

Original Article**The microbiological quality of water from dental unit waterlines in Malaysian Armed Forces dental centres**Ma Mei Siang ^{a*}, Zalini Yunus ^b, Ahmad Razi Mohammad Yunus ^b, Zukri Ahmad ^b, Haryanti Toosa^b^a Poliklinik Pakar Pergigian, Hospital Angkatan Tentera Tuanku Mizan, No 3, Jalan 4/27A, Seksyen 2, Wangsa Maju, 53300 Kuala Lumpur, Malaysia. ^b Institut Penyelidikan Sains dan Teknologi Pertahanan, Kompleks Induk STRIDE, Taman Bukit Mewah Fasa 9, 43000, Kajang, Selangor, Malaysia.

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Abstract Water quality in the dental unit waterlines (DUWLs) is important to the patients and dental health care personnel as they are at risk of being infected with opportunistic pathogens such as *Pseudomonas* or *Legionella* species. In this study, a total of 86 samples were collected from DUWLs of 19 dental units in 11 Malaysian Armed Forces dental centres (MAFDC). 350 ml water sample was collected in sterile thiosulphite bags from the outlets of 3-way syringe, high speed handpiece, scaler, cup filler, independent water reservoir or the tap of the same surgery respectively. Samples were transported to the laboratory within 24 hours and kept in the refrigerator at 4°C. 100ml of each sample was filtered through a 0.45 µm polycarbonate membrane filter. The filter was then inoculated onto plate count agar and incubated at 37°C for 24 hours, after which the formed colonies were enumerated. Another separate 100ml of water sample was poured onto buffered charcoal yeast extract agar and cetrimide agar to culture *Legionella* and *Pseudomonas* respectively. Identification of these bacteria were confirmed by polymerase chain reaction and sequencing. *Pseudomonas aeruginosa* was detected in 9.5% of the samples but *Legionella* was not detected in any of the samples. 77% of the samples met American Dental Association (ADA) recommendation of less than 200 cfu/ml. The result of this study showed that it is difficult if not impossible to eliminate biofilm from the DUWLs. Regular monitor of water quality from DUWL is required to maximise the health of the dental patients and dental health care personnel.

Keywords: Dental unit water lines, *Legionella pneumophila*, *Pseudomonas aeruginosa*, water quality.**Introduction**

Biofilm and bacterial contamination of dental unit waterlines (DUWLs) was first reported in the literature nearly 50 years ago (Blake, 1963). The source of water in DUWLs could be from public water supply or an independent water reservoir (bottle) (Fig. 1). The quality of water from DUWLs is important not only to the patient, but also to dental health care personnel as these groups are regularly exposed to water and aerosols generated from the dental units (Liaqat and Sabri, 2011). Bioaerosols generated from DUWLs has been shown as a

potential source of indirect infection to dental health professionals (Szymańska and Dutkiewicz, 2008).

American Dental Association (ADA) recommended that water delivered to patients during non-surgical dental procedures should contain no more than 200 colony forming units per milliliter (cfu/ml), whereas Center for Disease Control (CDC) recommended that drinking water should contain ≤ 500 cfu/ml and the water used in dental procedures should at least meet this target if not better (ADA 1999, Kohn *et al.*, 2003). Exposing patients or dental health care personnel to water of

uncertain microbiological quality, despite the lack of documented adverse health effects, is inconsistent with generally accepted infection control principles (Sehulster and Chinn, 2003).



Fig. 1 The source of water in DUWLs from the independent water reservoir (bottle).

Tests and research done in various part of the world showed that traditional dental clinic using public water supply has an average of 375,000 cfu/ml of water sample whereas those with independent water reservoir averaged 1,200,000 cfu/ml (Barbeau *et al.*, 1996). Microbial loads as high as 1.6×10^8 cfu/ml has been reported in unmonitored DUWLs (Mayo *et al.*, 1990; Santiago *et al.*, 1994; Karpay *et al.*, 1999) and these microbial accumulations can contribute to objectionable odour (ADA, 1999).

Most of the bacteria isolated from DUWLs are Gram negative bacteria which can produce endotoxin such as *Pseudomonas aeruginosa* and *Legionella pneumophila*. *Pseudomonas aeruginosa* is a natural water-loving biofilm producer, that when aerosolized is almost confirmed to cause pneumonia-like disease in elderly or immunocompromized individuals (Atlas *et al.*, 1995, Barbeau *et al.*, 1996). *Legionella pneumophila* in the DUWLs is the most frequent cause of human legionellosis as was the case of a dentist in San Francisco, USA, who became seriously ill from the disease (Atlas *et al.*, 1995).

In the Malaysian Armed Forces Dental Service, there is no system that monitors the water quality of DUWL and no study has been done to assess the water quality which might poses hazard to the patient and dental health care personnel. Hence, it is the aim of this study to assess the water quality of DUWLs of MAFDC and the contamination of the water by *Pseudomonas aeruginosa* and *Legionella pneumophila*.

Materials and methods

This study was carried out in August 2008 and completed in March 2009. Eleven centres were chosen by convenient sampling. Dental centres without dental officer in charge were excluded from the study. The samples consisted of 3 dental departments from the Armed Forces Hospitals, eight dental centres (two from each military division) and one Armed Forces mobile dental clinic. The details of the location of dental centres, type of dental unit used and water supply in each dental centre is shown in Table 1.



Fig. 2 Collection of water samples from high speed handpiece using aseptic technique.

At each sampling centre, 350 ml water sample was collected separately from the outlet of the high speed handpiece, scaler, 3-way syringe, cup filler, independent water reservoir or the tap water (depends on the source of water supply of the particular dental unit) into a sterile thiosulphite bag (Whirl-pak) by using aseptic technique (Fig. 2). The water samples were placed in an ice box and transported to the laboratory within 24 hours and kept in

the refrigerator at 4°C. On the following day, each water sample was analyzed by using microbiological analysis that comprised of total viable counts, tests for detection of *Legionella* (modified from BS 6068-4.12:1998 and ISO 11731:1998 Water Quality-Part 4) and *Pseudomonas* using the membrane filter method (Milipore, Massachusetts, USA).

Table 1: Brands of dental unit and source of water supply

Dental Centres	Room	Brand	Source of water supply
Kem KEMANTAH Kuala Lumpur	Surgery 3	Anthos	public
	Surgery 5	Adec	reservoir
	Surgery 8	Eurodent	public
Kem Sungai Besi, Kuala Lumpur	Surgery 3	Adec	reservoir
	Surgery 4	Eurodent	public
	Surgery 5	Eurodent	public
Jabatan Pergigian HAT Terendak, Melaka	Surgery 1	Adec	reservoir
	VIP	Adec	reservoir
Kem TUDM Butterworth	Surgery 1	Adec	reservoir
Kem Lok Kawi, Sabah		Adec	reservoir
Kem Teluk Sepanggar, Sabah		Adec	reservoir
Jabatan Pergigian HAT Lumut, Perak	Surgery 1	Eurodent	public
	Surgery 2	Anthos	public
	Surgery 3	Adec	reservoir
Kem Batu 10 Kuantan		Adec	reservoir
Kem Desa Pahlawan, Kota Bharu		Adec	reservoir
Dental mobile Clinic		Anthos	public
Poliklinik Pergigian, HAT Gemas	Surgery 1	Eurodent	public
	Surgery 2	Adec	reservoir

Legend: public – public water supply, reservoir – independent water reservoir.

Total viable count (TVC)

100 ml of sample was filtered through a 0.45 µm polycarbonate membrane filter

using membrane filter method. The filter paper was inoculated onto Plate Count Agar (PCA) (Oxoid CM035, Cambridge, UK) and then incubated for 24 hours at 37°C (Smith *et al.*, 2002). The colonies growths were enumerated by Gel Imager (Biorad, California, USA).

Legionella test

100 ml of water was filtered through a 0.45µm polycarbonate membrane in Membrane Filtration System (Milipore, Massachusetts, USA). Then, the membrane was cut up into tiny pieces in 5 ml sterile water. The sample was shook and placed in a water bath at 50°C for 20 minutes. 0.1 ml of sample was spread onto a BCYE (Isolab, Shah Alam, Malaysia) plates. The plates were incubated at 37°C in 5% CO₂ incubator (New Brunswick Scientific, Connecticut, USA) and examined daily for 10 days (Atlas *et al.*, 1995). The colonies that grew in BCYE plate were subcultured onto BCYE and Blood Agar (BA) (Isolab, Shah Alam, Malaysia). Isolated colonies that grew on BCYE agar but failed to grow on BA were presumed to be *Legionella*. Various tests such as oxidase tests (Oxoid, Cambridge, UK), catalase tests (Liofilchem, Roseto degli Abruzzi, Italy) and a latex agglutination test (Microgen, London, UK) were conducted to confirm the presence of *Legionella pneumophilla*.

Pseudomonas test

100 ml of water was filtered through a 0.45 µm polycarbonate membrane in a Membrane Filtration System. The filtered membrane was put onto a cetrimide agar plate and incubated at 37°C for 48 hours (Al-Hiyasat *et al.*, 2007). The colonies that grew in the cetrimide agar plate were subcultured onto Mac Conkey (MAC) agar (Oxoid CM007, Cambridge, UK) and BA plate. The colonies that grew on both MAC and BA agar were confirmed to be *Pseudomonas aeruginosa*.

Polymerase chain reaction (PCR) analysis

The identification of positive samples from microbiological analysis was

confirmed by using polymerase chain reaction (PCR) analysis, adapted from Atlas *et al.* (1995). Genomic DNAs from the positive samples were extracted using an AquaPure Genomic DNA Kit (BioRad, California, USA). The primers (ITS1 regions) 16F945 5'-GGG CCC GCA CAA GCG GTG G-3' and 23R458 5'-CTT TCC CTC ACG GTA C-3' were used. In each reaction, 49 µl of PCR master mix was added to the target DNA to achieve a final volume of 50 µl. After heating the samples at 94°C for 5 minutes, the target DNA was amplified in 30 subsequent cycles under the following conditions: 94°C for 1 minute, 55°C for 1 minute, 72°C for 2 minutes, and after the 30th cycle, it was held at 72°C for 10 minutes to allow the extension polymerisation to finish. The PCR reactions were performed in the Bio-Rad iCycler (BioRad, California, USA). The amplified PCR of ITS1 regions were purified using a QIAquick gel extraction kit (Qiagen, California, USA) and sent to First Base Laboratories Sdn. Bhd. (Seri Kembangan, Selangor, Malaysia) for sequencing.

Results

A total of 86 water samples were collected from the DUWLs of 19 dental units in 11 dental centres of Malaysian Armed Forces. The PCR results showed all the positive samples of the *Pseudomonas* tests were identified as *Pseudomonas aeruginosa*. Six of the 11 centres studied were free from *P. aeruginosa* contamination. No *Legionella pneumophila* was detected in any of the samples. *P. aeruginosa* was detected in the water sample from the independent water reservoir of one of the centre. 77% of the samples met ADA's recommendation of less than 200 CFU/ml. 21% of the water samples have the total bacteria count exceeded 200 cfu/ml with water sample from the 3-way syringe as the highest with 6 (29%) samples; there was only 1 sample from independent water reservoir with total bacteria count of more than 200 cfu/ml.

P. Aeruginosa were detected in 9 (10%) of the water samples with 6 (46%) from the scaler (Table 2). All water samples from the tap water were within the recommended quality of drinking water by CDC (Kohn *et al.*, 2003).

Table 2 Total bacteria count and presence of *Legionella pneumophila* and *Pseudomonas aeruginosa* in water sample

Site	Total bacteria count (cfu/ml)		Legionella test		Pseudomonas test	
	> 200	< 200	+ve	-ve	+ve	-ve
HHP*	5	13	0	18	2	16
SC	5	14	0	19	6	13
SW	6	13	0	19	1	18
CF	5	14	0	19	0	19
WRD	1	10	0	11	0	11
Total	22	64	0	86	9	77

Legend: HHP (high speed handpiece), SC (scaler), SW (3-way syringe), CF (cup filler), WRD (independent water reservoir).

*One sample could not be obtained because the high speed handpiece was under repair.

Discussion

Detachment of microorganisms from dental unit biofilm could theoretically infect the patient by flushing into the oral cavity. Splatter and aerosols from dental procedure may possibly infect health care personnel (Szymańska and Dutkiewicz, 2008). Frequently, water entering DUWLs is of good microbiological quality, but after shedding of microorganisms from the biofilm, it becomes contaminated over the acceptable level (Barbeau *et al.*, 1996).

This biofilm is protected from the effect of heat and chemicals thus reducing their susceptibility to disinfection processes. Two possible sources of microorganism that present in the biofilm of DUWLs are municipal water piped that into the dental units and suck-backed of patients' saliva into

the waterlines due to lack of anti-retraction valves (Barbeau *et al.*, 1996).

In the present study, 30% (6/20) of the dental units were contaminated with *P. aeruginosa* in comparison to a previous study (Al Hiyasat *et al.*, 2007) where 86.7% (26/30) of the dental units were contaminated. This is probably due to difference in the source of the water that supplied the dental units and the types of disinfectant that have been used.

Majority of microorganisms isolated from DUWLs are of low pathogenicity. However, the public health significance of these pathogens is still unclear. Studies done emphasized the need for effective mechanisms to reduce the microbial burden within DUWLs, and highlight the risk of occupational exposure and cross infection in general dental practice (Walker *et al.*, 2004).

Modern methods aiming to reduce DUWLs contamination concentrate on two aspects, which are treatment of dental water and improvement of dental unit design. These include: (1) filtration, (2) flushing, (3) antiretraction valves and retrograde aspiration of oral fluid, (4) using biocides and chemical disinfectants, (5) chlorination, (6) peroxide, ozone and ultraviolet light, (7) independent clean water system, (8) autoclavable systems, (9) electrochemically activated water and (10) drying.

In Malaysian Armed Forces Dental Centers, Dental Surgery Assistants (DSA) are trained to flush high speed hand-piece, scalers, and 3-way syringe for 20-30 seconds before starts surgery in the morning and in between patients. Two main models of dental units that are used in the Malaysian Armed Forces Dental Centers are Adec (Orgeon, United States) and Eurodent (Bologna, Italy). For Adec dental units, A-dec ICX waterline treatment tablets are placed into the independent water reservoir each time fresh distilled water is replaced whereas disinfectant called Calbenium is used for Eurodent dental units.

Meiller *et al.* (2004) evaluated A-dec ICX waterline treatment tablets in

a series of experiments for prevention of biofilm formation, microbial spectrum activity, minimum inhibitory time determination and treatment of established biofilms. They concluded that A-dec ICX waterline treatment tablets is effective in maintaining the effluent within the ADA and the CDC's recommendation. In our study, 5 out of 11 (45%) samples from Adec dental units that have been treated with A-dec ICX tablets have cfu/ml that exceed the recommended level by ADA. This could be as a result of low turnover of water in the independent water reservoir, hence reducing the effectiveness of the tablets.

Twenty three percent of the samples in this study that did not meet the ADA recommendation are probably due to the DSAs did not comply with the guidelines given, or non-compliance of the DSAs in following the manufacturer's guidance for disinfectant or the use of disinfectant that were unable to reduce the total viable bacteria counts to the safe level as recommended.

The other possibility is the dental tubing of DUWLs in the establishment were only replaced in the occasion of leakage (usually 5 years and above), compared to three months as recommended by ADA.

The contamination may be correlated with the age of the dental unit (Watanabe *et al.*, 2008). However, this information was not collected during the data collection.

Only one sample from the independent water reservoir that served as control has total bacteria count of more than 200 cfu/ml, therefore the heavy bacterial load from the samples of dental tubing are most likely from the waterlines itself. Walker *et al.* (2004) monitored the water emitted from dental units without independent water reservoir and found out that dental units attached to centralized combined water distillation-cleaning solution distribution systems can produce water with less than 200 cfu/ml; and missing of one weekly cleaning did not negatively affect the water quality.

However, study by Walker *et al* (2004) also indicates that independent water reservoir can reduce the numbers of micro-organisms released from DUWLs compared to central water source. Independent water reservoirs have the advantage of able to add in disinfectant. However, care must be taken to ensure that before the water bottle is used, it has been disinfected by a non-toxic solution. The water that is to be added to the bottle should either be sterile or distilled and there is proper maintenance. Otherwise, the water bottle itself would become another reservoir for microorganism as shown in the present study. Effective cleaning and maintenance of the tubing cannot be overemphasized and is essential for success (Douglas and van Noort, 1993).

Conclusions

Based on the result and within the limitations of this study, it was concluded that DUWLs in MAFDC are not totally free from *Pseudomonas* contamination. 23 % of the DUWLs in the MAFDC do not meet the ADA's recommendation of less than 200 cfu/ml although disinfectant is in use.

Contamination of DUWL is universal. It is difficult if not impossible to eradicate the biofilm in these tubing and prevent its regrowth. Nevertheless, every attempt has to be taken to minimize the contamination of the tubing in order to maximize the health of the dental health care personnel and the patient. Although the number of published cases of infection resulting from exposure to water from contaminated DUWLs is limited, there is a medico-legal requirement to comply with potable water standards and to conform to public perceptions on water safety (Sehulster and Chinn, 2003).

Dentists are encouraged to follow manufacturers' instruction in maintaining the DUWLs and use disinfectant whenever possible. Until ideal guidelines for maintaining DUWLs is released by a professional body, flushing water for 20-30 seconds before

starting the morning session and in between patient treatments, remains the most economic way of reducing bacterial load in DUWLs.

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