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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*.**

Legionella infections are caused by the inhalation of aerosols generated from water sources contaminated with *Legionella* bacteria, particularly in immunocompromised patients. *Legionella* species are ubiquitous in many water systems (6, 8, 9, 17, 22, 27)—including hospital water systems (30, 31, 39)—with *Legionella pneumophila* and *Legionella* species other than *L. pneumophila* isolated together (3, 11) or alone (19, 37). The most frequent species are *L. pneumophila* and *L. anisa* (5, 12, 16, 18, 36). The distribution of clinical cases differs for *L. pneumophila* and *Legionella* species other than *L. pneumophila*, with *L. pneumophila* accounting for most clinical cases (12, 28). *L. anisa* accounts for rare clinical cases (12, 38)—mostly of Pontiac fever (14, 15, 21)—and may be hospital acquired, as in reported cases of pleural infection and Legionnaires' disease due to *L. anisa* (4, 13, 35).

There is some controversy about the relationship between the presence of *Legionella* species other than *L. pneumophila* in hospital water systems and hospital-acquired legionellosis. Given the rarity of clinical infections caused by *Legionella* species other than *L. pneumophila*, it is unclear how water contamination with *Legionella* species other than *L. pneumophila* should be managed in hospitals (29, 39). Some guidelines recommend routine environmental cultures and, if cases of hospital-acquired Legionnaires' disease are uncovered, recommend the eradication of all *Legionella* species if such species are recovered (34). Other guidelines make routine environmental cultures mandatory in hospitals and focus on the eradication of *L. pneumophila* when this species is recovered from the water system (1). We report here findings suggesting that *L. anisa* may mask water contamination by *L. pneumophila*, demonstrating that the risk of *L. pneumophila* infection should be taken into account if water is found to be contaminated with *Legionella* species other than *L. pneumophila*.

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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 cardiac surgery 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 cardiac 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 (Genesystems, 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

Sample collection point	*Legionella* count(s) (CFU/liter) on:				
	09/09/04	01/07/05	03/25/05	05/23/05	06/29/05
Hot water loop (entry point)	<50 *Legionella* sp.	<50 *Legionella* sp.	<50 *Legionella* sp.	<50 *Legionella* sp.	<50 *Legionella* sp.
Burn ICU (bath tap)	<50 *Legionella* sp.	<50 *L. pneumophila* 4,000 *L. anisa*	<50 *Legionella* sp.	<50 *L. pneumophila* 1,400 *L. anisa*	<50 *L. pneumophila* 100 *L. anisa*
Cardiovascular surgery ICU (shower point)	<50 *Legionella* sp.	<50 *L. pneumophila* 500 *L. anisa*	<50 *Legionella* sp.	<50 *Legionella* sp.	<50 *Legionella* sp.
Cardiac surgery unit (shower point)	<50 *Legionella* sp.	<50 *L. pneumophila* 2,000 *L. anisa*	<50 *Legionella* sp.	<50 *L. pneumophila* 4600 *L. anisa*	<50 *Legionella* sp.
Hot water loop (exit point)	<50 *Legionella* sp.	<50 *Legionella* sp.	150 *L. pneumophila*	<50 *Legionella* sp.	<50 *Legionella* sp.
Actions for dealing with the risk of legionellosis:					
Replacement of equipment (showers) Descaling and chlorination (tap) Thermal shock in the water system			03/18/05	06/06/05	
Microfiltration (showers and water taps)			04/01/05 →		

FIG. 1. Results of environmental testing for *Legionella* spp. and actions to deal with *Legionella* contamination of the water system from January to June 2005. Dates are given in the form month/day/year.

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 the 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).

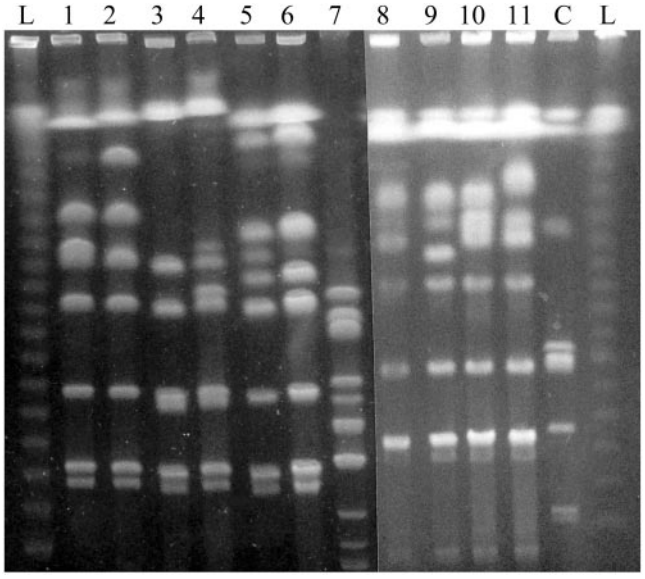

FIG. 2. PFGE SfiI patterns of 10 *L. anisa* isolates (lanes 1 to 10, respectively) and 1 *L. pneumophila* serogroup 1 isolate (lane 11). Lane L, molecular mass ladder; lane C, control.

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 less of 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.

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