Legionella anisa, a Possible Indicator of Water Contamination by Legionella pneumophila

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Legionella anisa is one of the most frequent species of Legionella other than Legionella pneumophila in the environment and may be hospital acquired in rare cases. We found that L. anisa may mask water contamination by L. pneumophila, suggesting that there is a risk of L. pneumophila infection in immunocompromised patients if water is found to be contaminated with Legionella species other than L. pneumophila.

MATERIALS AND METHODS

Setting. In 2003, a new wing was built at the teaching hospital of Tours, France. This 90-room building consists of four different wards: a burn unit, a cardiovascular surgery intensive care unit, and an emergency care unit. Most rooms are fitted with nontouch water taps and a conventional water tap for use by health care workers and patients, respectively. Forty-six showers and a bath are installed in the building for use by patients, and water to all of these is supplied by a central pipe system.

Bacteriology. According to French national recommendations (1, 10), measures used to prevent hospital legionellosis include routine sampling of water for Legionella in all departments of the hospital. Since the opening of the new building, we have tested the following series of six water samples (1,000 ml each) every 3 months: from one shower in the cardiovascular surgery unit; another shower in the cardiovascular surgery intensive care unit; a third shower in the emergency care unit; a bath in the burn unit; and two other points, the entry and exit points of the hot water loop, respectively. Legionella was isolated from water samples by culture, according to the recommendations of the French Standard method, AFNOR NF T90-431 (2), which conforms to international standard method ISO 11731 (20). Several colonies isolated from each positive sample were used for species and/or serogroup determination by latex slide agglutination tests with polyclonal antisera against L. pneumophila serogroup 1, L. pneumophila serogroups 2 to 14, and Legionella spp. (Oxoid s.a., Dardilly, France). Real-time PCR with the GeneDisc Legionella pneumophila kit and GeneDisc cycler (GeneSyste-ems, Bruz, France) was conducted with three water samples, according to the manufacturer’s recommendations. The GeneDisc kit detects and quantifies Legionella pneumophila in water, based on the recognition of a specific genetic sequence in the microorganism.

Epidemiological typing. Fourteen Legionella sp. strains were genotyped by pulsed-field gel electrophoresis (PFGE) with SfiI, as described previously (24). The patterns obtained were compared by eye.

RESULTS

In 2003, a newly built wing of the CHRU Trousseau hospital was opened. The new wing includes burn, cardiac surgery, cardiovascular surgery intensive care, and emergency care units. During the first 2 years, the results of routine water sampling for Legionella remained negative. In January 2005, water samples tested positive for Legionella at two shower points and the bath in the burn unit, with contamination levels of 500 to 4,000 CFU/liter (Fig. 1). L. anisa was identified by the National Reference Center for Legionella. Six L. anisa isolates were genotyped by SfiI macrorestriction analysis, which revealed considerable diversity, as the PFGE patterns obtained were not identical (Fig. 2, lanes 1 to 6). Given the presence of immunocompromised patients at high
risk for Legionnaires' disease in the wards, measures were implemented to eradicate Legionella spp. from the hot water system of the new building (Fig. 1). Showerheads and flexible pipes from the showers were replaced, the faucet was replaced in the burn unit bath, and the pipes were descaled and treated with chlorine. We then heated the central water pipe system and allowed water at 70°C to flow through each faucet and shower for 30 min (1).

Following treatment, L. anisa was no longer detected in water samples, but L. pneumophila serogroup 1 was found in a sample from the hot water loop (Fig. 1; Fig. 2, lane 7). In accordance with national recommendations and by taking into account the presence of immunocompromised patients in the building, preventive measures were taken. We installed a water microfiltration system in each of the showers used by severely immunocompromised patients—mostly heart transplant patients—and in the faucet of the bath in the burn unit (1).

Two months later, one of the two showers initially contaminated and the burn unit bath again tested positive for L. anisa (Fig. 1; Fig. 2, lanes 8 to 11), whereas L. pneumophila was not detected by microbiological methods in any of the samples. However, PCR detected genomic material from L. pneumophila in the water samples at one point of the water loop and at one shower point, with more than 3,000 genomic units/liter at both points. Aggressive eradication measures were implemented again, namely, replacement of shower equipment and descaling and chlorination of the burn unit bath system, followed by application of a thermal shock to the hot water system (Fig. 1).

No nosocomial infections epidemiologically related to this contamination episode were identified, as none of the patients in this building gave clinical swabs positive for Legionella or positive serological results for Legionella infection.

**DISCUSSION**

In a 2-year-old building housing patients at high risk for legionellosis, we detected water system colonization by Legionella species other than L. pneumophila in the first instance and by L. pneumophila serogroup 1 and Legionella species other than L. pneumophila in the second instance, following thermal shock.

We observed no nosocomial infections epidemiologically re-
lated to the water contamination episode. This may be due to the measures implemented, which included the replacement of equipment, disinfection of the water system, and the systematic installation of a water microfiltration system in the bath and shower units used by severely immunocompromised patients.

There are two possible reasons for the detection of *L. pneumophila* after the thermal shock. First, as the various bacterial components of the water flora interact according to their intrinsic characteristics and relative abundance, the removal of *L. anisa* from the system may have favored the establishment of *L. pneumophila* as a result of bacterial competition. However, this seems unlikely, as *L. pneumophila* was detected immediately after heat treatment, with no time lag.

Second, *L. pneumophila* may have been present before the thermal shock. The available evidence is consistent with this second hypothesis, although bacterial competition may have played a role in the emergence of more resistant *L. pneumophila*. (i) *L. pneumophila* is often found with Legionella species other than *L. pneumophila* in water (5, 8, 9, 17, 25). (ii) An outbreak of Legionnaires’ disease caused by *L. pneumophila*, despite the identification of only *L. anisa* in tap water, was reported in a previous study (26). In that case, *L. pneumophila* must have been present in the water but was not detected due to technical limitations relating to the detection of a minority population (*L. pneumophila*) in the presence of a much more abundant population (*L. anisa*). Indeed, as *L. anisa* contamination levels were high, despite careful observation of each suspect colony, the rarer *L. pneumophila* colonies may have been masked.

(iii) The high frequency of *L. pneumophila* serogroup 1 among clinical isolates may be due to the higher infectivity or more efficient intracellular growth of this species (7). Low densities of *L. pneumophila* serogroup 1 may therefore be responsible for legionellosis.

(iv) *L. pneumophila* has also been reported to be resistant to chemical and physical treatments (33). Heat shock may therefore have had an effect on *L. pneumophila* than on *L. anisa*, abolishing bacterial interference within samples and making it easier to detect *L. pneumophila* microbiologically. The detection of *L. pneumophila* genomic units by PCR, even though microbiological tests detected only *L. anisa*, is also consistent with this hypothesis.

(v) As described in a previous hospital outbreak of Legionnaires’ disease (32), the heat shock applied to the water system may have disrupted the biofilm, leading to the circulation of previously sessile bacteria and *L. pneumophila*-infected amoebae, causing the release of their intracellular contents.

Once Legionella is established within a system, it is difficult to eradicate (23, 33). The replacement of equipment followed by thermal shock was more effective—with Legionella becoming undetectable in cultures of hospital water—than the descaling and chemical disinfection applied to the faucet of the burn unit bath. Furthermore, the detection of *L. anisa* 1 month after the thermal shock—with similar PFGE patterns before and after the thermal shock—demonstrates that the lack of *L. anisa* detection in water samples after the thermal shock indicated only a temporary decrease in contamination to levels below the limit of detection of the method used (50 CFU/liter).

**Conclusion.** We suggest that (i) the thermal shock applied to the whole water system revealed the presence of previously undetected *L. pneumophila* contamination and (ii) the detection of *L. anisa* in water samples should be considered an indication that the water system was colonized by *Legionella* species, including *L. pneumophila*. Consequently, as recommended by the Centers for Disease Control and Prevention (34), when *Legionella* is detected in environmental samples, action should be taken to eradicate all *Legionella* contamination of the water distribution system to prevent *L. pneumophila* infection in immunocompromised patients.

**REFERENCES**


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