Dental Unit Water Lines: A propitious environment for bacterial colonisation.

Summary Contamination of dental unit water lines is not new to dentistry, but this problem takes on a new dimension when considering immuno-deficient patients and existing infection control measures. This study identifies the bacteria involved in the contamination process, estimates the contamination levels and reviews the methods that may be used to control the contamination.

Introduction:

Controlling the spread of infectious agents in dental offices has for several years been a major preoccupation of dentists concerned about improving the current state of affairs. As a result of this concern, certain rules and procedures have been established to control the risks of cross contamination. The success of this effort remains a major subject of discussion, principally due to the fears about possible infections by the AIDS and hepatitis B viruses. Various sources of contamination in the dental office setting that must be controlled have been reported in the literature: patients, dentists and assistants, ambient air, as well as the compressed air and water used in dental units.

Biofilms: a source of contamination. Several authors have shown that the water systems of dental units are propitious sites for the development of certain microbial populations. According to these studies, bacterial species found in aquatic environments, although present in low numbers in drinking water, can be found in very high numbers in the water lines of dental units.

The network of small diameter water lines feeding dental handpieces and air-water pistols is coated with a fine layer of bacteria called a biofilm. This biofilm forms naturally due to the stagnation of contaminated water inside these small, flexible tubes. The adherence of certain species is facilitated by microscopic imperfections on the internal surfaces of the water lines which allow bacterial adsorption and which in turn initiates the formation of the biofilm. The closely packed bacteria are held in place by a matrix of polysaccharides and glycoproteins. The attached bacteria, which are not easily flushed out, proliferate and produce a matrix which in turn allows the establishment of other species of bacteria. The biofilm becomes populated with a greater and greater variety of bacteria and it also protects the bacteria during their growth by retaining nutritional material as well as allowing a higher level of metabolic activity all the while protecting the bacteria from biocidal substances. Mayo made an interesting analogy between the composition of this type of biofilm and that represented by dental plaque. The biofilm gradually becomes visible to the naked eye and can eventually partially obstruct the lumen of the water line. This type of biofilm can be observed, in various degrees, in all dental units, old and new, and even in units that have never been used to treat patients.
Colonization of dental unit water lines

Several studies\textsuperscript{10,11,12,15,16,23} have reported that it is not the microorganisms aspirated from the oral cavity that are the main colonizers of water lines, but are those bacteria normally found in drinking water.

Moreover, oral bacteria from patients may also enter the water lines of units equipped with a water suction system and prevent handpieces from ejecting water. Some researchers have demonstrated the presence of oral streptococci in dental unit water lines\textsuperscript{9,15}. For the past several years, manufacturers have added check valves to prevent water from being reaspirated by the handpiece\textsuperscript{24,25} in order to minimize the risk of microorganisms being retracted into water lines\textsuperscript{26}. Certain modern dental units, using even more advanced technology, allow manual regulation of the water distribution module and volume controlled reaspiration\textsuperscript{25}. Very few studies have shown the presence of oral bacteria in the water lines of dental units equipped with check valves\textsuperscript{13,15}. It is thus likely that the bacteria that colonize water lines do not come from the patients, even if this is theoretically possible, but from the water itself.

Water from dental units can also transmit opportunistic pathogens such as Pseudomonas aeruginosa\textsuperscript{12,16} and Legionella pneumophila\textsuperscript{18,27}.

Bacterial counts and drinking water

Counting colony forming units (CFU: Colony Forming Units) is a simple way to verify the presence of a biofilm. This total bacterial count is obtained by sampling the water at the outlet of the dental unit water line as a large number of microorganisms are detached from the biofilm when water is circulated in the line.

The absence of coliforms has long been considered as a basic criterion for drinking water. In 1952, the American army established a standard\textsuperscript{28} in which the number of colony forming units must be less than 500, i.e., 500 bacteria per ml of water. Presently, public health standards consider water to be safe for human consumption when it contains a maximum of 500 CFU/ml, when it is free of coliforms and when its nephelometric turbidity is less than two (2)\textsuperscript{29}, while in Japan, the acceptable standard is 100 CFU/ml\textsuperscript{30}. However, several authors have indicated that the water coming out of dental unit water lines rarely meets these microbiological standards. Cabot Abel et al.\textsuperscript{9} observed bacterial counts ranging from 300 to 1,000,000 CFU/ml. Other studies\textsuperscript{11,13,15,20} have also shown that bacterial contamination of dental unit water lines is always superior to what is considered acceptable for drinking water. Moreover, Health and Welfare Canada is in the process of drawing up a document to establish standards for drinking water that would propose a maximum of 500 CFU/ml, 10 coliforms/100 ml of water, of which none must be fecal in origin, as well as a maximum nephelometric turbidity of 5 UTN\textsuperscript{31,32}.

Although the bacterial counts reported\textsuperscript{9,11,13,15,20} are not necessarily synonymous with a danger to the health of patients in general as these bacteria are not necessarily pathogenic, it nevertheless remains a problem that cannot be ignored in the case of immuno-deficient patients\textsuperscript{12,29,33,34} and because these bacterial counts may hide the presence of real pathogens such as Pseudomonas aeruginosa\textsuperscript{12,16} and Legionella.
pneumophila. Such high concentrations of bacteria pose a potential source of infection for these patients as it seems reasonable to assume that the transient presence of these pathogenic bacteria increases as a proportion of the total bacterial population.

Immuno-deficient patients

The importance of studying the colonization of water lines was recently raised by Lewis et al., even if previous studies had already reported the microbiological composition of this biofilm. The bacterial species in the biofilm are mainly composed of aquatic organisms such as Pseudomonads, Klebsiellae and Caulobacter sp. These bacteria, however, generally have a low pathogenic potential for most patients with a healthy immune system.

Martin et al. have even reported the case of two immuno-deficient patients that developed a localized Pseudomonas infection following dental operations. These "high risk patients" are not only those who suffer from acquired immune deficiency syndrome, but also those who are suffering from cystic fibrosis and diabetes, elderly patients, those who are undergoing radiotherapy or chemotherapy, those who are receiving massive doses of broad spectrum antibiotics or corticosteroids as well as alcoholics and drug addicts, in short, any patient with a condition that lowers their immune defenses. These patients will continue to increase in numbers in the coming years due to the aging of our population which is why this problem is of special importance today.

This study was aimed at evaluating the quality of the water coming out of dental units, as this water ends up in the mouths of the patients as well as in the ambient air due to the aerosols generated by the handpieces.

Material and methods

Water sampling and microbiological analyses

In order to sample various dental units, we divided them into three classes: private offices, old units in the clinics of the Faculté de médecine dentaire at the University de Montréal and new units in the clinics of the Faculté that were installed for this study. Samples were taken using the same protocol at the beginning of the work day and after a 2-minute purge in 13 private offices in Laval, on the Ile de Montreal and on the south shore and for the units at the Faculté. Two 5-ml samples of water were removed from the outlets of the water lines of each of the following instruments: the air/water pistols of the assistant and the dentist, the turbine, and the ultrasonic scaler. We then proceeded as follows: a first sample of 5 ml designated T0 (time zero) was removed, followed by a second sample of 5 ml (T2) taken after draining each water line for two minutes. This procedure was used at the beginning of the work day before any other clinical activity. It is interesting that a two minute drainage is equivalent to an average of 125 ml of water for the turbine, 170 ml for the ultrasonic scaler and 300 ml for the air/water pistols. It should be noted that there was a large variability in the results depending on the dental unit and the instrument as well as the variable adjustment of the water flow for each model of instrument.
A control sample was also removed from a tap in the operating room or from a nearby tap. The water samples were then diluted in sterile water (1:10 and 1:100). One hundred microlitres (100 µl) of these dilutions was plated on Brain Heart Infusion agar plates (BHI) supplemented with vitamin K and haemin and on a heterotrophic medium38 (Table 1). The agar plates were then incubated at 37°C for 48 hours in the presence of oxygen. For each dilution, the CFUs were counted and expressed as CFU/ml of water.

The new dental units acquired for this project possessed an independent water supply system and were equipped with a check valve. These units received a new bottle of water before each treatment. The water came from the tap in the operating room. Samples from these units therefore sampled water that had remained stagnant in the water lines since the last treatment.

Results

Our results showed that the water lines contain high levels of bacteria and that these microorganisms are highly concentrated in the water ejected by the handpieces and the air/water pistols. The bacterial counts varied among 500 and 3,330,000 CFU/ml at the beginning of the day and were much higher than the limits generally accepted for drinking water. After flushing the water lines for two minutes, although the bacterial levels dropped significantly, they never dropped below the limit of 500 CFU/ml required for drinking water28,29,32. The results of the bacterial counts for the dental units are shown in Tables 2. Tap water gave average bacterial counts of 660 CFU/ml, with results varying between 0 and 3,500 CFU/ml. There was, however, a median of 15 CFU/ml which allowed us to estimate that the quality of the tap water was well within the maximum limit of 500 CFU/ml. The results of several control samples pushed the average slightly over the recognized limit.

Discussion

Bacterial counts in the water lines of the various handpieces can attain values in the order of 3,000,000 CFU/ml. The average concentration varied between 200,000 and 600,000, with a standard deviation often larger than the average. Under these conditions, the median gave a more accurate estimate of the tendencies expressed by these measurements of bacterial concentrations. The dispersion of the results is thus more accurately expressed by the interquadratic deviation which represents the difference between the bacterial concentrations of the 25th and 75th percentiles. These bacterial concentration medians exceeded by 113 to 620 times the maximum standard of 500 CFU/ml at time 0 T0 and by 16 to 64 times at time T2, depending on the water line evaluated. We were able to establish that flushing the water lines for two minutes generally lowered the bacterial counts approximately 7 to 10 times, which represents a decrease of 85 to 90% in the counts. These results are in agreement with other studies1,11,13,30,39 which examined bacterial counts before and after flushing. It is, however, important to underline that a decrease of 85 to 90% in the bacterial concentrations found in most dental units, 193,000 CFU/ml on average, leaves bacterial levels of approximately 22,500 CFU/ml on average, a concentration that is still 45 times the maximum limit. According to these studies, flushing the water lines for 4.25 to 20 minutes, which represents one to two litres of water, can reduce bacterial counts to acceptable levels19,40. If such times are interesting for a kinetic study of the
phenomenon, they are out of question in the clinical setting, if only for practical reasons. Flushing thus appears to be an impracticable solution for eliminating viable bacteria from water lines.

Also, it was interesting to note that the water lines of the air/water pistols of the assistants are generally four times more contaminated than those of the dentist and they gave results that had two times more variability than the water lines of the dentist's pistol. It is possible that the pistol of the assistant, for certain units, is used less frequently than the other instruments. The ultrasonic scaler gave the highest bacterial counts. The bacterial counts from the ultrasonic scalers were also highly variable which could be due to a lower level of utilization. Furthermore, our results indicated a high degree of variability in bacterial counts between the ultrasonic scalers, the air/water pistols and the turbines. We could hypothetically explain these variations by the differences in pressure and water flow as well as by variable diameters of the water lines. These hypotheses will have to be verified by further investigations.

Our study also demonstrated that the age of the dental units had little influence on the bacterial counts. Even the water lines of the new units, which had been installed in the Faculty less than a week before the beginning of our study, and which had never been used to treat patients, were highly contaminated. This supports the comments of Whitehouse on this subject. These observations demonstrate two points: first, the main source of water line contamination cannot be the patient, the new units which were contaminated had never been used to treat patients; second, the colonization of the water lines is a rapid process which reaches a plateau within a short period of time. Cabot Abel et al. have demonstrated that bacterial counts return to normal levels only two hours after turning off the unit. It is reasonable to think that the water entering the unit, even if it meets normal standards (0-500 CFU/ml), becomes contaminated in the water lines by contact with biofilm. The microbiological quality of the water feeding the unit can thus not be used as an indicator of the quality of the water coming out of the unit. We have identified two possible sources, that are non-exclusive, which are responsible for the contamination. The water lines of the dental units provided by the manufacturer are not sterile and the bacteria present could contribute to the colonization of the lines when they are filled with water. However, the major source of contamination is, in our opinion, the water feeding the unit. This water is not sterile as it naturally contains certain species of microorganisms. Microscopic imperfections on the internal surfaces of the water lines could also allow the microorganisms in the water to induce the formation of colonies which in turn would initiate the establishment of a biofilm. This biofilm is firmly attached to the surface of the water line due to the adherent properties of certain microorganisms and by a polysaccharide matrix which provides cohesion to the biofilm. This biofilm which covers the internal surfaces of the water lines of dental units thus becomes the source of the large number of bacteria found at the outlet of handpieces as the flushing action of the circulating water removes superficial layers of bacteria which are then ejected with the water. This property of biofilms also explains why using sterile water to feed dental units is not effective. The water feeding the dental units normally contains fewer than 100 CFU/ml (median: 15 CFU) while the water coming out of the unit contains between 500 and 3,000 times more bacteria. The contamination of the water occurs, therefore, from a reservoir of microorganisms: the biofilm.
We were not able to detect the presence of coliforms in the water of the dental units that we tested. This absence of coliforms, although reassuring, should not surprise us. We believe that the presence of such bacteria would indicate a contamination of the water by fecal material and a contamination of the units. Furthermore, as Geldreich29 reported, a bacterial concentration greater than 500 CFU/ml can mask the presence of coliforms by bacterial competition during the growth of the cultures. Moreover, although certain authors have reported the presence of oral bacteria in the water of dental units9,13,15,26, we eliminated the possibility of any significant contamination by oral bacteria by plating the samples on selective media and culturing them in the absence of oxygen. Such contamination remains possible although minimal due to the incorporation of check valves in the water lines which prevent a reaspiration of oral fluids. The sterilization of handpieces carried out after each patient contributes to eliminating the risk of contaminating water lines by oral microorganisms. The use of ultrasensitive techniques such as the polymerase chain reaction (PCR) has nevertheless allowed certain researchers to establish that tissue fragments and viruses have the potential to contaminate water lines5.

The Pseudomonads constituted more than 60% of the bacteria sampled and Pseudomonas paucimobilis alone accounted for more than 50% The other bacteria identified included P. putida, P. vesicularis, Acinetobacter calcoaceticus and Rhodobacter sp. as well as other unidentified Gram negative bacterial species. Variations exist between the units of different clinics, in the water of the same unit as well as in the time (results not shown). Bacteria from the genus Pseudomonas are well known in the medical field as important opportunistic pathogens in cases of nosocomial infections. We did not try to detect the presence of bacteria from the genus Legionella. Certain authors have mentioned the presence of Legionella pneumophila, albeit in small numbers, in the water of certain dental units18,27.

Control measures

The literature is full of measures and recommendations for controlling the quality of the water of dental units. Flushing water lines considerably reduces bacterial counts but does not allow levels to fall below those acceptable for drinking water11,19,30. Furthermore, such flushing must be carried out in such a fashion to avoid misting which can contaminate the ambient air13. Nevertheless, flushing the water lines for a period of two minutes can decrease the number of microorganisms in the water of dental units. This flushing is, however, insufficient to reduce bacterial counts below the 500 CFU/ml limit unless unrealistically long clinical times are used. The use of sterile or distilled water is of no use in solving this problem. Even sterile water circulating in a tube covered with a biofilm will become as contaminated as normal tap water. Biocides are effective at eliminating bacteria in suspension in the water but do not work efficiently in the biofilm, which is the source of the contamination9,14,16. Freezing the water lines could eliminate the biofilm but this technique cannot be applied to dental units. The use of antibacterial filters near the outlets of the water lines only serves to avoid the problem and such filters must be changed frequently. Tubes with antimicrobial properties33 or composed of materials that do not promote the formation of a biofilm could be an interesting solution, but no studies have demonstrated the effectiveness of such tubes over the long term.

Conclusions and recommendations
The water lines of all the dental units sampled in our study were highly contaminated with microorganisms, some of which are opportunistic pathogens. We believe that very few dental units are free of this phenomenon. The formation of a biofilm is a normal phenomenon that is inherent to all systems containing tubes where the water frequently remains stagnant. Dental units are particularly vulnerable to this type of contamination. The problem, however, does not lie with the presence of bacteria in the water but with the number of bacteria and the opportunistic pathogens which may be present. The stagnation of the water in these lines, which allows the bacteria to multiply, increases the number of bacteria to unacceptable levels for medical treatments as they sometimes exceed standards for drinking water by more than 1000 times after a two-minute flushing period. With the growing number of immuno-deficient patients that may be treated in a dental office and the aging of the population, this problem is becoming one of considerable importance. Eliminating contaminated water must be an integral part of a complete infection control program in dental offices.

In our opinion, the only way to lower bacterial counts in the water that feeds handpieces to acceptable levels is to eliminate the biofilm and to prevent its return.

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The Problem of Contaminated Dental Unit Water Lines.

Newspaper, radio and television reports of unsafe, disease-causing water dispensed from dental unit water lines (DUWL’s) is causing concern among patients...and in some cases, keeping them out of your dental chair.

The problem of contaminated dental unit water is virtually universal, with some studies showing that more than 90% of tested dental units deliver grossly substandard water..." Dental waterlines provide an optimal breeding ground for microorganisms. This is not surprising considering the physical laws governing water flow in tubing. Biofilm formation results from initial bacterial adhesion. Because of the high surface area-to-volume ratio, water flows faster in the center of the tube than it does along the surface of the tube. So dental lines become home to a complex microbial matrix that adheres to the walls of the tubing. Micro-organisms that cling to the matrix break off into the flowing water, where they are dispersed by high speed handpieces, water syringes, and scalers.

Experts stress that the microbial presence in dental unit water may be a real threat to medically compromised patients, like the elderly, the immune-surpressed, and HIV-positive patients. Indeed, studies show that contaminated dental water also poses a risk to dental professionals. Seroconversion rates to legionella, a waterborne pathogen that can cause Legionnaires’ disease, are significantly higher among dental workers than the general population.
Recognition of the problem has, of course, brought about regulatory interest. The CDC, in formulating infection control guidelines for dentistry, recommended specific practices. Because dental waterlines were classified as medical devices, a 1993 interagency agreement between the EPA and the FDA gave the FDA responsibility for premarket approval or clearance of devices that claim to impact the performance of dental waterlines. And, on August 9, 1996, the California State Board of Dental Examiners officially mandated that, "Sterile coolants/irrigants shall be used for surgical procedures involving soft tissue or bone. Sterile coolant/irrigants are deemed to be sterile when delivered using a device or process that has a Federal Drug Administration (FDA) marketing clearance for delivery of sterile/coolant irrigants to the patient."


Legionella bacteria are ubiquitous in freshwater aquatic systems, and humans are infected by them primarily through inhalation of contaminated aerosols. This study analyzed a total of 47 water samples from dental lines in private dental offices and university and hospital dental clinics for Legionella using the polymerase chain reaction, direct fluorescent antibody staining and culture techniques. The typical temperature of dental waterlines (23 C) combined with Legionella's ability to form biofilms, stagnation of the water in the lines and a low chlorine residual all potentially create a unique niche for this microorganism.

Links for information

http://www.aquatechnology.net/dental_articles_3.html

http://www.aquatechnology.net/dental_articles_4.html

Prevalence of Legionella-specific IgG and IgM antibody in a dental clinic population. Fotos PG, Westfall HN, Snyder IS, Miller RW, Mutchler BM

This study was undertaken to determine the frequency of Legionella infection in a dental clinic setting. Serum samples from 270 dental clinic personnel were evaluated using an enzyme-linked immunosorbent assay to detect Legionella-specific IgM and IgG antibodies. The pooled-species whole-cell-antigen preparation used in these assays was derived from six Legionella pneumophila strains and one strain each from Legionella bozemanii and Legionella micdadei. Significant levels of IgG and IgM antibodies were found in 20% and 16%, respectively, of the samples. This compares with 8% and 10%, respectively, for a randomly selected non-clinical group from the region (P less than 0.005). Samples from clinic personnel with significant IgG titers (greater than 1:128) were also evaluated for activity to each of the
eight single-species antigens, with the following results: L. pneumophila, 45% (combined six strains); L. micdadei, 37%; and L. bozemanii, 18%. Comparing individuals' "years spent in the clinic environment" with the incidence of significant antibody levels strongly suggests that the risk of Legionella infection increases proportionately with increased clinic exposure time (P less than 0.05). Analysis of these data implies that Legionella may be present in the dental clinic environment, thus creating an increased risk for clinical personnel or patients.

Epidemiol Infect 1987 Aug;99(1):159-66
Widespread Legionella pneumophila contamination of dental stations in a dental school without apparent human infection.
Oppenheim BA, Sefton AM, Gill ON, Tyler JE, O'Mahony MC, Richards JM, Dennis PJ, Harrison TG

Following isolation of Legionella pneumophila from a special dental station water circuit, used primarily to cool high-speed dental drills which produce fine aerosols, a case finding and environmental survey was undertaken. Widespread colonization of the dental stations was found and the results suggested that amplification of the background levels of L. pneumophila was taking place within the stations. However there was no evidence for transmission causing human infection.

Serological examinations for antibodies against Legionella species in dental personnel.
Reinthaler FF, Mascher F, Stunzner DH
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Serum samples from 107 dentists, dental assistants, and dental technicians were examined with an indirect immunofluorescence test for antibodies to Legionella pneumophila SG1-SG6, L. micdadei, L. bozemanii, L. dumoffii, L. gormanii, L. jordanis, and L. longbeachae SG1 + 2. Thirty-six (34%) employees from dental personnel from 13 practices showed a positive reaction for antibodies to Legionella pneumophila. Only five samples (5%) from a control group (non-medical workers) were positive. Of the 36 positive serum samples, 13 (36%) reacted with Serogroup 6, 12 with SG 1 (33%), 12 with SG 5 (33%), and three with SG 4 (8%), and eight samples were positive for antibodies to other Legionella species. Dentists had the highest prevalence (50%) of L. pneumophila antibodies, followed by assistants (38%) and technicians (20%). These results indicate that dental personnel are at an increased risk of legionella infection.

J Hosp Infect 1990 Jul;16(1):9-18 Related Articles, Books Published erratum appears in J Hosp Infect 1990 Nov;16(4):393
The efficacy of chlorination and filtration in the control and eradication of Legionella from dental chair water systems.
Pankhurst CL, Philpott-Howard JN, Hewitt JH, Casewell MW
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The apparent failure of hyperchlorination and continuous dosing with chlorine to eliminate legionellae from a dental teaching hospital water supply prompted a prospective study to evaluate charcoal filters as a means of decontamination. Legionella pneumophila serogroup 10 and L. bozemanii serogroup 2 were isolated from dental units yielding 10(1)-10(3) colony forming units (cfu) ml-1 with total bacterial counts in the range 10(2)-greater than 10(4) cfu ml-1. After chair-side installation of charcoal filters bacterial contamination of the dental unit water was prevented and legionellae were initially not detected, but after 7 days the total count returned to pre-filtration levels of greater than 10(4) cfu ml-1; L. pneumophila serogroup 10 was eliminated but L. bozemanii serogroup 2 persisted. These results suggest that neither chlorination nor charcoal filtration deal adequately with the potential hazard of Legionella spp. in dental water.


Microbial contamination of dental unit waterlines: the scientific argument.
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The quality of dental unit water is of considerable importance since patients and dental staff are regularly exposed to water and aerosols generated from the dental unit. The unique feature of dental chair water lines is the capacity for rapid development of a biofilm on the dental water supply lines combined with the generation of potentially contaminated aerosols. The biofilm, which is derived from bacteria in the incoming water and is intrinsically resistant to most biocides, then becomes the primary reservoir for continued contamination of the system. Dental water may become heavily contaminated with opportunistic respiratory pathogens such as Legionella and Mycobacterium spp. The significance of such exposure to patients and the dental team is discussed. There is at the present time, no evidence of a widespread public health problem from exposure to dental unit water. Nevertheless, the goal of infection control is to minimise the risk from exposure to potential pathogens and to create a safe working environment in which to treat patients. This paper evaluates the range of currently available infection control methods and prevention strategies which are designed to reduce the impact of the biofilm on dental water contamination, and are suitable for use in general practice. Bacterial load in dental unit water can be kept at or below recommended guidelines for drinking water (less than 200 colony forming units/ml) using a combination of readily available measures and strict adherence to maintenance protocols. Sterile water should be employed for all surgical treatments.


Molecular techniques reveal high prevalence of Legionella in dental units.
Williams HN, Paszko-Kolva C, Shahamat M, Palmer C, Pettis C, Kelley J Department of Oral and Craniofacial Biological Sciences, Baltimore College of Dental Surgery, Dental School, University of Maryland at Baltimore, USA.

Legionella bacteria are ubiquitous in freshwater aquatic systems, and humans are infected by them primarily through inhalation of contaminated aerosols. This study analyzed a total of 47 water samples from dental lines in private dental offices and university and hospital dental clinics for Legionella using the polymerase chain
reaction, direct fluorescent antibody staining and culture techniques. The typical temperature of dental waterlines (23 C) combined with Legionella's ability to form biofilms, stagnation of the water in the lines and a low chlorine residual all potentially create a unique niche for this microorganism.