



FIRST REPORT OF *LEGIONELLA PNEUMOPHILA* SEROGROUP 1 ISOLATE FROM PUBLIC-SUPPLY WATER IN BANGLADESH

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ABSTRACT

Legionella pneumophila infection is generally recognized to be transmitted from aquatic sources to human. In Bangladesh, although suspected cases of legionellosis were reported, epidemiology of *Legionella* infection as well as water resource contamination by *Legionella* spp. was almost unknown. In the current study, we screened water samples from Dhaka, Bangladesh, for *Legionella* spp. One *L. pneumophila* isolate was recovered from tap water of a residential hotel. This isolate was identified as serogroup 1 by serum agglutination reaction, and sequence type 1 (ST1) by sequence based typing. Analysis of lipopolysaccharide synthesis cluster genes by PCR revealed *lag-1* gene was not detected in this isolate, and its predicted monoclonal antibody subgroup was OLDA/Oxford. This is the first report of isolation and characterization of *L. pneumophila* from aquatic environment in Bangladesh.

Keywords: *Legionella pneumophila*, Public-supply water, Environment, Cooling-tower, Public-health, Bangladesh.

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Contribution/ Originality

This study documents, as first, the existence of *Legionella* spp. in water in Bangladesh that contributes to understanding possible sources of contamination of aquatic environment by rapid urbanization in this territory. The objective of the study is to guide to develop an effective infection control policy in this country.

1. INTRODUCTION

Legionellosis, acquired by inhalation or aspiration of *Legionella* spp., represents a range of infections from flu like syndrome to fatal pneumonia. This bacterium is known to be present in natural environment and artificial aquatic sources. Especially, artificial aquatic sources including potable water, cooling towers, fountains, storage tanks, and tap waters are important sources for both nosocomial and community-acquired *Legionella* infections [1]. Since contamination of the aquatic environment by this bacterium could lead to outbreaks of *Legionella* infections, surveillance study of *Legionella* microbes in water resources is important for controlling legionellosis.

Bangladesh is a tropical Asian country with rapid urbanization including cooling tower construction where pneumonia is one of the leading causes of death in children and adults. Although suspected cases of legionellosis in Bangladesh children were reported, the source of this infection was almost unknown there [2]. Recent research revealed that *Legionella* spp. is a causative agent of respiratory tract infection in Singapore, Thailand and India, which are also tropical monsoon countries in Asia [3-6]. Moreover, no data regarding *Legionella* contamination in

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Bangladesh water were available. Accordingly, we screened environmental and public- supply water samples from central Dhaka, the capital of Bangladesh.

A total of 12 environmental and public-supply water samples were collected on July 16-17, 2012 (Figure 1). As of environmental sampling, the longest lake (about 4.5 Km) passing through the middle of Dhaka city, which receives waste water from the surrounding residents and commercial buildings, was selected. Four water samples those collected from this lake, at i) an opening of a pipe-line carrying waste water to the lake at Banani area, ii) at the edge of lake adjacent to the pipe-line opening, iii) at the middle of lake (approx. 50 m away from lake edge), iv) at the edge of the lake around 1 km away from the 2nd sampling point adjacent to a slum and market places where direct contact of human and animal to the lake water was evident, were screened. A fifth, iv) environmental water sample was collected from an artificial fountain in central Dhaka. Three samples (1~3) of supply or drinking water were collected from a dental clinic, nearest to the lake. Two samples (4 and 5), each from supply or drinking water, were collected from intensive care unit of a teaching hospital at central Dhaka. Two supply water samples were collected from a residential hotel (6) and from a mosque (7). In all cases, fifty milliliters of each sample was collected in disposable syringe and was filtered through sterile 0.45µm-pore-size hydrophilic PTFE plain membrane filters (Millipore). The membranes were cut into small pieces and were suspended in 3 ml of distilled water in sterile tubes. Tubes were shaken and kept for 5 min at room temperature. One milliliter water from each tube was aliquoted into 1.5 ml sterile microcentrifuge tube and was centrifuged at 8000 rpm for 20 min. Supernatants were discarded leaving 50 µl of concentrated precipitate. Fifteen microliters of 0.2M KCl-HCl (pH 2.0) was added in each tube, and was incubated for 10 min at room temperature. Thereafter, one hundred microliters of suspension from each tube was spread over the selective agar media of Wadowsky-Yee-Okuda (WYO). The agar plates were incubated at 37°C and were monitored for 1 week.

One out of seven (14.3%) public-supply water, namely tap water from an air-conditioned residential hotel in Dhaka, yielded colonies with typical appearance of *Legionella* spp. As anticipated, transfer of single colony from WYO plate to blood agar media yielded no growth despite incubation at 37°C for several days. This isolate (TUM13563) was identified as *L. pneumophila* by further molecular techniques, namely positive PCR amplification by using *Legionella*-specific primers and nucleotide sequences of 16S rRNA identical to the reference sequence of *L. pneumophila* (GenBank/EMBL/DDBJ Accession No. FQ958210) [7]. Meanwhile, no natural environmental water samples yielded colony growth on WYO media.

We further performed molecular characterization of the isolate TUM13563. Agglutination reaction using *L. pneumophila* antisera (Denka Seiken Co. Ltd. Tokyo, Japan) revealed it belonged to serogroup-1 (SG1). Sequence-based typing according to the European Working Group for *Legionella* Infections revealed it as sequence type 1 (ST1) [8]. Analysis of lipopolysaccharide synthesis cluster genes by PCR revealed *lag-1* gene was not detected in this isolate, and its predicted monoclonal antibody (mAb) subgroup was OLDA/Oxford (Table 1) [9].

To our knowledge, this is the first report of isolation of *L. pneumophila* from aquatic environment in Bangladesh. This seems concordant with the presence of *Legionella* spp. in water sources from neighboring tropical Asian countries, although their genotypes were not sufficiently documented [3, 4, 6].

Clearly, *L. pneumophila* SG1 is the most common and important among clinical isolates of legionellosis [10]. In addition, reactivity to a specific monoclonal antibody (mAb) referred as mAb2 (or also called as mAb3/1), typically due to the presence of *lag-1* gene, is known as a virulence marker for legionellosis [11]. Meanwhile, ST1 lineage without reactivity to mAb2 (presumably lacking *lag-1*), like the isolate in the current study, was reported to be prevalent among *L. pneumophila* SG1 isolates from environmental samples, typically from artificial water distribution systems including cooling tower [10, 12-14]. Although pathogenicity of mAb2-negative ST1 lineage remains controversial, this subgroup was reported as a substantial cause of sporadic legionellosis [10, 13, 14]. This

subgroup was also reported as a cause of outbreak and of a fatal nosocomial infection [13, 15]. Although whether this environmental isolate leads to clinical legionellosis remains unclear, the current research provided, partly at least, a clue to explain largely unknown epidemiology of respiratory tract infection among Bangladesh community.

This study has several limitations. One is the small size and number of water sampling leading to potential underestimation of the prevalence of this microorganism. Moreover, we are unable to further discuss the relationship between this environmental isolate and clinical setting due to the lack of the matched clinical isolates. Despite of these limitations, first isolation of possible pathogenic bacteria within a small number of water samples has a great importance to lead the public health authority in Bangladesh for appropriate management of water reservation-distribution systems.

In conclusion, water in Bangladesh is associated with risk of *Legionella* contamination and urbanization including cooling systems may be one of the major contributory factors behind this contamination. This is worth to conduct further epidemiological study with large number of water sample to confirm the prevalence of *Legionella* spp. in Bangladesh aquatic environment.

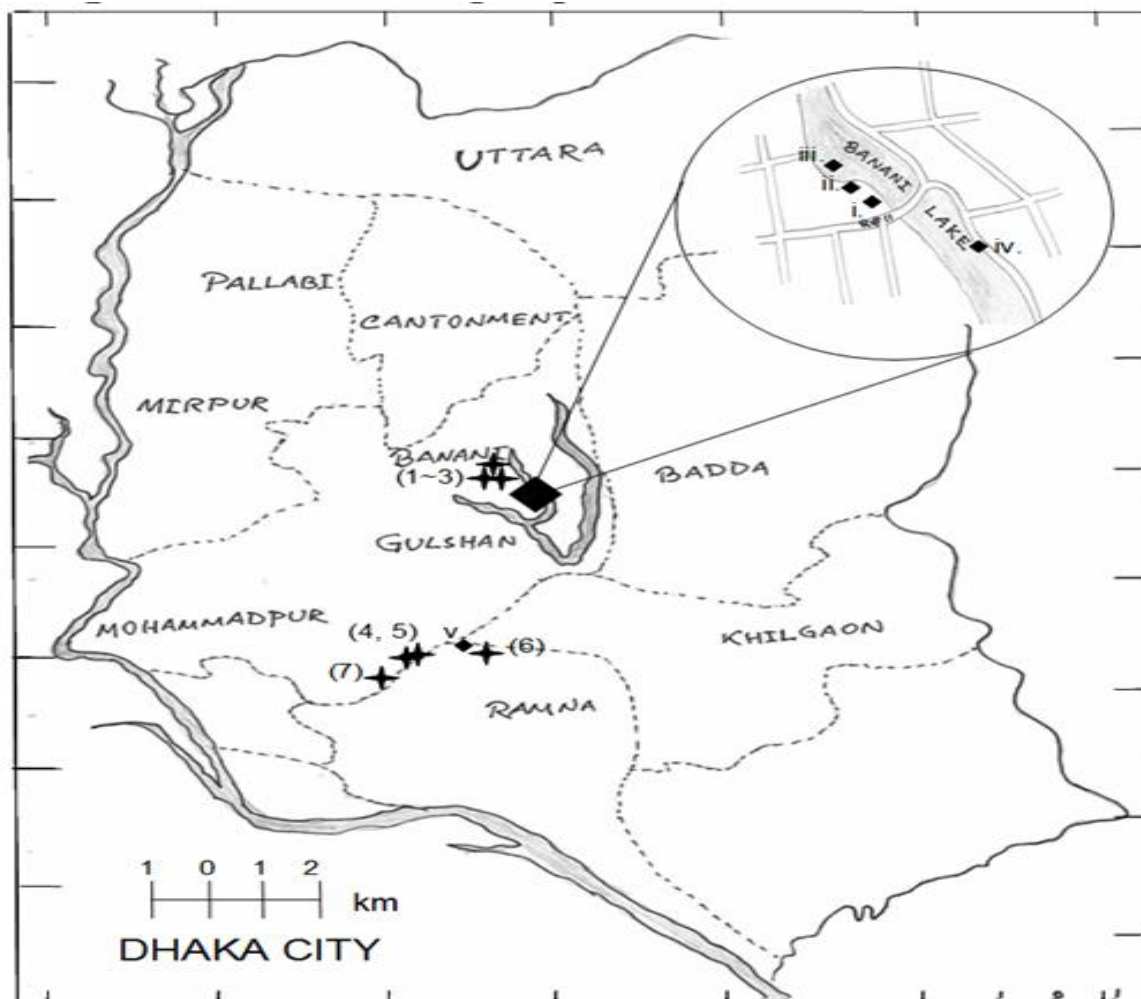


Figure-1. Water Sampling Locations

- ◆ represents environmental water collection points,
- ✦ represents public-supply water collection points in Dhaka.

Table-1. PCR analysis of lipopolysaccharide synthesis gene cluster of *L. pneumophila* Serogrupe 1 strain TUM13563, an isolate from tap water of residential hotel in Dhaka, Bangladesh

PCR results					
subgroup		Genetic		mAb	
<i>Lag-1</i>		ORF		Intergenic region	
All	-	ORF 9	+	ORF 7-9	-
Philadelphia	-	ORF 11	+	ORF 6-8	-
Knoxville	-				OLD/ Oxford
Allentown	-				

mAb; monoclonal antibody, ORF; open reading frame

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