Legionella species isolated from cooling towers in Kuala Lumpur

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Summary

Three building complexes in Kuala Lumpur were surveyed for the presence of legionellae in cooling towers. The organisms were grown from 12 out of 46 samples of water collected from 30 towers. *L. penumophila* serogroups 1 and 7 were the commonest serogroups isolated. None belonged to the Pontiac subgroup of *L. pneumophila* serogroup 1.

Key words: Legionella isolation, cooling towers.

Introduction

Bacteria belonging to the genus Legionella are known to be ubiquitous Gram-negative organisms in natural and man-made aquatic environments. They have been isolated from soil and surface waters, swimming pools, whirlpool baths, water from cooling towers, air-conditioning systems, hot water tanks and chlorinated tap water.[1-4] These organisms can cause sporadic or epidemic human infections and are a major cause of both community and nosocomial pneumonias in many European countries and the USA.[5,6] The epidemiology of sporadic legionellosis is poorly understood but in epidemics, the infecting bacteria have often been traced to water contained in air-conditioning systems or domestic hot water supplies of large buildings such as hospitals and hotels.

A number of hospital outbreaks have been associated with contaminated cooling towers.[7,8] Cooling towers are designed to cool water by evaporation. Warm water from a building's heat exchanger enters the tower from the top and is sprayed down from a nozzle over a fill pack. As the water droplets pass down, air is drawn up through the falling droplets by a fan, resulting in evaporative cooling. The cooled water collects in a pond at the bottom of the tower and is conducted to the heat exchanger and thence back to the cooling tower. As a result of evaporation, organic and inorganic impurities concentrate in the pond where algae, protozoa and bacteria proliferate. These bacteria may be blown out with the cloud of moist air discharged into the atmosphere from the top of the tower thus posing a potential health hazard to the susceptible.

Very few reports of legionellosis have come from South-East Asia although legionella organisms have been shown to be present in the environment.[9] This paper describes a survey of a sample of cooling towers in Kuala Lumpur for legionellae.

Materials and methods

Two surveys were carried out, the first in April and May, 1989 and the second, a year later in May 1990. A total of 46 samples of water were collected from 30 cooling towers in 3 building complexes in Kuala Lumpur. Samples were collected in sterile 5 litre flasks (at least 2 litres of water from each tower) and

filtered by negative pressure through 47mm cellulose nitrate membrane filters of 0.22 um pore size (Advantac, Japan). The filter membranes were then suspended in 10ml sterile distilled water which was then heated at 50°C for 30 min. Two aliquots of 0.1 ml heated filtrate were inoculated onto buffered charcoal yeast extract agar (Oxoid) supplemented with cysteine and ferric ions (Oxoid) and made selective with polymyxin, anisomycin and vancomycin (BCYEs agar). The plates were incubated in a moist chamber at 35°C up to 14 days and examined daily. Suggestive colonies were subcultured onto BCYEs agar and blood agar. Any organisms growing on BCYEs but not on blood agar were deemed legionella-like organisms. They were gram-stained, tested for biochemical characteristics and then kept in liquid nitrogen until preliminary identification by immunofluorescence (Legionella DFA, Organon Teknika, Belgium) or sent to a reference laboratory.

At the time of sampling, the water pH was determined with pH indicator papers (Macherey-Nagel, Germany) and the water temperature was measured with a thermometer. The free chlorine content of the water was obtained with a chlorine test kit (Hanna instruments, Italy).

Total bacterial counts were obtained for each water sample (kept at 4°C and processed within 2 hours of collection) by the pour plate method.

Results

Twelve isolates of legionella-like organisms were recovered in the two surveys. Ten were subsequently confirmed to be legionella species by Dr TG Harrison at the PHLS Legionella Reference Unit, Central Public Health Laboratories, Colindale, London, UK. The first two isolates were also confirmed by Associate Professor PD Meers at the National University of Singapore. Confirmation was by immunofluorescence with monoclonal antibodies. Of the two isolates which were non-viable when they reached the reference laboratory in Colindale, both had identical growth, microscopic and biochemical characteristics as the strains identified and one of them gave 3+ fluorescence in our fluorescent antibody staining test using a polyvalent antiserum to *L pneumophila* serogroups1-6. Serogroups 1 and 7 were the commonest isolates obtained (Table I). All isolates grew on BCYEs agar after two to eight days' incubation (average four days). All produced oxidase, catalase, gelatinase and beta-lactamase but not urease.

Table I
Legionella serogroups isolated from cooling towers

Isolate		No.
L. pneumophila		
serogroup 1	subgroup Camperdown	3
	subgroup Bellingham	1
serogroup 7		4
serogroup 13		1
L. parisiensis		1
Legionella-like organisms*		2
Total		**12**

* Identified by growth and biochemical characteristics and positive fluorescence with a polyvalent antiserum for *L. pneumophila* serogroups 1-6.

Table II shows the rate of isolation from the cooling towers. Twelve of the cooling towers were sampled on more than one occasion over the one year study period; four were sampled three times each and eight twice each. Of these,six were consistently negative, two became spontaneously negative, three became negative after biocide treatment and one became positive one week after biocide treatment. The twelve isolates came from twelve different cooling towers.

Table II
Detection of legionellae from various cooling towers on one or more occasions

Sampling site	No. of cooling towers	No. of water samples	No. (%) positive
Complex 1	3	9	0 (0)
Complex 2	15	20	5 (25)
Complex 3	12	17	7 (41)
Total	**30**	**46**	**12 (26.1)**

Total bacterial counts, temperature and pH were similar for samples positive and negative for legionellae (Table III). With one exception, all samples tested had < 0.5 mg/l of free chlorine.

Table III
Comparison of water samples positive and negative for legionellae

Water quality parameters	Mean values	
	Samples positive for legionellae (n = 12)	samples negative for legionellae (n = 34)
Total bacterial count (cfu/ml)	1.5×10^3	1.4×10^3
pH	6	6
Free chlorine (mg/l)	< 0.5	< 0.5
Temperature	29.2°C	28.8°C

Discussion

This paper documents the presence of legionellae in cooling towers in Kuala Lumpur. Although the number of samples studied is not large, the isolation rate of 40% (12/30) from cooling towers is similar to that reported from Singapore[10] and UK[11]. The detection rate might have been higher if we had sampled other parts of the cooling tower besides the water in the pond. Sludge, pipes and rubber grommets have all been associated with the persistence of legionellae in cooling towers.[7] Subsequent to this study, a larger survey of cooling towers and water distribution systems located in various parts

of the Klang Valley has been initiated to obtain a more accurate determination of the prevalence of legionellae in our environment.

At least 35 species and 54 serogroups of legionellae have been described. *L. pneumophila* is the species most commonly isolated from water[12] and the serogroup 1 subgroup Pontiac 1a has been most frequently associated with human infections[13] particularly epidemics. At least 9 of our 12 isolates were *L. pneumophila* and 4 of these were serogroup 1 but none belonged to the Pontiac subgroup. It is convenient to postulate that the infrequent occurrence of this subgroup may be a reason for the rarity of legionella epidemics in Malaysia despite the widespread occurrence of the organism in the environment but a larger study is required to ascertain this. The extent of endemic legionellosis in Malaysia is not known. It is probably prudent to consider treatment for legionella infection in a case of atypical pneumonia particularly in a patient who is elderly, immunosuppressed, an alcoholic or a chronic smoker.

Since contaminated cooling towers are a potential source of infection, although the risk is small, the question often raised is whether these towers should be kept free of legionellae. The origin of legionellae in cooling towers is not known but one can expect the towers to be seeded from time to time from contaminated air, aerosols and input water. Unfortunately, low levels of contamination cannot always be prevented even with the use of costly biocides. One of the towers in our study yielded legionellae hardly a week after adequate biocide treatment. Since infection risk is determined, among other factors, by the infective dose for man[3] and host susceptibility, it would probably be sufficient to keep the number of organisms below the level which would constitute a health hazard, minimize the dissemination of contaminated aerosols by constructing closed cooling towers and only attempt to eradicate the organism in high risk areas like hospital intensive care and transplant units. It has been shown that sediment and environmental microflora act synergistically to improve the survival of the organism.[14] Amoebae in particular, have been suspected to be the reservoirs supporting the survival and replication of virulent legionellae in cooling towers.[15] Hence, regular cleaning of cooling towers to rid them of slime, organic and inorganic sediments may be the single, most practical and inexpensive measure to reduce the infection risk posed by these towers.

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