Ann Agric Environ Med 2007, 14, 137-140

BACTERIAL CONTAMINATION OF WATER IN DENTAL UNIT RESERVOIRS

Jolanta Szymańska

Department of Paedodontics, Medical University of Lublin, Poland

Szymańska J: Bacterial contamination of water in dental unit reservoirs. *Ann Agric Environ Med* 2007, **14**, 137-140.

Abstract: The aim of this study was bacteriological assessment of water in dental unit reservoirs – concentration and composition of the aerobe and facultative anaerobe bacterial microflora. Reservoir water samples were taken from 25 units. Bacterial flora were determined with the plate culture method. Bacteria were identified with biochemical microtests: API 20E, API 20NE (bioMérieux, France) and GP2 MicroPlateTM (BIOLOG, USA). The concentration of total bacteria isolated from one site was 201,039 cfu/ml, on average; the minimum was 22,300 cfu/ml, and the maximum – 583,000 cfu/ml. The following bacteria were identified: Gram-negative bacteria – *Brevundimonas vesicularis*, *Moraxella lacunata*, *Moraxella* spp., *Ralstonia pickettii*, *Sphingomonas paucimobilis*, *Stenotrophomonas maltophilia*; Gram-positive cocci – *Micrococcus luteus*, *Micrococcus lylae*, *Staphylococcus cohnii*, *Staphylococcus hominis* ss *novobiosepticus*, *Staphylococcus* spp., *Streptococcus* spp.; actinomycetes – *Streptomyces albus*. The prevailing bacteria were: *Ralstonia pickettii* (96.46%), found in all the units. *Sphingomonas paucimobilis* (1.32%) and *Brevundimonas vesicularis* (1.07%) were the next most frequently occurring bacteria. Bacteria concentration in dental unit reservoirs reached excessive values, and the bacterial flora were composed of the bacteria characteristic for water supply systems, opportunistic pathogens, and bacteria of the oral cavity flora. Continuous microbiological monitoring of the DUWL water, including application of a disinfecting procedure, is necessary.

Address for correspondence: Jolanta Szymańska, DMD, Department of Paedodontics, Medical University of Lublin, ul. Staszica 11, 20-018 Lublin, Poland.
E-mail: adpunctum@adres.pl

Key words: dental unit waterlines, water, bacterial contamination.

INTRODUCTION

Handpieces in dental units – a high-speed and a low-speed handpiece, air-water syringe and scaler to remove dental deposits – are supplied with water through a system of thin plastic tubes which constitute the dental unit waterlines (DUWL). The source of water, in the case of an open water system, is a municipal water supply, and in a closed water system – water from a container (reservoir) belonging to a unit.

The aim of this study was bacteriological assessment of the dental unit reservoirs water – concentration and qualitative composition of aerobe and facultative anaerobe of the bacterial microflora.

MATERIAL AND METHODS

The study included 25 dental units located in public dental clinics. The water samples were collected aseptically from the water reservoirs of self-contained dental unit water systems.

Concentration and qualitative composition of bacterial microflora were determined with the plate dilution method using surface culture on the appropriate agar media: blood agar to determine the total number of bacteria and meso-philic actinomycetes, and eosine methylene blue (EMB) agar to identify Gram-negative rods. Ten-fold dilutions with a sterile solution of physiological salt were prepared from the initial water samples. Next, 0.1 ml of the

Received: 4 December 2006
Accepted: 15 May 2007

examined liquid was taken from each dilution and evenly spread on the surface of the agar media in 2 parallel repetitions. The cultures on blood agar and EMB agar were incubated for 24 hours at 37ºC, for 3 days at room temperature (22ºC), and 3 days at 4ºC. Prolonged incubation at lower temperatures was to enable growth of some meso- and psychrophilic species.

After incubation, preliminary identification of bacteria grown on each medium was performed following instructions of the manuals (*Bergey's Manual…*) [7, 16, 26]: the colonies grown were first assessed macroscopically, considering such characteristics as size, shape, structure, colony colour, etc., and next with microscopic methods, staining the bacterial preparations with the Gram method. The total number of bacteria and number of particular morphological types were determined, and their concentration reported as colony forming units in 1 ml of water – cfu/ml.

Next, the strains of most frequently occurring bacteria were isolated and identified to the level of species or genus with biochemical microtests: API 20E test (bioMérieux, Marcy l'Etoile, France), which is used to identify Gram-negative bacteria of Enterobacteriaceae family and other fermenting Gram-negative rods, API 20NE test (bioMérieux, Marcy l'Etoile, France) to identify non-fermenting Gram-negative rods, and GP2 MicroPlate™ test (BIOLOG, Inc., Hayward, CA, USA) used to determine Gram-positive bacteria. All the tests were performed according to the manufacturer's instructions.

API 20E, API 20NE and GP2 MikroPlate™ test technique have been described in a previous paper [18].

RESULTS

The identified bacteria were found in all the water samples taken from unit reservoirs.

The following Gram-negative bacteria were present: *Brevundimonas* (*Pseudomonas*) *vesicularis*, *Moraxella lacunata*, *Moraxella* spp., *Ralstonia* (*Pseudomonas*) *pickettii*, *Sphingomonas paucimobilis*, *Stenotrophomonas* (*Xanthomonas*) *maltophilia*; Gram-positive cocci: *Micrococcus luteus*, *Micrococcus lylae*, *Staphylococcus cohnii*, *Staphylococcus hominis* ss *novobiosepticus*, *Staphylococcus* spp., *Streptococcus* spp. and actinomycetes: *Streptomyces albus*.

Ralstonia pickettii were the most frequently occurring bacteria – present at all operative sites. *Micrococcus luteus* – at 9 sites, *Staphylococcus cohnii* – at 7 sites, *Sphingomonas paucimobilis* – at 6 sites and *Brevundimonas vesicularis* – at 5 sites (Tab. 1).

The concentration of total bacteria isolated from one site was 201,039 cfu/ml on average, the minimum was 22,300 cfu/ml, and the maximum – 583,000 cfu/ml (Tab. 2).

Ralstonia pickettii prevailed, and in all samples it constituted 96.5% of the total isolated bacteria. *Sphingomonas paucimobilis* (1.32%) and *Brevundimonas vesicularis* (1.07%) were the next most frequently found bacteria (Tab. 2).

Table 1. Bacteria identified in water from dental unit reservoirs at individual operative sites.

Bacteria	Site number
Gram-negative bacteria	
Brevundimonas vesicularis	5, 7, 11, 18, 19
Moraxella lacunata	21
Moraxella spp.	7, 21
Ralstonia pickettii	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25
Sphingomonas paucimobilis	9, 14, 18, 21, 24, 25
Stenotrophomonas maltophilia	9, 21, 25
Gram-positive cocci	
Micrococcus luteus	1, 2, 3, 4, 7, 9, 11, 23, 25
Micrococcus lylae	1, 4, 25
Staphylococcus cohnii	1, 3, 5, 9, 11, 15, 24
Staphylococcus hominis ss *novobiosepticus*	6
Staphylococcus spp.	9
Streptococcus spp.	7
Actinomycetes:	
Streptomyces albus	25

Table 2. Average concentration (cfu/ml) and proportion of particular genera/species of bacteria in water samples from dental unit reservoirs.

Bacteria of genus/species	cfu/ml	%
Micrococcus luteus	360.00	0.1791
Micrococcus lylae	17.20	0.0086
Moraxella lacunata	9.20	0.0046
Moraxella spp.	207.20	0.1031
Pseudomonas pickettii	193,924.00	96.4610
Pseudomonas vesicularis	2,160.00	1.0744
Sphingomonas paucimobilis	2,652.00	1.3191
Staphylococcus cohnii	1,124.00	0.5591
Staphylococcus hominis ss *novobiosepticus*	40.00	0.0199
Staphylococcus spp.	92.00	0.0458
Streptococcus spp.	400.00	0.1990
Streptomyces albus	4.00	0.0020
Xanthomonas maltophilia	49.20	0.0245
mean	201,039	100.0000
min	22,300	
max	583,000	

DISCUSSION

Determination of concentration and composition of microflora in the unit water is the basis for evaluation of DUWL microbial contamination. In 1963, Blacke was the first to inform about DUWL microbial contamination [3]. The contamination may reach extremely varying values. Some researchers reported DUWL water contamination at

the levels from 1.5×10^2 to 1×10^6 cfu/ml (Beierle 1993, Furuhashi and Miyamae 1985, Gross *et al.* 1976, Robert *et al.* 1994) [4]. According to others, the contamination range was from 1×10^3 to 1.6×10^8 cfu/ml [6, 15, 24].

The study by Souza-Gugelmin *et al.* [17] shows that bacteria concentrations found in dental unit water ranged from 0 to 1.52×10^6 cfu/ml, where plate count agar was used as the medium and samples were incubated for 48 hours at 32°C. This means that water in some of the reservoirs was not contaminated with bacteria, while in others bacterial contamination many times exceeded the recommended contamination level [1]. In our own research, water in all the reservoirs was contaminated, yet the average contamination level was slightly lower than the highest level in the cited study. In the research by Tuttlebee *et al.* [21], however, the average bacteria concentration amounted to 6.6×10^4 which was lower than in our own study.

The high bacterial contamination of water in all the reservoirs, found in our study, does not meet the standards recommended either for potable water or for the water used in dental conservative treatment [1, 12]. This is especially negative due to the fact that water from a reservoir, after having passed through DUWL, flows from handpieces during treatment and forms aerosol and splatter.

It should be stressed that the fact of the significant concentrations of Gram-negative bacteria that are the main source of endotoxin – an important factor in inflammations – is an advantage [19].

Both the research by Barbeau *et al.* [4], and our study, show that motile Gram-negative rods, a majority of which belong to the Pseudomonadaceae family, prevail in DUWL water. In the cited studies, *Sphingomonas paucimobilis* species constituted 41% of the total isolated bacteria, and *Acinetobacter calcoaceticus* – 23%, and both bacterial species were present in all water samples from 121 units. Moreover, in 24% of DUWL water samples *Pseudomonas aeruginosa* was found. The DUWL water contaminated with this bacteria showed a significantly higher total number of bacteria in comparison to DUWL free from this bacterial species. The presence of *Pseudomonas aeruginosa* in DUWL water is confirmed by the results obtained in other studies [2, 10, 11, 14, 22]. Monarca *et al.* [10] demonstrated high values of this bacteria concentration, especially in the water from the turbine and micromotor. In the research by Sacchetti *et al.* [14], *Pseudomonas aeruginosa* was detected in only one sample of supply water at very low levels, while it was isolated in 11.1% of samples taken from the turbine. The frequency of isolation of *Pseudomonas aeruginosa* was similar to that reported by Barbeau [2] but higher than that of a recent study carried out by Walker *et al.* [22]. In our own research, this bacteria was not isolated.

In our studies, *Ralstonia pickettii* was present in all samples from the unit reservoirs, and also constituted the highest proportion of the total isolated bacteria. Research by other authors confirm the presence of this bacterial species in DUWL water [9, 25].

Ralstonia pickettii – aerobe, Gram-negative, non-fermenting, oxidase-positive rods, may be isolated from environmental and clinical samples. They are believed to be of little clinical importance; the literature, however, reports numerous cases of infection with this microorganism, especially in hosts with immunity impaired as a result of an underlying disease [7, 13, 23]. *Sphingomonas paucimobilis* – aerobe Gram-negative rods, may be isolated from different environments and human-related sources; they are associated with infections connected with the use of catheters [20]. *Brevundimonas vesicularis* – aerobe, Gram-negative, slightly oxidase-positive rods, rarely isolated from environmental (water) and clinical (blood) samples. A case of bacteraemia caused by this bacteria in a child with sickle cell anaemia, fever and pneumonia was described, and this is the first report on the invasive form of this bacteria species in a child [5, 7, 20].

In the examined water samples from the dental unit reservoirs, bacteria of the Pseudomonadaceae family were the most common: they are widespread in the environment, their presence is related to water supply, and a part of these bacterial microorganisms are opportunistic pathogens [7, 8]. It should be noted that the bacteria of the *Pseudomonas* genus include species that are potentially pathogenic for immunocompromised individuals.

Our research showed that only a small percentage of the total isolated bacteria were the bacteria of *Streptococcus* and *Staphylococcus* genera, which form the physiological flora of the oral cavity [7, 8, 16, 26]. They were present in DUWL probably as a result of sucking back fluids from patients' oral cavities, and subsequent multiplication in the unit reservoirs. This may be a potential source of cross infections.

CONCLUSIONS

Bacteria concentration in dental unit reservoirs reached excessive values, and the bacterial flora was composed of the bacteria characteristic for water supply systems, opportunistic pathogens, and the bacteria of the oral cavity flora. Continuous microbiological monitoring of the DUWL water, including application of a disinfecting procedure, is necessary.

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