Influence of material and tube size on DUWLs contamination in a pilot plant

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SUMMARY

Numerous studies have shown that the water discharged from dental unit waterlines (DUWLs) contains high densities of bacteria, especially non-fermenting Gram negative bacteria.

The aim of the present study was to investigate how the material (polyethylene-PE and polytetrafluorethylene-PTFE) and size (1.6 and 4.0 mm) of 4 waterlines in a pilot plant influence the level of contamination in the output water. The water contamination was assessed by analyzing the trend of the heterotrophic plate counts at 22°C as a function of time and by testing for non-fermenting Gram negative bacteria.

In all waterlines, the bacterial density increased exponentially during the first months and thereafter remained between 10^4 and 10^6 cfu/ml. However, the plate count at 22°C was lower in the water from PTFE tubes and from larger size tubes.

Comamonas acidovorans, Pseudomonas aeruginosa and *Pseudomonas fluorescens* were isolated. *Pseudomonas aeruginosa,* responsible for infections associated with dental practice, was never isolated in the output water from PTFE tubes.

In order to control bacterial contamination the results suggest the use of waterlines made of PTFE on account of their ability to inhibit the colonization and growth of *Pseudomonas aeruginosa.*

KEY WORDS: Dental unit, Tubing material, Tube size, Water contamination

Received September 26, 2006 *Accepted* December 12, 2006

INTRODUCTION

Many studies have demonstrated that the output water from dental unit waterlines (DUWLs) contains high densities of bacteria which may have sloughed from biofilm formed inside the tubes (Mills 2000; Walker *et al.* 2000; Lee *et al.* 2001; DePaola *et al.* 2002; Montebugnoli *et al.* 2004; Szymanska 2006). It has also been shown that opportunistic pathogens such as *Pseudomonas aeruginosa, Legionella pneumophila*, *Comamonas acidovorans* are frequently present in water from DUWLs (Stampi *et al.* 1999, Zanetti *et al.* 2000; Tuttlebee *et al.* 2002; Szymanska 2005; Dutil *et al.* 2006; O'Donnell *et al.* 2006).

Various methods have been proposed for preventing or limiting the microbial contamination in DUWLs. A common practice, first proposed by Barbeau *et al.* (1996), is water flushing for some minutes before starting work. This method may effectively reduce the concentration of planktonic bacteria, but is not able to remove the biofilm (Smith *et al.* 2001; Smith *et al.* 2002; Ozcan *et al.* 2003; Walker *et al.* 2003). Chemical treatments have also been proposed, but the bacteria present within the biofilm are resistant to most biocides (Codony *et al.* 2003). Some chemical agents (sodium hypochlorite, chlorexidine, glutaraldehyde, etc.) are generally effective but present drawbacks such as the corrosion and

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deterioration of the dental unit's internal components (Zanetti *et al.* 2003). In addition, the aerosolization of these substances may lead to the chronic exposure of dental staff, especially if the chemical treatment is systematic (Meiller *et al.* 1999).
A simple measure suggested to prevent biofilm formation and reduce bacterial outflow from DUWLs is the use of tubes made of materials that inhibit bacterial accumulation (Yabune *et al.* 2005).
This study aimed to investigate the influence of the material and tube size in limiting bacterial contamination in DUWLs. Water contamination was assessed by analyzing the trend of the heterotrophic plate count (HPC) at 22 °C as a function of time and by counting and identifying non-fermenting Gram negative bacteria that commonly colonize DUWLs.

MATERIAL AND METHODS

Sampling
For the purposes of the study an Italian firm specializing in the production of dental units created a pilot plant equipped with 4 waterlines (scheme). The tubes had the following characteristics:

a) Polyethylene tube (PE), internal diameter of 1.6 mm, medium flow rate =1 ml/s.
b) Polytetrafluorethylene tube (PTFE), internal diameter of 1.6 mm, medium flow rate=1 ml/s.
c) Polyethylene tube (PE), internal diameter of 4 mm, medium flow rate =2 ml/s.
d) Polytetrafluorethylene tube (PTFE), internal diameter of 4.0 mm, medium flow rate =2 ml/s.

The length of the tubes (A and B = 1550 mm; C and D = 510 mm) was established so that the extent of the internal surface was the same in all tubes and the water flow during sampling was as close as possible to that of dental units normal working conditions.
The pilot plant used chlorinated municipal water. Before commencing the study the waterlines were disinfected with 3% hydrogen peroxide (contact time = 60 min). No further chemical treatment was carried out throughout the period of research.
Water sampling was performed periodically until 30 months after installation. On each occasion all the stagnant water present in the tubes (respectively 3.1 ml in A and B, and 6.3 ml in C and D) was removed through special taps and collected in graded sterile test tubes using aseptic techniques. In order to neutralize the residual chlorine in the water, 1-2 drops of 10% sodium thiosulphate were added. The samples were processed within 1-2 hours.
After each sampling the waterlines were refilled and the water remained stagnant until the next collection.
During the study 20 collections were made, amounting to a total of 80 output water samples. In each sample the residual chlorine, heterotrophic plate count at 22°C and non-fermenting Gram negative bacteria were determined. Since the pilot plant was never used for dental treatment, no search was made for bacteria normally originating from the oral cavity of patients.
The same parameters were examined for the incoming water each time the circuit was refilled.

Microbial and chemical analysis
22°C HPC was made by the pour plate method (APHA, 1998) using Plate Count Agar (Oxoid). The mean value of three replicates was calculated. Non-fermenting Gram negative bacteria were determined by the membrane filtration technique: 100 ml of incoming water and suitable aliquots of the outgoing water samples, diluted with sterile distilled water, were filtered through a 0.45 µm pore-size membrane (Millipore). Membranes were placed on Pseudomonas CFC agar (Oxoid) and incubated at 30°C for 48 h. The colonies were counted and differentiated and at least three isolates of each type of colony were subcultured on Tryptone Soy Agar (Oxoid) and identified by the API 20NE system (bioMérieux). The DPD Colorimetric Method (APHA, 1998) was used to measure the residual chlorine.

Presentation of results
The bacteriological data were converted into $Log_{10}x$. The t-test (paired) was used to compare the non-fermenting Gram negative bacteria contamination of the four water lines. The simple correlation test was used to compare the Pseudomonadaceae contamination level of the supply water with that of the water leaving the

four water lines. The significance of difference was assumed at $p<0.05$.
All descriptive and statistical calculations were carried out using the StatView program (Abacus Concepts Inc., Berkley, CA, USA) on an Apple Macintosh computer.

RESULTS

The incoming water showed residual chlorine: 0.02 mg/l (0.01 to 0.12 mg/l) and 22°C HPC: 1.65 Log cfu/ml (0.30 to 1.94 Log cfu/ml). The residual chlorine could not be determined (density below the limit of detectability) in water samples from DUWLs.
Figures 1 and 2 show the trend of the HPC at 22°C over time in the different types of waterlines.
In all waterlines bacterial contamination of the water increased progressively until around 4-4.5 months from the start of the study (reaching maximum values of 10^7 cfu/ml); thereafter the levels remained between approx. 10^4 and 10^6 cfu/ml.
In 72.5% of samples, the lowest counts were observed in water from the PTFE tubes (Figure 1).
In both the PTFE and PE tubes the HPC were often lower when the tube size was greater (Figure 2).
Three species of non-fermenting Gram negative bacteria were isolated from DUWL water samples: *Comamonas acidovorans, Pseudomonas fluorescens* and *Pseudomonas aeruginosa* (Table 1).
Comamonas acidovorans was detected in all the samples of water discharged from PTFE tubing. In samples from PE tubing the frequency was 50% and 100% when the size was 1.6 mm and 4 mm respectively (Table 1).
Pseudomonas fluorescens was detected in 60% of the samples from PE tubes and in 25% of the samples from the PTFE tubes. Occasionally (28.3%) it was isolated in association with *Comamonas acidovorans*.

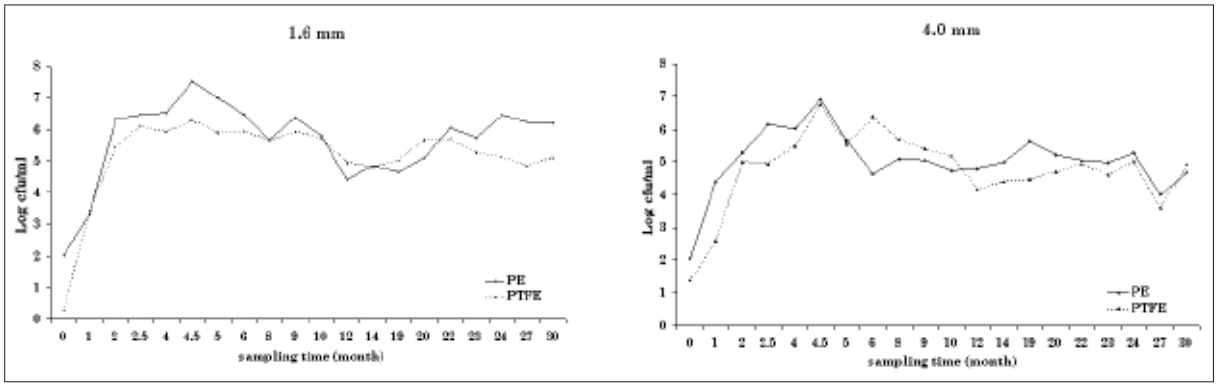

FIGURE 1 - Heterotrophic plate count at 22°C in different size and material tubings.

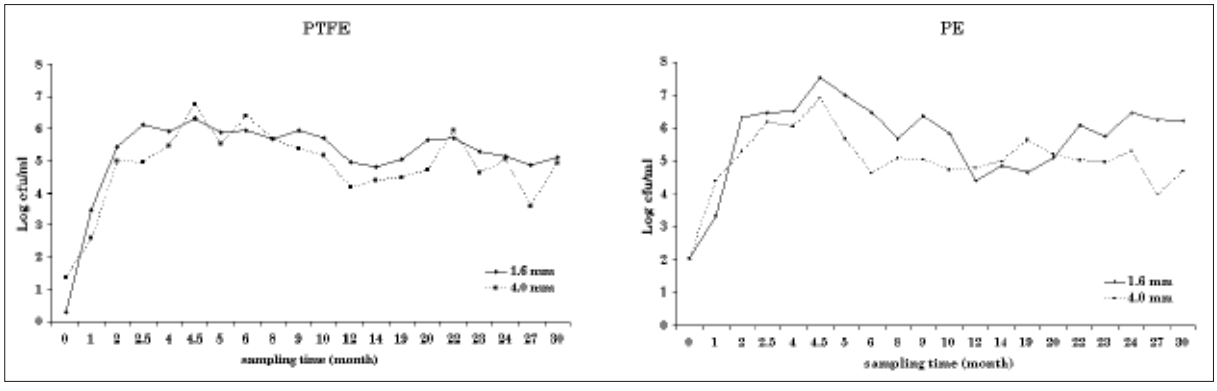

FIGURE 2 - Heterotrophic plate count at 22°C in different material and size tubings.

TABLE 1 - Percentage of positive samples and mean values (Log cfu/100 ml) of bacteria detected from *Pseudomonas* CFC agar

	Pseudomonas aeruginosa		*Pseudomonas fluorescens*		*Comamonas acidovorans*	
	% positive samples	*mean**	*% positive samples*	*mean**	*% positive samples*	*mean**
Incoming water	5.0	0.30	10.0	1.34	35.0	2.24
Output water						
PE (1.6 mm)	15.0	6.74	85.0	6.39	50.0	5.44
PTFE (1.6 mm)	-	-	-	-	100.0	6.17
PE (4.0 mm)	15.0	5.00	35.0	5.47	100.0	6.41
PTFE (4.0 mm)	-	-	50.0	4.00	100.0	5.43

*Mean of concentrations detected in positive samples; PE = polyethylene; PTFE = polytetrafluorethylene

Pseudomonas aeruginosa was isolated from 15% of samples from PE tubes and was never detected in water from PTFE tubes.
The mean values of the bacterial concentrations found in positive samples were high (Table 1).
The same species were detected in the incoming water, though less frequently and at much lower concentrations (Table 1). No statistically significant correlation in the counts of non-fermenting Gram negative bacteria was found between the incoming water and the water leaving the four water lines (p=NS).
Paired t-test revealed a statistically significant difference between the counts of *Pseudomonas fluorescens* in the water leaving the 1.6 mm PE tube and the 4.0 PE tube (t-test =2.82; $p<0.05$) and also between the counts of *Comamonas acidovorans* in the water leaving the 1.6 mm PTFE tube and 4.0 PTFE tube (t-test =3.09; $p<0.01$).

DISCUSSION

The waterlines of the pilot plant in question showed a high level of bacterial contamination already one month after installation. Although the levels found in the incoming water were always within the limits set by Italian law for drinking water (HPC less than 100 cfu/ml at 22 °C) (Legislative Decree no. 31, 2001), the water discharged from the DUWLs, irrespective of the type of tubing, showed values of HPC that were consistently higher than those recommended by the same law and by the guidelines of the American Dental Association (ADA) (HPC less than 200 cfu/ml) (Anonymous, 1996). All the tubes were seen to be suitable for the growth of bacteria when the water is stagnant. The fluctuations observed can probably be explained by the sloughing of fragments of biofilm from the inner surfaces of the tubes.
The lowest levels were found in the PTFE tubes and in the larger tubes.
The data obtained are in partial agreement with those reported by Schonen and Weshe (1988), who found a high level of microbial growth in PE tubes but negligible growth in PTFE tubes. More recently Yabune *et al.* (2005) showed that fluoridated resins were effective in inhibiting biofilm formation in the tubes and in reducing bacterial outflow from DUWLs. Comparison with our results is nevertheless limited by the fact that no details of the tube size (and thus of the flow) were given by the authors quoted.
The higher bacterial densities detected in the water from the smaller size tubes could be explained by the greater surface/volume ratio which may favour waterborne bacterial colonization of the inner walls (Barbeau 2000). Moreover, the water within the tubes of smaller diameter flows more slowly at the internal surface than that at the centre of the tube and thus allows the bacteria to proliferate before eventually dispersing in the waterlines as plancktonic forms (O'Donnell *et al.* 2006).
Finally, as previously reported by the present authors (Stampi *et al.* 1996) and by others (Barbeau *et al.* 1996; Szymanska 2005) the water from DUWLs may also be contaminated by opportunistic pathogens such as *Pseudomonas*

aeruginosa, which can be responsible for infections associated with dental practice (DePaola 2002). In the present study *Pseudomonas aeruginosa* was detected only in the circuits made of PE, never in those of PTFE.

In conclusion, under our experimental conditions, the use of different size tubing and material was not sufficient to reduce bacterial outflow from DUWLs to within acceptable limits. Nevertheless, PTFE tubing is preferable to PE on account of its ability to inhibit the colonization and growth of *Pseudomonas aeruginosa*. This aspect is of great importance since even after disinfection treatment *Pseudomonas aeruginosa* is able to recolonize the DUWLs during periods of rest and stagnation, as reported in our previous study (Zanetti *et al.* 2003).

ACKNOWLEDGEMENTS

The Authors wish to thank Ing E. Scalzotto and OMS Spa for their assistance.

This work was supported by grants for Local Projects provided by the Ministry of University and Scientific Research.

REFERENCES

ANONYMOUS (1996). ADA Statement on dental unit waterlines. *J Am Dent Assoc*, **127**, 186-186.

A.P.H.A. (1998). Standard Methods for the Examination of Water and Wastewater, 20th edn Washington, DC: APHA-AWWA-WEF.

BARBEAU, J., TANGUAY, R., FAUCHER, E., AVEZARD, C., TRUDEL, L., COTÉ, L., AND PRÉVOST, A.P. (1996). Multiparametric analysis of waterline contamination in dental units. *Appl Environ Microb,* **62**, 3954-3959.

BARBEAU, J. (2000). Waterborne biofilms and Dentistry: the changing face of infection control. *J Can Dent Assoc*, **66**, 539-541.

CODONY, F., DOMENICO, P., AND MAS, J. (2003). Assessment of bismuth thiols and conventional disinfectants on drinking water biofilms. *J Appl Microbiol*, **95**, 288-293.

D. Lgs. 2 febbraio 2001, n° 31. Attuazione della Direttiva 98/83/CE relativa alle acque destinate al consumo umano. G.U. della Repubblica Italiana. Supplemento Ordinario n° 52 del 3 marzo 2001.

DEPAOLA, L.G., MANGAN, D., MILLS, S.E., COSTERTON, W., BARBEAU, J., SHEARER, B., AND BARLETT, J. (2002). A review of the science regarding dental unit waterlines. *J Am Dent Assoc*, **133**, 1199-1206.

DUTIL, S., TESSIER, S., VEILLETTE, M., LAFLAMME, C., MERIAUX, A., LEDUC, A., BARBEAU, J., AND DUCHAINE, C. (2006). DETECTION OF *LEGIONELLA* SPP. BY FLUORESCENT in situ hybridization in dental unit waterlines. *J Appl Microbiol*, **100** (5), 955-963.

LEE, T.K., WAKED, E.J., WOLINSKY, L.E., MITO, R.S., AND DANIELSON, R.E. (2001). Controlling biofilm and microbial contamination in dental unit waterlines. *J California Dent Assoc*, **29**, 679-684.

MEILLER, T.F., DEPAOLA, L.G., KELLEY, J.I., BAQUI, A.A., TURNG, B.F., AND FALKLER, W.A. (1999). Dental unit waterlines: biofilm, disinfection and recurrence. *J Am Dent Assoc*, **130**, 65-72.

MILLS, S.E. (2000). The dental unit waterline controversy: defusing the myths, defining the solutions. *J Am Dent Assoc*, **131**, 1427-1441.

MONTEBUGNOLI, L., CHERSONI, S., PRATI, C., AND DOLCI, G. (2004). A between-patient disinfection method to control water line contamination and biofilm inside dental units. *J Hosp Infect*, **56**, 297-304.

O'DONNEL, M.J., SHORE, A.C., AND COLEMAN, D.C. (2006). A novel automated waterline cleaning system that facilitates effective and consistent control of microbial biofilm contamination of dental chair unit waterlines: A one-year study. *J Dent*, **23**, 1-14.

OZCAN, M., KULAK, Y., AND KAZAZOGLU, E. (2003). The effect of disinfectant agents in eliminating the contamination of dental unit water. *J Oral Rehabil*, **30**, 290-294.

SCHONEN, D., AND WEHSE, A. (1988). Microbial contamination of water by pipe and hose material. 1. Detection of colony count changes. *Zbl Bakt-Mykr Hyg B*, **186**, 108-117.

SMITH, A.J., BAGG, J., AND HOOD, J. (2001). Use of chlorine dioxide to disinfect dental unit waterlines. *J Hosp Infect*, **49**, 285-288.

SMITH, A.J., MCHUGH, S., MCCORMI,C.K.L., STANSFIELD, R., MCMILLAN, A., AND HOOD, J. (2002). A cross sectional study of water quality from dental unit water lines in dental practices in the West of Scotland. *Br Dent J*, **193**(11), 645-648.

STAMPI, S., ZANETTI, F., DE LUCA, G., ROMANO, G., PISTACCHIO, E., AND TONELLI, E. (1996). Effect of water softening and heating on microbial contamination of dental unit systems. *Zbl Hyg Umweltmed*, **198**, 522-530.

STAMPI, S., ZANETTI, F., BERGAMASCHI, A., AND DE LUCA, G. (1999). *Comamonas acidovorans* contamination of dental unit waters. *Lett Appl Microbiol*, **29**, 52-55.

SZYMANSKA, J. (2005). Endotoxin level as a potential marker of concentration of gram-negative bacteria in water effluent from dental units and in dental aerosols. *Ann Agric Environ Med*, **12**, 229-232.

SZYMANSKA, J. (2006). Bacterial decontamination of DUWL biofilm using Oxygenal 6. *Ann Agric Environ Med*, **13**, 163-167.

Tuttlebee, C.M., O'donnell, M.J., Keane, C.T., Russell, R.J., Sullivan, D.J., Falkiner, F., and Coleman, D.C. (2002). Effective control of dental chair unit waterline biofilm and marked reduction of bacterial contamination of output water using two peroxide-based disinfectants. *J Hosp Infect*, **52**, 192-205.

Walker, J.T., Bradshaw, D.J., Bennet, A.M., Fulford, M.R., Martin, M.V., and Marsh, P.D. (2000). Microbial biofilm formation and contamination of dental-unit water systems in general practice. *Appl Environ Microb*, **66**, 3363-3367.

Walker, J.T., Bradshaw, D.J., Fulford, M.R., and Marsh, P.D. (2003). Microbiological evaluation of a range of disinfectant products to control mixed-species biofilm contamination in a laboratory model of a dental unit water system. *Appl Environ Microb*, **69**, 3327-3332.

Yabune, T., Imazato, S., and Ebisu, S. (2005). Inhibitory effect of PVDF tubes on biofilm formation in dental unit waterlines. *Dent Mater*, **21**(8), 780-786.

Zanetti, F., Stampi, S., De Luca, G., Fateh-Moghadam, P., Bucci Sabattini, and A.M., Checchi, L. (2000). Water characteristics associated with the occurrence of *Legionella pneumophila* in dental units. *Eur J Oral Sci*, **108**, 22-28.

Zanetti, F., De Luca, G., Tarlazzi, P., and Stampi, S. (2003). Decontamination of dental unit water systems with hydrogen peroxide. *Lett Appl Microbiol*, **37**(3), 201-206.