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Original

Can technical, functional and structural characteristics of dental units predict *Legionella pneumophila* and *Pseudomonas aeruginosa* contamination?

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Abstract: ***Legionella pneumophila*** **and** ***Pseudomonas aeruginosa*** **are common colonizers of water environments, particularly dental unit waterlines. The aim of this study was to assess whether the technical, functional and structural characteristics of dental units can influence the presence and the levels of opportunistic pathogens. Overall, 42 water samples were collected from dental units in a teaching hospital in Palermo, Italy, including 21 samples from the 21 taps supplied by the municipal water distribution system and 21 samples from oral rinsing cups at 21 dental units. *L. pneumophila* was present in 16 out of 21 water samples (76.2%) from dental units, and the median concentration was higher in samples from oral rinsing cups than in those from taps ($P < 0.001$). *P. aeruginosa* was equally distributed in water samples collected from oral rinsing cups and from taps. Some characteristics of dental units (age, number of chairs per room, number of patients per day and water temperature) were slightly associated with the presence of *P. aeruginosa*, but not with contamination by *L. pneumophila*. Our experience suggests that *L. pneumophila* is frequently detected in dental units, as reported in previous studies, whereas *P. aeruginosa* is not a frequent contaminant. As a consequence, microbiological control of water quality should be routinely performed, and should include the detection of opportunistic pathogens when bacterial contamination is expected. (J Oral Sci 52, 641-646, 2010)**

Keywords: opportunistic pathogens; dental unit waterlines; risk assessment.

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Introduction

Many studies have examined the bacterial contamination of dental unit waterlines and have demonstrated the presence of opportunistic pathogens such as *Legionella pneumophila* and *Pseudomonas aeruginosa* (1-4). These organisms can proliferate in water environments and in artificial habitats, depending on the presence of biofilm (5) and the availability of nutrients (6). Dental settings represent a particular risk due to the presence of stagnant water with the increased internal temperature of water tanks.

Moreover, the generation of harmful aerosols and the flushing out of water from dental ablators, syringes and turbines carry the potential risk of inhalation, ingestion and/or direct inoculation to wounds (7). Although several papers have been already published on this topic, evidence of biologic risks to patients remains scarce, and exposure to highly contaminated water may affect immuno-compromised patients (8,9). In addition, dentists, who are exposed to high bacterial loads on a daily basis, are at increased occupational risk of contracting infections; several studies have reported high rates of respiratory infections in dentists and dental personnel (10,11), and there

has been at least one case in which a dentist died after being infected with *Legionella* from a dental unit (12). Furthermore, increased levels of antibodies against *Legionella* have been detected in dental personnel when compared with non-dental personnel (1). Considering the risks to human health posed by *L. pneumophila* and *P. aeruginosa* contamination, the aim of the present study was to assess whether technical, functional or structural characteristics can influence the presence and the levels of opportunistic pathogens in dental unit waterlines.

Materials and Methods

From October 2008 to March 2009, a total of 42 water samples were examined, including 21 samples from the 21 taps supplied by the municipal water distribution system and 21 samples from the 21 dental units at a major teaching hospital in Palermo, Italy. All dental units were used regularly for dental care activities and were also supplied from a cold water storage tank connected to municipal water. None of the dental units used antimicrobial apparatus to prevent biofilm formation and microbial contamination. Dental units lacking the above-mentioned inclusion criteria were excluded from the study. All samples were treated with sodium thiosulfate (20 mg/l) to neutralize the residual chlorine present in the water. Free chlorine concentration was determined in tap water, and temperature was recorded in both tap water and water used for oral rinsing.

At the beginning of each week, before the working day (generally 8.00 am), 2 samples were taken: one from an oral rinsing cup and one from the respective supply tap. For each dental chair, practitioners were requested to complete a record card with technical data (year of manufacture, make, disinfection system if present), structural data (room size, number and size of windows, number of doors, number of dental units in the room, building floor) and functional data (type of dental care, number of patients per day, organoleptic characteristics of water as stated by dentist and patients).

Two samples of water from the storage tank were also collected at the beginning and at the end of the study. In all samples, the following parameters were examined:

1) Total vial counts (TVC): Serial dilutions of water samples were plated onto Plate Count Agar for 72 h of incubation at 22°C and for 48 h of incubation at 37°C. As recommended by Italian law, threshold values of 20 CFU/ml at 37°C and 100 CFU/ml at 22°C were used.
2) Total coliform presence: Water samples (100 ml) were filtered through a 0.45-μm pore size sterile membrane (Millipore). Filters were transferred to m-ENDO agar LES (OXOID) in a Petri dish, and were incubated for 24 h at 37°C. Absence in 100 ml was required.
3) Presence of *Escherichia coli*: Water samples (100 ml) were filtered through a 0.45-μm pore size sterile membrane (Millipore). Membranes were transferred to tryptone bile X-glucuronide agar (OXOID) in a Petri dish, and were incubated at 44 ± 1°C for 24 h. Absence in 100 ml was required.
4) Presence of *Enterococci*: Water samples (100 ml) were filtered through a 0.45-μm pore size sterile membrane (Millipore). Filters were transferred to Slanetz-Bartley agar (OXOID) in a Petri dish, and were incubated at 37°C for 48 h. Absence in 100 ml was required.
5) Presence of *P. aeruginosa*: Water samples (250 ml) were filtered through a 0.45-μm pore size membrane (Millipore). Filters were placed on *Pseudomonas* CN selective agar (OXOID), and were incubated at 37°C for 48 h. Absence in 250 ml was required.
6) Presence of *Legionella* spp. and *L. pneumophila*: Water samples (1,000 ml) were filtered through a 0.2-μm isopore polycarbonate membrane. Filter membranes were then suspended in 10 ml of the same water sample and vortexed. Five milliliters of the suspension was placed in a 50°C water bath and incubated for 30 min. Heat-treated samples and the remaining 5 ml of suspension were seeded (0.1 ml) on agar BCYE (Oxoid; selective agar with supplement and antibiotics) for 10 days at 36 ± 1°C in a damp environment under 2.5% CO_2. Suspect colonies were subcultured on charcoal yeast extract (CYE) and on agar BCYE, and those consistent with *Legionella* morphologies were serologically identified.

Moreover, 21 air samples and 42 surface samples were collected. Microbial air contamination was assessed by Microflow Active Sampler (AQUARIA srl, Lacchiarella, Italy). Air (2,000 l) was actively sampled at 100 l per minute, near a dental unit at 130 cm above the floor. All rooms were naturally ventilated by periodically opening the windows. TVC were detected using Plate Count Agar after incubation for 48 h at 36 ± 1°C. The number of CFU was adjusted using a conversion table provided by the producer (AQUARIA srl), and are reported per cubic meter. As recommended for conventionally ventilated environments (13), a threshold limit value of 180 CFU/m^3 was used.

Microbial analysis of surfaces was performed pressing contact slides (OXOID) containing Plate Count Agar and MacConkey. The slides were pressed for 15 s on each surface to be controlled and, within 3 h, were incubated at 37°C for 48 h. Less than 1 CFU/cm^2 was considered to

indicate good hygiene (14).

All statistical analyses were performed using the R software package (15). The significance level chosen for all analyses was 0.05, two-tailed. Absolute and relative frequencies were calculated for qualitative variables, while quantitative variables are summarized as means ± standard deviation if normally distributed; otherwise, they are given as medians (interquartile range). All bacterial counts were evaluated assuming log-normal distributions. Two-tailed *t*-test was applied to compare the logarithms of bacterial loads. Linear regression analysis was performed between the number of structural and functional risk parameters, and logarithms of microbial concentration. If a threshold was reached, prevalence was calculated as the proportion of values exceeding the cut-off. Independence between categorical variables was tested using Fisher's exact test.

Results

Table 1 shows the functional and structural characteristics of 21 dental units that were analyzed in the study. All dental units were older than 3 years (average age: 11.9 ± 4.2 years). The main activities were equally distributed between surgical (42.9%) and medical duties (57.1%). A large majority of dental units were sited above the ground floor, and about 60% were in rooms with more than 2 dental units. On average, rooms were 39.4 ± 24.5 m^2. For each dental chair, a mean of 6.2 ± 2.4 patients per day visited for about 38.3 ± 13.5 min. Finally, oral rinsing cup water had an average temperature of 22.2 ± 4.3°C, whereas average tap water temperature was 19.9 ± 1.6°C. Tap water chlorination was 0.24 ± 0.12 ppm (data not shown).

Average levels of bacterial contamination of water collected from oral rinsing cups are shown in Fig. 1. *L. pneumophila* was present in 16 of 21 water samples (76.2%) from oral rinsing cups: 43.7% were positive for *L. pneumophila* serogroup 1; 43.7% were positive for *L. pneumophila* serogroup 2-14; and 12.6% were contaminated with both *L. pneumophila* serogroups (data not shown). Moreover, *L. pneumophila* was found in 3 out of 21 samples of water (14.3%) from taps: 33.3% were positive for *L. pneumophila* serogroup 1, whereas 67.7% were positive for *L. pneumophila* serogroup 2-14 (data not shown). Median concentration of *L. pneumophila* was higher in samples from oral rinsing cups than in those from taps (600 vs. 0 CFU/1,000 ml; $P < 0.001$). Nine of 21 water samples (42.9%) from oral rinsing cups were found to have *L. pneumophila* loads exceeding 10^3 CFU/l, while one (4.8%) exceeded 10^4 CFU/l (data not shown). *P. aeruginosa* was equally distributed in samples of water collected from oral rinsing cups and from taps (7/21 vs 6/21, respectively).

The median TVC at 22°C was higher, but not significantly, in water samples from oral rinsing cups than from taps (50 vs. 10 CFU/ml; $P = 0.27$). Samples from oral rinsing cups had also increased median TVC at 37°C with respect to tap water samples (25 vs. 10 CFU/ml; $P = 0.02$). The absence of total coliform, *E. coli* and *Enterococci* was confirmed in all water sample, while *L. pneumophila* and *P. aeruginosa* were absent in water samples from storage tanks supplied by municipal water (data not shown). With regard to air contamination, 3 of 21 samples exceeded the threshold levels and a median

Table 1 Functional and structural characteristics of dental units

	n = 21
Average age in years (±SD)	11.9 (±4.2)
Main activity (%)	
- surgical	9 (42.9)
- medical	12 (57.1)
Floors above ground (%)	
- 0	5 (23.8)
≥ 1	16 (76.2)
Number of dental units in a same room (%)	
≤ 2	8 (38)
> 2	13 (62)
Room size in mq (±SD)	39.4 (±24.5)
Number of patients per day (±SD)	6.2 (±2.4)
Mean of visit duration in minutes per day (±SD)	38.3 (±13.5)

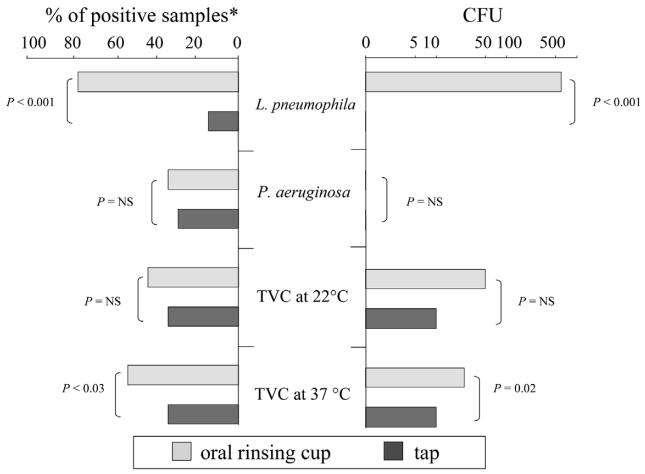

Fig. 1 Presence and median levels of *L. pneumophila* (CFU/1,000 ml), *P. aeruginosa* (CFU/250 ml), TVC at 22°C (CFU/ml) and TVC at 37°C (CFU/ml) in water samples obtained from oral rinsing cups and taps.
*Positive samples: presence of *L. pneumophila*, presence of *P. aeruginosa*, TVC at 22°C > 100 CFU/ml and TVC at 37°C > 20 CFU/ml.

count of 95 CFU/m^3 (interquartile range: 53-144) was recorded (data not shown). Eleven of 21 investigated surfaces showed the presence of bacteria with a median load of 0.1 CFU/cm^2 (interquartile rage: 0-0.9), but none had levels above those recommended (data not shown).

Univariate statistics between the presence of structural, functional and physicochemical risk factors for microbial contamination, and levels of *L. pneumophila* and *P. aeruginosa* contamination are shown in Table 2. Median *L. pneumophila* counts did not differ significantly between groups. Higher contamination values were observed in dental units having surgical duties (1,620 vs. 500 CFU/1,000 ml), with more than 2 chairs per room (1,200 vs. 250 CFU/1,000 ml) and with fewer than 6 patients per day (1,600 vs. 150 CFU/1,000 ml). Median *P. aeruginosa* counts were significantly higher in older dental units (2 vs. 0 CFU/250 ml) and in oral rinsing cup samples from rooms with at least 2 dental units (0 vs. 1 CFU/250 ml). Fewer than 6 patients per day and a water temperature higher than 20°C were significantly associated with increased bacterial loads (2 vs. 0 CFU/250 ml in both cases). Finally, water samples exceeding threshold values for TVC at 22°C and at 37°C did not show higher *L. pneumophila* loads ($P = 0.66$ and $P = 0.53$, respectively), whereas median *P. aeruginosa* loads were significantly higher in samples exceeding threshold values recommended for TVC at 37°C (0 vs. 1 CFU/250 ml; $P < 0.05$) (data not shown).

Discussion

Legionella pneumophila and *Pseudomonas aeruginosa* are considered common colonizers of water environments,

Table 2 Univariate analysis between characteristics of dental unit water systems and median concentrations of *L. pneumophila* (CFU/1000 ml) and *P. aeruginosa* (CFU/250 ml)

	L. pneumophila Median (interquartile range)	*P* value	*P. aeruginosa* Median (interquartile range)	*P* value
Age				
< 10 years	1,700 (300-2,100)	0.70	2 (0-6)	<0.05
≥ 10 years	550 (75-1,750)		0 (0-1)	
Main activity				
medical	500 (0-1,300)	0.16	0 (0-1)	0.31
surgical	1,620 (300-2,500)		0 (0-2)	
Floor				
Groundfloor	1,600 (500-2,500)	0.56	0 (0-1)	0.61
Above the groundfloor	500 (75-1,800)		0 (0-1)	
Number of dental units per room				
≤2	250 (0-925)	0.20	0 (0)	<0.05
>2	1,200 (400-2,500)		1 (0-2)	
Room size				
<40 mq	650 (625-1,675)	0.99	0 (0-2)	0.53
≥40 mq	500 (200-2,500)		0 (0-1)	
Number of patients per day				
< 6	1,600 (500-2,200)	0.23	0 (0-2)	<0.05
≥ 6	150 (0-475)		0 (0)	
Visit duration				
<40 min	550 (75-1750)	0.72	0 (0-1)	0.62
≥40 min	1,700 (300-2,100)		0 (0-1)	
Water temperature				
<20 °C	700 (250-2,300)	0.48	0 (0)	<0.05
≥20 °C	900 (175-2,275)		2 (0-6)	
Tap water chlorination (ppm)				
<0.20	650 (474-925)	0.28	1 (0-3)	0.54
≥0.20	500 (0-2,200)		0 (0-1)	

particularly dental unit waterlines as a result of their stagnant water. In this study, *L. pneumophila* was detected with a high frequency in dental units with a prevalence lower than the 86.7% reported by Ma'ayeah et al. (1), but higher than those reported by other authors (16). This variability is difficult to explain, and many other authors have recorded prevalences that are correlated with geographic location [58% in Luck et al. (17), 33.3% in Montagna et al. (18), 21.8% in Zanetti et al. (19)]. In this study, several structural, functional and physicochemical parameters within dental offices were analyzed, but none of these were found to play a significant role in determining microbial colonization of *L. pneumophila*. Moreover, as suggested by the literature, water chlorination has low efficacy with regard to *Legionella* control (20), and TVC at 22°C and 37°C are not associated with *L. pneumophila* proliferation. It is likely that unknown or unanalyzed factors, such as the presence of biofilm or free-living amoebae, may be the main factors responsible for *Legionella* colonization of dental units. The absence of *L. pneumophila* in water samples from storage tanks supplied by municipal water can be ascribed to its probable presence in lower loads from which larger relative water volumes are taken as samples.

Our data show that up to 40% of dental units reached values of *L. pneumophila* $>10^3$ CFU/l, which are considered to represent a human health hazard according to the Italian guidelines for legionellosis prevention and control (21). The lack of specific disinfection programs for dental units may be the reason for the high levels of biocontamination by both opportunistic bacteria and TVC at 22°C and at 37°C.

In contrast to what was observed for *L. pneumophila*, *P. aeruginosa* was not a frequent contaminant in dental units and its prevalence, in both oral rinsing cups and taps in dental offices, was similar with that reported by other authors (22). Values of $>1.5 \times 10^6$ CFU/ml, which are considered to be the infective dose in health subjects, were not seen (5). *P. aeruginosa* loads were associated with structural characteristics (age and number of dental units per room), functional duties (number of patients per day) and physicochemical properties of water (temperature). TVC at 37°C was also linked with *P. aeruginosa* proliferation. Generally, microbiological water quality of waterline systems, as well as technical, functional and structural characteristics of dental units were somewhat predictive of *P. aeruginosa* contamination.

Finally, the major limitation of this study was the small sample size, in part due to the strict inclusion criteria. On the other hand, the choice to restrict analysis to 21 dental units allowed us to remove confounding factors due to quality of water supplied to dental units and the presence of disinfection protocols.

In conclusion, the present study confirmed that opportunistic pathogens may have different behaviors in dental unit water systems, in which they reach increased concentrations with respect to municipal and tap water. Some characteristics of dental unit water systems were slightly associated with *P. aeruginosa* loads but none of these were able to exhaustively predict the presence and levels of colonization by *L. pneumophila*. These findings are very informative considering that factors such as age of dental unit, performance of medical activities that are associated with blood contamination, seeing few patients per day or being supplied by chlorinated water may lead dentistry workers into a false sense of security with regard to microbiological safety. Thus, microbiological control of water quality may be arbitrarily reduced with regard to the frequency and/or number of investigated microbiological parameters, thereby increasing the health risks of patients and workers. In this way, our experience suggests that microbiological controls of water quality should also be routinely performed also when low bacterial loads are expected, particularly focusing on *L. pneumophila* contamination.

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