THE MICROBIAL PROFILES OF DENTAL UNIT WATERLINES IN A DENTAL SCHOOL CLINIC

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ABSTRACT

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Background: The microbiological quality of water delivered in dental units is of considerable importance since patients and the dental staff are regularly exposed to aerosol and splatter generated from dental equipments. Dental-Unit Waterlines (DUWLs) structure favors biofilm formation and subsequent bacterial colonization. Concerns have recently been raised with regard to potential risk of infection from contaminated DUWLs especially in immunocompromised patients.
Objectives: The study aimed to evaluate the microbial contamination of DUWLs at RAK College of Dental Sciences (RAKCODS) and whether it meets the Centre of Disease Control's (CDC) recommendations for water used in non-surgical procedures (≤500 CFU/ml of heterotrophic bacteria).
Materials and Methods: Ninety water samples were collected from the Main Water Source (MWS), Distilled Water Source (DWS) and 12 random functioning dental units at RAKCODS receiving water either directly through water pipes or from distilled water bottles attached to the units. Bacterial enumeration and molecular identification were performed.
Results: The MWS had the lowest bacterial count (499 CFU/ml).The bottled units contained significantly higher numbers of CFU (2632±1231.783) compared to non-bottled units (1484.75±1395.093), $p<0.02$. *Ralstonia spp.* was the most common bacteria present in the MWS and DWS (in 96% of the samples). Other bacteria were *Sphingomonas paucimobilis* 88.8% and *Leifsonia spp*. 73.5%.
Conclusion: There is a need for regular water monitoring at dental clinics, in addition to regular maintenance and disinfection programs to ensure quality water delivery that meets the CDC guidelines for non-surgical water.
Keywords: Maintaining dental unit waterlines, microbial contamination, biofilm formation, non-surgical water.

1. Introduction

In the dental office, infection control in terms of self-protection, instrument sterilization and surface disinfection is given great importance due to its huge impact on the patient's health. The microbiological quality of water running in the Dental-Unit Water Lines (DUWLs) however is mostly overlooked.

Contaminated water in DUWLs causes a health threat to both patients and dental staff who are regularly exposed to aerosol and splatter.[1] The patients with the highest risk of infection from contaminated water are immunocompromised patients, elderly patients and patients with recent surgeries and open wounds.

Bacteria responsible for DUWL contamination can originate from municipal water piped into the dental chair unit or from patients' oral cavities through a process known as back-siphonage. Back-siphonage is the process of aspiring oral fluids as a result of the temporary negative pressure produced when the drill stops rotating while still in the patient's mouth[2,3] due to lack of anti-retraction valves.[4] In certain conducted studies, it has been observed that about 1mL of oral fluids is retracted in old as well as some new dental equipments.[3] This process increases the risk of cross infection as oral fluids are retracted from one patient's oral cavity, grown within the DUWL, and spread through aerosol or splatter to other patients or healthcare personnel.

Dental unit water systems' narrow lumens and small bores, in conjunction with the long periods of stagnant water favor the formation of biofilms which adhere to the inner surfaces of the lines and serve as a haven for pathogens protecting

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the bacteria both from being washed away by the water flow and from many types of antimicrobial water treatments.[4] Although the microorganisms found in biofilms are predominantly harmless, gram-negative water bacteria, opportunistic pathogens such as Pseudomonas aeruginosa, Legionella pneumophila, and non-tuberculous mycobacteria may also be found.[5]

The opportunistic pathogens of Pseudomonas spp. were found, in a number of cases, to be the predominant species isolated from DUWL.[2,6] In a study conducted by Dr. Barbeau et al. (1998), it was stated that pathogens like Pseudomonas aeruginosa, Legionella pneumophila and nontuberculous mycobacteria do not merely survive in DUWL, but also proliferate over time with enhancing resistance by the inhabited biofilm as they wait for a susceptible host.[7] This kind of contamination is especially dangerous, and crucial enough not to be overlooked or taken lightly, particularly when treatment of immunocompromised patients is considered, such as cases of cystic fibrosis or AIDS.[8,9,10]

In 1995, the American Dental Association (ADA) Board of Trustees and ADA council on Scientific Affairs adopted a statement on DUWLs. The statement recommended improving the dental unit design so that by the year 2000, water delivered to patients during non-surgical dental procedures would contain no more than 200 colony forming units (CFU)/ml of aerobic mesophilic heterotrophic bacteria.[1,11,12] This was equivalent to the standard for dialysate fluid.

In 2003, the Center for Disease Control's (CDC) guidelines for infection control in dental health care settings stated that coolant/irrigant water used in non-surgical dental procedures should meet the Environmental Protection Agency (EPA) regulatory standards for drinking water which is less than or equal to 500 CFU/ml of heterotrophic bacteria.[1,13]

RAK College of Dental Sciences (RAKCODS), UAE moved into a new building in 2011. The main water supply to the college is through the Municipality network. The water is collected in ground reservoir then pumped to the roof tank. From the latter, water is delivered through water pipes network to the whole building including the dental units (Fig. 1). The majority of dental units (90%) receive water directly through the water pipes. A limited number (10%) of dental units receive distilled water via bottles attached to the units, which are filled frequently from the water distilling machine as required (Fig. 1).

As part of the sanitary measures taken by the administration, the main water (Municipality water) is regularly examined for microbial load and other chemical ingredients to ensure that it meets the recommended standard by the local authority. However, the performed water analysis never included samples from the dental units. Before water is pumped to the roof tank a small device "Solenoid-Driven Metering Pump" is fixed with the main pipe, which ejects dosed chemicals into the water stream (Fig. 1). These non-toxic chemicals are commercially available under the name "MembraClean Plus Disinfectants", which presumably have antimicrobial action, including bacteria, fungus and algae, and prevent scale or biofilm formation. The supplier of the chemicals never discloses the actual chemical composition. Nevertheless, the product is approved by the local authority for drugs and chemicals control, UAE. Further search for the chemicals details was tried but to no avail.

The main aim of this study was to evaluate the microbial contamination of DUWLs in RAKCODS by determining composition as well as concentration of microflora and whether it meets the Centre of Disease Control's (CDC) recommendations for water used in non-surgical procedures. The research highlights on the importance of regular water monitoring as well as antimicrobial water treatments to assure quality water delivered.

2. Materials and Methods

2.1. Sample collection

The study material included water samples from the main water source, distilled water source and 12 functioning dental units at RAKCODS randomly. The main age of the dental units is 3 years of service. From each collecting point, 3 samples were collected at interval over a period of 6 weeks. Three water samples were collected from the Main Water Source (MWS) before entering RAKCODS water pipe-lines, 3 water samples from the Distilled Water Source (DWS) (water distilling machine) and 3 samples were collected from each point of the dental units; including Distilled Water Bottles (DWB) and dental units' Water Line Tubes (WLT) connecting the Hand pieces and Ultrasonic scaler tips (H/S). Care was taken to collect the samples in aseptic condition to avoid any external microbial contamination. Most of the samples were collected between 10.30 am and 12.30 pm using sterile air-tight containers. All dental units included in the study were operating at the time of sample collection. Approximately, 15 ml of water was collected from each collecting point in pre-labelled, air-tight sterile containers. The containers were labeled according to the point of water collection and reference number of the dental unit. The water outlet, like hand pieces, scaler tips, water line tubes were flushed for few seconds before taking the sample. In total 90 samples were collected successfully. The water samples were then transferred to the microbiology department, RAK Medical and Health Sciences University (RAKMHSU) within 3 hours from collection time for microbial analysis.

2.2. Laboratory procedures

• Pour plate technique for bacterial enumeration (Standard Plate Count):

In the RAKCOMS microbiology lab, pour plate technique for bacterial enumeration was performed as follows:

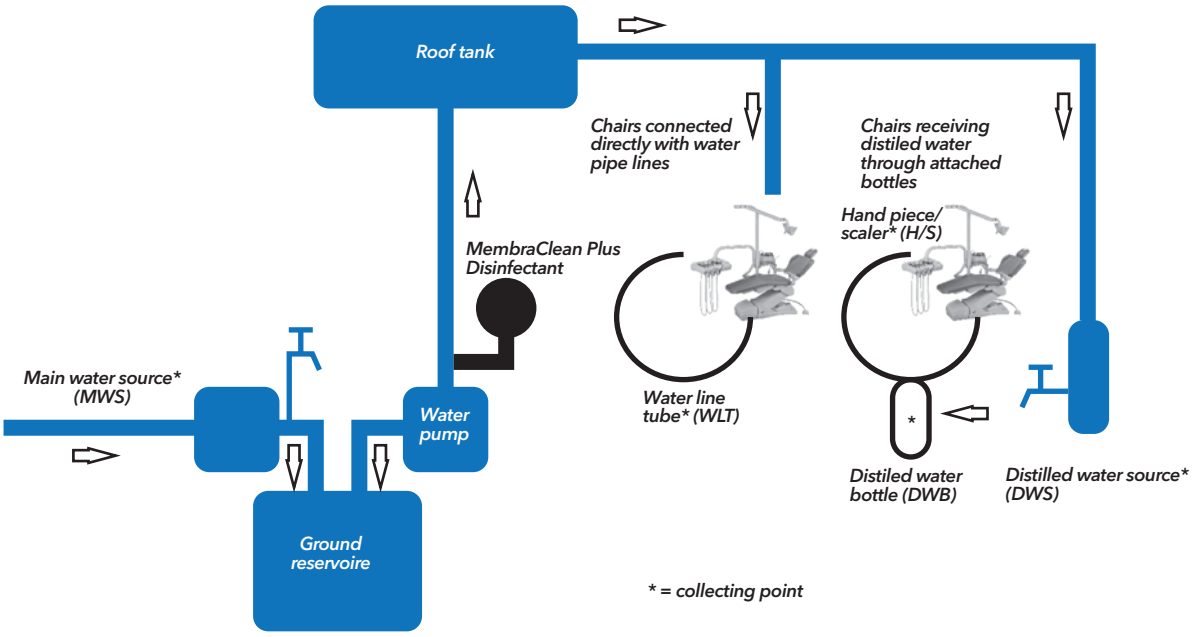

Figure 1. The diagram of the RAKCODS Dental Units' water supply system and the points of samples collection.

Plate Count Agar (HiMedia, India) was prepared according to standard procedure and then cooled at 44-46°C. Serial dilutions were prepared from the water samples in addition to undiluted sample (1:1, 1:10, 1:100). One ml of each sample or dilution was transferred to the properly labeled sterile Petri dish. Approximately 15ml of the cooled agar medium was then poured into each Petri dish. The sample and agar were then mixed by rotating the plate several times. After the media has solidified, the plates were inverted and incubated at 35°C for 48-72 hours. After incubation, the count of colonies, mean and standard deviation were calculated.

• Molecular identification of bacteria:

Pure cultures of the isolated bacteria were sent for molecular identification in AccuVis Bio laboratories in Abu Dhabi University Campus, Abu Dhabi, UAE. **Bacterial 16S rRNA gene sequencing** was performed according to the following protocol: Bacterial DNA isolation AccuVis Bio's Bacterial Genomic DNA Isolation Kit (AV1003). PCR amplification uses PCR Primers (Universal), forward: 27F - 5'- AGAGTTTGATCMTGGC TCAG - 3', Reverse: 1492 R - 5'- TACGGYTACCTTGTTACGACTT - 3'. DNA sequencing using BigDye® Terminator v1.1 Cycle Sequencing Kit, Sequencing reaction for Forward (518F) and Reverse (800R), Data analysis - Sequencing Analysis Software v5.2. Bioinformatics tools used Fasta format conversion of both sequences – NCBI, Pairwise sequence alignment – LALIGN software, trimming of final sequence, NCBI blast search, Similar sequence identification, identification of bacteria.

3. Results

Table 1 shows the total count of bacteria (CFU/ml) in water samples collected from the Main Water Source, the Distilled Water Source and 12 dental units of RAKCODS, counted according to ADA guidelines (Table 1).

Based on the ADA guidelines, which state that water used in dental treatment should contain a bacterial level of ≤200 CFU/ml, the majority of samples collected in our study showed CFU above the standard.

Table 1. Bacterial count in the water samples taken from different collecting points

Site of collection	Total number of collected samples	Number (%) of samples with 0-200 CFU*/ml	Number (%) of samples with >200 CFU/m	Mean number of CFU/ml±SD
Main Water Source	3	0 (0%)	3 (100%)	499±345
Distilled Water Source	3	0 (0%)	3 (100%)	1538±1165
Distilled Water Bottle	12	0 (0%)	12 (100%)	2397±1403
Water Line Tube	36	5 (14%)	31 (86%)	1867±1434
Handpiece/Ultrasonic Scaler	36	2 (6%)	34 (94%)	2000±1535

*CFU: colony forming units

Table 2. The bacterial distribution of the water samples according to the collecting points and isolated bacteria

Source/site of sample collection	Total number of collected samples	Number (%) of samples in which the following bacteria were isolated				
		Ralstonia spp.	*Sphingomonas paucimobilis*	*Leifsonia spp.*	*Brevundimonas aurantiaca*	*Pseudomonas aeruginosa*
Main Water Source	3	1 (33.3%)	2 (66.7%)	3 (100%)	0 (0%)	0 (0%)
Distilled Water Source	3	3 (100%)	3 (100%)	2 (66.7%)	1 (33.3%)	0 (0%)
Distilled Water Bottle	12	12 (100%)	10 (83.3%)	10 (83.3%)	6 (50%)	3 (25%)
Handpiece/Ultrasonic Scaler	36	35 (97%)	33 (91.7%)	24 (66.7%)	14 (38.9%)	3 (8.3%)
Water Line Tube	36	36 (100%)	32 (88.8%)	27 (75%)	13 (36.1%)	3 (8.3%)
Total	90	87 (96%)	80 (88.8%)	66 (73.5%)	34 (37.8%)	9 (10%)

The CDC recommended that non-surgical dental water should have a heterotrophic plate count (HPC) of ≤500 CFU/ml. The only samples that fulfilled this criterion were the Main Water Source samples (499 CFU/ml) which is equal to the levels of HPC in drinkable water.

Since the dental units' water supply systems were of two types as shown in Fig. 1, it was found that the bottled units contained significantly higher numbers of CFU (2632±1231.783) compared to the non-bottled units (1484.75±1395.093), $p<0.02$.

RAKCODS had a prescheduled plan to replace all of the distilled water bottled dental units with new units receiving direct water connection. The units were installed on time (September 2015) and were allowed to work for 4 months. Random water samples from 7 of the newly installed dental units. Two samples from each water outlets (water/air syringe and hand piece tubes) were collected in the same manner as described earlier and the bacterial colonies per ml were counted. The average CFU/ml of these samples were compared with the average counts of water line tubes of the previous bottled units. The newly installed dental unit counts showed remarkable reduction in the number of CFU/ml (720, SD±969).

Table 2 shows the isolated bacteria and number of water samples contaminated with each type of bacteria out of the total number of samples collected from the Main Water Source, the Distilled Water Source and the 12 dental units (Table 2).

Ralstonia spp. was the most common bacteria in the MWS, DWS and dental units' WLT, as it was found in 96% of the collected samples. The other common isolated bacteria were *Sphingomonas paucimobilis* 88.8%, *Leifsonia* spp.(73.5%), *Brevundimonas aurantiaca* (37.8%) and *Pseudomonas aeruginosa* (10%).

4. Discussion

The majority of the collected samples in this study showed CFU above the standards for drinking water or water used for dental procedures according to the CDC guidelines. The only samples that fulfilled this criterion were the MWS samples with an HPC of (499 CFU/ml), which is equal to the levels of HPC in drinkable water. The fact that MWS samples contained significantly lower CFU/ml of bacteria compared to the DWB, WLT or H/S clearly indicates that the dental water pipelines provide good environment for bacteria to thrive.

When bottled dental units were replaced with new dental units, the average CFU/ml was reduced dramatically. This result substantiates the assumption that the DWB was the main source of contamination.

In the examined water samples from the dental units, bacteria of the *Pseudomonadaceae* family were the most common. These obligate aerobic, motile, gram negative bacilli are widely spread and have the ability to survive and grow almost in any environment. Their presence is associated with the main water supply and failure of disinfection methods to eradicate them totally or even reduce their counts. The isolated bacteria tend to categorize as non-fermenting gram-negative bacilli (NFGNB) which are a group of organisms that either do not utilize glucose as a source of energy or utilize it oxidatively.[14]

Pseudomonas aeruginosa, species of *Pseudomonas* genus can be recovered from the oral cavity of 4% of healthy individuals[4] and this indicates the possibility of these microorganisms getting aspirated into the DUWLs through a defective check valve and colonized in the waterlines. This is a drawback due to the fact that water after having passed through DUWL, flows through hand pieces during treatment and forms aerosol and splatter therefore increasing the chances of cross infection especially in immunocompromised patients.

Following is a list of bacteria tested for in our study, in the order of their prevalence:

4.1. Ralstonia spp.

Ralstonia spp. was the most common type of bacteria present in the MWS, DWS and dental units' WLT. It was found in 96% of the collected samples. This finding is in accordance with many of the previous studies.[15,16,17] This bacterium is known to be isolated from water regardless of its source. It could be isolated from municipal drinking water, bottled water, dental waterline tubes, hospital water supplies, standard purified water, laboratory-based high-purity water systems and industrial ultra-pure/high purity water.[18]

Ralstonia (named after the American bacteriologist E. Ralston) is a genus of Proteobacteria, previously included in the genus *Pseudomonas* and contains 13 species *(R. basilensis, R. campinensis, R. eutropha, R. gilardii, R. insidiosa, R. mannitolilytica, R. metallidurans, R. paucula,R. pickettii, R. respiraculi,R. solanacearum, R. syzygii, R. taiwanensis).* Most of these bacteria are environmental bacteria with no clinical significance. However some of the species like *Ralstonia pickettii* can cause bacteraemia and serious infections e.g. sepsis contaminating injection solutions and aqueous chlorhexidine solutions.[19] These bacteria were also documented to be related to infection in cystic fibrosis patients.[19] *R. paucula* and *R. gilardii* have only been isolated from human clinical samples including cerebrospinal fluid, bone marrow, wounds, and the respiratory tract.[21] Previous studies stated that the majority of the *Ralstonia* isolates showed susceptibility to most of the tested antibiotics.[18]

4.2. Sphingomonas paucimobilis

The second most common contaminant of the MWS, DWSand dental units' WLT was an aerobic bacterium found in soil and water known as *Sphingomonas paucimobilis*. Although it rarely causes infection it has been reported as a causative agent of healthcare-associated infection especially in immunocompromised patients. In the current study it was found in 88.8% of the collected samples. These findings are similar to previous studies.[22,23]

Sphingomonas paucimobilis was reported to cause outbreaks of bacteremia among immunocompromised patients in hematology and oncology units due to bacterial contamination of hospital water systems.[24] It is now emerging as an opportunistic pathogen that is frequently reported in clinical settings.[25] It can be isolated from hospital environments such as distilled water, nebulizers, and multiple equipments used in medical care. It has been associated with a few cases of continuous ambulatory peritoneal dialysis and is notorious for its resistance to the commonly used antibiotics.[26] Some reports stated that *S. paucimobilis* can cause infections in healthy as well as immunocompromised individuals where infection caused by *S. paucimobilis* can lead to septic shock.[27] Although this organism is a gram negative bacteria it lacks the lipopolysaccharide components in the outer membrane of the cell wall which is associated with endotoxin activity.[28]

A recent study showed that *S. paucimobilis* isolates from cancer patients were fairly sensitive strains, with resistance observed only against ceftazidime and aztreonam.[14] This organism tends to show unpredictable antibiotic sensitivity attributed to the antibiotics' therapeutic failure.[26]

4.3. Leifsonia

The third most common contaminant *Leifsonia* was found in 73.5% of the samples collected from the MWS, DWS and dental unit's WLT. It is an aquatic bacterium typically found in environmental water habitats and is a usual finding in dental water lines as shown in previous studies.[29,30,31]

This bacterium is catalase and oxidase positive. *L. aquatica* was once classified as a species of the Corynebacterium genus. However, because of the chemotaxonomic and genetic differences from corynebacteria, it has been reclassified.[32] Infection due to *L. aquatica* is rare, and is commonly catheter associated in immunocompromised patients. Serious infections in healthy people however, have also been reported.[33]

4.4. Brevundimonas aurantiaca and Pseudomonas aeruginosa

Out of the 90 samples 34 (37.8%) showed the presence of *Brevundimonas aurantiaca*. This bacteria was present in water from all sources except the MWS. The highest contamination rate was in DWB (50%).

The pattern of contamination was the same with *Pseudomonas aeruginosa*, which was not present in MWS and DWS, but present in 25% of the samples taken from DWB, 8.3% ofH/S water samples and 8.3% of samples taken from WLT. The total number of samples positive for *Pseudomonas aeruginosa* was 9 (10%).

Brevundimonas (Pseudomonas) aurantiaca is a gram-negative soil bacterium which can synthesize antimicrobial compounds that have the same structure of compounds produced by other members of pseudomonades. These include phenazines, proteins, phloroglucinols and Mycolytin (an antifungal biopesticid).[34] These bacteria showed remarkable intrapopulation phenotypic variability observed during their germination. This is an important survival strategy under unfavorable environmental conditions.[35]

Pseudomonas aeruginosa is a gram-negative bacteria that is citrate, catalase and oxidase positive. It has the ability to grow in plumping fixtures and survive in distilled water.[36]

Pseudomonas aeruginosa in samples taken from DWB, WLT or H/S need not infer an oral source of the bacteria. In this study, *Pseudomonas aeruginosa* was present in 8.3% of H/S water samples which represent the point of contact to the oral cavity. This, compared to the 25% of DWB samples suggests that back-flow of the concerned bacteria from the oral cavity is unlikely. Therefore, we can only assume but not confirm the presence of fairly effective mechanisms that prevent sucking back fluids from patients' oral cavities, and subsequent multiplication in our university's dental clinic units. This eliminates a potential source of cross infections.

Our investigation showed that there were no bacteria of *Streptococcus* and *Staphylococcus* genera. Nevertheless, the presence of these microorganisms in distilled water reservoir of dental units has been reported.[37]

5. Conclusion

The bacterial concentration in majority of the collected water was relatively higher than the standard counts. The study revealed that the bottled units contained significantly higher numbers of CFU and had more chances of contamination with serious bacteria. The bacterial flora in the water samples comprised of bacteria characteristic for water supply systems and opportunistic pathogens, with no bacteria of the oral cavity flora. Nevertheless, microbial counts of water samples collected from dental units after replacement of all bottled dental units (causing the major contamination) demonstrated substantial reduction in the counts. In addition, this study's determination

of contamination sources and evaluation of microbial load in RAKCODS could contribute to the development of quality control methods in the future.

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Questions

Presence of which of the following bacteria in a water sample suggests an oral source?

❑a. Staphylococcus;
❑b. Leifsonia;
❑c. Pseudomonas aeruginosa;
❑d. A and C.

The most commonly present bacteria in the main water source, distilled water source and dental units' water line tubes was:

❑a. Leifsonia;
❑b. Sphingomonas paucimobilis;
❑c. Ralstonia spp.;
❑d. A, B and C above.

Samples from which of the following sources met the CDC recommendations for non-surgical dental water which have a heterotrophic count of ≤500 CFU/ml?

❑a. Main Water Source samples;
❑b. Distilled water source;
❑c. Both A and B;
❑d. None of the samples.

Which of the following statements is CORRECT?

❑a. Non-bottled units contained significantly higher numbers of CFU compared to bottled units;
❑b. Bottled units contained significantly higher numbers of CFU compared to non-bottled units;
❑c. The difference in bacterial count between bottled and non-bottled units was not statistically significant;
❑d. None of the statements is correct.