

**CONTAMINATION OF DENTAL WATERLINE AND ITS CONTROL MEASURES**RAYMOND BRITTO.P\*<sup>1</sup>, N.P. MURALIDHARAN<sup>2</sup><sup>1</sup>IIIyear Bds Saveetha Dental College, <sup>2</sup>Asst. prof of microbiology, Saveetha dental college, Chennai. Email: pio03davis@hotmail.com*Received: 1 August 2013, Revised and Accepted: 22 August 2013***ABSTRACT**

The purpose of this review is to analyse organism growth and type, and biofilm development on the inner surfaces of the dental unit water line. Outlined the complications faced in the dental clinics with references. It also elaborates in detail about the steps taken and the disinfection processes that had minimized the risk in the patients as well as the dentists

**Keywords:** Dental waterline, biofilms, microorganism, control measures

**INTRODUCTION**

The presence of bacterial biofilms in the dental unit waterlines (DUWLs) has been well documented and recognized as an undisputed source of contamination in dental treatment. Furthermore, as most DUWL treatment methods have limitations, biofilms are challenging to eliminate. Numerous studies have shown that DUWL biofilms harbor a diverse population of organisms and at least forty genera of bacteria have been identified at the molecular level. Although earlier identification techniques were culture-based, certain organisms, such as *Pseudomonas* spp. and *Sphingomonas* spp., have been commonly identified in studies across the globe. The phylogenetic group  $\alpha$ -Proteobacteria has been shown to be the predominant survivor in chlorinated water distribution systems and *Sphingomonas* spp. are closely aligned with these genera.

The majority of studies on DUWL biofilm tested dental units that used source water from the municipal water supply. Some studies tested units with source distilled water and demonstrated that distilled water alone did not prevent biofilm formation without a concurrent, regular intermittent DUWL cleaning scheme.

**BIOFILM**

Biofilm is a community of bacterial cells and other microbes that adhere to surfaces and form a self-protective slime layer. Found in virtually all places where moisture meets a suitable solid surface, biofilm can contain many types of bacteria as well as fungi, algae, protozoa, and nematodes. The polysaccharide slime produced by many microbial inhabitants protects the cells from physical and chemical challenges, while water channels within the biofilm carry nutrients to the cells inside the film. Individual organisms, or even portions of the biofilm near the surface, break off into flowing water.

Although biofilm can form in all non-sterile fluid environments, dental waterlines provide particularly well-suited conditions. The tubing has a very narrow bore (1/8- to 1/16-inch), which provides a high internal surface-area-to-volume ratio. Low water pressure, low flow rates, and frequent periods of stagnation also encourage any bacteria introduced from the public water supply to accumulate within the tubing. The result is output water that is often many times more contaminated than tap water from the faucet in the same treatment room.

Water heaters and pre-filters in dental units further exacerbate bacterial proliferation and colonization of dental unit waterlines. Heating water to near body temperature may enhance the number of microorganisms adapted for growth within a warm-blooded human host. Although they are intended to remove particles from municipal water as it enters the dental unit, pre-filters have pores that are too large to trap bacteria. They not only slow the flow of

water but also may provide additional surface area for microbial colonization. (1)

**The health implications of waterline biofilm**

Since the first report in 1963, dozens of researchers have investigated dental waterline contamination. Despite the high levels of organisms found in dental unit water, no outbreaks of disease have been reported. In fact, few clinical case reports have been associated with waterline contamination. To date, no published scientific evidence confirms a risk of serious health problems for patients or dental personnel from contact with dental water.

However, numerous studies conducted over the past 30-plus years have identified the presence of waterborne opportunistic pathogens in dental unit water, and these findings provide reason for cautious concern. Many environmental organisms identified in dental treatment water have been associated with opportunistic infections in hospitalized or immune-compromised patients. For example, *Pseudomonas* species, non-tuberculous mycobacteria, and *Legionella* species all have been isolated from dental unit water. *Legionella*, the causative agent of Legionnaires' disease, may pose a particular concern, as it appears to be transmitted by inhaling aerosols or aspirating water contaminated with the bacteria.

One study suggests that aerosols produced by contaminated water from high speed hand pieces were associated with altered nasal flora in 14 of the 30 dentists studied. Nine of the dentists with altered nasal flora (1) were positive for the same species of waterborne *Pseudomonas* isolated from the dental units. Several other studies have found higher titres of *Legionella* antibodies among dental personnel than in control populations, likely due to chronic exposure to *Legionella*-contaminated aerosols of dental unit water. Despite the higher antibody titres, however, no cases of *Legionella* pneumonia among the exposed workers have been documented.

A recent paper discussed the finding that high levels of the bacterial by-product known as endotoxin may be present in dental unit water. Exposure is known to exacerbate respiratory conditions such as asthma and may delay wound healing.

**Reports of death from Contaminated Dental Waterlines Demonstrates Life & Death Importance of Monitoring Water Quality**

The dental community is on a high-alert after an 82-year-old Italian woman died in February due to complications related to Legionnaires' disease—a life-threatening type of pneumonia caused by *Legionella* bacteria—which she acquired while receiving dental care.

In the Dimensions of Dental Hygiene Journal, the elderly woman was hospitalized after becoming ill with a fever and respiratory distress. After chest X-rays revealed lung consolidation, the patient tested positive for Legionella pneumophila and was promptly diagnosed with Legionnaires' disease. Despite medical efforts, the patient died 2 days later, and an investigation into the source of the bacterial infection was launched.

Because the patient had left her house only twice over the prior 2-week period to seek dental care, it was clear that the infection originated from either the dental office or her home. Samples taken from the dental office's tap and high-speed dental turbine contained positive traces of L. pneumophila, while the samples obtained from the woman's home were negative. A naturally occurring Gram-negative bacterium found in man-made and natural water systems, L. pneumophila is most infectious when inhaled from aerosolized water, such as that created by high-speed dental turbines. Once inhaled, this contaminated water has the potential to cause Legionnaires' disease, especially among people with compromised immune systems.(2)

**This case emphasizes the life-and-death importance of implementing—and monitoring—effective infection control protocols for cleaning and maintaining dental waterlines.**

To minimize this potential source of contamination, waterlines must be aseptically maintained with the same thoroughness as other aspects of the dental operator.

Let's work together to make sure this doesn't happen to your office and to one of your patients. First, the basics: bacteria and other microorganisms form a biofilm that adhere to the inside of the tubing that supplies water to dental instruments. There are 5 stages of biofilm development, with bacterial populations doubling every 20 minutes. As water moves through the waterline / tubing, microorganisms slough off into the water, thus contaminating it.

Stage 1 – initial attachment	Stage 2 –
irreversible attachment	Stage 3 –
maturation phase I	Stage 4 –
maturation phase II	Stage 5 –
dispersion	

A recent study documented in the Journal of the American Dental Association concluded that: "microbial contamination of dental unit water appears widespread and extensive, and the organisms populating the water lines include many with pathogenic potential which can cause serious illness and death, especially when the immune systems are down.

The source of these maverick bacteria which inhabit the dental unit water lines is two fold.

**First**, research indicates that the majority of the organisms originate in the municipal water system. Conventional municipal water treatment procedures are proving inadequate in dealing with a wide variety of these "super bugs".

**The second** source is known as the "suck back" effect, caused by imperfect anti-retraction valves in dental instruments, thus permitting the withdrawal or "suck back" of blood, saliva and other materials from a patient's mouth into the waterline.

## CONTROL MEASURES

**STEP 1 – Given your source water supply, re-examine your current infection control procedures.**

If you are using filters installed in line near the point of use to prevent the passage of microorganisms to the patient, these filters must be replaced with the frequency dependent on the amount of bio-film accumulation in your waterlines.

If you are using chemicals to remove, inactivate or prevent formation of bio-film, they should either be continuously infused

into or added intermittently to the dental unit water, in order to maintain the bacteriostatic effect

If you are using water purifiers that treat the source water entering dental unit waterlines by filtration, heat, UV light and other methods that kill and/or remove microorganisms, for these systems to deliver clean water at the point of use (i.e., to the patient) a chemical treatment must be used to remove bio-film in addition to intermittent chemical treatments to maintain source water quality.

The advantage of purified water systems is that they may delay formation of bio-film or enhance the effectiveness of the other treatment methods. However, these systems will not result in delivery of purified water should the water pass through waterlines containing bio-film.

## STEP 2 – MONITOR

Regardless of the source water you are using, the only way to know that your dental waterline cleaning regimen is effective is to monitor the water coming out of the unit — not the source water but the point-of-use water — using either in-house monitoring products or commercial water-testing laboratories.

CDC's Infection Control Guidelines for dental healthcare settings state that dentists should consult with the manufacturer of their dental unit or water delivery system to determine the best method for maintaining acceptable water quality (i.e., <500 CFU/mL) and the recommended frequency of monitoring. And, as noted above, the ADA recommendation regarding safe water quality is < 200 CFU/mL.

Types of chemicals can we use to treat and maintain our waterlines

Chlorine compounds have been fairly extensively studied, with published reports on the efficacy of dilute sodium hypochlorite, chlorine dioxide, chloramine T, and elemental chlorine. In fact, several manufacturers authorize weekly water system treatment with a 1:10 solution of household bleach.

Some other active agents scientifically evaluated for treatment of dental unit waterlines include hydrogen peroxide, chlorhexidine gluconate, and iodophors. Commercial products employing hydrogen peroxide, chlorhexidine gluconate, iodine, or citrus botanicals also are being marketed. Active ingredients in currently available continuous chemical treatments include chlorhexidine gluconate, citric acid, hydrogen peroxide, iodine, and ozone and silver. Although waterline antimicrobials now must also be registered with the Environmental Protection Agency (EPA), the agency has not yet formulated regulatory guidance for intermediate- and low-level germicides used in dental waterlines. The ADA and the American National Standards Institute currently are developing a national specification for waterline antimicrobials that is expected to serve as the basis for EPA evaluation.

Because of concern over compatibility with equipment components, always consult the dental unit manufacturer before introducing any chemical into the water system. Issues of waterline chemical compatibility with various dental materials (for example, dental adhesives) also have recently come to light, and questions regarding disinfectant by-products and their effects on oral tissues have been raised.(5)

## Filters effective in controlling water quality

Usually positioned on each water-bearing line near the hand-piece or air-water syringe, microfilters typically use a 0.2-micron membrane to trap free-floating microorganisms before they can be released in the effluent. One currently marketed product also releases small quantities of iodine intended to discourage biofilm formation; another is purported to trap bacterial endotoxin. Some filters incorporate anti retraction features.

The few studies conducted to date suggest that in-line microfilters can produce water that meets or exceeds water-quality goals. In fact, results of independent studies report that 80% of the filtered water samples tested were bacteria-free. Although in-line filters can improve dental water quality, they have no effect on the biofilm

within the waterlines. Without treating the biofilm, waterlines are at risk of biofouling, clogging, and release of bacterial byproducts into treatment water. As such, it may be necessary, at minimum, to periodically treat the post-filter segment of the waterlines to control biofilm(6)

Invest in a sterile water delivery system

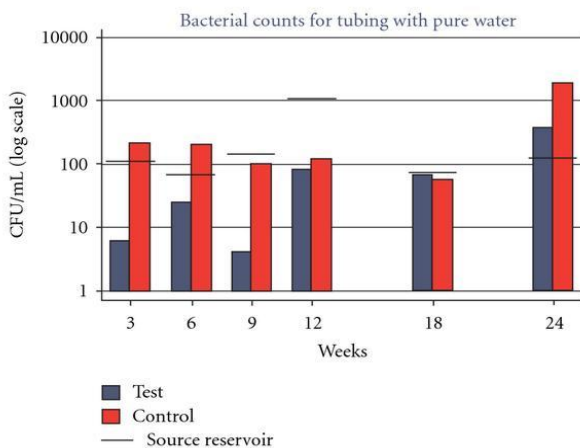
Sterile water delivery systems address the issue of biofilm by

offering disposable or autoclavable waterline tubing that bypasses the dental unit's water supply. Although many systems of this type are oral-surgery and implantology handpieces, ultrasonic scalers and retrofit devices for restorative handpieces also are available.

Sterile water cannot be delivered through a standard dental unit. For practices that perform surgery with instruments that are connected to the dental unit water system, a sterile water delivery system would be a worthwhile investment.

Logarithmic mean CFU/mL of bacteria dislodged from inside surfaces of test and control tubing

Week	Treatment	N	Log mean	Log Std Dev	Geometric mean	t-value	value
3	Test	3	0.784	0.719	5.1	3.48	0.025
	Control	3	2.321	0.263	208.3	Test < control	
6	Test	3	1.397	0.338	23.9		
	Control	3	2.31	0.851	203.4	1.73	0.159
9	Test	3	0.627	0.737	3.2	2.78	0.05
	Control	3	2.007	0.443	100.7	Test < control	
12	Test	3	1.9	0.747	78.4		
	Control	3	2.079	0.374	119	0.37	0.729
18	Test	3	1.838	0.923	67.8		
	Control	3	1.762	1.229	56.8	0.09	0.936
24	Test	3	2.58	0.296	379.4	3.69	0.021
	Control	3	3.28	0.144	1905.6	Test < control	
All Weeks	Test	6	1.521	0.738	32.2		
	Control	6	2.293	0.526	195.5	2.09	0.063



**Organism Identification**

There are four most commonly discussed bacteria within biofilms of Dental Water Line. They are Legionella pneumophila, Mycobacterium spp., Pseudomonas aeruginosa, Acinetobacter baumannii and Staphylococcus spp..

**Legionella pneumophila**

L. pneumophila is a gram-negative bacterium which spreads via the air-conditioning system. They are called chemo-organotrophs because they are capable of using certain amino acids as primary carbon and energy sources. A respiratory trace and the lungs are the main sites of infections. Legionnaire's disease is an atypical lung infection caused by Legionella pneumophila

**Mycobacterium spp**

It is a bacterial genus which contains a vast number of species. The best known species are M. leprae and M. Tuberculosis which cause leprosy and tuberculosis respectively. In their cell wall, it consists of special fatty molecules called mycolic acids, and these complexes make the cell walls less permeable. They are aerobic, non-motile rods

**Pseudomonas aeruginosa**

It is a heterogeneous genus comprising gram-negative, aerobic, rod-shaped bacteria. They exhibit motility since they have one or more polar flagella. They also contain fimbriae as an attachment to surfaces of other organisms. They grow fastest at the room temperature. Aeruginosa infections are relatively rare

**Staphylococcus spp**

They are small cocci that exist in irregular clumps or grape-like clusters. There are three main species of Staphylococcus that share

morphological and biochemical features. The pathogenic organisms produce many extracellular products known to play as a factor that leaves effects on humans

**Acinetobacter baumannii**

Acinetobacter baumannii is a Gram-negative opportunistic nosocomial pathogen. This microorganism survives in hospital environments despite unfavorable conditions such as desiccation, nutrient starvation and antimicrobial treatments. Pili assembly and production of the Bap surface-adhesion protein play a role in biofilm initiation and maturation after initial attachment to abiotic surfaces. regulatory processes associated with biofilm formation include sensing of bacterial cell density, the presence of different nutrients and the concentration of free cations available to bacterial cells

BLASTn results for the 16S ribosomal bacterial region returned the following highest % identities. (7,8,9)

**Testtubing:**

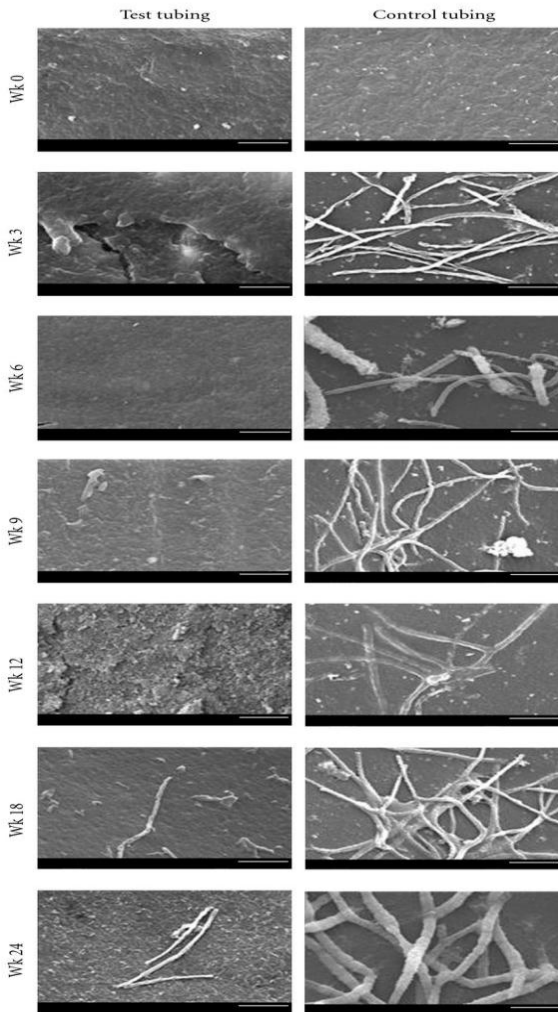
- (1)isolate 25B1 Sphingomonas spp. Identities 977/977 (100%);
- (2)isolate 25B2 Blastobacter spp. 956/956 (100%);
- (3)isolate 25B2b Erythromonas ursincola 956/956 (100%);
- (4)isolate 25B2c Sphingomonas natatoria 956/956 (100%);
- (5)isolate 25B-3 Erythromonas ursincola 1369/1389 (99%);
- (6)isolate 25B-3b Sphingomonas natatoria 1369/1389 (99%).

**Controltubing**

- (7) isolate 27A-1 *Sphingomonas* spp. 1350/1352 (99%),  
 (8) isolate 27A-1b *Proteobacterium* symbiont 1350/1352 (99%),  
 (9) isolate 27A-2 *Sphingomonas* spp. 982/982 (100%);  
 (10) isolate 27A-3 *Sphingomonas natatoria* 977/980 (99%).

#### Source reservoir

- (11) isolate 8C-1 *Sphingomonas* spp. 980/980 (100%),  
 (12) isolate 8C-2 *Proteobacterium* symbiont of *Nilaparvata lugens* 959/960 (99%)



#### DISCUSSION

The water organisms grew exponentially within an hour when Type I ultrapure water was contained in a clean, nonsterile, polycarbonate reservoir bottle that was refreshed at the beginning of every working day. Organism growth originated in the clean, nonsterile collection flask, or the reservoir, or both, with subsequent biofilm formation on the inside surfaces of untreated control DUWL tubing. Early biofilm colonizers were well established on the control tubing by Week 3, as seen on SEM images.

In a study conducted water samples were collected from air/water syringe and high-speed handpiece. Generally, 100-200 ml water samples were collected aseptically in sterile containers with sodium thiosulfate at the beginning of the day after a 2 minute purge. Samples viable heterotrophic bacteria and fungi. (ref <http://www.ups.upenn.edu>.)

The results shows heterotrophic plate count levels were significantly exceeded the American Dental Association recommendations for DUWL water quality (< 200 CFU/ml), in both air/water syringe

(84%, CFU/ml: 500-20000) and high-speed handpiece (96%, CFU/ml: 710-36800) samples. However, there was no significant difference between the level of contamination in the air/water syringe and high-speed handpiece. Fungi were found in 28% and 36% of air/water syringe and high-speed handpiece samples, respectively; and filamentous fungi were the most frequently isolated fungi.

Type I ultrapure water from a nanofiltration- /uv-treated water purifier that was collected in a nonsterile flask became contaminated after transfer to a reservoir within an hour, and within a six-month period, formed a dense biofilm on the untreated control waterline. The biofilm-controlling N-halamine test tubing prevented biofilm formation throughout the study period. However, some scattered organisms were visible on the test tubing by the end of the study period and were identified as a variation of the genera *Proteobacteria* found in the source carboy. This may be explained by one or all of the following reasons. (1) the biofilm-controlling properties of the N-halamine test tubing may have become exhausted by the end of the study period and should have been recharged within that time period; (2) the organisms may have become resistant to chlorine (10,11)

#### CONCLUSION

DUWLs should be subjected to routine microbial monitoring and to a decontamination protocol in order to minimize the risk of exposure to potential pathogens from dental units. The protocol should be designed depending upon the facilities available and the complications encountered in the clinic or the hospital. It is mandatory to have day to day maintenance protocol which should be displayed in all clinics. The water should be sourced only from a protected reservoir. Disinfection should be done everyday after the work of the day is over.

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