

Evaluation of Bacterial Contamination in Dental Unit Waterlines of Qazvin' Dental School, Iran

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Background: Contamination of dental unit waterlines is a well-known issue in the field of dentistry.

Objectives: This study was designed to evaluate contamination of dental unit waterlines by common gram positive and negative bacteria in Qazvin's dental school during the year 2012.

Materials and Methods: This survey was performed on 24 dental units routinely used in the dental school of the city of Qazvin. Four samples of 100 mL were obtained in sterile bottles from each unit at different work stages as follows: the beginning of daily activity, after flushing for 30 seconds, following two minutes of flushing, and after the end of daily practice. The number of bacterial colonies was determined for each sample and the mean colony number was calculated.

Results: Bacterial contamination was found in all 24 units at the beginning of daily activity with a mean colony count of 17850. The number of colonies decreased to 3250 following 30 seconds of flushing in 16 units and later to 1837 after flushing for two minutes in 13 units. In addition, the number of contaminated units at the end of daily activity was 19 with a mean colony number of 11170.

Conclusions: Flushing dental unit waterlines are an effective measure in reducing the number of bacterial contaminants.

Keywords: Gram Positive; Gram Negative; Biofilm; Colony Forming Unit assay

1. Background

Infection control in dentistry is one of the most important topics, which was initially introduced to address hepatitis and AIDS as two major global public health problems. Undoubtedly, specialists in the field of dentistry believe that investigation and research on the subject of infection transmission play a crucial role in designing better infection control measures and prevention strategies. Contamination of dental unit waterlines is a well-known concern, and can originate from either the patients or water supplies. Dental unit waterlines are suitable places for rapid formation and growth of various bacteria which accumulate on the inner surface of waterlines in high-speed hand pieces, turbines, air/water syringes, and ultrasonic scalers, leading to the generation of a thin layer of microorganisms called biofilm(1, 2). Biofilms are adherent colonies of bacteria, fungi, and protozoa, which are usually surrounded by a polymeric matrix (3, 4).

The formation of biofilm along dental unit waterlines was initially identified almost 40 years ago (5). The number of microorganisms present in biofilms is enormously high with most being pathogens. Biofilms contain a deli-

cate protective coating, called glycocalyx, for the attachment of microorganisms and once generated for the first time, they act as a storage source for extensive growth of free microorganisms in waterlines. Biofilms are resistant to most antimicrobial agents, leading to the appearance of serious problems in controlling such organisms. Thus, the formation of biofilms eventually causes contaminated water flows into the waterlines of dental units (2). To prevent the transmission of infection to personnel and patients, improvement in the quality of output water associated with plumbing system or flush tank and dental unit waterlines must be vigorously considered (6).

2. Objectives

In this research, the contamination level of dental unit waterlines at four different departments of Qazvin Dental School was evaluated.

3. Materials and Methods

The bacteriological media used for the primary identification of bacteria were blood agar (BA) (Merck, Germany)

and eosin methylene blue (EMB) agar (Merck, Germany), which were prepared according to the manufacturers' instructions. The bacteria isolated on BA and EMB were further confirmed by Gram staining. Samples were collected from the waterlines of dental units at Qazvin Dental School under sterile conditions. A total of 96 samples (four samples per unit), obtained from the turbine output water of 24 units located at four different departments (pediatrics, restorative dentistry, endodontic and periodontics) were used in this study (six randomly selected units from each department). Also, four samples were collected from drinking water supplies within the city of Qazvin and used as environmental control samples. Four samples, of 100 mL each, were collected in sterile bottles from each unit at different work stages, including: the beginning of daily activity, after flushing for 30 seconds, following two minutes of flushing, and after the end of daily practice. Samples were immediately transferred to the department of microbiology and following initial centrifugation at 3500 rpm for 20 minutes, the bacterial sediment was resuspended in 1 mL sterile of dH₂O, cultured on BA and EMB media, incubated at 37°C for 24 - 48 hours, and the number of colonies grown on media were counted, and the final volume of each sample was calculated and reported as CFU/100mL.

4. Results

Of the 24 collected samples from the Department of Periodontics, 20 samples (83.5%) were contaminated with microorganisms and the remaining four were not contaminated. Similarly, regarding the Endodontic, Restorative Dentistry, and Pediatrics Departments bacterial contamination was observed in 19 (79%), 18 (75%), and 15 (62.5%) samples, respectively. All 24 dental units at the four different departments were found to have bacterial contamination before the beginning of daily activity. The mean number of bacterial colonies was 18750 with a minimum of 500 and a maximum of 200000 colonies. This contamination level decreased to 16 units and 3250 colonies after flushing for 30 seconds (minimum: 200; maximum: 65000). The number of contaminated units following flushing for two minutes reached 13 with a mean number of 1837 colonies (minimum: 200; maximum: 30000). Of the 24 dental units, at the end of daily activities, 19 showed bacterial contamination with a mean number of 11170 colonies (minimum: 400; maximum: 11,000). The difference observed between the numbers of colonies in samples obtained at different time intervals was found to be statistically significant ($P < 0.05$).

5. Discussion

According to the findings of this study, the highest level of contamination was observed in the waterlines of dental units used in Endodontic (83.5%) and Periodontics (79%) departments. This could be associated with the use of older dental units in these departments. Therefore,

considering new methods for better detection of bacterial contamination, conducting further research with more recent equipment and facilities on dental units of Qazvin Dental School is necessary. Also, performing preventive practice against biofilm formation is of prime importance and essentiality.

In addition, the observation of a considerable decrease in the level of bacterial contamination at different stages of daily practice (flushing for 30 seconds, two minutes, and at the end of daily work) indicates that continuous water flow (flushing) before the beginning of daily activities and within working hours can be effective for preventing bacterial infection.

In similar studies carried out in the USA, 20% of West Virginia students and staff of dentistry department were found to be positive in terms of antibodies in their serum (7). These studies indicate the increased risk of exposure of dentistry personnel to pathogenic gram positive and negative bacteria and that the dental unit waterlines can be the source of infection. In the present study, similar to the study mentioned above, bacterial contamination of water samples, obtained from different departments of Qazvin Dental School, was investigated. The findings of the current study which were obtained based on bacteriological cultivation of the collected samples on BA and EMB agar media showed many similarities with the study mentioned earlier (7). Currently, the PCR method is used as the method of choice for bacteriological identification at CDC, by which a higher level of contamination in collected samples, is revealed. Also, the results obtained by fluorescent antibody methods are similar to that of the PCR technique (8). Therefore, the results of most studies, in which samples are cultured on bacteriological media, may not be precise. In our study, the level of bacterial contamination observed was higher than the recommended international standards. According to our findings and also the results of other research, factors such as geographical location, quality of water, quality of maintenance protocol used for dental equipment, the effective use of infection control measures, use of modern turbines equipped with anti-suction devices, renewing the structure of the dentistry department, use of standard pipe and fittings in plumbing systems of dental units, and the application of new dental units are important and have a direct effect on reducing the prevalence of microbial contamination (9). Zanetti et al. investigated the effect of chemical and physical properties of water on the prevalence of gram (positive and negative) bacteria, and showed that water temperature has a reverse relationship with the presence of bacterial contamination whereas the amount of minerals and residual chlorine in dental waterlines has a direct association with the concentration of gram positive and negative bacteria. In this study (similar to the present study) the direct cultivation method was used to identify the presence of bacteria (9). Walker et al. in 2000 performed a study based on bacteriological cultivation of 55 sam-

ples from various units that used different water sources. They concluded that the type of water source feeding the dental units affect the type of bacteria and the level of contamination (8).

In a study by Singh et al. (10) in 2003, the main techniques used was advanced fluorescent in situ hybridization (FISH) and electron microscopy. They investigated the biofilm obtained from dental unit water lines (DUWL) and reported that the high contamination prevalence in dental unit water samples could be the result of high amounts of microbial biofilms enriched with bacteria along the narrow waterlines of these units.

Similarly, Castiglia et al. (11) evaluated the presence of contamination in water and air systems of 102 dental units in eight Italian cities. The results showed that microbial contamination was present in all dental unit waterlines used in all dental surgeries. Also, the level of contamination in water samples was reduced during working hours, a finding compatible with the results found by the present study.

Likewise, Pasquarella et al. (12) investigated the contamination level of dental unit water systems in dental clinics of Italian cities. Sampling was performed during the first five work days (before and during work-shifts). The results showed that in 50% of cases, there was a considerable reduction in microbial contamination of dental unit waterlines during working hours.

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Authors' Contributions

1- Study concept and design: Dr. Saffarpour. 2- Acquisition of data: Dr. Peymani. 3- Analysis and interpretation of data: Dr. Ebrahimi. 4- Drafting of the manuscript: Dr. Rahmani. 5- Critical revision of the manuscript for important

intellectual content: Dr. Saffarpour. 6- Statistical analysis: Dr. Rahrotaban. 7- Administrative, technical and material support: Dr. Peymani. 8- Study supervision: Dr. Rahmani.

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