

Research Paper

Mycological contamination in dental unit waterlines in Istanbul, Turkey

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Abstract

Studies on dental units (DUs) are conducted either for the prevention or the reduction of the density of bacterial contamination in dental unit waterlines (DUWLs). However, the existence of fungi in these systems requires more attention. During dental treatment, direct contact with water contaminated with fungi such as *Candida*, *Aspergillus*, or inhalation of aerosols from high-speed drill may cause various respiratory infections, such as asthma, allergies, and wounds on mucose membranes, especially on immunocompromised patients and dentists. The aims of this study are to investigate the number and colonization of fungi in DUWLs in the city of Istanbul, Turkey. Water samples were collected from air-water syringes, high-speed drills, and inlet waters from 41 DUs. The aerobic mesophilic fungi count in high-speed drills was higher than inlet waters and air-water syringes. Non-sporulating fungi were found in 7 DUs. The isolated fungi were identified as *Penicillium waksmanii*, *Cladosporium spp.*, *Penicillium spp.*, *Candida famata*, *Cryptococcus laurentii*, *Candida guilliermondii*, *Penicillium verrucosum*, *Aspergillus pseudoglaucus*, *Penicillium decumbens*, and *Acremonium sp.* Some of these fungal genera are known as opportunistic pathogens that led to respiratory diseases such as allergic rhinitis. This study shows the importance of regular control of mycological contamination on water at DUs.

Key words: dental unit waterlines, dental equipments, filamentous fungi, mycological contamination, yeast.

Introduction

Water used in dental practices for activities such as oral rinsing and the cooling of dental equipment is generally supplied from the municipal water system. Dental unit waterlines (DUWLs), however, provide a suitable environment for microbial multiplication and the formation of biofilm which may be comprised of bacteria, fungi, and protozoa. A high number of microorganisms have been found in DUWLs (Walker *et al.*, 2004; Göksay *et al.*, 2008; Dogruöz *et al.*, 2012). Although most microorganisms are harmless, potential pathogens such as *Legionella*, *Pseudomonas*, and *Candida* also have been detected (Walker *et al.*, 2004; Türetgen *et al.*, 2009). These microorganisms are of particular concern because of their ability to cause pneumonia, and other respiratory infections while wound infections are immunocompromised. Consequently, the quality of

dental unit (DU) water is extremely important both for patients and dentists.

There have been many studies concerning the high levels of bacterial contamination, but little is known about fungal contamination in DUWLs (Williams *et al.*, 1993; Barbeau *et al.*, 1996; Genc *et al.*, 1997; Walker *et al.*, 2000; Walker *et al.*, 2004; Szymanska, 2005a; Göksay *et al.*, 2008; Türetgen *et al.*, 2009). *Candida* spp. which can cause both superficial and systemic diseases have been recovered occasionally from DUWLs (Genc *et al.*, 1997; Walker *et al.*, 2004; Szymanska, 2005a). The fungi isolated from DUs water contained several yeasts, but mostly filamentous fungi (Pankhurst *et al.*, 1998; Araujo and Contreras, 2004; Pitt, 2000; Walker *et al.*, 2004; Szymanska, 2005a; Göksay *et al.*, 2008). *Aspergillus* and *Penicillium* are the prevalent genera which have been isolated in DUWLs. It is known that, the spores of these genus members can cause allergic

reactions, asthma, and other respiratory problems. The research has suggested that fungal spores and hyphal fragments may be aerosolized into the air when contaminated water passes through dental equipments. In addition, several filamentous fungi are also potential toxin producers, and exposure to small amounts of toxins over several years may have negative effects on the immune system (Szymanska, 2005a). Owing to the hazardous effects of these microorganisms, we wanted to investigate the distribution of fungi in 41 DUWLs in 21 private and 20 public dental clinics. The aims of this study, then, were to determine the number and composition of the colonized mycobiota in the water from 41 DUWLs.

Materials and Methods

Water sampling

123 water samples were randomly collected from 41 DUs in the city of Istanbul, Turkey. 21 DUs were selected from different Private dental Clinics, whereas the other 20 DUs were at the same Public hospital's dental Clinics. 32 of the DUWLs were supplied with municipal water (containing chlorine), two DUWLs with distilled water, and seven DUWLs with commercial bottled water in polycarbonate containers. Water samples (250 mL) were taken from air-water syringes, high-speed drills, and inlet waters in the morning before patients arrived for treatment (Veronesi *et al.*, 2007). Water temperature, pH, and free chlorine values were measured.

Mycological analysis

For yeast definition, 100 μ L diluted samples (10^0 - 10^{-2}) were spread-plated in triplicate onto Sabouraud Dextrose Agar (SDA) plates with streptomycin, and incubated at 30 °C for 48-72 hours (Walker *et al.*, 2000). After incubation, the yeast colonies were counted and were subcultured on SDA to identify colonies.

In order to determine the filamentous fungi, 100 μ L diluted samples (10^0 - 10^{-2}) were spread-plated in triplicate onto SDA with streptomycin, and incubated at 25 °C for 7 - 10 days (Pereira *et al.*, 2009). After incubation, the colonies were counted and were subcultured on Potato Dextrose Agar to identify colonies.

Enumeration and identification of yeast and filamentous fungi

The quantities of yeast and filamentous fungi in each original sample were calculated using colony forming units per milliliter (cfu/mL). Gram staining, germ-tube formation and biochemical tests, including carbohydrate assimilation (API 20C AUX system) were applied for yeast identification (Arvanitidou *et al.*, 1999).

Filamentous fungi were inoculated into various media (Czapek Yeast Autolysate Agar, Czapek Dox Agar, Czapek Yeast Autolysate Agar with 20% Sucrose, 25%

Glycerol Nitrate Agar, Malt Extract Agar, and Potato Dextrose Agar) and then identified as to genus and species level by microscopical and macroscopical methods according to generally accepted standards (Ellis, 1971; Klich, 2002; Porteus *et al.*, 2003).

Statistical analysis

The mean of aerobic mesophilic fungi (AMF) counts and standard deviations were calculated. The Mann-Whitney U test was employed to detect statistically significant changes in the mean AMF of each water point (air-water syringes, high-speed drills, inlet waters). Statistical analysis were carried out using Spearman's Correlation Coefficient Test (SPSS, version 11, 5: SPSS Inc, Chicago, IL, USA) to examine the relationship between AMF counts at the different water points and pH, free chlorine and temperature. Significant differences are reported at the $p < 0.01$ level.

Results and Discussion

Studies conducted during the early years of microbial contamination in DUWLs focused mainly on bacterial colonization (Blake, 1963; Fitzgibbon *et al.*, 1984; Furuhashi and Miyamae, 1985; Martin, 1987), in which the importance of fungal colonization was not completely understood. Although the presence of fungi in DU water has not been shown to present a demonstrable health risk either to patients or dentists. The existence of opportunistic pathogens in DU water is not consistent with accepted infection control principles. Although there are suggested standards for the bacterial control of DU waters and provision of unit hygiene, no internationally recommended standard concerning fungi has yet been put forward (Anonymous, 1996; CDC, 2003). To the best of our knowledge, this is the first study reporting on mycological contamination in DUWLs in Istanbul, Turkey.

In this study, the number of AMF in the collected water samples ranged from 10- 130 cfu/mL in outlet water from air-water syringes, 10-813 cfu/mL in outlet water from high speed drills and 10-160 cfu/mL in the water from units' inlet waters. The mean of AMF counts in air-water syringes, high-speed drills, and inlet waters were 36 cfu/mL, 101 cfu/mL and 45 cfu/mL respectively. A previous study conducted on 25 DUs reported the mean counts of fungi in reservoirs and, high-speed hand-pieces were 410 cfu/mL and 578 cfu/mL. Researcher have generally preferred to use Malt Extract Agar as a medium (Szymanska, 2006), but as there is no internationally accepted standardized method for the isolation and determination of fungi in water environments various medias have been used in previous studies (Hageskal *et al.*, 2006), which is why fungi counts may differ. Consequently, an internationally accepted standardized method for the isolation of fungi in water is necessary.

The mean of the AMF counts was higher in high-speed drills than in air-water syringes. The Mann-Whitney U analysis revealed that there was a difference in the mean of the SDA plate counts obtained from air-water syringes and high-speed drills ($p < 0.01$). We did not detect any significant difference between the mean of the AMF counts in the air-water syringes and inlet waters. The mean of the AMF counts was significantly higher in high-speed drills than inlet waters. The Mann-Whitney U analysis revealed that there was a difference in the mean of the SDA plate counts obtained from high-speed drills and inlet water ($p < 0.001$). We also found that there was a difference between the mean counts of AMF in high-speed drills' outlet water and inlet waters ($p < 0.001$), suggesting microbial accumulation shows the formation of biofilm. Although some

fungal genus was established in inlet water and different outlet points of DUWLs, some was determined only in the outlets of DUWLs (Table 1). This would suppose that these fungi entered and attached themselves into the system earlier and were then absorbed into the biofilm, which may protect fungi from disinfection. Biofilm also acts as conidia reservoirs. Biofilm breaks into fragments, and subsequently these may be released into the water flow intermittently. As a result, contamination spreads throughout water systems (Hageskal *et al.*, 2006). To remove biofilm and reduce the microbial counts are required daily or weekly continuous treatment of peroxide based disinfectant such as sterilex ultra, oxigenal, sanosil in DUWS (Walker *et al.*, 2003).

Table 1 - The number of fungal species isolated in samples of water from air-water syringes, high-speed drills, and inlet water.

Dental unit (code)	Source of dental unit	Names of isolated fungi	A-W Syringe (cfu/mL)	H-S Drill (cfu/mL)	IW (cfu/mL)
1	P	<i>Cryptococcus laurentii</i> (Kuff.) C.E. Skinner	10 ± 0	17 ± 3	0.0
2	P	Filamentous fungi or yeast	0.0	0.0	0.0
3	P	<i>Penicillium waksmanii</i> K.M. Zalessky	0.0	0.0	10 ± 0
4	P	<i>Penicillium waksmanii</i> K.M. Zalessky	0.0	27 ± 6	0.0
5	P	<i>Candida famata</i> E.K. Novák & Zsolt	0.0	47 ± 19	0.0
6	P	<i>Penicillium waksmanii</i> K.M. Zalessky	25 ± 7	0.0	0.0
		<i>Candida guilliermondii</i> Castell. Langeron & Guerra	0.0	17 ± 0	0.0
7	P	<i>Penicillium verrucosum</i> Dierckx	0.0	67 ± 8	0.0
8	P	<i>Cladosporium sp. Link ex Fries</i>	0.0	0.0	73 ± 15
9	P	<i>Cladosporium sp. Link ex Fries</i>	15 ± 4	0.0	160 ± 17
10	P	<i>Penicillium sp. Link ex Gray</i>	0.0	20 ± 0	20 ± 10
		<i>Penicillium verrucosum</i> Dierckx	45 ± 10	0.0	0.0
11	P	<i>Candida famata</i> E.K. Novák & Zsolt	0.0	20 ± 5	10 ± 0
12	P	<i>Cryptococcus laurentii</i> (Kuff.) C.E. Skinner	0.0	67 ± 21	20 ± 0
13	P	Filamentous fungi or yeast	0.0	0.0	0.0
14	P	Filamentous fungi or yeast	0.0	0.0	0.0
15	P	<i>Cladosporium sp. Link ex Fries</i>	0.0	16 ± 1	0.0
16	P	Filamentous fungi or yeast	0.0	0.0	0.0
17	P	<i>Cryptococcus laurentii</i> (Kuff.) C.E. Skinner	27 ± 5	0.0	0.0
18	P	Filamentous fungi or yeast	0.0	0.0	0.0
19	P	Filamentous fungi or yeast	0.0	0.0	0.0
20	P	Filamentous fungi or yeast	0.0	0.0	0.0
21	PB	Non-sporulating fungi	25 ± 7	130 ± 0	0.0
22	PB	Filamentous fungi or yeast	0.0	0.0	0.0
23	PB	Non-sporulating fungi	30 ± 0	0.0	0.0
24	PB	Filamentous fungi or yeast	0.0	0.0	0.0
25	PB	Non-sporulating fungi	0.0	200 ± 53	0.0
26	PB	Filamentous fungi or yeast	0.0	0.0	0.0
27	PB	<i>Acremonium sp. Link ex Fries</i>	130 ± 17	0.0	0.0
		Non-sporulating fungi	0.0	13 ± 2	0.0
28	PB	Non-sporulating fungi	0.0	500 ± 46	0.0
29	PB	Non-sporulating fungi	0.0	13 ± 6	0.0
30	PB	<i>Cladosporium sp. Link ex Fries</i>	0.0	100 ± 17	0.0

Table 1 (cont.)

Dental unit (code)	Source of dental unit	Names of isolated fungi	A-W Syringe (cfu/mL)	H-S Drill (cfu/mL)	IW (cfu/mL)
31	PB	<i>Aspergillus pseudoglaucus</i> Blochwitz	0.0	10 ± 0	0.0
		Non-sporulating fungi	130 ± 14	65 ± 7	0.0
32	PB	<i>Cladosporium sp. Link ex Fries</i>	0.0	813 ± 40	0.0
33	PB	<i>Cladosporium sp. Link ex Fries</i>	20 ± 0	10 ± 0	0.0
34	PB	Non-sporulating fungi	0.0	207 ± 15	0.0
35	PB	<i>Penicillium decumbens</i> Thom	0.0	45 ± 21	0.0
36	PB	Non-sporulating fungi	0.0	40 ± 14	0.0
37	PB	Non-sporulating fungi	0.0	45 ± 7	0.0
		<i>Cladosporium sp. Link ex Fries</i>	10 ± 0	0.0	0.0
		<i>Penicillium sp. Link ex Gray</i>	10 ± 0	0.0	0.0
38	PB	Non-sporulating fungi	0.0	20 ± 3	0.0
39	PB	<i>Penicillium waksmanii</i> K.M. Zalessky	10 ± 0	0.0	0.0
		<i>Cladosporium sp. Link ex Fries</i>	10 ± 0	0.0	0.0
40	PB	Filamentous fungi or yeast	0.0	0.0	0.0
41	PB	Non-sporulating fungi	0.0	27 ± 6	0.0

A-W Syringe: Air-Water Syringe H-S Drill: High-Speed Drill IW: Inlet Water cfu/mL: Colony Forming Units per Millilitre P: Private dental clinic PB: Public dental clinic ± : Standard Deviation.

Because of the negative effects of fungi on public health, we believe that further research, especially research associated with fungal biofilms in DUWLs, is required.

We investigated the temperature, pH, and free chlorine of DU' influence on the fungal load of its water. And so the physical and chemical characteristics of DUs' outlet water were investigated. Water temperatures varied between 15-24 °C. The pH values of outlet waters varied between 6.8-7.71. In the supply waters of two DUs' low levels of free chlorine were detected (0.3 and 0.5 ppm). Even if these conditions are suitable for AMF growth, it was found that there was no correlation between the AMF counts at the different water points and water temperature, pH, and free chlorine, according to Spearman's correlation coefficient test.

The analysis of the water samples from the air-water syringes, detected fungi in 13 out of 41 DUs (31.70%). Fungi isolated from these samples were identified as *Cryptococcus laurentii*, *Penicillium waksmanii*, *Cladosporium spp.*, *Penicillium verrucosum*, *Acremonium sp.*, and *Penicillium sp.* In the water flowing from high-speed drills, fungi were found in 23 out of 41 DUs (56.09%). These identified fungi were: *Cryptococcus laurentii*, *Penicillium waksmanii*, *Candida famata*, *Candida guilliermondii*, *Penicillium verrucosum*, *Cladosporium spp.*, *Aspergillus pseudoglaucus*, *Penicillium decumbens*, and *Penicillium sp.* The water samples from DUs' inlet waters revealed the presence of fungi in 6 out of 41 DUs (14.63%). The fungi were identified as: *Penicillium waksmanii*, *Cladosporium spp.*, *Penicillium sp.*, *Candida famata*, and *Cryptococcus laurentii*. Non-sporulating fungi were found in (29.26%) 12 out of 41 DUs (Table 1).

Water contaminated with yeast can be a risk if there is direct contact with open wounds. Many researches have found the species *Candida* in DUWLs (Genc *et al.*, 1997; Walker *et al.*, 2000; Araujo and Contreras, 2004; Szymanska, 2005b). Opportunistic pathogen yeast in the genus *Candida* can cause skin and systemic disease. While *Candida albicans* is generally considered to be the most pathogenic of the *Candida* species, a variety of other members of this genus have been cited as the causative agents of an increasing number of infections (Szymanska, 2005b). In this study, *Candida guilliermondii*, *Candida famata*, and *Cryptococcus laurentii* were isolated from the different water points of DUs. Aerosols originating from DU water below 2.5 µm are considered dangerous. These small aerosols which contain microorganisms can induce asthma, rhinitis, allergic alveolitis, or other respiratory problems (Gorny, 2004). In previous studies on DU water, *Fusarium*, *Cladosporium*, *Alternaria*, *Aspergillus*, *Penicillium*, and *Scopulariopsis* have been determined (Szymanska, 2005a; Göksay *et al.*, 2008). *Aspergillus* and *Penicillium* are the most common genera of those found in aquatic environmental studies. These spores are known to cause allergic reactions (Asan *et al.*, 2003; Asan, 2004). Moreover *Cladosporium* and *Alternaria* are known to provoke respiratory and asthmatic symptoms in immunocompromised people. From our study, isolated fungi such as *Aspergillus*, *Penicillium*, and *Cladosporium spp.* could also create these harmful effects. To our knowledge, the potential allergic and infectious characteristics of fungi have not been studied in municipal water systems such as in DUWLs. Hence there is no detailed information about fungal infections in

the human population. Epidemiological researches, therefore, needs to be conducted in the future.

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