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IS WATER IN DENTAL UNITS MICROBIOLOGICALLY SAFE?

CZY WODA W UNITACH DENTYSTYCZNYCH JEST BEZPIECZNA?

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ABSTRACT

Background: Water supplied to the dental units must be of sufficient quality. The article presents the results of the microbiological analysis of cold municipal water which flows into a patient's disposable mouthwash cup, and demineralized water which flows through a waterline into the tool panel of a dental unit from the tank placed in the water group. **Material and Methods:** In order to assess the degree of purity (impurities) of water used in dental units, 2 series of microbiological tests were carried out in 6 dental surgeries from April to June, 2013. The water samples for microbiological testing were collected into sterile microbiological bottles in accordance with the current methodology. The water for the tests was collected from a sterile cup-filling tap (municipal water) and from an air/water syringe (demineralized water). The bacteria were cultured according to the Polish Standards – PN-EN ISO 6222, PN-EN ISO 9308-1, and PN-EN ISO 16266. **Results:** In the tested samples of water numerous psychrophilic bacteria (max 29 100 CFU/ml) and mesophilic bacteria (max 24 700 CFU/ml), including single coliforms, were found. **Conclusions:** The results show that water delivered to a dental unit should be periodically tested bacteriologically and in terms of physical and chemical properties. Water systems of dental units should also be periodically disinfected to eliminate bacteria and biofilm. *Med Pr* 2015;66(6):763–770

Key words: water quality, dental unit, bacteriological features, dental equipment, mesophilic and psychrophilic bacteria, coliforms

STRESZCZENIE

Wstęp: Woda doprowadzana do unitów dentystycznych musi być odpowiedniej jakości. W artykule przedstawiono wyniki mikrobiologicznej analizy zimnej wody wodociągowej, która służy do napełniania jednorazowego kubka dla pacjenta, oraz wody demineralizowanej, która do panelu narzędzi unitu dopływa przewodem ze zbiornika umieszczonego w grupie wodnej. **Materiał i metody:** W celu oceny stopnia czystości (zanieczyszczenia) wody wykorzystywanej w unitach stomatologicznych przeprowadzono w 6 gabinetach stomatologicznych w okresie od kwietnia do czerwca 2013 r. dwie serie badań mikrobiologicznych. Próbkę wody do badań mikrobiologicznych pobierano zgodnie z obowiązującą metodyką, do jałowych butelek bakteriologicznych z wydezynfekowanej wylewki zasilającej kubek (woda wodociągowa) oraz ze strzykawko-dmuchałki (woda demineralizowana). Bakterie hodowano zgodnie z zaleceniami Polskich Norm – PN-EN ISO 6222, PN-EN ISO 9308-1, PN-EN ISO 16266. **Wyniki:** W badanych próbkach wody stwierdzono liczne bakterie psychrofilne (maks. 29 100 CFU/ml) i mezofilne (maks. 24 700 CFU/ml), w tym m.in. pojedyncze bakterie z grupy coli. **Wnioski:** Wyniki wskazują, że woda dostarczana do unitu stomatologicznego powinna być okresowo badana pod względem fizykochemicznym i bakteriologicznym. Systemy wodne unitów należy również okresowo dezynfekować w celu wyeliminowania bakterii i biofilmu. *Med. Pr.* 2015;66(6):763–770

Słowa kluczowe: jakość wody, unity stomatologiczne, cechy bakteriologiczne, urządzenie stomatologiczne, bakterie mezofilne i psychrofilne, bakterie grupy coli

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Received: October 29, 2014, accepted: March 16, 2015

INTRODUCTION

At present, Poland or the European Union (EU) have no explicit requirements for the quality of water which is supplied to dental units. There is a regulation of the Minister of Health of 29 March 2007 [1] with subsequent amendments dated 20 April 2010 [2],

governing the quality of water intended for human consumption. It defines, among others, the requirements for water taken from water equipment and installations as well as water drawn from individual water intakes, regardless of the amount of supplied water, if the water is used for commercial or public activity.

It may, therefore, be assumed that the regulations also determine the quality of water supplied to dental units in dental surgeries from a water system as well as from private wells which may be located, for example, in rural areas or in places where tap water does not reach.

The guidelines for basic microbiological and chemical requirements and additional requirements, such as microbiological, organoleptic, physical and chemical parameters, radiological and chemical, by which water should be characterized may be found in appendices to the Regulation. The Regulation states that water is safe for human health if it is free from pathogenic microorganisms and parasites in the number constituting a potential threat to human health, and it is free from chemicals in quantities endangering health, has no aggressive corrosive properties, and meets the basic and the additional requirements specified in the relevant appendices. Similar requirements for the quality of water intended for human consumption may be found in the Council Directive 98/83/EC of 3 November 1998 [3]. There are no specific European Union rules concerning the quality of water which is supplied to dental units.

However, such guidelines were set out by the American Dental Association (ADA) [4] and by the Centers for Disease Control and Prevention (CDC). In 1995 they adopted a statement on dental unit waterlines. By the year 2000, water delivered to patients during non-surgical dental procedures contained no more than 200 colonies forming units of mesophilic, aerobic, and heterotrophic bacteria per milliliter (CFU/ml). In addition, the CDC recommends that coolant water used in non-surgical procedures meets the Environmental Protection Agency's (EPA) [5] regulatory standards for drinking water, which is less than or equal to 500 colonies forming units of heterotrophic bacteria per milliliter of water (CFU/ml). The Centers for Disease Control and Prevention's recommendations from 2003 report that sterile solutions such as sterile saline or sterile water should be used in the performance of oral surgical procedures. However, according to the recommendations of the CDA (Canadian Dental Association), in order to preserve the purity of water flowing through dental units, all treatments eliminating germs should be used, especially if drinking water is used, and in Canada there can be no more than 500 CFU/ml of heterotrophic bacteria in drinking water [4,6–11].

In dental unit waterlines numerous bacteria naturally occurring in water (autochthonous) and unfamiliar microflora (allochthonous), mainly bacteria

coming from humans, that is indigenous bacteria, mainly non-pathogenic heterotrophic bacteria may be found. These bacteria, however, with immunocompromised patients may be pathogenic. They may also initiate the development of a biofilm in dental unit waterlines. To a lesser extent, filamentous fungi, yeasts, protozoa, and viruses may also be present in water of a dental unit. Among the most common bacteria found in dental unit waterlines are, among others, *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Ralstonia pickettii*, *Arthrobacter woluwensis*, *Microbacterium testaceum*, and bacteria of the genus *Brevibacterium*, *Corynebacterium*, *Enterococcus* and *Micrococcus*. Human – derived bacteria may get into dental unit waterlines most often as a result of secondary contamination, e.g., by aspiration of patient material back into the handpiece or water lines or they can get into dental unit waterlines from water bottles [12–16].

In dental units compressed air and water are used. Water is pumped through small diameter waterlines made, most often, of plastic. Both in Poland and in other countries there are no specific recommendations regulating the hygiene of the air and water supplied to a dental unit. However, the most important problem is the quality of water delivered to dental unit components, such as a turbine, micromotor, syringe, scaler, and a disposable cup. These are items, in which water is present for a longer period of time.

There are numerous impurities, coming from water supply pipes, for example, calcium deposits (Ca) and magnesium (Mg), ferric hydroxide ($\text{Fe}(\text{OH})_3$) formed in the oxidation of dissolved iron, which are present in conduits supplying water to dental units. A biological membrane (biofilm) with a number of microorganisms may also be formed. The vast majority of dental surgeries use 2 types of water: municipal water for filling disposable cups, and for the other devices it is distilled or demineralized water [7,12,17–21].

The use of such a water system in a dental unit protects the equipment against the negative influence of physicochemical factors of water (hardness, iron, color, physical contaminants, etc.). However, using distilled or more frequently, demineralized water, we do not provide microbiological purity of the water which we usually buy in shops. Such water (demineralized or distilled) is not subject to any microbial controls, it is often poorly stored and yet it is used in everyday life by many users, e.g., to refill the batteries, used in irons, coolers, aquariums, etc.

With a view of the above facts, it was decided to examine water in several dental surgeries. During the study, basic parameters of microbiological water were determined. In each of the examined surgeries municipal water which is used to fill a disposable cup, and demineralized water which is supplied to a dental unit water panel, to an air/water syringe, among others, were taken separately.

MATERIAL AND METHODS

Construction of a dental unit

A basic dental unit includes a dental chair, a water group, which is the central unit of a dental unit, spittoon with a draw-off tap located on a water group of a unit, a tool panel, and an arm supporting a light source (Figure 1).

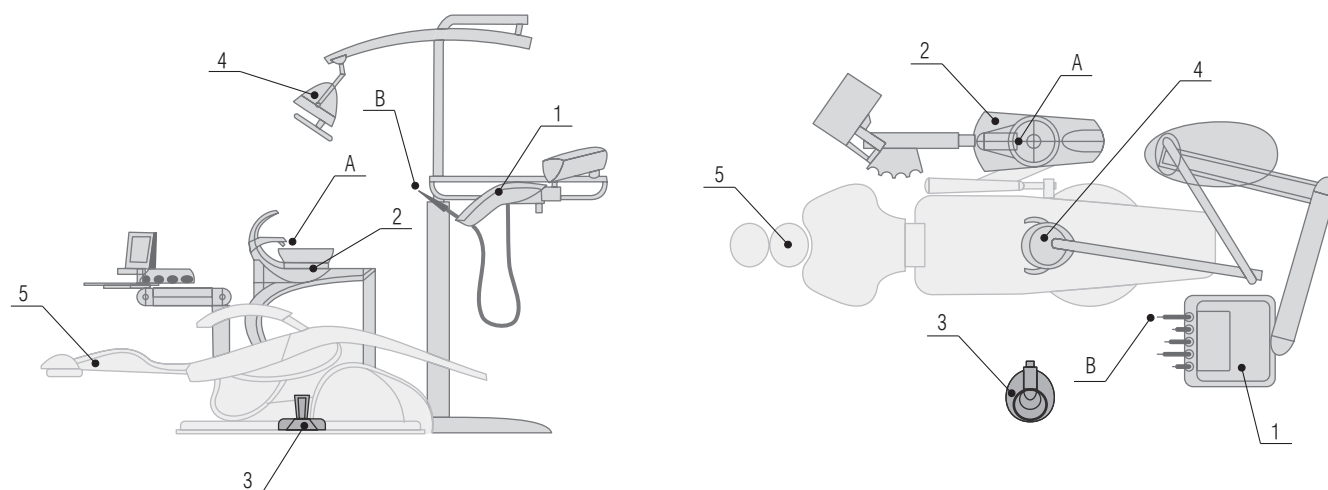
The water group is a control panel of a dental unit, to which not only water is supplied, but it has also got a lot of waterlines and automation system cables and conduits discharging water into drains. On the cover of the water group, or next to it, there is a spittoon set mounted, with water to rinse. The spittoon set is connected to the water supply from the mains by a conduit. The same conduit also supplies a valve which fills a disposable cup with water for rinsing the oral cavity. This part of the water group is supplied only with cold municipal water. Wastewater from the dental unit is discharged through a conduit to the sewer system of the building. A saliva ejector and an air/water suction tube are also connected to the conduit.

Water supplied to a tool panel of a dental unit comes from a demineralized or distilled water tank which is placed in the water group. In old models of dental units, tool panels were supplied with tap water, but based on many years of experience, manufacturers decided to apply more modern solutions that are safer for the equipment. There are 2 dental waterlines coming to the demineralized and distilled water tank (hereinafter a demineralized water tank the term referring to both demineralized and distilled water). The water lines are: the one carrying water to the tool panel and the air-spray cable. The compressed air is to push water from the tank, that is, to maintain the required pressure in the tank, which enables to supply water to handpieces.

Workstations and research methods

In order to assess the degree of purity (impurities) of water used in dental units, 2 series of microbiological tests were carried out from April until June 2013. The tests concerned cold municipal water supplied to a disposable cup (12 water samples) and demineralized water which flows through a conduit to a tool panel of a unit (turbine, scaler, air/water syringe and micromotor) from a tank placed in the water group (including 12 water samples). The sampling points are indicated in the Figure 1.

Water samples for microbiological testing were collected – in accordance with the current methodology – into sterile bottles from a disinfected spout supplying a cup (municipal water) and from the air/water syringe (demineralized water). Six dental surgeries, which agreed to such tests, were chosen. These are dental surgeries that



1 – tool panel / panel narzędzi, 2 – spittoon with draw-off tap / słuwalka z zaworem czerpalnym, 3 – chair foot control / sterownik nożny, 4 – dental light / lampa oświetleniowa, 5 – dental chair / fotel stomatologiczny.
A, B – the sampling points / punkty poboru próbek.

Fig. 1. Construction of a dental unit and sampling points

Ryc. 1. Budowa unitu dentystycznego z zaznaczonymi punktami poboru prób

work during the week, from Monday to Saturday, 8–10 h a day on average. According to the information obtained from the dentists who work in these dental surgeries, the only routine that involves cleaning water lines in dental unit water systems is draining the water before the next patient and disinfection of tips, e.g., an air/water syringe tip. The entire water system for demineralized water (from the bottle to the tips of the panel) is disinfected from time to time (several times a year) with disinfectants used in dentistry (e.g., Steridial Forte, Oxygenal, Alpron, Bilpron), while the very municipal water system which supplies the mouth rinsing cup is not additionally disinfected.

The cup is supplied with municipal water, which meets the requirements for drinking water in places where the tests were taken. The water samples for the tests were collected from the tap that supplies the mouth rinsing cup (located next to the spittoon) and is powered from a municipal water system as well as from the air/water syringe tip which is supplied with demineralized water from the bottle (container) located at the base of the panel. Both tips (the tap and the air/water syringe) prior to collection of water samples were thermally ster-

ilized by flaming (cotton wool soaked with alcohol) and the drained water for about 1 min, after which the water samples were collected in sterile bacteriological bottles. The samples were taken into sterile bottles with sodium thiosulfate, which was used to inactivate the disinfecting agent (chlorine). All the samples were taken on Monday, just before the first patient's visit.

In microbiological testing, the following were determined: the overall number of the microorganisms cultured at $36\pm 2^\circ\text{C}$ for 48 h (mesophilic bacteria in 1 ml of water sample), the number of microorganisms cultured at $22\pm 2^\circ\text{C}$ for 72 h (psychrophilic bacteria in 1 ml of water sample), the number of *Escherichia coli*, total coliforms and *Pseudomonas aeruginosa* in 100 ml of water sample. These bacteria were cultured according to the Polish Standards (PN-EN ISO 6222, PN-EN ISO 9308-1, PN-EN ISO 16266) [22–24].

RESULTS

The Table 1 summarizes the results of microbiological tests of municipal water supplying a mouth rinsing cup,

Table 1. Microbiological tests of municipal water for filling a disposable cup

Tabela 1. Badania mikrobiologiczne wody wodociągowej zasilającej kubek do płukania jamy ustnej

No. of dental surgery Nr gabinetu stomatologicznego	MB [CFU/1 ml]	PB [CFU/1 ml]	CB [CFU/100 ml]	E.c. [CFU/100 ml]	Pa. [CFU/100 ml]
1					
test 1 / próba 1	228	148	0	0	0
test 2 / próba 2	85	139	0	0	0
mean / średnia	157	144	0	0	0
2					
test 1 / próba 1	0	26	0	0	0
test 2 / próba 2	0	24	0	0	0
mean / średnia	0	25	0	0	0
3					
test 1 / próba 1	2	13	0	0	0
test 2 / próba 2	1	15	0	0	0
mean / średnia	1	14	0	0	0
4					
test 1 / próba 1	0	0	0	0	0
test 2 / próba 2	1	82	0	0	0
mean / średnia	1	41	0	0	0
5					
test 1 / próba 1	0	645	0	0	0
test 2 / próba 2	0	162	1	0	0
mean / średnia	0	404	1	0	0
6					
test 1 / próba 1	0	0	0	0	0
test 2 / próba 2	1	15	0	0	0
mean / średnia	1	8	0	0	0

MB – mesophilic bacteria / bakterie mezofilne, PB – psychrophilic bacteria / bakterie psychrofilne, CB – total coliform bacteria / bakterie grupy coli, E.c. – *Escherichia coli*, Pa. – *Pseudomonas aeruginosa*.

Table 2. Microbiological testing of demineralized water from the air/water syringe water line
Tabela 2. Badania mikrobiologiczne wody zdemineralizowanej z przewodu

No. of dental surgery Nr gabinetu stomatologicznego	MB [CFU/1 ml]	PB [CFU/1 ml]	CB [CFU/100 ml]	E.c. [CFU/100 ml]	P.a. [CFU/100 ml]
1					
test 1 / próba 1	108	3 100	0	0	0
test 2 / próba 2	0	3	0	0	0
mean / średnia	54	1 552	0	0	0
2					
test 1 / próba 1	304	4 800	0	0	0
test 2 / próba 2	27	153	0	0	0
mean / średnia	166	2 477	0	0	0
3					
test 1 / próba 1	332	3 500	0	0	0
test 2 / próba 2	2	1 400	0	0	0
mean / średnia	167	2 450	0	0	0
4					
test 1 / próba 1	226	1 780	0	0	0
test 2 / próba 2	46	295	0	0	0
mean / średnia	136	1 038	0	0	0
5					
test 1 / próba 1	6 850	24 400	0	0	0
test 2 / próba 2	24 700	16 400	0	0	0
mean / średnia	15 775	20 400	0	0	0
6					
test 1 / próba 1	16 300	29 100	0	0	0
test 2 / próba 2	7 150	10 400	1	0	0
mean / średnia	11 725	19 750	1	0	0

Abbreviations as in Table 1 / Objasnienia jak w tabeli 1.

and in the Table 2 there are the results of water from the tool panel of the dental unit (air/water syringe), which comes from the demineralized water tank.

Escherichia coli and *Pseudomonas aeruginosa* were not found in none of the tested water samples. Isolated total coliform bacteria were found in one sample of municipal water supplying a mouth rinsing cup (dental surgery No. 5) and in one sample of demineralized water supplied to an air/water syringe (dental surgery No. 6). Much worse situation occurred in the case of mesophilic and psychrophilic bacteria, their much higher concentrations occurred in demineralized water taken from the air/water syringe waterline. In the water, the number of mesophilic bacteria ranged 0–24 700 CFU/ml (at the mean values ($M = 1-15\ 775$ CFU/ml), and the psychrophilic ones 0–29 100 CFU/ml ($M = 41-20\ 400$ CFU/ml). Whereas, in the municipal water flowing into the cup, the number of mesophilic bacteria in 5 dental surgeries was very small, ranging 0–2 CFU/ml, and in one dental surgery it strongly deviated from the other samples and reached the number of 157 CFU/ml (range: 85–228 CFU/ml). Psychrophilic bacteria con-

centrations were significantly higher and ranged 0–645 CFU/ml ($M = 8-404$ CFU/ml).

Discussion of the results

According to the rules concerning the quality of water intended for human consumption listed in the Regulation of the Minister of Health [1,2] in 100 ml of water *Escherichia coli* and total coliform bacteria, among others, must not be present. The amendment to the regulation of 2010 stated that the total number of microorganisms at $22\pm 2^\circ\text{C}$ after 72 h should remain with no abnormal changes (in 2007 identified 100 CFU/ml), and the determination of the number of microorganisms at $36\pm 2^\circ\text{C}$ after 48 h (in 2007 identified 50 CFU/ml) was abolished [1,2]. In addition, the regulation states that other types of water intended for human consumption should not contain *Pseudomonas aeruginosa* bacteria in 100 ml of water sample.

Therefore, comparing the results of microbiological tests to the applicable rules, it may be stated that all water samples met the requirements of water for human consumption in terms of the presence of *Escherichia coli*

and *Pseudomonas aeruginosa*. In 2 water samples (one supplying a mouth rinsing cup, the other supplying an air/water syringe), which came from 2 different dental surgeries, one of total coliform bacteria in each sample was found, which may disqualify water as safe. Choosing the maximum value of 100 CFU/ml of bacteria at $22\pm 2^\circ\text{C}$ after 72 h (psychrophilic bacteria) as an assessment criterion, it was found that 33.3% of the samples of municipal water and 91.7% of the demineralized water samples showed non-compliance with the acceptable parameters of drinking water. However, as far as mesophilic bacteria are concerned (at $36\pm 2^\circ\text{C}$ after 48 h), with the assumption of 50 CFU/ml as the prescribed value, it was found that 2 samples (16.7%) of municipal water and 8 samples (66.7%) of demineralized water did not meet the criteria of safe water defined in the Regulation of the Minister of Health [1,2].

Summing up the results of microbiological tests, it may be concluded that 8 samples of municipal water flowing into the cup (per 12 samples tested) in 4 dental surgeries (66,7%) met the criteria for water intended for human consumption. The remaining water samples were exceeding the permissible concentrations of microorganisms. In contrast, the demineralized water flowing to the panel, in only 1 period of research (from 12 samples from 2 series) was consistent with the guidelines of the Minister of Health [1,2] on water intended for human consumption.

DISCUSSION

Water supplied to dental units must be of sufficient quality. Microbiologically contaminated water in dental units may infect patients and dental health-care workers. The water system in a dental unit does not favor maintenance of appropriate water quality parameters. On the inner surface of thin tubes supplying water to a cup and to the tool panel of a dental unit, biofilm may develop, which is a reservoir for bacteria, which consequently affects the continuous contamination of the water flowing through the water system of a dental unit. In addition, the biofilm is resistant to chemical disinfection [8,12,15,25]. In the tested samples of municipal water and the water flowing into the syringe, it was found that the number of psychrophilic and mesophilic bacteria, including single total coliform bacteria occurred.

The number of bacteria, which is introduced into a dental unit with tap water, should not be dangerous for people's health because the water usually meets the

standards set for drinking water. The analysis of samples of tap water indicates that in most cases it meets the recommendations of the EPA and the stringent requirements of the European Union. It is possible that water supplied to dental units may contain a small number of microorganisms, which could in time lead to the development of a biofilm that is difficult to destroy [12,26]. The Polish regulations concerning the quality of water intended for human consumption are rigorous and reflect the standards of the EU. Monitoring and control tests of drinking water in the places where the studies were conducted, show that municipal water meets all the criteria for drinking water. Additional studies of distilled and demineralized water supplying the water panel system showed the presence of 0–2 CFU/ml mesophilic or psychrophilic bacteria. Hence, the possible contamination of water samples that were collected in the dental surgeries must be connected with secondary contamination of the inner installation of a dental unit.

The presence of numerous bacteria in a biofilm is also indicated by Walker and co-workers' studies [17]. They cultured from the biofilm present in a syringe waterline as follows: *Oral streptococci*, *Oral anaerobes*, *Pseudomonas aeruginosa*, *Legionella pneumophila* and bacteria of the genus *Mycobacterium*. They also indicate that the water samples from air/water syringes and turbines of the units contained traces of blood, which can be a source of contamination. Numerous studies indicate that when a unit is operating, the flow of water through the unit causes the detachment of biofilm fragments with individual bacterial cells, which may cause the development of a biofilm in another part of the installation, and they may be transferred directly into the patient's mouth or patients and dental health-care workers may be exposed to aerosols generated by dental units. In addition, factors contributing to the development of a biofilm are: elevated temperature, water stagnation during periods when patients are not treated and the presence of inorganic compounds in water, which are often found in significant concentrations mainly in municipal water [12,18,27].

Literature provides numerous examples of water contamination in dental units. In an Italian hospital in Palermo units were connected to municipal water, which was used for rinsing the mouth during dental examination. In these samples *Legionella pneumophila* and *Pseudomonas aeruginosa* were among others detected. It may be assumed that they came from the installation supplying a cup with water [6]. However, according to the research done by Bzdęga et al. [28]

Legionella pneumophila and faecal *Escherichia coli* were found in water flowing into the cup, while the number of psychrophilic and mesophilic bacteria was high and exceeded the limits for drinking water. Similarly, water from the tool panel (from the turbine, micromotor, syringes, and the scaler) was highly contaminated with mesophilic and psychrophilic bacteria, and it finds the presence of faecal *Escherichia coli*. Contaminated water or biofilm developing in the waterlines could have been the direct source of those bacteria.

Numerous units, which are equipped with bottles supplying water to the installation of the dental units, may be contaminated with bacteria that are located directly in the added water, or are secondarily contaminated, such as during careless handling when filling bottles. To avoid such contamination, the bottles should be kept clean and disinfected, and preferably should be sterilized in an autoclave.

CONCLUSIONS

Studies have shown that in all the analyzed dental surgeries municipal water was used to fill a cup and demineralized water – to supply the tool panel of a dental unit. Unfortunately, this water (demineralized) does not guarantee microbiological purity, and even single bacterium occurring in water may be a source of formation of a biofilm in a dental unit installation. Another source of water contamination may be stagnant water in waterlines of a dental unit, and the lack of water draining from the tool panel – both between patients' visits and after completing a day's work. This is confirmed by some water samples, in which very high concentrations of psychrophilic bacteria (29 100 CFU/ml) and mesophilic ones (24 700 CFU/ml) were found. Psychrophilic bacteria which are present in water are not pathogenic. Mesophilic bacteria are microflora associated with humans and warm-blooded animals (mammals, birds). In addition to natural symbiotic microflora, pathogenic forms may occur among mesophilic bacteria.

On the basis of the analyses, in the case of the dental surgeries where water was collected, the procedures of correct dealing with water and dental unit waterlines were implemented.

REFERENCES

- [Regulation of the Minister of Health of 20 April 2010 on concerning the quality of water intended for human consumption. J Laws 2007, No. 61, item 417]. Polish.
- [Regulation of the Minister of Health of 20 April 2010 amending regulation on the quality of water intended for human consumption. J Laws 2010, No. 72, item 466]. Polish.
- Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption. Off J Eur Communities L 330/32, 3.11.1998 (Nov 3, 1998) [cited 2014 Aug 29]. Available from: <http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:31998L0083&from=PL>.
- American Dental Association (ADA). Statement on Dental Unit Waterlines [cited 2014 Aug 29]. Available from: <http://www.ada.org/1856.aspx>.
- Epa.gov [Internet]. United States Environmental Protection Agency [cited 2014 Aug 29]. Available from: <http://www.epa.gov>.
- Aprea L, Cannova L, Firenze A, Bivona MS, Amodio E, Romano N. Can technical, functional and structural characteristics of dental units predict *Legionella pneumophila* and *Pseudomonas aeruginosa* contamination? J Oral Sci. 2010;52(4):641–6, <http://dx.doi.org/10.2334/josnusd.52.641>.
- Barbeau J. Waterborne biofilms and dentistry: The changing face of infection control. J Can Dent Assoc. 2000;66(10):539–41.
- Bednarsh HS, Eklund KJ, Mills S. Dental unit waterlines: Check your dental unit water IQ. Am Dent Hyg Assoc. 1997 [cited 2014 Sep 25];10(9). Available from: http://www.osap.org/?page=Issues_DUWL_7.
- Szymańska J. Risk of exposure to *Legionella* in dental practice. Ann Agric Environ Med. 2004;11(1):9–12.
- Cda-adc.ca [Internet]. Canadian Dental Association [cited 2014 Oct 2]. Available from: <http://www.cda-adc.ca>.
- Cdc.gov [Internet]. Centers for Disease Control and Prevention [cited 2014 Oct 2]. Available from: <http://www.cdc.gov>.
- O'Donnell MJ, Boyle MA, Russell RJ, Coleman DC. Management of dental unit waterline biofilms in the 21st century. Future Microbiol. 2011;6(10):1209–26, <http://dx.doi.org/10.2217/fmb.11.104>.
- Szymańska J, Sitkowska J. Evaluation of activities aimed at preventing microbiological risks in dental practice. Med Pr. 2013;64(1):11–7, <http://dx.doi.org/10.13075/mp.5893/2013/0002>.
- Liaqat I, Sabri AN. Biofilm, dental unit water line and its control. Afr J Clin Exp Microbiol. 2011;12(1):15–21.
- Smith AJ, McHugh S, Aitken I, Hood J. Evaluation of the efficacy of Alpron disinfectant for dental unit water lines. Br Dent J. 2002;193(10):593–6, <http://dx.doi.org/10.1038/sj.bdj.4801635a>.

16. Coleman DC, O'Donnell MJ, Shore AC, Russell RJ. Bio-film problems in dental unit water systems and its practical control. *J Appl Microbiol.* 2009;106(5):1424–37, <http://dx.doi.org/10.1111/j.1365-2672.2008.04100.x>.
17. Walker JT, Bradshaw DJ, Finney M, Fulford MR, Frandsen E, Østergaard E, et al. Microbiological evaluation of dental unit water systems in general dental practice in Europe. *Eur J Oral Sci.* 2004;112(5):412–8, <http://dx.doi.org/10.1111/j.1600-0722.2004.00151.x>.
18. Kolwzan B. [Analysis of biofilms – Their formation and functioning]. *Ochr Środ.* 2011;33(4):3–14. Polish.
19. Singh R, Stine OC, Smith DL, Spitznagel JK Jr, Labib ME, Williams HN. Microbial diversity of biofilms in dental unit water systems. *Appl Environ Microbiol.* 2003;69(6):3412–20, <http://dx.doi.org/10.1128/AEM.69.6.3412-3420.2003>.
20. Szymańska J, Sitkowska J. Bacterial hazards in a dental office: An update review. *Afr J Microbiol Res.* 2012;6(8):1642–50, <http://dx.doi.org/10.5897/AJMR11.1002>.
21. Szymańska J, Sitkowska J. Bacterial contamination of dental unit waterlines. *Environ Monit Assess.* 2013;185(5):3603–11, <http://dx.doi.org/10.1007/s10661-012-2812-9>.
22. PN-EN ISO 16266:2009P. [Water quality – Detection and enumeration of *Pseudomonas aeruginosa* – Method by membrane filtration]. Warszawa: Polish Committee for Standardization; 2009. Polish.
23. PN-EN ISO 6222:2002. [Water quality. Determination of living organisms. Specify the total number of colon nutrient agar spread plate method or plunge]. Warszawa: Polish Committee for Standardization; 2002. Polish.
24. PN-EN ISO 9308-1:2004/AC:2009. [Water quality – Detection and enumeration of *Escherichia coli* and total coliform bacteria – Part 1: Membrane filtration method]. Warszawa: Polish Committee for Standardization; 2009. Polish.
25. Pareek S, Nagaraj A, Sharma P, Atri M, Walia S, Naidu S, et al. Disinfection of dental nit water line using aloe vera: *In vitro* study. *Int J Dent.* 2013;2013:618962, <http://dx.doi.org/10.1155/2013/618962>.
26. Schel AJ, Marsh PD, Bradshaw DJ, Finney M, Fulford MR, Frandsen E, et al. Comparison of the efficacies of disinfectants to control microbial contamination in dental unit water systems in general dental practices across the European Union. *Appl Environ Microbiol.* 2006;72(2):1380–7, <http://dx.doi.org/10.1128/AEM.72.2.1380-1387.2006>.
27. Stypułkowska-Misiurewicz H, Pancer K. [Legionellosis – A risk of the new infection in Poland]. *Przegl Epidemiol.* 2002;4:567–76. Polish.
28. Bzdęga W, Trykowski J, Bzdęga J. [The disinfection of water and compressed air systems of a dental unit]. *J Med Sci.* 2007;5:436–9. Polish.