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The importance of biofilm contamination control for dental unit waterlines: a multicenter assessment of the microbiota diversity of biofilm in dental unit waterlines

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ABSTRACT

Background: The biofilm formation in Dental Unit Waterlines (DUWLs) could become an important cause of infection during dental care, which could put immunocompromised individuals at risk of cross-infection. The aim of this study was to characterize the microbial communities of biofilms among DUWLs using high-throughput sequencing technology.

Methods: Twenty-nine biofilm samples were obtained from 24 dental chair units at 5 hospitals and 2 dental clinics. The genomic DNA of the samples was extracted, then 16S rDNA and ITS2 gene were amplified and sequenced. Alpha-diversity and Beta-diversity were calculated with QIIME2 and the Kruskal – Wallis H-test was adopted for statistical analysis.

Results: Microbial communities with a high diversity of bacteria (377 genera) and fungi (83 genera) were detected in the biofilm samples. The dominant phylum of bacteria was Proteobacteria (93.27%) and that of fungi was Basidiomycota (68.15%). Potential human pathogens were detected including 7 genera of bacteria (Pseudomonas, Stenotrophomonas, Hafnia-Obesumbacterium, Burkholderia-Caballeronia-Paraburkholderia, Ralstonia, Enterobacter, Klebsiella) and 6 genera of fungi (Malassezia, Candida, Alternaria, Cryptococcus, Rhodotorula, Rhinocladiella).

Conclusions: This multicenter assessment revealed the infectious risk during dental care. It emphasized the importance of biofilm control due to biofilm accumulation and multiple kinds of opportunistic pathogens in DUWLs.

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Background

As crucial components of dental chair units (DCUs), Dental Unit Waterlines (DUWLs) provide water for handpieces, air/water syringes, and ultrasonic scalers, by narrow-bore plastic tubing. A variety of reasons make DUWLs prone to biofilm formation, such as the long, small-diameter tubing, low flow rates used in dentistry, and frequent periods of stagnation [1]. Microorganisms adhere to the inner surface of the tube and colonize to form biofilms, which play an important role in continued DUWL contamination and act as reservoirs of opportunistic pathogens [2]. Some biofilm fragments and endotoxins released by pathogenic bacteria can enter the oral cavity of patients directly via handpieces or three-in-one air/water syringes, or spread with aerosols produced during dental treatments, which could put immunocompromised individuals at risk of cross-infection [3].

There is serious microbial contamination observed in DUWLs, of which more than 30% is due to opportunistic pathogens including *Legionella pneumophila*, *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis* [4]. Some studies reported that pneumonia caused by *Legionella pneumophila* [5,6] and odontogenic infections in nine children caused by *Mycobacterium abscessus* [7] was associated with DUWLs. Seventy-one children were diagnosed with odontogenic nontuberculous *Mycobacterium* infection after pulp removal at a pediatric dental clinic in 2016 [8]. Recently, one report informed that the facial cutaneous sinus tract infection was linked with *Mycobacterium fortuitum*, *M. abscessus*, and *M. peregrinum* in the DUWL [9]. Therefore, it is significant to assess bacterial contamination and take measures to prevent it.

Numerous studies have been performed to investigate the bacterial communities in DUWLs by bacterial culture, Laser scanning Confocal Microscopy and Scanning Electronic Microscopy, etc [10,11]. However, these traditional methods are insufficient

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to detect the actual microbial diversity and community structure in the DUWLs [12]. As a result, the risk of cross-infection by unknown bacteria in the DUWLs might be ignored. It's a serious challenge to the prevention and control of DUWL infection due to the unique characteristics and biofilm diversity of DUWLs. It seems that the current procedures applied could not stop biofilms from accumulating and detaching. Despite current infection control measures such as disinfecting treatments and the flushing process, it verified that biofilms with a high bacterial and fungal diversity remained in the DUWLs [13]. Thus, it is imperative to find more effective methods to identify microbiota diversity and explore better measures to control biofilms present in DUWLs.

The bacterial 16S rDNA high-throughput sequencing platform this study used enables the production of highly accurate sequencing data, which can provide new insights into studying the diversity of microbial communities in DUWLs [14]. Bacterial community structure in DUWL may be influenced by various factors such as the age of DCUs, the number of patients and different dental treatments [15,16]. Therefore, the diversity and composition of microorganisms in DUWLs would be diverse between different hospitals and specialties. The purpose of this study was to conduct a multicenter assessment of the microbiota diversity of biofilm in DUWLs systematically. In this study, we used high-throughput sequencing technology to comprehensively characterize the microbial communities. The findings would help us to evaluate the potential risks of microbial contamination in DUWLs and propose reasonable strategies to control biofilm.

Table 1. Characteristics of sampling dental chair units.

	Biofilm samples ($n = 29$)	
	n	%
Origin		
Stomatological hospitals	14	48
General hospitals	10	34
dental clinics	5	17
Sample		
Biofilm of Water tubes	22	76
Biofilm of air tubes	7	24
Departments		
General Dentistry	11	38
Orthodontics	7	24
Endodontics	4	14
Oral surgery	3	10
Prosthodontic	2	7
Implant	2	7
water supply		
purified water	19	66
Municipal water system	10	34
Age		
1 year	5	17
2 years	5	17
3 years	1	3
5 years	3	10
6 years	11	38
9 years	2	7
11 years	2	7
Treatment of incoming water		
Filter	29	100
Treatment of DUWL		
Chemical disinfection	29	100
Disinfection cycle		
All the time/cycle	10	34
Once Every day	12	41
Once every two days	3	10
Once a week	4	14
Handpieces sterilization		
After each patient	29	100

Methods

Biofilm sample collection

Samples selected in this study were from water tubes which provide dental water for treatments and air tubes that provide compressed air for dental treatments. Twenty-nine biofilm samples (22 samples from water tubes and 7 samples from air tubes) were obtained from 24 DCUs at 5 hospitals and 2 dental clinics in the region of Zhejiang Province (China) with random selection (Table 1). Flushing the plastic tube concatenated to a high-speed handpiece for 30 seconds and sterilization of handpieces were all conducted before sampling. Also, the characteristics of each sample were collected (Supplementary material, Table AS1). During sampling, the sampler has adopted protective measures including sterile gloves and facial masks. Tubes were exposed after an engineer removed the control panel of DCU, and the tube concatenated to the high-speed handpiece was separated from the control device. After the separated tube was disinfected, a 0.5 cm part in the front of the tube was cut with a sterile scissor and discarded, then 1.5 cm-2 cm of the tube was cut as a sample. The interdental brush, which was sterilized and slender enough (brush of kernel: diameter 0.5 mm, length 1 cm), was inserted into the tube sample, then rotated and traversed 10 times to collect as much biofilm as possible. Then, the kernel of the brush was broken with a sterilized scissors and preserved in an EP tube. All the EP tubes were immediately transported and restored at – 80°C within half an hour.

DNA extractions

The biofilm samples with ddH2O were centrifuged at 8,000 g for 3 min, and the sediment was collected. Then, DNA from biofilm samples was extracted using the Cetyltrimethylammonium Bromide (CTAB) kit (Hangzhou Zeheng Biotechnology Co., Ltd, China) according to the manufacturer's instructions. Meanwhile, the quality of DNA extraction was measured by agarose gel electrophoresis, and the DNA was quantified by ultraviolet spectrophotometer. The total DNA was eluted in 50 µL of

Elution buffer and stored at −80°C until measurement.

PCR amplification and high-throughput sequencing

The extracted genomic DNA was amplified with a set of primers, which targeted the V3-V4 hypervariable regions of bacterial 16SrRNA genes (341F: 5'-CCTACGGGNGGCWGCAG-3", 805 R: 5"-GACTACHVGGGTATCTAATCC-3"), and the fungal ITS2 gene (ITS1FI2: 5"-GTGARTCATCGAATCTTTG-3", ITS2: 5"-TCCTCCGCTTATTGATATGC-3'). The 5'ends of the primers were tagged with specific barcodes per sample and sequencing universal primers. PCR amplification was performed in a total volume of 25 μL reaction mixture containing 25ng of template DNA, 12.5 μL PCR Premix (Shanghai Yitao Biological Instrument Co., Ltd, China), 2.5 μL of each primer, and PCR-grade water to adjust the volume. The PCR conditions to amplify the prokaryotic 16S fragments consisted of an initial denaturation at 98°C for 30 seconds; 32 cycles of denaturation at 98°C for 10 seconds, annealing at 54°C for 30 seconds, and extension at 72°C for 45 seconds; and then final extension at 72°C for 10 minutes. The PCR products were confirmed with 2% agarose gel electrophoresis. The PCR products were purified by AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) and quantified by Qubit (Invitrogen, USA). The amplicon pools were prepared for sequencing and the size and quantity of the amplicon library were assessed on Agilent 2100 Bioanalyzer (Agilent, USA) and with the Library Quantification Kit for Illumina (Kapa Biosciences, Woburn, MA, USA), respectively. The libraries were sequenced on the NovaSeq PE250 platform.

Paired-end reads were assigned to samples based on their unique barcode and truncated by cutting off the barcode and primer sequence. Paired-end reads were merged using FLASH. Quality filtering on the raw reads was performed under specific filtering conditions to obtain high-quality clean tags according to the fqtrim (v0.94). Chimeric sequences were filtered using Vsearch software (v2.3.4). After dereplication using DADA2, we obtained amplicon sequence variants (ASVs) feature table and feature sequence. Alpha diversity and beta diversity were calculated by normalized to the same sequences randomly. According to SILVA (release 138) classifier, feature abundance was normalized using relative abundance of each sample.

Statistical analysis

The microbial community was analyzed in terms of descriptive statistics. Alpha diversity indices (including Chao1, Observed outs, Goods coverage, Shannon, and Simpson indices) and Beta diversity [principal coordinates analysis (PCoA)] were calculated by QIIME2 (2019.07). The differences of Alpha diversity indices among the groups were evaluated by means of Kruskal – Wallis test in R package (v3.5.2). The graphs of PCoA were also drawn by R package (v3.5.2). A p-value <0.05 was considered statistically significant.

Results

Overview of sequencing results

A total of 1,883,753 bacterial reads were obtained from the 29 dental unit biofilm samples with an average of 64,957 ± 2,215 sequences per sample (range: 60952–69,030), representing 5,791 amplicon sequence variants (ASVs). On average, 200 ASVs were detected in each sample (range: 163–269). Additionally, a sum of 1,749,165 fungal reads with an average of 60,316 ± 20367 sequences per sample (range: 59927–110,364) were obtained from the 29 samples and represented 550 ASVs with an average of 19 ASVs detected in each sample. Both the rarefactions reached saturation when the number of bacterial sequences analyzed exceeded 10,000 reads and the number of fungal sequences analyzed exceeded 5,000 reads (Supplementary material Figure A1). Furthermore, the coverage index of all samples was 100%. All these findings indicated a high microbial diversity in the DUWLs.

Microbial community diversity and structure of the DUWL biofilm

The calculated alpha-diversity indices which measured the bacterial and fungal richness and diversity, are presented in Table 2. The Observed outs and Chao 1 indices reflect the species richness, while the Shannon and Simpson indices usually indicate the microbial community diversity. In this study, there were no significant differences in diversity indices among the samples of water pipes and air pipes ($P > 0.05$). Also, the alpha diversity analysis showed no significant differences between samples of different departments or different hospitals ($P > 0.05$) (Supplementary material, Table AS3 and Table AS3). Meanwhile, there were no significant differences in bacterial and fungal community structure among the 29 samples according to PCoA (Supplementary material Figure A2).

Microbial community composition of the DUWL biofilm

The 16S rDNA gene sequencing showed that the bacterial communities of all samples covered 18

Table 2. Alpha-diversity indices and good coverage for microbial community among biofilm samples.

Microbial community	sample	Observed outs	chao1	Shannon	Simpson	Goods coverage	p
bacteria	Water tubes	203.86±28.40	203.94±28.43	4.34±0.13	0.86±0.01	1.000.00	>0.05
	Air tubes	186.4314.92	186.44±19.92	4.27±0.12	0.85±0.01	1.000.00	
fungi	Water tubes	18.23±10.81	19.65±11.10	1.76±1.02	0.56±0.26	1.000.00	>0.05
	Air tubes	14.43±5.50	15.86±5.85	1.23±0.87	0.43±0.28	1.000.00	

phyla, 36 classes, 85 orders, 167 families, 377 genera, and 514 species. The ITS2 gene sequencing showed that the fungal communities covered 5 phyla, 15 classes, 32 orders, 60 families, 83 genera, and 118 species. The relative abundance of microbial community compositions at genus levels is illustrated in Figure 1.

The overall relative abundances (%) of the top 5 bacteria and fungi at phylum, class, order, family, and genus level were shown in Supplementary material, Table AS4. The main phylum of bacteria was Proteobacteria (93.27%) and that of fungi was Basidiomycota (68.15%) in all samples. Other phyla of bacteria including Actinobacteriota (2.25%), Firmicutes (2.15%), Bacteroidota (1.40%) and Verrucomicrobiota (0.31%), as well as the fungi were Ascomycota (31.55%), Glomeromycota (0.14%), Zygomycota (0.10%) and unclassified fungi (0.50%). At the class level, Gammaproteobacteria (82.21%) was the dominant class of bacteria, and Ustilaginomycotina (33.36%) was the dominant class of fungi. The most abundant genus of bacteria and fungi were *Herminiimonas* (31.24%) and Malassezia (33.36%) respectively.

Amount of potential human pathogens were detected in the biofilm samples, including 7 genera of bacteria and 6 genera of fungi (Table 3). The potentially human-pathogenic genera of bacteria with relative abundance over 1% were *Pseudomonas* (35.0%±2.4%), *Stenotrophomonas* (5.7%±0.7%), *Hafnia-Obesumbacterium* (4.8%±0.8%), *Burkholderia-Caballeronia-Paraburkholderia* (3.0%±0.5%), *Ralstonia* (2.5%±0.6%), *Enterobacter* (1.8%±0.4%), *Klebsiella* (1.6%±0.3%). The potentially human-pathogenic genera of fungi with relative abundance exceeding 1% were Malassezia (36.7%±40.2%), Candida (6.9%±14.2%), Alternaria (5.6%±17.0%), Cryptococcus (3.5%±7.1%), Rhodotorula (2.4%±9.3%) and Rhinocladiella (1.4%±4.4%). In this study, we also detected opportunistic pathogens including bacteria of *Stenotrophomonas maltophilia*, and fungi of *Malassezia restricta* and *Malassezia globose*.

Discussion

The findings of this study revealed that DUWLs are heavily colonized by bacterial and fungal communities and evaluated the risk of exposure of both patients and dental staff to potential human pathogens. According to the published studies, the water in DUWLs appeared to be regularly contaminated with microorganisms ranging from 10^5 to 10^6 colony-forming units per milliliter (CFU/ml) [17,18], which exceed the standard of no more than 200 CFU/ml for DUWLs established by the American Dental Association (ADA). Also, the research we once performed observed that bacterial concentrations reached 900 CFU/mL of the output water, and the composition of microbes was different between the output water samples and the biofilm samples attached to the inner surface of tubes [11]. Another article reported that the relative abundance of Proteobacteria was significantly higher in the biofilm

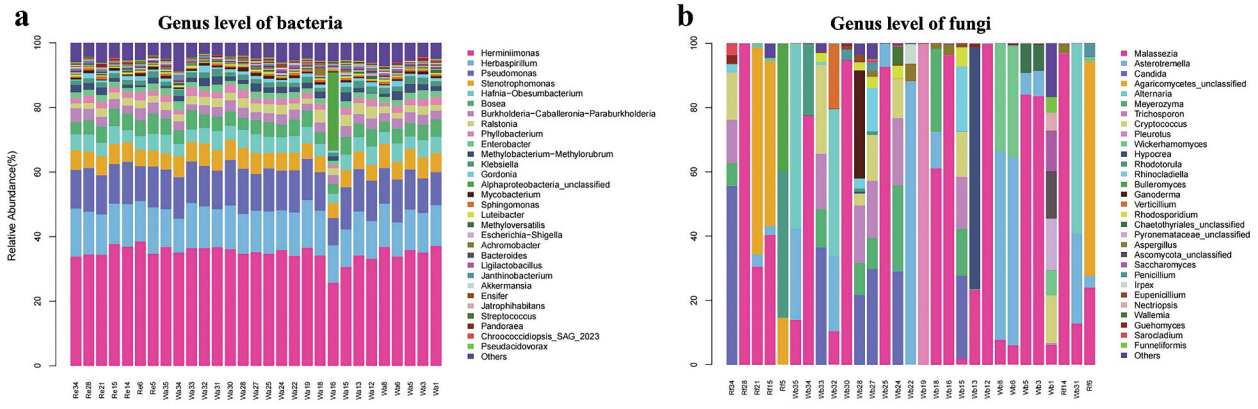

Figure 1.Relative abundance of bacterial and fungal community at genus level (Re: samples from air tubes for 16SrRNA, Rf: samples from air tubes for ITS2, Wa: sample from water tubes for 16SrRNA, Wb: sample from water tubes for ITS2, the Arabic numbers indicate the corresponding DCUs).

Table 3. The overall relative abundance (%) of potential pathogenic microorganism at genus level.

Pathogen	Biofilm samples from water tubes	Biofilm samples from air tubes	overall relative abundance
Bacteria			
Pseudomonas	12.30±1.30	12.34±0.92	35.00±2.40
Stenotrophomonas	5.78±0.77	5.63±0.64	5.70±0.70
Hafnia-Obesumbacterium	4.80±0.85	4.71±0.64	4.80±0.80
Burkholderia-Caballeronia-Paraburkholderia	2.87±0.39	3.33±0.54	3.00±0.50
Ralstonia	2.59±0.67	2.15±0.39	2.50±0.60
Enterobacter	1.86±0.40	1.80±0.32	1.80±0.40
Klebsiella	1.65±0.37	1.53±0.21	1.60±0.30
Fungi			
Malassezia	35.11±40.61	41.66±40.52	36.70±40.20
Candida	6.50±12.50	7.96±21.05	6.90±14.20
Alternaria	7.39±15.16	0.00±0.01	5.60±17.00
Cryptococcus	3.96±7.32	2.11±5.57	3.50±1.70
Rhodotorula	1.10±4.67	6.57±17.38	2.40±9.30
Rhinocladiella	1.71±5.05	0.36±0.96	1.40±4.40

sample compared to the water sample, the reason may be attributed to the release of microbiota from biofilm to flowing water [19]. Thereby, the biofilm sample might be more representative rather than the water sample. In addition, there are other reasons to demonstrate the significance of investigation in DUWL biofilms. The existing biofilms are difficult to eliminate totally because of the complex structure, and as the resistance to antibiotics of bacterial cells in mature biofilms is 10 to 1000 times higher than that of planktonic bacteria, biofilms play an important role in cross-infection in dentistry [20]. Thus, this study was conducted using biofilm samples from the inner surface of tubes among DUWLs to provide more accurate data on the microbial community of DUWL.

Previous reports have informed several environmental factors associated with biofilm accumulation, such as the age of dental unit [21], disinfectants [10,13], and specialty and daily dental practices [15]. However, in this study, despite the 29 samples compared in different groups, no significant differences among these samples as shown by alpha diversity indices and the PCoA. These results confirmed the similarity in the composition of biofilm samples obtained from different DUWLs. Certainly, further verification is needed in the future due to the insufficient sample size and certain unknown factors. Published reports verified that disinfecting treatments and the flushing process could remove part of planktonic bacteria and biofilms [10,22]. It might suggest that microorganisms with strong colonization ability or resistance to antibiotics were more likely to colonize the inner surface of DUWLs as an important part of biofilms. Meanwhile, biotic factors could be involved in the process of biofilm formation. The number of nutrients could be able to modulate the initial attachment of microbial cells [23], and gene regulation as well as quorum sensing may affect the different steps involved during biofilm development [24]. Thereby, it is essential to evaluate the microbial community composition in terms of exploring the biofilm formation and control strategy for biofilm contamination of DUWLs. Additionally, it is noted that the air samples selected in this study were also observed in biofilms. The reason might be related to the dental air compressors bringing water and microorganisms from the air into the air system while producing air. The issue of biofilm contamination in air tubes should attract more attention and further study in the future. To sum up, based on our results on the similarity of the microbial composition of the biofilm samples, we could identify the dominant bacteria and keystone bacteria in biofilm formation more explicitly and find general control methods for biofilm contamination.

In our study, the dominant bacteria at the phylum level were Proteobacteria representing over 91% of the total sequences, which was similar to the results of previous reports [14,15,18]. Unsurprisingly, Proteobacteria was a commonly detected phylum in water distribution systems, owing to high tolerance to chlorine [25]. Members of Actinobacteriota, Firmicutes and Bacteroidota were also observed as previously found [10,15]. Additionally, the abundant fungi in this study were Basidiomycota (68.15%) and Ascomycota (31.55%) at the phylum level and Ustilaginomycotina (33.36%), Tremellomycetes (22.46%) and Saccharomycetes (15.92%) at the class level. It was slightly different from other studies that showed the pattern of Ascomycota and Basidiomycota at phylum level and the Saccharomycetes at class level [13], or Ascomycota at the phylum level and Saccharomycetes at the class level [15]. These distinctions are possibly caused by a variety of factors, whereas we could still obtain some information as support for monitoring and controlling biofilm contamination.

Despite the difference in the ratios and diversity of opportunistic pathogens found between studies, *Pseudomonas* and *Acinetobacter* were previously described as prevalent. In this study, *Pseudomonas* was also detected with a high relative abundance

(35.0%±2.4%) which could form biofilm in DWULs [26] and exhibit resistance to antimicrobial agents and disinfectants, most notably *Pseudomonas aeruginosa* [27]. It was reported that Pseudomonas aeruginosa caused lung infections associated with brain abscess [28] and cystic fibrosis [29]. In contrast, *Acinetobacter* was not detected in our samples. Moreover, *Stenotrophomonas* (5.7%±0.7%) found in our research was also reported previously [10,15], and *Stenotrophomonas maltophilia* was also identified in our biofilm samples. Importantly, *S. maltophilia* is a multidrug-resistant global opportunistic pathogen, which can be recovered from polymicrobial infections, especially from the respiratory tract of cystic fibrosis patients [30]. Other potentially pathogenic bacteria including *Mycobacterium* and *Escherichia-Shigella* in this study were rarely detected compared with similar research [10,15]. Research has informed etiological links between these pathogens and a number of diseases, such as *Mycobacterium tuberculosis* infection in the central nervous system after dental extraction [31] and infectious hypoxia-induced within foci of infection during *Shigella* infection [32]. Regarding the opportunistic fungal pathogens, Malassezia (36.7%±40.2%), Candida (6.9%±14.2%), Alternaria (5.6%±17.0%), etc. detected may also lead to infections. Hitherto, studies have established that Malassezia yeasts were associated with immunocompromised individuals and sepsis of neonates, and play a role in aggravating seborrheic dermatitis, dandruff, folliculitis, and onychomycosis [33]. Candida, recognized as a major agent of hospital-acquired infection, can cause respiratory infections including asthma, allergies and wounds on mucous membranes, even resulting in serious systemic disease [34,35]. Absolutely, these opportunistic pathogens could bring multifarious infectious risks to patients and dental staff during dental treatments, and endotoxins released from gram-negative pathogens in the DUWLs might cause allergic airway reactions [36].

Compared with cultivation techniques, High-throughput sequencing was more time-efficient, and could provide a huge amount of biological information. However, this technology also has the disadvantage of expensive analysis costs and short reads which are difficult to identify at the species level. Although 29 biofilm samples from 7 dental institutions were analyzed, the sample size is still not large enough. Thus, subsequent research should be carried out with more samples and performed to identify species accurately. These results highlighted the potential infectious risk of DUWL biofilms and the necessity of control approaches to microbiome contamination. Disinfection protocols to reduce DUWL contamination presently include physical measures (e.g.: flushing, anti-suction device and filtration), chemical disinfectants (e.g.: chlorhexidine, hydrogen peroxide, chlorine dioxide, sodium hypochlorite, and superoxidized water), and new materials for DUWLs [37,38]. However, all these current measures are inadequate, such as physical measures are unable to remove forming biofilms and chemical disinfections could not remove all biofilms effectively due to resistance to disinfectants of biofilms [39] as well as DUWL might be damaged by the corroded internal components [37]. Hence, validated disinfection protocols should be established to control DUWL contamination. In addition, the scheduled monitoring of microbial contamination of dental units is equally important. Altogether, our results may suggest that more effective disinfection strategies are needed to improve the microbial quality of the DUWL. The pathogenic mechanisms, independent factors, and drug resistance of pathogenic bacteria should be explored further to provide a basis for DUWL disinfection in the future.

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Disclosure statement

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References

[1] O'donnell MJ, Boyle MA, Russell RJ, et al. Management of dental unit waterline biofilms in the 21st century. Future Microbiol. 2011;6(10):1209–1226. doi: 10.2217/fmb.11.104
[2] Dahlen G. Biofilms in Dental Unit Water Lines[J]. Monogr Oral Sci. 2021;29:12–18.
[3] Hikal W, Zaki B, Sabry H. Evaluation of ozone application in dental unit water lines contaminated with pathogenic acanthamoeba. Iran j parasitol. 2015;10 (3):410–9.
[4] Spagnolo AM, Sartini M, Cristina ML. Microbial contamination of dental unit waterlines and potential risk

of infection: a narrative review. Pathogens. 2020;9 (8):651. doi: 10.3390/pathogens9080651
[5] Ricci ML, Fontana S, Pinci F, et al. Pneumonia associated with a dental unit waterline. The Lancet. 2012;379(9816):684. doi: 10.1016/S0140-6736(12)60074-9
[6] Schonning C, Jernberg C, Klingenberg D, et al. Legionellosis acquired through a dental unit: a case study. J Hosp Infect. 2017;96(1):89–92. doi: 10.1016/j.jhin.2017.01.009
[7] Peralta G, Tobin-D'angelo M, Parham A, et al. Notes from the field: mycobacterium abscessus infections among patients of a pediatric dentistry practice–georgia, 2015. MMWR Morb Mortal Wkly Rep. 2016;65 (13):355–356. doi: 10.15585/mmwr.mm6513a5
[8] Singh J, O'donnell K, Nieves DJ, et al. Invasive mycobacterium abscessus outbreak at a pediatric dental clinic. Open Forum Infectious Diseases. 2021;8(6): ofab165. doi: 10.1093/ofid/ofab165
[9] Perez-Alfonzo R, Poleo Brito LE, Vergara MS, et al. Odontogenic cutaneous sinus tracts due to infection with nontuberculous mycobacteria: a report of three cases. BMC Infect Dis. 2020;20(1):295. doi: 10.1186/s12879-020-05015-5
[10] Costa D, Mercier A, Gravouil K, et al. Pyrosequencing analysis of bacterial diversity in dental unit waterlines. Water Res. 2015;81:223–31. doi: 10.1016/j.watres.2015.05.065
[11] Zhu C, Hong FH, Yu X. Anti biofilm effect of low concentration chlorine containing disinfectant assisted by multi enzyme detergent in dental unit waterlines. New Microbiol. 2021;44(2):117–124.
[12] Ditommaso S, Giacomuzzi M, Ricciardi E, et al. Colonization by Pseudomonas aeruginosa of dental unit waterlines and its relationship with other bacteria: suggestions for microbiological monitoring. J Water Health. 2019;17(4):532–539. doi: 10.2166/wh.2019.240
[13] Costa D, Mercier A, Gravouil K, et al. Occurrence and diversity of both bacterial and fungal communities in dental unit waterlines subjected to disinfectants. Pathog Dis. 2016;74(7):ftw094. doi: 10.1093/femspd/ftw094
[14] Dang Y, Zhang Q, Wang J, et al. Assessment of microbiota diversity in dental unit waterline contamination. PeerJ. 2022;10:10. doi: 10.7717/peerj.12723
[15] Fan CC, Gu HJ, Liu LM, et al. Distinct microbial community of accumulated biofilm in dental unit waterlines of different Specialties. Front Cell Infect Microbiol. 2021;11. doi: 10.3389/fcimb.2021.670211
[16] Omran EAH, Abbass AAG, Abaza AF, et al. Study of some risk factors for fungal contamination of dental unit waterlines in Alexandria, Egypt. J Infect Developing Countries. 2021;15(8):1197–1204. doi: 10.3855/jidc.13810
[17] Ji XY, Fei CN, Zhang Y, et al. Three key factors influencing the bacterial contamination of dental unit waterlines: a 6-year survey from 2012 to 2017. Int Dent J. 2019;69(3):192–199. doi: 10.1111/idj.12456
[18] Yoon HY, Lee SY. An assessment of the bacterial diversity found in dental unit waterlines using the illumina MiSeq. Biocontrol Sci. 2019;24(4):201–212. doi: 10.4265/bio.24.201
[19] Paramashivaiah R, Prabhuji ML, Narayanan R. Dental unit water lines decontamination with the aid of nanotechnology. Indian J Dent Res. 2016;27(1):91–9. doi: 10.4103/0970-9290.179838
[20] Wroblewska M, Struzycka I, Mierzwinska-Nastalska E. Significance of biofilms in dentistry. Przegl Epidemiol. 2015;69(4):739-44, 879–83.
[21] Petti S, Moroni C, Messano GA, et al. Detection of oral streptococci in dental unit water lines after therapy with air turbine handpiece: biological fluid retraction more frequent than expected. Future Microbiol. 2013;8(3):413–421. doi: 10.2217/fmb.12.151
[22] Barbot V, Robert A, Rodier MH, et al. Update on infectious risks associated with dental unit waterlines. FEMS Immunol Med Microbiol. 2012;65 (2):196–204. doi: 10.1111/j.1574-695X.2012.00971.x
[23] Arnaouteli S, Bamford NC, Stanley-Wall NR, et al. Bacillus subtilis biofilm formation and social interactions. Nat Rev Microbiol. 2021;19(9):600–614. doi: 10.1038/s41579-021-00540-9
[24] Huang R, Li M, Gregory RL. Bacterial interactions in dental biofilm[J]. Virulence. 2011;2(5):435–44. doi: 10.4161/viru.2.5.16140
[25] Huang K, Zhang XX, Shi P, et al. A comprehensive insight into bacterial virulence in drinking water using 454 pyrosequencing and Illumina high-throughput sequencing. Ecotoxicology And Environmental Safety. 2014;109:15–21. doi: 10.1016/j.ecoenv.2014.07.029
[26] Bayani M, Raisolvaezin K, Almasi-Hashiani A, et al. Bacterial biofilm prevalence in dental unit waterlines: a systematic review and meta-analysis. BMC Oral Health. 2023;23(1):158. doi: 10.1186/s12903-023-02885-4
[27] Rodrigues S, Suvarna S, Suvarna J, et al. Microbial assessment of dental unit waterlines in an institutional setup in Karnataka, South India. Indian J Dent Res. 2017;28(5):555–559. doi: 10.4103/ijdr.IJDR_775_16
[28] Pereira RS, Bonardi JP, Ferreira A, et al. An unusual case of dental infection by Pseudomonas aeruginosa causing a brain abscess: case report[J]. Aust Dent J. 2017;62(4):523–527. doi: 10.1111/adj.12539
[29] Schick A, Kassen R. Rapid diversification of Pseudomonas aeruginosa in cystic fibrosis lung-like conditions. Proc Natl Acad Sci U S A. 2018;115 (42):10714–10719. doi: 10.1073/pnas.1721270115
[30] Brooke JS. Stenotrophomonas maltophilia: an emerging global opportunistic pathogen. Clin Microbiol Rev. 2012;25(1):2–41. doi: 10.1128/CMR.00019-11
[31] Liao Y, Wu F, Dai F, et al. Mycobacterium tuberculosis and Pseudoramibacter alactolyticus coinfection in brain after dental extraction: A case report[J]. Medicine (Baltimore). 2019;98(50):e18289. doi: 10.1097/MD.0000000000018289
[32] Arena ET, Tinevez JY, Nigro G, et al. The infectious hypoxia: occurrence and causes during Shigella infection. Microbes And Infection. 2017;19 (3):157–165. doi: 10.1016/j.micinf.2016.10.011
[33] Gaitanis G, Magiatis P, Hantschke M, et al. The malassezia genus in skin and systemic diseases. Clin Microbiol Rev. 2012;25(1):106–41. doi: 10.1128/CMR.00021-11
[34] Kadaifciler DG, Okten S, Sen B. Mycological contamination in dental unit waterlines in Istanbul, Turkey. Braz J Microbiol. 2013;44(3):977–81. doi: 10.1590/S1517-83822013000300049
[35] Douglas LJ. Candida biofilms and their role in infection. Trends Microbiol. 2003;11(1):30–6. doi: 10.1016/S0966-842X(02)00002-1
[36] Coleman DC, O'donnell MJ, Shore AC, et al. Biofilm problems in dental unit water systems and its practical

control. Journal Of Applied Microbiology. 2009;106 (5):1424–1437. doi: 10.1111/j.1365-2672.2008.04100.x

[37] Hong FR, Chen PP, Yu XF, et al. The application of silver to decontaminate dental unit waterlines-a systematic review. Biol Trace Element Res. 2022;200 (12):4988–5002. doi: 10.1007/s12011-022-03105-w

[38] Sun X, Hill P, Liu J, et al. Marine-source quorum quenching enzyme YtnP to improve hygiene quality in dental units. Mar Drug. 2021;19(4):225. doi: 10.3390/md19040225

[39] Yuan L, Sadiq FA, Wang N, et al. Recent advances in understanding the control of disinfectant-resistant biofilms by hurdle technology in the food industry. Critical Reviews In Food Science And Nutrition. 2021;61(22):3876–3891. doi: 10.1080/10408398.2020.1809345