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EXPOSURE TO AIRBORNE FUNGI DURING CONSERVATIVE DENTAL TREATMENT

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Abstract: The aim of the study was a mycological assessment of bioaerosol forming during conservative dental treatment, taking into account concentration and type of fungal microflora, and evaluation of the influence of DUWL disinfecting protocol on the fungal contamination of the bioaerosol. The research was conducted on 25 operative sites located in public dental clinics. The air contained in the space between a patient and a dentist during conservative dental treatment with the use of a high-speed handpiece was examined. Air samples were taken using the portable RCS PLUS Air Sampler (BIOTEST AG, Dreieich, Germany) and ready-to-use agar YM Strips for yeast and mould fungi culture. The volume of the sampled air was 100 litres. Before disinfection, the concentration of fungi in the collected air samples at individual operative sites ranged from 4×10^1 cfu/m^3 to 34×10^1 cfu/m^3. The most common species was *Penicillium herquei* (62.17% of the total count), followed by other fungi: *Alternaria alternata* - 12.68%, *Penicillium roseopurpureum* - 9.41%, *Rhizopus nigricans* - 5.93%, *Aspergillus terreus* - 3.89%, *Geotrichum candidum* - 2.25%, *Aspergillus glaucus* group - 2.04%, *Cladosporium cladosporoides* - 1.23% and *Penicillium diversum* - 0.41%. The concentration of *Penicillium herquei* at individual operative sites ranged from 0 to 34×10^1 cfu/m^3, mean 121.6 cfu/m^3, *Penicillium roseopurpureum* - from 0 to 11×10^1 cfu/m^3, mean 18.4 cfu/m^3 and *Alternaria alternata* - from 0 to 18×10^1 cfu/m^3, mean 24.8 cfu/m^3. After disinfection, like before disinfection procedures, the prevailing species of fungi were: *Penicillium herquei*, *Penicillium reseopurpureum* and *Alternaria alternata*, which amounted to 62.6%, 18.28% and 11.36% of the isolated fungi, respectively. The recorded levels of total airborne fungi were lower after DUWL disinfection compared to those before disinfection.

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INTRODUCTION

Dental unit handpieces, generally used in dental practice, are a source of microbial aerosol, which is a potential threat to health of patients and a dental team. Microbial pollution of air seems especially important in the case of immunologically deficient patients because of the risk of causing opportunistic infections; it is also significant for a dental team, as a occupational hazard, due to its continual exposure to bioaerosols at the workplace [4].

The aim of the study was a mycological assessment of bioaerosol forming during conservative dental treatment, taking into account concentration and type of fungal microflora, and evaluation of the influence of DUWL disinfecting protocol on the fungal contamination of the bioaerosol.

MATERIAL AND METHODS

The research was conducted on 25 operative sites (dental units) located in public dental clinics. The air

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contained in the space between a patient and a dentist during conservative dental treatment with the use of a high-speed handpiece was examined. Air samples were taken using the portable RCS PLUS Air Sampler (BIOTEST AG, Dreieich, Germany) and ready-to-use agar YM Strips for yeast and mould fungi culture. The volume of the sampled air was 100 litres. The strips were incubated for 5 days at the temperature of 30ºC. After incubation, colonies were counted and identified to the level of species with macro- and microscopic methods [7]. The concentration of fungi (cfu) in a cu m was calculated according to the following formula: cfu/m^3 = cfu counted on an agar strip/sample volume (litre) × 1000 (litre).

The research was carried out twice: before the procedure of DUWL disinfection and after application of the procedure previously described [6].

Statistical analysis. The obtained results were processed using Microsoft Excel 2000, Statistica 5.1. The assumed error risk was 5%.

RESULTS AND DISCUSSION

Fungi in air samples before disinfection. The concentration of total fungi in the collected air samples before of DUWL disinfection at individual operative sites ranged from 4 × 10^1 cfu/m^3 to 34 × 10^1 cfu/m^3 (Tab. 1, 4). The following fungal species were found: *Alternaria alternata* (Fr.) Keissl, *Aspergillus glaucus* group, *Aspergillus terreus* Thom, *Cladosporium cladosporioides* (Fresen.) G.A. de Vries, *Geotrichum candidum* Link ex Pers., *Penicillium diversum* Raper et Fennel, *Penicillium herquei* Bainier and Sartory, *Penicillium* (*carmino-violaceum*) *roseopurpureum* Diereckx (=*Penicillium sanguifluus* Sopp), *Rhizopus nigricans* (=*Rhizopus stolonifer* (Ehrenb.: Fr) Vuill.) by M.A.A. Schipper (Tab. 3).

In the examined air no yeast-like fungi belonging to the *Candida* species, which prevailed among fungi identified in DUWL tests, were found [7]. In the bioaerosol, mould fungi were isolated among which there were alergising and/or immunotoxic species.

The most common species was *Penicillium herquei* (62.17% of the total count), followed by other fungi: *Alternaria alternata* - 12.68%, *Penicillium roseopurpureum* - 9.41%, *Rhizopus nigricans* - 5.93%, *Aspergillus terreus* - 3.89%, *Geotrichum candidum* - 2.25%, *Aspergillus glaucus* group - 2.04%, *Cladosporium cladosporoides* - 1.23% and *Penicillium diversum* - 0.41% (Tab. 2). The concentration of *Penicillium herquei* at individual operative sites ranged from 0 to 34 × 10^1 cfu/m^3, mean 121.6 cfu/m^3, *Penicillium roseopurpureum* - from 0 to 11 × 10^1 cfu/m^3, mean 18.4 cfu/m^3 and *Alternaria alternata* - from 0 to 18 × 10^1 cfu/m^3, mean 24.8 cfu/m^3 (Tab. 4).

Fungi in air samples after disinfection. The concentration of total fungi in the studied air samples after DUWL disinfection ranged from 1 × 10^1 cfu/m^3 to 34 ×

Table 1. Concentration of total fungi in air at the individual operative sites before and after DUWL disinfection (cfu/m^3).

Unit No.	Before disinfection	After disinfection
1	200	130
2	200	100
3	300	200
4	290	190
5	330	170
6	290	60
7	170	70
8	250	200
9	180	220
10	220	90
11	210	200
12	110	110
13	100	120
14	200	70
15	40	80
16	140	330
17	40	30
18	240	120
19	250	10
20	40	340
21	330	120
22	340	180
23	140	200
24	120	60
25	160	210
Mean	195.6	144.4

Table 2. Mean concentration of fungal species in air before and after DUWL disinfection (cfu/m^3).

Fungal species	Before disinfection		After disinfection	
	cfu/m^3	%	cfu/m^3	%
Alternaria alternata	24.8	12.68	16.4	11.36
Aspergillus glaucus group	4	2.04	2.8	1.94
Aspergillus terreus	7.6	3.89	2.4	1.66
Cladosporium cladosporioides	2.4	1.23	0	0
Geotrichum candidum	4.4	2.25	3.2	2.22
Penicillium diversum	0.8	0.41	0	0
Penicillium herquei	121.6	62.17	90.4	62.6
Penicillium roseopurpureum	18.4	9.41	26.4	18.28
Rhizopus nigricans	11.6	5.93	1.6	1.11
Rhodotorula rubra	0	0	1.2	0.83
Total	195.6	100.00	144.4	100.00

Table 3. Fungi isolated from the air during dental treatment with the use of a high-speed handpiece at the individual operative sites before DUWL disinfection.

Alternaria alternata (9, 10, 11, 12, 13, 14, 15, 16), *Aspergillus glaucus* group (8, 14, 24), *Aspergillus terreus* (3, 9, 11, 25), *Cladosporium cladosporioides* (8, 9), *Geotrichum candidum* (8), *Penicillium diversum* (20), *Penicillium herquei* (1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 13, 18, 20, 21, 22, 23, 24, 25), *Penicillium roseopurpureum* (1, 2, 3, 10, 16), *Rhizopus nigricans* (17, 19).

Table 4. Concentration of fungal species in air before and after DUWL disinfection (cfu/m^3).

Fungi	Air before disinfection			Air after disinfection		
	Mean	Min	Max	Mean	Min	Max
Penicillium herquei	121.6	0	340	90.4	0	340
Penicillium roseopurpureum	18.4	0	110	26.4	0	100
Alternaria alternata	24.8	0	180	16.4	0	90
Total identified fungi	195.6	40	340	144.4	10	340

mean - mean concentration for all sites, min - minimum concentration at a site, max - maximum concentration at a site.

Table 5. Statistical analysis of DUWL disinfection influence on fungi concentration in the air (Wilcoxon test).

Fungi	T	Significance	p
Penicillium herquei	86.5	—	0.194082
Penicillium roseopurpureum	28.0	—	0.388193
Alternaria alternata	51.5	—	0.629264
Total identified fungi	71.0	*	0.024006

T - Wilcoxon test value for groups: $n \leq 25$; p - significance level for Wilcoxon test; * - $p<0.05$, — - no statistic significance.

10^1 cfu/m^3 (Tab. 1). The same species of fungi were present as before disinfection, except for *Cladosporium cladosporioides* and *Penicillium diversum*. Fungi of the *Rhodotorula rubra* species, however, were found. Like before disinfection procedures, the prevailing species of fungi were: *Penicillium herquei*, *Penicillium reseopurpureum* and *Alternaria alternata*, which amounted to 62.6%, 18.28% and 11.36% of the isolated fungi, respectively (Tab. 2). The concentration of the three mentioned fungi species equalled, respectively, 0–34 × 10^1 cfu/m^3, on average 90.4 cfu/m^3; 0-10 × 10^1 cfu/m^3, on average 26.4 cfu/m^3, and 0–9 × 10^1 cfu/m^3, on average 16.4 cfu/m^3 (Tab. 4).

Influence of DUWL disinfection procedure on concentration of fungi in bioaerosol. Analysis of the fungi concentration in bioaerosol was performed before and after DUWL disinfection. The Wilcoxon test was used because the study results did not show the normal distribution, which was checked with the aid of the Kołmogorow test.

Due to the too small number of operative sites where the identified fungi species were found in the air, only the most frequently occurring fungi, i. e. *Penicillium herquei*, *Penicillium roseopurpureum*, and *Alternaria alternata* were analysed statistically. The distinct decline in the number of fungi colonies in the air samples collected after application of DUWL disinfection, was shown to be statistically insignificant when every species was examined individually. When all the identified species were analysed, the actual concentration of fungi was significantly lower ($p<0.05$) (Tab. 4, 5).

CONCLUSION

The examination of dental bioaerosols showed: 1. presence of fungal species which occur in air and are cosmopolitan in various climatic zones; 2. prevalence of fungi belonging to *Penicillium* genus which reveal allergenic properties; 3. presence of other fungal species reported to be allergenic and/or immunotoxic (*Alternaria alternata*, *Aspergillus terreus*, *Rhizopus nigricans*) [2].

The concentration of fungi in the examined dental aerosols is difficult to evaluate because: 1. no research on fungal contamination of dental bioaerosols is reported; 2. there are no referential methods for examination of fungal contamination of room air, including medical rooms [1, 3, 5]. The results may only be compared with few data concerning medical centres rooms, or with the suggested referential limits for residential rooms (RLV) and occupational exposure limit (OEL), which equal, respectively, 5 × 10^3 cfu/m^3 and 50 × 10^3 cfu/m^3 [3].

Because dental bioaerosol is intensely emitted in the breathing space of a patient and a dentist, it seems necessary to monitor its microbiological quality. Identification of the contamination sources, evaluation of air quality and of a potential threat constituted by contamination of the air, and also development of quality control methods, are the problems which should be addressed by scientific research in the future.

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