any respiratory pathogenic bacterial isolate on routine culture and were negative for AFB, were collected and processed, clinical samples included sputum, BAL and pleural fluid. In this study a total number of clinical samples collected were 2000 in which comprised of sputum 1498, BAL 164 and pleural fluid 338. Maximum isolation noted in this study was from sputum samples (1.68%), (Chart No.1).

Chart NO, 1: Samples Processed for Culture and Positivity



### Identification of the Organism

The clinical isolates were confirmed as *Legionella* by observing features like colonies which grew only on BCYE, but not on blood agar (as *Legionella* spp. requires cysteine for their growth) and colonies that were glistening, convex, circular margin when examined through a dissecting microscope, ageing colonies lost most of their iridescence and developed grayish white centers average number of colonies per plate was 14. Conventional and modified Grams staining was done to the colonies. Gram negative faintly stained pleomorphic rods were presumptively identified as belonging to *Legionella* spp, Medical records were referred to find out the clinical features and associated risk of legionellosis.

A total of 32 such isolates were obtained out of the 2000 respiratory samples were analyzed, which gives positive percentage of 1.6%. Isolates were further identified based on the biochemical tests. Oxidase, Catalase and Hippurate hydrolysis test were positive for all these 32 isolates which corresponds the species *Legionella pneumophila*.

Most of the clinical isolates were from males (75%) and >50 years of age. Modified Grams staining improved the visibility of *Legionella* cells under light microscope. The clinical samples that were treated with acid (KCl-HCl buffer pH-2.2) and heat (50°C/30min) were grown more *Legionella* colonies and samples which were not treated with acid and heat were grown heavy contaminants it was not possible to isolate *Legionella* from such plates. Among the risk factors alcohol and/or smoking habit were found in 10 patients. Ex-smokers were 3 (9%) (7) while 21.8% of the patients had diabetes mellitus, while 9% patients did not have any underlying risk factor.

Co-morbid illness like heart disease 4 (12.5%), asthma 4 (12.5%), renal disease 3 (9%), seizures 3 (9%) and COPD 05 (15.6%) were also seen. Diabetes mellitus 7 (21.8%) and end stage renal disease 3 (9%), were also noted as co-morbid illnesses in this study. Legionellosis can easily be confused with symptoms of flu and is clinically indistinguishable from other causes of pneumonia, so the diagnosis of Legionnaires' disease requires specific diagnostic tests. The World Health Organization observed almost two decades back that the morbidity and mortality due to sporadic and epidemic legionellosis was under-reported in most health statistics (13). Prognostic factors in legionellosis are not well described but delay in appropriate antibiotic therapy has been

associated with increased mortality. Other potential prognostic factors include: Delayed hospitalization, long duration of symptoms prior to ICU admission and hyponatremia (14).

A limited number of studies have focused on prognostic factors in *legionellosis* (15). Marston et al., (1994) found that the likelihood of death from *Legionella pneumonia* increased in patients who were elderly or male. Similarly we found that the two deaths occurred in male patients and there was no death among the females who were positive for *Legionella*. Similar observations were also made by Sanne Jespersen et al., (16). Four tests are predominantly used to detect the presence of *Legionella*; culture of specimens, direct fluorescent antibody test, urine antigen test, and serum antibody assay. Among these available tests, culture remains the gold standard for the diagnosis of *legionellosis*. In our study, *legionellae* were isolated from the respiratory tract samples(17). The clinical manifestations of Legionnaires' disease or the signs and symptoms are nonspecific and are similar to those of an atypical pneumonia, but more severe. Multiple systems may be affected in some patients. Liver and renal dysfunction have been noted. Hematologic disturbances like high and low platelet count, higher neutrophilic count, and higher ESR have been associated with progression of Legionnaires' disease. Other abnormalities included elevated serum levels of liver enzymes, and moderate elevation.

Serum levels of creatinine. Elevated ESR was noted in 93.7% among In order to evaluate the actual burden of *Legionella* in community acquired pneumonia, further studies are required. A better understanding of clinical presentation and prognostic factors for *legionellosis* may optimize our therapeutic approach and management of *legionellosis*, and thus reduce mortality and morbidity. The early recognition of infection due to *Legionella* plays a major role in its treatment and preventing mortality in patients with any underlying disease (5). In our study majority (43.7%) of the patients were treated with clarithromycin, 40.6% were treated with levofloxacin, and 15.6% were treated with azithromycin. According to Victor L. Yu (2004) levofloxacin is an effective drug against Legionnaires' disease (19). Macrolides have been used in many hospitals as the standard treatment of LD and these agents have been proved clinically effective even if used as a single agent therapy (20). Majority of patients (59%) in our study were treated with macrolids (clarithromycin & azithromycin)(20).

This study using two thousand patient population having community acquired lower respiratory tract infection is the first study from India where *Legionella* detection by culture method has been attempted in such a large number of subjects. The finding of *Legionella pneumonia* among 1.6% of the patients goes on to prove that this disease is not an exotic one if we look for it systematically. Worldwide prevalence varies from 1- 9% among CAP patients and it may be concluded at this juncture that disease rate is towards the lower side in this part of India. However unless and until similar large studies are initiated from other parts of our country, we may not be able to get the true scenario for India.

In conclusion it may be stated that this study has thrown considerable light on *Legionella* and Legionellosis which remain an almost unknown entity in the health care settings in this country. It

is imperative that the medical community in India becomes more aware of this condition and at least some simple test like urinary antigen detection may be initiated in the laboratories as part of the diagnosis of respiratory tract infection. The clinicians need to be aware of the management of Legionellosis particularly in hospital acquired pneumonias. This will go a long way in understanding the *Legionella* scenario.

## CONCLUSION

1. The present study was carried out from March 2006 to May 2010 Majority of the clinical isolates were from sputum (1.6%). But there was no drastic variation between the clinical samples (sputum, BAL, Pleural fluid)

2. modified Gram's staining improved the visibility.

3. Overall positivity for clinical isolates was 1.6%.

4. Among clinical features, fever was the most common finding in this study, followed by respiratory symptom like cough and dyspnoea and gastrointestinal symptoms like vomiting and diarrhoea. Chest pain was also a notable finding of this study among the patients.

5. Associated risk factors included advanced age, cigarettesmoking, alcohol consumption, chronic lung disease, and male sex.

6. Co-morbidities like diabetes mellitus, heart disease, asthma, end stage renal disease, seizures and COPD were also found in this study.

7. Multi-organ dysfunction was noted in 3% of the study subjects.

8. In our study, majority (84%) had some radiological abnormality, which is a common feature of *Legionella pneumonia*. Hence other confirmative methods like isolation and biochemical identification of *Legionella* are needed to diagnose Legionellosis.

9. Two deaths were noted in this study which gives significant mortality rate of 6% in this study.

10. Medical records of patients were shone these investigations 93.7% had raised ESR, 90% had higher WBC count, 18.7% had higher neutrophil count and variation in LFT was found in 75% of the patients in this study, Pleural effusion was noted in 37.5%, S1S2 variation was noted in 25%. High creatinine was noted in 12.5% indicating kidney dysfunction.

11. Acid treatment of the specimen yielded more number of *Legionella* colonies indicating acid buffer treatment is required to enhance the recovery of *Legionella*.

12. In this study majority (53%) of patients were treated with quinolones and 43.7% were treated with macrolides. One death was noted among macrolide treated individuals but no death was noted in quinolone treated patients.

This study may be considered as a wakeup call for clinicians and microbiologists of any hospital in this country. From the paucity of data from India, it can be safely inferred that so far no attention has been given to this organism or the disease.

**Acknowledgements:**

Authors thank the Rajiv Gandhi National Fellowship (RGNF) for providing financial support (JRF), and also thank the physicians in the department of medicine and the Emergency department for their co-operation, and acknowledge Dr Sophie Jarraud of the French National *Legionella* Reference Centre, Lyon, France, for providing the reference strain.

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