

JOLANTA SITKOWSKA<sup>1</sup>, JOLANTA SZYMAŃSKA<sup>2</sup>, GRAŻYNA CHOLEWA<sup>1</sup>

## Badania skażenia mikrobiologicznego wody z unitów dentystycznych w wiejskich gabinetach stomatologicznych

### Streszczenie

**Wstęp.** Z wyników badań naukowych wynika, że woda używana w gabinetach stomatologicznych, która pochodzi z unitu dentystycznego jest skażona mikroorganizmami – bakteriami, grzybami i pierwotniakami, może stanowić potencjalne źródło zakażenia dla pacjentów i personelu stomatologicznego.

**Cel.** Celem badań było określenie stężenia i składu gatunkowego mezofilnych bakterii występujących w wodzie unitów dentystycznych w wiejskich gabinetach stomatologicznych, w aspekcie potencjalnego narażenia zdrowia pacjentów i personelu stomatologicznego.

**Materiał i metody.** Materiał do badań stanowiła woda pobrana z 20. zbiorników unitów dentystycznych, które znajdowały się w wiejskich gabinetach stomatologicznych na terenie województwa lubelskiego. Do hodowli mezofilnych bakterii Gram-dodatnich i Gram-ujemnych użyto pożywki wzbogaconej 5% krwią baranią. Do izolacji i identyfikacji pałeczek Gram-ujemnych zastosowano agar wybiórczo-różnicujący z eozyną i błękitem metylenowym. Identyfikację wyizolowanych mikroorganizmów przeprowadzono testami API 20E i API 20NE oraz systemem BIOLOG.

**Wyniki.** We wszystkich próbach wody stwierdzono obecność mezofilnych bakterii Gram-dodatnich i Gram-ujemnych. Najliczniejszą grupę stanowiły pałeczki Gram-ujemne z gatunków *Ralstonia pickettii* i *Sphingomonas paucimobilis* z frekwencją po 35%. W grupie bakterii Gram-dodatnich najczęściej izolowane były pałeczki Gram-dodatnie z gatunku *Cellulomonas cellasea* i rodzaju *Staphylococcus* spp. Całkowite średnie stężenie wszystkich mikroorganizmów wyizolowanych z badanej wody wyniosło 84 450,75 cfu/ml.

**Wnioski.** Powszechny charakter skażenia bakteriologicznego i obecność mikroorganizmów oportunistycznych w wodzie używanej w leczeniu stomatologicznym może stanowić potencjalne zagrożenie dla zdrowia pacjentów i zespołu stomatologicznego. Konieczne wydaje się monitorowanie skażenia mikrobiologicznego wody w zbiornikach unitów dentystycznych - stosowanie zalecanych metod i dostępnych środków chemicznych w celu jej dekontaminacji.

**Słowa kluczowe:** unity dentystyczne, bakterie, skażenie wody, zagrożenie zdrowotne.

## Studies of microbiological contamination of water from dental units in rural dental practices

### Abstract

**Introduction.** Scientific studies show that the water used in dental practices, which comes from the dental unit is contaminated with microorganisms – bacteria, fungi and protozoa – and may be the potential source of infections in patients and dental staff.

**Aim.** The objective of the study was the determination of the concentration and species composition of mesophilic bacteria present in the water from dental units in rural dental practices, from the aspect of potential health risk for patients and dental staff.

**Material and methods.** The material for the study was the water sampled from 20 dental unit reservoirs placed in rural dental practices in the Lublin Region. Mesophilic bacteria Gram-positive and Gram-negative were cultured on plating medium enriched with 5% sheep blood. Gram-negative rods were isolated and identified with the use of the selective-differential Eosin-Methylene Blue Agar. The identification of the isolated microorganisms was performed by means of the API 20E and API 20NE test technique, and the BIOLOG system.

**Results.** In all water samples the presence of mesophilic Gram-positive and Gram-negative bacteria was observed. The most abundant group were Gram-negative rods of the species *Ralstonia pickettii* and *Sphingomonas paucimobilis*, with the frequency of 35% each. In the group of Gram-positive bacteria, Gram-negative rods of the species *Cellulomonas cellasea* and genus *Staphylococcus* spp. were most frequently isolated. The total mean concentration of total microorganisms isolated from the water examined was 84,450.75 cfu/ml.

**Conclusions.** The common character of bacteriological contamination and the presence of opportunistic microorganisms in the water used in dental treatment may constitute a potential risk for the health of patients and dental team. It seems necessary to monitor the microbiological contamination of water in dental units – the application of the recommended methods and chemical agents available for the purpose of decontamination.

**Key words:** dental units, bacteria, water contamination, health hazard.

<sup>1</sup>Institute of Agricultural Medicine, Lublin, Poland

<sup>2</sup>Medical University of Lublin, Poland

## INTRODUCTION

Based on the review of literature available, the water used in dental practices, which is present in a dental unit reservoir and waterlines, is microbiologically contaminated (bacteria, fungi, and protozoa), and may constitute a potential source of infections for patients and the dental team [1-8]. The main cause of the microbiological contamination of water in dental units is the biofilm present on the inside walls of water supply tubes, which is formed by microorganisms, their metabolites and extra-cellular polymers, as well as microbes penetrating the inside of the unit from a patient's oral cavity and the environment of the practice [9].

During the operation of handpieces there occurs the emission of microbiologically contaminated aerosol and splatter of liquids. This emission is composed of water from the dental unit, secretion from nasal and oral cavities, elements of blood, fragments of teeth tissues and fillings [10]. Aerosol is present not only at the surgical site and the space between the patient and the dentist, it is also dispersed to nearly the same extent in other parts of the practice, reaching a distance of even up to 2 meters. The aerosol is inhaled both by the patient receiving treatment and the dental staff of the practice – the dentist and assistants.

Health hazards due to microbiological contamination of water in dental units are undoubtedly a serious public health problem, particularly in the case of patients with decreased immunity – especially the elderly, patients who had undergone transplants, got infected with HIV, are chronically ill or receiving immunosuppressive treatment [11-17], as well as the dental staff, for whom water constitutes one of the sources of hazardous biological agents occurring at the workplace [2, 10].

## AIM

The objective of the study was determination of the concentration and species composition of mesophilic bacteria occurring in the water of dental units applied in the treatment performed in rural dental practices from the aspect of potential health risk for patients and dental staff.

## MATERIAL AND METHODS

The material analyzed was the water sampled from 20 dental units located in selected rural dental practices in the area of the Lublin Region. In order to obtain the same conditions of taking water samples and eliminate the possibility of its occasional microbiological contamination, water was collected in early spring, during the heating season (similar values of room temperature inside practices), prior to patients' visits, into sterile, tightly closed tubes. Each water sampling from a dental unit required the obtaining of consent from the supervisor of the health care facility or the owner of the dental practice. Scientific character of the studies conducted obliged keeping total anonymity of the data from the sampling site, which was associated with the necessity of double marking of the tubes containing the collected material.

In order to assess the total number and species composition of mesophilic aerobic or anaerobic Gram-negative and Gram-positive bacteria, conventional methods used in bacteriology were applied. For the cultivation of mesophilic bacteria, and Gram-negative and Gram-positive microorganisms with increased nutritional requirements, the nutrient agar was used enriched with 5% defibrinated sheep blood. Gram-negative rods were isolated and identified with the use of the selective-differential Eosin-Methylene Blue Agar. The water samples examined were cultured simultaneously on both media, by the method of double dilution on plates with the use of surface culturing. After 24-hour incubation at the temperature of 35-37°C, the samples were subjected to room temperature (22°C) for 3 days, and refrigerator temperature (4°C) for 3 days. After the termination of incubation, the preliminary macro- and microscopic assessment was carried out of the bacterial colonies which grew on the media, which were subsequently identified with respect to species or genus by means of biochemical micro-tests. Gram-negative rods isolated from the EMB agar were determined by the API 20E and API 20NE test techniques (BioMérieux, Marcy l'Etoile, France), whereas Gram-positive bacteria from blood agar – by the test GP2 MicroPlate™ (BIOLOG, Inc., Hayward, USA). All the determinations were performed according to the procedures indicated by manufacturers. The total concentration of individual bacterial species and genera in 1 ml of the water examined was expressed as colony forming units - cfu.

## RESULTS AND DISCUSSION

It was as late as in the last decade that special attention was paid to the problem of the microbiological quality of water in waterlines of dental units. The data from literature show that in Polish conditions the mean concentration of microorganisms in the water from dental units reservoirs remained at the level of 317,000 cfu/ml [18]. In own studies, the mean total concentration of total microorganisms isolated from dental units was 84,450.75 cfu/ml, with a minimum of 735 cfu/ml and a maximum of 548,000 cfu/ml; thus, it was lower. In all 20 water samples the presence of mesophilic bacteria was found, with Gram-negative rods constituting the most abundant group among Gram-positive and Gram-negative; their mean concentration from blood agar was 80,900.79 cfu/ml, the lowest – 665 cfu/ml, and the highest – 548,000 cfu/ml. Gram-negative rods of the species *Ralstonia pickettii* and *Sphingomonas paucimobilis* were most frequently isolated at individual sites, and the frequency of their occurrence was 35% each (Table 1,2). The following Gram-negative rods were isolated from single water samples: *Acidovorax delafieldii*, *Brevundimonas vesicularis* and *Burkholderia cepacia* (Table 1). The species *Acidovorax delafieldii* reached the highest concentration in the group of Gram-negative rods – 288,000 cfu/ml (Table 1), which constituted 39.09% of the cfu value for individual species of bacteria cultured on the EMB agar. The species *Ralstonia pickettii*, *Ralstonia paucula* and *Sphingomonas paucimobilis* constituted 21.41%, 18.25% and 13.66% of isolated Gram-negative rods, respectively (Table 3). In earlier studies

TABLE 1. Compilation of results obtained from cultures of dental units water on EMB agar.

Sample	Total cfu/ml	<i>Acidovorax delafeldii</i>	<i>Burkholderia cepacia</i>	<i>Brevundimonas vesicularis</i>	<i>Pseudomonas chlororapae</i>	<i>Pseudomonas fluorescens</i>	<i>Pseudomonas stutzeri</i>	<i>Ralstonia pauca</i>	<i>Ralstonia pickettii</i>	<i>Sphingomonas paucimobilis</i>
1	25,000								25,000	
2	5,000								5,000	
3	75,000									
4	1,800									75,000
5	15,880				10				20	1,800
6	376,500	288,000							88,500	15,850
7										
8	405									
9	6,525			25						405
10	7,190					6,860	330			6,500
11	38,000				38,000					
12	75						75			
13	1,950							1,950		
14	1,050		750							
15	132,500							132,500		300
16	9,800								9,800	
17	20,550								20,550	
18	8,850								8,850	
19	815									
20	9,900					9,900				815

TABLE 2. Numerical and percentage data of water samples from dental units, where individual species/genera of bacteria were isolated (EMB agar).

	Total cfu/ml	<i>Acidovorax delafeldii</i>	<i>Brevundimonas vesicularis</i>	<i>Burkholderia cepacia</i>	<i>Pseudomonas chlororapae</i>	<i>Pseudomonas fluorescens</i>	<i>Pseudomonas stutzeri</i>	<i>Ralstonia pickettii</i>	<i>Ralstonia pauca</i>	<i>Sphingomonas paucimobilis</i>
Number	19	1	1	1	2	2	2	7	2	7
%	95	5	5	5	10	10	10	35	10	35

TABLE 3. Percentage cfu/ml for individual species of bacteria isolated from dental units water on EMB agar.

	Total cfu/ml	<i>Acidovorax delafeldii</i>	<i>Brevundimonas vesicularis</i>	<i>Burkholderia cepacia</i>	<i>Pseudomonas chlororapae</i>	<i>Pseudomonas fluorescens</i>	<i>Pseudomonas stutzeri</i>	<i>Ralstonia pauca</i>	<i>Ralstonia pickettii</i>	<i>Sphingomonas paucimobilis</i>
total	736,790	288,000	25	750	38,010	16,760	405	134,450	157,720	10,0670
%	100.00	39.09	0	0.10	5.16	2.27	0.05	18.25	21.41	13.66

of dental units water conducted by other researchers, Gram-negative rods of the species *Sphingomonas* spp. were isolated [19, 20], the presence of bacteria of the species *Ralstonia pickettii* and *Sphingomonas paucimobilis* was found [10, 21], and also the prevalence of occurrence of Gram-negative bacteria of the genus *Pseudomonas* spp. [22].

The most abundant group of microorganisms isolated from the water of dental units were Gram-negative rods, among which the bacteria of the genus *Ralstonia pickettii* obtained the highest mean concentration value.

The presence of Gram-positive granulomas of the species *Staphylococcus* spp. in the water of dental units had been observed in earlier studies [10, 22]. In own studies, Gram-

TABLE 4. Numbers and percentage of water samples from dental units from which individual species/genera of bacteria were isolated (blood agar).

	Total cfu/ml	Gram-negative rods	<i>Actinomyces</i> spp.	<i>Bacillus</i> spp.	<i>Cellulomonas cellasea</i>	<i>Corynebacterium</i> spp.	<i>Micrococcus</i> spp.	<i>Rhodococcus coprophilus</i>	<i>Rhodococcus</i> spp.	<i>Staphylococcus lentus</i>	<i>Streptococcus mitis</i>	<i>Staphylococcus sciuri</i> ss rodentium	<i>Staphylococcus</i> spp.	<i>Streptococcus</i> spp.	<i>Streptomyces</i> spp.	Other Gram-positive rods
Number	20	19	1	2	3	1	2	1	1	1	1	2	4	2	2	1
%	100	95	5	10	15	5	10	5	5	5	5	10	20	10	10	5

negative bacteria of the species *Staphylococcus* spp. and the genus *Cellulomonas cellasea* were most frequently isolated - 20% and 15%, respectively (Table 4). The highest cfu/ml concentration values concerned bacteria of the species *Corynebacterium* spp. - 50,000 cfu/ml and *Actinomyces* spp. - 20,000 cfu/ml and the genus *Rhodococcus coprophilus* - 42,500 cfu/ml and *Staphylococcus lentus* - 22,500 cfu/ml (Table 5).

It is noteworthy that in the water samples examined the presence of pathogenic microorganisms of the species *Staphylococcus* spp., *Micrococcus* spp., *Streptococcus* spp., *Corynebacterium* spp., *Rhodococcus* spp., *Actinomyces* spp., and *Streptomyces* spp. were observed, which may be the etiologic factor of infectious and invasive diseases.

## CONCLUSIONS

1. The common character of bacteriological contamination and the presence of opportunistic microorganisms in water used in dental treatment creates a potential health risk for patients and dental staff.

**TABLE 5. Compilation of results obtained from culturing water samples from dental unit reservoirs on blood agar.**

Sample	Total cfu/ml	Gram-negative rods	Actinomyces spp.	Bacillus spp.	Cellulomonas cellulasea	Corynebacterium spp.	Micrococcus spp.	Rhodococcus coprophilus	Rhodococcus spp.	Staphylococcus lentus	Streptococcus mitis	Staphylococcus sciuri ss rodentium	Staphylococcus spp.	Streptococcus spp.	Streptomyces spp.	Other Gram-positive rods
1	24,000	24,000														
2	23,000	23,000														
3	119,500	119,500														
4	64,510	43,500	20,000										10	1,000		
5	40,230	40,000			215											5
6	11,650	11,650														
7	4,400				135			4,150			90		25			
8	51,000	8,000			500			42,500								
9	65,030	6,530		4,500		50,000								4,000		
10	548,000	548,000														
11	396,500	396,500														
12	12,240	10,550		1,650								40				
13	1,145	1,020											120			5
14	735	665										70				
15	143,000	120,500							22,500							
16	17,285	17,250					35									
17	29,270	29,250											20			
18	117,000	117,000														
19	5570	5,550					20									
20	14,950	14,650														300

2. It seems necessary to monitor the microbiological contamination of water in dental units and apply the recommended methods and available chemical agents for its decontamination.

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## Informacje o Autorach

Dr n. med. JOLANTA SITKOWSKA – asystent, Zakład Biologicznych Szkodliwości Zawodowych, Instytut Medycyny Wsi w Lublinie; dr hab. n. med. JOLANTA SZYMAŃSKA – adiunkt, Katedra i Zakład Stomatologii Wieku Rozwojowego, Uniwersytet Medyczny w Lublinie; inż. GRAŻYNA CHOLEWA – pracownik inżynieryjno-techniczny, Zakład Biologicznych Szkodliwości Zawodowych, Instytut Medycyny Wsi w Lublinie.

## Adres do korespondencji

Dr n. med. Jolanta Sitkowska  
Instytut Medycyny Wsi  
21-090 Lublin, ul. Jaczewskiego 2  
tel. 81 7184403