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# ENDOTOXIN LEVEL AS A POTENTIAL MARKER OF CONCENTRATION OF GRAM-NEGATIVE BACTERIA IN WATER EFFLUENT FROM DENTAL UNITS AND IN DENTAL AEROSOLS

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**Abstract:** Gram-negative bacteria concentration in water effluent from a dental unit, and in dental aerosol forming during the work of a dental handpiece, was assessed. The study was conducted on 25 dental units before and after a 2-week period of using a disinfecntant for water in dental units waterlines (DUWL). The contamination of water with Gram-negative bacteria before disinfection was 18–398 × $10^3$ cfu/ml, and after disinfection, bacteria were not found. The concentration of Gram-negative bacteria in the air before disinfection was 0–23 × $10^1$ cfu/$m^3$, and after disinfection - 0–8 × $10^1$ cfu/$m^3$. Simultaneously, the water and air were sampled to determine bacterial endotoxin. The statistical analysis did not show correlation between endotoxin concentration and Gram-negative bacteria concentration for the water before disinfection, and for the air before and after disinfection of DUWL water. Because the number of bacteria in the water after disinfection dropped to zero, statistical methods could not be used. The performed analysis suggests that bacterial endotoxin concentration is not indicative of Gram-negative bacteria contamination. Thus, bacterial endotoxin determination is not recommended as a method of monitoring the microbiological quality of DUWL water and dental aerosols.

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Endotoxins are biologically-active lipopolysaccharides (LPS) which occur in the external layer of the Gram-negative bacterial cell wall. Toxicity is associated with the lipid component (Lipid A) and immunogenicity is associated with the polysaccharide components. The release of LPS from bacteria takes place after death and lysis of the cells. Endotoxins play an important role in the course of infections with Gram-negative bacteria. In monocytes and macrophages 3 types of events are triggered during their interaction with LPS: 1) Production of cytokines, including IL-1, IL-6, IL-8, tumour necrosis factor (TNF), and platelet-activating factor. These are powerful mediators of inflammation and septic shock that accompanies endotoxin toxemia. 2) Activation of the complement cascade C3a and C5a cause histamine release (leading to vasodilation) and effect neutrophil chemotaxis and accumulation. The result is inflammation. 3) Activation of the coagulation cascade. The net effect is to induce inflammation, intravascular coagulation, hemorrhage and shock. Typical clinical symptoms caused by endotoxin include: fever and shock, typical diseases - septicaemia and endotoxin shock. This is closely related to the biological characteristics of endotoxins [20]. It is also known that endotoxin might act as a co-allergen

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facilitating sensitization to other allergens [10], or it may increase the severity of allergic disease [9].

Bacterial endotoxins belong to the group of biological harmful agents to which, along with prions, bacteria, fungi and other bacterial substances, a dental team is exposed at their workplace. Aerosols and droplets which are produced during many dental procedures constitute a significant source of biological hazards; they are transmitted via air-dust or air-droplet routes, enter the organism through skin, mucous membranes, and less frequently - to the respiratory or digestive systems. It should be stressed that in the case of dentists, the air-droplet route includes both the saliva-droplet route, because they work in the operative field of the oral cavity, in the presence of saliva and tissue fluids, and the water-droplet route relates to the presence of water in dental units waterlines (DUWL) [1, 5, 11, 14, 15, 17, 18, 19].

The aim of the study was to measure concentrations of Gram-negative bacteria in water effluent from dental unit waterlines and in dental aerosol during conservative treatment. The intention was to assess correlations between endotoxin concentration and Gram-negative bacteria concentration in order to use the level of endotoxin concentration as a possible marker of microbiological contamination of water and air at a dentist's workplace.

## MATERIAL AND METHODS

The study included 25 dental units located in public dental clinics. All studied units were retrofitted with self-contained reservoirs filled with distilled water. At each operative site, water and air were collected: the water effluents from a high-speed handpiece of a dental unit prepared for a new patient, and the air during conservative treatment of a patient with the use of the same handpiece of a dental unit. Water and air samples were collected twice: before application of a DUWL chemical disinfection (KaVo OXYGENAL 6) and after a 2-week period of disinfection procedure. The method of testing water and air to determine bacterial endotoxin and results were presented in a previous paper [16]. The water and air for both analyses - of endotoxin level and of Gram-negative bacteria concentration - were sampled simultaneously.

**Microbiological examination of the water.** 1 ml samples of water effluent from a high-speed handpiece of a dental unit were taken aseptically. To determine the concentration and species composition of Gram-negative bacteria in the water samples, the plate dilution method was applied. The 0.1 ml aliquots of each dilution were spread on duplicate sets of blood agar. The blood agar medium was chosen because it ensures much better isolation of *Pseudomonadaceae* strains from DUWL water than specific media for recovery of Gram-negative bacteria (as the eosin methylene blue agar). Blood agar plates were incubated for 1 day at 37ºC. The grown colonies were counted and differentiated, and the data reported as cfu per 1 ml of water (cfu/ml). Bacterial isolates were identified with microscopic and biochemical methods, as recommended in Bergy's Manual [6, 13, 22]. Additionally, the selected isolates were identified with microtests: API System 20E and NE (bioMérieux, Marcy l'Etoile, France).

**Microbiological examination of the air.** To determine microbiological composition of the air, Biotest Air Sampler RCS Plus, with ready-to-use culture media on flexible strips (Biotest AG, Dreieich, Germany), was used. The tested air was sampled in the space between a patient and a dentist during conservative treatment with a high-speed handpiece of a dental unit. The volume of air samples taken was 100 litres. The strips were incubated for 2 days at the temperature of 32±2.5ºC. After incubation, the same procedure as in microbiological assessment of water was followed to isolate and identify species of cultured microorganisms. The concentration of microorganisms (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 (litres).

**Statistical analysis.** Statistical calculations were performed with software package Statistica 6.0 for Windows. Since most data were not normally distributed, Spearman rank correlation was used.

## RESULTS AND DISCUSSION

Gram-negative bacteria concentration in water effluent from DUWL ranged before disinfection from 18–398 × $10^3$ cfu/ml, with the mean value 143,600.4 cfu/ml. After disinfection, Gram-negative bacteria were not detectable (Tab. 1). Gram-negative bacteria concentration in the air ranged from 0–23 × $10^1$ $cfu/m^3$ with the mean value 52.0 $cfu/m^3$ before disinfection, and after disinfection from 0–8 × $10^1$ $cfu/m^3$, with the mean value 12.8 $cfu/m^3$ (Tab. 1).

Bacterial endotoxin concentration in the examined water and air samples before and after using a disinfectant was the subject-matter of a previous paper [16]. In the water before disinfection it amounted to 15.625–3,125.00 µg/ml, with the mean value 620.0 µg/ml, and after disinfection - 0.0078–0.78 µg/ml, with the mean value 0.3295 µg/ml. In the air, it was 0–0.0625 $\mu g/m^3$, with the mean value 0.0218 $\mu g/m^3$ before DUWL disinfection, and after disinfection - 0–0.0625 $\mu g/m^3$ with the mean value 0.01988 $\mu g/m^3$. The above given values of endotoxin concentration refer to the same samples of water and air, which were tested for Gram-negative bacteria concentration, and were taken simultaneously.

The statistical analysis did not show significant correlation between bacterial endotoxin level and Gram-negative bacteria concentration in water flowing from a unit before DUWL disinfection. Because the quantity of bacteria after using a disinfectant dropped to zero at all operative sites, and no value changes occurred, it was

**Table 1.** Concentration of Gram-negative bacteria in water (cfu/ml) from high-speed handpiece of a dental unit and in air (cfu/m$^3$) during conservative dental treatment before and after disinfection of DUWL.

| Unit No. | Gram-negative bacteria in water (cfu/ml) | | Gram-negative bacteria in air (cfu/m$^3$) | |
|---|---|---|---|---|
| | before disinfection | after disinfection | before disinfection | after disinfection |
| 1 | 77,000 | 0 | 80 | 0 |
| 2 | 47,000 | 0 | 130 | 0 |
| 3 | 58,000 | 0 | 50 | 0 |
| 4 | 61,000 | 0 | 40 | 0 |
| 5 | 76,000 | 0 | 60 | 80 |
| 6 | 122,000 | 0 | 20 | 20 |
| 7 | 112,000 | 0 | 90 | 50 |
| 8 | 69,000 | 0 | 20 | 20 |
| 9 | 328,000 | 0 | 30 | 80 |
| 10 | 135,000 | 0 | 30 | 10 |
| 11 | 156,000 | 0 | 230 | 20 |
| 12 | 295,000 | 0 | 0 | 0 |
| 13 | 79,000 | 0 | 90 | 0 |
| 14 | 79,000 | 0 | 0 | 0 |
| 15 | 18,000 | 0 | 0 | 0 |
| 16 | 90,000 | 0 | 0 | 20 |
| 17 | 103,000 | 0 | 80 | 0 |
| 18 | 93,000 | 0 | 20 | 0 |
| 19 | 199,000 | 0 | 0 | 0 |
| 20 | 95,000 | 0 | 0 | 0 |
| 21 | 332,000 | 0 | 110 | 0 |
| 22 | 398,000 | 0 | 20 | 0 |
| 23 | 336,000 | 0 | 90 | 0 |
| 24 | 141,000 | 0 | 30 | 0 |
| 25 | 91,010 | 0 | 80 | 20 |
| mean | 143,600.4 | 0 | 52.0 | 12.8 |

impossible to use statistical methods. No significant statistical relation between bacterial endotoxin level and Gram-negative bacteria concentration in the air was found, neither before nor after DUWL water disinfection (Tab. 2).

It seems that the absence of the analysed correlation indicates that endotoxin concentration cannot be used as a marker of Gram-negative bacteria concentration in water and air at a dentist's operative site, which has also been shown in other studies [2, 12].

Culture on media still remains the method to evaluate microbiological quality of water and air in a dental surgery. Microbiological tests showed that in all studied units, the concentration of Gram-negative bacteria in the water was high, several times higher than the limit for output water from DUWL for all dental procedures suggested by ADA - bacterial loads ≤ 200 cfu/ml and in the EU – of less than 100 cfu/ml [21].

It is well known that Gram-negative bacteria are only a part of bacterial flora in DUWL water. It is positive, however, that application of a disinfection procedure allowed - in a 2-week period - the reduction of contamination with Gram-negative bacteria to zero level at all workplaces, which confirms the effectiveness of the used disinfectant with respect to Gram-negative bacteria.

**Table 2.** Analysis of the correlation of endotoxin level and Gram-negative bacteria level in water flowing from a dental unit and in air before and after disinfection of water in DUWL.

| | Spearman correlation coefficient | t | p |
|---|---|---|---|
| water before DUWL disinfection | 0.09 | 0.43 | 0.6713 |
| water after DUWL disinfection | – | – | – |
| air before DUWL disinfection | 0.10 | 0.48 | 0.6364 |
| air after DUWL disinfection | -0.11 | -0.55 | 0.5870 |

Nevertheless, further research is required to test the effect of disinfection on other kinds of bacteria and microorganisms. Despite the fact that after disinfection Gram-negative bacteria were not found in the water, endotoxin was present in the water at all workplaces, although its level was significantly reduced. The water free of Gram-negative bacteria after disinfection is still harmful, as it contaminates air with bacterial endotoxins. DUWL water disinfection improves the microbiological quality of the water, reducing its infectivity, but the risk factor in the form of bacterial endotoxin remains present.

It should be noted that the use of the device applied in this study for air quality testing, with strips containing appropriate media, considerably simplifies the procedure of air sampling and culture. Sampling air in the space between dentist and patient during treatment with the use of this apparatus, allows the assessment of actual microbiological contamination of a dentist's breathing space. In the case of the dentist's workplaces tested in this study, the amount of Gram-negative bacteria in the air was significantly lower, especially after DUWL water disinfection, in comparison to the occupational hazard threshold value for Gram-negative bacteria in air according to Malmros *et al.*, which is $1.0 \times 10^3$ cfu/m$^3$ [8].

According to the list of standards and proposed normative (referential) values for bacterial endotoxins present in the air, compiled by Górny on the basis of literature, these values range from $1.0 \times 10^{-1}$ µg/m$^3$–$5.0 \times 10^{-3}$ µg/m$^3$ [3].

The proposal for residential limit value (RLV) for dwellings and communal premises for bacterial endotoxin concentration is 5 ng/m$^3$ (50 EU/m$^3$). The proposed value for occupational exposure limit (OEL) for industrial settings contaminated with organic dust is 200 ng/m$^3$ (2000 EU/m$^3$) [4]. In this study, in several samples of the air before disinfection, endotoxin was not found, and in the great majority of samples where it was found, the level was 12.5–625.0 EU/m$^3$ with the mean value 218.0 EU/m$^3$; also after DUWL disinfection, in several samples, endotoxin was not found, and in the majority of samples where it was present its level was 3.125–625.0 EU/m$^3$, with the mean value 198.8 EU/m$^3$.

There are no internationally recognized criteria for assessing exposure to biological factors in air, among others - at the dentist's workplace. Clinical activity,

because it produces bacterial aerosols, has a negative influence on microbiological quality of air, and with every treated patient, the air pollution changes depending on different factors [7].

In the study of Puttaiah and Cederberg [12] mean endotoxin level in dental unit effluent water was 80.7 EU/ml. In the study by Putnins *et al.* [11], the mean LPS levels in water samples collected from high-speed lines and air/water lines was 480 EU/ml and 1,008 EU/ml, respectively, and in Fulford *et al.* study [2] - free endotoxin ranged from 25–600 EU/ml. In this study, mean endotoxin level in water before disinfection was 6,200,000 EU/ml and after disinfection - 3,295.0 EU/ml; it showed a very poor micorbiological quality of DUWL water.

## CONCLUSIONS

The performed analysis suggests that bacterial endotoxin concentration is not indicative of Gram-negative bacteria contamination. Thus, bacterial endotoxin determination is not recommended as a method of monitoring DUWL water microbiological quality and dental aerosols.

The DUWL water after disinfection, free of Gram-negative bacteria, is still harmful as it contains bacterial endotoxins.

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