

Legionella contamination in the water system of hospital dental settings

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Abstract. *Background and aim of the work:* Among hospital facilities the dental unit is an environment that is at major risk of Legionella due to equipment such as the air/water syringe, the turbine, the micromotor and the scaler which generate potentially harmful aerosols that may be a source of exposure to *Legionella* spp. particularly in immunodeficient patients, and those affected by chronic diseases, and also in dental personnel. Therefore, an examination of the extent of *Legionella* spp. contamination in the dental chairs waterlines and the incoming water supply of some public dental units is the subject of the present study. *Methods:* From February 2002 to March 2004, a total of 208 water samples were collected: 160 samples from the water supply of 4 dental chair and 48 samples from the cold incoming tap water of 2 units. *Results:* *Legionella* spp. was detected in 46 samples (22.1%): 19 of them (41.3% of *Legionella* spp.; 9.1% of the total) were *Legionella pneumophila*; *Pseudomonas aeruginosa* was detected in 86 samples (41.4%) and both microorganisms were detected in 2 samples (0.96%). *Conclusions:* Our results show a microbiological condition in dental settings, that is not at all satisfactory due to the presence of *Legionella* in concentrations that are considered to be a health hazard ($\geq 10^3$) in certain cases. Given the extent of the health risk in these surroundings, the difficulty in its assessment, and also considering the wide diffusion of general dental care, our investigation has confirmed the need to regularly monitor the microbiological condition of water in dental units (www.actabiomedica.it)

Key words: Legionella, dental units, dental chairs, water supply, hospital settings

Introduction

An increasing number of studies have shown the spread of *Legionella* in water environments such as springs, public and private supply systems (1-5), and in hospital and dental institutions (6, 7). The association between isolated strains in the environment, strains that are responsible for human diseases and the number of reported cases of Legionellosis is steadily increasing (8-16).

In natural environments *Legionella* is present in a low density but its concentration can significantly increase in artificial habitats depending on the type of material (17), on the presence of biofilms (18), on the

presence of available nutrients (1, 19, 20), and on the microbial condition of the water.

Given the particular traits of these opportunistic pathogens, some environments are particularly at risk and among these, dental units are at risk due to the use of equipment such as the air/water syringe, the turbine, the micromotor and the scaler which generate potentially harmful aerosols, especially in immunodeficient patients affected by chronic illness, and in dental personnel (1, 21, 22).

Therefore, an examination of the extent of *Legionella* spp. contamination in the dental chair waterlines and the incoming water supply of some public dental units is the subject of the present study.

Methods

The study was carried on between February 2002 and March 2004. Four dental units of Parma hospital were involved in the study: 208 water samples were examined, 160 were collected from the water supply of 4 dental chairs and 48 samples from the cold incoming potable water supply of 2 units.

Water was collected at the beginning of the week, before the start of the working day. One litre of water was analysed; the quantity collected from each dental chair was of 4 aliquots of 250 mL collected from the scaler, the air/water syringe, the micromotor, and the turbine. In the laboratory the samples were filtered through a polycarbonate filter (pore size 0.2 μ); the filter membranes were immersed in 5 mL of the water sample and vigorously agitated with a vortex to re-suspend the adhered bacteria (23). Each sample was doubly plated onto *Legionella* agar: (a) 0.2 mL of concentrate, in agar BCYE (Oxoid; selective agar with supplement and antibiotics); (b) 0.2 mL of concentrate after heat treatment (at 50° for 30 min), in agar BCYE supplemented with more concentrated antibiotics (GVPC Oxoid). The plates were incubated at 36°C (90% humidity, 3% of CO₂) for 10 days; typical legionella-type bacteria colonies appeared after 2-3 days followed by the detection of legionella type bacteria through isolation in a medium without supplement (agar CYE) and in a supplemented medium (agar BYCE); only micro-organisms which were grown in a medium with supplements, being catalase +, oxidase +, arginine +, were considered as suspect and underwent identification by latex agglutination tests and molecular biology (PCR). The latex agglutination test identified *Legionella pneumophila* (serogroup 1 and 2-14) and several other *Legionella* spp. (*Legionella* Latex test kit-Oxoid). The identification was confirmed by genic amplification (PCR). DNAzol Reagent (Invitrogen) was used to extract genetic bacterial material and various primers were used to amplify nucleic acids: L5SL9 and L5SR93 (24) to amplify a region of the 5S rRNA gene, present in all the species of *Legionella* corresponding to a 104-bp segment. In order to identify *Legionella pneumophila* (semi-nested PCR) primers Lpn0901F (forward) and Lpn0941P and Lpm1011R (reverse) were used for a region of the mip

gene present only in the different *Legionella pneumophila* serogroups (product of 60-bp) (25).

In parallel, an investigation on the presence of *Pseudomonas aeruginosa* was conducted, filtering 250 mL of water. To detect this micro-organism, the membrane was placed on Centrimide Agar Base (Difco) plates and the resulting suspect oxidase-positive colonies, which were able to replicate at 42°, were characterized with the API 20 NE (BioMerieux-Italia) system.

Microbic loads at 22°C and 36°C were also studied with Standard plate count agar (Oxoid).

For each dental chair, a record card was filled out (disinfection system, year of manufacture, type of water supply) and the nature and frequency of dental operations (number of patients per day) was recorded.

Results

The results of the investigation of 208 water samples, 160 from dental chairs (scaler, micromotor, turbine, syringe) and 48 from the incoming potable water supply of the dental units are summarised in Tables 1 and 2.

Overall, 46 samples (22.1%) were *Legionella* positive and in 19 of these (41.3% of positive samples and 9.1% of the total) *Legionella pneumophila* was detected; 86 samples (41.4%) were *Pseudomonas aeruginosa* positive and 2 samples were positive for both micro-organisms.

Legionella spp. was detected in 11.9% of dental chairs (19/160), *Pseudomonas aeruginosa* was detected in 53.8% (86/160); in one sample both *Legionella* and *Pseudomonas* were detected (0.6%). In potable water (48 samples), *Legionella* was detected in 56.3% of samples (27/48) and in 39.6% of these (19/48) *Legionella pneumophila* was also detected; in one sample both *Legionella pneumophila* and *Pseudomonas aeruginosa* (2.1%) were detected (Tables 1 and 2).

Concentrations of *Legionella* spp. >10³ cfu/mL were detected in 7.5% of dental chairs water samples and *Pseudomonas aeruginosa* in 36.3% of cases; the 39-55% of *Legionella* spp.-positive samples (from dental chairs and supply water respectively) showed up in high concentrations as shown in Figure 1 (a and b),

Table 1. Results of Legionella investigation in dental chairs and potable water samples from dental units

Samples	Positive Legionella		Positive Legionella non pneumophila		Positive Legionella pneumophila		Positive Legionella and Pseudomonas			
	N.	(%)	N.	(%)	N.	(%)	N.	(%)		
Dental chairs	160	(76.92)	19	(11.88)	19	(11.88)	0	1	(0.62)	
Potable water	48	(23.08)	27	(56.25)	8	(16.67)	19	(39.58)	1	(2.08)
Total	208		46	(22.11)	27	(12.98)	19	(9.13)	2	(0.96)

where the results are divided according to concentration: low ($< 2 \times 10^2$) medium ($2 \times 10^2 \geq x < 10^3$ cfu/mL) high ($\leq 10^3$ cfu/mL).

Table 3 shows the total bacterial load at 36°C and at 22°C; it shows that the microbial concentration in tap water was low at 36°C (from 0 to 71 cfu/mL) with a wider range at 22°C (max 684 cfu/mL), whereas in different sections of the dental chair, particularly in the turbine, rather high microbial concentrations at both temperatures were found. ($4.8 \cdot 10^3$ at 36°C and $9.6 \cdot 10^3$ at 22°C). No significant difference in contamination levels was found in the 4 monitored dental chair, even though one of the units was used for patients with infections.

The main type of dental work was conservative, surgical and prostheses, with an average of 80 patients per week. All the dental chairs, which used demineralized water, had aspiration systems (anti retraction valves) and the filters and circuits were disinfected weekly with phenol-based products. No clear difference emerged in contamination levels between units installed in different years: 2 units had been operative for 8 years and 2 units for 2 years.

Conclusions

Water derived from high powered aerosolizing instruments in dental units represents a potential source of infection by Legionella, an important opportunistic respiratory pathogen. The rich microbic biofilm frequently found along the length of the fine-bore flexible water hoses favours the colonisation of Legionella and justifies the high count observed by several researchers (4, 26).

Atlas et al. (6) report the presence of Legionella

Table 2. Results of *Pseudomonas aeruginosa* investigation in dental chairs and potable water samples from dental units

Samples	Positive <i>Pseudomonas aeruginosa</i>		Positive Legionella and Pseudomonas	
	N.	(%)	N.	(%)
Dental chairs	160	(76.92)	86	(53.75)
Potable water	48	(23.08)	0	
Total	208		86	(41.35)

in 68% of dental unit water samples, concurring with the extent of the contamination (61%) found by Zanetti et al. (26), and also in accordance with the data of Luck et al. (50%) (27). The values suggest that both patients and dental personnel are exposed to *Legionella* spp. and are at risk of contracting the disease. However, there are no recorded cases of Legionella disease caused by dental unit water, contrary to the many documented observations of the correlation between strains detected in the environment and those detected in the patient (14, 15, 28). In the case of the death of a Californian dentist, from Legionnaire's disease, the occupational exposure seems likely but unconfirmed. On the other hand, the source of infection in many cases of "community acquired Legionellosis" has not been identified and dental exposure may represent the un-recognised element in the medical history of several clinical cases or even the predominant risk factor (29). An interesting fact emerges from studies of seroconversion in subjects who work in contaminated hospitals and in dental units. Significant rates of Legionella pneumophila seroconversion were recorded by Fotos (30) and Reinthaler (31): 45% and 34% of dental personnel respectively, had antibodies

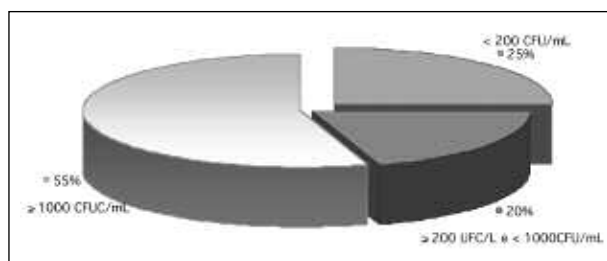


Figure 1a. Concentration of *Legionella* spp in water supply

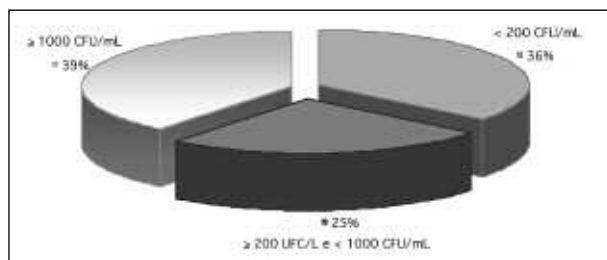


Figure 1b. Concentration of *Legionella* spp in dental chair

for *Legionella pneumophila*, compared with the low rates recorded in the control groups. On the other hand, Pankhurst (32) reports a very low prevalence of *Legionella pneumophila* antibodies in a recent survey conducted in the UK. The seroconversion could result, at least partially, from nonpneumonic forms of *Legionella pneumophila* (Pontiac fever) and is indistinguishable from the flu-like episodes that commonly affect the general population.

The results of our investigation clearly show a si-

gnificantly lower level of *Legionella* spp. contamination in dental unit water lines compared with results reported by other researchers from Italy and other countries (31.5% and 68%) (26, 6). Contrary to other investigations, *Legionella pneumophila* was never isolated in dental chairs water lines, although it was detected in 39.58% of incoming water supply samples. Moreover, there have been frequent reports of high loads of *Pseudomonas aeruginosa* which is likely to have masked the presence of *Legionella*, leading to an underestimation in some cases. Many authors agree that different microbic species as well as high microbic loads can play an important role in limiting or even inhibiting the growth of *Legionella* (4, 26). The presence of *Pseudomonas aeruginosa*, often at high concentrations, as evidenced by our study involves an added risk to our dental environment, as described in the Barbeau's study (21), in which he records the cases of two patients infected with *Pseudomonas aeruginosa* from dental chairs water lines and the detection of high concentrations of this bacteria on the nasal mucosa of some dentists. Concerning isolation, *Legionella* spp. appeared more frequently in the samples; *L. pneumophila* was never isolated in dental chairs whereas it was frequently detected in the incoming supply water of dental units. *L. pneumophila* always represented the serogroup 2 - 14 which in 22.92% of cases reached values of $\geq 10^3$, which is considered to be a "health hazard" level in the Italian national guidelines for Legionellosis prevention and control. (23) Although se-

Table 3. Determination of bacterial loads at 36°C and 22°C: minimum and maximum values

Sample	Bacterial load at 36°C cfu/ml							
	scaler		syringe		micromotor		turbine	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Dental chairs	0	2.7x10 ³	2	19	52	5x10 ³	130	4.8x10 ³
Potable water		Min. 0				Max. 71		
Sample	Bacterial load at 22°C cfu/ml							
	scaler		syringe		micromotor		turbine	
	Min.	Max.	Min.	Max.	Min.	Max.	Min.	Max.
Dental chairs	3.8x10 ³	4.7x10 ³	606	2.2x10 ³	3.4x10 ³	8x10 ³	3x10 ³	9.6x10 ³
Potable water		Min. 4				Max. 684		

serogroup 1 is considered to be the serogroup that is most responsible for human disease, in the last few years there have been numerous recorded cases of more or less serious diseases caused on by other Legionella strains including the 2-14 serogroup (8, 10, 13, 14, 29, 33, 34).

Although *Legionella pneumophila* strains are not the most common strains in the water supplies of dental facilities, as shown, this does not preclude the danger of infection, nor does a negative report preclude infection; in fact, it has been shown that the presence of these opportunistic pathogens (as with other waterborne pathogens) can be sporadic and not finding them does not preclude their presence in the water supply.

Overall, the analysis of our results shows a microbiological condition in dental chairs waterlines, that is not at all satisfactory with the presence of Legionella in 11.9% of samples with concentrations that are considered to be a health hazard ($\geq 10^3$ in 55% of these) and the presence of *Pseudomonas aeruginosa* in more than 50% of the samples with high concentration ($>10^3$) in 1/3 of the cases; neither the presence of medium high bacterial loads nor the detection of *L. pneumophila* in 39.6% of water supply samples should be underestimated. These conditions require prompt purification measures.

Given the extent of the health risk in these surroundings, and also considering the wide diffusion of general dental care, our investigation has confirmed the need to regularly monitor the microbiological condition of water in every dental unit.

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