SHORT PAPER

Oral pathogens in duwl's: risk of infection and strategies for their control

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Water lines are an important source of potential contamination. Every dental unit is equipped with small-bore flexible plastic tubing to bring water to different hand pieces, such as the air/water syringe, the ultrasonic scaler or the high-speed hand piece. Most dental units are connected directly to municipal distribution systems for potable water and chlorinated or not, this water contains diverse microflora that include viruses, bacteria, yeast, fungi, protozoa, unicellular algae and nematodes. Free-floating (planktonic) microorganisms are vulnerable to environmental stress, biocide activity and microscopic predators. However, once inside the dental unit, such microorganism can settle on the inner turbine surface, initiating a chain of events that results in colonization, microcolony formation and, eventually, biofilm formation [1]

Whereas only water with less than 500 colony-forming units (cfu)/ml, free of coliforms and nephelometric turbidity lower than 2 can be considered potable [2], dental water lines present colony counts ranging from 1,000 to 100,000 cfu/ml (sometimes as high as 200,000 cfu/ml). There are different reasons that provide the opportunity for the development of bacterial biofilm: (i) the high area-to-volume ratio of DUWLs (6:1), which offers a high surface area on which microorganism can settle; (ii) periods of stagnation of water in lines when the dental unit is not in use; (iii) and laminar flow conditions with low shear forces near the lumen wall of the waterlines[3]. American Dental Association (ADA) in 1995 established a goal for dental unit waterlines in order to reduce the level of bacteria to $\leq$ 200 cfu/ml. The problem is that no state or local laws or regulations exist and the same regulations used for drinking water should be applied.

The formation of the biofilms can be regarded as a bacterial strategy for survival in dental units in which a consortium of microbes are enclosed in an exopolysaccharide (EPS) matrix and are attached to either biotic or abiotic surfaces. This EPS provides added protection to the biofilm microorganims by limiting the diffusion of surfactants, biocides and antibiotics as well as by acting as a nutrient source for the bacterial community[3]
The well-established biofilm constitutes a reservoir of bacteria; in most cases saprophytic, heterotrophic, Gram-negative,—aerobic or facultative. At first, these microorganisms are being considered as non-pathogenic in dentistry. Biofilm includes a high diversity of bacterial groups from symbionts to predators. For example, *Geobacter*, a strict anaerobe involved in the reduction of Fe (III), may benefit from close association with *Leptothrix* involved in the oxidation and chelation of iron, and common in water distribution systems. On the other hand, *Bdellovibrio*, a predatory organism, prey upon a wide range of gram-negative bacteria [3]
Persistent presence of microorganisms in dental units water lines (DUWLs) has been demonstrated by different authors[4,5]. The range of microorganisms isolated from samples obtained from DUWLs include bacteria and eukaryotic microorganisms. However, to date, viruses have not been detected in DUWL's [6]. It has been shown that DUWL's are densely colonized during routine dental practice with both environmental (e.g. *Moraxella* spp. and *Flavobacterium* spp.) as well as opportunistic and true human pathogens (e.g. *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Mycobacterium* spp., and *Staphylococcus* spp.) [6] mainly originated from incoming municipal water and to a lesser extend sucked back into the lines during dental procedures due to inappropriate work of anti-retraction mechanisms included in dental units.

Risk of infection from dental unit water lines
Some organisms found in DUWL's may suppose a health risk to some patients. *Leptospira*,a common organism detected in biofilm can invade any susceptible mucosal membrane and cause leptospirosis. *Sphingomonas* and *Legionella* are easily spread via aerosols and have been found in hospital environments, including devices as mechanical ventilators, catheters and bronchofiberoscopes [7,8.] *Sphingomonas* spp.strains secrete viscous polysaccharides which help in the development of the biofilm and *Legionella* species are known to cause respiratory infections. Besides, *L. pneumophila* can survive within amoeba which can protect the bacteria from chlorination. Their presence could suppose a potential risk to patients and dental personnel due to the aerosols generated from dental handpieces during treatment. In fact, it has been demostrated that dental personnel have a significantly higher antibody titer to *L. pneumophila* than other person [9] .

Infections of non-tuberculosis mycobacteria (NTM), which are defined as those mycobacteria that are not part of the *Mycobacterium tuberculosis* complex, are increasing in both industrialized and developing countries not only in immunocompromised but also in immunocompetent people . Various NTM (*M fortuitum, M. chelonae* and *M. phlei*) have been associated experimentally with biofilm formation under different conditions and it could result in an increase in resistance to conventional biocides used in clinical and industrial settings[10]. Although all of this experiments have been realized with non pathogenic NTM, these results may be extrapolated to pathogenic NTM, as the mechanism of biocide killing are independent of virulence factors and grow rate.

The interest in the study of DUWL's stable biofilms and the eventual colonization of DUWL's by human oral bacteria is mainly due to the concern originated by the increasing number of immunocompromised patients as well as the emerging awareness of occupational hazards in the dental offices). Only *P. aeruginosa* derived from DUWL has been shown to cause oral infection in patients. [11]. *P. aeruginosa* is considered an opportunistic pathogen highly virulent in some particular conditions such as in immunocompromised patients. Most of these patients require odontologic assistance since one of the clinical manifestations of their disease is periodontitis, in these cases exceptional caution should be exercised in order to prevent occasional infections.

However, there is little additional epidemiological evidence that microbial contamination of dental unit water lines constitutes a significant risk of infection to either patients or their dentists, probably due to difficulties in collecting appropriate data. Conventional culture methods do not provide a representative profile of the true composition of microbial comunities in DUWLs. When enrichment procedures were applied the percentage of positive microorganisms in DUWLs was dramatically increased. In general it is assumed that human pathogens cannot multiply in external media and that when these microorganisms are in the environment they can occasionally survive although they do it in a stressed state. Moreover, several authors have pointed out that it is feasible to detect the presence of bacteria still alive but unable to form colonies on conventional culture media. These microorganisms so called VBNC (Viable but non culturable) can be active as infectious agents but, in general, microbiologists cannot detect them by conventional methods. Among culturable pathogenic bacteria surviving in cold and, theoretically, clean water such as that of the DUWLs it is feasible that most of them are in low proportion when compared with bacteria forming biofilms. Additionally it should be stressed that the viability checked by direct platting on selective media appears considerably reduced. However, when a pre-enrichment step in a rich medium such as thioglycolate is used the successful detection of human pathogens increases drastically. Thus, it is clear that in order to evaluate the actual risk of infections spread by DUWLs the inclusion of a step of pre-enrichment is highly recommended.

Strategies to control risk of infection in DUWL's

American Dental Association (ADA) and Centers for Disease Control and Prevention (CDC) make some recommendations[12].

- Flushing waterlines for several minutes before the first patient of the day and for 20-30 s between patients to remove material that may be retracted during treatment. Although flushing can reduce the numbers of bacteria in dental treatment water, the effects are transient and has no effect on the biofilm.
- Improve the quality of dental unit water, installing filters before the water reaches the dental unit or use an independent water reservoir system with a periodic disinfectant treatment protocol, ultra-violet radiation disinfection and the use of in-line filters
- Install filters near the handpieces to barrier the passage of microorganisms.
- Install anti-retraction valves in dental lines (installed inside the handpieces or inside the DUWL's) to prevent retrograde aspiration of oral secretions into the water supply line.
- Autoclave or sterilize solutions and handpieces, or replace handpiece between patients.
- Use chemical products (disinfectants) to reduce bacterial counts in dental units water lines. In general, disinfectants remain in the lines overnight and they are flushed from the lines next morning.

However, the problem of microbial contamination of DUWLs still exists due to the intricacy and complexity of dental units. It seems that immediate solutions wouldn't appear yet . The long-term solution to the problem could lie in redesigning the water supply system within dental units to eliminate stagnant areas and to low the biofilm build up. In shorter term, disinfectants may have a role to play in controlling the levels of microbial contamination.

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