ENVIRONMENTAL CULTURES AND HOSPITAL-ACQUIRED LEGIONNAIRES’ DISEASE: A 5-YEAR PROSPECTIVE STUDY IN 20 HOSPITALS IN CATALONIA, SPAIN

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ABSTRACT
OBJECTIVE: To determine whether environmental cultures for Legionella increase the index of suspicion for legionnaires’ disease (LD).

DESIGN: Five-year prospective study.

SETTING: Twenty hospitals in Catalonia, Spain.

METHODS: From 1994 to 1996, the potable water systems of 20 hospitals in Catalonia were tested for Legionella. Cases of hospital-acquired LD and availability of an “in-house” Legionella test in the previous 4 years were assessed. After the hospitals were informed of the results of their water cultures, a prospective 5-year study was conducted focusing on the detection of new cases of nosocomial legionellosis and the availability and use of Legionella testing.

RESULTS: Before environmental cultures were started, only one hospital had conducted active surveillance of hospital-acquired pneumonia and used Legionella tests including Legionella urinary antigen in all pneumonia cases. Only one other hospital had used the latter test at all. In six hospitals, Legionella tests had been completely unavailable. Cases of nosocomial LD had been diagnosed in the previous 4 years in only two hospitals. During prospective surveillance, 12 hospitals (60%) used Legionella urinary antigen testing in house and 11 (55%) found cases of nosocomial legionellosis, representing 64.7% (11 of 17) of those with positive water cultures. Hospitals with negative water cultures did not find nosocomial LD.

CONCLUSIONS: The environmental study increased the index of suspicion for nosocomial LD. The number of cases of nosocomial LD increased significantly during the prospective follow-up period, and most hospitals began using the Legionella urinary antigen test in their laboratories (Infect Control Hosp Epidemiol 2004;25:1072-1076).

Since Legionella pneumophila was first identified as the causative agent of legionnaires’ disease,1,2 this microorganism has been associated with both community-acquired and nosocomial pneumonia. More than 300 reports of nosocomial Legionella pneumonia have appeared in peer-reviewed journals and public health bulletins. It is now recognized that many cases of nosocomial Legionella pneumonia go undiagnosed. Thus, many authorities actually agree that Legionella could be an important cause of nosocomial pneumonia.3-8

However, there is no general consensus regarding the prevention of legionellosis in hospitals. The position of the Centers for Disease Control and Prevention (CDC),9 which recommends environmental investigation only after detecting cases of nosocomial Legionella pneumonia, is controversial. The CDC argues that negative environmental cultures may give a false sense of security and that legionnaires’ disease could thereby be underestimated by physicians. On the other side, the Allegheny County Health Care Department and the Maryland Scientific Working Group have recommended routine environmental cultures for Legionella in acute care hospitals10,11; if the microorganism is detected in the water, the Legionella test should be available in the laboratory and applied to all cases of hospital-acquired pneumonia. These groups argue that knowing that the water is colonized by Legionella, clinicians are more aware of legionnaires’ disease and, consequently, more Legionella tests are requested and the possibility of achieving the diagnosis of nosocomial legionellosis increases. The CDC has recommended periodic cultures for Legionella in water samples from high-risk wards housing patients undergoing stem cell transplant, even in the absence of previous cases of legionnaires’ disease.12

In a previous study, L. pneumophila was isolated from potable hot water systems in 17 of 20 hospitals in Catalonia.13 After all of the hospitals had been informed about the environmental data collected, a 5-year follow-up study was performed to detect cases of hospital-acquired legionnaires’ disease and incorporate Legionella tests, especially Legionella urinary antigen, in the laboratories of the hospitals.

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METHODS
Sites

Twenty hospitals in Catalonia, an autonomous region of 32,000 km² located in northeastern Spain, were studied. Five of these hospitals were located in the city of Barcelona. The sizes of the hospitals ranged from 200 to 2,000 beds. Nineteen were acute care hospitals and one was a psychiatric hospital.

Environmental Study

From November 1994 to April 1996, 196 water samples were taken from the 20 hospitals. The water samples were concentrated, decontaminated by acid treatment, and inoculated on selective modified Wadowsky-Yee buffered charcoal yeast extract plates (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) in duplicate. Isolates of *Legionella* were identified by demonstrating growth on buffered charcoal yeast extract but not on sheep blood agar plates (bioMérieux, Paris, France) and by Gram staining. *L. pneumophila* was determined by the Monofluo *L. pneumophila* IFA Test Kit (Genetic Systems Corp., Redmond, WA) and then classified into two groups (serotype 1 or serotypes 2–14) according to the reaction with the immunooagglutination serotyping by the MicroScreen *Legionella* Latex Kit (Microkit Iberica, Madrid, Spain). A complete description of the points sampled and the methodology applied may be found elsewhere.¹³

First Questionnaire

At the time of the environmental sampling, a questionnaire was completed by the staff involved in the control of nosocomial infection. Information about surveillance programs for nosocomial pneumonia, tests available for *Legionella* in the hospital laboratory, and cases of nosocomial legionnaires’ disease diagnosed in the 4 years prior to sampling was collected.

Information to Hospitals

At the end of 1996, each hospital received a written report of its water culture results. In addition, a general information session was held in one of the hospitals and the heads of most of the hospitals studied attended. By request, this session was also held in some of the other hospitals. During these sessions, the results of the study were presented with emphasis on the problem of nosocomial legionnaires’ disease. These results were also reported at the community, state, and international levels.

Second Questionnaire

From December 1997 to December 2001, the information requested by the first questionnaire was collected annually.

The timing of the study and the data requested on the first and second questionnaires are detailed in the figure and Table 1, respectively.

Definitions

The diagnosis of pneumonia caused by *L. pneumophila* was based on the following: isolation of *L. pneumophila* from respiratory samples, a fourfold increase in antibody titers to greater than 1/128 in the paired serum samples, or positive results for urinary antigen. Nosocomial legionnaires’ disease was as defined by the CDC.⁹

RESULTS

Environmental Study

Positive Samples. *Legionella* was isolated from 73 (37.2%) of 196 water cultures, corresponding to 17 of the 20 hospitals. The isolates observed ranged from 200 to 74,250 colony-forming units/L. More than 30% of the samples from the peripheral points were positive in 11 hospitals. In hospital 3, negative water cultures were performed a few months after water disinfection procedures.

Microbiological Typing. *L. pneumophila* was the species detected in all of the positive isolates. *L. pneumophila* serogroup 1 was present in 8 hospitals, whereas *L. pneumophila* serogroups 2–14 were present in 11. *L. pneumophila* serogroup 1 and other serogroups coexisted in two hospitals.
First Questionnaire

According to the first questionnaire, only one hospital (hospital 6) had been performing active surveillance of hospital-acquired pneumonia in the entire institution (ie, intensive care unit and general wards). This same hospital used tests for *Legionella*, including *Legionella* urinary antigen, in all cases of hospital-acquired pneumonia. Only one other hospital (hospital 3) had the *Legionella* urinary antigen test. No *Legionella* tests had been available in the laboratories of six hospitals. Cases of nosocomial legionnaires’ disease had been diagnosed in the 4 years prior to the questionnaire in only two centers (hospitals 3 and 6).

Prospective Follow-Up Study

According to the second questionnaire, the situation of active surveillance of hospital-acquired pneumonia had not changed. However, in 12 (60%) of the 20 hospitals the *Legionella* urinary antigen test was available in house and in 11 (55%) of the 20 hospitals cases of nosocomial legionellosis had been diagnosed, representing 64.7% (11 of 17) of those that had shown positive results on the environmental study and 63.6% (7 of 11) that had more than 30% of the peripheral points colonized by *Legionella*. The hospitals with negative results on the environmental study did not diagnose cases of nosocomial legionnaires’ disease. One of the hospitals that reported cases on the first questionnaire (hospital 3) did not diagnose cases during the prospective follow-up period.

Table 2 provides the results of the environmental study, the percentage of peripheral points positive for *Legionella*, and the data regarding the availability of the *Legionella* urinary antigen test and the number of cases of nosocomial legionnaires’ disease diagnosed during both periods for each hospital.

DISCUSSION

*L. pneumophila* can infect anyone, but it has a clear predilection for immunosuppressed patients. Patients
who have neoplasms, are receiving immunosuppressive therapy or organ transplants, or are elderly with chronic lung disease are the most susceptible hosts.\textsuperscript{14,15} Several studies have demonstrated that \textit{Legionella} is a frequent inhabitant of hot water systems in hospitals,\textsuperscript{16-19} and thus legionnaires' disease should be a common cause of pneumonia in this setting. However, the prevalence of nosocomial legionellosis remains unknown.\textsuperscript{20}

When environmental cultures are performed routinely, hospitals report more cases of nosocomial legionnaires' disease. A Canadian study found that the presence of \textit{Legionella} in the water supply led to a significant number of hospitals discovering cases of nosocomial legionnaires' disease. However, the study was performed for only 9 months.\textsuperscript{21} If the study had been performed for a longer period, it might have uncovered more cases in the uninvolved hospitals. In three studies conducted in Pittsburgh, the discovery of a contaminated water supply led to the subsequent discovery of nosocomial legionnaires' disease.\textsuperscript{22-24} However, the number of hospitals was small: the first study had two hospitals,\textsuperscript{22} the second study had three hospitals,\textsuperscript{23} and the third study included four hospitals.\textsuperscript{24} Our study of 20 hospitals is the largest to correlate the notification of contamination of hospital water with the subsequent discovery of nosocomial legionnaires' disease. Furthermore, our study lasted longer (5 years) than any such study conducted to date.

The results of our environmental study increased the index of suspicion for nosocomial legionnaires' disease among clinicians. This is supported by the fact that the number of cases of nosocomial legionnaires' disease increased significantly during the prospective follow-up period and many hospitals later used the \textit{Legionella} urinary antigen test in their laboratories. Thus, although only two hospitals (10\%) had reported cases of nosocomial legionnaires' disease previously, 55\% did so in the prospective follow-up period (rising to 63\% for hospitals colonized with \textit{Legionella}). We also confirmed that hospitals not colonized by \textit{Legionella} found no cases of legionnaires' disease. This is consistent with five other prospective studies in which no cases of nosocomial legionellosis were observed in any hospital that did not have a contaminated water supply.\textsuperscript{20}

Many of the hospitals included in our study have since incorporated the use of \textit{Legionella} urinary antigen testing and this has undoubtedly contributed to the increase in the number of cases of nosocomial legionellosis. Approximately 66\% of the hospitals using the \textit{Legionella} urinary antigen test had diagnoses of nosocomial legionnaires' disease. This method has been shown to be highly useful for diagnosing legionnaires' disease, with a sensitivity ranging from 60\% to 100\% and a specificity of 100\%.\textsuperscript{25,26} However, it is mainly useful for the diagnosis of \textit{L. pneumophila} serogroup 1. Hospital 14 of our study is a clear example of this. The environmental study demonstrated the presence of \textit{L. pneumophila} serogroups 2-14, yet all of the cases diagnosed were attributed to \textit{L. pneumophila} serogroup 6 and were diagnosed through sputum cultures. When environmental cultures are positive for species other than \textit{L. pneumophila} or for \textit{L. pneumophila} serogroups other than serogroup 1, specialized culture techniques using selective media should be reinforced to identify legionnaires' disease.\textsuperscript{22,27}

Despite the communication with the other hospitals and the intention of performing active surveillance for nosocomial legionnaires' disease, only one hospital (hospital 6) was successful in applying the \textit{Legionella} diagnostic test to almost all of the cases of hospital-acquired pneumonia. This hospital also discovered the greatest number of cases of legionnaires' disease. These limitations are important because they reinforce the possibility of under-diagnosis of nosocomial legionellosis in our study. The fact that no further environmental survey has been performed since the 1994-1996 period is another limitation of this study. It is unknown whether hospitals with negative environmental cultures became colonized by \textit{Legionella} or whether those colonized by \textit{Legionella} had negative cultures later. Complete elimination of \textit{Legionella} from a water system is difficult to achieve, even using complementary disinfection methods.\textsuperscript{28} On the other hand, hospitals that were colonized by \textit{Legionella} continued reporting cases of nosocomial legionnaires' disease in the prospective follow-up study, indicating that they remained colonized. In September 1999, a copper–silver ionization system was installed in hospital 6 after many years of failure using the hyperchlorination and superheat-and-flush methods. Thereafter, the number of cases of nosocomial legionnaires' disease dropped significantly in this hospital (Figure). Currently, the copper–silver ionization system is in use in five of the hospitals included in this study.

There are increasing reports of unrecognized cases of nosocomial legionellosis in hospitals colonized by \textit{Legionella} over long periods.\textsuperscript{8,9} In these hospitals, patients may be incorrectly treated and the mortality rate may thus be high.

Discovery of a single case of nosocomial legionnaires' disease is an important sentinel of the possibility of additional undiscovered cases.\textsuperscript{7} Thus, the search for these microorganisms in hospital water systems seems irrefutable. If they are found, measures of primary prevention should be applied, ranging from the introduction of adequate diagnostic tests and the incorporation of antibiotics active against \textit{Legionella} in the therapeutic protocols to the implementation of complementary disinfection measures for the potable water system of the hospital.

REFERENCES

1. Fraser DW, Tsai T, Orenstein W, et al. Legionnaires' disease: description of an epidemic of pneumonia. \textit{N Engl J Med} 1977;297:1189-1197.
2. McDade JE, Shepard CC, Fraser DW, Tsai TR, Redus MA, Dowdle WR. Legionnaires' disease: isolation of a bacterium and demonstration of its role in other respiratory disease. \textit{N Engl J Med} 1977;297:1197-1203.
3. Sabria M, Yu VL. Hospital-acquired legionellosis: solutions for a preventable infection. \textit{Lancet Infect Dis} 2002;2:368-373.
4. Yu VL. Nosocomial legionellosis. \textit{Curr Opin Infect Dis} 2000;13:385-388.
5. Kohler JR, Maiwald M, Luck PC, Helbig JH, Hingst V, Sonntag HG.
They performed an unmatched case-control study in a 20-45 years; P < .0001, infection. Patients with MRSA infection were older (56 vs 64 patients with MSSA included in the study. Twenty-four patients with MRSA bed medical intensive care unit from 1994 to 2001. All institutions. Lepelletier et al. from the Laboratoire de MRSA and methicillin-susceptible S. aureus (MSSA).

Sent a growing problem and a challenge for healthcare (cases) or MSSA (controls) nosocomial infections were compared with 676 INFECTION CONTROL AND HOSPITAL EPIDEMIOLOGY December 2004

11. State of Maryland, Department of Health and Mental Hygiene. 10. Allegheny County Health Department. More than 10 years of unrecog­ nexto­nized nosocomial transmission of legionnaires' disease among transplant patients. Infect Control Hosp Epidemiol 1998;19:999-1006. 2001;11:673-676.

10. Centers for Disease Control and Prevention. Guidelines for prevention of nosocomial pneumonia. MMWR 1997;46:31-34. 11. State of Maryland, Department of Health and Mental Hygiene. Report of the Maryland Scientific Working Group to Study Legionella in Water Systems in Health Care Institutions. Baltimore, MD: Department of Health and Mental Hygiene; 2000. Available at www.dhmh.state.md.us/html/Legionella.htm.

12. Centers for Disease Control and Prevention. Guidelines for environ­ mental infection control in healthcare facilities. MMWR 2003;52(RR-10):1-42. 13. Sabria M, Garcia-Nunez M, Pedro-Bolet ML, et al. Presence and chro­ nosomal subtyping of Legionella species in potable water systems in 20 hospitals of Catalonia, Spain. Infect Control Hosp Epidemiol 2001;22:673-676. 14. Chow JW, Yu VL. Legionella: a major opportunistic pathogen in transplant recipients. Semin Respir Infect 1998;13:132-139. 15. Carratala J, Gudiol F, Pallares R, et al. Risk factors for nosocomial Legionella pneumophila pneumonia. Am J Respir Crit Care Med 1994; 149:625-629.

16. Patterson WJ, Hay J, Seal DV, McLuckie JC. Colonisation of trans­ plant unit water supplies with Legionella and pathogenic bacteria. Clin Pathol (Paris) 1998;36:64-69. 17. Goetz AM, Stout JE, Jacobs SL, et al. Nosocomial legionnaires' disease discharged in community hospitals following cultures of the water sys­ tem: seek and ye shall find. Am J Infect Control 1998;26:6-11. 18. Dominguez JA, Gali N, Pedroso P, et al. Comparison of the Binax Legionella urinary antigen enzyme immunoassay (EIA) with the Biostest Legionella urinary antigen EIA for detection of Legionella anti­ gen in both concentrated and nonconcentrated urine samples. J Clin Microbiol 1998;36:2718-2722. 19. Yu VL, Beam TR, Lunish RM, et al. Routine culturing for Legionella in the hospital environment may be a good idea: a three hospital prospec­ tive study. Am J Med Sci 1997;304:97-99.

20. Yu V. Resolving the controversy on environmental cultures for Legionella: a modest proposal. Infect Control Hosp Epidemiol 1998;19: 895-897. 21. Joly J, Alary M. Occurrence of nosocomial legionnaires' disease in hospitals with contaminated potable water supply. In: Barbaric JD, Breiman RF, Dufour A. eds. Current Status and Emerging Perspectives. Washington, DC: American Society of Microbiology; 1994:39. 22. Muder RR, Yu VL, McClure J, Kominos S. Nosocomial legionnaires' disease uncovered in a prospective pneumonia study: implications for under­ diagnosis. JAMA 1983;249:3184-3188. 23. Yu VL, Beam TR, Lunish 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.

24. Goetz AM, Stout JE, Jacobs SL, et al. Nosocomial legionnaires' disease discovered in community hospitals following cultures of the water sys­ tem: seek and ye shall find. Am J Infect Control 1998;26:6-11. 25. Dominguez JA, Gali N, Pedroso P, et al. Comparison of the Binax Legionella urinary antigen enzyme immunoassay (EIA) with the Biostest Legionella urinary antigen EIA for detection of Legionella anti­ gen in both concentrated and nonconcentrated urine samples. J Clin Microbiol 1998;36:2718-2722. 26. Murdock DR. Diagnosis of Legionella infection. Clin Infect Dis 2003;36: 64-69. 27. Kirsch CA, Jakob K, Schoonmaker D, et al. An outbreak of Legionella micdadei pneumonia in transplant patients: evaluation, molecular epi­ demiology, and control. Am J Med 2000;108:290-295. 28. Lin YE, Stout JE, Yu VL, Vilec RD. Disinfection of water distribution systems for Legionella. Semin Respir Infect 1998;13:147-149.

Detecting legionellosis by unselected culture of respiratory tract secretions and developing links to hospital water strains. J Hosp Infect 1999;41:293-305.

Methicillin-Resistant Staphylococcus aureus Nosocomial Infections in an Intensive Care Unit: Risk Factors, Morbidity, and Cost

Methicillin resistance and infections caused by methicillin-resistant Staphylococcus aureus (MRSA) repre­ sent a growing problem and a challenge for healthcare institutions. Lepelletier et al. from the Laboratoire de Bacteriologie-Virologie, Hygiene Hospitaliere, Hopital Laennec, Nantes, France, evaluated the risk factors, mor­ bidity, and cost associated with infections caused by MRSA and methicillin-susceptible S. aureus (MSSA). They performed an unmatched case–control study in a 20-bed medical intensive care unit from 1994 to 2001. All patients with pneumonia, bacteremia, and urinary MRSA (cases) or MSSA (controls) nosocomial infections were included in the study. Twenty-four patients with MRSA infection were compared with 64 patients with MSSA infection. Patients with MRSA infection were older (56 vs 45 years; P < .01), had a longer stay (47 vs 35 days; P < .05), and were infected later (22 vs 10 days; P < .0001) than patients with MSSA infection. No difference was observed between the two groups according to the Omega index or mortality. MRSA infection involved extra cost due to antimicrobial treatment (184 vs 72; P < .005) and length of stay (37,278 vs 27,755; P < .05). The authors concluded that patients infected by MRSA in this relatively small study seemed to be different from patients infect­ ed by MSSA but without effect on the Omega index or mortality. Methicillin resistance did involve extra costs due to antimicrobial treatment and length of stay.

FROM: Lepelletier D, Ferreol S, Villers D, Richet H. Methicillin-resistant Staphylococcus aureus nosocomial infections in ICU: risk factors, morbidity and cost. Pathol Biol (Paris) 2004;52:474-479.