



Research Article

PREVALENCE OF *LEGIONELLA PNEUMOPHILA* AMONG LRTI PATIENTS AND ENVIRONMENTAL WATER SAMPLE FROM A TERTIARY CARE HOSPITAL

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Abstract- Legionella an important respiratory pathogen which is often neglected and under reported. It is known to be the second most common cause of Community acquired pneumonia. Hence, the present study was undertaken to know the prevalence of Legionella from hospital environmental water samples and from patients admitted with lower respiratory tract infection. Objective: To know the prevalence of *Legionella pneumophila* and to study about water as a source of Legionella in tertiary care hospital. Method: Out of 100 samples collected 82 were sputum samples & 9 were Broncho alveolar lavage, 5 was pleural fluid. The samples were processed according to standard protocol. 21 Water samples were collected from taps of various ICU's and wards. The samples were streaked on both legionella agar medium with supplement and BA without supplement and incubated at 37 C at 5 -10 %CO₂ for 3- 7 days presence of colonies on legionella agar medium alone were confirmed as legionella. Results: Out of 100 samples collected 96 (96%) samples were included in the study. Of 96 samples 82(85.4%) were sputum samples in which 12 (14.6%) grew legionella in medium with supplement. Out of 21 water samples collected from various wards and ICU's only 3 (14.3%) were positive for the growth of legionella. Conclusion: Our study emphasis the need for further studies involving multi centers throughout state. Our study also urges a need to fill the control gaps and Implement surveillance on environmental systems in Hospitals.

Keywords- Infection, Pathogen, Pneumonia

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Introduction

India is the Legionella an omnipresent bacterium seen in aquatic environment is now being recognized as a main cause for community acquired pneumonia (CAP) & Hospital acquired pneumonia (HAP) [1]. Legionella infection is an uncommon but important infection of the respiratory tract with significant mortality and morbidity rate. More than three decades ago it was recognized as a cause of Pneumonia [2]. The disease 'Legionnaires' was named after the place Legion where it was identified and reported first. Lack of appropriate assays are the main reason for the bias in identification of the bacterium [2]. Other countries like Australia, Scotland and New Zealand uses PCR to identify all the species of legionella cases [3]. The underlying cause associated with the morbidity of Legionnaires disease is its widespread occurrence emphasize the urgent need for the research in diagnosis the bacterium earlier and to prevent outbreak of pneumonia caused by this bacterium [4,5]. Legionella an important respiratory pathogen which is neglected & under reported [6]. Water is the source of both nosocomial & community acquired pneumonia [7]. Legionellosis can be diagnosed with recent modalities [8]. Very scarce data are available on legionella from developing countries. In many countries Legionnaires' disease are not recognized due to lack of defined surveillance and diagnostic systems. Since very less data are reported on this bacterium it is difficult to compare between the countries with the available data. Globally, the age and sex distribution of cases are similar between countries. The legionnaires' disease occurs more commonly among the elderly people with 74-91% of reported cases in above 50 years of age. Male preponderance was observed with 1.4-4.3 male for every female patient [9]. Legionnaires' disease shows a seasonal pattern, being high in summer till autumn. Many studies have correlated this increase to warm and wet weather conditions, and higher relative humidity in these seasons [10-14].

Whereas in Australia and New Zealand peak infections was observed with *L. longbeachae* which is linked with the compost widely used for gardening [11,13]. The detection of this organisms in water signifies risk factors for spread of legionella infection [4].

Predisposing Risk factors and transmission of Legionnaires disease

Many data's suggest the route of infection for the legionella is by inhalation of the aerosols or by aspirating the water containing Legionella to be the source of transmission of the infection. Till date no Person to person transmission was reported. Among the legionella species *L. longbeachae* is the only species which is linked to spread by compost used for gardening and person exposed to those compost pose a high risk of getting legionella infection [15, 16]. The other risks found are smoking, person working near water dripping plant pots, person not following proper hand washing after working in garden are at greater risk of getting legionella infection [17].

In some cases, legionella occurs sporadically requiring point source investigation emphasizing a burden on public health [18]. The outbreak of legionella has been associated with Hot and cold-water systems, cooling towers, whirlpool spas which generates aerosols are the potential source to transmit legionella [18-20]. In hospitals instruments, equipment's, contaminated with the water from cooling or hot water systems or aerosols are the source for nosocomial infections [21, 22]. Higher mortality rate of 8-12% was observed in elderly people with preexisting health conditions, smoking, Nosocomial cases was reported in many studies. In Europe 10% case fatality was reported in USA 8% were reported but if legionella occurs as Nosocomial infections the case fatality rate is very high of 15-34% [9,10].

Serogroup 1 is the predominant serogroup causing about 84% of human infection across the globe [3]. *Legionella pneumophila* are known to cause life threatening CAP. The infection usually acquired by the aerosol's inhalation. *Legionella* are known to cause both epidemic & sporadic infections though no reported causes of known epidemics was observed [8]. The pneumonia caused by *Legionella pneumophila* cannot be differentiated from other pneumonia causing bacterial infection. 5-30 % of legionnaires disease leads to death [5]. This study was undertaken to determine the prevalence of legionella from patients with pneumonia & also hospital environmental water samples was done to detect the risk factors of presence of legionella.

Materials and Methods

Study Design

The study will be conducted in department of microbiology in a tertiary care Hospital.

Study Period: 2 Months

Sample Size: 100 sample from patients with lower respiratory tract infection such as sputum, broncho alveolar lavage and pleural fluid were collected.

• 21 Water samples were collected from taps of various ICU's and wards.

• The study was approved by institutional ethical committee board.

The Inclusion Criteria

Patients with symptoms of LRTI.

Hospitalized patients with other respiratory conditions.

Exclusion Criteria

Patients with URI

Patients on prior treatment for LRTI will be excluded from the study.

Methods

Out of 100 samples collected 82 were sputum samples & 9 were Broncho alveolar lavage, 5 was pleural fluid. 4 sputum samples were rejected as samples had salivary contamination. So total of 96 samples were included in the study. The samples were processed according to standard protocol. All the samples obtained were processed and decontamination procedure for sputum were followed by plating the samples on to legionella agar base medium with legionella supplement containing L-cysteine to make the plates selective for the growth of legionella. The samples were inoculated on to BA without legionella supplement & both the plates were incubated with 5 - 10% CO₂ at 37°C for 3- 7 days after incubation the plates were looked for the presence of colonies. After 3 days of Incubation the plates were observed for the presence of tiny grey colonies on legionella agar base medium with supplement whereas BA showed no growth of the organisms. *Legionella* grows only in the presence of L-cysteine.

Processing of Water Sample

Out of 21 water samples were collected. Before Collection the tap tip was wiped with spirit & the nozzle of the tap was heated to avoid other contamination. The water sample collected are streaked on to the legionella agar medium with supplement & BA without supplement and plates were incubated with 5- 10% CO₂ at 37°C for 3- 7 days. After 3 days the presence of growth was observed in both plates. Only colonies grown on legionella agar medium were taken for further processing. *Legionella* are inert for all biochemicals weak catalase & oxidase were observed. On gram staining this gram-negative bacillus weakly stained are observed. These colonies were again streaked on both legionella agar medium with supplement and BA without supplement and incubated at 37°C at 5 -10 %CO₂ for 3- 7 days presence of colonies on legionella agar medium alone were confirmed as legionella.

Statistical Analysis

The data's collected were analyzed using SPSS software package version 20.0. chi square test was used to calculate b/w variables mean and standard deviation were calculated. The significance level of p value more than 0.05 (p <0.05) are taken as significant.

Observation and Results

Out of 100 samples collected 96 (96%) samples were included in the study out of 96 samples 82(85.4%) were sputum samples, 9(9.4%) were BAL, 5(5.20%) were pleural fluid. All the patients were in the age group of 20-70 years [Table-1] [Fig-1].

Table-1 Shows various samples collected from patients with LRI

SN	Samples	Number Collected (N=96)
1	Sputum	82 (85.4%)
2	Broncho Alveolar lavage	9 (9.4%)
3	Pleural fluid	5 (5.2%)

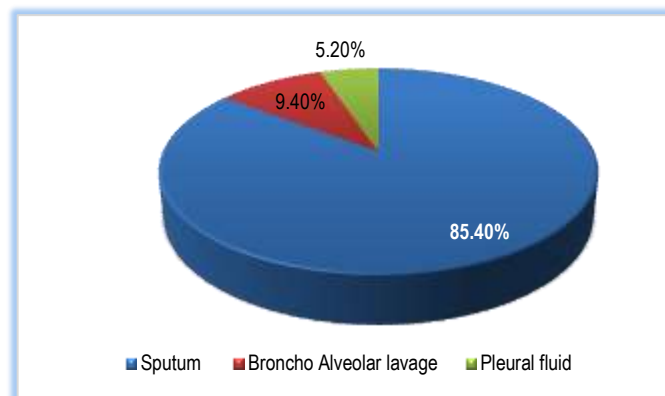


Fig-1 Shows various samples collected from patients with LRI

Table-2 Shows sex distribution among the patients

SN	Sexes	No (%)
1	Male	59 (61.5%)
2	Female	37 (38.5)

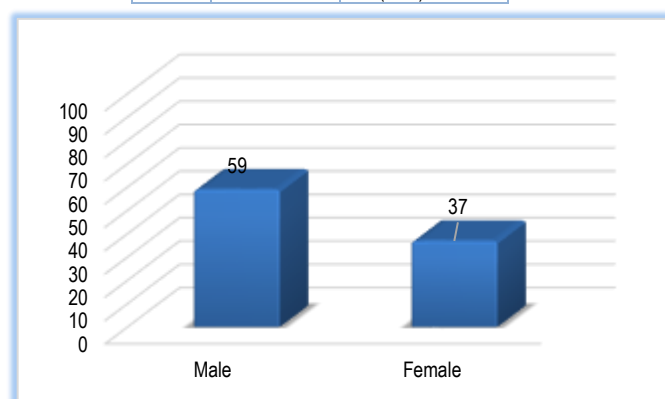


Fig-2 Shows sex distribution among the patients

Table-3 Shows Number of positive *Legionella* among various samples

SN	Samples	Number Positive (%)
1	Sputum (N=82)	12 (14.6%)
2	BAL (N=9)	0 (0%)
3	Pleural fluid (N=5)	0 (0%)

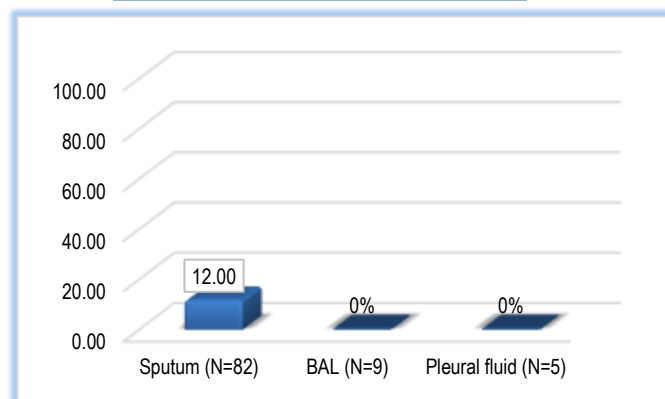


Fig-3 Shows Number of positive *Legionella* among various samples

Out of 96 patients 59 (61.5%) were male and 37 (38.5%) were female patients. The mean age of patients were 38 ± 6.2 years [Table-2] [Fig-2]. Out of 96 patients samples 9 (9.4%) were BAL fluid which showed no growth of legionella, similarly 5(5.20%) pleural fluid was negative for legionella [Table-3] [Fig-3].

Out of 82 sputum samples collected and processed 12 (14.6%) grew legionella medium with supplement [Table-3] [Fig-3]. Among 12 (14.6%) legionella 8 (66.7%) were male & 4(33.3%) were female patients with mean age of 45 ± 4.7 years [Table-4] [Fig-4]. Male preponderance was observed in our study. Only legionella was taken under consideration other pathogens were neglected in our study.

Table-4 Sex distribution among Positive Legionella cases

SN	Sexes	Number of Positive (N=12)
1.	Male	8 (66.7%)
2.	Female	4 (33.3%)

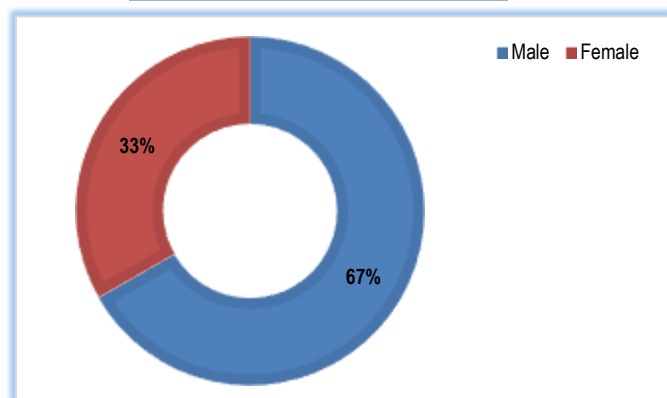


Fig-4 Sex distribution among Positive Legionella cases

Out of 21 water samples collected from various wards and ICU 's only 3 (14.3%) were positive for the growth of legionella. All the others showed no growth both in legionella medium with supplement & blood agar [Table-5].

Table-5 Shows places of water sample collection in the Hospital wards and ICUs

SN	Wards/ICUs (N=21)	No of Water sample positive for Legionella
1	Special Ward (N=4)	1
2	PICU (N=1)	0
3	RICU (N=1)	0
4	ICCU (N=1)	0
5	MICU (N=1)	1
6	Male Medical Ward (N=1)	0
7	TB & Chest Ward (N=4)	1
8	Medical Ward (N=4)	0
9	OG ward (N=2)	0
10	Female Medical ward (N=1)	0
11	Emergency ward (N=1)	0

Statistical analysis showed no significant correlation among the age and sex of patients. No level of significance was observed when age, sex and LRI infections were compared. This shows that legionella infection is seen in patients of all age group but predominant in age above 35 years and in patients suffering from LRI.

Discussion

In developing countries studies on legionella are scarce and rare. *Legionella pneumophila* serogroup 1 is the most common pathogen causing human infection among the legionella spp. If favourable condition arise other spp of legionella are known to cause infection by cellular invasion and intracellular proliferation [45]. The detection of legionella infection can be done in numerous ways with culture being more specific. But culture takes long time and technically difficult. So other test like Direct fluorescent antibody test (DFA) is rapid but it has very low sensitivity. Other tests like Radio immunoassay, ELISA and latex agglutination are done but these tests detect only serogroup 1 of *L. Pneumophila* with emergence of new molecular techniques diagnosis of legionella are made easy. Real time PCR are more rapid and sensitive method for detection [46]. In our study we have isolated 12 (14.6%) of Legionella. In a study by Kohler *et al* [47] reported six cases of legionella emphasizing the importance of Culture. This study correlates with our study with 12 (14.6%) isolation rate of Legionella from culture. From our study we

suggest that culture for Legionella must be done for patients with LRI, Pneumonia. Especially in Immunocompromised and immunosuppressed patients. Legionella may emerge as significant pathogen due to unrelated clinical practice where it was previously insignificant [48]. The Comparison of Patient isolates with water isolates can yield better observation which is the lacunae of this study. Since our study is a preliminary study on prevalence of legionella. Further research on serogroup identification will be done in future to identify the strains. In a study 28.03% of legionella growth was reported from water samples collected which is high compared to our study with only 14.3% isolation [46]. In another study they reported 78.70% of legionella from water samples which is higher than our isolation percentage [49]. In a research by Legnani *et al* [50] reported that isolation rate of legionella was high during June to October months which is similar to our study. Our study also conducted between the month of August to September.

In a preliminary study by Agarwal *et al* [51] 76% of environmental sample and 9% of patients with pneumonia were positive for Legionella this is contradictory to our study. In our study isolation percentage is 14.3% in environmental sample which is very less compared to their study whereas in patients sample we got 14.6% which is slightly higher than the reported data [51]. In our study most of the patients positive for legionella were in the mean age group 45 ± 4.7 years which is concordant with a study who reported *L. pneumophila* infection in >50 years of age [52]. In our study male preponderance was observed 8 (66.7%) of male were positive for legionella which is due to the predisposing factors like smoking, occupational exposure etc., which is similar to other studies reported by European center for disease prevention and control reports that predominant legionella infection was seen in male with 1.4-4.4 male for every female patients. Our study well correlates with this study [52-55]. Our study highlights the importance of environmental surveillance on routine basis and also emphasize laboratories to look for uncommon bacterium. With Greater focus on aetiology and early diagnosis can prevent infection caused by legionella. Also suggest that referral laboratories to be setup in every state to identify the pathogen.

Conclusion

From our study it is clear that very less data is available and less research done on legionella. Hence, there is an urgent need for introducing newer methods to identify these pathogens. Further studies involving multi center throughout state to know the prevalence rate in other geographical distributions. New Diagnostic modalities, Vaccines for Legionella should be focused to encounter these pathogen which can end up as a public health threat if left unnoticed. Our study urges a need to fill the control gaps on surveillance system on environmental systems in Hospital which need to be implemented immediately.

Application of Research: New Diagnostic modalities, Vaccines for Legionella should be focused to encounter these pathogens which can end up as a public health threat

Research Category: Medical Microbiology

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Study area / Sample Collection: Meenakshi Medical college Hospital and Research Institute, Enathur, Kanchipuram, 631552, India

Conflict of Interest: None declared

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Ethical Committee Approval Number: Nil

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