

# Evaluation of Microbial Profile in Dental Unit Waterlines and Assessment of Antimicrobial Efficacy of Two Treating Agents

Mungara J\* / Dilna N C\*\*/ Joseph E\*\*\*/ Reddy N \*\*\*\*

**Objective:** The quality of water in a dental unit used for cooling and flushing the high and low speed handpiece, air/water syringes and the scalers is of considerable importance. The present study was carried out to enumerate and identify the microorganisms present in water samples collected from dental unit waterlines of different dental specialty clinics and to find out the efficacy of two treating agents in disinfecting dental unit waterlines. **Study design:** Sample included 70 dental unit waterlines from different speciality dental clinics which were checked for microbial contamination. From these dental units 40 units were randomly selected and divided into two groups of 20 each. Group A, treatment was done in 20 dental units with 0.2% Chlorhexidine gluconate solution and Group B, treatment was done in 20 dental units with 10% Povidone iodine solution and the reduction in the microbial levels were assessed. Five dental units were randomly selected and checked the microbial contamination using mineral water, sterile distilled water, fresh tap water as a water source in the dental unit reservoir bottles. Also from the test group, five from each group were checked for the duration of efficacy of treating agent for one week by analyzing the water samples collected on 3, 5 and 7 day intervals. **Results:** Most of the identified microorganisms are Gram negative and pseudomonas predominating up to 98.59% of the total isolates. Usage of disinfectants 0.2% Chlorhexidine and 10% Povidone Iodine were found to be very effective in reducing the microbial contamination and 10% Povidone iodine was found to be more efficient (97.13%) and active for a period of 3 days and gradually losing its efficacy by 7th day. No significant difference were found in microbial contamination of water samples collected from different water outlets such as handpiece outlets, air water syringe outlets, scaler lines. **Conclusion:** To continue maintaining the sterility of the Dental unit waterlines and to complete the infection control measures adopted in the dental clinics, suitable disinfectants like 0.2% Chlorhexidine on daily basis or 10% Povidone iodine on every 3rd day basis intermittently maintain the sterility of dental unit waterlines it is essential to have a good water source and an effective disinfectant.

**Keywords:** Dental unit waterline, microbial profile, Povidone Iodine, Chlorhexidine

## INTRODUCTION

A successful treatment requires sterile environment which in turn renders infection control a major importance in routine daily procedures in the dental office. The goal of infection control is to prevent the spread of infection from one patient to another and to the treating health care worker. This can be achieved by a series of actions such as hand washing and gloving, protection against aerosol and splatter with the use of facemasks, eye

wear, protective clothing and Instrument processing. Many of the infection control measures called 'Universal precautions'<sup>1</sup> is recommended by national dental associations and for effective infection control, every possible source of contamination should be submitted to these actions before, during and after dental intervention. The quality of water in a dental unit used for cooling and flushing the high and low speed handpiece, air/water syringes and the scalers is of considerable importance because patients and dental staff are regularly exposed to water and aerosol generated from dental unit. The Source of water supply to these dental units can be through open system from pipe lines or through closed system which is also called as independent water system that is when water is poured into a reservoir attached to the dental unit.

The presence of microbial contamination of the water coming from dental units was first reported by Blake.<sup>2</sup> A recommendation has been issued by the American Dental Association<sup>3</sup> that by the year 2000, water for non surgical procedures should contain no more than 200cfu/ml of aerobic, mesophilic, heterotrophic bacteria in the unfiltered output of dental unit waterline. The recommendations of the U.S. Centers for Disease Control and Prevention (CDC), the American Dental Association (ADA) and the British Dental Association (BDA) are that waterlines should be flushed through for "several minutes" at the beginning of each clinical day to expel the

\* Jayanthi Mungara, MDS, Professor and Head, Ragas Dental College and Hospital, India

\*\* Dilna NC, Post graduate student, Department of Pedodontics and Preventive Dentistry, Ragas Dental College and Hospital, India

\*\*\* Elizabeth Joseph, MDS, Professor, Department of Pedodontics and Preventive Dentistry, Ragas Dental College and Hospital, India

\*\*\*\* Nilaya Reddy, MDS, Reader, Department of Pedodontics & Preventive Dentistry, Ragas Dental College and Hospital, India

Send all correspondence to: Dr. Jayanthi Mungara, Department of Pedodontics and Preventive Dentistry, 2/102, East Coast Road, Uthandi, Chennai- 600119, Tamil Nadu, India

Phone+91-9840849850

E-mail: jayanthi\_1963@yahoo.com

overnight build up of microbial load in stagnant areas and for 20–30 seconds between patients to remove material that may have been retracted during treatment.<sup>4</sup> The safety of dental treatment requires a good quality of the water used. Transmission of microbial pathogens from biofilm within dental unit waterlines to the patients is a concern because it is difficult to maintain the sterilization in these areas. Two problems can arise from the presence of biofilms in a distributing aqueous system. First, the biofilm can clog pipes and tubing or interfere with the proper function of mechanical devices. Second, bacterial populations living in this protected mode of growth produce planktonic cells that contaminate fluids and alter their properties or, in the case of pathogens, can result in food poisoning or infections. In addition, biofilm bacteria are substantially resistant to surfactants, biocides, and antibiotics. These concerns are now being realized in the dental profession.

It is important to disinfect the source of water supply to the dental units for which knowledge about the bacterial load in dental unit waterlines is necessary. With this background, the present study was undertaken to evaluate the microorganisms present in the water samples collected from dental unit waterlines of different specialities which were randomly selected to find out the efficacy of two commonly available treating agents in disinfecting the dental unit waterlines. The aims of the present study were:

1. To enumerate and identify the microorganisms present in water samples collected from dental unit waterlines of different dental speciality clinics.
2. To find out the efficacy of two treating agents in disinfecting dental unit waterlines.

### MATERIALS AND METHOD

The present study was conducted in the department of Pedodontics and Preventive dentistry, Ragas Dental College to assess the bio-film of Dental unit waterlines from various speciality dental clinics and to check the effect of treating agent used to disinfect the Dental unit waterline. Sample included 70 dental unit waterlines from different speciality dental clinics which were checked for microbial contamination. From these dental units 40 units were randomly selected and divided into two groups of 20 each. Group A, (20 dental units) was treated with 0.2% Chlorhexidine gluconate solution and Group B, (20 dental units) was treated with 10% Povidone iodine solution and the reduction in the microbial levels was assessed. Five dental units were randomly selected and checked for the microbial contamination using mineral water, sterile distilled water, fresh tap water as a water source in the dental unit reservoir bottles. Five dental units were randomly selected to collect water samples from three different water outlets such as handpiece lines, air/water syringe and scaler lines and microbial contamination was assessed. The duration of efficacy of treating agent was checked in 5 samples from each group for one week at 3, 5 and 7day intervals.

#### Inclusion criteria<sup>5</sup>:

- Units that had been in daily use for more than one year.
- Units that have not been treated for removal of bio-film or reduction of planktonic bacteria.

### Sample collection at baseline

Water samples were collected from the end of operator's water syringe line of 70 dental units from different specialities using sterile techniques.<sup>6</sup> (The sterile techniques include the use of sterile gloves, wiping the external surfaces of water line with sterile cotton gauze soaked in 70% alcohol and collection of waterline samples in sterile bottles). Before sample collection the reservoir bottle on each unit was washed and disinfected, then filled with fresh tap water and reattached to the dental unit. Fresh tap water was collected in a bottle before sample collection to assess the microbial levels for baseline evaluation. Lines were flushed for 20 seconds if dental unit was in use that day or for 2 minutes if the unit was not in use. 20ml water was collected from water syringe line in a sterile bottle. Water splashing was minimized when filling the container and any contact between air/water syringe and the container was avoided. The samples were transported immediately to the laboratory for microbial evaluation.

### Laboratory procedure

Ten fold dilutions of each unit sample were made in sterile phosphate buffer solution. Autoclaving was done to get the sterile solution. 1/10 dilution was made by mixing 1ml of sample with 9 ml of sterile phosphate buffer solution.

Samples were vigorously agitated by vortex for 15 seconds. 0.1ml of one tenth millilitre of each dilution was plated on R2A agar using spread plate method and kept in the incubator at 35° C for 5 days.

### Preparation of medium

Bacterial culturing was done using R2A agar medium. Enumeration was done with the help of magnifying glass by counting the total colony forming units irrespective of the type and genera. Each colony was assessed for the identification of the microorganisms and confirmed by using Gram Staining<sup>7</sup> and biochemical tests. Gram stain was used in identification of bacteria which helps differentiate Gram positive organisms and Gram negative organisms.<sup>8</sup>

Oxidase Strips were used to detect the presence of the enzyme Cytochrome Oxidase produced by a number of bacteria. Positive result was indicated within a few seconds by smeared area turning deep purple. Triple sugar Iron agar slant was used to confirm the presence of the bacterias such as *E.Coli*, *Pseudomonas* and *Proteus* based on their sugar fermenting capacity. Two or three colonies of test organism on agar medium were touched by using a loop, inoculated onto the agar slants. Identification of bacterias was done based on the color changes and gas production that was detected within 18-24hrs.<sup>8</sup>

### Treatment with 0.2% Chlorhexidine and 10% Povidone Iodine

The self contained reservoir bottle were filled with 25ml of treating agent either 0.2% Chlorhexidine or 10% Povidone iodine solution and run through the waterlines for 30 seconds and left in the lines overnight. The following morning, self contained reservoir bottle was removed and filled with fresh tap water and the product was flushed out until clear water could be seen. Water samples were collected from water syringe lines and microbiological analysis was done by following the same procedure as the baseline.

**Table I.** Comparing the efficacy of 0.2% Chlorhexidine and 10% Povidone Iodine

Treating agent	No.	Pretreatment	Post treatment	Percentage reduction	Mean $\pm$ S.D.	P value
0.2% chlorhexidine	20	23075+ <sub>-</sub> 17912.47	2695+ <sub>-</sub> 1773.63	86.2%	86.2+ <sub>-</sub> 8.20	<0.001**
10% Povidone Iodine	20	7125+ <sub>-</sub> 3978.88	275+ <sub>-</sub> 286.31	96.14%	96.17+ <sub>-</sub> 4.21	

**Table II.** Baseline contamination with different water reservoir sources

Different water sources	Mean values before passing through lines	No. of dental units	Cfu/ml after passing thru lines	Mean values + <sub>-</sub> S.D. after passing thru the lines	P Value
Mineral water	500	5	4900 5600 4400 6300 5800	5400 <sup>a</sup> + <sub>-</sub> 751.66	<0.001**
Sterile distilled water	0	5	3300 4500 3800 2200 5400	3840 <sup>a</sup> + <sub>-</sub> 1209.55	
Fresh tap water	1300	5	13800 12600 9800 11200 11000	11680 <sup>b</sup> + <sub>-</sub> 1546.61	

## RESULTS

The bacterial profile of the water samples collected from the 71 dental unit waterlines. The cultures from water samples showed the presence of following microorganisms in the order of descending frequency- Pseudomonas 70 (98.59%), Staphylococci 65 (91.55%), Klebsiella 26(36.62%), Candida 25 (35.21%), Bacillus 24(33.8%), Serratia22 (30.99%), Proteus14 (19.72%), Methylobacterium Mesophilicum 5 (7.04%), E. Coli 4 (5.63%). The colony count varied from 2800cfu/ml to a maximum of 68000cfu/ml with a mean colony forming units of 18380.28/ml. The variation is statistically significant (P<0.001).

Table I shows the comparative evaluation of the antimicrobial efficacy of 0.2% Chlorhexidine and 10% Povidone Iodine which was represented by mean percentage colony forming unit reduction. Microbial efficacy of Chlorhexidine and Povidone Iodine were analyzed by T-tests followed by Mann-Whitney showed significant difference (P <0.001).

Table II shows the effect of different types of water when used as reservoir source on the baseline contamination of dental unit waterlines. According to this table either mineral water or sterile distilled water can be preferred to fresh tap water as reservoir source in dental unit waterlines

According to Table III no significant difference were found in mean baseline contamination of water collected from different outlets of dental units such as Handpiece outlets, Air/ Water syringe outlets and scaler outlets.

Table IV compares the duration of the efficacy of 0.2% Chlorhexidine versus 10% Povidone iodine. Immediate post treatment values with both the agents, showed no significant difference. Differences were observed between 3rd, 5th and 7th day sample mean contamination, 10% Povidone iodine was found to be more

efficient (97.13%) and active for a period of 3 days and gradually losing its efficacy by 7th day.

## DISCUSSION

The provision of dental unit water that is safe for use with all categories of patients is now an issue world wide. Dental unit waterlines are considered an integral part of dental units as they supply water to air turbines and ultrasonic scalers as a coolant which are very small in diameter; present a very high surface-to volume ratio with relatively low flow rates, intermittent patterns of use and overnight stagnation that are ideal for colonization with aquatic bacteria, leading to biofilm formation<sup>9</sup> if not intervened might lead to the exposure of patients and dental professionals to opportunistic and pathogenic organisms originating from the various components of the dental unit, which might be potential for human impact.<sup>10</sup>

The Dental unit waterlines biofilm is a mixture of living bacteria, extracellular carbohydrates and biological debris, once established, bacterial cells are continuously recruited to and released from the biofilm into the walls flowing through or standing in the tubing lumen.<sup>11</sup> Biofilm bacteria are substantially resistant to surfactants, biocides, and antibiotics. As a result, microbial biofilms constitute major industrial and medical concerns. The source of bacteria for biofilm formation is either from water itself or from the operating environment as result of reaspiration of contaminated fluids.<sup>12</sup> Two problems can arise from the presence of biofilms in a distributing aqueous system. First, the biofilm can clog pipes and tubings or interfere with the proper function of mechanical devices. Second, bacterial populations living in this protected mode of growth produce planktonic cells that contaminate fluids and alter their properties or, in the case of pathogens, can result in various bacterial infections that were believed to have originated from dental

**Table III.** Baseline contamination from different water outlets

Outlets	Fresh tap water mean value	No.of dental units	After passing through the lines	Mean values after passing through the lines ± S.D.	P value
Hand piece lines	1500 cfu/ml	5	7800 9700 5400 11200 5600	7940±2533.38	0.996
Air/water syringe lines	1500 cfu/ml	5	6300 10200 6800 9600 7500	8080±1728.29	
Ultrasonic scaler lines	1500cfu/ml	5	7300 8600 6200 12600 5400	8020±2828.78	

**Table IV.** Comparing the duration of efficacy of 0.2% Chlohexidine & 10% Povidone Iodine

Treating agent	Baseline	After treatment	3rd day	5th day	7th day
0.2% chlorhexidine	9880±2838.49	900±316.23 (90.89%)	3320.00 ±1047.38 (66.39%)	6900± 1894.73 (30.16%)	9140 ±1395.71 (7.48%)
10%Povidone Iodine	7680±5799.31	220±130.38 (97.13%)	280.00 ±130.38 (96.35%)	680.00 ±319.37 (91.145%)	4320.00 ±2096.90 (43.75%)
P value		0.661	0.0152	0.000	0.000

unit water.<sup>11</sup> People considered to be at risk are elderly people and immuno compromised persons like HIV/AIDS patients, patients with chronic auto immune diseases/ organ transplant recipients, patients on prolonged radiotherapy ,patients with multiple blood transfusions, patients exposed to immunosuppressive agents.<sup>13</sup>

If untreated, the microbial populations in dental unit waterlines often exceed 104 to 105 colony forming units /ml of water but there are several methods of reducing the numbers of colony forming units in the Dental unit waterlines, including flushing lines with water, intermittent or continuous use of bactericidal chemicals, radiation, self-contained independent water reservoirs, and filtration.<sup>4</sup> Studies conducted using these methods show their inability to control the biofilm to acceptable level.<sup>6,14</sup> The intermittent use of bactericidal agents proves to be an effective way in controlling biofilm to some extent.<sup>4,11</sup>

This study was undertaken to enumerate and identify the micro-organisms present in water samples collected from 40 dental unit waterlines of different specialty clinics and to find out the efficacy of two commonly available treating agents in disinfecting dental unit waterlines. As a part of laboratory procedure, evaluation of the number of heterotrophic microorganisms in each water sample was done as suggested by Noce.<sup>15</sup> Disinfectants were selected in the study according to their ability to kill microbial cells and remove biofilm from the inner surfaces of Dental unit waterlines tubing according to the study outcome of Walker.<sup>16</sup>

Most of the cleaners and disinfectants do not effectively remove the biofilm because the biofilm carry a net negative charge which results in repulsion or non interaction of materials. Chlorhexidine was selected as one of the treating agents, which is a positively charged organic antiseptic agent belongs to the bis –biguanides group. Second treating agent selected was 10% Povidone Iodine which is a highly efficient microbicide to a wide variety of bacterial, fungal and viral infections. Even though it has disadvantages of generating iodophor laden aerosols and elevation of dissolved mercury levels in dental unit waste water,<sup>17</sup> because of its known antibacterial efficacy and relative lack of toxic or irritating properties, Povidone Iodine was selected.

The water samples of the study showed bacterial colony count varied from 2800 colony forming units/ml to a maximum of 68000 colony forming units/ml with a mean colony forming units of 18380.28/ml, which was found to be higher than the American Dental Association recommendation level of 200 colony forming units /ml and none of the dental units under study delivered water that could meet the accepted standard for potable water. Similar findings were seen in the studies done at the various parts of the world by many researchers<sup>11,18</sup> which shows the bacterial concentration ranging from 7x10<sup>3</sup> to 5x10<sup>5</sup>. The variations in the results can be due to the heterogenous distribution of bacterial cells within a given water sample. Bacterial cells in the water obtained from Dental unit waterlines are thought to be released from the biofilm formed inside the tubing. During sampling, small pieces of biofilm

or microcolonies may be released. This is likely to result in a bias toward higher or lower counts or toward the predominance of a given bacterial species in the sample.

The cultures from water samples showed the presence of following microorganisms like *Pseudomonas*, *Staphylococci*, *Klebsiella*, *Candida*, *Bacillus*, *Serratia*, *Proteus*, *Methylobacterium*, *Mesophilicum*, *E.coli* in descending frequency. Similar microorganisms were found in the studies done by researchers in other countries.<sup>2,7,11,12</sup>

Reduction in microbial contamination after using the treating agent 0.2% Chlorhexidine was found to be statistically significant. Similar findings were seen in the study done by Walker *et al*<sup>16</sup> and Porteous.<sup>19</sup>

The treatment with 10% Povidone iodine showed the reduction in microbial contamination ranging from 83.60% to 100%. The mean value was 96.14% which was found to be significant. Similar result was noted in the study done by Mills *et al*<sup>18</sup>. 0.2% Chlorhexidine showed less antimicrobial efficacy than 10% Povidone Iodine with statistically significant difference ( $P < .001$ ).

When different types of water was used as reservoir source on the baseline contamination of dental unit waterlines, no significant difference were found in mean baseline contamination using sterile distilled water and mineral water as a reservoir source while mean baseline contamination using fresh tap water as reservoir source showed significant difference in baseline contamination when compared with other two groups ( $P < .001$ ). According to the study results either mineral water or sterile distilled water can be preferred to fresh tap water as reservoir source in dental unit waterlines. Similar findings were seen in the study done by Kettering *et al*.<sup>20</sup>

The evaluation of baseline contamination of water collected from different water outlets of dental units showed no significant difference in mean baseline contamination of water collected from different outlets of dental units such as Handpiece outlets, Air/ Water syringe outlets and scaler outlets. A research done by Szymanska *et al*<sup>21</sup> showed similar findings to study but a study done by Hiyasat *et al*<sup>22</sup> showed significant difference in various water lines.

The results of the present study suggests that daily use of 0.2% Chlorhexidine and every 3 day use of 10% povidone iodine can be done to maintain the biofilm within the recommendations of ADA.

The present study included the evaluation of efficacy and duration of the action of irrigants for one week period and also different reservoir water sources. However due to practical difficulties effect on biofilm coverage, adverse effect on the waterline tubing, byproduct formation when treating agents are used intermittently, the effect of Chlorhexidine and Povidone Iodine on the enamel and dentin bond strength of dental adhesive materials and the development of resistance to these treating agents when used for prolonged period of time could not be assessed. Future clinical research in this field can be undertaken to overcome these limitations and to arrive at more specific recommendations for maintenance of the sterile environment in the dental clinic. To continue maintaining the sterility of the Dental unit waterlines and to complete the infection control measures adopted in the dental clinics, suitable disinfectants like 0.2% Chlorhexidine on daily basis or 10% Povidone iodine on every 3rd day basis intermittently are recommended.

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