

Epidemiological investigation of a case of nosocomial Legionnaires' disease in Taiwan: implications for routine environmental surveillance

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Abstract

An epidemiological investigation with *Legionella* and molecular subtyping was conducted to determine the source of a case of nosocomial Legionnaires' disease (LD) who was hospitalized in three hospitals within a month. *Legionella pneumophila* serogroup 3, an uncommon serogroup for infection, was isolated from the patient's sputum. Environmental surveillance revealed *Legionella* colonization in all three hospitals; the patient isolate matched the isolate from the first hospital by molecular typing. Culturing the hospital water supply for *Legionella* is a pro-active strategy for detection of nosocomial LD even in hospitals experiencing no previous cases.

Keywords: Epidemiological investigation, hospital water supply, nosocomial legionellosis, PFGE, routine environmental cultures

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Nosocomial Legionnaires' disease (LD) is rarely reported in Taiwan [1–4]. To our knowledge, Chen *et al.* [5] were

the first to discover that the hospital water supply was responsible for cases of nosocomial LD in a Taiwan hospital. Because of this experience, several hospitals initiated routine environmental surveillance of the water supply, and found *Legionella* colonization in their hospital water supplies [6]. In this study, we report an epidemiological investigation of a case of nosocomial LD in which the patient was hospitalized in three hospitals within a month. None of the three hospitals had ever experienced a known case of nosocomial LD. The DNA subtyping technique was used to determine the source of the infection.

Three hospitals were involved in this study. Hospital A was an 1100-bed teaching hospital. Hospital B was a 260-bed community hospital with a long-term-care facility. Hospital C was a 700-bed military hospital. Hospital environmental cultures were conducted by taking swab samples of faucet outlets (both hot and cold water). The environmental samples were processed as previously described [7]. The media plates were incubated at 37°C for 5 days. Colonies that grew after subculture on buffered charcoal yeast extract medium but not on a blood agar plate were tested with a latex test (Oxoid Ltd, Basingstoke, UK) and confirmed using a monoclonal direct fluorescent antibody for serogroup identification (m-Technologies, Inc., Alpharetta, GA, USA). The patient's sputum samples were processed as previously described [8]. The molecular subtyping of chromosomal DNA for pulsed-field gel electrophoresis (PFGE) was performed using a CHEF MAPPER system (Bio-Rad Laboratories, Richmond, CA, USA) [9], and criteria for interpretation of PFGE patterns were as previously published [10,11].

The patient was hospitalized at hospital A for 1 month, because of productive cough. The patient received no antimicrobial therapy and was later discharged without a definitive diagnosis. After staying home for 5 days, the patient was admitted to the long-term-care unit at hospital B. Four days later at hospital B, acute shortness of breath and fever ensued. The patient was transferred to the intensive-care unit at hospital C, where pneumonia was diagnosed on admission on the basis of chest radiography. Cefpirome was prescribed initially, but fever persisted for 3 days. On day 4 at hospital C, erythromycin, meropenem and ampicillin–sulbactam were administered. Given the negative test result for *Legionella pneumophila* serogroup 1 urinary antigen, LD was not considered initially. Thus, although the sputum specimen was taken on day 1 at hospital C, the sputum specimen was not processed for *Legionella* by the hospital microbiology laboratory.

The patient's sputum was processed for LD on day 14 during an ongoing *Legionella* pneumonia study, in which sputum specimens from all pneumonia patients were processed weekly for *Legionella*. *L. pneumophila* serogroup 3 was isolated

from the sputum culture on day 17. Moxifloxacin was immediately initiated on day 17 for 3 weeks, and the patient was discharged.

The water supply of the patient's home was negative for *Legionella* (0/4). All three hospital water supplies were positive for *L. pneumophila*; 60% (6/10) of distal sites were positive for *L. pneumophila* serogroups 1, 3 and 6 in hospital A. One site (1/2) was positive for *L. pneumophila* serogroup 1 in the patient's room in hospital B. Twenty-five per cent (5/25) of distal sites were positive for *L. pneumophila* serogroups 1 and 3 in hospital C. PFGE showed that the *L. pneumophila* serogroup 3 from hospital A matched the patient's isolate (Fig. 1).

Physicians in Taiwan tend to overlook nosocomial LD, as it is rarely reported. In this study, the patient's sputum was tested for *Legionella* at hospital C because: (i) the hospital's environmental surveillance revealed that 27% of distal sites of hospital water supply were positive for *Legionella* [6]; and (ii) the hospital had an ongoing prospective study in which every patient with nosocomial pneumonia was screened for *Legionella*. Coincidentally, water supplies in all three hospitals were positive for *Legionella*, and were thus potential sources of infection. Molecular subtyping established that hospital A was probably the source.

Our finding confirmed the hypothesis that cases of nosocomial LD can be found through pro-active culturing of the hospital water distribution system. In four studies conducted in the USA and Canada, all hospitals colonized with *Legionella*

reported nosocomial LD following subsequent clinical surveillance [12–16]. In a Spanish study of 12 hospitals, 92% of hospitals (11/12) found cases of nosocomial LD following prospective clinical surveillance [17]. Culturing of the hospital water supply for *Legionella* as a pro-active measure for prevention of nosocomial LD has been adopted in France, Denmark, Germany, The Netherlands, Spain, Italy, Norway, Portugal, and Switzerland [18].

Our experience with *L. pneumophila* serogroup 3 has relevance for a recent report. Leoni *et al.* [19] found no cases of nosocomial LD, despite the fact that 60% of the hospital water samples were positive for *L. pneumophila*. The investigators used *Legionella* urinary antigen as a screening test, and found no cases, and concluded that monitoring of hospital water is 'of little clinical significance'. However, we note that their water was colonized with *L. pneumophila* serogroup 3, which cannot be detected by urinary antigen test [20]. Had these investigators used culture, cases of LD might have been found as in our report.

In summary, environmental monitoring followed by clinical surveillance revealed a case of nosocomial LD due to a serogroup of *L. pneumophila* that is not commonly associated with infection. The infection had been acquired from hospital A, which had no previous knowledge or experience with LD. Advocates of a pro-active approach for prevention by using environmental cultures recommend that respiratory tract culture for *Legionella* should be adopted if the hospital water supply is colonized with *L. pneumophila* other than serogroup 1.



FIG. 1. The pulsed-field gel electrophoresis pattern showed that the *Legionella pneumophila* serogroup 3 of the patient matched the *L. pneumophila* serogroup 3 from hospital A, but not hospital C. ATCC, American Type Culture Collection.

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Transparency Declaration

All authors report no conflicts of interest.

References

1. Liu YC, Cheng DL, Shi FW, Huang WK, Wang JH. Legionnaires' disease—a case report. *Taiwan Yi Xue Hui Za Zhi* 1985; 84: 1180–1185.
2. Wang LS, Chin TD, Liu C. Nosocomial legionnaires' disease. *Zhonghua Yi Xue Za Zhi (Taipei)* 1989; 44: 242–248.

3. Pan TM, Yea HL, Huang HC, Lee CL, Horng CB. Legionella pneumophila infection in Taiwan: a preliminary report. *J Formos Med Assoc* 1996; 95: 536–539.
4. Chang FY. Multilobar consolidation with abscess formation caused by Legionella pneumophila: an unusual chest radiographic presentation. *J Microbiol Immunol Infect* 1998; 31: 200–202.
5. Chen YS, Liu YC, Lee SS et al. Abbreviated duration of superheat-and-flush and disinfection of taps for Legionella disinfection: lessons learned from failure. *Am J Infect Control* 2005; 33: 606–610.
6. Yu PY, Lin YE, Lin WR et al. The high prevalence of Legionella pneumophila contamination in hospital potable water systems in Taiwan: implications for hospital infection control in Asia. *Int J Infect Dis* 2008; 12: 416–420.
7. Ta AC, Stout JE, Yu VL, Wagener MM. Comparison of culture methods for monitoring Legionella species in hospital potable water systems and recommendations for standardization of such methods. *J Clin Microbiol* 1995; 33: 2118–2123.
8. Lin A, Stout JE, Rihs JD, Vickers RM, Yu VL. Improved Legionella selective media by the addition of fluconazole: results of in vitro testing and clinical evaluation. *Diagn Microbiol Infect Dis* 1999; 34: 173–175.
9. Drenning SD, Stout JE, Joly JR, Yu VL. Unexpected similarity of pulsed-field gel electrophoresis patterns of unrelated clinical isolates of Legionella pneumophila, serogroup I. *J Infect Dis* 2001; 183: 628–632.
10. Tenover FC, Arbeit RD, Goering RV et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995; 33: 2233–2239.
11. Hunter SB, Vauterin P, Lambert-Fair MA et al. Establishment of a universal size standard strain for use with the pulsenet standardized pulsed-field gel electrophoresis protocols: converting the national databases to the new size standard. *J Clin Microbiol* 2005; 43: 1045–1050.
12. Muder RR, Yu VL, McClure JK, Kroboth FJ, Kominos SD, Lumish RM. Nosocomial legionnaires' disease uncovered in a prospective pneumonia study. *JAMA* 1983; 249: 3184–3188.
13. Yu VL, Beam TR Jr, Lumish RM et al. Routine culturing for Legionella in the hospital environment may be a good idea: a three-hospital prospective study. *Am J Med Sci* 1987; 294: 97–99.
14. Johnson JT, Yu VL, Best MG et al. Nosocomial legionellosis in surgical patients with head-and-neck cancer: implications for epidemiological reservoir and mode of transmission. *Lancet* 1985; 2: 298–300.
15. Goetz AM, Stout JE, Jacobs SL et al. Nosocomial legionnaires' disease discovered in community hospitals following cultures of the water system: seek and ye shall find. *Am J Infect Control* 1998; 26: 8–11.
16. Joly J, Alary M. Occurrence of nosocomial legionnaires' disease in hospitals with contaminated potable water supply. In: Barbaree JD, Breiman RF, Dufour AP, eds. *Legionella: current status and emerging perspectives*. Washington, DC: American Society for Microbiology, 1994; 39.
17. Sabria M, Modol JM, Garcia-Nunez M et al. Environmental cultures and hospital-acquired legionnaires' disease: a 5-year prospective study in 20 hospitals in Catalonia, Spain. *Infect Control Hosp Epidemiol* 2004; 25: 1072–1076.
18. The European Surveillance Scheme for Travel Associated Legionnaires' Disease and the European Working Group for Legionella Infections. References for national guidelines for control and prevention of Legionnaires' disease. In: *EWGLI: The European guidelines for control and prevention of travel associated Legionnaires' disease: 2005; Supplement I, Part C*. London: The European Working Group for Legionella Infections, 79–81, 2005.
19. Leoni E, Sacchetti R, Aporti M et al. Active surveillance of legionnaires disease during a prospective observational study of community- and hospital-acquired pneumonia. *Infect Control Hosp Epidemiol* 2007; 28: 1085–1088.
20. Stout JE, Yu VL. Legionellosis. *N Engl J Med* 1997; 337: 682–687.

First isolation of *Legionella* species, including *L. pneumophila* serogroup I, in Greek potting soils: possible importance for public health

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Abstract

A total of 21 *Legionella* isolates were recovered from six out of 22 samples of potting soil from the Athens area, Greece. *Legionella pneumophila* (serogroups I and 2–15) and species and serotypes included in the group of *L. longbeachae* serogroups I and 2, *L. bozemanii* serogroups I and 2, *L. dumoffii*, *L. gormanii*, *L. jordanis*, *L. micdadei* and *L. anisa* were isolated on BCYE α agar containing cysteine, GVPC and natamycin and on BCYE α agar containing cysteine, Wadowsky Yee supplement and natamycin. The bacterial load was 4000–120 000 CFU/g of potting soil. The isolation of *L. pneumophila* serogroup I from Greek potting soils is reported here for the first time.

Keywords: Environmental exposure, *Legionella pneumophila* serogroup I, Legionnaires' disease, public health, soil

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