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## ABSTRACT

The purpose of this study was to assess water samples from a hospital dental clinic to determine whether a disinfectant/coolant irrigant containing chlorhexidine (Lines,™ Micrylium Laborator-ies) affects the presence of microbial organisms in dental unit waterlines. Water samples from three hospital dental operatories were collected at baseline and after overnight treatment with a disinfectant-containing irrigant followed by sterile water irrigation. Saliva of treated patients and sterile water rinse specimens were collected from the waterlines of these operatories for three consecutive days, then weekly for eight weeks after treatment. Specimens were cultured to identify total heterotrophic plate counts as well as presence of *Pseudomonas aeruginosa* and *Candida* species. Baseline organism counts varied from 10<sup>3</sup> to 10<sup>5</sup> colony-forming units per milliliter. After treatment, no organisms were detected in waterline discharge. Decontamination of dental unit waterlines is possible using a disinfectant/irrigant followed by sterile water irrigation. The potential for contamination of the lines from patients' saliva may have been reduced due to use of anti-retraction valves and the disinfectant/sterile water irrigation, as conducted in this study.

**KEY WORDS:** dental unit waterlines, disinfection

# The effect of a disinfectant/coolant irrigant on microbes isolated from dental unit water lines

## INTRODUCTION

Several authors have recognized the contamination of dental unit waterlines (DUWL) with microorganisms from source water and/or patients, and the subsequent development of biofilm in the waterline as a potential source of infection for dental patients.<sup>1-15</sup> Despite the lack of epidemiological evidence of infectious outcomes associated with such biofilms, concerns about the microbial colonization of small-diameter waterlines have led to the development of some guidelines.<sup>8,10</sup> However, concern may be warranted for people who are immunocompromised and immunosuppressed, who may be exposed to microorganisms in community water supplies as well as those in water used during dental treatment. During dental treatment, rubber dam placement and high-volume suction could play an important role in reducing the theoretical exposure of such patients to microorganisms that may colonize and proliferate in the DUWL and in biofilms within these lines. The development of guidelines has led to initial studies of disinfecting/sterilizing protocols for DUWL contaminated by biofilm.<sup>1-14,16-22</sup> Documentation of the need for intervention should be based on outcomes studies, since at this time it appears prudent to consider microbial colonization of DUWL as a potential source of infection, particularly among patients who are immunocompromised.

Biofilms form more readily in small diameter tubing with a high ratio of surface area to water volume.<sup>13,15,23-25</sup> Organisms are then shed from the biofilm into the lumen. The organisms in DUWL are typically heterotrophic, gram-negative rods, some of which are considered medically significant.<sup>25,26</sup> Organisms that may be of importance include *Pseudomonas*,<sup>27,28</sup> *Legionella* species<sup>29-31</sup> and *Mycobacterium*,<sup>32</sup> all of which have been considered important in nosocomial infections.<sup>33</sup> However, contamination of DUWL by *Legionella* reportedly is not common.<sup>30,34</sup>

One article<sup>28</sup> has documented potential exposure to *Pseudomonas aeruginosa* from DUWL in two patients who were immunocompromised, and another describes a case of *Legionella* infection following dental treatment.<sup>30</sup> Other articles also have documented such water-related nosocomial infections in people who are immunosuppressed.<sup>33,35,36</sup> In addition, some authors suggest that DUWL, which occasionally are contaminated by *non-pneumophila Legionella* and by *L. pneumophila*, may be related to a higher rate of *Legionella* antibodies in dental care workers,<sup>37,38</sup> although no cases of Legionnaires disease have been documented in this population.<sup>29,39</sup>

Anti-retraction valves have been recommended for placement in dental handpieces for a number of years to reduce "suck-back" of discharged water into the drive and waterlines.<sup>16-18,20</sup> The goal of this measure is to reduce the risk of contamination of the lines with microorganisms originating from the oral cavity. However, municipal water supplies are not sterile, and organisms

found in the small diameter lines of dental units could enter the units from the water supply as well as from a patient source. Biofilms establish themselves as the growth of organisms occurs, regardless of the source.<sup>9,10,20</sup> Coliform growth is used to define whether water is potable; in one study of DUWL,<sup>40</sup> the authors documented coliform counts that met the limit for drinking water. Heterotrophic microbial growth below 500 colony forming units per milliliter (cfu/ml) has been considered acceptable.<sup>41,42</sup>

Developed for use in DUWL, Lines™ (Micrylium Laboratories, Toronto, ON and Phoenix, AZ) is a disinfectant/coolant/irrigant solution that contains chlorhexidine in a polyor irrigant base with mint flavoring. We selected this disinfectant for our study because of its ease of use, safety, U.S. Food and Drug Administration clearance and its ability to kill *P. aeruginosa*. The solution was assessed to determine whether it could disinfect DUWL. In addition, the potential source(s) of the recolonization of waterlines once they had been disinfected also was observed.

## MATERIALS & METHODS

Water samples were collected from three different dental operatories, which included 11 DUWL, and analyzed to establish baseline counts for total heterotrophic plate counts (THPC). Duplicate baseline, pretreatment collections were collected by passing sterile water through the waterlines. These DUWL were then treated with the disinfectant overnight and sterile water was passed through the lines the next day. After treatment of each patient during a single day, waterline specimens were collected by flushing the lines with sterile water. Saliva specimens were collected from each of the treated patients using oral saline rinsing for one minute and collection of the expectorated saline. Saliva and sterile water rinse samples were collected for three consecutive days, after which the lines were allowed to sit unused for a week to allow the potential build-up of biofilm. After one week, a sample of sterile water rinsed through the DUWL was collected. Such collections continued on a weekly basis for eight weeks. Specimens from untreated operatories were also collected. The specimens were refrigerated and sent in batches with frozen packs via overnight express to the microbiological laboratory for analysis.

### Microbiologic methods

R2A agar (Difco Laboratories, Detroit, MI) was used to assess THPC. M-PA-C (Becton Dickinson Microbiology Systems, Cockeysville, MD) was used in culturing *Pseudomonas aeruginosa* and Sabourauds Agar Modified (SAM) (Difco Laboratories, Detroit, MI) was used to assess *Candida* species isolated from patients at each dental visit.

The samples were labeled and mixed on a vortex for 30 seconds. In the first dilution, 50 microliters ( $\mu$ l) of each sample was added to 100 ml of sterile deionized water and mixed. A second dilution was made by taking 100  $\mu$ l from Dilution 1 and adding it to 100 ml of sterile deionized water (Dilution 2). Using Dilution 2, the process was repeated to obtain Dilution 3. All dilutions were done in triplicate – once for each culture medium (R2A, M-PA-C and SAM agar).

Samples for R2A and M-PA-C agar cultures were filtered

(nanometer pore size) then rinsed with 100 ml deionized water. Alcohol/flame-sterilized forceps were used to remove the membrane that was placed on the agar for each of the dilutions. Samples for SAM agar were filtered using Gelman magnetic reusable filter funnels using Metrical filter membrane (0.8 micrometer pore size). Dilution 3 was filtered and rinsed with 100 ml sterile deionized water after which the filter was removed and placed on SAM. New, sterile Metrical filter membranes were used to filter Dilutions 2 and 1.

The petri plates with the membranes were left in the upright position for 15 minutes before being inverted and incubated. R2A cultures were kept at room temperature (23 °Celsius [C]) for seven days, M-PA-C plates were incubated at 41.5 °C for two days, and SAM plates were incubated at 37 °C for two days. To determine colony-forming units per milliliter, the plates were examined and counted (Dilution 1, counts multiplied by 20; Dilution 2 counts multiplied by 20,000; and Dilution 3 counts multiplied by 20,000,000).

The morphology of the colonies from the baseline water and saliva samples were noted with respect to size, color, texture, and consistency. Stock cultures of *P. aeruginosa* (ATCC 15442) and *C. albicans* (ATCC 10231) were used for comparison to aid in identification of these organisms in the sample cultures. Gram stain also was used to aid in identification of yeast. The results were analyzed using the Wilcoxon rank sum test.

## RESULTS

The pretreatment samples from the DUWL showed baseline microorganism counts that ranged from  $6.16 \times 10^3$  to  $4.6 \times 10^5$ , with a median count of  $6.29 \times 10^4$ . The median counts for the control samples collected from untreated lines were  $6.23 \times 10^4$ . No microorganisms were detected after treatment of the lines, indicating that there was no contamination in the waterlines (Table 1).

The THPC in patient saliva samples varied from  $2 \times 10^4$  to  $1.82 \times 10^6$  cfu/mL, with several samples containing *C. albicans*. No *P. aeruginosa* were identified in these samples. After patient treatments, no organisms were recovered from the weekly culture specimens from the DUWL samples collected during the first three weeks after the lines were disinfected. The change in bacterial counts with treatment of the lines was highly statistically significant at all follow-up assessments (Table 1) ( $p = 0.004$ ). At Week 4, bacteria were identified from the DUWL samples, although the detected organisms were not the same as those identified in any of the patient saliva specimens. No *C. albicans* or *P. aeruginosa* were identified in any of the specimens from the treated DUWL.

High THPC (total counts) were observed initially before disinfectant was flushed through the DUWL; however control samples collected from DUWL of operatories that were not treated with the disinfectant were heavily contaminated with microorganisms. The level of bacteria cultured using samples from these DUWL was similar to the original baseline DUWL samples of the test group (Tables 1 and 2;  $p = 0.96$ ). Treated DUWL had no detectable microorganisms in the samples.

Microorganisms were detected in saliva samples including *C. albicans*, but no *P. aeruginosa* were found. No microorganisms were detected in water samples collected

water samples were analyzed weekly for approximately two months after patients were treated to assess recolonization of waterlines. No organisms were detected for approximately three weeks after treatment with the disinfectant; at Week 4 40 cfu/ml were recorded. A mean count of colonial morphology of these bacteria was not similar to those isolated from the patients' saliva samples, and no *C. albicans* or *P. aeruginosa* were detected.

Table 1. Effect of disinfectant irrigation of dental unit waterlines.

DUWL #	Initial THPC (cfu/ml)	Post-treatment THPC (cfu/ml)							
		Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
1	1.63 x 10 <sup>5</sup>	0	0	0	40	80	160	60	500
2	6.27 x 10 <sup>4</sup>	0	0	0	0	0	0	0	0
3	6.27 x 10 <sup>4</sup>	0	0	0	0	0	0	0	0
4	2.34 x 10 <sup>4</sup>	0	0	0	0	0	0	0	0
5	3.20 x 10 <sup>5</sup>	0	0	0	0	0	0	0	0
6	8.00 x 10 <sup>4</sup>	0	0	0	0	0	0	0	0
7	6.00 x 10 <sup>4</sup>	0	0	0	0	0	0	0	0
8	4.60 x 10 <sup>5</sup>	0	0	0	0	0	0	0	0
9	6.16 x 10 <sup>3</sup>	0	0	0	0	0	0	0	0
10	1.21 x 10 <sup>5</sup>	0	0	0	0	0	0	0	0
11	1.20 x 10 <sup>5</sup>	0	0	0	0	0	0	0	0

THPC - total heterotrophic plate count; cfu/ml - colony forming unit per milliliter; DUWL - dental unit waterline

## DISCUSSION

Approaches for the management of biofilms have included recommendations for flushing the lines with water, use of sterile water during dental/oral and maxillofacial surgery, and the placement of anti-retraction valves. The effectiveness of flushing the lines with water has been variable and in some cases has resulted in increased microbial counts.<sup>3,5,13,25,40,42</sup> Use of in-line filters close to the site of patient treatment require frequent changing to effectively reduce organism growth.<sup>14,19,39</sup> Numerous disinfectants have been considered for rinsing DUWL, including: dilute alcohol based antiseptics,<sup>20</sup> chlorine compounds,<sup>4,43</sup> iodine compounds,<sup>44</sup> polyoxyethylenesorbitan (Tween 80),<sup>11,26</sup> chlorhexidine<sup>11</sup> and sodium hypochlorite.<sup>11,21</sup> However, even when effective, these agents have not prevented rapid recolonization of the waterline. Disinfectant products must be compatible with dental materials and free from toxic and carcinogenic chemicals.<sup>14</sup> Another technique that has been studied<sup>22</sup> includes use of electrochemically activated water, which when compared with distilled water and the use of conventional disinfectants, was found more effective in controlling biofilm.

Disinfection of waterlines with sodium hypochlorite, glutaraldehyde or isopropanol was assessed using cultures of DUWL water samples and by assessing the inner lumen of the lines using scanning electron microscopy.<sup>1</sup> It was found that effluent water contained an average of 10<sup>4</sup> cfu/ml bacteria and that the biofilm matrix harbored an average of 10<sup>9</sup> cfu/ml organisms in the lumen. Overnight treatment with the above disinfectants resulted in elimination of recoverable microorganisms, with a return to pretreatment levels that varied according to the antiseptic agent used (sodium hypochlorite and isopropanol: 6-15 days; glutaraldehyde: 3 days). Repeated treatment resulted in elimination of recoverable organisms. The authors suggested that residual effects occurred in the lumen of small waterlines but expressed concern that retained disinfectant could represent a risk to patients. The irrigant used in this study (Bio 2000) differs from other products as it has been approved by the U.S. Food and Drug Administration for intraoral use.

A recent study<sup>21</sup> assessed two concentrations of sodium

Table 2. Total heterotrophic plate counts in the dental unit waterlines not treated with disinfectant irrigation.

DUWL Line #	Final THPC (cfu/ml)
1	2.05 x 10 <sup>4</sup>
2	4.46 x 10 <sup>4</sup>
3	1.20 x 10 <sup>5</sup>
4	8.00 x 10 <sup>4</sup>
5	2.00 x 10 <sup>4</sup>
6	4.60 x 10 <sup>5</sup>

DUWL - dental unit waterlines; THPC - total heterotrophic plate count; cfu/ml - colony forming units per milliliter, mean

hypochlorate (5,000 and 1,500 parts per million; no difference in effect on recovered colony forming units (<200cfu/ml) was recorded for either solution. One study<sup>20</sup> assessed the use of dilute mouthwash solutions (Scope, Cepacol) or equivalent ethanol in independent reservoirs, and found that the system resulted in no growth of aerobic bacteria. The authors note that the study was limited in that the DUWL sample was small (<1ml) and the researchers only assessed the presence of aerobic flora due to the probability of oxygen within the DUWL. In addition, there is concern that mouthwashes do not have proven ability to kill *P. aeruginosa* and other pathogens. Another study<sup>12</sup> assessed the potential of chlorine (at 50x concentration in tap water) and found a lack of effect on cultures from DUWL samples. This lack of effect was attributed to the inability of chlorine compounds to penetrate the biofilm. Based on preliminary data,<sup>20</sup> it appears that ethanol and other alcohols may be more effective in such an environment. In a study<sup>20</sup> of ethanol-based irrigants, no viable aerobic organisms were isolated from treated DUWL. The authors suggest that further study of low concentration ethanol-based products is warranted.

In the current study, a disinfectant/coolant/irrigant with chlorhexidine was shown to be effective in eliminating existing bacterial and fungal colonization, and likely to influence established biofilm in DUWL. In addition, we did not observe recolonization of the organisms in DUWL samples for several weeks when treatment was followed by sterile water irrigation. The use of sterile water in the disinfectant lines may have reduced the rate of recolonization or residual action of the disinfectant may have persisted after DUWL treatment. Even after two months, the numbers of bacteria collected from the DUWL samples were within the limit that define potable water. It is possible that in clinics which do not use distilled water, recolonization would occur much more rapidly. These findings are similar to those of another recently reported<sup>14</sup> irrigant trial.

The potential concern of "suck-back" causing contamination of the lines from patient saliva did not appear to be a significant problem in the present study, as none of the bacteria detected in the saliva samples were found in samples collected from the DUWL after each patient was treated. Furthermore, the colony forming units recorded after treatment with the study irrigant were within the limit that defines potable water as recommended by the Food and Drug Administration and Health Canada (40-500 cfu/ml), although the American Dental Association guideline is <200 cfu/ml.<sup>10</sup>

## CONCLUSION

This study suggests a means of controlling growth of bacteria and *Candida* species in DUWL. The results also document that recolonization of DUWL after disinfection is not derived from patient sources when anti-retraction valves are present.

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