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Isolation of *Legionella pneumophila* from Tehran Hospital Water Samples

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ABSTRACT

Over a Period of three months (summer), 108 samples of water collected from four hospitals in Tehran. They were taken from numerous potable and non-potable water, including main supply cisterns, calorifiers, hot and cold tap water, shower, cooling tower and drain of air condition system. Culturing of concentrated and non-concentrated water samples on Buffered Charcoal Yeast Extract (BCYE) Agar and Selective BCYE agar yielded one strain of *Legionella pneumophila.* It was found in cooling tower drain of one hospitals. The strain isolated belonged to sero-group 4 (Portland-1 Strain). It was also atypical being hippurate negative.

Keywords: Legionella pneumophila, Water contamination, BCYE agar

INTRODUCTION

Legionella pneumophila is known to be a cornmon cause of pneumonia [1-3]. Cooling towers and potable water supplies have been implicated as a source of infection for both nosocomial and community-acquired outbreaks of legionnaires' disease [4]. Since *Legionella* is chlorine-tolerant [5], the organism survives the water treatment process and only a small number passes into the water distribution system [6]. Subsequent growth and proliferation occur in man-made habitants, including cooling tower, cooler and water distribution system which provide favorable water temperature, physical protection and nutrients. The water system of the hospitals with endemic *legionella* may be the main source of nosocomial legionellosis in these institutions. In this study we describe the isolation of organism from water system of four hospital in Tehran in summer.

MATERIALS AND METHODS

Water sampling: 108 water samples were taken from numerous potable and non-potable water sites, including main supply, calorifiers hot and cold cisterns, hot and cold tap water, shower, cooling tower and drain of air conditioning systems at the hospitals in July, August and September 1992. For isolation of *Legionella* species, three liters of water were concentrated by filtration through 0.22 µm pore size filter membrane. After filtration the filter membrane was placed in 50 ml of distilled water in a suitable container and shaken vigorously until the deposit on the membrane was re-suspended. This concentrated sample was then used as the inoculum. Aliquots (0.1 ml) of concentrated and unconcentrated water samples were plated on Buffered Charcoal Yeast Extract (BCYE) agar [7] and selective BCYE agar (with antibiotics; polymixin 39600/IU, vancomycin 0.5 mg, cycloheximide 40 mg) and incubated at 37°C. For each water sample a further two set of selective plates were inoculated; one after pretreating 10 ml of the concentrated sample with heat (50°C for 30 minutes) and other set inoculated after acid (HC1-KC1 buffer, pH 2.2) pretreatment. (9 ml of buffer was added to 1 ml of concentrated sample and mixture gently was shaken for five minutes). The treated sample (0.1 ml) is spread on BCYE and selective BCYE medium. Plates were incubated for up to 10 days. Isolates that failed to grow on the blood agar, were small, poorly stained Gram-negative rods were presumptively identified as *Legionella.* Specific identification was achieved by immunofluorescence.

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RESULTS

In July, numerous sample taken from cold and hot water, drain of cooling tower and air-conditioning systems within the building of department of four hospitals and from hospital water supplies did not yield any *Legionella.* However, in August after concentration of 3 liters of drain of cooling tower of one hospital by 60-fold ~17 cfu of *L. pneumophila* serogroup 4 (portland-1) per liter was cultured. from 0.1 ml of concentrate. This strain was atypical in being hippurate negative. Positive result was confirmed by the Environmental Microbiology Reference unit, London, England. In September, all of water samples taken from multiple sites of building of hospitals did not yield any *Legionella.* However *L. pneumophila* was isolated from drain of cooling tower of the same hospital again. Direct Culturing (un-concentrated) of water sample of all hospitals on to selective media did not yield any *Legionella.* Culturing of concentrated and un-concentrated water of cooling tower on the non-selective media yielded a heavy overgrowth of bacterial organisms on these media which prevents the possible recovery of *Legionella.*

DISCUSSION

Our investigation provided microbiological evidence that Legionellaceae may found in the water supply systems of hospitals in Tehran. The Serogroup 4 *L. pneumophila* was recovered from cooling tower water but not other potable and non-potable water samples. The positive culture from cooling tower water in August and September and negative one in July compatible with previous evidence that increasing temperature of water provided a favorable environment for *L. pneumophila* in nature [8]. This may related to the following reasons: (a) the thermophilic properties of *L. pneumophila,* (b) the possible presence of host amoebae for *L. pneumophila* in warm water systems [9-11], (c) the reduced levels of free chlorine in this water.

In one study exposure to 0.65 ppm of hypochlorite for ore hour was required to inhibit the growth of *L. pneumophila* [12]. This level may be approached in some cold tap water system, but much lower levels were found in hot water and circulating cooling tower at our hospitals and elsewhere [13]. Successful use of artificial media to detect *Legionella* species [14] may prompt hospitals to test their tap water every month. Testing appears to be unnecessary in the absence of a recognized infection problem because *L. pneumophila* has been isolated from plumbing systems in hospitals and hotels not associated with known cases of legionnaire's disease [15, 16].

Although our study did not demonstrate a hazard in chlorinated tap water, earlier evidence from many different studies showed that routine chlorination of tap water does not protect against contamination of *L. pneumophila* [13, 15, 16]. It appears prudent to avoid the use of tap water in aerosol generating respiratory devises and room humidifier at least when immunosuppressed patient are treated with those devices.

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