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Microbiological evaluation and antibiotic susceptibility of dental unit water systems in general dental practice

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Abstract: *Objective:* The microbial quality of water in a dental unit water systems (DUWS) is of considerable importance because patients and dental staff are regularly exposed to water and aerosol generated from the dental units. The objective of this study was to evaluate the 20 DUWS in general dental practices and to determine the antibiotic susceptibility of the colonizing bacteria. *Methods:* Three water and one biofilm samples from each DUWS were investigated for total viable count (TVC), oral streptococci, *Pseudomonas* spp., *Enterobacteria*, *Candida albicans* and *Legionella pneumophila*. *Results:* A total of 44 morphologically different colonies were obtained from water samples and 20 types of colonies (45.5%) could be identified using API test strips. The mean TVC values were 4.36 log CFU ml^{-1} for source waters, 4.95 log CFU ml^{-1} for 3-in-1 syringe samples, 4.91 log CFU ml^{-1} for air rotor samples and 3.66 log CFU cm^{-2} for biofilm samples. Susceptibilities of the isolates were tested against piperacillin, ampicillin, ceftazidime, meropenem, gentamicin, tetracycline, ofloxacin and chloramphenicol by using microdilution method according to NCCLS. The meropenem and ofloxacin have shown the broadest spectrum against to the tested isolates. *Conclusion:* The study emphasizes the need for effective mechanisms to reduce the microbial contamination in DUWS, and highlights the risk for cross-infection in general dental practice.

Key words: antibiotic susceptibility; biofilm; dental unit water systems

Introduction

Microbial quality of water in dental unit water systems (DUWS) is of considerable importance as patients and dental staff are regularly exposed to water and aerosol generated by the unit. Water used in dentistry comes from either the municipal water system or an independent distilled or sterile water reservoir (1). DUWS may contain opportunistic and true pathogenic bacteria, such as *Pseudomonas* spp., *Mycobacterium* spp., *Staphylococcus* spp., *Candida* spp. and *Legionella pneumophila* (2, 3). All these microorganisms may pose a risk to the patients, especially immunocompromised patients, children as well as to the practice staff (4, 5). Exposure of dental personal to such pathogens is also important; as dentists practicing in dental schools have a significantly higher antibody titre to *L. pneumophila* than other equivalent employment sectors. Furthermore the carriage of the pathogens by asymptomatic patients can cause cross-infections (6).

Design and the material used at the units, ambient temperature, source of the water and biofilm formation can affect microbial number and diversity. Presence of the biofilm is one of the most effective factors responsible for high number of bacteria (1). This contamination mainly comes from the water supply, such as municipal water. Once the bacteria attached at the lumen surface of dental waterline tubing, environmental factors and relatively high surface area/volume ratio associated with the DUWS tubing provide the optimum conditions for biofilm generation (7, 8). When the biofilms are formed in the DUWS, it may be difficult to remove from surfaces, and the bacteria within biofilms are more resistant to antimicrobial agents. Biofilms may also enhance the survival of fastidious pathogens, such as *L. pneumophila*, in water distribution systems. Up to 25% of DUWS have been shown to be contaminated with this bacterium (9).

Currently, dentists all over the world have no evidence-based guidelines to control bacterial numbers in DUWS. In the USA, a standard of $\leq$200 CFU ml^{-1} for the maximum microbial count delivered by DUWS settled by American Dental Association (ADA). In European Union (EU), there are no specific standards for DUWS but guidelines recommend that tap water should be delivered at <100 CFU ml^{-1} at 22°C and <20 CFU ml^{-1} at 37°C (10). In Turkey the Ministry of Health of Turkey has set a standard similar to the EU.

The objective of this study was to evaluate the 20 DUWS in general dental practices and to determine the antibiotic susceptibility of the colonizing bacteria.

Materials and methods

Microbiological sampling

Twenty randomly selected different DUWS that have independent reservoirs supplied with distilled water were investigated in this study. All investigated dental offices were in the city of Izmir and all they were connected to the same municipal water source. Three water and one biofilm samples were taken from each DUWS after operating 2 h in the morning: (i) water line samples taken from 3-in-1 syringe; (ii) the air rotor water samples taken from the outlet of the air rotor; (iii) source water samples that were supplied the DUWS and (iv) biofilm samples taken from a 3-cm part of the water line tubing supplied to 3-in-1 syringe and air rotor. All water samples and the biofilm containing tube samples were placed in sterile bottles. All samples were assessed within 2 h in the microbiology laboratory.

The air samples were also taken from 1 m^2 around of each unit; 100 l for total viable count (TVC) and 100 l for *L. pneumophila* with the aid of the air sampler Mas-100 (Merck, Damstadt, Germany).

Detection of microorganisms

All the water samples (100 ml) were filtered through a 0.22-μm nitrocellulose filters and the membranes were placed in a sterile, screw-capped containers containing 0.5 g of glass beads with 10 ml of the sterile water and vortexed for 1 min except for *L. pneumophila*. Biofilm samples were obtained by cutting the tubing with a sterile lancet to obtain a 1-cm^2 surface under aseptic conditions. The surface was rinsed with sterile PBS to remove the planktonic bacteria and the biofilms were scraped with a sterile dental probe into 1 ml of PBS. After decimal dilutions from water and biofilm samples 0.1 ml of the appropriate dilutions were spread onto duplicate plates of a range of selective and non-selective agar media. The level of detection was 10 CFU ml^{-1}. R2A medium was used for detection of TVC at 22°C and 37°C, and incubated 72 h. Mitis Salivarius Bacitracin agar (MSB) for oral streptococci, incubated under anaerobic conditions at 37°C for 72 h. *Pseudomonas* agar base with CFC supplement SR103 (Oxoid, Basingstoke, UK) (PA) used for *Pseudomonas* spp. and incubated at 37°C for up to 48 h. Mc Conkey Agar CM7 for *Enterobacteria* and incubated aerobically at 37°C for 48 h. *Candida* ID agar (Biomerioux, Marcy l'Etoile, France) for *Candida albicans* and incubated at 37°C for 72 h. *Legionella pneumophila* occurrence in water samples were investigated by concentration of 100 ml water samples using sterile 0.22 μm porosity nitrocellulose filter. After

concentration, the membranes were aseptically removed, cut into smaller pieces and placed into sterile, screw-capped containers containing 0.5 g of glass beads with 10 ml of the original filtrate. These were vortexed vigorously for 5 min and treated as below for selective isolation of *L. pneumophila*. Heat treatment; 5 ml were placed in a water-bath at 50°C for 30 min. Acid treatment; 1 ml sample was acidified with 9 ml buffered HCl–KCl solution (pH 2.2) for 15 min and neutralized by adding 1.0 ml alkaline neutralizer agent. The remaining concentrated sample was untreated. For each water sample, 0.1 ml was spread on duplicate plates of GVPC agar (BCYE agar with the addition of glycine, vancomycin, polimyxin B, cycloheximide supplement; Oxoid) from each of the three above treatments. These plates were incubated at 35°C in a humidified atmosphere and examined after 2, 5, 7 and 10 days of incubation.

For air samples only R2A agar, *Pseudomonas* agar and GVPC agar were used for detection of TVC, *Pseudomonas* spp. and *L. pneumophila* respectively.

Identification and antimicrobial susceptibility of the microorganisms

The total number of colonies was recorded and the number of colony forming units in the original water, biofilm and air samples was calculated. A description of the different colony types present was recorded and selected colonies of each type were purified by subculture for subsequent identification. These were tested for oxidase and catalase production, grown on Mc Conkey agar, Gram stained and identified by using API 20 E and API 20 NE, API 50 CHB and API Staph test strips (Biomerioux).

The minimum inhibitory concentration (MIC) of the isolates were determined by means of the broth microdilution method described by the National Committee for Clinical Laboratory Standards (11) against a panel of antibiotics; piperacillin, ampicillin, ceftazidime, meropenem, gentamicin, tetracycline, ofloxacin and chloramphenicol. All tests were performed in Mueller–Hinton Broth (Oxoid) in triplicate. Control organisms used for susceptibility assays were: *P. aeruginosa* ATCC 27853 and *E. coli* ATCC 25922. Isolates were grouped as susceptible or resistant according to the NCCLS.

Statistical analysis

All data were analysed using the SPSS 13.0 software program (SPSS Inc., Chicago, IL, USA) with univariate analysis of variance and student's *t*-test. Log_{10} CFU values were used for statistical analysis. The significance was set as $P < 0.05$.

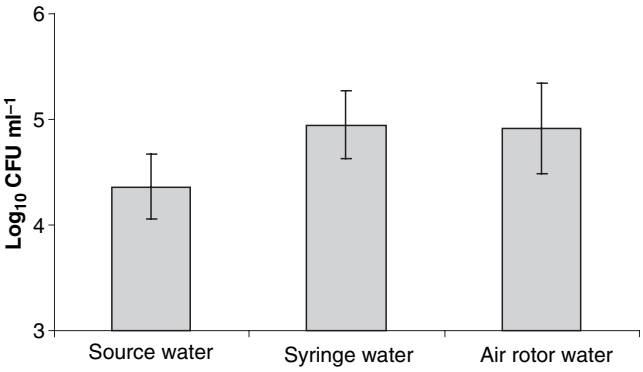

Fig. 1. Mean TVC values of different sample types.

Results

All three types of water samples obtained from 20 units were found to have higher TVC values than EU guidelines. The lowest and highest TVC values were: 3.61–4.89 log CFU ml^{-1} for source waters, 4.32–5.36 log CFU ml^{-1} for 3-in-1 syringe samples, 4.14–5.47 log CFU ml^{-1} for air rotor samples and 2.67–4.23 log CFU cm^{-2} for biofilm samples. The mean TVC values for the air rotor and syringe water samples were relatively high comparing to mean TVC values for unit tanks ($P < 0.05$) (Fig. 1). The mean TVC value for air samples was 2.83 log CFU m^{-3} and *Pseudomonas* spp. and *L. pneumophila* have not been isolated from air samples.

R2A, *Pseudomonas* agar and GVPC plates from water and biofilm samples produced bacterial colonies. Colonies typical for oral streptococci, *Enterobacteriaceae* and *C. albicans* were not observed on selective media. Colonies with similar morphology to that of *Legionellaceae* (grey, glistening, convex and circular with a uniform edge) were observed on GVPC agar from water and biofilm samples. These colonies were tested for their cystein requirements on blood agar plates without cystein and found that none of the isolates from GVPC plates belong to *Legionella* spp. Selected isolates of each colony type present on R2A and *Pseudomonas* agar plates were purified by subculture for subsequent identification. Only biofilm and air rotor isolates were attempted to identification. A total of 44 morphologically different colonies were obtained, but only 20 types of colonies (45.5%) could be identified using API test strips as follows: *Acinetobacter calcoaceticus*, *Aeromonas hydrophila*, *Aeromonas sobria*, *Alcaligenes denitrificans*, *Bacillus licheniformis*, *Bacillus subtilis*, *Brevundimonas vesicularis*, *Burkholderia cepacia*, *Burkholderia gladioli*, *Chryseomonas luteola*, *Comamonas acidovarans*, *Methylobacterium mesophilicum*, *Ochrabactrum anthropi*, *Pseudomonas aeruginosa*, *P. fluorescens*, *P. putida*, *P. studzerii*, *Raltsonia pickettii*, *Sphingomonas paucimobilis* and *Staphyloccus cohnii*. *Sphingomonas*

Table 1. **Susceptibility results of isolates**

Microorganisms	Isolates			% Susceptibility							
	WI (n = 14)	BI (n = 14)	Total isolates (357)	PIP	AMP	CAZ	MER	GEN	TET	OFX	CHL
Acinetobacter calcoaceticus	11	9	20	30.0	30.0	95.0	95.0	5.0	15.0	85.0	10.0
Aeromonas hydrophila	9	6	15	86.7	86.7	100.0	100.0	100.0	80.0	100.0	100.0
Aeromonas sobria	12	10	22	86.4	81.8	95.5	95.5	90.9	86.4	100.0	95.5
Alcaligenes dentrificans	7	7	14	14.3	14.3	85.7	100.0	21.4	21.4	42.9	85.7
Bacillus licheniformis	4	nd	4	100.0	75.0	100.0	100.0	25.0	75.0	100.0	100.0
Bacillus subtilis	3	nd	3	ni	ni	ni	ni	ni	ni	ni	ni
Brevundimonas vesicularis	8	4	12	ni	ni		ni	ni	ni	ni	ni
Burkholderia cepacia	14	11	25	52.0	28.0	80.0	88.0	12.0	100.0	56.0	24.4
Burkholderia gladioli	13	10	23	52.2	26.1	82.6	91.3	21.7	100.0	73.9	34.8
Chryseomonas luteola	14	13	27	25.9	22.2	59.3	88.9	33.3	81.5	100.0	44.4
Comamonas acidovarans	4	nd	4	100.0	100.0	100.0	100.0	0.0	100.0	100.0	100.0
Methylobacterium mesophilicum	12	7	19	73.4	52.6	68.4	89.5	100.0	94.7	100.0	89.5
Ochrabactrum anthropi	8	6	14	7.1	7.1	7.1	100.0	92.9	85.7	100.0	71.4
Pseudomonas aeruginosa	10	12	22	86.4	18.2	77.3	90.9	63.6	13.6	72.3	72.7
Pseudomonas fluorescens	14	14	28	75.0	60.7	89.3	67.9	82.1	50.0	82.1	46.4
Pseudomonas putida	11	13	24	75.0	66.7	87.5	66.7	83.3	54.2	87.5	41.7
Pseudomonas studzerii	12	12	24	100.0	91.7	100.0	100.0	100.0	100.0	100.0	100.0
Raltsonia pickettii	14	13	27	66.7	66.7	25.9	22.2	40.7	70.4	96.3	44.4
Sphingomonas paucimobilis	14	14	28	46.4	46.4	75.0	96.4	82.1	96.4	100.0	67.9
Staphyloccus cohnii	2	nd	2	100.0	100.0	50.0	100.0	50.0	100.0	100.0	100.0

WI, water isolates; BI, biofilm isolates; *n*, number of the samples; nd, not detected; ni, MIC results could not be interpretable as susceptible according to the NCCLS for *Bacillus* spp.; PIP, piperacillin; AMP, ampicillin; CAZ, ceftazidime; MER, meropenem; GEN, gentamicin; TET, tetracycline; OFX, ofloxacin; CHL, chloramphenicol.

paucimobilis were the prevalent species in water samples but no dominance was detected in biofilm samples.

A total of 357 isolates belonging to the 20 different species were tested for their antibiotic susceptibility against eight antibiotics. The results of the antibiotic susceptibility tests for identified bacteria are shown in Table 1. Susceptibility results of water and biofilm isolates were given in one group, as statistically no differences ($P > 0.05$) are found for susceptibility rates between water isolates and biofilm isolates. The isolates were grouped as susceptible, intermediate susceptible and resistant according to the MIC breakpoints listed in the NCCLS data sheets and susceptibility rates were shown in Table 1.

Discussion

Twenty DUWS in general dental practices were evaluated for the type and level of microbial contamination. As the water from the DUWS is used to irrigate the oral cavity during dental treatment, the water quality should be acceptable according to the drinking water standards. All of the dental units in this study exceeded the current EU potable water standard (<100 CFU ml^{-1}) indicating a heavy contamination.

Previous studies showed that the total bacterial counts of the DUWS could be variable in a wide range between 0.84 log CFU ml^{-1} and 6.0 log CFU ml^{-1} in different units. The mean level of contamination of the studied units was relatively high compared with the other studies (8, 12–14). Water from air rotor lines and from 3-in-1 syringe was contaminated to a similar degree, but significantly ($P < 0.05$) higher than the source water. This finding indicates that there is an increase in the bacterial count from source water to outlet possibly due to biofilms. Although relatively high levels of bacteria found on DUWS, these values probably underestimate the true microbial load to which a patient is exposed, as only 3% of the microscopically visible bacteria produced colonies on agar plates (15).

The predominant bacterial species recovered from the dental unit water samples were motile Gram-negative rods (85%). These findings are in accordance with the literature (16, 17). *Burkholderia cepacia*, *C. luteola*, *P. fluorescens*, *R. pickettii* and *S. paucimobilis* were the most prevalent species and recovered from all of the DUWS. However, Gram-positive species were the least prevalent bacteria recovered from DUWS and *S. cohnii* was found only in two DUWS samples. Some of our isolates were pigmented bacteria. It has been suggested that pigmented bacteria may be more chlorine tolerant than non-pigmented forms (18). Among our isolates, *A. calcoaceticus*, *A. hydrophila*, *A. sobria*, *B. cepacia*, *B. vesicularis*, *M. mesophilicum*, *P. aeruginosa*, *P. fluorescens*, *P. putida* and *S. paucimobilis* are known as opportunistic human pathogens (19, 20). Dental patients may be exposed to these bacteria through inhalation

of aerosols, ingestion of contaminated treatment water or inoculation into oral wounds. These bacteria can pose a health threat to patients who have cancer or diabetes and to those who are immunocompromised. Young children, as well as the elderly, are also at an increased risk of infection by these pathogens. Dental staffs are also at risk of exposure to aerosols. Furthermore the carriage of the pathogens by asymptomatic patients and staffs can cause cross-infections (21).

Some studies highlight the nature of contamination of DUWS (14, 16, 17). However, in spite of isolation of many true or opportunistic pathogens from DUWS there is no information about the antibiotic susceptibilities of the contaminated bacteria. It may be useful to know the susceptibility patterns as some of the bacteria isolated from DUWS are pathogens. According to the best of our knowledge, this is the first study to describe the antibiotic susceptibility profiles of bacteria which harbour DUWS.

It was not possible to define primary antimicrobial agents for all DUWS isolates due to the great variation of the susceptibility among these pathogens. The meropenem and ofloxacin have shown the broadest spectrum against the tested isolates. However, meropenem have significantly reduced activity against *P. putida* and *R. pickettii* and ofloxacin have also reduced activity against *A. denitrificans*, *B. vesicularis* and *B. cepacia*. For *B. licheniformis* and *B. subtilis* no susceptibility rates were shown in Table 1 because MIC results could not be interpretable as susceptible or resistant according to the NCCLS standards for *Bacillus* spp. Although, we can conclude that gentamicin is the most effective antibiotic for tested *Bacillus* species based on MIC results.

Water that does not pass drinking water standards should not be used in DUWS. Effective control of the microorganisms in DUWS can be achieved by using several methods; using an appropriate disinfectant or a filtration device, flushing and drying of DUWS, using autoclavable or disposable water delivery systems and using an independent sterile water reservoirs that bypass the municipal water. This study emphasizes the need for effective mechanisms to reduce the microbial contamination in DUWS, and highlights the risk for cross-infection in general dental practice.

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