

# Isolation of Legionella from the Environment

Illuminado P. Livel, M.T.,\* Ofelia T. Monzon, M.D.,\* Mediadora C. Saniel, M.D.\* and Keizo Yamaguchi, M.D.\*\*

(\*The Department of Health Research Institute for Tropical Medicine, Alabang, Muntinlupa, Metro Manila; \*\*The 2<sup>nd</sup> Department of Internal Medicine, Nagasaki University Hospital, Nagasaki, Japan)

No Abstract Available [*Phil J Microbiol Infect Dis* 1988; 17(2):37-40]

*Key Words:* Legionella pneumophila, Legionnaire's disease, legionellosis, environmental surveillance

## INTRODUCTION

The genus Legionella, family Legionellaceae, consists of mesophilic, fastidious, gram negative rod-shaped or filamentous bacilli.<sup>1</sup> This organism was first recognized in 1977, following an outbreak of pneumonia during the 1976 American Legion State Convention in Philadelphia. Investigation of the lung tissue from the four fatal cases yielded a previously unidentified gram-negative, organism initially named Legionnaires' disease bacterium.<sup>2</sup> Previously unidentified gram-negative bacteria were subsequently reinvestigated, and were shown to have the same characteristics as Legionnaires' disease bacterium. In November 1978, the name *Legionella pneumophila* was introduced at the first International Symposium on Legionnaires' Disease in Atlanta, Georgia. Table 1 shows the 23 species of Legionella known as of 1987.

Legionella species are unique from other bacteria in three ways; First, the presence of DNA, second, the presence of cellular fatty acid, and third, their dependence for growth on L-cysteine on primary isolation. Studies have shown that Legionella infection is an important, cause of adult pneumonia. Despite the many new species of legionellaceae identified, the organism largely responsible for human disease is still *Legionella pneumophila* serogroup.<sup>1</sup> Persons particularly at risk include smokers, alcoholics, diabetics, those with chronic illness or those receiving corticosteroids. Inasmuch as Legionnaires' disease can occur in endemic form, it is important that clinicians should be aware of this disease especially since the common antimicrobial agents in use for pneumonia are not effective against legionellosis. The prompt administration of erythromycin for this disease often leads to cure.

This paper describes the results of attempts to isolate Legionella from the environment by the Research Institute for Tropical Medicine (RITM).

**Table 1. Legionella Species**

L. pneumophila	L. saintelensis
L. Micdadei	L. anisa
L. bozemanii	L. steigerwaltii
L. dumoffii	L. parisiensis
L. longbeachae	L. spiritensis
L. gormanii	L. hackeliae
L. Jordanis	L. maceachernii
L. wadsworthii	L. Jamestowniensis
L. oakridgensis	L. cherrii
L. feelei	L. rubrilucens
L. santicrucis	L. erythra
L. israelensis	

## MATERIALS AND METHODS

### Materials

The environmental samples used in this study were obtained from the cooling towers of various hospitals and hotels in Metro Manila. Four hundred (400) ml of water samples were collected in sterile screw-capped containers and brought directly to RITM. Table 2 shows the number of samples collected from various sources. Three water samples from the supply towers of three hospitals were also tested. In addition, a sample from the showerhead of one hospital was collected.

**Table 2. Source of Water Samples**

Place	Cooling Tower	Water Supply Tower
Hotel 1	Sample 1 Sample 2	
Hotel 2	Sample 1 Sample 2	
Hospital 1	Sample 1	
Hospital 2	Sample 1	
Hospital 3	Sample 1	
Hospital 4	Sample 1 Sample 2 Sample 3	
Hospital 5	Sample 1 Sample 2	Sample 1
Hospital 6		Sample 1
Hospital 7		Sample 1

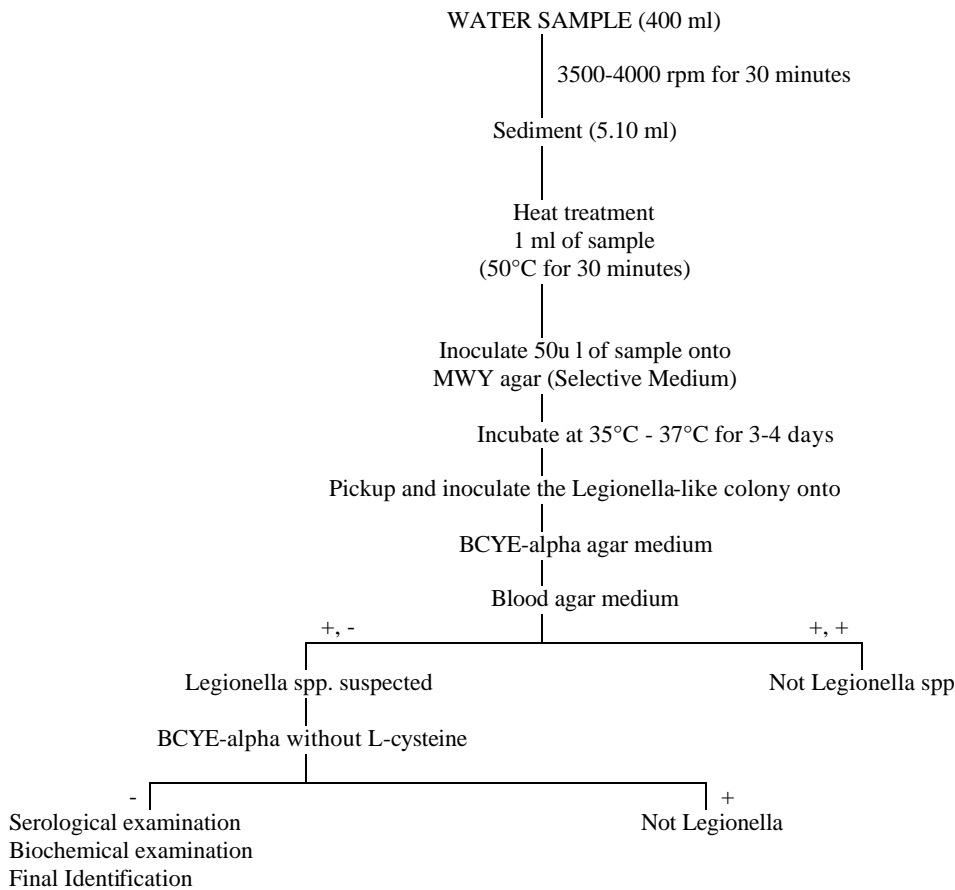
### Methods

The heat treatment technique was used to isolate *Legionella* from the environmental specimens in this study. This procedure is quite simple, inexpensive and does not require sophisticated equipment. The media used included MWY agar medium, BCYE-alpha medium and blood agar medium. Both MWY and BCYE-alpha medium contain L-cysteine and ferric pyrophosphate.<sup>5</sup> Cysteine is the amino acid required in the growth metabolism of *Legionella pneumophila*.<sup>6</sup> MWY agar medium contains glycine, which enhances the growth of environmental legionella organisms. It also contains antibiotics, which inhibit the growth of other bacteria.<sup>7</sup> Deviation from pH 6.9  $\pm$  .05 will compromise the growth of legionella from this medium.

Water samples were centrifuged at 4000 rpm for 30 minutes. The supernate was discarded and 10 ml of the sediment were taken. In discarding the supernatant portion, care should be exercised so as not to disturb the sediment. One (1) ml of the sediment was subjected to heat treatment at 50°C for 30 minutes. The remaining 9 ml were stored at 20°C for possible use later. Fifty microliters of the heated water samples were inoculated onto MWY agar medium and incubated at 35°C under humidified air for 3-4 days. Suspicious growth from MWY medium were counted and then inoculated into BCYE-alpha agar medium and blood agar plate and incubated at 35°C-37°C for another 3-4 days. The presence of growth on BCYE-alpha medium and absence of growth on blood agar plate suggested legionella species. Suspected colonies ranged in size from 1-4 mm and appeared glistening, convex and speckled.<sup>8</sup>

Figure 1 shows the schema used in isolating legionella species. For identification purposes, the following antisera and biochemical tests were used: *L. pneumophila* serotypes 1,2,3,4,5, and 6, *L. dumoffii*, *L. bozemanii*, *L. gormanii*, *L. micdadei*; and oxidase, catalase, gelatin, b-lactamase, and hippurate hydrolysis. Isolates suspected to be legionella species were

emulsified into .05 ml of the antisera. Any agglutination occurring within 1 minute was considered legionella positive to that particular species.



**Figure 1. Schematic Diagram of Procedure in the Isolation of Legionella**

## RESULTS

Ten out of 16 samples or 62.5% were found to be positive for legionella. These samples were collected from both hotels and hospitals (Table 3-5). The most frequent serotype isolated was *L. pneumophila* 1. Between 1-60 colony-forming units (CFU)/ml water were noted. The isolation of legionella specie was not uniformly observed in all tested samples from any particular test site and the sources of the tested water samples were located in geographically separated districts in Metro Manila.

## DISCUSSION

Legionella organisms are gram-negative bacteria that are widely present in the environment and appear to be able to survive for varying periods at temperatures ranging from 15°C or higher. Epidemic legionellosis can result from exposure of susceptible individuals to aerosols from environmental water containing Legionella. Environmental sources that have been implicated in outbreaks include domestic hot water system in large buildings, cooling water system used for air conditioning purposes, cooling water system used for industrial purposes, spas/whirlpools, industrial coolant used for grinding/machine lubrication (88099 percent water and 1-12 percent oil), and respiratory therapy materials.<sup>3</sup>

**Table 3. Legionella Isolates from the Cooling Tower Water Samples**

Place	Sample No.	CFU/ml	Serotype
Hotel 1	1	3	L. pneumophila 1
	2	13	L. pneumophila 1
Hotel 2	1	0	
	2	1	L. pneumophila 1
Hospital 1	1	7	L. pneumophila 3
Hospital 2	1	0	
Hospital 3	1	60	L. pneumophila 1
Hospital 4	1	4	Not determined
	2	0	
	3	0	
Hospital 5	1	1	L. pneumophila 1
	2	4	L. pneumophila 1

**Table 4. Legionella Isolated from Supply Tower Water Samples**

Place	Sample No	CFU/ml	Serotype
Hospital 5	1	0	
Hospital 6	1	0	
Hospital 7	1	1	L. pneumophila 1

**Table 5. Legionella Species Isolated from the Shower Head**

Place	Sample No.	CFU/ml	Serotype
Hospital 5	1	1	L. pneumophila 6

Decontamination measures used to eliminate Legionella from contaminated water supply systems include continuous hyperchlorination of the water system, intermittent flushing with hot (70°C) systems and by increasing the temperature of hot water storage from 45°C to 65°C.

Although testing of potable water systems or other sources is not routinely performed because of its wide spread distribution, no local studies demonstrating the presence of Legionellaceae in the Philippines have been reported. This report documents the ubiquitous nature of these organisms and its apparent presence in several areas in Metro Manila. Thus, legionellae should also be considered in the etiology of pneumonia among patients seen locally. Antimicrobial agents commonly used for the treatment of pneumonias do not generally include erythromycin, the drug of choice for this organism. Furthermore, studies need to be done to demonstrate clinical cases of legionella infection in the Philippines.

## CONCLUSION

Using the heat treatment technique, *Legionella pneumophila* was isolated from several environmental water samples in Metro Manila. The presence of legionellae in the environment demonstrates the need to investigate the role this organism plays in clinical disease in the Philippines. Because of these findings, RITM has created a Task Force Committee with the objectives of monitoring the occurrence of sporadic cases and/or outbreaks of legionella infection in Metro Manila and conducting further environmental investigation. The results of such studies will establish the need for guidelines for the prevention and control of legionella infection.

## Acknowledgements

Sincere thanks and appreciation are extended to the following: Dr. Kazunoli Tomono, and Dr. Kazuhiro Tateda of the 2nd Department of Internal Medicine, Nagasaki University Hospital, for their valuable help and

suggestions; the staff of the Research Institute for Tropical Medicine Bacteriology Section and Rally Calve for help in the collection and processing of the water samples.

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