Opportunistic Bacteria in Dental Unit Waterlines. Assessment and Characteristics

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Disclosures
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Table 1. Number and percentage shares of pathogenic genera/species.

Group (total n = 53)	Pathogenic species (n)	Share in pathogenic group (%)	Share in total isolated species (%)
Gram-negative rods (n = 16)	8	50.00	14.54
Gram-positive rods (n = 23)	13	56.52	23.64
Gram-positive cocci (n = 12)	8	66.67	14.54
Actinomycetes (n = 2)	1	50.00	1.82

Table 2. The cfu/ml values and percentage shares of pathogenic bacteria isolated from dental unit water samples.

Group	Genus/species	CFU/ml	Share in pathogenic group (%)
Gram-negative rods	*Pseudomonas aeruginosa*	20.00	0.00
	Burkholderia cepacia	5.00	0.00
	Brevundimonas vesicularis	21,550.00	0.03
	Ralstonia pickettii	5,814,815.00	89.90
	Sphingomonas paucimobilis	564,620.00	8.74
	Sphingomonas paucimobilis B	25,500.00	0.39
	Sphingobacterium spiritovorum	41,150.00	0.64
	Stenotrophomonas maltophilia	310.00	0.00
	Total CFU/ml	6,467,970.00	
	Average CFU/ml	808,496.25	
Gram-positive rods	*Arthrobacter* spp.	90.00	0.01
	Arthrobacter woluwensis	45.00	0.00
	Aureobacterium spp.	715.00	0.06
	Brevibacterium epidermidis	132,620.00	11.64
	Brevibacterium otitidis	1435.00	0.12
	Brevibacterium spp.	687,400.00	60.35
	Brevibacterium spp. (CDC. B-1/3)	29,500.00	2.59
	Corynebacterium auris	2215.00	0.19
	Corynebacterium spp.	67,010.00	5.88
	Corynebacterium urealyticum	202,500.00	17.78
	Brevibacterium mcbrellneri	1650.00	0.14
	Microbacterium spp.	7700.00	0.67
	Microbacterium spp. (CDC. A-5)	6190.00	0.54
	Total CFU/ml	1,139,070.00	
	Average CFU/ml	87,620.77	
Gram-positive cocci	*Enterococcus casseliflavus*	1600.00	3.18
	Micrococcus spp.	25,620.00	50.94
	Staphylococcus lentus	95.00	0.19
	Staphylococcus lugdunensis	700.00	1.39
	Staphylococcus saprophyticus	985.00	1.96
	Staphylococcus spp.	14,995.00	29.82
	Stomatococcus mucilagenosus	880.00	1.75
	Streptococcus spp.	5415.00	10.77
	Total CFU/ml	50,290.00	
	Average CFU/ml	6286.25	
Actinomycetes	*Actinomyces* spp.	1,562,450.69	100.00
	Total CFU/ml	1,562,450.69	

Average CFU/ml	1,562,450.69

Table 3. Number and percentage shares of allergizing bacteria groups isolated from dental unit water samples.

Group	n	Share in allergizing group (%)
Gram-negative rods (n = 16)	1	6.25
Actinomycetes (n = 2)	1	50

Table 4. The CFU/ml values and percentage shares of allergizing actinomycetes isolated from dental unit water samples.

Species	CFU/ml	Share in allergizing group (%)
Streptomyces albus	505.00	100
Average CFU/ml	4.72	

Table 5. The CFU/ml values and percentage shares of immunotoxic Gram-negative rods isolated from dental unit water samples.

Species	CFU/ml	Share in immunotoxic group (%)
Alcaligenes faecalis	85,025.00	1.25
Pseudomonas aeruginosa	20.00	0
Burkholderia cepacia	5.00	0
Pseudomonas fluorescens	181,240.00	2.67
Pseudomonas putida	623,055.00	9.2
Pseudomonas stutzeri	47,000.00	0.69

Abstract and Introduction

Abstract

Aim: The study aimed to determine qualitative and quantitative contamination of dental unit reservoir water with aerobic and facultative anaerobic bacteria, with regards to health risk to dental staff and patients.

Materials & methods: The study material included water samples from 107 unit reservoirs. Conventional microbiological methods were used. The isolated bacteria were divided into three groups according to pathogenic mechanisms.

Results: Dental unit water contamination was widespread. The isolated bacteria average concentration was 1.1×10^5 CFU/ml, with *Ralstonia pickettii* as the prevailing species (49.33%). The total potentially pathogenic bacteria were 54.54% of all the isolated bacteria. Bacteria causing infectious and invasive diseases constituted over one-half of this group, while allergizing and immunotoxic bacteria occurred in smaller quantities.

Conclusion: The presence of over 50% potentially pathogenic microorganisms among the isolated bacteria and their very high concentrations call for the daily use of effective methods to reduce dental unit water contamination and health risk.

Introduction

The appropriate microbiological quality of water used in dental treatment within a dental unit is extremely important for health reasons. Patients and staff are exposed to microorganisms from dental unit waterline (DUWL) output water in addition to contaminated aerosols generated during the work of dental handpieces.[1] In addition, the generated bioaerosol affects the microbiological conditions of the dental surgery environment.[2,3] The aforementioned factors are particularly significant owing to the threat

of cross-infections.[4] Water present in DUWLs may be contaminated with microorganisms from the biofilm, formed due to water stagnation in the narrow-bore DUWL tubings.[5] Therefore, apart from the level of DUWL water contamination, the type of contamination – in other words, the kind of microorganisms present in DUWLs – is important. The assessment of infection risk created by microorganisms from DUWL water therefore seems necessary.

The aim of this study was to determine the qualitative and quantitative contamination of dental unit reservoir water with aerobic and facultative anaerobic bacteria, with regards to health risks to dental staff and patients resulting from exposure to the isolated bacterial microflora.

Materials & Methods

The study material included 15 ml water samples taken from 107 reservoirs of dental units located in randomly selected dental surgeries of public health centers in the Lublin Voivodeship, Poland. A water reservoir is an independent bottle that attaches to DUWLs, and has no connection to the municipal water supply. Distilled water is added to it manually once it has been used. In the studied surgeries, no DUWL disinfection method was used. In order to guarantee identical sampling conditions and to avoid accidental microbiological contamination of water, all samples were taken successively in winter (heating season), at the beginning of a working day and before patient consultations had started. No water samples taken from the general water supply were involved in the study. Water samples were obtained in sterile, airtight test tubes and transported to the laboratory immediately after sampling in an insulated container at the temperature not exceeding 4–6°C. Depending on the distance, the samples reached the laboratory within 1–3 h and were inoculated into the media on the same day.

Considering the scientific character of the study and the necessity to warrant anonymity (protection of data related to health centers), the method of double coding samples was used. The consent of the surgery owners or health center directors was obtained before each sampling.

Microbiological Examination of Water Samples

Microbiological examination of water aimed to assess the concentration and qualitative composition of aerobic and facultative anaerobic bacterial flora present in a reservoir built in a dental unit. In order to isolate and identify microorganisms, conventional microbiological methods were used. Mesophilic Gram-positive and -negative bacteria with increased nutritional requirements were cultured on nutrient agar with 5% sheep blood. Eosin methyl blue (EMB) agar was used for isolation and initial identification of Gram-negative rods. The examined samples were inoculated on both media simultaneously, using the plate dilution method with surface inoculation. A quantity of 0.1 ml of the initial water samples and their tenfold dilution in sterile physiological salt solution (0.85% NaCl) were introduced twice, parallell to each of the two media, and distributed evenly on the agar surface with a sterile glass spreader. The water inoculations on blood and EMB agar were incubated for 24 h at 35–37°C, then 3 days at room temperature (22°C) and 3 days at refrigerator temperature (4°C). The prolonged culture at a low temperature favored the growth of some of mesophilic and psychrophilic microorganisms. After incubation, the initial identification of microorganisms cultured on both media was performed. The assessment of the growth of

bacterial colonies on the media included their macroscopic morphological characteristics, such as the size and form of colonies, surface and margin, color, opacity and texture. Microscopic preparations were made from the colonies differing in appearance with the use of Gram-staining methods. The analysis of their microscopic image estimated the color of bacterial cell staining, shape, size, arrangement of the neighboring cells and spore presence. Next, considering the previously described characteristics, the number of morphological types was determined, as well as their concentration, expressed in colony forming units in 1 ml of water (CFU/ml) according to the formula $x = a \times r/0.1$, where: 'x' is the concentration of bacteria in water expressed as CFU/ml; 'a' is the average number of colonies on a plate; and 'r' is the reverse of the dilution.

In order to obtain a reliable number of bacterial colonies on the plates, the count was performed when 100–300 microorganisms were present.

Subsequently, bacterial colonies that more frequently occurring in inoculations on each of the media were isolated and identified to the genus or species level using biochemical microtests. Gram-negative rods from EMB agar were identified with API 20E and API 20NE tests (bioMeriéux, France), while Gram-positive bacteria from blood agar were identified with GP2 MicroPlate™ test (Biolog, Inc., CA, USA). Gram-negative rods that proved impossible to determine with the API kit were identified with the analogous test GP2 MicroPlate. All the tests were used according to the procedures recommended by the manufacturers.

API Test Technique

The initial identification of aerobic Gram-negative rods was performed by testing the ability to produce cytochrome oxidase by the examined strains. The bacterial mass cultured within 24 h was applied onto the reactive surface of the test strip (Bactident® Oxidase, Merck, Germany) and after 20–60 s the result was read. Blue or purple–blue color of the strip indicated an oxidase-positive strain and the absence of color indicated an oxidase-negative strain. Oxidase-positive strains were identified with the API 20NE test, oxidase-negative strains were identified with API 20E. The strips of both API tests, consisting of 20 microtubes containing dehydrated substrates, were filled according to the manufacturer's manual, with an appropriate density of the previously prepared bacterial suspension, in sterile physiological liquid (in some microtubes anaerobic conditions were created by covering their surface with liquid sterile paraffin). The strips were placed in humid chambers and incubated for 24 h at 35–37°C (in the case of API 20E) and 24–48 h at 30°C (in the case of API 20NE). The final, specific results of API 20NE were readings after a total of 48 h. Metabolic processes during incubation caused color changes in microtubes, either spontaneously or due to added reagents. The results of those reactions, in the form of seven-digit numeric code, were used to identify the examined strain.

GP2 & GN 2 MicroPlate Test Technique

The Biolog GP2 and GN2 MicroPlate system is a standardized micromethod used to identify aerobic and facultative anaerobic Gram-positive and Gram-negative bacteria on the basis of their metabolic pattern. The test determines the ability of microorganisms to participate in biochemical reactions with substrates contained in reaction wells. The suspension (18 ml) of the strains selected for identification in gelled 0.40% NaCl was prepared; the appropriate cell

density, different for Gram-negative and Gram-positive bacteria, was determined using a turbimeter.

A total of 150 µl of the suspension was added to each of the 96 wells in the reactive plate (one of which was a negative control, containing only indicator substance). Subsequently, the microplates were incubated for 24 h at 30 or 35°C, according to the microorganisms to be determined. As a result of the reaction, the color indicator in each well (terazolium violet), responded with a change of color to purple in the positive wells containing a given strain. Results were read after 6 and 24 h, comparing the color of liquid in individual wells with the negative control. The final identification was made with the MicroLog software, provided by the manufacturer (Biolog, Inc.), determining the conformity and probability coefficient for an identified microorganism.

Assessment of the Percentage Shares of Bacteria According to Their Pathogenicity

Bacteria considered as pathogenic were found among the mesophilic bacterial microflora isolated from the tested dental unit water samples. For the purpose of analysis, the bacteria were divided into three groups: the bacteria causing infectious and invasive diseases (pathogenic bacteria/pathogens); bacteria causing allergic reactions leading to respiratory system diseases (allergizing bacteria); and bacteria causing inflammatory reactions in the lung tissues as a result of releasing immunotoxic endotoxins (immunotoxic bacteria).[6] This threefold classification emphasizes the diversity of the pathological influence that bacteria may exert on the organism and indicates the dominating type of this pathological effect.

Results

In all 107 tested water samples, mesophilic bacteria were found. The average concentration of total bacteria isolated from the reservoir water was 1.1×10^5 CFU/ml (the minimum concentration was 3×10^1 CFU/ml and the maximum was 1.2×10^6 CFU/ml). The highest concentration was found for *Ralstonia pickettii* and reached 5.4×10^4 CFU/ml.

Gram-negative rods were represented by the following species: *Acidovorax avenae* spp. *cattleyae*, *Alcaligenes faecalis*, *Brevundimonas vesicularis*, *Bulkholderia cepacia*, *Pseudomonas aeruginosa, Pseudomonas chlororaphis*, *Pseudomonas fluorescens*, *Pseudomonas huttiensis* (Burkholderia-like), *Pseudomonas putida*, *Pseudomonas stutzeri*, *Pseudomonas syringae pathovar aptata*, *R. pickettii*, *Sphingomonas paucimobilis*, *Sphingomonas paucimobilis B*, *Sphingobacterium spiritovorum* and *Stenotrophomonas maltophilia*. Bacteria of the species *R. pickettii* were identified at 52 workstations (48.6% of all the tested units). The second most numerous Gram-negative rods, according to the number of samples, were *S. paucimobilis* and *P. fluorescens*: 27 (25.23%) and 16 (14.95%) workstations, respectively. Other species were identified at individual workstations.

The isolated Gram-positive rods belonged to the following genera and species: *Arthrobacter histidinolovorans*, *Arthrobacter* spp., *Arthrobacter woluwensis*, *Microbacterium flavescens*, *Aureobacterium* spp., *Microbacterium testaceum*, *Brevibacterium epidermidis*, *Brevibacterium otitidis*, *Brevibacterium* spp., *Brevibacterium* spp. (CDC. B-1/3), *Clavibacter michiganensis synonimus insidiosus*, *Corynebacterium auris*, *Corynebacterium* spp., *Corynebacterium urealyticum*, *Corynebacterium variabile*, *Brevibacterium mcbrellneri*, *Arthrobacter ilicis*, *Microbacterium laevaniformans*, *Microbacterium* spp., *Microbacterium* spp. (CDC. A-5), *Rhodococcus fascians* and *Rhodococcus* spp. Among them, the most

frequently found were: *M. laevaniformans*, present at eight workstations (7.48%) and *Corynebacterium* spp., present at seven workstations (6.54%). *B. epidermis*, *Microbacterium* spp., *Microbacterium* spp. (CDC. A-5) and other unidentified Gram-negative rods occurred at five workstations (4.67%). *Brevibacterium* spp. was found in four examined water samples (3.74% of all the units). Other previously mentioned species were identified at individual workstations.

The following Gram-positive cocci were identified: *Enterococcus casseliflavus*, *Micrococcus* spp., *Pediococcus pentosaceus*, *Staphylococcus arlettae*, *Staphylococcus haemolyticus*, *Staphylococcus lentus*, *Staphylococcus lugdunensis, Staphylococcus saprophyticus*, *Staphylococcus sciuri synonimus rodentium*, *Staphylococcus* spp., *Stomatococcus mucilaginosus*, *Streptococcus acidominimus* and *Streptococcus* spp. At 32 workstations *Staphylococcus* spp. (29.91%) was found, and at 20 work stations *Micrococcus* spp. (18.69%) was found. Other species were found at individual workstations.

Among spore-forming Gram-positive bacteria, *Bacillus* spp. was found at ten workstations (9.34%) and *Bacillus halodurans* was identified at three workstations (2.8%).

Actinomycetes of the genus *Actinomyces* spp. were isolated from 21 samples (19.63%) of the tested unit water, and *Streptomyces albus* from two samples (1.87%).

Pathogenic Bacteria

Among all the isolated 53 mesophilic bacteria, species causing infectious and invasive diseases (pathogenic bacteria/pathogens) were identified. Eight pathogens were found in the group of Gram-negative rods (16 isolated species). Their percentage share in Gram-negative rods was 50.00%, and was 14.54% in all the isolated bacteria. In the group of Gram-positive rods (23 isolated genera/species), there were 13 pathogens, which constituted 56.52% of all Gram-positive rods and 23.64% of all the found aerobic and facultative anaerobic bacteria. Gram-positive cocci (12 isolated genera/species) included eight pathogens, which constitutes 66.67% of all Gram-positive cocci and 14.54% of the total number of mesophilic bacteria. The number of pathogenic actinomycetes (two: one genus and one species) was one, which is 50.00% of the group and 1.82% of all the isolated mesophilic bacteria (Table 1).

The CFU/ml values and percentage shares of pathogenic bacteria isolated from dental unit water samples are presented in Table 2.

Allergizing Bacteria

In the group of Gram-negative rods one species was known to cause allergic reactions; *A. faecalis* (1.12% of the group). In the group of Gram-negative rods, the proportion of allergizing rods was 6.25% of the species identified in this group and 1.82% of all the isolated bacterial species (Table 3).

In the group of actinomycetes there was one species of bacteria known to cause allergic reactions: *S. albus*. This bacterial species demonstrated 5.05×10^2 CFU/ml, constituting 0.03% of the number of actinomycetes (Table 4). The proportion of allergizing actinomycetes was 50% of the species identified in this group and 1.82% of all the isolated bacterial species (Table 3).

Immunotoxic Bacteria

The species of Gram-negative immunotoxic rods are presented in Table 5. The percentage share of *R. pickettii* was the largest among the immunotoxic bacteria (85.85%).

Discussion

Patients and dental teams are exposed to water emitted from the dental water unit (bioaerosol) and the presence of opportunistic pathogens creates health risks. The aim of this study was to examine, in detail, the microbiological quality of aerobic and facultative anaerobic bacteria and to discuss possible risks related to their presence, in particular to opportunistic microorganisms that were found in the highest concentrations.

Opportunistic pathogens are present in the human life environment and are commensals of the skin and mucosa. In favorable conditions they may cause infections in patients with deficient natural immune mechanisms and weak immunological systems. The risk groups included small children, the elderly, patients with chronic generalized diseases and hospitalized patients. The predisposing factors include the patient's age, long-term stay in hospital or at the intensive therapy department, wide-range therapy with antibiotics, steroids or immunotherapy, primary diseases, neoplasms, multiorgan trauma, HIV infections, and also invasive diagnostic and therapeutic procedures involving breaking tissue continuity. As long as the immunological system functions correctly, microorganisms remain neutral to their host. However, in favorable conditions, opportunistic microorganisms become pathogens.[7]

Among the Gram-negative rods isolated and identified from the tested water samples, species associated with nosocomial infections (*P. aeruginosa*, *B. cepacia*, *R. pickettii*, *Sphingomonas paucimobilis* and *Stenotrophomonas maltophilia*), skin infections (*Pseudomonas* spp.), and patients with mucoviscidosis (*B. cepacia* and *P. aeruginosa*) were found.[8]

Bacteria of the species *R. pickettii* have been a cause of many clinical problems, including septicemias caused by injection of solvents contaminated with those microorganisms. Some of these infections have been described as invasive and severe, leading to meningitis, septic arthritis and osteomyelitis.[9,10] In 55 out of 366 examined patients, the presence of *R. picekttii* or infection with this bacteria was confirmed. It seems that this microbial species is more widespread and pathogenically significant than previously believed.[11] Earlier laboratory tests showed that a small quantity (1–10 CFU/ml) of *R. pickettii* introduced to 0.9% NaCl solution is sufficient for the bacteria to multiply in a wide range of temperatures from 15 to 42°C.[12] It should be stressed that in the current study, *R. picekttii* populated almost half of the examined unit reservoirs (49.95%) and showed the highest concentration in water samples (average concentration: 5.4×10^4 CFU/ml).

S. paucimobilis is a bacterial species widespread in the natural environment. It also contaminates water supplies, hospital equipment and indwelling devices such as mechanical ventilators or catheters, causing nosocomial infections. *S. paucimobilis* can cause a variety of infections including bacteremia/septicemia.[13,14] Nosocomial infections caused by *S. paucimobilis* are mild and can be successfully treated with antibiotics.[15] Bacteria of that species were also considered a cause of urinary tract infections, meningitis, wound

infections and septicemia. A case of late onset eyeball infection with *S. paucimobilis* after cataract removal in a patient without a systemic disease was also described.[16] In the present study, bacteria of this species were isolated from approximately one-quarter of the reservoir water samples, the average concentration being slightly over ten-times lower than the concentration of *R. pickettii*.

Among the Gram-positive rods isolated from dental unit water in the current study, there were microorganisms of the coryneform group. These are bacteria of varying morphological structure, belonging to the physiological human microflora, which are increasingly more frequently believed to be causes of opportunistic infections. They include the genera: *Arthrobacter* spp., *Brevibacterium* spp., *Corynobacterium* spp. and *Microbacterium* spp. The coryneform bacteria of these genera are widespread in the natural environment (e.g., in soil and organic fluids) and, to an increasing extent, they are also considered causes of serious infections in patients with decreased immunity.[17,18]

Among the isolated Gram-positive rods of the genus *Brevibacterium* spp., *Brevibacterium otitidis* is a known cause of otitis in humans. The cases of bacteremia caused by this pathogen were also reported. For the first time, the case of peritonitis caused by this bacterial species in a patient after outpatient peritoneal dialysis was confirmed.[19,20]

Other rods identified in the water of the tested unit, belonging to the genus *Brevibacterium*: *B. epidermis*, *Brevibacterium mcbrellneri* and *Brevibacterium* spp. being commensals, are also identified in clinical materials and classified as opportunistic pathogens.[21] Species of the genus *Brevibacterium* spp. were isolated from blood, cerebrospinal fluid, joint fluid, bone marrow, pleural fluid, spleen, urine, throat swab and dialysate.

Gram-positive rods *C. urealyticum*, isolated from the water samples and belonging to the physiological flora of the human skin, occur primarily in armpits, groin, anus and abdominal folds. Bacteria of this species are recognized to be etiological factors of nosocomial infections, most frequently responsible for urinary tract infections (cystitis and pyelonephritis), occurring mostly in patients with lowered immunity, after catherization, and with diseases or traumas in the urinary system. Wound infections, pneumonias, bone and joint infections, as well as endocarditis involving an implanted valve and bacteremia after kidney transplantation were also reported. Cases of bacteremia unrelated to urinary tract infections, necrotizing soft tissue infections in children with neutropenia and a cyst in a nonhospitalized woman that are causally connected to *C. urealyticum*, were also described.[22]

Gram-positive cocci of the species *Enterococcus casseliflavus* may cause various infections in humans, including bacteremia and endocarditis among others, primarily in patients with a weakened immune system. It is currently assessed that *E. casseliflavus* and *Enterococcus gallinarum* (not found in this study) are responsible for 45% of the cases of bacteremia.[23] A case of meningitis – which is rarely due to enterococci, but if so, it is usually severe – caused by *E. casseliflavus* was also described.[24]

Micrococcus spp. (~18% unit reservoirs were populated by these bacteria) is mostly considered as nonpathogenic; however, in immunodeficient patients, it may cause various opportunistic infections.[25]

Gram-positive cocci of the *Staphylococcus* genus: *S. lentus* and *Staphylococcus* spp. are considered as opportunistic pathogens, especially in immunodeficient patients. *S. lentus* was described as a cause of urinary tract infections and splenic abscess, while cocci *Staphylococcus* spp. may be responsible for suppurative systemic infections, as well as infections in the oral cavity.[26,27]

Streptococci isolated from water samples in the current study (*Streptococcus* spp.) may be a cause of infection of soft tissues surrounding partially erupted teeth and of tooth abscesses. The bacteria are considered as harmful biological factors that may be pathogenic for humans, but usually can be coped with by effective prophylactic and therapeutic methods.[6,28] In the present study, similarly to other European research, relatively low concentrations of *Streptococcus* spp. were detected.

In one-fifth of the samples, the presence of actinomycetes of the genus *Actinomyces* spp. was found. They show low virulence, but may cause opportunistic infections if the mucous membrane is broken as a result of a dental procedure or trauma. Furthermore, they are etiological factors in actinomycosis, dental caries and periodontitis.[6,28]

Among aerobic and facultative anaerobic bacteria isolated from water samples in the current study, there are two species that may cause allergic reactions: Gram-negative rods *A. faecalis* and mesophilic actinomycetes *S. albus*. The latter was identified as a cause of allergic alveolitis.[6]

Our study found a large group of Gram-negative rods causing inflammatory reactions in the lung tissue as a result of endotoxin release. The group included: *A. faecalis*, *P. aeruginosa*, *B. cepacia*, *P. fluorescens*, *P. putida*, *P. stutzeri*, *Brevundimonas vesicularis*, *R. pickettii* and *Stenotrophomonas maltophilia*. Bacterial endotoxin is a biologically active compound present in the most external layer of the cell wall in Gram-negative rods. It is easily released into the external environment upon destruction of the bacteria. Experiments on animals and studies of infections caused by Gram-negative rods in humans show that effects of the inhalation of bacterial endotoxins (even minimum quantities) are inflammatory foci in the lungs and bronchial spasms leading to respiratory failure.[29] It is also known that asthma may be triggered or exacerbated as a result of indoor endotoxin exposure. However, the review of 1996–2007 literature shows that the number of published cases of infection or respiratory symptoms resulting from exposure to water from contaminated DUWLs is limited.[30]

The results of the current study allow certain generalizations. It should be noted that over a half of the bacteria contaminating water in dental unit reservoirs were opportunistic bacteria associated with various pathogenic mechanisms, and the percentage of Gram-negative bacteria, which are the source of immunotoxin, was very high. The literature review shows that high levels of bacterial contamination in DUWLs were reported by other authors,[31–33] while research concerning endotoxin demonstrated that dental unit water contains high concentrations of endotoxin, and that there is a statistically significant positive correlation between endotoxin and the bacterial load present. At the same time, it was stressed that exposure to either the endotoxin-laden water or the aerosolized endotoxin

represents a potential health threat,[34–36] especially to immunodeficient patients or, if the exposure is prolonged, to members of the dental team.

Researchers studying the problem of DUWL water contamination indicate the need for investigating and raising awareness of the risk of occupational exposure and cross-infection in general dental practices.[37] They also stress that it is necessary to develop and use effective methods of reducing DUWL microbiological load.[8,38,39]

Conclusion

The presence of over 50% potentially pathogenic microorganisms among the isolated aerobic and facultative anaerobic bacteria, and their very high concentrations, indicate the necessity to use effective methods to reduce bacterial contamination of DUWL water in daily clinical practice in order to reduce health risks.

Future Perspective

Taking into consideration the risk of crossinfection among patients through DUWL water, a detailed microbiological analysis of microflora composition and, in particular, of its pathogenicity, seems extremely important and useful. The extensive analysis and determination of pathogenicity of bacterial microorganisms, which in this paper covered aerobic and facultative anaerobic bacteria (a large portion of the bacteria colonizing DUWL) is a starting point for work towards a more effective monitoring protocol of DUWL water microbiological quality. Developing effective decontamination protocols is an activity directed against the most frequently found and most pathogenic microorganisms living in DUWLs. From the perspective of daily clinical practice, there is still a need for an easy-to-use, cheap and safe protocol for DUWL water disinfection.

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Papers of special note have been highlighted as: * of interest