Environmental Cultures and Hospital-Acquired Legionnaires' Disease. A Five-Year
Prospective Study in Twenty Hospitals in Catalonia. Spain.

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ABSTRACT

OBJECTIVE: To determine if environmental cultures for Legionella increase the index of suspicion for Legionnaires’ Disease (LD).

DESIGN: Five-year prospective study.

SETTING: 20 hospitals in Catalonia, Spain.

METHODS. From 1994-1996 the potable water system of twenty hospitals in Catalonia were tested for Legionella. Retrospective analysis of cases of hospital-acquired LD and availability of an “in house” Legionella test in the previous four years were performed when sampling. After informing the Infection Control staff of each hospital of the results of the environmental cultures a prospective follow-up study focused on the discovery of new cases of nosocomial legionellosis and the availability and applicability of Legionella testing in hospitals over a 5-year period was made.

RESULTS: Before making environmental cultures only one hospital performed active surveillance of hospital-acquired pneumonia and applied Legionella tests including Legionella urinary antigen in all the cases. Only one other hospital performed this later test. In six hospitals Legionella tests were not available. Cases of hospital-acquired LD had been diagnosed in the previous four years in only two centers. In the prospective follow-up 12/20 hospitals (60%) had Legionella urinary antigen test in house and in 11/20 hospitals (55%) cases of nosocomial legionellosis had been diagnosed, representing 64.7% (11/17) of those with a positive environmental study. Hospitals with a negative environmental study did not diagnose cases of hospital-acquired LD.

CONCLUSIONS: The environmental study increased the index of suspicion for hospital-acquired LD. Discovery of cases of hospital-acquired LD increased significantly during the prospective follow-up period and many hospitals later incorporated the Legionella urinary
antigen test to their laboratories.
INTRODUCTION

Since "Legionella pneumophila" was first identified as the causative agent of Legionnaires' Disease (LD), this microorganism has been associated with both community and nosocomial pneumonia. More than 300 reports of hospital-acquired pneumonia have appeared in peer-review literature and public-health reports. It is now recognized that many cases of hospital-acquired LD (HALD) may go undiagnosed. Thus, many authorities actually agree that HALD could be an important cause of hospital-acquired pneumonia.

There is, however, no general consensus regarding the prevention of legionellosis in hospitals. The position of the Centers for Disease Control adopted by the health care authorities of many European countries, including Spain, which recommends environmental investigation only on detection of cases of HALD is controversial. The CDC argues that negative environmental cultures may prompt a sense of false security and LD could thereby be underestimated by physicians. To the contrary, the Allegheny County Health Care Department (Pittsburgh, USA) and the Maryland Scientific Working Group recommend routine environmental cultures for Legionella in acute-care hospitals. 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. They argue that knowing that the water is colonized by Legionella, clinicians are more aware of LD, and consequently, more Legionella tests are requested, and the possibility of achieving the diagnosis of nosocomial legionellosis increases.

In a previous study L. pneumophila was isolated from potable hot-water systems in 17 out of 20 hospitals in Catalonia. After informing all the centers about the environmental data collected a five-year follow-up study was carried out aimed at the detection of cases of HALD and the incorporation of Legionella tests, especially Legionella urinary antigen, in the hospital laboratories.
MATERIAL AND METHODS:

Site
Twenty hospitals in Catalonia, an autonomous region of 32,000 km² located in the northeast of Spain, were studied. Five of these hospitals were located in the city of Barcelona. The size of the hospitals ranged from 200 to 2000 beds. Nineteen hospitals attended acute patients and one corresponded to 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 then inoculated on a selective MWY-BCYE plate per duplicate. Isolates of Legionella were identified by demonstrating growth on BCYE but not on sheep blood agar plates (BioMerieux, Paris, France) and by Gram staining. Legionella pneumophila species was determined by the Monofluo Legionella pneumophila IFA test kit (Genetic Systems Corporation, Redmon, USA). Then L. pneumophila were classified into two groups (serotype 1 or 2-14) according to the reaction with the immunoagglutination serotyping by MicroScreen Legionella Latex Kit (Microkit Iberica, Madrid, Spain). A complete description of the points sampled and the methodology applied may be found elsewhere. 14

First questionnaire
At the time of the environmental sampling a questionnaire was filled out by the staff involved in the control of nosocomial infection. Information about hospital-acquired pneumonia surveillance programs, tests available for Legionella in the hospital laboratory, and cases of HALD diagnosed in the four years prior to the sampling data, were collected.

Information to hospitals
At the end of 1996 each hospital received a written report of the environmental results. In addition, a general information session was held in the reference hospital (HUGTiP) with the attendance of the heads of most of the hospitals studied. By request, these sessions were also held in some of these hospitals. In these sessions the results of the study were presented with emphasis on the problem of HALD. These results were reported at a community, state and international level.

**Second questionnaire**

From December 1997 to December 2001, the information requested in the first questionnaire was collected yearly.

The timing of the study and the data requested in the first and second questionnaire are shown in figure 1 and table 1, respectively.

**Definitions**

The diagnosis of pneumonia by *Legionella pneumophila* was achieved by the following:

- isolation on respiratory samples,
- a fourfold increase in antibody titers to >1/128 in the paired serum samples or a positive antigenuria.

Nosocomial infection was considered when the appearance of the pneumonia was after 72 hours of hospital admission or within ten days following discharge.

**RESULTS**

**Environmental study**

Positive samples. *Legionella* was isolated from 73 (37.2 %) of the 196 sites analyzed, corresponding to 17 out of the 20 hospitals. The isolates observed ranged from 200 CFU/L to 74,250 CFU/L. More than 30 % of the samples from the peripheral points were positive in 11
hospitals. In hospital 3 the environmental study (negative) was made a few months after the disinfection procedures.

**Microbiological typing.** *L. pneumophila* was the species detected in all the positive isolates. *L. pneumophila* serogroup 1 was present in 8 hospitals while *L. pneumophila* serogroups 2-14 were present in 11. Both serogroups coexisted in 2 hospitals.

**First questionnaire**

In the first questionnaire, only one hospital (hospital 7) performed active surveillance of hospital-acquired pneumonia in the whole institution (intensive care unit and area of general hospitalization). This same hospital applied tests for *Legionella* including *Legionella* urinary antigen in all the cases of hospital-acquired pneumonia. Only one other hospital (hospital 3) had the *Legionella* urinary antigen test. In six hospitals no *Legionella* tests were available in their laboratory. Cases of HALD had been diagnosed in the four years prior to the questionnaire in only two centers (hospitals 3 and 6).

**Prospective follow-up study**

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

Table 2 shows the results of the environmental study, the percentage of positive peripheral points for *Legionella*, and the data regarding the availability of *Legionella* urinary antigen and the
number of cases of HALD diagnosed in both periods for each hospital.
DISCUSSION

Legionnaires disease may affect any individual but it has a clear predilection for immunosuppressed patients. Patients with neoplasms or receiving immunosuppressive therapy, organ transplant recipients, or elderly patients with chronic lung disease are the most susceptible hosts. On the other hand, several studies have demonstrated that *Legionella* is a frequent inhabitant of potable hot water systems in hospitals, and thus, LD should be a common cause of pneumonia in this setting. However, the prevalence of nosocomial legionellosis remains unknown.

When environmental cultures are performed routinely, hospitals report more cases of LALD. A Canadian study found that the presence of *Legionella* in the water supply led to a significant number of hospitals discovering cases of HALD. However, the study was carried out for only nine months. If the study had been continued over a longer period, probably would have been 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 HALD.

However, the number of hospitals was small; one study had two hospitals, the second study had 3 hospitals, and the third study included 4 hospitals. Our study of 20 hospitals is the largest study correlating the notification of contamination of hospital water with the subsequent discovery of HALD. Furthermore, the duration of our study was 5 years, which is also the longest follow-up period for any such study conducted to date.

The results of our environmental study increased the index of suspicion of clinicians for HALD. This is supported by the fact that the discovery of cases of HALD increased significantly during the prospective follow-up period and many hospitals later incorporated the *Legionella* urinary antigen test to their laboratories. Thus, while only 2 (10%) hospitals had reported cases of
HALD previously, this percentage rose to 55% in the prospective follow-up period (rising to 65% for hospitals colonized with *Legionella*). We also confirmed that hospitals not colonized by *Legionella* found no cases of LD. This is consistent with five other prospective studies in which no cases of nosocomial legionellosis were observed in hospitals that did not have a contaminated water supply.\(^{21}\)

Many of the hospitals included in our study have since incorporated the use of *Legionella* urinary antigen and this has undoubtedly contributed to the increase in the number of diagnoses of nosocomial legionellosis. Note that approximately 66% of the hospitals applying the *Legionella* urinary antigen made the diagnoses of HALD. This method has shown to be of high profitability for the diagnosis of this disease, with a sensitivity ranging from 60-100% and a specificity of 100%.\(^ {26,27}\) However, it should be kept in mind that it is mainly useful for the diagnosis of *L. pneumophila* serogroup 1. Hospital 14 of our study is a clear example of this. The environmental study demonstrated the presence of *L. pneumophila* serogroup 2-14 and all the cases diagnosed were attributed to *L. pneumophila* serogroup 6 and were diagnosed through sputum cultures. It should be highlighted that when environmental cultures are positive for non-*L. pneumophila* species or for *L. pneumophila* serogroups other than serogroup 1, specialized culture techniques in selective media should be reinforced to identify LD.\(^ {23,28}\)

Despite the communication with the other hospitals and the intention of performing active surveillance for HALD, it must be conceded that only one hospital (hospital 6) was successful in applying the *Legionella* diagnostic test to almost all the cases of hospital-acquired pneumonia. It is noteworthy that this hospital also discovered the greatest number of LD. These limitations are important since they reinforce the possibility of underdiagnosis of nosocomial legionellosis in
our study. The fact that no further environmental survey has been performed since 1994-1996 period is another limitation of this study. It’s unknown whether hospitals with negative environmental cultures became colonized by *Legionella* or if those colonized by *Legionella* became culture-negative later. Complete elimination of *Legionella* from a water system is difficult to achieve even using complementary disinfection methods. On the other hand, hospitals that were colonized by *Legionella* continued reporting cases of HALD in the prospective follow-up study, indicating that there were still colonized by this microorganism. In September 1999 a copper-silver ionization system was installed in hospitals 6 and 14 after many years of failure using the hyperchlorination and superheat-and-flush methods. Thereafter, the number of cases of HALD has dropped significantly in these hospitals. At present, the copper-silver ionization system is in use in five of the hospitals included in this study.

There are increasing reports of unrecognised cases of nosocomial legionellosis in hospitals colonized by *Legionella* over long periods of time. In these hospitals patients may be incorrectly treated and the mortality related to hospital-acquired pneumonia may increase. These cases may represent the tip of an iceberg the dimensions of which have not been considered for years. Discovery of a single case of HALD is an important sentinel of the possibility of additional undiscovered cases. Thus, the search for these microorganisms in hospital water systems seems irrefutable. If found, measures of primary prevention should be applied, ranging from the introduction of adequate diagnostic tests and the incorporation of active antibiotics against *Legionella* in the therapeutic protocols, to the implementation of complementary disinfection measures to the hospital potable water system.
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Table 1.

Data requested in the questionnaire to the hospitals included in this study.

1. Characteristics of the hospital (number of beds, university hospital, transplant units, potable water system, cooling towers).

2. Surveillance for hospital-acquired pneumonia (Infection control program, incidence vs prevalence studies, local vs hospital-based surveillance)

3. Available tests for Legionella (BCYE, direct fluorescent antibody test, serology, Legionella urinary antigen).

4. Cases of HALD diagnosed during the corresponding period
Table 2.
Results of the environmental survey, availability of *Legionella* urinary antigen and cases of hospital-acquired LD diagnosed in the two periods.

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<td>HOSPITAL</td>
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*Detection of *Legionella pneumophila*

† Environmental study was made few months after disinfection procedures

‡ Yes: presence of *Legionella pneumophila* in > 30 % of the peripherical points