A Comparison of Different Disinfectants on the Microbiological Quality of Water from the Dental Unit Waterlines of a Military Hospital

(Perbandingan antara Disinfektan yang Berlainan terhadap Kualiti Mikrobiologi Air daripada Paip Air Unit Pergigian di Sebuah Hospital Angkatan Tentera)

MEI SIANG MA*, ZALINI YUNUS, AHMAD RAZI MOHAMMED YUNUS, ZUKRI AHMAD & FARIZAH ABDUL FATAH

ABSTRACT
Water from the dental unit waterlines (DUWLs) is known to contaminate with microbial from the biofilm that formed in the tubing system. The water quality from DUWLs is important to patients and dental health care professionals as they could be infected either directly from the contaminated water or aerosol that is generated during dental procedures. Suppliers claimed that dental units supplied to the hospital can only use a specific disinfectant which is uneconomic compared with the others. The aims of this study were to evaluate and compare the efficacy of different disinfectant on the water quality of DUWLs. Four disinfectants (Calbenium, A-dec ICX tablet, Dentel 5, Metassys) and distil water were evaluated. 350 mL water sample was collected separately, from the outlet of high-speed handpiece, scaler, 3-ways syringe and cup filler into a sterile thiosulfate bag on the 1st, 2nd, 4th, 8th, 12th and 24th weeks of the study. The samples were tested on the following day for total viable count (TVC). There is significant difference in the efficacies of the different disinfectants. Only one disinfectant consistently produces water quality within the recommended level of American Dental Association (ADA). Within the limitation of this study, it was found that there is alternative disinfectant that can reduce the TVC to the level recommended by ADA. However, the water qualities produced with these disinfectants were not consistent although they did not cause any technical problem to the dental units during the period of study.

Keywords: Dental unit water line; disinfectant; microbial; total viable count

INTRODUCTION
Dental unit waterlines (DUWLs) are the small-bore flexible plastic tubing that connects the high-speed handpiece, three ways syringe and ultrasonic scaler to the water supply (Figure 1) which could be from an independent water reservoir or the public water supply. Water that passes through DUWLs not only cools the tooth that undergoes dental treatment but also irrigates the working field and improves the vision of dentist.

Researches that have been done in many countries showed that microorganism contamination of DUWLs is a problem (Al-Hiyasat 2007; Blake 1963; Ma et al. 2012;...
Mavridou et al. 2006; Schel et al. 2006; Singh et al. 2003). Various microorganisms have been isolated from DUWLs, including environmental bacteria and human pathogens such as Pseudomonas (Al-Hiyasat et al. 2007; Ma et al. 2012; Mavridou et al. 2006), Legionella pneumophila (Mavridou et al. 2006; Walker et al. 2000), Mycobacterium spp. (Mavridou et al. 2006; Walker et al. 2000) and Staphylococcus spp (Singh et al. 2003).

Good water quality from DUWLs is important especially to the dental health care professionals and patients as they are regularly exposed to the water and aerosols generated from the dental units (Pankhurst 2003; Szymanska & Dutkiewicz 2008). This issue has been a great concern lately because of an increased awareness of potential occupational hazards and the increasing numbers of immunocompromised patients seeking dental care. American Dental Association (ADA 1999) recommended that water delivered to patients during non-surgical dental procedures should contain no more than 200 colony forming units (cfu) of bacteria for each milliliter (mL) of water.

AIM
Supplier claimed that the dental units supplied can only use a specific disinfectant which is solely distributed by the same supplier. As a result, the price of the disinfectant is uneconomic. Therefore the aims of this study were to evaluate and compare the efficacy of different disinfectants on the microbiological quality of water from DUWLs and their compatibility with the dental unit.

MATERIALS AND METHODS
The study was done at the dental department of Tuanku Mizan Armed Forces Hospital, Kuala Lumpur, Malaysia. It was a six months study that started in October 2011 and completed in April 2012. Five dental units (Absolute, Eurodent, Italy) of the same age, design and treatment methods for DUWLs were used for the study. A week before the study started, the routinely used disinfectant was changed to either one of the following four different disinfectants, Calbenium (Airel, France), A-dec icX tablet (A-dec, Newbury, United States), Dentel 5 (Tristel, United Kingdom) or Metassys (Medizintecnik GmbH, Austria). Distilled water was used in the fifth surgery to serve as control. Each disinfectant was prepared per manufacturer’s instructions. At each studied dental unit, 350 mL water sample was collected separately from the outlet of the high speed handpiece, scaler, 3-ways syringe, cup filler and water reservoir and tap into a sterile thiosulphite bag (Whirlpak) by using aseptic technique. The water samples were collected on the 1st, 2nd, 4th, 8th, 12th and 24th weeks of the study period. The collected samples were placed in an ice box and transported to the laboratory within 24 h and kept in the refrigerator at 4°C. They were tested on the following day for total viable count whereby 100 mL of sample was filtered through a 0.45 μm polycarbonate membrane filter using a membrane filter system (Milipore). The filter paper was inoculated onto Plate Count Agar (PCA) (Oxoid CM035) and then incubated for 24 h at 37°C. The colonies growths were enumerated by Gel Imager (Biorad, California, USA).

ANOVA was used for Statistical analysis. P value of less than 0.05 is considered as significant.

All the dental units were observed throughout the study period to make sure there was no breakdown reported. Any breakdown of dental unit would be investigated for the cause to ascertain that it was not due to the disinfectant.

RESULTS
During the period of the study, significant difference between the log_{10} cfu/mL were constantly found on water samples treated with Calbenium compared with water...
samples treated with the other disinfectants when p value is set at 0.05 (Table 2). The microbiological quality of water from DUWLs that use other disinfectants and distilled water were not consistent. This is in contrast with water treated with Calbenium which has consistently showed to be within the recommended level of ADA (1999). Table 1 shows the number of samples that exceeded 200 cfu/mL.

The dental unit was compatible with all the disinfectants as there was no technical breakdown of dental unit during the period of the study.

**DISCUSSION**

Biofilm is a community of microorganism that attached irreversibly to a surface. DUWLs is prone to biofilm formation because of its small diameter and water stagnation. According to Tall et al. (1995), biofilm forms in 8 h once DUWLs is connected to the main water supply even it is not used for patient treatment. Microorganism that forms the biofilm in DUWLs can either comes from the water that was piped into the dental unit or the saliva that has been suck back from patients’ saliva as a result of lack of preventive valve (Szymanska 2003). Theoretically, these microorganisms could shed into the water that passes through the dental unit and cause contamination (Walker et al. 2000). Literature has shown that water quality from the main water supply is always within recommended level, however this is not so when it has gone through the DUWLs.

Currently, there is no evidence based protocol for dentist to control the microbial quality of water from DUWLs (Liaqat & Sabri 2011; Schel et al. 2006; Walker et al. 2000). Distilled water does not prevent biofilm formation in DUWLs without proper and regular maintainence (Kettering et al. 2002).

Exposure of dental health care professionals to contaminated water from DUWLs is evidenced from studies by Fots et al. (1985) and Reinthaler et al. (1988) which showed that dental personnel have higher antibody titre towards Legionella than the equivalent population of the other sectors. In addition, Pankhurst et al. (2005) showed that total aerobic counts of more than 200 cfu/mL from DUWLs at 37°C may be associated with developing asthma in the early career pathway of a dentist.

Attempts that have been done to improve water quality from DUWLs includes flushing, individual water reservoir, chemical disinfection, daily draining, air purging regimes, water line filters, anti-retraction valves and use of sterile water. However, there is insufficient data to establish the effectiveness of each of these methods (ADA 1999). In fact, the present data suggests that none of these methods permanently remove biofilms from DUWLs.

Biofilms that develop in different tubing system has different size, texture and resistance to antibiotic/ biocides (Mayo et al. 2002). The effectiveness of chemical germicide in removing the contamination has been tested by Blake about 50 years ago. Various disinfectants are available in the market. An ideal disinfectant, other than effective in improving the microbial water quality, should also be biocide at low concentration, non-toxic, non-corrosive, biodegradable, compatible with dental units and restorative material and does not cause microbial resistance with its optimum contact time. In addition, it

**TABLE 1. Number of samples exceeded 200 cfu/mL (total 20 for each disinfectant)**

<table>
<thead>
<tr>
<th>Disinfectant</th>
<th>Number of samples exceeded 200 cfu/mL (total 20 for each)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dentel 5</td>
<td>11 (55%)</td>
</tr>
<tr>
<td>Distilled water</td>
<td>10 (50%)</td>
</tr>
<tr>
<td>Calbenium</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>ICX Tablet</td>
<td>3 (15%)</td>
</tr>
<tr>
<td>Metassys</td>
<td>9 (45%)</td>
</tr>
</tbody>
</table>

**TABLE 2. Mean different of colony forming unit between surgeries**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Mean different (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgery 1(Dentel 5)</td>
<td>Surgery 2(Distill water)</td>
<td>0.32 (-0.99, 1.63)</td>
</tr>
<tr>
<td></td>
<td>Surgery 3(Calbenium)</td>
<td>1.96 (-0.56, 3.36)</td>
</tr>
<tr>
<td></td>
<td>Surgery 8 (ICX tablet)</td>
<td>1.34 (0.05, 2.63)</td>
</tr>
<tr>
<td></td>
<td>Surgery 9 (Metassys)</td>
<td>0.66 (-0.72, 2.03)</td>
</tr>
<tr>
<td>Surgery 2(Distill water)</td>
<td>Surgery 3(Calbenium)</td>
<td>1.64 (-0.41, 2.86)</td>
</tr>
<tr>
<td></td>
<td>Surgery 8 (ICX tablet)</td>
<td>1.02 (-0.10, 2.13)</td>
</tr>
<tr>
<td></td>
<td>Surgery 9 (Metassys)</td>
<td>0.34 (-0.86, 1.53)</td>
</tr>
<tr>
<td>Surgery 3(Calbenium)</td>
<td>Surgery 8 (ICX tablet)</td>
<td>-0.62 (-1.11, -0.13)</td>
</tr>
<tr>
<td></td>
<td>Surgery 9 (Metassys)</td>
<td>-1.30 (-1.54, -1.07)</td>
</tr>
<tr>
<td>Surgery 8 (ICX tablet)</td>
<td>Surgery 9 (Metassys)</td>
<td>-0.68 (-1.14, -0.22)</td>
</tr>
</tbody>
</table>

Repeated measures ANOVA between group analysis was applied: F-stat (df)= 26.79 (4), p value < 0.05; Assumption of normality and compound symmetry were checked and were fulfilled; and assumption of homogeneity of variances were not met and dunnett’s T3 was applied
should have an acceptable shelf life and safe with the working dose. Dental unit might needs some modification before disinfectant can be used. For example, disinfectant that contains hydrogen peroxide could burst a plastic bottle when it is activated (Kathariya et al. 2013).

The specific feature of Absolute dental unit is that it has a separate plastic bottle that served as container for the disinfectant (Figure 2). Water from public water supply is mixed with the disinfectant in DUWLs before it was discharged during dental treatment.

The disinfectants, namely Calbenium, A-dec ICX tablet, Dentel 5 and Metassys, were chosen for the study because there is no budget involvement. Calbenium and A-dec ICX tablet were purchased earlier per manufacturers’ advice as disinfectant for Eurodent and Adec dental units. Dentel 5 and Metassys were given free by supplier to test its efficacy on DUWLs and compatibility with Eurodent dental unit.

In the present study, TVC of the water from DUWLs treated with Calbenium appears to be consistently below the level recommended by ADA (1999). For others, occasional high values occurred probably due to contamination of water sample by detachment of biofilm from the inner surface of the dental tubing or non-compliance of dental staff in following the manufacturer’s instruction when using the disinfectant. Study by Williams et al. (1994) had shown that compliance of staff is critical in maintaining the microbiological quality of water from DUWLs when non-automated procedures were used. This is especially so with Dentel 5 which only active/function for 24 h once it is mixed, compared to A-dec ICX tablet that can last for 2 weeks, whereas Metassys and Calbenium only need refill when it has finished. Hence, using Dentel 5 demands high compliance from the user, failing which would result in regrowth of the biofilm.

Metassys contains hydrogen peroxide with 0.0235% dilution. It is an oxidant with unspecific action that prevents the settlement of biofilm within DUWLs. Metassys performance would be less satisfactory if there is a high bacterial loading. The advantage of using hydrogen peroxide as disinfectant is it can be added continuously into the waterlines during patient treatment (Liaqat & Sabri 2011). This is important because research have shown that the microorganism from DUWLs shed continuously into the water while the film is incontact with the water (Liaqat & Sabri 2011).

Dentel 5 is a liquid disinfectant that contains chlorine dioxide as the active ingredient. Study done by Wirthlin and Marshall (2001) has showed that chlorine dioxide cause a significant reduction in heterotrophic plate count when used in private dental clinic and also as a lavage with ultrasonic scaler. In addition, it does not cause corrosion to metal. However, Dentel 5 as a disinfectant for DUWLs needs daily changes as it only active for 24 h.

On the other hand, Calbenium is a mixture of EDTA, chloramines, benzalkonium, allantoin, aspartame and sorbitol. It is stable and active till its expiry date. In this study, it has constantly given satisfactory quality water from treated dental units.

A-dec ICX tablet contains sodium percarbonate, silver nitrat and cationic surfactants. Handling of this tablet is simple, ie: a tablet is dropped into a self-contained bottle before each filling and it continues to work in the systems for 2 weeks. However, this also means that in order to use this disinfectant, the dental unit needs a separate water bottle reservoir. McDowell et al. (2004) showed that A-dec ICX tablet is significantly effective in preventing biofilm formation in DUWLs compared with untreated units for a 16-week study.

Researches have shown that disinfectants were able to achieve a zero CFU but were unable to remove the biofilm.
from the surface (Liaqat & Sabri 2008), hence do not have a long lasting effect.

CONCLUSION

Within the limitation of this study, disinfectant suggested by the supplier appears to be the best in controlling the microbiological quality of water from DUWLs. The dental unit is compatible with the other disinfectants as there is no technical breakdown of dental unit during the period of the study. Other disinfectants may be able to control the CFU of DUWLs to the level recommended by ADA (1999) provided manufacturer’s instruction is strictly followed.

Antimicrobial efficacies of different disinfectant varied in its ability to control the microbiological quality of water from DUWLs. The disinfectants that tested do not eliminate the microbial totally. Biofilm elimination and prevention need to be done through some other means. Further research with more DUWLs should be carried out in order to obtain more conclusive data.

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REFERENCES


Mei Siang Ma*
Dental Specialist Polyclinic
Tuanku Mizan Armed Forces Hospital
No 3, Jln 4/27A, Seksyen 2, Wangsa Maju
53300 Kuala Lumpur
Malaysia

Zalini Yunus, Ahmad Razi Mohamed Yunus,
Zukri Ahmad & Farizah Abdul Fatah
Science and Technology Research Institute for Defence
Kompleks Induk STRIDE
Tmn Bukit Mewah, Fasa 9
43000 Kajang, Selangor D.E.
Malaysia

*Corresponding author; email: drliana@gmail.com
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