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EXPOSURE TO BACTERIAL ENDOTOXIN DURING CONSERVATIVE DENTAL TREATMENT

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Abstract: The aim of the study was to determine bacterial endotoxin concentration in the water flowing from a high-speed handpiece of a dental unit and in the air contained in the bioaerosol formed during dental conservative treatment. The air was collected in the space between the patient and dentist. The study was conducted on 25 operative sites (units) and had two stages: before application of a dental unit waterline (DUWL) disinfectant and after a 2-week application of disinfection procedure. The research showed that the mean concentration of bacterial endotoxin in the water flowing from high-speed handpieces was significantly reduced after the use of a disinfectant. The mean concentration of bacterial endotoxin in the air was similar at both stages – before and after application of waterline decontamination procedure. The study showed that in dental air-water aerosol, water is the main source of bacterial endotoxin contaminating the aerosol during the work with dental handpieces. Application of a user-friendly water disinfectant to significantly decrease endotoxin concentration in the DUWL water and in the aerosol, is one of recommended methods to reduce health risk.

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INTRODUCTION

During dental procedure, several infectious diseases could be transmitted to staff and patients by airborne bacterial and other contaminations in a dental clinic. There are at least three potential sources of airborne contamination during dental treatment: dental instrumentation, saliva and respiratory sources, and the operative site. Contamination from dental instrumentation is a result of the presence of organisms on instruments and in the waterlines of dental units (DUWL). Routine cleaning and sterilisation procedures should eliminate contamination of all dental instruments, except those currently used to treat a patient. Application of DUWL treatment methods should also minimise or eliminate the airborne contamination from DUWL [2].

During work with high-speed dental instruments, air-water aerosols, composed of particles varying in size, are produced. Particles greater than 100 µm in diameter disperse and deposit quickly on the surface of objects due to gravitation forces. Most of the produced particles are droplets smaller than 100 µm in diameter. After water has evaporated, they form the so called "droplet nuclei", not larger than 5 µm in diameter, composed of dried saliva and serum excretion with microorganisms. The droplet nuclei may float in the air for many hours and become a source of viral, bacterial and fungal infections. Infection occurs by way of contact of the infectious material

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dispersed in the air with mucous membranes of the conjunctiva, nose and oral cavity, and also by inhalation [3].

It would be interesting to discover whether the application of a disinfectant to the water in the DUWL would affect the quality of the air in dental air-water aerosol.

MATERIAL I METHODS

The research was conducted in 25 dental units located in public dental clinics. In all studied units, the water was supplied from reservoirs filled with distilled water. At each operative site, water and air were collected: the water flowing from a high-speed handpiece of a dental unit prepared for a new patient, and the air during conservative treatment of the patient using the same dental unit. Water and air samples were collected twice: before application of a DUWL chemical disinfection and after a 2-week period of disinfection procedure. The disinfectant used in the study was a product licensed for sale and available for dentists. Before using a disinfectant in a working concentration, an intensive DUWL decontamination process was performed according to the producer's recommendations. The disinfecting solution was supplemented when necessary, and was continuously present in the DUWL.

Examination of the water. To determine bacterial endotoxin concentration in the DUWL water, 1 ml samples of the water flowing from a high-speed handpiece of a dental unit were collected aseptically. Bacterial endotoxin determination was performed using *Limulus* test, as described below. The initial sample was 0.1 ml of the DUWL water and 0.1 ml of *Limulus*.

Examination of the air. To determine the dust and endotoxin concentration, the air samples were collected on polyvinyl chloride filters with the use of an AS-50 one-stage sampler (TWOMET, Zgierz, Poland). The samples were taken during conservative dental treatment with high-speed and others handpieces in the space between the patient and dentist. The concentration of dust in the air was estimated gravimetrically. The concentration of bacterial endotoxin in the airborne dust was determined with the *Limulus* amebocyte lysate gel tube test (LAL) [4].

The filters were extracted for 1 hour in 10 ml of pyrogen-free water at room temperature, heated to 100°C in a Koch apparatus for 15 min. (to dissolve endotoxin better and to inactivate interfering substances), and after cooling, serial dilution was prepared. The 0.1 ml samples of the dilution were mixed with the "Pyrotell" Limulus reagent (Associates of Cape Cod, Inc., Falmouth, MA, USA) in equal proportion. The test was incubated for 1 hour in a water bath at 37ºC, using pyrogen-free water as a negative control and the standard lipopolysacharide (endotoxin) of *Escherichia coli* 0113:H10 (Difco) as positive control. The formation of a stable clot was regarded as a positive result. The estimated concentration of endotoxin in dust (ng/mg) was multiplied by the estimated concentration of dust in the air (mg/m^3), and the results were reported as micrograms of the equivalents of the *E. coli* 0113:H10 endotoxin per 1 m^3 of air. To convert to Endotoxin Units (EU), the value in nanograms was multiplied by 10.

Statistical analysis was carried out using t-Student test.

RESULTS AND DISCUSSION

The obtained results are presented in Tables 1 and 2.

Concentration of bacterial endotoxin in water before application of a DUWL disinfectant ranged from 15.625–3,125.00 µg/ml with a median value 31.25 µg/ml. Endotoxin concentration in water after intensive DUWL decontamination, and with the continuous application of the DUWL disinfectant ranged from 0.0078-0.78 µg/ml, with a median 0.3125 µg/ml. The differences between analysed groups were statistically significant ($p < 0.001$).

Table 1. Concentration of bacterial endotoxin in water from high-speed handpieces of dental unit before and after disinfection of DUWL water.

Unit No.	Endotoxin in water (µg/ml)	
	Before disinfection	After disinfection
1	31.25	0.78
2	15.625	0.78
3	31.25	0.0078
4	15.625	0.0078
5	31.25	0.156
6	31.25	0.156
7	156.25	0.156
8	156.25	0.078
9	156.25	0.78
10	15.625	0.0078
11	15.625	0.0078
12	312.5	0.0078
13	1,562.5	0.625
14	1,562.5	0.3125
15	3,125	0.3125
16	1,562.5	0.3125
17	31.25	0.625
18	31.25	0.3125
19	31.25	0.625
20	31.25	0.3125
21	312.5	0.3125
22	31.25	0.3125
23	3,125	0.625
24	1,562.5	0.3125
25	1,562.5	0.3125
mean	620	0.3295
standard deviation	946.7122	0.2555
median	31.25	0.3125

$p < 0.001$

Table 2. Concentration of bacterial endotoxin in air during conservative dental treatment before and after disinfection of DUWL water.

	Endotoxin in air (μg/m^3)	
Unit No.	Before disinfections	After disinfections
1	0.00625	0.00625
2	0.00625	0.0003125
3	0.0625	0.00625
4	0.0625	0
5	0.00625	0
6	0	0.00313
7	0	0.00625
8	0	0.00625
9	0	0.00625
10	0.0625	0
11	0.0625	0.0625
12	0.0625	0.0625
13	0	0.00625
14	0.00125	0.00625
15	0	0.00625
16	0	0.0625
17	0.0625	0.0625
18	0.0625	0.0625
19	0.00625	0.0625
20	0.0125	0.0625
21	0.0625	0.00625
22	0.00625	0
23	0	0
24	0	0
25	0	0
mean	0.0218	0.019888
standard deviation	0.028087	0.026696
median	0.00625	0.00625

$p > 0.05$

The strong positive statistical association confirms the effectiveness of a routine monitoring of the DUWL water in order to reduce bacterial contamination of the dentist's work place. The composition of bacterial flora in the DUWL water, and of the DUWL biofilm, which is considered a source of microbial water contamination, has been described in others publications [6, 7]. An active biofilm is a source of continuous DUWL water contamination. The DUWL colonising bacteria are typical for the environment related to water supply, with the prevalence of Gram-negative bacteria which are a potential source of bacterial endotoxin. The cell walls of Gram-negative bacteria are composed of an outer membrane of lipoproteins, phospolipids, and most notably lipopolysaccharide (LPS), i.e. endotoxin. In humans, at the cellular level, LPS stimulates the release of the pro-inflammatory cytokines, tumour necrosis factor (TNF)-alfa, interleukin (IL)-1 and IL-6.

Putnins *et al.* [5] have reported that the mean LPS levels in water samples collected from high-speed lines and air/water lines was 480 EU/ml (SD 294 EU/ml) and 1.008 EU/ml (SD 598 EU/ml) ($p < 0.05$), respectively. The differences between air/water and high-speed lines were not significant. It follows from the research by Fulford *et al.* [1] that in the studied water samples collected from DUWL, free endotoxin ranged from 25–600 EU ml^{-1}. Very few of the samples showed detectable levels of endotoxin, and in the majority of those that did, the level was found to be low.

The studied air contained trace amounts of bacterial endotoxin, and the mean values were similar at both study stages. The level of endotoxin in the air was low. Concentration of bacterial endotoxin in the air before and after DUWL decontamination ranged from 0–0.0625 μg/m^3 with a median value 0.00625 μg/m^3. The difference was not statistically significant ($p > 0.05$). In the available literature, there are no studies concerning bacterial endotoxin in dental aerosols.

CONCLUSIONS

The study showed that in dental air-water aerosol, water is the main source of bacterial endotoxin contaminating the aerosol during the work with dental handpieces. Application of a user-friendly water disinfectant which significantly decreases endotoxin concentration in the DUWL water and in the aerosol, is one of the recommended methods to reduce health risk.

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