Microbial Assessment of Dental Unit Waterlines in an Institutional Setup in Karnataka, South India

Abstract

Background and Aim: Biofilms in dental unit waterlines (DUWLs), suction hoses, and fittings are a potentially significant source of cross-contamination posing significant health risk as these may come into contact with patients during treatment. The purpose of this in vitro study was to identify the spectrum of bacterial flora colonizing the DUWLs and to detect pathogenic microorganisms present in such an environmental niche. Materials and Methods: Thirty DUWL samples were collected from in use dental units selected randomly from various clinical departments. Samples were collected from the following devices; 3-in-1 syringe waterline, section of waterline tubing supplying the 3-in-1 syringe, and the air rotor water. The samples were subjected to bacteriological analysis, and all bacterial isolates were tested for their ability to form biofilms. Results: A descriptive analysis of the results obtained was carried out, and it was observed that 7 out of 30 (23.3%) samples collected from DUWL were supplying water of unsatisfactory quality with species of low-pathogenicity bacteria isolated present in significant numbers; four of ten (40%) water supply lines contained bacterial biofilms; and the species with greatest capability to form biofilms were Enterobacter species (spp.). In addition, the results were also subjected to Chi-square test which revealed no statistical difference between the species and the location of collection of samples. Conclusion: Within the limitations of this study, it is concluded that DUWLs are not totally free of contamination. Microbial biofilms are a significant source of cross-contamination and cross-infection in the dental clinic environment.

Keywords: Biofilms, dental unit waterlines, water quality

Introduction

Biofilms in dental chair unit waterlines (DUWLs), suction hoses, and fittings are a potentially significant source of cross-contamination and pose a significant health risk as these may come into contact with the patient and the clinician during treatment.[1] The polluted water may enter the alimentary tract or respiratory tract of the patient through bioaerosols generated by high-speed handpieces.[2] This can be hazardous to susceptible individuals such as those with immunodeficiency, pregnant women, elders, children, smokers, and people undergoing a transplant operation or radiation therapy.[3] With an ever-increasing population of such individuals, both, due to physiological aging and as a cofactor of associated medical conditions and treatments, it is imperative to ensure a supply of good quality, sterile water in such DUWLs so as to prevent any opportunistic, iatrogenic infections.

The European Union Medical Devices Directive classifies dental chair units (DCUs) as medical devices as they are used for treatment of patients.[4] Microbial contamination of specific constituent parts can be a significant potential source of cross-infection, the source of which may be from the municipal water piped into the DCU or the suck back of patient’s saliva into the line due to the lack of anti-retraction valves.[5,6]

Each DCU is equipped with narrow-bore (i.e., mostly 2–3 mm internal diameter) flexible plastic tubing called DUWLs that supply water to cup-filler and bowl-rinse water outlets and all of the DCU-supplied instruments which activate or cool them.[7,8] Due to the texture and composition of the many meters of plastic tubing, microbial biofilms form readily, resulting in DCU output water that is often heavily contaminated with microorganisms. Biofilms are impervious to a wide range of chemical agents including detergents, disinfectants, and antimicrobial agents.[9,10] Biofilms in DUWLs have been...
measured to be 30–50 µm thick.\cite{11,12} During DCU operation, the shear force generated within DUWLs detaches pieces of biofilm along with planktonic forms of microorganisms, which can be deposited directly in the mouths of patients, can seed biofilm growth at other sites within the waterline network, or can be aerosolized and subsequently inhaled into the respiratory tracts of patients and dental staff when dynamic dental instruments such as ultrasonic scalers are used.\cite{7,8,13,14} Consequently, DUWL biofilm functions as a reservoir for continuous contamination of DUWL output water.

The goal of infection control is to minimize the risk from exposure to potential pathogens and to create a safe working environment for providing dental health care. The American Dental Association (ADA) recommends that water delivered to patients during nonsurgical dental procedures should contain no more than 200 CFU/ml, whereas the Center for Disease Control (CDC) recommends that drinking water should contain ≤500 CFU/ml.\cite{15}

Previous studies have confirmed high level of bacterial contamination that violates basic infection control principles.\cite{16-18} Despite this knowledge of high bacterial contamination, an exhaustive literature survey indicates very limited published reports from India. This in vitro study aims to investigate the microbial contamination of water in the dental unit (DU) water systems in the clinical departments of this institution which might pose a hazard to the patient specifically the elderly and dental health-care personnel. The null hypothesis tested was that the pathogenic bacterial flora does not colonize the DUWLs.

**Materials and Methods**

The study was conducted in Manipal College of Dental Sciences, Mangalore, India, following approval from the Institutional Ethics Committee in collaboration with the Department of Microbiology of the same institution.

**Sampling of dental unit waterlines**

Thirty DUWL samples (three samples from each chair) were collected from in use DUs (Clinix New Generatia; Confident Dental, Bengaluru, India) selected randomly from various clinical departments: two chairs each from the Department of Pedodontics, Prosthodontics, Conservative Dentistry and four chairs from the Department of Periodontics. Three samples were collected early morning from each chair after flushing with water for 3 min; water from 3-in-1 syringe designed to deliver air, water, or air/water into the mouth during dental treatment; a section of the waterline tubing supplied to the 3-in-1 syringe (internal diameter of approximately 1 mm) and air rotor water.

**Bacteriological analysis of the dental unit waterlines samples**

Analysis of the presence and the count of bacteria in the water samples was done according to the multiple tube method as described by BW senior.\cite{19}

**Isolation and identification of bacterial isolates from dental unit waterline samples**

Water samples collected as above were filtered through a membrane filter. Organisms were washed from the membrane by vortexing the container for 1 min in 10 ml of sterile phosphate-buffered saline (PBS). These samples were inoculated into an enriched medium such as brain heart infusion (BHI) broth and incubated aerobically at 37°C in the presence of 5% CO₂ for up to 48 h. Any sample which showed growth (as indicated by turbidity) was subcultured onto appropriate solid media (Sheep blood agar/MacConkey agar/Sabouraud’s dextrose agar) and after suitable incubation was further processed for the identification of the isolate by standard microbiological methods.\cite{20}

**Detection of biofilm formation on the dental unit waterlines**

External DUWL tubing surfaces were wiped with a sterile alcohol wipe and approximately 5 cm of the tubing cutoff with presterilized (121°C for 15 min) scissors. The tubing was sectioned to obtain a specimen representing 1 cm². The surfaces were rinsed in sterile PBS to remove planktonic cells. Using sterile dental probes, the surface biofilm was scraped into 1 ml of sterile PBS. These biofilm specimens were then inoculated into BHI broth to observe for bacterial growth.\cite{5} Any specimen which showed growth after suitable incubation was further processed for the identification of the isolate by standard microbiological methods.\cite{20}

**Determination of the capability of bacterial isolates to form biofilm**

Bacterial strains isolated from DUWL samples were used to determine their capacity to form biofilms by the microtiter plate method.\cite{21}

**Results**

A total of thirty samples were available for analysis from 10 DUs. The results of the microbiological investigation are listed in Table 1. A descriptive analysis of the bacteriological investigation of the water samples by the multiple tube test revealed that seven of thirty samples (23.3%) were of unsatisfactory quality (confirmed *Escherichia coli* count of one - >180 most probable number (MPN)/100 ml) as per the recommendations of the WHO (1971).\cite{22} These seven samples were from four DU, two from Pedodontics and one each from Prosthodontics and Conservative Dentistry Departments, respectively. It was observed that in all four DUs, it was the three in one syringe and the air rotor samples which were supplying contaminated water but the waterlines supplying these did not show any bacteria, indicating the existence of these bacteria within these units.

A total of six species (spp.) of bacteria were isolated from these samples, namely, *Acinetobacter* spp., *Bacillus*
spp., *E. coli*, *Enterobacter* spp., *Klebsiella* spp., and *Pseudomonas* spp. Although *E. coli*, *Klebsiella* and *Enterobacter* spp. (coliforms) indicated a human source of contamination, the others such as *Bacillus*, *Pseudomonas* and *Acinetobacter* spp. are found in the environment as saprophytes [Table 2].

The attempt to detect biofilms from the water tubings which supply the DUs showed that four out of the ten tubings (40%) did contain biofilms [Table 3]. Two of the water tubings from Periodontics and one each from Pedodontics and Prosthodontics contained the biofilm. The bacterial species forming biofilms were two Gram-negative saprophytic organisms, namely, *Acinetobacter* spp. and *Pseudomonas* spp. and one Gram-positive organism, namely, *Bacillus* spp.

Except for *E. coli*, all other five species were found to be capable of producing biofilms by at least 72 h (h) of incubation [Table 4]. Biofilm was established by *Enterobacter* spp. within 24 h of incubation. The same species also produced the highest density of biofilm within 72 h of incubation (optical density value of >0.5).

### Discussion

The results of this study confirm earlier work done by researchers[13-18,23-29] demonstrating that the microbiological quality of water emerging from DUWLs does not conform to accepted guidelines for potable water. Thus, the null hypotheses were rejected.

It was observed that in all DUs, it was the 3-in-1 syringe and the air rotor samples which were supplying contaminated water but the waterlines supplying these did not show any bacteria on culture, indicating the existence of these bacteria within these units. Bacterial counts of water samples from high-speed handpiece and the air/water syringe were higher than in the dental tubing. These results are in agreement with similar studies done by other researchers.[30,31] Clinically, this implies that water from the air rotor should be avoided in dental surgical procedures including implantology. The bacterial count obtained presumably underestimates the true microbial load to which a patient is exposed, since it has been demonstrated that only 5% of the microscopically visible bacteria can be recovered by conventional cultural techniques.[32] Other bacteria may be either in a temporarily nonculturable state or may represent the large fraction of the microflora from many natural habitats which remain “as yet uncultured.”[33]

The current investigation identified a total of six species of bacteria, namely, *Acinetobacter* spp., *Bacillus* spp., *E. coli*, *Enterobacter* spp., *Klebsiella* spp. and *Pseudomonas* spp. Other studies have identified a host of bacteria, which may be explained by the considerable differences in methods and technique used to identify these isolates including

### Table 1: Bacteriological analysis of water samples collected from dental unit waterlines

| Department       | DCU number | Bacterial counts, expressed as MPN/100 mL* (sample type) | Grade* |
|------------------|------------|----------------------------------------------------------|--------|
|                  | WLS        | WAR | WLT |
| Periodontics     | 1          | <1  | <1  | Excellent |
| Prosthodontics   | 1          | <1  | <1  | Excellent |
| Pedodontics      | 1          | <1  | <1  | Excellent |
| Conservative dentistry | 1 | <1  | <1  | Excellent |

*Presumptive coliform count; *Grading as per WHO (1971) standards. DCU=Dental chair unit, WLS=Syringe waterline, WAR=Air rotor water, WLT=Waterline tubing, MPN=Most probable number, WHO=World Health Organization

### Table 2: Bacterial species isolated from various samples of dental units

| Bacterial species            | Samples collected from | Total number of strains |
|------------------------------|-------------------------|-------------------------|
|                              | WLS | WAR | WLT |                     |
| *Acinetobacter* spp.         | 2   | 1   | 1   | 4                    |
| *Bacillus* spp.              | -   | -   | 1   | 1                    |
| *Escherichia coli*           | 2   | 1   | -   | 3                    |
| *Enterobacter* spp.          | 2   | -   | -   | 2                    |
| *Klebsiella* spp.            | 3   | 2   | -   | 5                    |
| *Pseudomonas* spp.           | -   | 1   | 2   | 3                    |
| Total                        | 9   | 5   | 4   | 18                   |

\( \chi^2=12.072, P=0.2802. \) There is no significant difference in the species and the location of collection of samples. WLS=Syringe waterline, WAR=Air rotor water, WLT=Waterline tubing

### Table 3: Bacterial species found to form biofilm on water tubing supplying dental units

| Department* (n=4) | *Bacillus* spp. | *Acinetobacter* spp. | *Pseudomonas* spp. | Total number of species isolated |
|-------------------|-----------------|----------------------|--------------------|----------------------------------|
| Periodontics*     | 2               | 1                    | -                  | 3                                |
| Prosthodontics*   | -               | 1                    | 1                  | 2                                |
| Pedodontics*      | -               | 1                    | 1                  | 2                                |
| Conservative      | -               | -                    | -                  | -                                |
| Total (n=10)      | 2               | 3                    | 2                  | 7                                |

*Biofilm detected (4 of 10; 40%), two from periodontics, one each from prosthodontics and pedodontics
Table 4: Production of biofilm by the bacterial species isolated from dental unit waterlines at different time intervals

| Bacterial species          | Density of biofilms at different incubation periods | Total number of strains producing biofilm |
|----------------------------|-----------------------------------------------------|------------------------------------------|
|                            | 24 h       | 48 h       | 72 h       |                                      |
| Acinetobacter spp.         | +          | ++         | +          | 5                                     |
| Bacillus spp.              | -          | +          | ++         | 2                                     |
| Escherichia coli           | -          | -          | -          | -                                     |
| Enterobacter spp.          | ++         | +++        | +++        | 2                                     |
| Klebsiella spp.            | +          | ++         | +          | 4                                     |
| Pseudomonas spp.           | -          | ++         | ++         | 5                                     |

+++High degree of biofilm produced; OD>0.5, +++=Moderate degree of production; OD between 0.5 and 0.1, +=Low producer of biofilm; OD<0.1

sampling time, culture medium, time, temperature and mode (aerobic/anaerobic) of incubation such as Columbia blood agar (for oral streptococci, Actinomyces, and Pseudomonas), R2A agar (for environmental isolates), MacConkey agar (for Enterobacteria), Sabouraud’s dextrose agar (for Candida), BCYE agar (for Legionella), and Middlebrook’s agar for Mycobacteria.[3,5-7] We have used the enriched liquid medium (BHI) for primary isolation of microorganisms as the best isolation rates are obtained only with liquid media (as in case of blood cultures). Waterborne pathogens such as fecal coliforms, for example, *E. coli* should always be absent from potable water supplies. Water with <1 fecal coliform/100 mL and <500 CFU/mL is considered potable. Although *E. coli*, *Klebsiella* and *Enterobacter* spp. (coliforms) indicated a human source of contamination, the others such as *Bacillus*, *Pseudomonas*, and *Acinetobacter* spp. are found in the environment as saprophytes. *Acinetobacter* found in the environment, is a well-known cause of nosocomial infections, and exhibits a high degree of drug resistance. *Bacillus* found in the environment and does not usually cause any infection and its presence in clinical samples is considered as a contaminant. *Pseudomonas* exhibits a high degree of drug resistance, is found in the environment, and is a well-known agent of nosocomial infections. *Pseudomonas* spp., principally *Pseudomonas aeruginosa*, grow in low nutrient environments and often exhibit resistance to antimicrobial agents, disinfectants, and biocides.

The presence of *E. coli* indicates fecal contamination as it is part of the normal flora of the gastrointestinal tract of both humans and animals and cannot survive for longer periods in the environment. It is a primary pathogen and responsible for hospital acquired infections causing a spectrum of infections ranging from urinary tract infection (UTI), diarrhea, respiratory tract infections, wound infections, surgical sepsis, sepsis, meningitis, etc. Also known for extended spectrum beta-lactamases production, *Klebsiella* and *Enterobacter* are also part of the normal flora of the gastrointestinal tract of humans but are able to survive for longer periods in moist environments. Both are known causes of sepsis, meningitis, wound infections, UTI, and respiratory infections.

In the present study, seven out of the thirty samples were contaminated with majority of the identified organisms of low pathogenicity. Despite their low pathogenic potential, they have increasingly been recognized as opportunistic pathogens mainly in immunocompromised patients and hospitalized patients.[27]

Most of the isolated bacteria from the tubing and from other sites were capable of producing biofilms. Biofilms protect the proliferating organisms embedded in a matrix of extracellular polymeric substance from the effects of heat and chemicals, thus reducing their susceptibility to disinfection processes. In addition, the complex design of dental chair equipment results in the stagnation of water within the equipment lines where bacteria could proliferate within a biofilm is a major factor affecting microbial contamination of waterlines.

According to the ADA and CDC guidelines, commercially available options for improving DU water quality include the use of independent reservoirs, source water treatment systems, chemical treatment regimens, daily draining, air purging and point-of-use filters.[28]

Both the ADA and the CDC endorse flushing waterlines for several minutes before the first patient visit and for 20–30 s between patients.[12] The effectiveness of flushing before the start of therapeutic services and after providing services to each patient has been proved.[28] The use of chemical germicides has been recommended for the removal or inactivation of biofilms in dental waterlines.[29] In our institution, in addition to flushing all the suction units, hoses are cleaned and disinfected using 2% solution of Orotol® Plus (dimethyl-dioctyl-ammonium chloride 50%, benzyl-dimethyl-dodecyl-ammonium chloride 50%), manufactured by Durr Dental AG; Germany on a regular basis. Chemical agents, though effective in reducing microorganisms in effluent water, do little to destroy the biofilm matrix in the DUWL, even with periodic treatments. Bacterial populations in the DU water rapidly recolonize the DUWL.

**Conclusion**

Based on the results and within the limitations of this study, it is concluded that DUWLs are not totally free of contamination. Microbial biofilms are a significant source of cross-contamination and cross-infection in the dental clinic environment, and evidence-based preventive maintenance strategies in the key areas associated with biofilm contamination are necessary. This is of paramount importance because of the increasing numbers of medically compromised and immunocompromised patients receiving regular dental treatment. Awareness of the threat of infection and steps to decrease this should be a priority for every dentist in a clinical practice.
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Conflicts of interest
There are no conflicts of interest.

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