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WATERLINE CONTAMINATION IN THE DENTAL PRACTICES IN IASI

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Abstract. Aim. The aim of the study was to determine the degree of contamination of the water in the dental unit and the risk of pathogens transmission on this route, in Iaşi dental practices in 2006. The water supply in dental units through oral rinses, air/water syringes, ultrasonic scalers, and high or low-speed hand pieces may be heavily contaminated with microorganisms that present potential risks of contamination for both medical team and patients. The microorganisms detected in Dental Unit Water System (DUWS) are mainly bacteria, but some protozoa and fungi may also be found. **Materials and methods.** A total of 93 water samples were collected of which 67 from the DUWS, in four distribution points. Contamination was determined using bacteriological indicators: Total Number of mesophilic Germs (TNG/ml) growing at 37^0C, coliform bacteria (CFU/100 ml), *Escherichia coli* (CFU/100 ml) and enterococci (CFU/100 ml). **Results.** The values for TNG/ml exceeded European Union drinking water guidelines *i*n 52.5% of DUWS water samples. Coliform bacteria were present in 30.3% of the samples collected from DUWS. *E. coli* was detected in one air-water syringe sample. **Conclusions.** The pathogenicity and metabolism of the biofilm in the DUWS, insufficiently evaluated till now, require further studies in this field in view of elaborating effective strategies for water supply quality control aiming the decrease of the risk for microorganisms transmission on this route.
Key words: contamination, biofilm, Dental Unit Water System, infection control

Rezumat. Obiectiv. Obiectivul acestui studiu a fost evaluarea nivelului de contaminare a apei din reţeaua unit-ului dentar şi a riscului de transmitere a agenţilor patogeni pe această cale în cabinetele dentare din Iaşi în anul 2006. Apa furnizată prin reţeaua unit-ului dentar pentru clătiri orale, răcirea pieselor de mână, dispozitivul de detartraj ultrasonic şi seringa de apă /aer este contaminată cu microorganisme provenite de la sursa de distribuţie publică şi din cavitatea orală a pacienţilor constituind, astfel, o potenţială sursă de contaminare atât pentru personalul medical cât şi pentru pacienţi. Microorganismele detectate în apa reţelei unit-ului dentar sunt mai ales bacterii, dar pot fi evidenţiate şi unele protozoare sau fungi**. Material si metodă.** Au fost recoltate un număr total de 93 de probe din care 67 de la reţeaua de apă a unit-ului dentar in patru puncte de distribuţie. Indicatorii bacteriologici utilizaţi au fost: Numărul Total de Germeni (NTG/ml*)* care se dezvoltă la 37^0C, bacteriile coliforme (UFC/100 ml*), E. coli* (UFC/100 ml*),* enterococi (UFC/100 ml*)*. **Rezultate.** 52,4% din probe au depăşit normele Uniunii Europene şi valorile admise privind calitatea apei potabile în Romania privind NTG. Bacteriile coliforme au fost prezente în 30,3% dintre probe iar *E. coli* a fost detectată într-o singură probă provenind de la o seringa de aer/apă. **Concluzii.** Potenţialul patogen şi metabolismul biofilmului depus în reţeaua de apă a unit-ului dentar, insuficient

evaluat până în prezent, impune elaborarea unor strategii eficiente de control al calității apei şi reducere a riscului de transmitere a infecției pe această cale.
Cuvinte cheie: contaminare, biofilm, rețeaua de apă a unit-ului dentar, controlul infecției

INTRODUCTION

Microbial contamination of dental unit water network was first reported in 1963 following the examination of the samples from hand pieces cooling water and water/air syringe (1). The water supply of the dental unit consists in a system of small caliber plastic tubes through which the water used for oral rinses and irrigation, cooling of hand pieces, ultrasound scaling device and water/air syringes circulates. For most dental units, the water source is the public water supply.

In the conditions of active and passive retraction phenomenon, the colonization of bacteria and fungi from natural water leads to the formation of microbial communities located on the walls of Dental Unit Water System (DUWS) as a *biofilm* (2). Its formation is favored by the stagnation of water in the supply system (low natural running rate and usage pressure), the permanent restoration of bacterial nutrients – the continuous entry of a small number of microbes, the small diameter of the tubes as compared to surface/volume. Microorganisms produce a polysaccharide matrix (glycocalyx) by means of which the adherence and retention to the inner surfaces of the water network and a 1500-times higher resistance to antimicrobial agents than that of the planktonic ones is possible (3). This resistance results from factors as: lack of penetration of the antimicrobial agents, location of inhibitory enzymes, low microbe growth rate, and selection of some resistant phenotypes due to surface growth. The release of microorganisms from the biofilm during clinical activities is a potential source of infection for both the medical personnel and patients, particularly for those with an affected immune system (4).

The microorganisms detected in DUWS are mainly bacteria but some protozoa and fungi may also be found. Most bacteria are opportunistic germs similar to those in the biofilms from pools, but pathogenic conditioned species as non-tuberculous *Mycobacteria* (NTM)*, Pseudomonas,* and *Klebsiela* can also be found. Biofilms may also determine the survival of some sensitive pathogens such as *Legionella pneumophila*, reported in almost 25% of the samples collected from dental unit water network (5).

Clinical implications

The potential of contamination through DUWS was demonstrated by the results of numerous investigations. Thus**,** *Pseudomonas* or NTM can be transmitted to susceptible patients by direct contact with the water or following exposure to contamination due to inadequate reprocessing of the medical instruments. It is believed that the data in the literature do not reflect the true extent of this phenomenon, as it is difficult to establish a relationship between clinical manifestations and the possible cause of disease The case of a well-known California dentist who died from an infection caused by

Legionella pneumophilia from the water system of his dental unit, as well as the case of a patient who died from endocarditis associated with the contamination of dental unit water during a dental scaling are mentioned (6).

The concentration of bacterial endotoxin from Gram-negative bacteria was proved to be, also, very high (1000 units/ml) as compared to the accepted level for the sterile oral irrigation water (0.25 units/ml). Although the consequences of acute or chronic exposure to these aerosolized endotoxins in dental practice have not been investigated, they were associated with the exacerbation of asthma and pulmonary hypersensitivity in medical units of a different profile (7, 8).

The effects of water-borne pathogens on human health differ in severity from mild forms of gastroenteritis to severe diarrhea, dysentery, hepatitis, typhoid fever, urinary infections, meningitis or septicemia (9) (table 1).

Tabel 1. The effects of water-borne pathogens on human health (9)

Water-borne pathogens	Human health effects
***Aeromonas* spp**	septicemia in immunocompromised patients, wound infections and respiratory tract infections
Bacillus spp	diarrhea, bacteraemia in immunocompromised patients, anthrax in humans and animals
Escherichia coli	urinary tract infections, bacteraemia and meningitis
Helicobacter pylori	chronic gastritis, with complications such as peptic and duodenal ulcer disease and gastric cancer
Klebsiella	nosocomial infections
Legionella spp	legionellosis
Nontuberculous *Mycobacterium* spp.	diseases involving the skeleton, lymph nodes, skin and soft tissues, as well as the respiratory, gastrointestinal and genitourinary tracts
Pseudomonas aeruginosa	destructive lesions, septicemia and meningitis
Salmonella	gastroenteritis, bacteraemia or septicemia, typhoid fever / enteric fever, carrier state in persons with previous infections
Shigella spp.	intestinal diseases, including bacillary dysentery
Staphylococcus aureus	nosocomial infections, enteric infections, septicemia, endocarditis, osteomyelitis and pneumonia

Water quality standards

According to the current standards, the amount of bacteria in drinking water is expressed in CFU (Colony Forming Units). The amounts of bacteria in drinking water is 100 CFU/ml at 22^0C and 20 CFU/ml at 37^0C in the European Union (EU 98/83 Directive) and 500 CFU/ml in USA (FDA 2000) (10). The Colony Forming Unit is defined

as a bacterial cell or group of bacterial cells that form a distinct colony when cultured on agar culture medium. Researches on water quality in dental units have demonstrated that the contamination of water collected from high-speed handpiece and water/ air syringe may reach levels hundreds or even thousands times higher than those accepted for drinking water (1,000,000 CFU/ml) (11).

In Romania, water quality standards are established by the Law no. 458/ 2002 (8). These standards refer to the drinking water in the public water supply system, local sources of water, transportable water tanks and domestic warm water (table 2).

Table 2. Microbiological parameters for the water bottled in glass or other recipients (12)

MICROBIOLOGICAL PARAMETERS	VALUE	METHODES
Escherichia coli /250 ml	0	ISO 9308-1
Enterococi (*Streptococcus fecalis*)/250 ml	0	STAS 3001/91 ISO 7899-2
Pseudomonas aeruginosa/250 ml	0	STAS 3001/91 pr EN ISO 12780
Colonies number /ml at 22^0 C	100	STAS 3001/91 pr EN ISO 6222
Colonies number /ml at 37^0C	20	STAS 3001/91 pr EN ISO 6222

The methods for decreasing the bacterial contamination of water from dental unit recommended by the guidelines regarding infection control in dentistry (Center of Disease Control and Prevention – CDC, Occupational Safety and Health Administration - OSHA and World Health Organization -WHO) (6,7,13). consist in:

- flushing water lines at the beginning of the day for 30 seconds and after periods of disuse, the effect being a temporary decrease in the number of bacteria;
- flushing air/water through hand pieces for 20 seconds after each patient (less microbes from that patient, microbes that can enter the hand pieces as a result of "aspiration" phenomena) (15);
- use of independent water reservoirs, separate from the public water supply (sterile water);
- use of independent water reservoirs and a concomitant periodic or continuous use germicide agents (iodophors, sodium hypochlorite, glutaraldehyde, ispropanol) according to dental unit type, water quality, amount of work in the practice, and predictive risk factors. However, treating the water implies medical personnel and patients' exposure to these substances that also act upon the equipments and materials used in clinical activity (16);
- use of anti-retraction devices: control valves, retraction testing devices, water spray devices;

- use of micro-filtration for retaining the microbes. Microbial filters are effective for decreasing the amount of bacteria but have no effect on the biofilm already present in the water network;
- use of water purification devices applied on dental unit system or independent (fig.1);
- periodical testing of water quality;
- personnel education in view of compliance with the measures for water quality control.

Fig. 1. Water purification devices

Surgical irrigations During surgical procedures the use of devices that supply sterile water is necessary. Conventional dental units are not provided with such devices, even when they have independent water reservoirs, because the water distribution system cannot be adequately sterilized. Sterile water systems avoid the dental unit and use an autoclavable tube network (17, 18). Hand pieces for oral surgery and implants, ultrasound scaling devices that produce water or other sterile solutions and use single use articles or tube systems that can be sterilized are available.

MATERIALS AND METHODS

The aim of this study was to determine the degree of contamination of the water in the dental unit and the risk of pathogens transmission on this route in Iasi dental practices in 2006.

Dental Practices

Water samples were taken from 29 dental practices in Iaşi selected according to the following criteria: location in various town areas (different water sources), number of patients, endowment (dental unit type, used devices and equipments) and daily work program. During the visits to the dental practices selected for this study a form regarding the clinical activity was filled out: daily work hours, daily number of patients, type of dental unit, other devices and equipments, clinical procedures performed, measures of water quality control.

Sample collection. A total of 93 water samples were collected of which 67 from the dental unit in four distribution points: the water source for oral rinses

(27 samples), water/air syringe (17 samples), high-speed hand piece (11 samples), and ultrasound scaling device (12 samples). Also, 26 samples of water were collected from the public water supply (the dental office sink). Samples were collected at approximately mid activity day. For collection sterile glass recipients (500 ml in volume), pretreated with 1.8% sodium thiosulfate solution (ISO – 8199/88) were used. The samples labeled and recorded were transported to laboratory in iceboxes (4^0C). Each sample received an identification number in order to facilitate data processing. Laboratory tests were performed at the Laboratory of Microbiology, Department of Environmental and Collectivities Hygiene of the Institute of Public Health Iaşi.

The used **bacteriological indicators** were**:**

a. ***Total number of mesophilic germs (TNG/ml).*** growing at 37^0C (ISA - 6222). The method of incorporation was used; measured sample volumes were incorporated into culture medium, placed on sterile Petri plates and incubated depending on specific conditions. The number of CFU/ml sample was then calculated.

According to Law 458/2002, the highest admitted value is 20 CFU/ml.

b. ***Coliform bacteria*** (CFU/100 ml). The membrane filtrated method was used (ISO 9308 –1): water samples were filtered through a hydrophilic membrane 47 mm in diameter, pore size 0.20 μm or 0.45 μm so that bacteria are retained on its surface. The membrane is then placed on a culture medium selective for lactose-positive colonies, used for detecting and counting the coliform bacteria and *Escherichia coli,* and then incubated for 24 hours at 37^0C. After incubation, the characteristic lactose-positive colonies were counted. Admitted value: 0.

c. ***Escherichia coli*** (CGU/100 ml*)*. The membrane filtrated method was used (ISO9308–1). Admitted value: 0.

For the coliform bacteria and *E. coli* subcultures were done for applying confirmation tests: oxidase and indole production. Oxidase-positive colonies were counted as being coliform bacteria and the indole-positive ones, as *E. coli.*

d. ***Enterococci*** (CFU/100 ml*).*The membrane filtrated method was used (ISO-7899-2).

The counting of intestinal enterococci was based on the filtration of a specific volume of water sample through a filter membrane with 0.45 μm pores which was then placed on a specific solid culture medium. After incubation at 37^0 C for 44 hours the suspected colonies were confirmed by membrane transfer to another medium, the esculine hydrolysis after 2 hours being followed. Admitted value: 0.

The statistical analyses of the results were carried out using SPSS 10.0. The statistical significance was established for $p<0.05$.

RESULTS AND DISCUSSIONS

Results of bacteriological tests

Of the 74 samples in which the *total number of germs (TNG/ml)* was determined, in 52.5% the values required regarding the quality of drinking water in Romania and EU were exceeded. The average value was

54.4 NTG/ml, ranging from 32.35 NTG/ml for the oral rinses source and 66.6 NTG/ml for the scaling device and high-speed hand piece (fig.2).
In 13 of the 29 dental practices (45.7%) for which TNG/ml was determined, a significant increase in its value at the collection points on the dental unit as compared to the source of public water supply was found (fig.3).

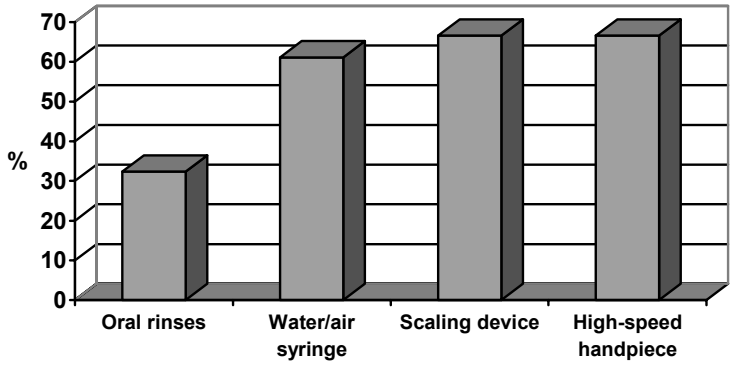

Fig. 2. The mean value of the TNG/ml according to water collection point

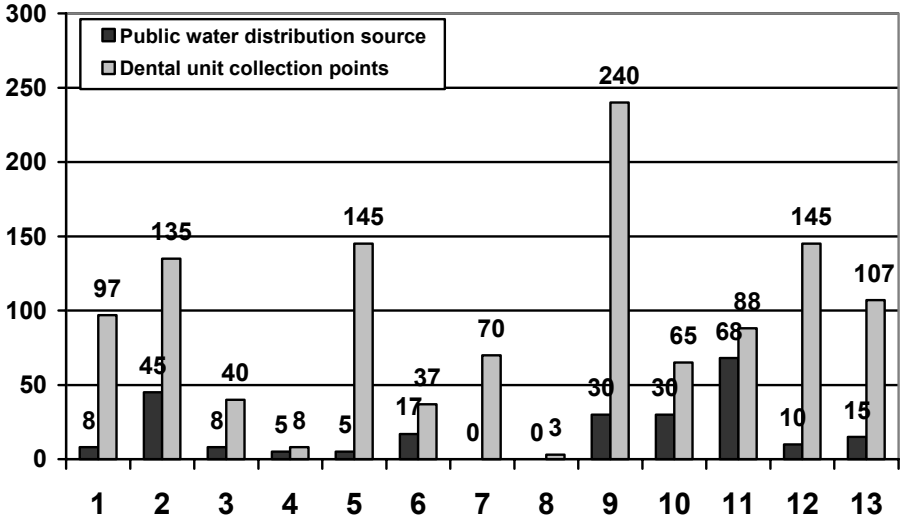

Fig. 3. Total number of germs at DUWS as compared to the source of public water supply in 13 dental practices

In order to determine the presence of coliform bacteria**,** 93 samples of which 66 from dental unit water system and 26 samples from the public water supply were processed. Coliform bacteria were present in 30.3% of the samples collected from DUWS.

Results according to the sample collection point:

I. Water source for oral rinses

- ***Total number of germs (TNG/ml).*** – ranged between 1 ÷ 152 TNG/ml, with an average of 33.35. The admitted norms regarding the quality of drinking water were exceeded in 40.7% of the samples (fig. 4).
- ***Coliform bacteria*** were present in 33.3% of the samples.

II. Air /water syringe

- ***Total number of germs (TNG/ml*** – 64.2% of the collected samples exceeded the admitted standards, the values ranging between 1 ÷ 193 TNG/ml.
- ***Coliform bacteria*** were present in 17.6% of the samples (fig.5).
- ***E. coli*** was detected in but one sample.

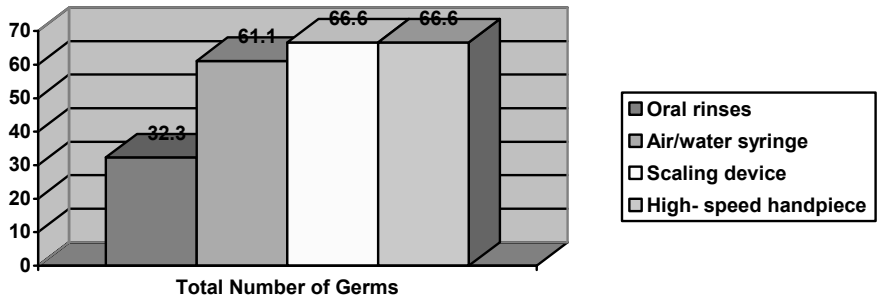

Fig. 4. The distribution of TNG exceeding the standard values related to water collection point

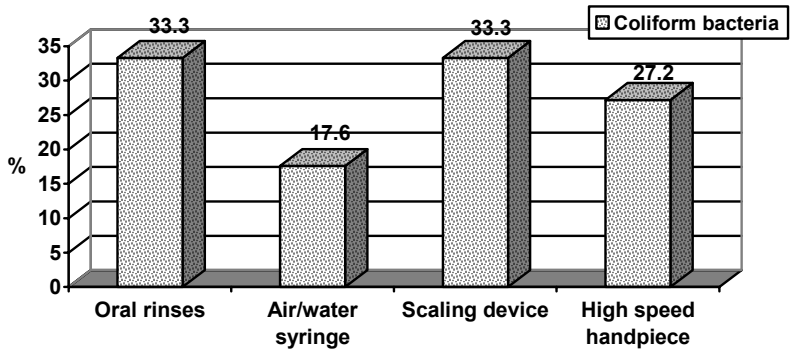

Fig. 5. The distribution of coliform bacteria related to water collection point.

III. Ultrasound scaling

- ***Total number of germs (TNG/ml*)** – ranged between 2 and 247 TNG/ml, 66.6% of the samples exceeding the standards. Depending on sample collection point, the highest TNG values were found in the ultrasound scaling device, thus proving the high level of contamination associated with this clinical procedure.
- ***Coliform bacteria*** were present in 33.3% of the samples.

IV. High-speed handpiece

- ***Total number of germs (TNG/ml*** – 66.6% of the collected samples exceeded standards.
- ***Coliform bacteria*** were present in 27.2% of the samples

V. Water samples from the water supply (sink).

- ***Total number of germs (TNG/ml)***– ranged between 3 and 147 TNG/ml, 52.3% of the samples exceeding the standards.
- ***Coliform bacteria*** were present in 22.2 % of the samples. In 52.2% of the dental practices under investigation in which coliform bacteria were found presented an increased value at the dental unit collection points as compared to public water supply.

Enterococci were absent in all collected samples.

Five of the sampled DUWS (all bottle fed). were reported to have been sanitized using alcohol solutions (Lisetol), chlorinated compounds (chloramine), and 2% chlorhexidine gluconate under the form of various trade products. No statistically significant relation between the degree of contamination of the water in the dental unit and the treatment of DUWS was established.

Twenty-three of the 29 dental practices (79.3%) use water from the public water supply and 6 (10.7%). use distilled water reservoirs for the water/air syringe, high-speed hand piece cooling and ultrasound scaling device. No significant differences in dental unit water contamination between the dental units using distilled water and those connected to public water supply were noticed. A single dental unit is provided with water sterilization equipment. In 8 of the 29 dental practices (27.58%). water in the dental unit is usually flashed at the beginning of every workday.

The average number of treated patients per day in the dental practices included in this study was 9.6. Significantly fewer bacteria were found in the dental practices in which less than 8 patients were treated during the workday (average value for TNG: 37,05) as compared to the dental practices which reported over 8 patients (average value for TNG: 50,93).

CONCLUSIONS

- The contamination of the dental unit water supply system by microbial biofilm deposits is associated with a significant risk of infection in patients during dental treatments as well as in the personnel working in oral health care.
- The results of the laboratory tests have demonstrated that in over 50% of the samples, the total number of germs (TNG/ml), considered the

most significant marker of contamination, exceeded the standards regarding the quality of drinking water required by Romania and European laws.

- A significant increase in NTG/ml values at dental unit collection points as compared to public water supply source was recorded in 45.7% of the dental practices.
- Significantly fewer bacteria were found in the dental practices which reported less than 8 patients treated during a workday.
- No significant differences in water contamination between the dental units using distilled water and those connected to public water supply were noticed, probably due to some differences within the procedure.
- The pathogenic potential and the metabolism of the biofilm in the dental unit, insufficiently evaluated till now, require further study in this field in view of elaborating effective strategies for water supply aiming the decreasing of the risk of infection transmission on this route.

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