Asian Journal of Medical Sciences 4(2): 99-102, 2012
ISSN: 2040-8773
© Maxwell Scientific Organization, 2012
Submitted: March 12, 2012 Accepted: March 26, 2012 Published: April 30, 2012

Detection of *Legionella* from Teaching Hospital Cooling Tower Water of Air Conditioning Systems in Eastern Province of Saudi Arabia

[1]Nasreldin Elhadi and [2]Hatem Qutub
[1]Department of Medical Laboratory Sciences, College of Applied Medical Sciences,
[2]Department of Internal Medicine, College of Medicine, University of Dammam, Dammam, Saudi Arabia

Abstract: The objective of this study was to investigate the frequency of contamination of *Legionella* in the King Fahd Hospital of the University (KFHU) cooling tower water of air conditioning system and other water supplies. A total of 300 samples were collected from various sites in KFHU. Water samples were collected from the cooling tower (88), hot-shower head (40), cold-shower head (59), engineering building (12) and drinking fountain (12). Swab samples were also collected from shower heads (44) and air ventilator (45). Overall *Legionella* was detected in only 5 (5.68%) out of 88 water samples and yielded 7 isolates. All other water samples that were collected from other sites were negative for the presence of *Legionella*. Culturing the hospital water supply for *Legionella* was the first step in the assessment of the risk for hospital-acquired Legionnaires' (LD). *Legionella* is a common cause of hospital-acquired pneumonia, especially for immunocompromised patients.

Key words: Cooling tower water, Eastern Province of Saudi Arabia, King Fahd Hospital of the University, *Legionella*

INTRODUCTION

Legionella is a facultative intracellular pathogen known to cause both community and hospital acquired pneumonia (Fields *et al*., 2002; Sabria *et al*., 2004). The genus *Legionella pneumophila* accounts for 90% of cases of legionellosis, (Joseph, 2004) and about 85% are due to serogroup (Grove *et al*., 2002) other *Legionella* spp. are rarely pathogenic in humans (Doleans *et al*., 2004). Community-Acquired Pneumonia (CAP) is commonly defined as an acute infection of the pulmonary parenchyma that is associated with at least some symptoms of acute infection, accompanied by the presence of an acute infiltrate on a chest radiograph or auscultatory findings consistent with pneumonia, in a patient not hospitalized or residing in a long-term care facility for 14 days before onset of symptoms (Bartlett *et al*., 2000).

In recent years, nosocomial Legionnaires' disease has been on the increase (Chien *et al*., 2004). Although cases of nosocomial *Legionella* pneumonia are increasingly recognized, there is no general consensus regarding the prevention of legionellosis in hospitals. The Centers for Disease Control and prevention (CDC) only recommend routine environmental investigation in water samples from high risk wards housing transplanted patients (Tablan *et al*., 2004). Legionella infection occurs mainly by inhalation of aerosols generated from water sources (Leoni *et al*., 2005). Legionnaires' disease is known to cause hospital acquired pneumonia and may occur as part of an outbreak or sporadically (Yu, 2003). Between 1980 and 2002, 4402 cases of Legionnaires' disease were identified in England and Wales, of which 264 were hospital acquired (Cooke *et al*., 2004). The proportion of hospital acquired pneumonia due to *Legionella* has been reported as ranging from 0 to 47% (Hutchinson, 1990). The incidence of hospital-acquired legionellosis is underestimated for a variety of reasons, including a lack of clinical awareness or a missed diagnosis, e.g. non-classical presentation especially in very ill or immunocompromised patients, infection at sites other than the respiratory tract, including soft tissue infections (Hart and Makin, 1991) and endocarditis, (Johnson *et al*., 1985) delayed seroconversion, or lack of specialized culture facilities or urinary antigen detection tests in diagnostic microbiology laboratories. It has been shown that when an active search for legionella infection is initiated, cases are frequently confirmed (Hutchinson, 1990). Forty-eight different species of *Legionella* have been identified to date, although less than half of these have been linked to disease in human (Stout and Yu, 1997). Thus, this the first study to investigate the frequency of *Legionella* contamination in hospital water systems in Eastern Province of Saudi Arabia, with a

Corresponding Author: Nasreldin Elhadi, Department of Medical Laboratory Sciences, College of Applied Medical Sciences, University of Dammam, P.O. Box 2435, Dammam 31451, Saudi Arabia, Tel.: + 966 3 8577 000 ext. 2751

Fig. 1: Cooling tower water system

Fig. 2: Water inside the cooling tower

purpose to understand the extent *of Legionella* contamination in the cooling tower water of air conditioning system.

MATERIALS AND METHODS

Sampling: This study was conducted in November, 2009 in King Fahd Hospital of the University of Dammam. A total of 300 samples were collected from cooling tower water system (88), swab from shower head (12), hot-shower head (40), cold-shower head (59), engineering building (12), drinking fountain (12) and air ventilator (45). A volume of 500 mL of water was collected immediately after opening the valve from each of sampling site (cooling towers, drinking fountain, cold and hot showers, and engineering building) as shown in Fig. 1 and 2. Swabs samples also were collected from ventilation units, air humidifiers and hot and cold showers.

Microbiological processing of water samples: Water samples were processed according to the Centers for Disease Control and prevention (2005) (CDC) procedures for the recovery of *Legionella* from the environment.

Water samples were filtered and concentrated in a biological safety cabinet by pouring the samples into a sterile 47 mm filter funnel assembly containing a 0.2 µm polycarbonate filter and were connected to a vacuum source. All water samples were allowed to pass through the filter and the filter papers were removed aseptically from the holder with sterile forceps. The filter studyies were folded and placed into a sterile 50 mL centrifuge tube containing 5 mL of sterile water. The centrifuge tubes were vortex for one 1 min to free bacteria and other organic material from the filter studies. (1.0 mL) of the vortex suspension were placed into a sterile 15 mL centrifuge tube containing 1.0 mL of acid buffer (acid treatment) and incubated for 15 min at room temperature. (0.1 mL) of the suspension were spread onto Buffered Charcoal Yeast Extract (BCYE) agar containing 0.1% alpha-ketoglutarate is the base medium used for the recovery of *Legionella* from environmental and clinical specimens. All plates were incubated at 35ºC for 3 to 10 days and examined for growth every 24 h.

Identification and confirmation: Suspect colonies were sub-cultured in parallel onto BCYE and blood agar plate media and incubated at 35ºC for another 3 to 4 days. The presence of growth on BCYE agar and absence of growth on blood agar plate suggested *Legionella* species. *Legionella* species were further confirmed by using hippurate hydrolysis test and were tested with a latex test (Oxoid Ltd, Basingstoke, UK).

RESULTS AND DISCUSSION

A total 5 (1.66%) out 300 water samples and swabs samples were found to be positive for *Legionella*. These samples were collected from different sites and wards in King Fahd hospital of the University (Table 1). In this study *Legionella* were detected only from water samples collected from cooling towers which yielded a total of 7 isolates. Hospital-acquired LD has rarely been reported from Saudi Arabia and environmental cultures of *Legionella* in hospitals water systems in Saudi Arabia have never been systematically performed. To our

Table 1: Surveillance of *legionella* in King Fahd Hospital of the University (KFHU)

Source of water samples	No. of samples	No. of positive samples (%)
Cooling towers	88	5 (5.68%)
Shower heads (hot)	40	0
Shower heads (cold)	59	0
Swabs from shower heads	44	0
Engineering building	12	0
Drinking fountain	12	0
Air ventilator (swabs)	45	0
Total	300	5 (1.66%)

knowledge, there was no documented epidemiological investigation in Saudi Arabia to determine the origin and sources of this organism. *Legionella* was found in 5.68% out 88 water samples of KFHU water systems in Eastern Province of Saudi Arabia. Cases of hospital-acquired Legionnaires' disease may have occurred in Eastern Province of Saudi Arabia but not been detected. This study recommends that the KFHU infection control unit consider mandating environmental surveillance of *Legionella* not only for KFHU but for all hospitals in Eastern province of Saudi Arabia, as is now implemented in many European countries (Anonymous, 2005) and elsewhere (Lin and Yu, 2006; Stout *et al*., 2007).

Hospital water systems are the primary reservoirs for hospital-acquired Legionnaires' disease (Farr *et al*., 1988). Prevention of hospital-acquired LD has been accomplished by disinfecting hospital water system (Best *et al*., 1983; Lin *et al*., 1998). To our knowledge no hospital in Eastern Province routinely cultures their water distribution system for *Legionella*; this policy was adopted following the discovery of the first case of hospital-acquired LD that was epidemiologically linked to the hospital water distribution system, not the cooling towers (Patterson *et al*., 1997). Prevalence and surveys of *Legionella* colonization in hospitals have been conducted and reported in in the UK, (Patterson *et al*., 1997), Canada (Alary and Joly, 1992; Marrie *et al*., 1994), USA (Vickers *et al*., 1987) and Spain (Sabria *et al*., 2004).

Cooling towers were originally thought to be the main reservoir for *Legionella*, but subsequent reports have identified the water distribution systems as the major source of LD in hospitals (Joseph *et al*., 1994). Culturing the hospital water supply for Legionella is the first step in the assessment of the risk for hospital-acquired LD. This approach is widely adopted in the national guidelines (Anonymous, 2005) for France, Denmark, Germany, Netherlands, Spain, Italy, Norway, Portugal and Switzerland and in other regional guidelines and recommendations (Anonymous, 1997). Environmental surveillance for *Legionella* in hospital water supplies can provide significant data that are useful for prevention of hospital-acquired LD. However, this the first study was conducted the first environmental surveillance of King Fahd Hospital of the University (KFHU) in Eastern Province of Saudi Arabia.

CONCLUSION

It can be concluded that *Legionella* were prevalent in KFHU cooling tower water of air conditioning system which may lead to nosocomial infection. This study recommends that the cooling tower and its water tank should be treated and physically cleaned at least by a weekly basis. In addition, the hospital water supply shall be routinely screened for *Legionella* bacteria by environmental culture that has been proven as an important strategy in prevention whereas an application of copper-silver ionization systems has emerged as the most successful long-term disinfection method for the hospital water systems (Stout and Yu, 2003).

ACKNOWLEDGMENT

This research study was supported by University of Dammam from the Deanship of Scientific Research. The authors would like to thank the Microbiology Laboratory technicians (Mr. Lauro Bartolome, Mr. Piodennis Dasal and Mr. Bader Sager) for their contribution.

REFERENCES

Alary, M. and J.R. Joly, 1992. Comparison of culture methods and an immunofluorescence assay for the detection of *Legionella pneumophila* in domestic hot water devices. Curr. Microbiol., 25: 19-23.

Anonymous, 1997. Approaches to Prevention and Control of *Legionella* Infection in Allegheny County Health Care Facilities. 2nd Edn., Pittsburgh, Allegheny County Health Department, PA.

Anonymous, 2005. European Guidelines for Control and Prevention of Travel Associated Legionnaires' disease. Decision No.2119/98/ EC. European Working Group for *Legionella* Infections, London, pp: 79-81.

Bartlett, J.G., S.F. Dowell, L.A. Mandell, T.M. File, D.M. Musher and M.J. Fine, 2000. Practice guidelines for the management of community-acquired pneumonia in adults. Clin. Infect. Dis., 31: 347-382.

Best, M., V.L. Yu, J. Stout, A. Goetz, R.R. Muder and F. Taylor, 1983. *Legionellaceae* in the hospital water-supply. Epidemiological link with disease and evaluation of a method for control of nosocomial legionnaires' disease and Pittsburgh pneumonia. Lancet., 2: 307-310.

Centers for Disease Control and Prevention, 2005. Procedures for the recovery of Legionella from the environment Atlanta GA: US department of health and human services. Public Health Serv., 20: 1-13.

Chien, M., I. Morozova and S. Shi, 2004. The genomic sequence of the accidental pathogen *Legionella pneumophila*. Science, 305: 1966-1968.

Cooke, R.P.D., 2004. Hazards of water. J. Hosp. Infect., 57: 290-293.

Doleans, A., H. Aurell and M. Reyrolle, 2004. Clinical and environmental distributions of *Legionella* strains in France are different. J. Clin. Microbiol., 42: 458-460.

Farr, B.M., J.C. Gratz, J.C. Tartaglino, S.I. Getchell-White and D.H. Groschell, 1988. Evaluation ofultraviolet light for disinfection of hospital water contaminated with *Legionella*. Lancet, 2: 669-672.

Fields, B.S., R.F. Benson and R.E. Besser, 2002. *Legionella* and legionaires' disease: 25 years of investigation. Clin. Microbiol. Rev., 15: 506-526.

Grove, D.I., P.J. Lawson and J.S. Burgess, 2002. An outbreak of Legionella longbeachae infection in an intensive care unit. J. Hosp. Infect., 52: 250-258.

Hart, C.A. and T. Makin, 1991. *Legionella* in hospitals: A review. J. Hosp. Infect., 18: 481-489.

Hutchinson, D.N., 1990. Nosocomial legionellosis. Rev. Med. Microbiol., 1: 108-115.

Johnson, J.T., V.L. Yu, M.G. Best, R.M. Vickers, A. Goetz and R. Wagner, 1985. Nosocomial legionellosis in surgical patients with head-and-neck cancer: Implications for epidemiological reservoir and mode of transmission. Lancet, 2: 298-300.

Joseph, C.A., J.M. Watson, T.G. Harrison and C.L. Bartlett, 1994. Nosocomial Legionnaires' disease in England and Wales, 1980-1992. Epidemiol. Infect., 112: 329-345.

Joseph, C.A., 2004. Legionnaires' disease in Europe 2000-2002. Epidemiol. Infect., 132: 417-424.

Leoni, E., G. De Luca and P.P. Legnani, 2005. *Legionella* waterline colonization: Detection of *Legionella* species in domestic, hotel and hospital hot water systems. J. Appl. Microbiol., 98: 373-379.

Lin, Y.S. and V.L. Yu, 2006. Underdiagnosis of hospital-acquired Legionnaires disease in Singapore. Am. J. Infect. Control., 34: 161-162.

Lin, Y.S., J.E. Stout, V.L. Yu and R.D. Vidic, 1998. Disinfection of water distribution systems for *Legionella*. Semin. Respir. Infect., 13: 147-159.

Marrie, T.J., W.M. Johnson, S.D. Tyler, G.S. Bezanson and S. Burbridge, 1994. Genomic stability of *Legionella pneumophila* isolates recovered from two cardiac transplant patients with nosocomial Legionnaires' disease. J. Clin. Microbiol., 32: 3085-307.

Patterson, W.J., J. Hay, D.V. Seal, J.C. McLuckie, 1997. Colonization of transplant unit water supplies with *Legionella* and protozoa: Precautions required to reduce the risk of legionellosis. J. Hosp. Infect., 37: 7-17.

Sabria, M., J.M. Modol, M. Garcia-Nunez, E. Reynaga, M.L. Pedro-Botet and N. Sopena, 2004. Environmental cultures and hospital-acquired Legionnaires' disease: A 5-year prospective study in 20 hospitals in Catalonia, Spain. Infect Control Hosp. Epidemiol., 25: 1072-1076.

Stout, J.E. and V.L. Yu, 1997. Current concepts: Legionellosis. N. Engl. J. Med., 337: 682-687.

Stout, J.E. and V.L. Yu, 2003. Experiences of the first 16 hospitals using copper-silver ionization for *Legionella* control: Implications for the evaluation of other disinfection modalities. Infect. Control Hosp. Epidemiol., 24: 563-568.

Stout, J.E., R.R. Muder, S. Mietzner, M.M. Wagener, M.B. Perri and K. De Roos, 2007. Role of environmental surveillance in determining the risk of hospital-acquired legionellosis: A national surveillance study with clinical correlations. Infect. Control Hosp. Epidemiol., 28:818-824.

Tablan, O.C., L.J. Anderson, R. Besser, C. Bridges and R. Hajjeh, 2004. Guidelines for preventing health-care-associated pneumonia, 2003: Recommendations of CDC and the healthcare infection control practices advisory committee. MMWR, 53: 1-36.

Vickers, R.M., V.L. Yu, S.S. Hanna, P. Muraca, W. Diven and N. Carmen, 1987. Determinants of *Legionella* pneumophila contamination of water distribution systems: 15-hospital prospective study. Infect. Control, 8: 357-363.

Yu, V.L., 2003. Nosocomial legionellosis, 2000. Curr. Opin. Infect Dis., 13: 385-388.