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Environmental surveillance of *Legionella pneumophila* in distal water supplies of a hospital for early identification & prevention of hospital-acquired legionellosis

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***Background & objectives*: *Legionella pneumophila*, a ubiquitous aquatic organism is found to be associated with the development of the community as well as hospital-acquired pneumonia. Diagnosing *Legionella* infection is difficult unless supplemented with, diagnostic laboratory testing and established evidence for its presence in the hospital environment. Hence, the present study was undertaken to screen the hospital water supplies for the presence of *L. pneumophila* to show its presence in the hospital environment further facilitating early diagnosis and prevention of hospital-acquired legionellosis.**

***Methods*: Water samples and swabs from the inner side of the same water taps were collected from 30 distal water outlets present in patient care areas of a tertiary care hospital. The filtrate obtained from water samples as well as swabs were inoculated directly and after acid buffer treatment on plain and selective (with polymyxin B, cycloheximide and vancomycin) buffered charcoal yeast extract medium. The colonies grown were identified using standard methods and confirmed for *L. pneumophila* by latex agglutination test.**

***Results*: About 6.66 per cent (2/30) distal water outlets sampled were found to be contaminated with *L. pneumophila* serotype 2-15. Isolation was better with swabs compared to water samples.**

***Interpretation & conclusions*: The study showed the presence of *L. pneumophila* colonization of hospital water outlets at low levels. Periodic water sampling and active clinical surveillance in positive areas may be done to substantiate the evidence, to confirm or reject its role as a potential nosocomial pathogen in hospital environment.**

Key words Environmental surveillance - hospital-acquired pneumonia - hospital water outlets - *Legionella pneumophila*

Legionella pneumophila is a ubiquitous bacterium commonly found in various natural and human-made aquatic environments. They can enter and multiply in hospital water systems in low or undetectable numbers and may result in the acquisition of infection by patients through aspiration of contaminated water or direct inhalation of aerosols. Water outlets, faucets, showers, humidifiers, respiratory devices and nebulizers that have been filled or cleaned with tap water have been reported as potential sources of infection in several cases[1].

Establishing diagnosis of *Legionella* infection considering clinical criteria alone is difficult and warrants some index of suspicion and laboratory diagnosis[2]. This index of suspicion can be raised by generating knowledge about the possible presence of the organism in the hospital water supplies and outlets[3]. *Legionella* colonization of hospital water supplies in >30 per cent of the sampled water outlets merits initiation of specialized laboratory tests for *Legionella* screening in all patients with hospital-acquired pneumonia[3]. There are only a few studies on environmental colonization by *Legionella* from India[4-7]. Considering this, the present study was planned to identify potential environmental sources of *Legionella* species in a tertiary care hospital so that necessary interventions related to early diagnosis and prevention of hospital-acquired legionellosis can be initiated.

Material & Methods

The study was conducted in the department of Microbiology of All India Institute of Medical Sciences, Raipur, a tertiary care hospital in central India over a period of two months from July to August 2016. The study protocol was approved by the Institutional Ethics Committee.

Thirty water outlets in various patient care areas of the hospital were sampled. From each outlet, water samples and swabs from the inner portions of the outlet pipe were collected amounting for a total of 60 samples. Swabs were collected before water samples from the same source.

The samples were processed as per the protocol[8] given by Centers for Disease Control (CDC), Atlanta. To describe briefly, filtrate of water samples and swabs were inoculated after acid buffer treatment on buffered charcoal yeast extract (BCYE) agar with and without antibiotics (polymixin B, cycloheximide, vancomycin). Cultures were examined at intervals until 14 days of incubation. Colonies of *Legionella* were identified as per the standard protocols[8]. Faintly stained Gram-negative pleomorphic bacilli (Figure) which failed to grow on blood agar or L- cysteine-free agar on subculture were suspected for *Legionella* species. The identification was further confirmed by latex agglutination against *L. pneumophila* antisera 1 and 2-15 by commercially available kit (LK04-HiLegionella Latex Kit; Himedia, Mumbai, India).

The isolates which were suspected to be *Legionella* but failed to agglutinate by any of the antiserum were subjected to identification by matrix-associated laser desorption ionization-time of flight (MALDI-TOF) at Post-graduate Institute of Medical Education and Research (PGIMER), Chandigarh.

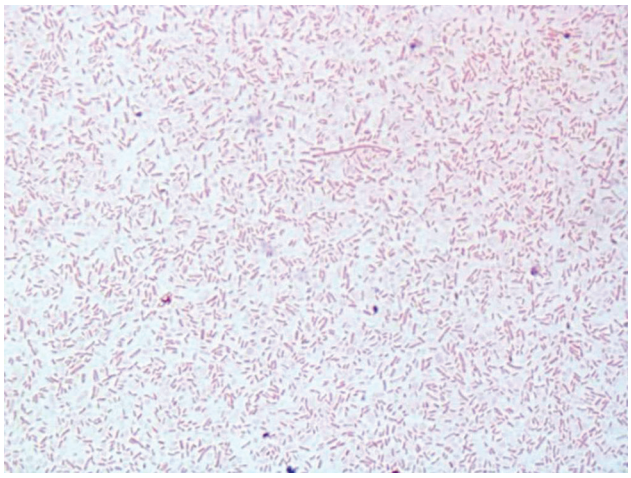

Figure. Gram-stained morphology of the isolate identified as *Legionella pneumophila* the figure shows pleomorphic Gram-negative rods after Gram staining using safranine as a decolourizer for prolonged time (10 min) (×1000).

Statistical analysis: Results were calculated in terms of percentage positivity of the water outlets tested positive for *Legionella*. The proportions were compared using Fisher's exact test.

Results & Discussion

Samples including one water and four swabs, each collected from five (16.6%) different sites (Table), grew colonies suspected of *Legionella* species. However, only two (both from swabs) of these could be confirmed as *L. pneumophila* serotype 2-15 giving percentage positivity of 6.66 per cent (2/30). None of the isolates was identified as *L. pneumophila* serotype 1. The remaining isolates tested by MALDI-TOF were identified as *Reyranella massiliensis.* The swabs yielded more number of organisms as compared to water samples.

Selective BCYE alone could inhibit the growth of environmental bacteria other than *Legionella* in 53.33 per cent (16/30) of water samples and 30 per cent (9/30) of swabs. Acid buffer treatment was able to limit the number of organisms up to 30 per cent (9/30) on plain BCYE agar (P<0.001). It was useful to limit the growth of organisms in 70 per cent (21/30) samples on BCYE with added antibiotics (P<0.001). Acid buffer treatment resulted in further growth inhibition of the *L. pneumophila* isolates.

Table. Hospital water outlets which were suspected for the presence of *Legionella* species

Sl. No.	Name of the site	Type of water source	Nature of sample	Organism isolated and identified as
1	Pre-operative room	Sink tap (number II)	Swab	*Legionella pneumophila* serotype 2-15
2	NICU	Sink tap	Swab	*Legionella pneumophila* serotype 2-15
3	Male medical ward	Sink tap	Swab	Non-*Legionella* species
4	Male medical ward	Water cooler	Swab	Non-*Legionella* species
5	Trauma and emergency ward	Water cooler (number II)	Water sample	Non-*Legionella* species

NICU, neonatal intensive care unit

Routine environmental surveillance for *L. pneumophila* has been a matter of debate over the years. The CDC does not support routine environmental culture for *Legionella* species in the absence of recognized disease, with the exception of transplant unit, because of the supposedly ill-defined relationship between the presence of the organism in the water system and risk of acquiring the infection[9]. However, this approach was countered by different prospective studies across the world in which cases of hospital-acquired legionellosis were discovered subsequent to the identification of *Legionella* colonization of the hospital water supply[10-17].

The present study reported positivity of 6.66 per cent for *L. pneumophila* which was less as compared to the previously reported positivity of 76 and 33 per cent from Indian hospitals[4,7] as well as in the western literature 38 per cent (range 5-83%)[2], 37[18] and 63 per cent[19]. Some of the studies have reported positivity rate of <30 per cent (12-18.7%)[20,21] or even zero[6]. The positivity cut-off of >30 per cent has been used and confirmed by many researchers to indicate the extent of colonization by *L. pneumophila* in any hospital warranting active clinical surveillance of legionnaire's disease[3,11,21]. Colonization in only 6.66 per cent of water outlets obtained in our study does not warrant active screening for the legionnaires' disease in the hospitalized patients. On the contrary of highest reported serotype 1 of *L. pneumophila*, we isolated serogroup 2-15 in our study that has been equally reported worldwide[2,18,19].

Sampling directly the biofilms using swabs was considered better compared to water in increasing the yield of isolation[3,4]. Acid buffer treatment of samples was effective in reducing the contamination; although, it was found to be detrimental to the growth of *L. pneumophila.* Hence, sampling of biofilms without acid buffer treatment, streaked on selective BCYE agar may be considered as the most sensitive method for recovering *Legionella*.

Quantification of environmental samples was tried on water samples by measuring the number of colony forming unit/ml of water cultured as doing so may provide information crucial for assessing the risk of transmission and identifying impending outbreaks. However, it did not prove much useful in our study because on most of the occasions, water culture grew confluent growth of mixed bacteria, making it very difficult to quantitate the slowly growing *Legionellae*. Another limitation of our study was that we did not include hospital-acquired pneumonia cases. Therefore, it was not possible to establish an association between the isolation of *Legionellae* with hospital-acquired pneumonia.

The extent of *Legionella* colonization in hospital water outlets was found to be very less precluding the necessity of incorporating specialized *Legionella* diagnostics in the routine workup of patients with hospital-acquired respiratory infections. However, repeated periodic environmental sampling and active case finding for hospital-acquired legionellosis in areas with positive reports need to be undertaken to confirm or reject its role as a potential nosocomial pathogen in hospital environments.

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***Conflicts of Interest*:** None.

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