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Microbial Contamination in the Waterlines of the Dental Units in Sari School of Dentistry in 2019

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Abstract

Background: Microbial contamination of water sources in dental units is one of the problems in dentistry as such contamination can lead to the occurrence of dangerous infections. Given the importance of infection control and creating a healthy environment for the treatment of patients, this study conducted a microbiological analysis of water in the dental units of Sari School of Dentistry in 2019.

Methods: In this descriptive-analytical and cross-sectional study, three units from each of the endodontic, restorative, surgical, pediatric, and prosthetic units of Sari School of Dentistry were randomly selected. Samples of 3-way syringe water, turbine water before and after flushing, and glass water were prepared and transferred to the microbiology department in sterile tubes. The samples were then cultured in Müller Hinton agar and blood agar and placed in an incubator at 37 °C for 24 hours. After incubation, gram staining was performed on the samples in which the bacterial colony had grown. The bacteria were examined for morphology and gram reaction.

Results: Out of 61 samples, 23 samples (37.7%) were infected with bacterial colonies. The highest frequency of bacterial infection was found in the mixed bacterial group (gram-positive cocci and gram-positive bacilli). The results of the chi-square test and Fisher's exact test showed no significant relation between the sampling site and contamination (P = 0.309).

Conclusion: Water contamination in the pediatric and endodontic units in Sari School of Dentistry is high. Thus, effective measures should be taken to reduce water pollution in these units and reduce the risk of infection in staff and patients.

Keywords: Dental unit, Dental unit waterline, Biofilm, Dental infection control

Background

The water path of the dental unit is part of the dental office equipment that provides the necessary water for the turbine, 3-way syringes, and the ultrasonic scaler. Water particles may be inhaled by the patient and the dental staff during dental procedures. A large volume of water produced by suction is drained, but some of it is swallowed by the patient (1). The handpieces used in dental procedures are also contaminated with oral microorganisms, and over time these microbes may accumulate and colonize in the aqueous systems of the dental unit. The presence of strains in the waterway causes infections in the lower respiratory tract (2). The prevalence of Legionella is higher among members of the dental team than the general population, confirming that aerosols created by dental equipment can be a source of infection (3).

Infection control aims to minimize the risk of contact with pathogenic organisms and to create a healthy environment for the treatment of patients. One of the contamination risks is the rapid formation of biofilms in water flow pipes along with the production of contaminated aerosols. A biofilm is a complex set of heterogeneous microbial masses that form on the surface in contact with the liquid. Due to the low flow and high static period, dental units provide a suitable place for biofilm formation. Low water velocity inside the pipe causes bacteria to bind and colonize. Although the biofilm remains attached to the walls of the pipe, the released microorganisms are often present and may be transmitted to the patient's mouth or space through 3-way syringes or other dental instruments (4-7). Besides, particle size and concentration of bioaerosols can cause sinusitis, allergic alveolitis, and other respiratory diseases. Hence, infection control is especially important in immunocompromised patients and transplant recipients (8, 9).

Since dental staff and patients are exposed to water and aerosols produced in the dental unit, the water quality of the dental unit should be controlled regularly. Thus, given the importance of infection control and creating a healthy environment for the treatment of patients, this study aimed to conduct a microbiological analysis of water supply in different units in the Sari School of Dentistry in 2019.

Methods

This cross-sectional descriptive-analytical study was conducted in 2019 at Sari School of Dentistry. Following Abbasi et al. (9) who reported the microbial contamination frequency of 6.7%, taking an accuracy of 0.06, and using the sample volume formula for a specific prevalence, the number of samples was determined to be 61.

In the present study, out of 30% of the total active units (50 units) in the endodontic, restorative, surgical, pediatric, and prosthetic units of Sari School of Dentistry, a total of 15 units were sampled. From each unit, 3 active and widely used units were selected. Sampling was performed on all three selected units, and in each unit on three different days of the week (Saturday, Monday, Wednesday). The samples contained 3-way syringe water, turbine water before flushing (before starting work), turbine water after two minutes of flushing, and glass water. A sample of the municipal used in the dental school water was also taken. From each unit, 50 ml samples were prepared and placed in coded sterile tubes. The sample sterile containers were kept in the vicinity of ice and immediately transferred to the Microbiology Research Laboratory of the School of Medicine. In the laboratory, the samples were cultured in Müller-Hinton agar and blood agar media and incubated at 37 ° C for 24 hours. After incubation, gram staining was performed on samples in which the bacterial colony had grown. The bacteria were examined diagnostically based on morphology and gram reaction. The catalase test was used to separate staphylococcal bacteria from streptococcus. Accordingly, Staphylococcus catalase-positive and Streptococcus catalase-negative bacteria were identified. To determine the type of staphylococci, coagulase tests were performed with rabbit plasma in the slides and tubes. The presence of agglutination in the slide and clot in the tube was detected as coagulase-positive and as Staphylococcus aureus; and coagulase-negative and mannitol-negative were detected and separated as staphylococci epidermidis and saprophyticus by nobiocin test. Novobiocin-susceptible species were

considered as Staphylococcus epidermidis and novobiocin-resistant species were identified as Staphylococcus saprophyticus. Furthermore, small gram-positive bacilli resembling Chinese letters and catalase-positive morphology were also considered as diphtheriae. Gram-negative bacilli were re-cultured in McConkey culture medium and an oxidase test was performed to determine bacterial species. Gram-negative and oxidase-negative bacilli were identified as bacteria from the Enterobacteriaceae family, and MRVP Urea, SIM, Simmon citrate, and TSI differential media were used to determine Enterobacteriaceae species. Gram-negative and oxidase-positive bacteria were considered non-Enterobacteriaceae.

The data were analyzed with SPSS software (version 16) and described using frequency and percentage. Moreover, mean and standard deviation were used to determine the number of colonies. Contamination frequencies were compared between different units using the chi-square test or Fisher's exact test. Values with a significance level of less than 0.05 were considered significant.

This research project was carried out after approval by the Vice-Chancellor for Research and Technology of Mazandaran University of Medical Sciences, the approval of the Biomedical Research Ethics Committee (code IR.MAZUMS.REC.1398.432), and compliance with the principles of ethics in biomedical research.

Results

Data analysis was performed on 300,209 bacterial colonies found in 61 samples, including 1 sample of municipal water and 60 samples taken from 3-way syringe water, turbine water before and after flushing, and glass water from the endodontic, restorative, surgical, pediatric, and prosthetic units. Out of 61 samples, 23 samples (37.7%) were infected with bacterial colonies and 38 samples (62.3%) were free of infection. Furthermore, mixed bacteria (gram-positive cocci and gram-positive bacilli) were most frequently found in glass waster of restorative and pediatric units. Gram-positive Staphylococcus epidermidis was also found with the lowest frequency (Table 1).

Table 1. The frequency of bacteria found in the water samples

Bacterial groups	Bacterial colonies	Frequency (%)
Mixed bacteria (gram-positive cocci and gram-positive bacilli)	20000	66.62
Diphtheroid gram-positive bacilli	100116	33.35
Spiral bacillus	62	0.02
Pseudomonas aeruginosa	16	0.005
Staphylococcus epidermidis	15	0.005

Water contamination in different units is shown separately in Table 2. The highest contamination level was found in the pediatric unit and the lowest contamination level was found in the surgical unit. Fisher's exact test showed a significant relation between different units in terms of water contamination ($P < 0.05$).

Table 2. Number of contaminated samples in different units

Unit	Percentage of contaminated samples (Total samples)	Percentage of non-contaminated samples (Total samples)
Pediatric	9 (75)	3 (25)
Endodontic	7 (58.3)	5 (41.7)
Restorative	6 (50)	6 (50)
Prosthetic	1 (8.3)	11 (91.7)
Surgical	0 (0)	12 (100)
Total	23 (38.3)	37 (61.7)

The contaminated samples in different parts of each unit are shown in Table 3. The chi-square test did not show a significant relation between the sampling site and the presence of contamination ($P=0.309$). The highest pollution level was found in the turbine water before flushing and the lowest pollution level was found in the 3-way

syringe and turbine water samples after two minutes of flushing. Moreover, there was no significant relation between turbine water contamination before and after flushing as indicated by the results of the chi-square test (P= 0.136).

Table 3. Number of contaminated samples in different locations

Sampling site	Percentage of contaminated samples (Total samples)	Percentage of non-contaminated samples (Total samples)
Pre-flushing turbine sample	8 (53.3)	7 (46.7)
Glass water	7 (46.7)	8 (53.3)
3-way syringe water	4 (26.7)	11 (73.3)
Turbine water after 2-min flushing	4 (26.7)	11 (73.3)
Total	23 (38.3)	37 (61.7)

Discussion

This study examined the presence of microbial contamination of water samples from different parts of 15 units from five different wards in the Sari School of Dentistry. The results showed that 37.7% of the samples were contaminated with bacterial colonies. Varying levels of water pollution in dental units have been reported in previous studies. For instance, Yazdanbakhsh et al. reported 64% water contamination in Shahroud Dental School, Malakootian and Nowroozi reported 25% microbial contamination in Kerman, Ghaem Maghami et al. found 50% water contamination in Shahid Beheshti Dental School, and Ghasempour et al. reported a microbial contamination of 33.3% in Babol (10-13). Güngör et al. reported bacterial contamination in 37 out of 50 water samples of dental units to be beyond the limits set by the American Dental Association (14).

An analysis of the samples collected in this study showed that the mixed bacteria group (gram-positive cocci and gram-positive bacilli) had the highest frequency, while the Staphylococcus epidermidis group had the lowest frequency. There was also a significant relation between the sampling unit and the presence of contamination. For instance, the highest contamination level was found in the pediatric unit and the lowest contamination level was found in the surgical unit. In a similar vein, contamination with gram-positive cocci and gram-positive and gram-negative bacilli was also reported by Abbasi et al. in dental units of Shahed Dental School (9). A study by Szymańska et al. at dental clinics in Lublin, the Netherlands showed that the most common bacteria in all samples were gram-negative bacilli, with Ralstoniaceae, Sphingomonadaceae, and Pseudomonadaceae being the most frequent gram-negative bacteria and Brevibacterium as the most common gram-negative bacteria. Gram-negative bacilli were also found in the samples of the present study but were not among the most common bacteria (15). According to Salam et al. the most common bacteria found in the water supply of Kerala Dental College units in India were gram-negative bacilli, especially Escherichia coli, Pseudomonas, and Klebsiella. Pseudomonas aeruginosa gram-negative bacilli were also observed in the present study (16). Abbasi et al. examined the water resources of the dental units of Shahid Beheshti Dental School and reported that the samples of the periodontal and prosthetic units were infected with enterococci and streptococci, respectively. Orthopedic specimens were also contaminated with Staphylococcus epidermis and gram-negative bacilli, which were observed in low frequency in the present study (17).

The highest pollution level was found in this study in the turbine water before flushing and the lowest pollution level was found in the 3-way syringe and turbine water samples after two minutes of flushing. However, there was no significant relation between turbine water contamination before and after flushing as indicated by the results of the chi-square test. Different studies have reported varying pollution levels. For instance, in a study conducted by Ghasempour et al. at Kerman Dental School, the highest mean bacterial count was reported in the glass filling service of the oral diseases unit, and the lowest mean bacterial count was reported in the 3-way syringe water of the surgical unit (13). Memarian et al. found the turbine head had higher levels of contamination than other parts

of the unit (18). Furthermore, Khorakian et al. investigated the contamination of Pseudomonas aeruginosa in the waterlines of the units of Mashhad Dental School and found high contamination of these species in the water supply of the active units, in the units studied at the beginning of the working day. In line with the present study, the water pollution of the units after 2 minutes of flushing was less than before. Following these findings, it can be concluded that flushing the unit water before work can play a role in reducing pollution (19). However, Szymanska showed that the pollution in high-speed turbines, low-speed turbines, and 3-way syringes were not statistically significant, but their pollution level was much higher compared to the water source (20).

In many studies, there was a higher level of contamination in different parts of the unit compared to the water source. Thus, it can be suggested that the main cause of water contamination in dental units is the formation of biofilms in the ducts of the water system and the difference in the amount of contamination in different parts of the unit can be related to the volume of water used and water flow in each part of the unit. Given the existing conditions and facilities, some measures should be taken to reduce water contamination levels as much as possible.

The small number of samples, the low number of units, and also the number of sampling times were some of the limitations of the present study.

Conclusion

The data in this study confirmed the high water contamination in the pediatric and endodontic units in Sari School of Dentistry. Thus, some measures need to be taken to reduce water contamination levels in these units and reduce the risk of infection in staff and patients. Furthermore, more extensive research is needed to determine the microbial contamination of water in dental units in each ward separately and evaluate ways to deal with these contaminants.

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Conflict of interest

The authors reported no conflict of interest.

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