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The effect of the use of disinfectants during COVID-19 pandemic on the bacterial contamination of dental unit waterlines

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Abstract

Background: The emergence and spread of SARS-CoV-2 has increased environmental disinfectant usage to reduce the transmission of this virus. Ethanol 70%-90% and 5% sodium hypochlorite have the highest consumption for disinfection of various environmental surfaces during the COVID-19 pandemic. Dental unit waterlines (DUWLs) are more susceptible to microbial contamination due to their particular structure. This study aimed to investigate the effect of increasing the use of disinfectants during the COVID-19 pandemic on the bacterial contamination of DUWLs.

Methods: During November (before the COVID-19 pandemic) and December (during the COVID-19 pandemic), a questionnaire was used to assess the frequency of disinfection of unit surfaces and other environmental surfaces and the type of disinfectants used. The water samples were collected from different parts of DUWLs. The gram staining method followed by the biochemical method was used to identify the desired bacteria.

Results: The results showed that the frequency of disinfection of dental units increased 8 times in December compared to November. There is a significant inverse relationship between the frequency of disinfection of dental units surfaces and the bacterial contamination of DUWLs.

Conclusion: The microbial load in different parts of the DUWLs was less than 200 CFU/mL. The American Dental Association (ADA) recommended and indicated the allowable microbial concentration and the appropriate quality and water used in these units.

Keywords: Dental clinics, Disinfectants, Biofilms, Bacteria, SARS-CoV-2

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Introduction

Dental units are the most important parts of dental clinics. In these units, water is delivered to different handpieces through thin plastic waterline tubes. In dental units, water is supplied through two types of systems, open and closed. Both open and closed systems, water supply sources, municipal water sources, and reservoirs belong to a unit. Different parts of dentistry can be contaminated with microorganisms. Dental unit waterlines (DUWLs) have a proper environment for the presence and growth of some organisms due to their more suitable conditions (1). Over the past two decades, the water used in dentistry has been high microbial counts, typically 10^4 to more than 10^6 CFU/mL (2). Environmental factors such as contaminated surfaces and objects are the primary sources of contamination of dental units, specially DUWLs (3). Therefore, there can be a significant relationship between environmental hygiene in dentistry and microbial contamination in DUWLs (4). The outbreak of the COVID-19 in December 2019 has had many effects on environmental health (5, 6). It has increased hygienic behaviors such as attention to surface cleanliness and personal equipment to reduce SARS-CoV-2 transmission (7-9). During the COVID-19 pandemic, the use of alcohol and other disinfectants to decontaminate various surfaces and environments, especially therapeutic environments such as dentistry, has increased (10-12). Expanding the use of disinfectants and paying attention to environmental health can effectively reduce other pathogenic microbes and reduce the SARS-CoV-2 virus (13-16). Many bacteria and potential opportunistic pathogens which cause pneumonia, other respiratory infections, or wound infections in immunocompromised individuals have been reported by various studies (17-19). All dental procedures, including the use of handpieces, create contaminants suspended by microorganisms, including potential pathogens, in the air (20). Given that dentists and patients can be exposed to these microorganisms through water and aerosols produced in these units, contamination of these surfaces with microorganisms can be a risk factor for them (21-23). According to current knowledge, in addition to identifying microorganisms, determining their number is essential. According to the ADA recommendation, water is unsuitable for human consumption if the CFU/mL exceeds 200 (24). Due to the high level of exposure of dentists and patients to water and aerosols produced by dental units, it is necessary to evaluate the microbial quality of water in these units to protect the health of these people. Hence, the present study was conducted to investigate the increase in the use of disinfectants on bacterial contamination of DUWLs and quantify *Acinetobacter* spp, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and Streptococcus spp bacteria from the DUWLs of the School of Dentistry in AJUMS during November and December.

Materials and Methods

Disinfection

In the present study, 28 questionnaires were used to assess the frequency of disinfections of unit surfaces and other environmental surfaces during the day and the type of disinfectants used. The questionnaires were completed by the cleaning team of the School of Dentistry.

Sample collection

This cross-sectional descriptive study was conducted in 2019. The samples were collected during November and December. As shown in Table 1, from 14 dental units in the School of Dentistry, 112 water samples of air/water syringe, mouth washing water, tap water, and handpiece of each unit were collected. Sampling was taken at the beginning of the day, before the start of dental activities. Sterile 100 mL containers were used to collect samples and transfer them to the laboratory under controlled conditions in light and temperature, and the analyses were started immediately (25,26).

Samples preparation and analysis

Petri plates were used to prepare culture media. After centrifugation of each sample, 0.1 mL of sediment was taken for analysis. Then, each sample was spread on Sabouraud Dextrose Agar (SDA) and MacConkey (MAC) agar culture media to perform a microbial culture. The samples were incubated. Gram staining followed by biochemical methods was used to identify the target bacteria. Then, the colony-forming units were counted.

Table 1. Number of samples taken from different parts of DUWLs in November and December

Samples Types	November	December
Mouth washing water	14	14
Tap water	14	14
Air/water syringe	14	14
Handpieces	14	14
Total	56	56

Gram staining

The gram staining method is based on the ability of microorganisms to preserve the color of the stain. This method divides bacteria into gram-negative and gram-positive bacteria. In this method, if the bacterium were gram-negative, after using alcohol for decolorization, the purple color of the primary stain would disappear. Still, if the bacterium were gram-positive, the use of alcohol would not decolorize the stain. After determining the gram type of the studied bacteria, the pink color was imparted to gram-negative bacteria using counterstain. After this step, a smear of the bacterial was prepared, and then, heat-fixed. The crystal violet has filled the slide for 1 minute. The excess stain was washed with running water. Gram's iodine was added for 1 minute and the smear was rinsed with water. By using 0.25% safranin, it was counterstained for 30 seconds and again washed with water. Finally, the drained and blotted smear was analyzed under an oil immersion microscope. Purple and pink bacteria were considered as gram-positive and gram-negative bacteria, respectively. For further biochemical identification, colonies were subcultured onto non-selective nutrient media.

Biochemical identification

Pseudomonas aeruginosa secretes pyrobin (reddish-brown), pyordin (yellow-green and fluorescent), pyocyanin (green-blue), and pigments. Mainly in the laboratory, this bacterium has a pearl-like appearance and a grape-like odor. Acinetobacters are oxidase negative, catalase-positive, exhibit twitching motility, and are identified based on the biochemical reactions; they can utilize many substrates for growth. On the gram stain, both species appear as coccobacilli. Although the colonies of this bacterium are colorless, they produce pale yellow to white-gray grains in a solid culture medium. Streptococci are immotile and gram-positive bacteria. Klebsiella is also a gram-negative bacterium that can ferment lactose. This bacterium can metabolize glucose through gas production. In MacConkey agar, these organisms appear as mucoid colonies. They show negative results for the citrate test and Vogues-Proskauer test and positive reactions for methyl red and indole.

Statistical analysis

Descriptive statistics, including the minimum, maximum, mean, and standard deviation, were determined using SPSS version 22 software. Kolmogorov-Smirnov (27) and Mann-Whitney U test (28) were used to evaluate data normality and compare the mean concentrations of CFU/mL of DUWLs in November and December, respectively. The Excel software was used to draw graphs.

Results

Consumption of disinfectants

As shown in Table 2, ethanol 70%-90% and 5% sodium hypochlorite were used to disinfect the surfaces of dental units and other peripheral surfaces. Ethanol 70%-90% is the most common disinfectant in hospitals, clean rooms, and medical device manufacturing. Different solutions, purity grades, concentrations, and different types of alcohol yield beneficial cleaning and disinfection properties when applied correctly. 5% sodium hypochlorite is most frequently used as a disinfecting agent. It is an effective broad-spectrum disinfectant against viruses, bacteria, fungi, and mycobacterium (29). In December, due to the outbreak of the COVID-19 and the need to increase environmental health, the use of disinfectant compounds to disinfect unit surfaces and other contact surfaces had quadrupled (30).

The questionnaires showed that in December compared to November, to prevent the transmission of the SARS-CoV-2 virus, the use of ethanol 70%-90% and 5% sodium hypochlorite for disinfection of dental equipment such as units increased 16 times.

Bacterial density

Table 3 presents the number of bacteria from air/water syringe, mouth washing water, tap water, and handpiece in dental units in November and December. According to the results, the dental units under study delivered water with bacterial quality lower than 200 CFU/mL during November and December, meeting the accepted ADA standards. Comparison of the results showed that the mean CFU/mL of microorganisms of the dental units in December (60.99) was lower than that in November (76.27) (Figure 1). The minimum CFU/mL of microorganisms was related to the samples taken from air/water syringes of dental units. In November, the CFU/mL of the air/water syringe for Acinetobacter spp (91.63), *Pseudomonas aeruginosa* (59.34), Streptococcus spp (50.48), and *Klebsiella pneumonia* (64.51) dropped to Acinetobacter spp (71.32), *Pseudomonas aeruginosa* (48.73), *Streptococcus* spp (44.61), and *Klebsiella pneumonia* (54.21) in December. The results obtained from the examination of the samples taken from the handpiece in November and December were almost similar to those obtained from the samples collected from the air/water syringe section of the dental units, Acinetobacter spp (99.21 CFU/mL), *Pseudomonas aeruginosa* (65.24 CFU/mL), *Streptococcus* spp (62.34 CFU/mL), and *Klebsiella pneumonia* (58.65 CFU/mL). Also, in November, the mean concentrations of microorganisms were related to the samples collected from the mouth washing water in the decreasing order, Acinetobacter spp (99.44 CFU/mL), *Pseudomonas aeruginosa* (71.48 CFU/mL), Streptococcus spp (66.27 CFU/mL), and *Klebsiella pneumonia* (65.21 CFU/mL), respectively, which were higher than the mean concentrations of Acinetobacter spp (82.41 CFU/mL), *Pseudomonas aeruginosa* (62.74 CFU/mL), Streptococcus spp (50.37 CFU/mL), and *Klebsiella pneumonia* (49.58 CFU/mL), respectively, in the samples collected from the mouth washing water in December. The mean concentrations of *Acinetobacter* spp, *Pseudomonas aeruginosa*, *Streptococcus* spp, and *Klebsiella pneumonia* in Tap water in November were 140.45, 88.89, 61.82, 78.49 CFU/mL, respectively, which were higher than the mean concentrations of *Acinetobacter* spp (118.57 CFU/mL), *Pseudomonas aeruginosa* (73.64 CFU/mL), *Streptococcus* spp (50.94 CFU/mL), and *Klebsiella pneumonia* (63.45 CFU/mL), respectively, in the samples collected in December. The final stage of bacterial culture is shown in Figure 2.

The minimum, maximum, mean, and standard deviation of the studied bacteria in November and December are presented in Table 4. Also, the results of statistical analysis showed that the statistical difference of CFU/mL of water samples taken from different parts of the dental unit was not significant ($P > 0.05$). The Kolmogorov-Smirnov test showed the non-normal

Table 2. Types of disinfectants used and mean frequency of disinfection

Month	Type of disinfection	Mean frequency of disinfection (per day)
November	5% sodium hypochlorite/ ethanol 70-90%	2
December	5% sodium hypochlorite/ ethanol 70-90%	16

Table 3. Mean CFU/mL of DUWLs water samples in November and December

Microorganisms	*Acinetobacter* spp		*Pseudomonas aeruginosa*		*Streptococcus* spp		*Klebsiella pneumonia*		Average bacterial contamination
	November	December	November	December	November	December	November	December	
Air/water syringe	91.63	71.32	59.34	48.73	50.48	44.61	64.51	54.21	60.60
Mouth washing water	99.44	82.41	71.48	62.74	66.27	50.37	65.21	49.58	68.43
Tap water	140.45	118.57	88.89	73.64	61.82	50.94	78.49	63.45	87.77
Handpiece	99.21	79.24	65.24	49.27	62.34	49.20	58.65	45.61	63.77

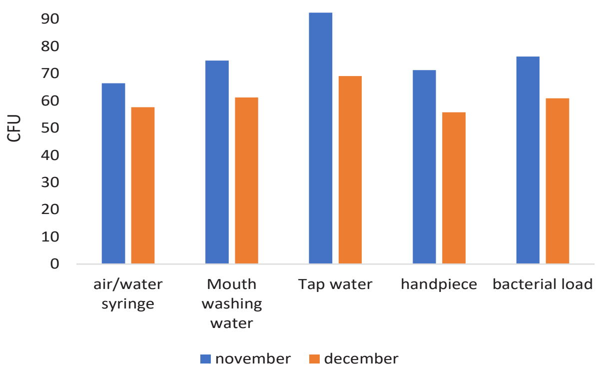

Figure 1. Comparison of CFU/mL of DUWLs in November and December.

Figure 2. The final stage of microbial culture of bacteria.

Table 4. The minimum, maximum, mean, and standard deviation of the studied bacteria in November and December (CFU/mL)

Microorganisms	Min	Max	Mean + SD		Acceptable level
			November	December	
Acinetobacter spp	12	229	107.68 ± 22.14	87.88 ± 20.98	200
Pseudomonas aeruginosa	15	183	71.23 ± 12.76	58.60 ± 11.94	200
Streptococcus spp	9	198	60.22 ± 6.79	48.78 ± 2.87	200
Klebsiella pneumonia	12	211	65.96 ± 8.69	53.21 ± 7.67	200

distribution of data in different samples and significant differences between the groups ($P < 0.05$). Also, the results of Mann-Whitney U test showed significant differences between CFU/mL of water samples and disinfectant usage in November and December ($P < 0.05$).

Discussion

The low chemical and microbial quality of water can affect consumers' health (31). In the Dentistry School of AJUMS, chlorinated disinfected municipal water is used to supply the required water to the dental units. The total dissolved solid of municipal water supply sources in most southern provinces of Iran, especially in Khuzestan province, is higher than the standard level (32). For this reason, water is first purified in the central softening system unit to remove particles that may damage the dental units, then, transferred to the dental units for consumption.

Infection control in dental offices is essential for the health care of dentists and patients. Disinfection of dental unit surfaces can have a positive effect on breaking the infection transmission chain.

The results of this study illustrated that bacterial contamination of DUWLs during the COVID-19 pandemic, due to the excessive use of disinfectants to disinfect surfaces and equipment, was less than that before the COVID-19 pandemic, which is consistent with the results of a study conducted by Tuladhar et al (33). In confirmation of the results of the present research, the results of the study of Rutala and Weber showed that the use of disinfectant compounds in environmental surfaces and equipment in health care facilities could reduce the amount of pathogens (34).

It is sufficient for the dental units' microbiological water quality to meet the drinking water standards. During the COVID-19 pandemic, increasing the disinfection of dental unit surfaces effectively has reduced the risk of infection transmission.

As shown in Figure 3, all units had colony-forming units under 200 CFU/mL, which is acceptable as the ADA recommendations. However, microbial proliferation inside DUWLs is inevitable, representing the low risk of infection (35). Investigated bacteria in this study can cause human diseases such as periodontitis, throat infection, and many other nosocomial diseases. Most of these microorganisms are found in high concentrations in the water distribution system of therapeutic environments such as hospitals and dentistry (36). Until now, no studies have been performed on the effect of disinfectant usage during the COVID-19 pandemic on the bacterial contamination of the dental units' waterlines. In a study conducted by Rahman Oleiwi, the bacterial contamination of the samples collected from air/water syringe, mouth washing water, tap water, and handpiece was 60, 50, 6, and 90 CFU/mL, respectively (37). In the present study, the increase in the frequency of disinfection of dental unit surfaces due to the COVID-19 pandemic may be the

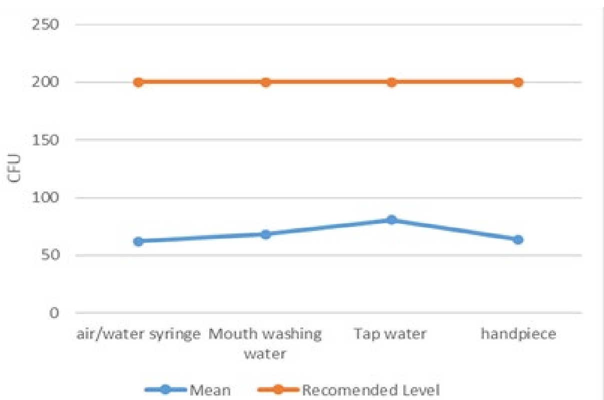

Figure 3. Comparison of the bacterial contamination of different parts of the DUWLs with the ADA recommendations.

reason for this difference. A similar study showed that the level of bacterial contamination in the water samples collected from handpieces of the dental unit is between 0 and 375 CFU/mL (23). In this study, the concentration of *Acinetobacter* spp in the air/water syringe, mouth washing water, tap water, and handpiece was 91.63, 99.44, 140.45, and 99.21 CFU/mL, respectively, which is similar to the findings of an investigation conducted by James et al where the concentration of *Acinetobacter* spp in air/water syringe, tap water, and handpiece was 95.75, 130.95, and 93.25 CFU/mL, respectively (25). *Pseudomonas aeruginosa* grows significantly in the DUWLs (36). The present study showed that different parts of dental units such as handpiece, air/water syringe, and tap water were contaminated with *Pseudomonas aeruginosa*, which is similar to the results of the study performed by Al-Hiyasat et al. They showed that DUWLs is mainly contaminated with *Pseudomonas aeruginosa* (38). A study by Rahman Oleiwi reported that 12 of the 60 (21%) samples taken from DUWLs were contaminated with *Pseudomonas aeruginosa* (37). *Pseudomonas aeruginosa* can be transmitted to the DUWLs through the oral cavity. Because patients with various infections go to the dentist and all parts of the dental unit are in some way in contact with patients, there was no significant difference between the level of contamination in the air/water syringe, mouth washing water, tap water, and handpiece. *Acinetobacter* spp was the predominant organism in the studied dental units that its concentration has reduced from 107.6 to 87.9 during the COVID-19 pandemic by increasing the use of disinfectants to promote hygiene at various levels of dentistry. Other previous studies have conflicting results with the results of the present study (20,39,40). Different parameters such as source quality of water supply, the degree of observance of hygienic measures by the dental team and patients, the level of environmental health, the use of disinfectants and infection control practices can be the reasons for differences in the results obtained from various studies conducted in this field.

Conclusion

The presence of pathogens, like most bacteria, in the water of dental units poses a severe risk to the health of patients and dentists. According to the results, during the COVID-19 pandemic, the frequency of disinfection of dental unit surfaces and other environmental surfaces in the dental clinic increased, and subsequently, it significantly reduced the bacterial contamination of DUWLs. The quantitative bacterial contamination analysis of water samples taken from different parts of the dental units indicated that the microbial quality of the studied DUWLs was lower than 200 CFU/mL and could meet the recommended standard by ADA. However, regular monitoring of DUWLs microbial quality and various treatment methods to disinfect this equipment is necessary.

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Ethical issues

The study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences (Ethical code: IR.AJUMS.REC.1398.893).

Competing interests

The authors declare that there is no conflict of interests.

Authors' contributions

NJHF conceptualized and designed the experiments. SJ contributed to writing-review and editing. MAZ contributed to writing-review. MPF performed the experiments, writing-original draft writing-review, and editing.

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