

## EVALUATION OF MYCOLOGICAL CONTAMINATION OF DENTAL UNIT WATERLINES

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**Abstract:** The quality of dental unit water is of great importance since patients and dental staff are regularly exposed to water from aerosols generated during work. The main purpose of this investigation was mycological evaluation of dental unit waterlines (DUWL). The author determined the number and species of fungi present in the water from a unit reservoir which is the source of water for a dental unit, in the water flowing from a high-speed handpiece of a unit, and in the biofilm samples collected from the wall of a waterline connecting a unit reservoir and dental handpieces. The following mould fungi were identified: *Aspergillus amstelodami*, *Aspergillus fumigatus*, *Aspergillus* spp. from *Aspergillus glaucus* group, *Aspergillus repens*, *Citromyces* spp., *Penicillium aspergilliforme*, *Penicillium pusillum*, *Penicillium turolense*, *Sclerotium sclerotiorum*; yeast-like fungi: *Candida albicans*, *Candida curvata*, and *Geotrichum candidum*. Some of them, in certain circumstances, especially in people with immunological disorders, may be a cause of opportunistic infections. Thus, it is necessary that the DUWL should be submitted to a decontamination protocol and to routine microbial monitoring to guarantee an appropriate quality of water used in dental treatment.

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In dental unit waterlines, bacteria, fungi, free living amoebae, protozoa and nematodes find favourable conditions to develop. The presence of various microorganisms is a potential source of microbial contamination of dental aerosols, and thus a potential threat to the health of patients and dental staff. In the conditions of decreased immunity of an organism, opportunistic infections with fungi constitute a health risk [13].

The aim of the study was to determine the number and species composition of the fungal microflora in the water from DUWL water reservoirs, in the water flowing from a high-speed handpieces of a dental units, and also in biofilm samples collected from the walls of a waterline connecting a unit reservoir and dental handpieces; the microflora was considered as a potential threat to health of patients and of a dental team.

### MATERIAL AND METHODS

The research was conducted on 25 operative sites – dental units located in public dental clinics. From each unit, the following material was collected aseptically: 1. water from the water reservoir of a self-contained dental unit water system; 2. biofilm sample from the wall of the main waterline providing water to dental handpieces; 3. water flowing from a high-speed dental handpiece. The reservoirs were filled with distilled water.

**Processing of samples for counting and identification of fungi.** In the case of water, 0.1 ml of the collected sample was taken and spread on a malt agar medium. The plate dilution method was used. For examining of the DUWL biofilm, a 15 mm-long fragment of the tubing was

aseptically taken from each unit. The tube fragments were immersed in 2 ml of the sterile buffered solution of physiological salt with calcium chloride and magnesium chloride, and shaken in order to obtain fungal suspension. The solution was inoculated in the same way as water samples. The malt agar plates were subsequently incubated for 4 days at 30°C and 4 days at 22°C. The prolonged incubation at lower temperatures was aimed at isolating as wide a spectrum of fungi as possible. After incubation, the colonies were counted and identified to species level by microscopic methods, according to different manuals by Barron [2], Larone [5], Litvinov [6], Ramirez [10], and Raper and Fennell [11]. The concentration of fungi was expressed as colony forming units (cfu) per 1 ml.

## RESULTS AND DISCUSSION

In the water and biofilm samples collected from the DUWL the fungi listed in Table 1 were found.

Analysis of the water samples from dental unit reservoirs showed the presence of fungi in the water from 12 out of 25 units. 3 species of fungi were found in the water from 1 reservoir, 2 species - in 3 reservoirs, 1 species - in 8 reservoirs. In the studied water samples, the following fungi were identified: *Aspergillus glaucus* group, *Sclerotium sclerotiorum*, *Candida albicans*, *C. curvata*.

In the studied biofilm from the DUWL main tubes, fungi were found in 11 out of 25 swab samples. 3 species of fungi were detected in 3 swab samples, 2 species in 2 sample, 1 species of fungi in 6 samples. The following fungi were identified: *Aspergillus amstelodami*, *A. fumigatus*, *Aspergillus glaucus* group, *Penicillium pusillum*, *P. turolense*, *Sclerotium sclerotiorum* and *Candida albicans*, *Candida curvata*, and *Geotrichum candidum*.

In the water flowing from high-speed handpieces, fungi were found in 16 out of 25 samples. 3 species of fungi were detected in 1 case, 2 species of fungi - in 4 cases, 1 species in 11 cases. The following fungi were identified: *Citromyces* spp., *Aspergillus fumigatus*, *A. repens*, *Aspergillus glaucus* group, *Penicillium frequentans*, *Penicillium pusillum*, *Sclerotium sclerotiorum* and *Candida albicans*, *Candida curvata*, and *Geotrichum candidum*.

The number of colony forming fungi (cfu/ml) in the collected water samples varied from  $0-645 \times 10^1$  cfu/ml in the water from unit reservoirs and from  $0-375 \times 10^1$  cfu/ml in water flowing from high-speed handpieces, and in the biofilm from  $0-311.39$  cfu/mm<sup>2</sup>. In the reservoir water, the most common were: *Candida curvata*, *Candida albicans*, and *Aspergillus glaucus* group, in the swab: *Aspergillus glaucus* group, *Candida albicans*, and *Sclerotium sclerotiorum* and in the water from a high-speed handpiece: *Candida albicans*, *Aspergillus glaucus* group, *Sclerotium sclerotiorum*.

**Table 1.** List of fungal species identified in samples of water from units reservoirs, water from a high-speed handpieces and biofilm from the DUWL wall.

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### Yeast-like fungi:

*Candida albicans*  
*Candida curvata*  
*Geotrichum candidum*

### Mould fungi:

*Aspergillus amstelodami*  
*Aspergillus fumigatus*  
*Aspergillus* spp. from *Aspergillus glaucus* group  
*Aspergillus repens*  
*Citromyces* spp.  
*Penicillium aspergilliforme*  
*Penicillium pusillum*  
*Penicillium turolense*  
*Sclerotium sclerotiorum*

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### Others yeasts

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The microflora that contaminate the waterlines can originate from two places: 1. municipal water piped into the dental unit (directly - in the case of an open system water supply, or after distillation - in the case of a closed system water supply) and 2. suck-back of a patient's saliva into the line due to the lack of antireaction valves [7]. Pederson *et al.* [9] believe that *Candida* species found in DUWL, which belong to the commonly reported oral flora, are most likely derived from suck-back events. Reaspiration of fluid from the oral cavity occurs when a negative pressure is generated on stopping equipment. Fungi from dental units occurring in the water: *Phoma* spp., *Penicillium* spp., *Cladosporium* spp., *Alternaria* spp., *Scopulariopsis* spp., are a confirmation of the presence of oral microflora [8].

It was interesting to note a variety of fungi species, which was 2-times greater in the case of the biofilm from the waterline wall and of the water flowing from a handpiece than in the water from a reservoirs. As the water in a reservoir is used up, it is filled with distilled water and disinfected once a month, which undoubtedly improves the microbial quality of the water. In public clinics, where the studied units are located, a permanent chemical DUWL decontamination procedure is not used, which may affect the composition of biofilm and fungal contamination of the water flowing from a handpiece.

The best conditions for the growth of fungi is provided by a biofilm forming on the waterline walls, which is periodically released and may contaminate the water flowing from handpieces. The water in bioaerosol which is produced during the work of dental handpieces, contains fungi and may be a threat to health for both patients and dental staff.

Airborn microorganisms may cause ill effects in humans ranging from mild irritation to disease. A moderate risk for both groups may be associated with the presence of potentially pathogenic fungal species having allergenic and/or immunotoxic properties. Generally, fungi relevant in medicine are not as virulent as bacteria. The exception is aflatoxin produced by fungi of

*Aspergillus* genus. Fungi usually cause slowly progressing, chronic infections, without acute symptoms which usually occur in bacterial and viral infections. However, in patients with compromised immunity they may cause acute, life-threatening infections [12].

Fungi of *Candida* genus and fungi of *Aspergillus* genus may be causes of the most frequent opportunistic mycoses - candidiasis and aspergillosis. The opportunistic fungi that normally would not cause diseases in humans, in favourable conditions, especially in immunologically deficient patients, may cause infections. The fungi species of the greatest clinical importance is *Candida albicans*, abundantly prevalent in the studied samples from DUWL. The fungus may lead to diseases, including septicemia of the mucous membranes, skin, subcutaneous tissue, internal organs and tissues. Among the biological risk factors in dentistry, *Candida albicans* and *Candida tropicalis* - the fungi showing infectious or invasive activity and allergenic activity - are mentioned.

Infections with *Aspergillus* fungi concern mainly lungs. The infection resulting from inhaling *Aspergillus* spores may cause bronchial asthma in patients allergic to antigens of these fungi. In immunocompromised patients the infection may take an invasive form and may lead to, among others, septicemia, aspergillosis of endocardium, central nervous system, skin and subcutaneous tissue.

*Aspergillus fumigatus* is a known fungal pathogen causing allergic alveolitis, asthma, pulmonary aspergillosis and possibly mycotoxicoses.

The fungi of *Penicillium* genus are characterised with strong allergenic properties and may cause bronchial asthma, asthma with lung eosinophilia (APE), and allergic rhinitis [3, 4, 12, 14].

The literature reports the prevalence of yeast-like fungi in the water and biofilms present in dental unit waterlines. Some yeasts and amoebae have been observed by direct microscopic observation by Barbeau *et al.* [1]. The yeast-like fungi strains *Candida parapsilosis* are supposed to form a biofilm in plastic tubes especially easily. In water, the following mould fungi were found: *Fusarium* spp., *Cladosporium* spp., *Alternaria* spp., *Penicillium* spp. and

*Scopulariopsis* spp. In particular, *Penicillium marneffeii* may be a serious risk factor for patients with immunity disorders. It is reported that a new threat for patients wearing contact lenses is contamination by sprayed aerosol. Among others, a mould fungus *Paecilomyces* spp. is mentioned as causing cornea infections in people using the lenses. Recently, attention has been given to infections with fungi of *Fusarium* genus in patients after traumas and surgeries, even if the patients' immune system is normal [14].

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