ORIGINAL ARTICLE

CONTENTS

Microbial contamination of dental unit waterlines systems in Ukraine: results a multicenter study (2020-2022)

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ABSTRACT

Aim: The aim of this study was to evaluate the microbial contamination of dental unit waterlines (DUWLs) and characterize the microbial communities of biofilms in dental chair units (DCUs) from different specialties in Ukrainian dental clinics.

Materials and Methods: A multicentre study was performed between January 1, 2020, to December 31, 2022. Dental water samples and biofilm samples were obtained from 191 DCUs at eighteen dental clinics from seven regions of Ukraine. The genomic DNA of the biofilm samples was extracted, then 16S rDNA were amplified and sequenced.

Results: A total of 1,146 dental water samples were collected, of which 57,4% samples did not meet microbiological parameters of Ukrainian National Standard on drinking water. Sequencing results showed significant differences in bacterial community structure between dental specialties. The largest specific weight of biofilm samples with high bacterial concentrations were detected from orthodontics (54.2%), prosthodontic (47.5%), and oral surgery (44,3%). The 16S rDNA gene sequencing showed high diversity of bacteria (311 genera) were detected in the biofilm samples. Amount of potential human pathogens were detected in the biofilm samples, including *Pseudomonas aeruginosa* (33.7%), *Escherichia coli* (27.3%), *Enterococcus faecalis* (17.4%), *Enterococcus faecium* (9.5%), *Serratia marcescens* (6.8%), *Stenotrophomonas maltophilia* (5.9%), *Staphylococcus aureus* (5.1%), *Burkholderia cepacia* (4.3%), *Acinetobacter lwoffii* (4.8%), *Enterobacter cloacae* (4.6%), *Klebsiella oxytoca* (4.2%), *Streptococcus pneumoniae* (3.9%), *Streptococcus pyogenes* (2.6%), and *Streptococcus* sp, (1.9%).

Conclusions: The most water quality of the DUWLs tested failed to reach the Ukrainian drinking water standard. Furthermore, most DCUs contained pathogens which poses a risk of infection for patients.

KEY WORDS: dental unit waterlines, contamination, microorganisms, biofilm, Ukraine

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INTRODUCTION

In the literature, the transmission of pathogens to patients during dental treatment has been a topic of discussion for many years. This is because using high-speed handpieces without cooling can cause irreversible damage to teeth, spraying water both to clean the working area and to cool the modern dental unit. A complex network of interconnected dental unit waterlines (DUWLs) supply water to dental instruments. Aerosols from dental instruments increase the risk of infection for both the patient and the dentist, due to the constant increase in the germs in the dental office.

Several previous studies showed that the water which reached the dental chair units (DCU) failed to meet national drinking water standards in various countries. The most studies on biofilm formation in DUWLs have focused on a range of individual DCUs. According to the literature, DUWL output water is often becomes contaminated by high densities of microorganisms, principally Gram-negative environmental bacteria including *Pseudomonas aeruginosa* [1, 2] and *Legionella* species, but sometimes contain *Staphylococcus aureus* [3], and *Acinetobacter* species [1]. A wide variety of approaches, many unsuccessful, have been proposed to control a complex network of interconnected plastic dental unit waterlines bacterial biofilm [3]. In Ukraine no studies focused on the water quality and formation of biofilm in dental chair unit waterlines system or individual dental chair units. Therefore, microbial contamination of water from DCUs and the prevalence of opportunistic microorganisms in this water in Ukrainian dental clinics is unknown.

AIM

The aim of this study was to evaluate the microbial contamination of dental unit waterlines (DUWLs) and characterize the microbial communities of biofilms in DCUs from different specialties in Ukrainian dental clinics.

MATERIALS AND METHODS

STUDY DESIGN AND SETTING

A multicentre study was performed between January 1, 2020, to December 31, 2022. Our study included eighteen dental clinics from seven regions (Kyiv, Zhytomyr, Kharkiv, Odesa, Mykolaiv, Kherson, and Lviv) of Ukraine. Of these, five were state-owned and seven were private dental clinics. Dental unit waterlines (DUWLs) include air/water syringe, ultrasonic scaler, and narrow-bore plastic tubing that carry water to the dental instruments. The dental chair and ancillary equipment at all dental clinics are used for routine general oral healthcare procedures. These procedures include oral examinations, dental extractions, endodontics, prosthodontic procedures, restorations and prophylaxis among others. All dental clinics used a closed system, where the reservoir bottles were filled with distilled water and attached to the DCUs or fitted at a designated location within the oral healthcare facility to ensure water supply. Inclusion criteria: all participants had to be perform routine dental procedures; dental clinics willing to partake in the study who completed the questionnaire were included in the study. Exclusion criteria: none. In this study a different number of DCUs were included in sampling.

SAMPLING PROTOCOL

All dental clinics participating in the study signed a letter of consent. The microbial contamination of water samples and presence of biofilm associated organisms from swab samples were collected at eighteen dental clinics from seven regions of Ukraine. All dental clinics used closed system DCUs and samples were collected from outlets of municipal taps, dental handpieces, distiller bottles and reservoir bottles. None of the DUWLs are treated with chlorine products or other disinfectants. However, in dental clinics surfaces and the handpieces are routinely treated with disinfectants between patients or are autoclaved.

COLLECTION OF WATER SAMPLES

In this study the exterior surfaces of taps, fast handpieces and DCU tubing were disinfected with an alcohol swab. Water samples were collected aseptically in wide-mouthed sterile glass bottles. The municipal water source water (tap) to the DCU in the dental clinic and water exiting the fast handpieces was allowed to run for one minute before a water sample was collected. Distilled water was used to fill reservoir bottles. Distilled water was used to fill reservoir bottles that were attached to the water system DCUs to supply water to the handpieces. Water samples were collected from distiller bottles as well as from reservoir bottles that were used for DCUs. Water samples were placed in a cooler box on ice and transported to the Zarifa Aliyeva International Center of Medical Science Laboratory and processed within 24 hours.

COLLECTION OF BIOFILM SAMPLES

In this study the conventional swabbing method of field testing with a cotton swab on surfaces for detecting pathogenic bacteria was used. The exterior surfaces of taps, fast handpieces and DCU tubing were disinfected with an alcohol swab. Swab samples were collected from the internal surfaces of distal outlets of taps and the fast handpieces of DCUs, and from internal surfaces of empty distiller bottles and reservoir bottles. Swabs of internal surfaces of sampling sites were collected aseptically using a pre-moistened cotton swab. Swab samples were placed in a cooler box on ice with a temperature not exceeding 4°C and were transported to the Zarifa Aliyeva International Center of Medical Science Laboratory and processed within 24 hours.

MICROBIAL ANALYSIS

In this study water and swab samples were measured against the Ukrainian National Standard, Drinking water, Requirements and control methods of quality DSTU 7525:2014 (2015) for heterotrophic plate counts (HPC) microbial drinking water quality and total coliforms. In this study the water sample was platted onto agar using the constant function of the ScanStation automatic plater (interscience) and spreading it evenly over the agar. This procedure was performed for all water samples. After incubated period all colonies were counted using the ScanStation Automatic colony counter (interscience) and reported as colony forming units, as stipulated in DSTU 7525:2014 (2015). In our study the membrane filtration technique was performed for the propagation and enumeration of total coliform bacteria. Each water sample was filtered through a Millipore Filtration assembly with a sterile membrane nitrocellulose grid filter. The membrane filters were placed on agar, which consists of a chromogenic medium and a

selective supplement. The samples were transported refrigerated to the Lab of the Zarifa Aliyeva International Center of Medical Science (Ukraine) and immediately analyzed. All samples were processed according to EN 12780:2002.

MOLECULAR ANALYSIS

In this study the biofilm samples were centrifuged and the sediment was collected. Then, DNA from biofilm samples was extracted using QIAmp® DNA Mini kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The quality of DNA extraction was measured by agarose gel electrophoresis. In this study DNA was quantified by ultraviolet spectrophotometer. Extracted genomic DNA was amplified using a set of primers targeting bacterial 16SrRNA genes. Molecular analysis was conducted in the Lab of the Zarifa Aliyeva International Center of Medical Science (Ukraine).

ETHICS

The Ethics Board of the Ukrainian Association of Infection Control and Antimicrobial Resistance has approved the protocols of this study. All participants (dental clinics) were assured of their privacy and anonymity throughout the study and in subsequent reports and articles.

STATISTICAL ANALYSIS

In this study microbiological data were collected in Microsoft® Excel (Microsoft Corporation, WA, USA).

Descriptive statistics were conducted. All the data are presented as numbers and percentages. Levels of microbial contamination were summarised using descriptive statistics (mean, standard deviation, minimum, median, maximum) separately by type, location, and system. A p-value <0.05 was considered statistically significant. Where the p-value was less than 0.05 (<0.05), the hypothesis was rejected.

RESULTS

WATER QUALITY

During the study period (2020-2022), a total of 1,146 dental water samples were collected, of which 57,4% (95% CI: 55.9-58.9) samples did not meet microbial parameters. Only 42.6% of the water samples had bacterial concentrations below the threshold of Ukrainian National Standard DSTU 7525:2014 (2015) and to requirements of the State Sanitary Norms and Rules of the DSanPiN 2.2.4-171-10, section "Indicators of Epidemiological Safety of Drinking Water" (heterotrophic plate counts <100 CFU/mL). In 2022, compared to the baseline level (2020) for the dental water quality there was an increase in the number of non-standard water samples for microbiological indicators. During study period the share of non-standard dental water samples on microbiological parameters in 2022, 2021, and 2020 was 57.4%, 29.7 %, and 30.4%, respectively. The largest specific weight of non-standard (on microbiological parameters) water samples from DUWLs systems was detected in Mykolaiv, Kherson, Zhytomyr region of Ukraine (Table 1).

In this study HPC in both water and swab samples indicated mean counts significantly exceeding the Ukrainian National Standard DSTU 7525:2014, which stipulates that water should not contain more than 1×10^3 CFU mL of heterotrophic bacteria. The mean HPC count for water quality in DUWLs system in Ukrainian dental clinics was the high, 1.94×10^4 CFU mL for municipal tap water and 6.91×10^4 CFU mL in water exiting the handpieces. The average values HPC of DUWLs system of distiller bottles in dental clinics was 4.71×10^4 CFU mL and 5.88×10^4 CFU ml for reservoir bottles.

The high HPCs values in the supply water of dental clinics create optimal conditions for the growth of biofilm and the proliferation of opportunistic microorganisms – potential pathogens of healthcare infections (HAIs). These were indicated by the microbial load detected in this study on the internal surfaces of municipal taps, distiller bottles, reservoir bottles, and handpieces in Ukrainian dental clinics. Number of HPCs of the inner municipal tap surfaces of DCUs (4.1×10^3 CFU mL) and inner surfaces of handpieces (3.93×10^4 CFU/mL) were high exceeded the Ukrainian National Standard DSTU 7525:2014 recommendation for HPC of less than 1×10^3 CFU/mL The inner surfaces of reservoir bottles and distiller bottles were also not compliant with the DCTU 7525:2014 recommendation for HPC and was 8.1×10^4 CFU mL and 2.4×10^4 CFU/mL, respectively.

In present study the total coliforms were determined as an indicator organism of contamination and possible detection of potentially pathogenic bacteria in the DCUs at the various sampling sites. The Ukrainian National Standard, DSTU 7525:2014, for total coliforms stipulates that water should not contain more than 1×100 CFU/100 mL. However, the findings this study indicated that municipal taps supplying DCUs (9.2×100 CFU/100 mL) and water exiting handpieces (8.63×100 CFU/100 mL) did not comply with Ukrainian National Standard, DSTU 7525:2014 (2015). In this study the handpieces (6.2×100 CFU/100 mL), reservoir bottles (6×100 CFU/100 mL), and distiller bottles (1.36×100 CFU/100 mL) also, exceeded total coliform counts the DCTU 7525:2014 recommendation of 1×100 CFU/100 mL.

Table 1. The general information of sampling Dental Chair Units in Ukrainian dental clinics.

Region	Specialty	Number of sample	Age of DUWL				
			<3	4-6	7-10	11-14	≥15
Kyiv	Endodontics	9	1	2	7	0	0
	Orthodontics	18	1	4	6	5	2
	Periodontic	5	0	1	4	0	0
	Oral surgery	27	2	6	16	2	1
	Prosthodontic	22	3	5	9	2	3
Kharkiv	Endodontics	3	0	2	1	0	0
	Orthodontics	4	0	1	1	2	0
	Periodontic	2	0	1	1	0	0
	Oral surgery	5	0	2	3	0	0
	Prosthodontic	3	0	2	1	0	1
Mykolaiv	Endodontics	2	0	0	2	0	0
	Orthodontics	4	1	0	2	1	0
	Periodontic	1	0	1	0	0	0
	Oral surgery	3	1	1	1	0	0
	Prosthodontic	2	0	1	1	0	1
Kherson	Endodontics	2	0	1	1	0	0
	Orthodontics	4	0	0	3	1	0
	Periodontic	1	1	0	0	0	0
	Oral surgery	4	0	2	1	0	0
	Prosthodontic	5	0	2	2	1	2
Odesa	Endodontics	2	0	1	1	0	0
	Orthodontics	8	1	3	4	0	0
	Periodontic	2	1	1	0	0	0
	Oral surgery	7	1	4	2	0	0
	Prosthodontic	6	0	2	4	0	0
Zhytomyr	Endodontics	1	0	1	0	0	0
	Orthodontics	6	0	1	3	1	1
	Periodontic	1	1	0	0	0	0
	Oral surgery	3	0	1	2	0	0
	Prosthodontic	3	0	1	1	1	0
Lviv	Endodontics	2	1	1	0	0	0
	Orthodontics	9	1	3	5	0	0
	Periodontic	2	1	1	0	0	0
	Oral surgery	6	0	2	4	0	0
	Prosthodontic	7	0	2	5	0	0
	Total	**191**	**17**	**58**	**93**	**16**	**11**

MICROBIAL COMMUNITY OF THE BIOFILM

A total of 764 biofilm samples from 191 DCUs of five specialties (i.e., prosthodontics, orthodontics, endodontics, oral surgery, and periodontics) in four time points (i.e., internal surfaces of distal outlets of taps and the fast handpieces of DCUs, and from internal surfaces of empty distiller bottles and reservoir bottles) were collected. The biofilm samples from dental specialties had significantly different bacterial concentrations. Sequencing results showed significant differences in bacterial community structure between dental specialties. The largest specific weight of biofilm samples with high bacterial concentrations were detected from orthodontics (54.2%), prosthodontic (47.5%), and oral surgery (44,3%). Older DCUs were affected more frequently than those under five years of age. Biofilm

Table 2. The overall relative abundance (%) of potential pathogenic microorganism - pathogens of healthcare associated infections (HAI) in dental unit waterlines (DUWLs) system, Ukraine, 2020-2022.

Pathogen	Overall relative abundance (%)
Pseudomonas aeruginosa	33,7
Escherichia coli	27,3
Enterococcus faecalis	17,4
Enterococcus faecium	9,5
Serratia marcescens	6,8
Stenotrophomonas maltophilia	5,9
Staphylococcus aureus	5,1
Burkholderia cepacia	4,3
Acinetobacter lwoffii	4,8
Enterobacter cloacae	4,6
Klebsiella oxytoca	4,2
Streptococcus pneumoniae	3,9
Streptococcus pyogenes	2,6
Streptococcus sp,	1,9

samples with high bacterial concentrations from periodontics and endodontics specialties, was 31.4% and 27.8%, respectively. The biofilm samples with high bacterial concentrations were detected from the fast handpieces of DCUs (43.1%), reservoir bottles (37.8%) the internal surfaces of distal outlets of taps (29.1%), and internal surfaces of empty distiller bottles (17.9%).

Genomic DNA of samples was extracted, and then 16S ribosomal DNA (rDNA) were amplified and sequenced. Microbial community with high diversity of bacteria. In this study the 16S rDNA gene sequencing showed that the bacterial communities of all samples covered 27 classes, 64 orders, 133 families, 311 genera, and 487 species. Microorganisms belonging to multiple genera involved in human diseases were detected including 25 genera of bacteria. Amount of potential human pathogens were detected in the biofilm samples, including 7 genera of bacteria. The potentially human-pathogenic genera of bacteria with relative abundance over 1% were *Pseudomonas aeruginosa* (33.7±2.2%), *Escherichia coli* (27.3±2.4%), *Enterococcus faecalis* (17.4±1.4%), *Enterococcus faecium* (9.5±1.8%), *Serratia marcescens* (6.8±1.7%), *Stenotrophomonas maltophilia* (5.9±1.4%), *Staphylococcus aureus* (5.1±1.3%), *Burkholderia cepacia* (4.3±0.9%), *Acinetobacter lwoffii* (4.8±1.2%), *Enterobacter cloacae* (4.6±1.6%), *Klebsiella oxytoca* (4.2±0.6%), *Streptococcus pneumoniae* (3.9±0.8%), *Streptococcus pyogenes* (2.6±0.7%), and *Streptococcus* sp, (1.9±0.4%). These pathogens were detected in al dental clinics of Ukraine. The overall relative abundances (%) of potential pathogenic microorganism - pathogens of healthcare associated infections (HAI) and that among different groups of DUWL biofilm samples were shown in Table 2.

DISCUSSION

The aim of this study was to evaluate the microbial contamination of DUWLs and characterize the microbial communities of biofilms in DCUs from different specialties in Ukrainian dental clinics. In present study for the first time in Ukraine, microbial contamination of DUWLs and biofilm in DCUs related to dental specialty was comprehensively evaluated, with more abundance of bacterial communities using high-throughput sequencing technology.

The findings of this study revealed that water from DUWLs is heavily colonized by microbial communities. 57,4% of the DUWLs tested samples did not meet microbiological parameters of Ukrainian National Standard on drinking water. Sequencing results showed significant differences in bacterial community structure between dental specialties. The largest specific weight of biofilm samples with high bacterial concentrations were detected from orthodontics (54.2%), prosthodontic (47.5%), and oral surgery (44,3%). The biofilm samples with high bacterial concentrations were detected from the fast handpieces of DCUs (43.1%), reservoir bottles (37.8%) the internal surfaces of distal outlets of taps (29.1%), and internal surfaces of empty distiller bottles (17.9%). The 16S rDNA gene sequencing showed high diversity of bacteria (311 genera) were detected in the biofilm samples. Amount of potential human pathogens were detected in the biofilm samples, including *Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis, Enterococcus faecium, Serratia marcescens, Stenotrophomonas maltophilia, Staphylococcus aureus*, *Burkholderia cepacia*, *Acinetobacter lwoffii*, *Enterobacter cloacae, Klebsiella oxytoca, Streptococcus pneumoniae, Streptococcus pyogenes*, and *Streptococcus* sp.

Several previous studies found that the water which reached the DUWLs failed to meet drinking water standards [4-8]. In our study a large proportion water samples of the DUWLs tested did not comply with the Ukrainian drinking water standards. According to the findings of a United Nations survey, Ukraine is ninety-fifth state in the ranking of drinking water quality [9]. According to the statistical report of the Centres for Disease Control and Prevention of the Ministry of Health of Ukraine (CDCP) and the Ukrainian Association of Infection Control and Antimicrobial Resistance, the situation with the quality of drinking water in healthcare institutions is not improving. In 2020, 2021 and 2022, the share of non-standard water samples for microbiological parameters was 21.2%, 29.7% and 43.5%, respectively [10].

Opportunistic pathogens such as *P. aeruginosa*, *E.coli* and Enterococci are prevalent in dental unit water and waterlines [5, 11-14]. In our study *P. aeruginosa* was detected in the source water and handpieces of DCUs, reservoir bottles and distiller bottles. It could be assumed that the waterlines of DCUs were colonised by *P. aeruginosa* to form biofilm. According to the literature, the origin of bacteria that contaminate DUWLs can be attributed to 2 factors: (1) contaminated municipal water that is used in DCUs, and (2) the suck back of patients' saliva into the DUWL because of ineffective or faulty anti-retraction valves [15]. Other authors reported that the contamination may be caused by the water supply [16], the retraction of biological fluids from the handpieces used in oral cavities of patients [17], or probably the continuous biofilm detachment or fragmentation in the narrow waterline tubes [18]. Opportunistic pathogens such as Legionella pneumophila, Pseudomonas aeruginosa, and Staphylococcus aureus have previously been revealed in water samples from DUWL [19, 20]. In addition, other genera such as Stenotrophomonas were also recovered in dental unit waters [21].

The biofilm was possibly sloughed off and was fed into the water supply of the fast handpieces where it contaminated the water at the distal outlets of these instruments. However, there are no legislated parameters relating to the presence of these organisms and microbial water quality. Their presence can encourage the growth of other microorganisms. According to requirements of the State Sanitary Norms and Rules of the DSanPiN 2.2.4-171-10, section "Indicators of Epidemiological Safety of Drinking Water", Appendix 1, "*E.coli*" and "Enterococci" indicators should not be listed. However, national statistical reports do not use data on contamination associated with *E. coli* and other microorganisms. According to statistical reporting, percentage shares of sub-standard samples of drinking water are to be reported in terms of bacteriological indicators. The water supply control and monitoring in healthcare institutions are conducted by various Central executive bodies, there is a need of interdepartmental coordination and unification of relevant reporting under water quality.

Legal regulation of drinking water in Ukraine is governed by legal acts. The main regulatory legal acts regulating the right to drinking water and its quality in Ukraine are Law on Drinking Water and Drinking Water Supply. Rationing of the quality and safety of drinking water is related to the area of subordinate legislation, but in Ukraine there are two standards at the same time: SanPiN 2.2.4-171-10 "Hygienic requirements for drinking water intended for human consumption" and State standards of Ukraine DSTU 7525: 2014 (2015) "Drinking water. Requirements and methods of quality control". According to the Law of Ukraine "On Technical Regulations and Conformity Assessment Procedures", the use of standards or their individual provisions is mandatory for healthcare facilities, if the standards are referred to in technical regulations. SanPiN 2.2.4-171-10 is a binding normative legal act, agreed with all interested ministries and departments. State SanPiN is applied to most water sources. At the same time National standards of Ukraine DSTU 7525:2014 (2015) are optional. Thus, SanPiN 2.2.4-171-10 remains the main valid normative document in the area of drinking water supply and drinking water quality in the Ukraine.

In European Union the quality and safety of drinking water is regulated by Directive 98/83 / EC. The Directive determines that the parametric values shall be complied with: in the case of water supplied from a distribution network, at the point, within premises or an establishment, at which it emerges from the taps that are normally used for human consumption. In order to reduce or eliminate the risk of non-compliance with a parametric value, the Directive requires strict compliance with the requirements of informing the public about changes in the quantity and quality of drinking water. However, in national legislation, insufficient attention is paid to controlling the quality of equipment and materials (pipes, containers, cranes, etc.).

The Ministry of Health of Ukraine currently has no explicit requirements for the quality of water, which is supplied to DCUs, and neither has it issued an infection control policy that regulates to protect their patients and healthcare personnel of dental practice. The Ukrainian National Health Policy also does not contain any regulations related to aspects to curb the transmission of healthcare associated infection or for infection control issues related to the oral healthcare practice.

STRENGTHS AND LIMITATION

In this study for the first time in Ukraine, biofilm in DCUs related to dental specialty was comprehensively evaluated, with more abundance of bacterial communities. For the first time, biofilm in DUWL related to dental specialty was comprehensively evaluated, with more abundance of bacterial communities. The findings of this study revealed that DUWLs are heavily colonized by bacterial communities. There is serious microbial contamination observed in DUWLs is due to opportunistic pathogens. Our findings could help better characterize and assess the cross-contamination risk of dental care.

A limitation of our study is that we studied water samples and biofilm samples deriving only from seven region (29.2%) of Ukraine and it cannot be representative of the overall Ukrainian situation. Further studies are required to address those limitations.In the future, research should be focused on the risks to patients and staff, surveillance of adverse events related to dental treatment and importance of following the advice of dental unit manufacturers. On the basis of comprehensive research on biofilms in Ukraine, further studies on prevention of biofilm accumulation are essential.

CONCLUSIONS

Results this study showed that both patients and dental staff are exposed to healthcare-associated infection risks due to inhalation or spreading of aerosols produced during dental cares. The present study contamination levels of DUWL water were high. The water quality of 57.4% of the DUWLs tested in the seven regions failed to reach the Ukrainian drinking water standard. Results of present study highlights the risk of contaminated source water that is supplied to DCUs, as well as the risk of contaminated water that exits distal outlets of fast handpieces of DCUs. Furthermore, most DCUs contained pathogens which poses a risk of infection for patients. Biofilm accumulation DCUs and multiple kinds of opportunistic pathogen emphasized the risk for healthcare associated infection during dental care and the importance of biofilm control.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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