

Detection of *Legionella* spp. and Other Pathogens in Water Systems of Nursing Homes and Spa Pools

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Abstract—*Legionella* spp. are gram-negative and non-spore-forming bacteria. The *Legionella* infections are caused by the contaminated aerosols from the water distribution systems. The objectives of this study were to detect *Legionella* spp. and other bacterial pathogens in water systems of nursing homes and spa pools and to determine the relationship between water parameters and the prevalence of *Legionella* spp. The water samples were collected from nursing homes and spa pools and examined for the presence of *Legionella* spp. and other bacterial pathogens by culture methods. *Legionella* spp. was found in the water systems; 6 from 30 (20%) of nursing homes and 13 from 30 (43.33%) of spa pools. Other bacterial pathogens were *Pseudomonas* spp., *Enterobacter* spp., *Acinetobacter* spp. and *Citrobacter* spp.. There were no statistical significant correlation between water parameters (pH and temperature) and *Legionella* detection.

Keywords— *Legionella* spp., Nursing homes, Spa pools, Water systems.

I. INTRODUCTION

The *Legionella* spp. are gram-negative bacteria and short rod-shaped cells. The major representative species of the genus is *Legionella pneumophila* that can cause the Legionellosis [1]. Legionellae are commonly found in natural water environments (e.g., rivers, lakes, lagoon and reservoirs) and human-made water systems (e.g., cooling tower, water heater tanks, fountain and spa pools). The Legionellosis is divided into two distinct clinical entities, Pontiac fever which is a self-limited flu-like illness and has a high rate of infection of about 95% and Legionnaires' disease which is a severe multisystem disease involving pneumonia with about 5% of infection but symptoms are more severe than Pontiac fever and may lead to death [2]. In Thailand, between 1984 to 2002, there were 17 patients reported to be infected with *Legionella*. Fourteen patients were infected by *L. pneumophila*, two patients were infected by *Legionella* spp. and another one was infected by *L. jordanis*. *Legionella* spp. has been isolated from human-made water systems and environmental samples in

several regions of Thailand [3]. In 2006, there were *Legionella* infections in travellers at Phuket province.

Total 5 confirmed cases and 1 presumptive case were detected among all Scandinavians staying in the hotel at Phuket province. The risk factors of infection were showers in the hotels which had *Legionella* and people aged more than 45 years old had increased risk for *Legionella* spp. infection [4]. Other microorganisms that may be found in the water systems and can cause problems to human are gram-negative bacteria that are commonly found in soil, water and natural environments and may be found in the hospitals causing nosocomial infections. Most frequently reported microorganisms are Enterobacteriaceae, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, coagulase-negative staphylococci and fungi which include *Flavobacterium*, *Alcaligenes* and *Acinetobacter* [5]. The purpose of this work is to study the prevalence of *Legionella* spp. and other bacterial pathogens in water systems of nursing homes and spa pools and to determine the relationship between water parameters and the prevalence of *Legionella* spp..

II. PROCEDURE FOR PAPER SUBMISSION

A. Samples collection and processing

Water samples from nursing homes and spa pools were collected approximately 500 ml in the sterile containers and stored samples at room temperature during transporting to the laboratory. Each sample of water was collected in a sterile container which had 1 ml of 10 mg/ml solution of sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$). The water temperature and pH were determined immediately after collection. The water samples were concentrated by filtration through 0.22 μm pore size cellulose acetate membrane filters (Millipore S.p.A., Milan, Italy) and the membrane filters were cut into small pieces with aseptic technique, then put into a sterile tube that containing 1.5 ml sterile distilled water and vortexed for 30 seconds to remove bacterial cells from the membrane filters.

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B. Detection of *Legionella species*

The 1.5 ml of acid solution (HCl - KCl solution pH 2.2) were added to the concentrated water samples for 5 minutes, then pipetted 1 ml to another tube that already contained 9 ml of sterile distilled water. The water samples were tested by spread plate technique at undiluted and 10^{-1} dilution, 0.1 ml of each sample was placed in duplicate on Buffered Charcoal Yeast Extract (BCYE) agar and BCYE agar + glycine, vancomycin, polymyxin B and cycloheximide (GVPC), then incubated at 37°C in the humid chamber for 3-4 days. The suspect colonies were cultured on BCYE and BCYE without L-cysteine for testing the requirement of cysteine by streak plate technique and incubated at 35°C for 4 days.

C. Isolation and quantitation of total heterotrophic plate count

The water samples were analyzed by 10-fold dilution series of the concentrate water samples. The samples were cultured duplicate on plate count agar (PCA) with spread plate technique. All plates were incubated at 35°C for 24 – 48 hours. The number of colonies were counted and reported as colony forming unit per ml (CFU/ml).

D. Isolation of gram - negative bacteria

The gram – negative bacteria were cultured by spreading 0.1 ml of sample on the Mac Conkey agar in duplicate. All plates were incubated at 35°C for 24 hours. The colonies of gram – negative were identified by morphology and biochemical tests.

E. Isolation of *Staphylococcus spp.*

The isolation of *Staphylococcus spp.* was analyzed by spread plate technique on the selective medium, Manitol salt agar in duplicate. All plates were incubated at 35°C for 24 hours.

F. Isolation of coliform and *Escherichia coli* bacteria

The determination of coliform bacteria was analyzed by inoculate sample water to lactose broth and incubated at 35°C for 24-48 hours. The positive tubes had turbidity and produced gas within durham tube. The isolation of *E.coli* was analyzed by inoculated the positive tube to EC medium, streak plate on eosin methylene blue agar (EMB), confirm with urea test, Gram stain and catalase test.

G. Relationships between water parameters and the prevalence of *Legionella spp.*

The microbiological analysis was extended to the other information by investigated the relationship between *Legionella spp.* and other water quality parameters. The relationship was statistical analyzed by Pearson's correlation analysis.

III. RESULTS

From total of 60 water samples from nursing homes (N=30) and spa pools (N=30), they were found *Legionella spp.* in the

water systems; 6 from 30 (20%) of nursing homes and 13 from 30 (43.33%) of spa pools. Total of 60 water samples sites were selected from showerheads, faucets and water tanks. The faucets were the most site that could be found *Legionella spp.* Other gram-negative bacteria were *Pseudomonas spp.*, *Enterobacter spp.*, *Acinetobacter spp.*, and *Citrobacter spp.* The mean value of total plate count of nursing homes and spa pools were 5.26×10^4 cfu/ml and 4.33×10^4 cfu/ml, respectively. The *Staphylococcus spp.* was found in the water systems; 9 from 30 (30%) of nursing homes and 1 from 30 (3.33%) of spa pools. The coliform bacteria, found in the nursing homes and spa pools, were 6 from 30 (20%) and 4 from 30 (13.33%), respectively and *E.coli* was found 2 from 30 (6.67%) of nursing homes only. The mean value of pH was 7.32 ± 0.07 of nursing homes and 7.61 ± 0.08 of spa pools and mean value of temperature was $28.33 \pm 0.54^\circ\text{C}$ of nursing homes and $27.10 \pm 0.50^\circ\text{C}$ of spa pools. The relationship between water parameters (pH and temperature) and the prevalence of *Legionella spp.* had no statistical significant correlation in nursing homes (pH, $r = -0.282$ and temperature, $r = -0.010$ at $p < 0.05$) and also in the spa pools (pH, $r = 0.255$ and temperature, $r = 0.089$ at $p < 0.05$).

IV. DISCUSSIONS

The total of 60 water samples from nursing homes and spa pools were cultured for *Legionella spp.* and 19 of them were positive (31.67%). The number of positive samples for *Legionella spp.* in this study was similarly to other studies in Thailand. The prevalence of *Legionella spp.* in water systems of hotels and resorts in the North-Eastern of Thailand was 24 from 75 (32.0 %) hotels and resorts [6]. During 2003-2007, the prevalence of *Legionella spp.* in various water resources from 33 provinces in Thailand were investigated and 256 *Legionella* strains were isolated, among these, 206 isolates (80%) were belonged to *L. pneumophila* and 50 isolates were identified as non-*pneumophila* by DNA tree analysis [7]. In 2004, Triassi *et al.* studied *Legionella* infection risk from domestic hot water [8] and found 22.6% (33/146) *Legionella spp.* and 38.4% (56/146) *Pseudomonas spp.*. Naturally occurring *L. pneumophila* multiplied at a temperature between 25°C - 37°C and pH levels of 5.5 - 9.2, [9] and the average mean of pH and temperature of this study was in standard value of Queensland health swimming and spa pool water quality and operational guidelines 2004 which were between 7.2 - 7.8 and 35°C - 37°C [10]. In part of nursing homes, the positive sites may be risk to the elderly or intendants that stay there. But the elderly age over 60 years included smokers have more risk than the other people because they have low immunity and the *Legionella* infection was found in male more than female [11]. In part of spa pools, the positive sites may be risk to the customers and spa pool keepers. The route of infection can transmit by inhalation of bacterial contaminated aerosols. Therefore, the result of this study should be concerned by the epidemiologists since *Legionella spp.* and other bacterial

pathogens may be cause of the possible outbreak of Legionellosis and other bacterial pathogens in the water systems.

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