

MICROBIAL CONTAMINATION OF DENTAL UNIT WATERLINES

Jolanta Szymańska¹, Jolanta Sitkowska², Jacek Dutkiewicz²¹Department of Paedodontics, Medical University of Lublin, Lublin, Poland²Department of Occupational Biohazards, Institute of Agricultural Medicine, Lublin, Poland

Szymańska J, Sitkowska J, Dutkiewicz J: Microbiological contamination of dental unit waterlines: characteristic. *Ann Agric Environ Med* 2008, **15**, 173–179.

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.

Address for correspondence: Associate Professor Jolanta Szymańska, DMD, Department of Paedodontics, Medical University of Lublin, Staszica 11, 20-018 Lublin, Poland. E-mail: szymanska.lublin@gmail.com

Key words: dentistry, dental unit waterlines, microbiological contamination.

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

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 xyloxi-*

dans, *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 plaque 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 suck-back occurs in high-speed handpieces without antiretraction 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 xyloxi-*

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

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

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.

REFERENCES

1. ADA Council on Scientific Affairs: ADA statement on dental unit waterlines. *J Am Dent Assoc* 1996, **127**, 185-189.
2. ADA Council on Scientific Affairs: Dental unit water lines: Approaching the year 2000. *J Am Dent Assoc* 1999, **130**, 1653-1664.
3. Adley CC, Ryan MP, Pembroke JT, Saieb FM: *Ralstonia pickettii* in high purity water. In: Mc Bain A, Alison D, Pratten J, Spratt D, Upton M, Verran J (Eds): Biofilms: Persistence and Ubiquity, 261-272. BioLine, Cardiff 2005.
4. Adley CC, Saieb FM: Biofilm formation in high purity water: *Ralstonia pickettii* a special case for analysis. *Ultrapure Water J* 2005, **Jan/Feb**, 14-17.
5. Adley CC, Saieb FM: Comparison of bioMérieux API 20NE and Remel RapID NF Plus, identification systems of type strains of *Ralstonia pickettii*. *Lett Appl Microbiol* 2005, **41**, 136-140.
6. Al-Saif KM, Assery M, Nahas MA: Microbial contamination of dental unit water systems in Saudi Arabia. *Saudi Dent J* 2007, **19**, 110-114.
7. Barbeau J, Buhler T: Biofilms augment the number of free-living amoebae in dental unit waterlines. *Res Microbiol* 2001, **152**, 753-760.
8. Barbeau J, Tanguay R, Faucher E, Avezard C, Trudel L, Côté L, Prévost AP: Multiparametric analysis of waterline contamination in dental units. *Appl Environ Microbiol* 1996, **62**, 3954-3959.

9. Blake GC: The incidence and control of bacterial infection in dental spray reservoirs. *Br Dent J* 1963, **115**, 413-416.
10. Centers for Disease Control and Prevention: Guidelines for Infection Control in Dental Health-care settings – 2003. *MMWR Rep* 2003, **52**, (No RR-17): 1-66.
11. Cobb CM, Martel CR, McKnight SA 3rd, Pasley-Mowry C, Ferguson BL, Williams K: How does time-dependent dental unit waterline flushing affect planktonic bacteria levels? *J Dent Educ* 2002, **66**, 549-555.
12. Chi CY, Fung CP, Wong WW, Liu CY: *Brevundimonas* bacteremia: two case reports and literature review. *Scand J Infect Dis* 2004, **36**, 59-61.
13. Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption: *Official J Eur Commun* 1998, **330**, 32-54.
14. Dutil S, Veillette M, Mériaux M, Lazure L, Barbeau J, Duchaine C: Aerosolization of mycobacteria and legionellae during dental treatment: low exposure despite dental unit contamination. *Environ Microbiol* 2007, **9**, 2836-2843.
15. Fiehn NE, Larsen T: The effect of drying dental unit waterline biofilms on the bacterial load of dental unit water. *Int Dent J* 2002, **52**, 251-254.
16. Franco FFS, Spratt D, Leao JC, Porter SR: Biofilm formation and control in dental unit waterlines. *Biofilms* 2005, **2**, 9-17.
17. Göksay D, Çotuk A, Zeybek Z: Microbial contamination of dental unit waterlines in Istanbul, Turkey. *Environ Monit Assess* 2008, Jan 22. Available from: <http://www.ncbi.nlm.nih.gov/pubmed18210208> (accessed 15 May 2008).
18. Göksay D, Çotuk A, Zeybek Z: Research of bacterial contamination in dental unit waterlines. *Clin Microbiol* 2006, **12(Suppl 4)**, R 2086.
19. Huntington MK, Williams JF, Mackenzie CD: Endotoxin contamination in the dental surgery. *J Med Microbiol* 2007, **56**, 1230-1234.
20. Jeon EH, Han JH, Ahn TY: Comparison of bacterial composition between human saliva and dental unit water system. *J Microbiol* 2007, **45**, 1-5.
21. Karpay RI, Plamondon TJ, Mills SE, Dove SB: Combining periodic and continuous sodium hypochloride treatment to control biofilms in dental unit water systems. *J Am Dent Assoc* 1999, **130**, 957-965.
22. Kettering JD, Munoz-Viveros CA, Stephens JA, Naylor WP, Zhang W: Reducing bacterial counts in dental unit waterlines: distilled water vs. antimicrobial agents. *J Calif Dent Assoc* 2002, **30**, 735-741.
23. Kettering JD, Stephens JA, Munoz-Viveros CA, Naylor WP: Reducing bacterial counts in dental unit waterlines: tap water versus distilled water. *J Contemp Dent Pract* 2002, **3**, 1-9.
24. Korvick JA, Rihs JD, Gilardi GL, Yu VL: A pink pigmented, oxidative, nonmotile bacterium as a cause of opportunistic infections. *Arch Intern Med* 1989, **149**, 1449-1452.
25. Linger JB, Molinari JA, Forbes WC, Farthing CF, Wingret WJ: Evaluation of a hydrogen peroxide disinfectant for dental unit waterlines. *J Am Dent Assoc* 2001, **132**, 1287-1291.
26. Ma'ayeh SY, Al-Hiyasat AS, Hindiyeh MY, Khader YS: *Legionella pneumophila* contamination of a dental unit water line system in dental teaching centre. *Int J Dent Hyg* 2008, **6**, 48-55.
27. Meiller TF, DePaola LG, Kelley JI, Baqui AAMA, Turng BF, Falkler WA Jr: Dental unit waterlines: biofilms, disinfection and recurrence. *J Am Dent Assoc* 1999, **130**, 65-72.
28. Mills SE: Waterborne pathogens and dental waterlines. *Dent Clin North Am* 2003, **47**, 545-557.
29. Mills SE, Karpay RI: Dental waterlines and biofilm – searching for solution. *Compendium* 2002, **23**, 237-240, 242, 244, 247-249, 252, 254, 256, quiz 258.
30. Monarca S, Garusi G, Gigola P, Spampinato L, Zani C, Sapelli PL: Decontamination del sistema idrico del riunito mediante disinfezione e filtrazione. *Minerva Stomatol* 2002, **51**, 451-459.
31. Monarca S, Grotto M, Feretti D, Gigola P, Zerbini I, Alberti A, Zani C, Sapelli PL: Monitoraggio ambientale dei rischi infettivi legati all'assistenza odontoiatrica. *Minerva Stomatol* 2002, **51**, 451-459.
32. Montebugnoli L, Dolci G: A new chemical formulation for control of dental unit water line contamination: an "in vitro" and clinical "study". *BMC Oral Health* 2002, **2**, 1. Available from: <http://www.biomedcentral.com/1472-6831/2/1> (accessed 28 April 2008).
33. Noce L, Di Giovanni D, Putnins EE: An evaluation of sampling and laboratory procedures for determination of heterotrophic plate counts in dental unit waterlines. *J Can Dent Assoc* 2000, **66**, 262-268.
34. O'Donnel MJ, Shore AC, Coleman DC: A novel automated waterline cleaning system that facilitates effective and consistent control of microbial biofilm contamination of dental chair unit waterlines: a one year study. *J Dent* 2006, **34**, 648-661.
35. Pankhurst CL, Coulter WA: Do contaminated dental unit waterlines pose a risk of infection? *J Dent* 2007, **35**, 712-720.
36. Pankhurst CL, Johnson NW, Woods RG: Microbial contamination of dental unit waterlines: the scientific argument. *Int Dent J* 1998, **48**, 359-368.
37. Porteous NB, Grooters AM, Redding SW, Thompson EH, Rinaldi MG, De Hoog GS, Sutton DA: Identification of *Exophiala mesophila* isolated from treated dental unit waterlines. *J Clin Microbiol* 2003, **41**, 3885-3889.
38. Porteus NB, Redding SW, Thompson EH, Grooters AM, De Hoog GS, Sutton DA: Isolation of unusual fungus in treated dental unit waterlines. *J Am Dent Assoc* 2003, **134**, 853-858.
39. Porteus NB, Redding SW, Jorgensen JH: Isolation of non-tuberculosis mycobacteria in treated in dental unit waterlines. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2004, **98**, 40-44.
40. Putnins EE, Di Giovanni D, Bhullar AS: Dental unit waterline contamination and its possible implications during periodontal surgery. *J Periodontol* 2001, **72**, 393-400.
41. Rautemaa R, Nordberg A, Wuolijoki-Saaristo K, Muerman JH: Bacterial aerosols in dental practice – a potential hospital infection problem? *J Hosp Infect* 2006, **64**, 76-81.
42. Ryan MP, Pembroke JT, Adley CC: *Ralstonia pickettii*: a persistent Gram-negative nosocomial infectious organisms. *J Hosp Infect* 2006, **62**, 278-284.
43. Sacchetti R, Baldissarri A, De Luca G, Lucca P, Stampi S, Zanetti F: Microbial contamination in dental unit waterlines: comparison between Er:YAG laser and turbine lines. *Ann Agric Environ Med* 2006, **13**, 275-279.
44. Santiago JI, Huntington MK, Johnston AM, Quinn RS, Williams JF: Microbial contamination of dental unit waterlines: short and long-term effects of flushing. *Gen Dent* 1994, **48**, 528-535.
45. Schel AJ, Marsch PD, Bradshaw DJ, Finney M, Fulford MR, Frandsen E, Østergaard E, ten Cate JM, Moorer WR, Mavridou A, Kamma JJ, Mandilara G, Stösser L, Kneist S, Araujo R, Contreras N, Goronczy-Bermes P, O'Mullane D, Burke F, O'Reilly P, Hourigan G, O'Sullivan M, Holman R, Walker JT: Comparison of the efficacies of disinfectants to control microbial contamination in dental unit water systems in general dental practices across the European Union. *Appl Environ Microbiol* 2006, **72**, 1380-1387.
46. Shepherd PA, Shojaei MA, Eleazer PD, Van Stewart A, Staat RH: Clearance of biofilms from dental unit waterlines through the use of hydroperoxide ion-phase transfer catalysts. *Quintessence Int* 2001, **32**, 755-761.
47. Singh T, Coogan MM: Isolation of pathogenic *Legionella* species and legionella-laden amoebae in dental unit waterlines. *J Hosp Infect* 2005, **61**, 257-262.
48. Smith AJ, Bagg J, Hood J: Use of chlorine dioxide to disinfect dental unit waterlines. *J Hosp Infect* 2001, **49**, 285-288.
49. Smith AJ, McHugh S, Aitken I, Hood J: Evaluation of the efficacy of Alpron disinfectant for dental unit water lines. *Br Dent J* 2002, **193**, 593-596.
50. Souza-Gugelmin MCM, Lima CDT, Lima SNM, Mian H, Ito IY: Microbial contamination in dental unit waterlines. *Braz Dent J* 2003, **14**, 55-57.
51. Szymańska J: Antifungal efficacy of hydrogen peroxide in dental unit waterline disinfection. *Ann Agric Environ Med* 2006, **13**, 313-317.
52. Szymańska J: Bacterial contamination of water in dental unit reservoirs. *Ann Agric Environ Med* 2007, **14**, 137-140.
53. Szymańska J: Bacterial decontamination of DUWL biofilm using Oxygenal 6. *Ann Agric Environ Med* 2006, **13**, 163-167.
54. Szymańska J: Biofilm and dental unit waterlines. *Ann Agric Environ Med* 2003, **10**, 151-157.
55. Szymańska J: Dental bioaerosols as an occupational hazard in a dentist's workplace. *Ann Agric Environ Med* 2007, **14**, 203-207.

56. Szymańska J: Exposure to bacterial endotoxin during conservative dental treatment. *Ann Agric Environ Med* 2005, **12**, 137-139.
57. Szymańska J: Microbiological risk factors in dentistry. Current status of knowledge. *Ann Agric Environ Med* 2005, **12**, 157-163.
58. Tambekar DH, Gulhane PB, Goyal KS, Gulhane SR: Prevalence of *Pseudomonas aeruginosa* in dental unit water-lines. *Res J Microbiol* 2007, **2**, 983-987.
59. Tuttlebee CM, O'Donnell MJ, Kean CT, Russell RJ, Sullivan DJ, Falkiner F, Coleman DC: Effective control of dental chair unit waterline biofilm and marked reduction of bacterial contamination of output water using two peroxide-based disinfectants. *Hosp Infect* 2002, **52**, 192-205.
60. Uzel A, Cogulu D, Oncag O: Microbiological evaluation and antibiotic susceptibility of dental unit water systems in general dental practice. *Int J Dent Hyg* 2008, **6**, 43-47.
61. Walker JT, Bradshaw DJ, Bennett AM, Fulford MR, Martin MV, Marsh PD: Microbial biofilm formation and contamination of dental-unit water systems in general dental practice. *Appl Environ Microbiol* 2000, **66**, 3363-3367.
62. Williams JF, Johnston AM, Johnson B, Huntington MK, Mackenzie CD: Microbial contamination of dental unit waterlines: prevalence, intensity, and microbiological characteristics. *J Am Dent Assoc* 1993, **124**, 59-65.
63. Williams JF, Molinari JA, Andrews N: Microbial contamination of dental unit waterlines: origin and characteristics. *Compend Contin Educ Dent* 1996, **17**, 538-540, 542, quiz 558.
64. Wirthlin MR, Marshall GW Jr, Rowland RW: Formation and decontamination of biofilms in dental unit waterlines. *J Periodontol* 2003, **74**, 1595-1609.
65. Zhang W, Onyango O, Lin Z, Lee SS, Li Y: Evaluation of Sterilox for controlling microbial biofilm contamination of dental water. *Compend Contin Educ Dent* 2007, **28**, 586-588, 590-592.