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MICROBIAL CONTAMINATION OF DENTAL UNIT WATERLINES

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Abstract: The specific structure of dental units favours the presence of biofilm and microbial contamination of the dental unit waterlines (DUWL) water. The ability of bacteria to colonize surfaces and to form biofilm in water supply tubes, including DUWL, is a common phenomenon, which has been well documented, just as with difficulties in biofilm removal and prevention of its regrowth. Microorganisms from contaminated DUWL are transmitted with aerosol and splatter, generated by working unit handpieces. On the basis of the detailed literature review, the state-of-the art knowledge of the microflora of dental unit waterlines is presented. Most of the microorganisms isolated from DUWL are of low pathogenicity. Nevertheless, the public health significance of many of the microorganisms found in DUWL is unknown. According to current knowledge, it is not the mere presence of bacteria that is important in DUWL contamination monitoring, but their number, the presence of potential pathogens, and patients' oral cavity microflora. Numerous studies emphasize the need for effective mechanisms to reduce the microbial contamination in DUWL and highlight the risk for cross-infection in general practice, especially in view of the ever-increasing number of immunocompromised persons who present at outpatient dental clinics.

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INTRODUCTION

The quality of water in dental units is of considerable importance because both patients and dental staff are regularly exposed to water and aerosol generated by the dental unit [55].

The cause of microbial DUWL contamination may be: water delivered to a unit, working handpieces of a unit, and the biofilm present inside DUWL. The source of water in dental units is municipal water in the case of an open water system, or water coming from a reservoir (container, bottle) built into a unit in the case of a closed water system. Microorganisms may penetrate into DUWL through unit working handpieces. Contamination occurs during suck-back of liquids from the patient's oral cavity, either as a result of protective valve malfunction, or when handpieces are removed and replaced incorrectly. Presence of the biofilm in DUWL is one of the most effective factors responsible for the high numbers of bacteria in dental units water [16, 28, 29, 54]. Water entering DUWL is usually free from pathogens, but after the shedding of bacteria from biofilm it becomes contaminated above the acceptable level [42].

In 1996, the American Dental Association (ADA) set a goal for dental water to contain no more than 200 colony-forming units per milliliter (cfu/ml) of heterotrophic unfiltered output [1, 2]. In 2003, the Centre for Disease Control and Prevention (CDC) recommended ≤ 500 cfu/ml for non-surgical dental procedures [10]. In the European Union (EU), there are no specific standards for dental unit waterlines (DUWL), but it was recommended in the guidelines that water should be delivered at <100 cfu/ml at 22°C and <20 cfu/ml at 37°C [13].

Determination of concentration and composition of microflora in water and in the biofilm in dental units is the

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basis for estimation of microbial contamination of the dental unit waterlines (DUWL). Identification of microorganisms, mainly of bacterial species, is based on standard laboratory criteria (colony morphology, haemolytic zones, production of catalase and coagulase), with the use of biochemical tests and modern methods of molecular biology.

CONCENTRATION OF MICROFLORA IN DUWL

The first report on microbial contamination of DUWL was published in 1963 [9]. DUWL contamination may assume considerably varying values. Researchers studying this problem reported contamination of DUWL water at the level from 1.5×10^2 to 1×10^6 cfu/ml [8]. According to others, contamination ranged from 1×10^3 to 1.6×10^8 cfu/ml [21, 44, 62].

Microbial contamination of DUWL is universal, and water in dental units is abundantly colonized by bacteria. In most cases, the values of bacterial contamination exceed the norms accepted for potable water both in USA and European Union, as well as recommendations for water used in conservative dental treatment.

There are few reports concerning the extent of fungal contamination of DUWL water. Detailed studies of concentration and composition of fungal flora in DUWL show that mycological contamination is less widespread than bacterial contamination, and that the mean concentration of the total identified fungi seems to be high: in water from dental unit reservoirs it amounts on average to 410 cfu/ml, and in the water from a high-speed handpiece – to 578.4 cfu/ml [51].

The concentrations of bacteria in the DUWL water samples reported by various authors are presented in Table 1.

COMPOSITION OF MICROFLORA CONTAMINATING DUWL

DUWL water microflora has usually been described after studies related to methods of DUWL contamination monitoring. It was demonstrated that bacteria form the dominant part of the microflora, while fungi and protozoa are less common.

A review of the literature made it possible to compile a list of bacteria and fungi identified in DUWL water samples, beginning with the 1960's up until the 1980's [64]. The list includes: *Streptococcus mitis*, *Streptococcus salivarius*, enterococci, *Staphylococcus cohnii*, *Staphylococcus warneri A*, *Klebsiella* (*Enterobacter*) *aerogenes*, *Bacillus subtilis*, *Pseudomonas* spp., *Streptococcus* (*Enterococcus*) *faecalis*, *Enterobacter cloacae*, *Alcaligenes faecalis*, *Cladosporium* spp., *Cephalosporium* spp., *Aeromonas* spp., *Acinetobacter* spp., *Flavobacterium* spp., *Moraxella* spp.

Pankhurst *et al.* [36] provide a list of microorganisms isolated from dental units which includes bacteria: *Achromobacter xyloxidans*, *Acinetobacter* spp., *Actinomyces* spp., *Alcaligenes denitrificans*, *Bacillus* spp., *Bacterioides* spp., *Caulobacter* spp., *Flavobacterium* spp., *Fusobacterium* spp., *Klebsiella pneumoniae*, *Lactobacillus* spp., *Legionella pneumophila*, *Legionella* spp., *Micrococcus* spp., *Moraxella* spp., *Mycobacterium avium*, *Mycobacterium* spp., *Nocardia* spp., *Pasteurella* spp., *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Streptococcus* spp., *Staphylococcus aureus*, *Xanthomonas* spp.; the fungi: *Phoma* spp., *Penicillium* spp., *Cladosporium* spp., *Alternaria* spp. and *Scopulariopsis* spp.; and the protozoa: *Acanthamoeba* spp., *Cryptosporidium* spp., *Microsporidium* spp. and *Giardia* spp.

At the beginning of the current century, Shepherd *et al.* [46] isolated from the DUWL water samples bacteria typical for potable water, using a R2A agar medium. Bacterial genera most frequently occurring in DUWL were: *Acinetobacter*, *Alcaligenes*, *Flavobacterium*, *Pseudomonas*, *Sphingomonas*, *Xanthomonas* (Gram-negative rods), *Bacillus* (Gram-positive endospore-forming rod), *Streptococcus* (Gram-positive coccus). Water samples from various handpieces (high speed-handpiece, air-water syringe used by dentists and assistants) at each station showed a relatively homogenous bacterial flora composition, and the contamination level was relatively invariable, regardless of the sampling site. Unexpectedly, 85% of the isolated bacteria were those of the *Streptococcus* genus, which belong to the physiological flora of the human oral cavity. *S. sanguis* and *S. mutans* typically occur in dental plaque, *S. intermedius* and *S. mitis* – in dental plague and on the mucous membrane, and *S. salivarius* – on the tongue and in saliva. The presence of cocci in DUWL water samples indicates that the bacteria whose source was the patient could have come from the working handpiece. It is believed that suckback occurs in high-speed handpieces without antiretracion valves; however, in the case of the cited study [46], most examined units were provided with such devices. Thus, the cause of retraction of bacteria from the oral cavity to the waterlines is not clear. Therefore, the authors assumed that the oral cavity cocci settled in the biofilm because they were never found in water samples after disinfection. If the temporary source of bacterial contamination was the previous patient, the oral cavity bacteria should be easily detected, regardless of the disinfection procedure, which was not the case.

On the basis of morphological and biochemical identification it was shown by Williams *et al.* [63] that the following bacteria occurred in DUWL: *Achromobacter xyloxidans*, *Acinetobacter* spp., *Alcaligenes denitrificans*, *Bacillus* spp., CDC group IVc-2, *Flavobacterium indologenes*, *Klebsiella pneumoniae*, *Legionella* spp., *Micrococcus luteus*, *Nocardia* spp., *Ochrobactrum anthropi*, *Pasteurella haemolytica*, *Pasteurella* spp., *Pseudomonas acidovorans*, *Pseudomonas aeruginosa*, *Pseudomonas cepacia*, *Pseudomonas fluorescens*, *Ralstonia pickettii*, *Pseudomonas paucimobilis*, *Pseudomonas stutzeri*, *Pseudomonas testosteroni*, *Brevundimonas vesicularis*, *Serratia marcescens*,

Table 1. Bacterial flora concentration in DUWL water according to literature and own studies.

Number of studied units	Mean bacteria concentration determined (cfu/ml)	Water sampling site	Culture conditions	Researchers
20	4.95 log	air/water syringe	22°C and 37°C, 72h, R2A	Uzel *et al.*, 2008 [60]
	4.91 log	air rotor	22°C and 37°C, 72h, R2A	
20	> 3.9×10^4	reservoir	22°C, 7 days, R2A	Zhang *et al.*, 2007 [65]
		air/water syringe	37°C, 48 h, TSB	
20 surgeries (59 water samples)	317–46,320	air/water syringe	R2A agar, NA	Göksay *et al.*, 2006 [18]
	370–52,240	high-speed handpiece	R2A agar, NA	
2 (38 samples each)	3.64 log; 3.53 log	high-speed handpiece	36ºC, PCA	Sacchetti *et al.*, 2006 [43]
	3.61 log; 3.41 log	high-speed handpiece	22ºC, PCA	
134	0- 5.41 log	syringe	selective and non-selective agar media	Schel *et al.*, 2006 [45]
25	2.01×10^5	reservoir	selective and non-selective agar media	Szymańska, 2006 [52, 53]
	1.5×10^5	high-speed handpiece	selective and non-selective agar media	
15	0–1.52×10^6	reservoir	32ºC, 48 h, PCA	Souza-Guelmin *et al.*, 2003 [50]
	0–3×10^8	air/water syringe	32ºC, 48 h, PCA	
	0–3×10^8	high-speed handpiece	32ºC, 48 h, PCA	
16	992–1,343		25ºC, 7 days	Wirthlin *et al.*, 2003 [64]
12	15.32×10^3	high-speed handpiece	37ºC, 72 h, glucose-enriched soya agar	Cobb *et al.*, 2002 [11]
18	$6.7–7.8 \times 10^4$	ultrasound handpiece	32ºC, 48 h, PCA	Fiehn & Larsen, 2002 [15]
	$3.3–7.7 \times 10^4$		21ºC, 72 h, KA	
60	178,100	air/water syringe	25ºC, 5 days, R2A	Kettering *et al.*, 2002 [22]
	350,130	high-speed handpiece	25ºC, 5 days, R2A	
75	781	air/water syringe	25ºC, 5 days, R2A	Kettering *et al.*, 2002 [23]
	762	high-speed handpiece	25ºC, 5 days, R2A	
1	810	air/water syringe	22ºC	Monarca *et al.*, 2002 [30]
	7.6×10^3	high-speed handpiece	22ºC	
	6.3×10^3	micromotor	22ºC	
20	244 (8:00) – 52 (13:00)	air/water syringe	36ºC	Monarca *et al.*, 2002 [31]
	280 (8:00) – 40 (13:00)	air/water syringe	22ºC	
6	3.45 ± 0.35 log	high-speed handpiece	22ºC, 7 days, R2A	Montebugnoli & Dolci, 2002 [32]
6	6–2,575	air/water syringe	22ºC, 72 h, agar medium	Smith *et al.*, 2002 [49]
	0-73	air/water syringe	37ºC, 24 h, agar medium	
	$5 \times 10^2–1 \times 10^5$	high-speed handpiece	22ºC, 72 h, agar medium	
	0–1×10^5	high-speed handpiece	37ºC, 24 h, agar medium	
18	6.6×10^4	reservoir		Tuttlebee *et al.*, 2002 [59]
23	8,440–9,760		37ºC, 7 days, R2A	Linger *et al.*, 2001 [25]
117	0 – over 1×10^6; mean 1.4×10^5	air/water syringe, high-speed handpiece	23-26ºC or 37ºC, 7 days, HPC, R2A (for all microorganisms), M-S (for *Streptococci* count), DGVP (selective agar for *Legionella* rods)	Shepherd *et al.*, 2001 [46]
7	3.52×10^2		37ºC	Smith *et al.*, 2001 [48]
	1.0×10^5		22ºC	
16 surgeries (1-9 units each)	$4.0 \times 10^2 – 3.2 \times 10^5$	air/water syringe	35ºC, 7 days, R2A	Noce *et al.*, 2000 [33]
	$1.3 \times 10^3 – 2.5 \times 10^5$	high-speed handpiece	35ºC, 7 days, R2A	
55	2.9×10^3	air/water syringe	selective and non-selective agar media	Walker *et al.*, 2000 [61]
	3.3×10^3	rotor	selective and non-selective agar media	

Media used in bacteria culture: PCA – plate count agar medium; KA – Kings Agar B medium; R2A – R2A agar medium; M-S – Mitis Salivarius agar – agar medium for *Streptococcus mitis* and *Streptococcus salivarius* count; HPC – heterotrophic plate count medium; NA – Nutrient Agar.

Staphylococcus spp., *Staphylococcus capitus*, *Staphylococcus saprophyticus*, *Staphylococcus warneri*, *Streptococcus* spp., *Stenotrophomonas maltophilia*.

The study of the prevalence of microorganisms in water samples from dental units in practices in Saudi Arabia showed that the most common bacteria were *Bacillus* spp. (29.6%) and *Pseudomonas* spp. (22.8%) [6].

The bacterial biota in DUWL water were characterized by direct sequence analysis of 16S rDNA clone libraries. The phylum *Proteobacteria* was the major group in both clone libraries at phylum level. DUWL clone library contained 80.0% *Proteobacteria*, 8.0% *Bacteroides*, 4.0% *Nitrospira*, 4.0% *Firmicutes*, 2.0% *Planctomycetes*, and 2.0% *Acidobacteria* [20].

The majority of the bacterial species isolated in the recent Turkish study by Göksay *et al.* [17] were identified as *Pseudomonas fluorescens*, *Pasteurella haemolytica*, *Photobacterium damsela*, *Ochrobacter anthropi* and *Moraxella* spp. A little earlier, it was found [34] that the most common bacterial species cultured from the mains water and the dental chair output water were *Micrococcus luteus* and *Sphingomonas* spp. respectively, the latter of which are known as opportunistic pathogens.

In most studies, the Gram-negative mesoheterotrophic water bacteria accounted for the majority of the microorganisms identified from DUWL. These bacteria produce endotoxin, a biologically active, macromolecular lipopolysaccharide (LPS) located in outer membrane. Dying and degenerating Gram-negative bacteria may release large amounts of endotoxin into the dental water. A high concentration of bacterial endotoxin in DUWL water significantly downgrades microbiological DUWL water quality [19, 40, 56, 65].

The prevalence of Gram-negative bacteria in DUWL was demonstrated by Barbeau *et al.* [8]. The authors proved the presence of following bacteria: *Sphingomonas paucimobilis*, *Acinetobacter calcoaceticus*, *Methylobacterium mesophilicum*, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Brevundimonas vesicularis*, *Pseudomonas acidovorans*, *Actinomyces* spp. and *Bacillus* spp.; it should be noted that the *Sphingomonas paucimobilis* amounted to 41% of the total isolated bacteria, and *Acinetobacter calcoaceticus* – to 23%. These two bacterial species were isolated from all 121 examined units. Most bacterial species isolated in the cited studies belong to the families related to water and soil. Among the isolates from DUWL, human opportunistic pathogens were present: *Pseudomonas putida*, *Pseudomonas aeruginosa*, *Sphingomonas paucimobilis*, *Acinetobacter calcoaceticus* and *Methylobacterium mesophilicum*.

Examination of water and biofilm samples from the units located in dental clinics at the University of Maryland Dental School was the basis for compiling by Meiller *et al.* [27] a list of bacteria prevailing at individual work stations. At station 1, dominated *Ralstonia pickettii*, *Psychrobacter phenylpyruvica*, coagulase-negative *Staphylococcus*; at station 2 – *Burkholderia cepacia*, *Ralstonia pickettii*; at station 3 – *Moraxella osloenis*, *Alcaligenes faecalis* (*odorans*); at station 4 – *Myroides odoratum*, coagulase-negative *Staphylococcus*, *Staphylococcus aureus*; at station 5 – *Alcaligenes faecalis* (*odorans*), *Brevundimonas vesicularis*; at station 6 – *Sphingomonas paucimobilis*, *Stenotrophomonas maltophilia*, *Bacillus* spp.; at station 7 – *Stenotrophomonas maltophilia*, *Sphingomonas paucimobilis*, *Pseudomonas stutzeri*; at station 8 – *Bacillus* spp., *Stenotrophomonas maltophilia*, *Pseudomonas stutzeri*.

It should be noted that most of the bacteria identified in DUWL belong to the Pseudomonadaceae family, among which environmental bacteria, widespread in nature, prevail. These are Gram-negative, aerobic bacteria, usually motile by monotrichous flagella. Some of these bacteria are opportunistic pathogens. It should be also noted that the bacteria of the *Pseudomonas* genus isolated from DUWL include species which may be potentially pathogenic for immunocompromised patients, while the cocci isolated from most of the units are components of the human oral cavity flora.

A recent study by Uzel *et al.* [60] confirms that the Pseudomonadaceae species, including *Burkholderia cepacia*, *Chryseomonas luteola*, *Pseudomonas fluorescens*, *Ralstonia pickettii* and *Sphingomonas paucimobilis* are the most prevalent bacteria in DUWL, having been recovered from all the examined sites.

Bacteria of the *Ralstonia pickettii* species deserve a special attention; they were previously found in DUWL water, but never in such a large number as reported in later studies which showed the prevalence of *Ralstonia pickettii* both in water and in DUWL biofilm. In the studies carried out by Szymańska [52, 53], bacteria of the *Ralstonia pickettii* species constituted 96.5% of the total bacteria identified in the water from unit reservoirs, 68.6% in the water from high-speed handpieces, and 78.6% in the biofilm. In the light of the latest literature, this aerobic, non-fermenting, oxidase-positive and Gram-negative rod proves to be an opportunistic pathogen which has been isolated both from clinical and environmental samples. Although the virulence of this bacterial species is low, it is the source of serious problems in the hospital environment as a widespread cause of nosocomial infections [3, 4, 5, 42]. It seems that the significance of *Ralstonia pickettii* as an opportunistic pathogen in the dental environment should be considered.

Among the isolates from DUWL, *Acinetobacter calcoaceticus*, *Aeromonas hydrophila*, *Aeromonas sorbia*, *Burkholderia cepacia*, *Brevundimonas vesicularis*, *Methylobacterium mesophilicum*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Sphingomonas paucimobilis* and *Staphylococcus cohnii* are known as opportunistic human pathogens [12, 24, 34, 59]. The obligatory human pathogens, such as *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Mycobacterium* species and *Staphylococcus* species, have been also reported from this environment [26, 39, 47, 57, 58]. Nevertheless, most of the microorganisms isolated from DUWL are of low pathogenicity [14, 35].

Table 2. Genus/species of bacteria and fungi identified in the dental unit waterlines [51, 52, 53].

BACTERIA	
Gram-negative bacteria	Gram-positive bacteria
Brevundimonas vesicularis	**Rods:**
Moraxella lacunata	*Brevibacterium epidermidis*
Moraxella spp.	
Ralstonia (Pseudomonas) pickettii	**Cocci:**
Sphingomonas paucimobilis	*Micrococcus luteus*
Stenotrophomonas maltophilia	*Micrococcus lylae*
	Staphylococcus cohnii
	Staphylococcus hominis ss novobiosepticus
	Staphylococcus lentus
	Staphylococcus pulvereri/vitulus
	Staphylococcus spp.
	Streptococcus spp.
	Actinomycetes:
	Streptomyces albus
FUNGI	
Yeast-like	Moulds
Candida albicans	*Aspergillus amstelodami*
Candida curvata	*Aspergillus fumigatus*
Geotrichum candidum	*Aspergillus glaucus*
	Aspergillus repens
	Citromyces spp.
	Penicillium frequentans
	Penicillium pusillum
	Penicillium turolense
	Sclerotium sclerotiorum

It is known that opportunistic and/or obligatory pathogens may constitute more than 30% of all bacteria present in the water distribution system and that they may be a cause of nosocomial infections related to water. In the research by Barbeau *et al.* [8], *Pseudomonas aeruginosa* was isolated from 24% of the studied units. The analysis revealed that the units contaminated with these bacteria showed a significantly higher total number of bacteria in comparison with the units where *Pseudomonas aeruginosa* was not found.

Tests of DUWL water for *Pseudomonas aeruginosa* carried out by Monarca *et al.* [30] showed that this bacterial species was present in 15-30% of all the samples taken from air-water syringes, while in the samples from turbines and microengines the concentration of these bacteria was very high.

A detailed study of the DUWL mycobiota, including quantitative and qualitative analysis of water and biofilm, indicates that yeast-like fungi of the *Candida* genus: *Candida albicans* and *Candida curvata* are the prevailing species [51]. In other research, *Aspergillus flavus* and *Penicillium expansum* were isolated [17]. Earlier, an unusual fungus *Exophiala mesophila* was isolated from units undergoing a continuous waterline treatment. Identification was performed by DNA sequencing. As previously mentioned, *Exophiala* organisms have been known to cause infection in immunocompromised people [37, 38].

Amoebae were also found in DUWL water. There are studies reporting free-floating amoebae in water samples from all tested units. The protozoan concentration was 330/ml and the most frequent were *Hartmanella*, *Vanella*, *Vahlkampfia* spp. In 40% of the samples *Naegleria* and *Acanthamoeba* spp. were identified [7].

In the DUWL water samples examined by Barbeau *et al.* [8], both yeasts and amoebae were present, yet they were not identified in detail.

The species/genera of bacteria and fungi identified in dental unit waterlines in the primary author's studies [51, 52, 53] are shown in Table 2.

CONCLUSIONS

The specific structure of dental units favours the presence of biofilm and microbial contamination of the dental unit waterlines (DUWL) water. The ability of bacteria to colonize surfaces and to form biofilm in water supply tubes, including DUWL, is a common phenomenon, which has been well documented, just as with difficulties in biofilm removal and prevention of its regrowth. Microorganisms from contaminated DUWL are transmitted with aerosol and splatter, generated by working unit handpieces.

Most of the microorganisms isolated from DUWL are of low pathogenicity. Nevertheless, the public health significance of many of the microorganisms found in DUWL is unknown. According to current knowledge, it is not the mere presence of bacteria that is important in DUWL contamination monitoring, but their number, the presence of potential pathogens, and patients' oral cavity microflora.

Numerous studies emphasize the need for effective mechanisms to reduce the microbial contamination in DUWL and highlight the risk for cross-infection in general practice, especially in view of the ever-increasing number of immunocompromised persons who present at outpatient dental clinics.

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