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MICROBIAL QUALITY OF WATER IN DENTAL UNIT RESERVOIRS

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Abstract: Microbial quality of water in a dental unit is of considerable importance since patients and dental staff are regularly exposed to water and aerosol generated by the unit. Water delivered to a dental unit by the so-called independent water system is the water coming from a reservoir which, at the same time, is an initial part of dental unit waterlines (DUWL). Thus, microbiological quality of this water is extremely important for the quality of water flowing from dental handpieces. The aim of the study was to assess microbiologically the water contained in dental unit reservoirs. Water samples were collected aseptically from the water reservoirs of 19 dental units. Results concerning microbial contamination: potable water quality indices, and detection and isolation of *Legionella* species bacteria, were presented. Over a half of the samples did not comply with the norms for potable water. In 63.1% of the cases, the number of colony forming units (cfu/ml) and of coliform organisms significantly exceeded acceptable values. *Enterococcus* was not detected in the samples of examined water. Similarly, no *Legionella* were found in the samples of dental unit reservoirs water. Reservoirs as water supplies and initial segment of DUWL should be subject to protocol to eliminate microbial contamination and routine monitoring to guarantee an appropriate quality of water used in dental treatment.

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Safety of dental treatment requires an appropriate high quality of water used for cooling and flushing high and low-speed handpieces, air/water syringes and scalers. Because of aerosols forming during the work of dental handpieces, microbiologically contaminated water may be a risk factor both for the dental team and patients with decreased immunity [4, 6, 12, 13].

The water supply, in the case of an open system, is municipal water, while in the case of a closed system – a water poured into a reservoir belonging to a dental unit. In this study, the microbial quality of water present in self-contained dental units was assessed to compare its quality to microbiological norms for potable water. In addition, a test to detect and isolate *Legionella* in the water was performed.

MATERIALS AND METHODS

Samples. 19 water samples were collected aseptically from the water reservoirs of self-contained dental unit water systems The water reservoirs deliver water by dental unit waterlines to dental handpieces. Some water

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reservoirs were built in the unit while others were placed outside it. The reservoirs were filled with distilled water. The dental units were located in public dental clinics.

Processing of samples for determination of the total number of bacteria (cfu/ml). To determine the total number of bacteria in 1 ml of water, pour plate method was used. To molten agar medium containing yeast extract (BTL Sp. z o.o., Zakład Enzymów i Peptonów, Łódź, Poland), cooled to the temperature close to solidification point, 1 ml of the studied water was added, mixed and left to set. All samples were incubated for 24 h at 37ºC. After incubation, the numbers of colony forming units (cfu) grown on each plate were counted up to 300.

Processing of samples for the presence of coliform organisms. Water samples of 100 ml were filtered through cellulose filters (pores 0.45 µm, Millipore, USA). Filters were placed on lactose agar TTC with Tergitol 7 medium (BTL Sp. z o.o., Zakład Enzymów i Peptonów, Łódź, Poland). All samples were incubated for 24 h at 37ºC [3]. After the incubation period, the presence or absence of coliform organisms was examined.

Processing of samples for the presence of *Enterococcus* spp. Water samples of 100 ml were filtered through cellulose filters (pores 0.45 µm, Millipore, USA), then placed on Slanetz and Bartley agar medium (BTL Sp. z o.o., Zakład Enzymów i Peptonów, Łódź, Poland). Bacteria were cultured for 24 h at 37ºC. After the incubation period, the presence or absence of *Enterococcus* spp. was examined.

Processing of samples for the presence of *Legionella*. Water samples of 300 ml were filtered through cellulose filters (pores 0.45 µm, Millipore, USA). Filters were washed for 10 min in acid buffer (pH 2.2), then rinsed in Ringer solution (Merck, Germany) and placed on isolation agar medium.

Isolation of *Legionella* strains. The buffered charcoal yeast extract (BCYE) agar medium supplemented with Growth Supplement SR 110 A and the Selective GVPC Supplement SR 152 E (Oxoid, England) was used for isolation of *Legionella*. Inoculated agar plates were incubated for 7 days at 37ºC with a daily check of growth. Colonies of Gram-negative bacteria grown after 4-7 days were isolated and examined for ability to grow on media with and without cysteine. Strains unable to grow on media without cysteine were considered as suspected *Legionella* strains. The isolates were identified to the species and serogroup level with the use of the *Legionella* Latex Kit (Oxoid, England) which, on the basis of microcoagulation with latex particles sensitised with specific rabbit antibodies, enables a separate identification of *Legionella pneumophila* serogroup 1, *Legionella pneumophila* serogroups 2-14, and *Legionella* spp. (a complex group including: *L. longbeache* serogroups 1 and 2, *L. bozemanii* serogroups 1 and 2, *L. dumoffii*, *L. gormanii*, *L. jordanis*, *L. micdadei* and *L. anisa*). Only isolates positively responding to the latex test were considered as strains of *Legionella*.

RESULTS AND DISCUSSION

The results of the study are presented in Table 1. According to Polish sanitary regulations [2], the total number of bacteria in 1 ml of potable or industrial water may not exceed 20 cfu after 24 h incubation of agar plates at 37ºC, and 100 cfu after 72 h incubation at 22ºC. The number of *Enterococcus* in 100 ml of water sample should be 0. The number of *Escherichia coli* and related coliform organisms (including *Enterobacter* spp., *Citrobacter* spp., *Klebsiella* spp.) in 100 ml water sample should also be 0. In 12 out of 19 samples (63.1%), the total number of bacteria exceeded the acceptable level more than 10 times. Only in 3 samples (15.8%), water was microbiologically clean. In the remaining samples (21.1%), 1-3 cfu/ml were found. The American Dental Association recommendation is that water for restorative procedures should contain no more than 200 cfu of heterotrophic, mesophilic bacteria per milliliter in DUWL [1].

The presence of *Enterococcus* spp. was not observed in any of the samples, which conforms with the norm for potable water. Confluent growth of coliform organisms was detected in 12 out of 19 samples (63.1%). In the remaining samples, the growth of these bacteria was not observed. The acceptable level was exceeded in the samples in which confluent growth of coliform organisms occurred (Fig. 1).

Table 1. Levels of microbial contamination of water in the reservoirs of 19 dental units.

No.	Average number of aerobic bacteria (cfu/ml)	*Enterococcus*	*Escherichia coli* and coliform organisms	*Legionella*
1	>300	Not isolated	Confluent growth	Not isolated
2	>300	Not isolated	Confluent growth	Not isolated
3	>300	Not isolated	Confluent growth	Not isolated
4	>300	Not isolated	Confluent growth	Not isolated
5	>300	Not isolated	Confluent growth	Not isolated
6	>300	Not isolated	Confluent growth	Not isolated
7	>300	Not isolated	Confluent growth	Not isolated
8	>300	Not isolated	Confluent growth	Not isolated
9	>300	Not isolated	Confluent growth	Not isolated
10	>300	Not isolated	Confluent growth	Not isolated
11	>300	Not isolated	Confluent growth	Not isolated
12	>300	Not isolated	Confluent growth	Not isolated
13	0	Not isolated	Not isolated	Not isolated
14	0	Not isolated	Not isolated	Not isolated
15	3	Not isolated	Not isolated	Not isolated
16	2	Not isolated	Not isolated	Not isolated
17	2	Not isolated	Not isolated	Not isolated
18	1	Not isolated	Not isolated	Not isolated
19	0	Not isolated	Not isolated	Not isolated

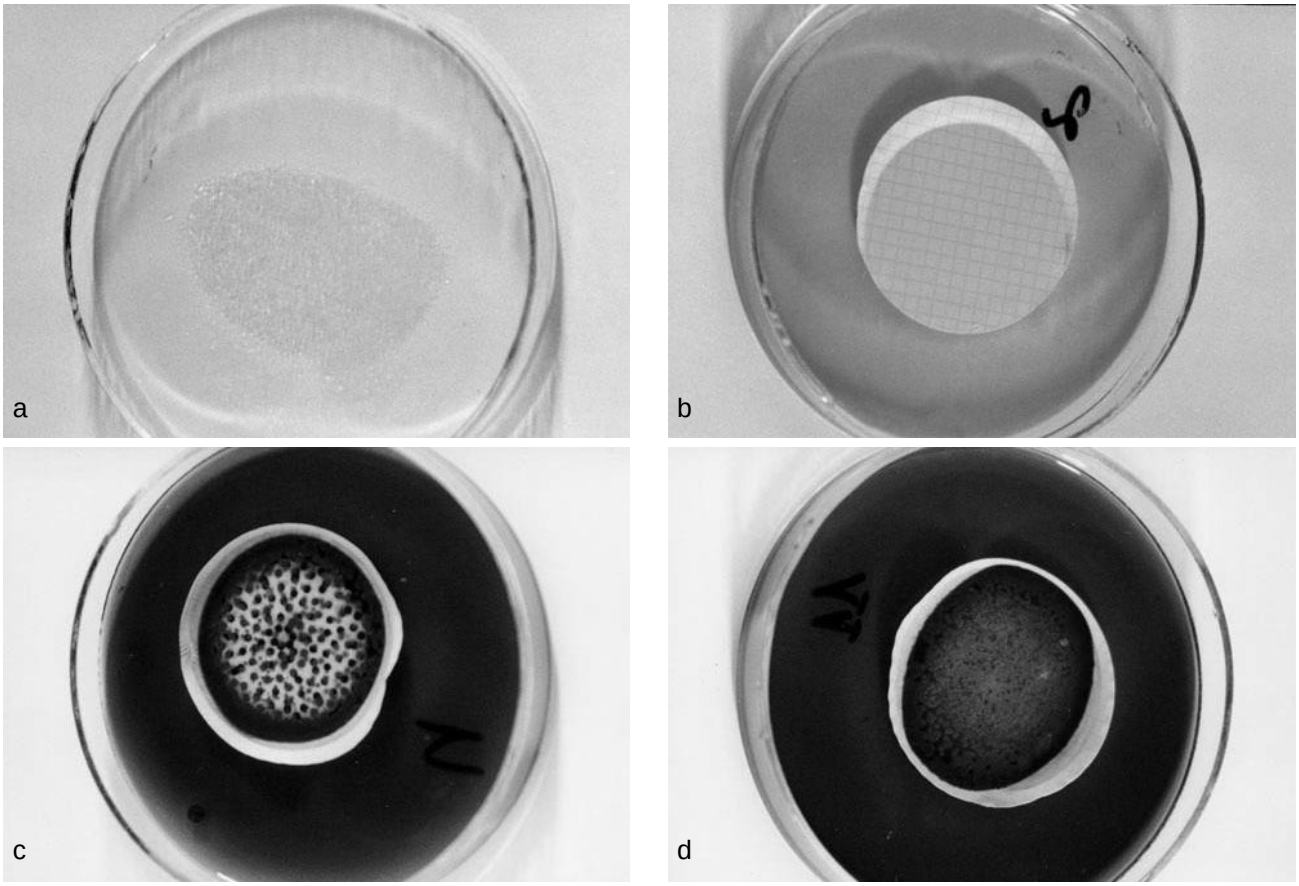

Figure 1. a. No growth of bacteria on agar medium with yeast extract; b. No growth of *Enterococcus* spp. on Slanetz and Bartley agar medium; c, d. Confluent growth of Gram-negative rods on lactose agar TTC with Tergitol 7 medium.

Water reservoirs are the initial part of waterlines in dental units with so-called independent system. Water from these reservoirs is provided to dental handpieces, therefore, its microbiological quality should be at least as high as that of potable water. In over half of the samples the norms were found to be considerably exceeded, which eliminates the water from use in treatment procedures.

The Brazilian quantitative microbiological analysis of samples collected in waterlines of 15 dental units indicated that 13 of 15 reservoirs were contaminated to different extents (15–1,520,000 cfu/ml) and analysis of the data showed that levels of contamination of the samples from handpieces were significantly higher than levels of the initial contamination detected in the reservoirs [8]. Biofilm persisting in thin DUWL tubing can additionally contaminate the water microbiologically and decrease its quality [10, 11]. Apart from this, dental aerosol is contaminated with microflora from the patient's oral cavity, which constitutes an extra risk for the dental team [4].

One of the methods postulated to reduce microbial contamination in DUWL is a water system with a reservoir, independent from municipal water, and the use of distilled water combined with liquids reducing microbial contamination [4, 5]. In this study, the reservoirs of dental units were supplied with distilled water which was, however, not sterile. Units 1-11 had built-in reservoirs placed inside the unit cover. Removing a reservoir to sample water for the study required dismantling the cover by a technician. To pour the distilled water, it was necessary to remove the lid. In the remaining units, the water reservoir is a bottle, placed outside the unit and fixed with the use of a screw-thread.

The results of the present study suggest that the microbial quality of water in the exterior bottles (No. 12–19) was better compared to build-in reservoirs (No. 1–11).

The water reservoir should be washed, disinfected, sterilised, and filled with sterile distilled water with appropriate frequency, and the temperature of water should not exceed 20ºC. In the case of reservoirs placed within the dental unit cover, however application of an appropriate disinfecting procedure is difficult. This could cause a poor microbiological quality of water taken from them.

The main infection route is droplet aerosol. The presence of *Legionella* in the aerosol is particularly dangerous to patients with decreased immunity [9, 13]. Matuszewska *et al.* [7] reported the presence of *Legionella* in 2 samples of water taken from 2 reservoirs with distilled water in Polish dental units. In the water we investigated, *Legionella* was not detected. Many researchers observed *Legionella* in DUWL – both in the water flowing from dental handpieces and in biofilm [13]. It is possible that the ecosystem of biofilm present in thin

DUWL tubes, which is difficult to eliminate, and water stagnation favour multiplication of *Legionella* in further parts of DUWL. Thus, the problem requires further examination.

In conclusion, the results of this preliminary study suggest that water from the dental unit water reservoirs does not comply with microbiological criteria for potable water, yet it does not represent a potential source of *Legionella* infection for dental patients and workers. Water reservoirs as water supplies and initial elements of DUWL should be submitted to a decontamination protocol and to routine microbial monitoring to guarantee an appropriate quality of water used in dental treatment [4, 6]. In addition, dental personnel should be trained in the proper use and maintenance of DUWL.

REFERENCES

1. ADA Council on Scientific Affairs. Dental unit waterlines: Approaching the year 2000. *J Am Dent Assoc* 1999, **130**, 1653-1664.

2. *Rozporządzenie Ministra Zdrowia z dnia 4 września 2000 r. w sprawie warunków, jakim powinna odpowiadać woda do picia i na potrzeby gospodarcze, woda w kąpieliskach, oraz zasad sprawowania kontroli jakości wody przez organy Inspekcji Sanitarnej*. Dziennik Ustaw z 2000 r., Nr 82, poz. 937.

3. ISO 9308-1:1990: *Water quality - Detection and enumeration of coliform organisms, thermotolerant coliform organisms and presumptive Escherichia coli.*

4. Jorgensen MG, Detsch SG, Wolinsky LE: Disinfection and monitoring of dental unit waterlines. *Gen Dent* 1999, **47**, 152-156.

5. Kettering JD, Muňoz-Viveros CA, Stephens JA, Naylor WP, Zhang W: Reducing bacterial counts in dental unit waterlines: distilled water vs. antimicrobial agents. *J Calif Dent Assoc* 2002, **30**, 735-741.

6. Linger JB, Molinari JA, Forbes WC, Farthing CF, William J, Winget WJ: Evaluation of a hydrogen peroxide disinfectant for dental unit waterlines. *J Am Dent Assoc* 2001, **132**, 1287-1291.

7. Matuszewska R, Krogulska B: Występowanie bakterii z rodzaju *Legionella* w wodzie z turbin dentystycznych. *Mag Stomat* 2000, **10**, 10-13.

8. de Souza-Gugelmin MCM, Lima CDT, de Lima SNM, Mian H, Ito IY: Microbial contamination in dental unit waterlines. *Braz Dent J* 2003, **14**, 55-57.

9. Stojek NM, Dutkiewicz J: *Legionella* in sprinkling water as potential occupational risk factor for gardeners. *Ann Agric Environ Med* 2002, **9**, 261-264.

10. Szymańska J: Biofilm and dental unit waterlines. *Ann Agric Environ Med* 2003, **10**, 151-157.

11. Szymańska J: Control methods of microbial water quality in dental unit waterlines. *Ann Agric Environ Med* 2003, **10**, 1-4.

12. Szymańska J: Occupational hazards of dentistry. *Ann Agric Environ Med* 1999, **6**, 13-19

13. Szymańska J: Risk of exposure to *Legionella* in dental practice. *Ann Agric Environ Med* 2004, **11**, 9-12.