Detection of Bacterial Contamination in Dental Unit Water Lines and Testing the Effectiveness of Disinfectants against these Contaminants

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Authors' contributions

This work was carried out in collaboration among all authors. Author SL provided concept, designed the study, collected and assembled the data, performed statistical analyses as well as did final approval of the manuscript. Author PK analysed and interpreted the data. Authors Sapna, OP, ZAM, WAG and AAU wrote original draft of the manuscript. Author AAM and KU did critical revision of the article for important intellectual content. All authors read and approved the final manuscript.

Article Information

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ABSTRACT

Aim: Contaminated dental unit water lines (DUWLs) are a possible source for spreading microorganisms in dental practices. The aim of this study was to detect the bacterial contamination of dental unit water and investigate the effectiveness of the disinfectants.
Methodology: Bacterial contamination was detected by a) using bacterial culture of heterotrophic bacteria, total coliforms and *Pseudomonas aeruginosa* and b) Scanning electron microscopy (SEM) of DUWLs tube. Subsequently, dentists were suggested to treat the DUWLs with disinfectants to eradicate bacterial contaminants and its effectiveness was tested after three months.

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Results: Bacterial contamination of the water samples ranged from not detected to 2.38×10^6 CFU/mL. Out of 34 DUWLs water samples tested, 30 (88.24%) samples exceeded the Centers for Disease Control and Prevention (CDC) and Environmental Protection Agency (EPA) recommended threshold of ≤500 CFU /mL, whereas only 4 (11.76%) samples met the standards. Contamination by total coliforms and *P. aeruginosa* was detected in 52.94% and 64.7% of samples respectively. SEM displayed a dense biofilm on DUWLs tubing confirming the bacterial contamination. The intervention for disinfection of DUWLs resulted more than 50% samples with acceptable bacterial count in test performed after three months.

Conclusion: The high rate of bacterial contamination of dental unit water highlights the need to disinfect and monitor the quality of DUWLs periodically.

Keywords: Dental unit waterlines; Bacterial contamination; Biofilm.

1. INTRODUCTION

Dental chair unit comprises of channels of narrow flexible plastic tubes (2-3nm internal diameter) known as Dental unit waterlines (DUWLs). DUWLs are connected with dental instruments including an air-water syringe, high-speed dental handpieces (drills), and ultrasonic scalers. DUWLs are used to irrigate dental instruments and teeth via hydraulic system while working. DUWLs are highly susceptible to microbial contamination and formation of biofilm due to reduced velocity of water at the periphery of the narrow flexible plastic tube [1,2]. The bacterial contamination of DUWLs water was first reported by Blake in 1963 [3].

Biofilm is an aggregate of same or different microorganisms living together in self-producing extracellular polymeric substances [4]. There are several factors responsible for contamination and subsequent formation of biofilm in DUWLs. For example, water stagnation during off-hours, failure of anti-retraction valves (fitted in dental hand devices), contaminated water supply and the presence of water heaters [5,6]. As soon as the biofilm is formed in DUWLs, it becomes continuous source of bacteria in DUWLs [7,8].

Majorly DUWLs are responsible for disseminating bacteria although some reports have also revealed the presence of protozoa and fungi [9,10]. The most predominant contaminants of DUWLs are Gram-negative non-pathogenic environmental bacteria, however; they can be harmful to immunocompromised people. These bacteria include but not limited to *P. aeruginosa*, *Legionella pneumophila* and non-tuberculosis *Mycobacterium* that cause respiratory diseases [4,5]. During dental procedures, aerosols are generated which are also responsible for dissemination of bacteria [5].

In this field, a very few epidemiological studies have been conducted such as Martin [11], reported that two cancer patients got infected with *P. aeruginosa* originating from DUWLs. Two other studies have reported increase in antibody titer in dental staff compared to general public [12]. Gungor et al. [13] have reported immune system suppression in patients and dentists who were exposed to DUWLs contaminated water aerosols from dental unit. In another study transmission of *L. pneumophila* from a contaminated dental unit to patient has been reported. The patient developed a sudden onset of Legionnaires' disease and died from septic shock [14].

Internationally there are no unique guidelines for the acceptable limit of heterotrophic bacteria in DUWLs and application of disinfectant for eliminating the biofilm. However, the American dental association suggests that DUWLs output water must contain ≤ 200 colony-forming units (CFU)/mL of heterotrophic bacteria [15], whereas Centers for Disease Control (CDC) and Environmental Protection Agency (EPA) recommends ≤500 CFU/mL of heterotrophic bacteria [16]. CDC also recommends regular disinfection and monitoring of DUWLs. However, a number of studies have reported the failure of dental practices to achieve these recommended limits [17,18]. There could be various reasons of disinfection protocols failure, such as bacterial resistance towards disinfectants, staff negligence for proper application of disinfection protocol, old dental units, anti-retraction valves failure and low dose of disinfectant use [19,20].

Although DUWLs contamination is a universal problem, the dimension of this problem in our country is less studied and thus patients and dental staff are at risk in acquiring infection from contaminated DUWLs water and aerosols generated during dental procedures. The objective of current study was to investigate

bacterial contamination of DUWLs output water in private dental clinics of district Khairpur, Sindh, Pakistan. The significance of this research is to prevent potential occupational/public health outbreaks.

2. MATERIALS AND METHODS

2.1 Recruitments of Dental Surgeries and Ethical Approval

In this study, 34 different dental practices located in district Khairpur, Sindh, Pakistan were designated for collection of DUWLs water samples. Before laboratory investigation, information sheet of project and consent letter was delivered to each dental practice by hand along with verbal discussion.

2.2 Sample Collection

In designated dental practices source of water for their dental units was distilled water or municipal/ground water stored in storage tanks made up of polyethylene, steel or cement. Thirty-four DUWLs water samples were collected from consented dental clinics. Before collecting, water from air-water syringe (connected with DUWLs) was flushed for two minutes to release the stagnant water. Water samples (50mL) were collected in sterilized glass bottles (containing 100μL of 10% sodium thiosulfate solution to neutralize residual chlorine) during the working hours of dental practices. While collecting the water samples, another glass bottle containing sterilized water was exposed (as a control for splashes and aerosols). All the samples were labelled and transported to laboratory in a cool box within two hours for further analysis.

2.3 Determination of Total Bacterial Count (TBC)

The water samples in triplicates from each dental practice (N=34) was serially diluted in sterilized physiological saline (0.85%) followed by inoculation (0.1mL) on 90 mm R2A agar (Oxoid) by using aseptic techniques. All the samples were incubated at 22°C for 7 days [21]. Plates displaying bacterial colonies in the range of 30-300 were used to calculate the final number of CFU/mL by using following formula.

CFU/mL = (No. of colonies x dilution factor) / volume of culture plate (mL)

2.4 Qualitative Assessment of Water Samples

This study focused on the detection of total coliforms (TC) and *P. aeruginosa* in DUWLs water samples.

2.4.1 Detection of TC

Water sample (0.1mL) was inoculated in lactose broth (Oxoid) tube containing Durham tube and phenol red (a pH indicator). Subsequently, the test tubes were kept in incubator at 37°C for 48 h [22].

2.4.2 Detection of *P. aeruginosa*

DUWLs water samples (0.1mL) were inoculated on cetrimide agar (Oxoid) plates and placed in incubator at 37°C for 48 h [23]. Green colored colonies (due to pyocyanin pigment) were observed. The pure culture was prepared on nutrient agar (Oxoid) plates followed by identification based on cultural, Gram staining and conventional biochemical tests [24].

2.5 Scanning Electron Microscopy (SEM)

DUWLs tube (connected with air-water syringe) was obtained as a gift from a dentist who was planning to change the DUWLs of one of the dental units of his clinic due to blockage in the tube (connected with a high-speed dental drill). This dental unit was in use for 7 years. The portion of the tube was cut (1cm) with a sterilized scalpel and transferred to the microbiology lab in refrigerated temperature (4°C) for processing. In lab, tube was further split lengthwise to expose its lumen and immerse fixed in 2.5% glutaraldehyde (sigma) up to 4h at 4°C. Subsequently tube was washed in PBS and then left in new PBS for 8h at 4°C. This was followed by fixation in 2% osmium tetroxide solution (sigma) for 2h and then washed in distilled water. The specimen was then dehydrated in ethanol of various concentrations (70% to absolute). The specimen was placed in glass desiccator for overnight [25]. Finally, the specimen was sent to University of Punjab for commercial imaging of specimen.

2.6 Follow up Test for DUWLs Water Samples

Results of TBC count were reported to the dentists and they were advised to follow CDC

guidelines for flushing and disinfection of DUWLs to eradicate biofilm and keep the bacterial count within the accepted limit. After three months, water samples from all the dental surgeries were retested for TBC as per method described earlier in this report.

2.7 Statistical Analysis

Data was analyzed using XLSTAT365-Freemium. Where necessary, experiments were performed in triplicates. Results were displayed as mean ± standard division. Since data of TBC in first and follow up study was skewed, the nonparametric Mann-Whitney U test was performed to detect statistically significant differences (p<0.05). Pearson's correlation was performed to investigate the relation of bacterial contamination with the old age of dental unit.

3. RESULTS

3.1 Bacterial Contamination of DUWLs Water

The bacterial contamination of the DUWLs water samples ranged from not detected to 2.38×10^6 CFU/mL. Out of 34 DUWLs water samples analyzed, 30 (88.24%) samples surpassed the CDC/EPA recommended value of ≤500 CFU /mL (Fig. 1), whereas only 4 (11.76%) samples were able to meet the EPA standards (Fig. 1). Sterilized water samples (control) exposed

during collection of DUWLs water samples displayed no growth. Pearson's correlation a statistical test was applied to see the relation of bacterial contamination with the old age of dental unit and statistical analysis showed a negative correlation (-0.027) between contamination level and age of dental chair. This indicated that bacterial contamination level was not directly proportional to the age of dental unit (Table 1).

3.2 Qualitative Assessment of Water Samples

Out of 34 water samples tested, 22 (64.7%) samples were positive for *P. aeruginosa*, whereas 18 (52.94%) samples were positive for total coliform test as confirmed by acid (yellow coloration) and gas production (in Durhams' tube) (Fig. 2). This indicated that 4 samples which were negative for total coliform test showed presence of *P. aeruginosa*. The macroscopic, microscopic, biochemical and sugar fermentation profile for identification of *P. aeruginosa* is shown in Fig. 3 and Table 2.

3.3 SEM Analysis

SEM image of tubing showed a thick biofilm on the lumen of tubing (Fig. 4). Bacteria especially rod shaped could be observed in biofilm. The cracks seen in the image could be due to the stress on the sample during SEM preparation. However, these cracks reveal the thickness of biofilm and indicate the maturity of biofilm.

Table 1. Contamination level of water samples and age of dental chairs
(Correlation coefficient= -0.027)

| Sample ID	Average log CFU/mL	Dental chair age since installation (Months)	Sample ID	Dental chair age since installation (Months)	Average log CFU/mL
DUWL1	4.5×10^4	13	DUWL 2	60	1.1×10^5
DUWL 3	2.2×10^5	85	DUWL 4	35	8.2×10^5
DUWL 5	2.5×10^5	50	DUWL 6	55	2.3×10^5
DUWL 7	3.6×10^3	48	DUWL 8	59	3.0×10^3
DUWL 9	8.4×10^4	11	DUWL 10	61	2.3×10^5
DUWL 11	0	5	DUWL 12	84	2.4×10^4
DUWL 13	3.4×10^5	26	DUWL 14	36	2.3×10^6
DUWL 15	7.5×10^5	73	DUWL 16	65	1.3×10^5
DUWL 17	3.4×10^2	2	DUWL 18	108	3.4×10^4
DUWL 19	2.9×10^6	80	DUWL 20	81	2.1×10^5
DUWL 21	2.6×10^5	25	DUWL 22	36	8.8×10^5
DUWL 23	8.7×10^5	8	DUWL 24	21	2.3×10^6
DUWL 25	2.6×10^5	12	DUWL26	15	7.3×10^5
DUWL 27	3.7×10^2	3	DUWL 28	3	4.4×10
DUWL 29	1.1×10^3	40	DUWL30	20	1.9×10^6
DUWL 31	2.0×10^5	49	DUWL 32	40	1.1×10^6
DUWL 33	2.8×10^5	36	DUWL 34	49	4.0×10^5
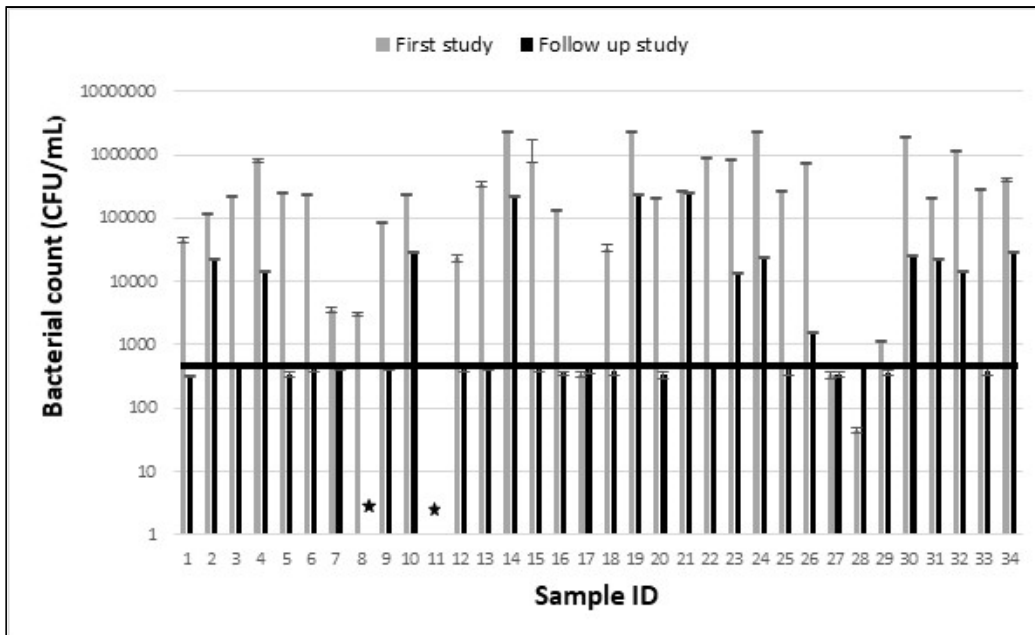
Fig. 1. Total bacterial count from dental unit water samples
Black line within graph indicates acceptable limit as suggested by CDC/EPA (≤500 CFU/mL)
*= No growth

Fig. 2. Total Coliform test (Yellow coloration in tube indicates positive test)
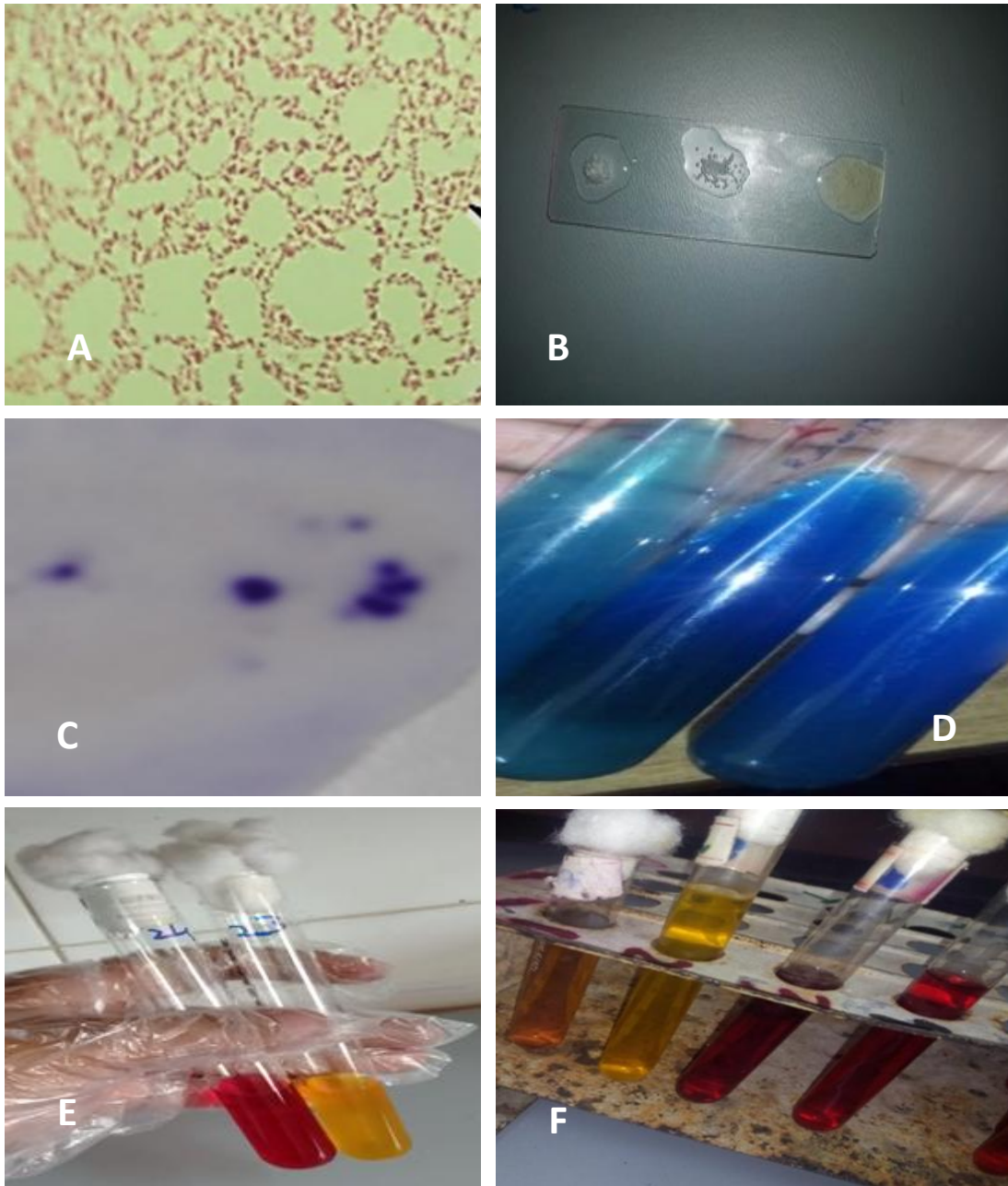
Fig. 3. Microscopic and Biochemical test results for *P. aeruginosa*, A) Gram staining image showing Gram negative rods, B) Catalase test (Positive, as indicated by appearance of bubbles, C) Oxidase test (Positive, as indicated by blue coloration of oxidase reagent on filter paper, D) Citrate Utilization test (Positive, as indicated by blue coloration of media, E) Nitrate Reduction test (Positive test is indicated by red coloration compared to yellow in negative control, F) sugar fermentation tests (Yellow coloration in tubes indicates positive glucose fermentation test)

Table 2. Microscopic, biochemical and sugar fermentation profile for confirmation of
P.   aeruginosa

	Characteristics	Result
Cultural characteristics	Growth on Cetrimide agar	Positive (Yellow-green colonies)
Microscopy	Gram's staining	Gram Negative, rods
	Motility	Motile
	Flagella staining	Polar flagella
	Capsule staining	Non-capsulated
	Spore staining	Non- sporing
Biochemical tests	Catalase	Positive
	Oxidase	Positive
	Indole	Negative
	Methyl red	Negative
	Voges Proskauer	Negative
	Citrate	Positive
	Urease	Negative
	Nitrate reduction	Positive
Sugar fermentation tests	Glucose	Positive
	Maltose	Negative
	Lactose	Negative
	Sucrose	Negative
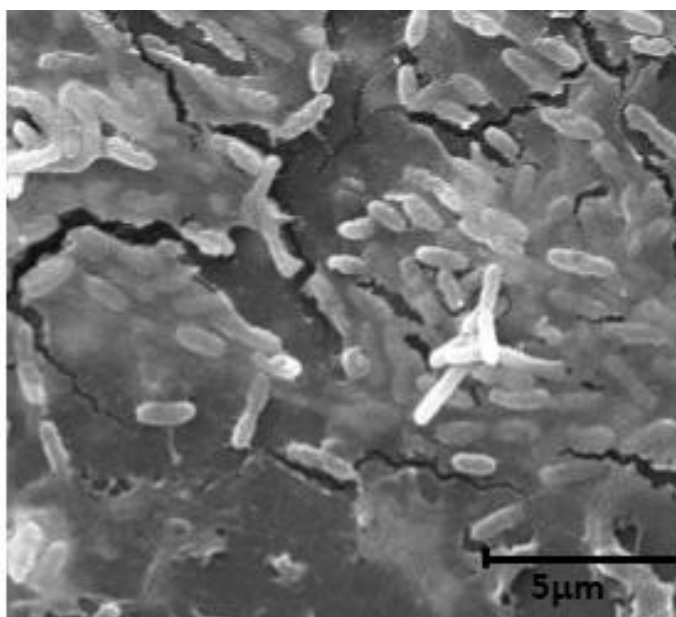
Fig. 4. SEM image of biofilm produced on DUWL tube lumen (x7000)

3.4 Follow up Test of DUWLs Water Samples

After intervention of researchers of this study, although all previously tested samples (N=34) were retested but out of 30 dental units that were previously contaminated with high bacterial count (above CDC/EPA standards), 17 (56.67%) samples revealed acceptable bacterial count ($\leq$500 CFU/mL) and 13 (43.33%) revealed low bacterial count than previous test although above the standard threshold (Fig. 1). Four dental units that showed acceptable limit in 1st test revealed the acceptable limit once again. Mann-Whitney U test indicated that there was a statistically significant difference (P= 0.04) in contamination level during 1st study and follow up study.

4. DISCUSSION

Although DUWLs contamination was identified more than 55 years ago, this issue still persists as evidenced by a vast number of research articles being published internationally on DUWLs contamination and its control. Before commencing this research, the interviews regarding DUWLs contamination were undertaken (as a part of undergraduate student assignment) from 50 dentists running their private clinics. The response of dentists (unpublished data) revealed that many of them were unaware of microbial contamination of DUWLs and its negative impact on health of dental staff and patients. Therefore, this study was designed to monitor the quality of DUWLs water used in private dental clinics. In this study, the majority of the DUWLs did not meet the CDC/EPA standards as they were highly contaminated with heterotrophic bacteria. These results agree with already published studies [12,26]. Small number of dental practices water samples showed acceptable limit possibly because their dental units were newly installed (2-5 months old). However, statistical analysis showed a negative correlation (-0.027) between contamination level and age of dental chair. These surprising statistical results could be due to low sample size. Therefore, in future more study should be carried out with a large sample size to authenticate statistical analysis.

In this study, TBC was evaluated on R2A plates since inoculation of water samples on low-nutrient medium (such as R2A) and incubation for longer time is suggested for enumerating bacterial cells from water sources in which disinfectants have been used such as municipal water. R2A was introduced by Reasoner and Geldreich [27] and since then number of authors have used R2A for counting heterotrophic bacteria in water [21,28,29]. In this study one sample showed no growth that indicates presence of less than 100CFU/ml, bacteria were not detected due to inoculation of 0.1 mL water sample on the R2A plates. However, membrane filtrate method could be used to detect the less number (<100CFU/ml) of bacteria.

Since *P. aeruginosa* has notable ability to form biofilms in many environments including DUWLs [30] and the presence of coliform bacteria in water is considered as an indicator of non-potable water [7], this study also investigated the presence of these bacteria. The dental surgeries whether they were using distilled water or overhead tank water showed contamination by *P. aeruginosa* and TC. In contrast with these results, the research performed by other researchers [31,32] showed absence of TC, whereas, in similar to these results, multiple studies have revealed the presence of *P. aeruginosa* in DUWLs water samples [20,33,34]. High prevalence of *P. aeruginosa* in water samples is alarming.

There is a drawback of determining TC in this study since presence of TC does not confirm the presence of faecal coliforms or *Escherichia coli*. Detection of *P. aeruginosa* in 4 samples that were negative for coliforms was surprising. It suggests that traditional indicators of drinking water quality may not be sufficient for regulatory monitoring of drinking water samples. The prior contaminated distilled water, addition of distilled water in residual water, improper cleaning the storage tank may have contributed for TC and *P. aeruginosa* contamination. However, only the use of water with an initial low contamination level cannot prevent the high number of bacteria in high-speed and the air-water syringe, if the efforts are not taken for reducing or eliminating biofilm in DUWLs.

In this research, a thick biofilm on DUWLs lumen agrees with the high planktonic bacterial count found in DUWLs water samples. However, SEM imaging shows both live and dead bacteria. Alternatively, live/dead assay by using propidium iodide [25] followed by confocal microscopy can be a good method to observe both live and dead cells. Live/dead assay can be more useful while evaluating disinfection strategies to eradicate biofilm.

When the results of TBC were reported to the dental staff, they were also suggested to follow CDC guidelines for flushing and disinfection. Due to the intervention of researchers, more than 50% dental units met the CDC recommended standards for DUWLs water quality in follow up study.

However, even after applying disinfectants some dental units did not met the standards. This could be due to various reasons such as dental chair unit (DCU) type, DCU age, DCU supply water, incompliance by dentists, DUWL disinfection frequency, DCU anti-retraction valve validity.

Additional measures and repeated involvement were needed to achieve acceptable levels in all other dental units. This follow-up study highlights

the importance of using biocides (Alpron and ICX) to clean DUWLs. In literature, the use of various biocides (Hypochlorous acid, Alpron, Sterilox, Bio 2000, Dentosept, Oxygenal and sodium hypochlorite) have been suggested to disinfect DUWLs [35-38]. However, to meet the CDC/EPA recommended standards (≤500 CFU/mL), dentists should follow the specified protocols for using biocides as suggested by manufacturers of biocides/dental unit.

This study highlights the importance of routine monitoring the DUWLs water quality by using a microbiological test that provides valid results. Till now this can be done by testing DUWLs water samples by using conventional microbiological techniques, which involves the culturing on R2A or similar media plates. However, for dental practitioners it can be laborious and expensive to send the DUWLs water samples to microbiology laboratory for conventional microbiological testing. To overcome this problem, various authors have tested in-office tests such as Petrifilm[TM] test, Heterotrophic Plate Count Sampler[TM] [21,39]; Aquasafe[TM] water test [39]; Dip slide[TM] [40] for monitoring the quality of DUWLs water samples. According to their suggestions, although these in-office tests are not very sensitive, their specificity values are very high and show gross bacterial contamination level of water samples. This will help the dentists to inspect the failure of disinfection protocols.

5. CONCLUSION

For the first time, DUWLs have been evaluated in this study for bacterial contamination in district Khairpur, Sindh, Pakistan. High level of bacterial contamination and presence of *P. aeruginosa* and Total Coliform in DUWLs water samples highlights the need for effective disinfectant treatment of DUWLs and regular monitoring the bacterial quality of DUWLs output water. Further research is needed to investigate the risk of bacterial transmission to patients and dental staff. Local and National Health department should take measures to provide guidelines to dental staff for using disinfectants for the eradication of biofilm in DUWLs and routinely monitoring the quality of water. Subsequently, the health department should ensure the compliances with guidelines by the dental practices. Manufacturers of the dental units should also take efforts for developing biofilm resistant DUWLs.

REFERENCES

1. Abdouchakour F, Dupont C, Grau D. *Pseudomonas aeruginosa* and *Achromobacter* sp. clonal selection leads to successive waves of contamination of water in dental care units. Appl. Environ Microbiol. 2015;81(21):7509-24.
2. Ampornaramveth RS, Akeatichod N, Lertnukkhid J. Application of D-amino acids as Biofilm dispersing agent in dental unit waterlines. Int J Dent. 2018;1-7.
3. Blake GC. The incidence and control of bacterial infection in dental spray reservoirs. Br Dent J. 1963;115:412-416.
4. Pankhurst CL, Coulter WA. Do contaminated dental unit waterlines pose a risk of infection? J Dent. 2007;35(9): 712-20.
5. Szymańska J. Biofilm and dental unit waterlines. Ann Agric Environ Med. 2003;10(2):151-7.
6. Szymanska J, Sitkowska J, Dutkiewicz J. Microbial contamination of dental unit

waterlines. Ann Agric Environ Med. 2008; 15(2).

7. O'Donnell MJ, Boyle MA, Russell RJ. Management of dental unit waterline biofilms in the 21st century. Future Microbiol. 2011;6:1209-1226.

8. Alkhulaifi MM, Alotaibi DH, Alajlan H. Assessment of nosocomial bacterial contamination in dental unit waterlines: Impact of flushing. Saudi Dent J. 2019; 32(2):68-73.

9. Meiller TF, Kelley JI, Baqui AA. Disinfection of dental unit waterlines with an oral antiseptic. J Clin Dent. 2000;11(1): 11-5.

10. Putnins EE, Di Giovanni D, Bhullar AS. Dental unit waterline contamination and its possible implications during periodontal surgery. J Periodontol. 2001;72(3): 393-400.

11. Martin MV. The significance of the bacterial contamination of dental unit water systems. Br Dent J. 1987;163(5):152.

12. Borella P, Bargellini A, Marchesi I. Prevalence of anti-legionella antibodies among Italian hospital workers. J Hosp Infect. 2008;69(2):148-55.

13. Gungor ND, Kadaifçiler DG, Peker OÖ. Investigation of the bacterial load and antibiotic susceptibility of dental units. Environ Monit Assess. 2014;186(3): 1847-53.

14. Ricci ML, Fontana S, Pinci F, Fiumana E, Pedna MF, Farolfi P, Sabattini MA, Scaturro M. Pneumonia associated with a dental unit waterline. The Lancet. 2012;379(9816):684.

15. ADA. American Dental Association: Statement on Dental Unit Waterline. 2004. Available:https://www.ada.org/en/member-center/oral-health-topics/dental-unit-waterlines Accessed: 5 November 2018

16. William G, Kohn AS, Collins MPH. Centers for Disease Prevention and Control (CDC). Guidelines for Infection Control in Dental Health-Care Settings Recommendations and Reports; MMWR. 2003;52:1-76. Available:http://www.cdc.gov/mmwr/PDF/rr/rr5217.pdf Accessed: 5 November 2018

17. Arvand M and Hack A. Microbial contamination of dental unit waterlines in dental practices in Hesse, Germany: A cross-sectional study. Eur J Microbiol Immunol. 2013;3(1):49-52.

18. Leoni E, Dallolio L, Stagni F. Impact of a risk management plan on *Legionella* contamination of dental unit water. Int J Environ Res Public Health. 2015;12(3): 2344-58.

19. Ditommaso S, Giacomuzzi M, Ricciardi E. The role of chemical products at low doses in preventing the proliferation of bacteria in dental unit waterlines: the ICX® experience. J Water Health. 2018;16(1): 150-8.

20. Lizzadro J, Mazzotta M, Girolamini L. Comparison between two types of dental unit waterlines: how evaluation of microbiological contamination can support risk containment. Int J Environ Res Public Health. 2019;16(3):328.

21. Morris BF, Vandewalle KS, Hensley DM. Comparison of in-office dental unit waterline test kits. Military Medicine. 2010;175:901-906.

22. Park JE, Ahn TS, Lee HJ. Comparison of total and faecal coliforms as faecal indicator in eutrophicated surface water. Water Sci Technol. 2006;54(3):185-90.

23. Al-Hiyasat AS, Ma'Ayeh SY, Hindiyeh MY. The presence of *Pseudomonas aeruginosa* in the dental unit waterline systems of teaching clinics. Int J Dent Hyg. 2007;5(1): 36-44.

24. John GH, Noel RK, Peter HAS. Bergey's Manual of Determinative Bacteriology Williams & Wilkins, USA; 1993. ISBN: 0-683-00603-7

25. Lal S, Pearce M, Achilles-Day UE. Developing an ecologically relevant heterogeneous biofilm model for dental-unit waterlines. Biofouling. 2017;33(1):75-87.

26. Ji XY, Fei CN, Zhang Y. Evaluation of bacterial contamination of dental unit waterlines and use of a newly designed measurement device to assess retraction of a dental chair unit. Int Dent J. 2016; 66(4):208-14.

27. Reasoner DJ, Geldreich EE. A new medium for the enumeration and subculture of bacteria from potable water. Appl Environ Microbiol. 1985;49(1):1-7.

28. Zhang W, Onyango O, Lin Z. Evaluation of Sterilox for controlling microbial biofilm contamination of dental water. Comp Conti Edu Dent. 2007;28:590-592.

29. Watanabe A, Tamaki N, Matsuyama M. Molecular analysis for bacterial contamination in dental unit water lines. New Microbiol. 2016;39(2):143-5.

30. Bjarnsholt T. The role of bacterial biofilms in chronic infections. APMIS. 2013;121:1-58.

31. Aprea L, Cannova L, Firenze A. Can technical functional and structural characteristics of dental units predict *Legionella pneumophila* and *Pseudomonas aeruginosa* contamination? J Oral Sci. 2010;52(4):641-646.

32. Watanabe E, Agostinho AM, Matsumoto W. Dental unit water: bacterial decontamination of old and new dental units by flushing water. Int J Dent Hyg. 2008;6(1):56-62.

33. Barben J, Kühni C, Schmid J. Water quality in dental chair units. A random sample in the canton of St. Gallen. Schweiz Monatsschr Zahnmed. 2009; 119(10):976-85.

34. D'ovidio C, Carnevale A, Pantaleone G. First report of an acute purulent maxillary sinusitis caused by *Pseudomonas aeruginosa* secondary to dental implant placement in an immunocompetent patient. Br Dent J. 2011;211(5):205.

35. Walker JT, Marsh PD. A review of Biofilms and their role in microbial contamination of dental unit water systems (DUWS). IBB. 2004;54(2-3):87-98.

36. Martin MV, Gallagher MA. An investigation of the efficacy of super–oxidised (Optident/Sterilox) water for the disinfection of dental unit water lines. Br Dent J. 2005;198(6): 353.

37. Schel AJ, Marsh PD, Bradshaw DJ. Comparison of the efficacies of disinfectants to control microbial contamination in dental unit water systems in general dental practices across the European Union. Appl Environ Microbiol. 2006;72(2):1380-7.

38. Shajahan IF, Kandaswamy D, Srikanth P. Dental unit waterlines disinfection using hypochlorous acid-based disinfectant. J Conserv Dent. 2016;19(4):347.

39. Momeni SS, Tomline N, Ruby JD. Evaluation of in-office dental unit waterline testing. General Dentistry. 2012;60(3): 142-7.

40. Lal S, Singhrao SK, Bricknell M. Monitoring dental-unit-water-line output water by current in-office test kits. Curr Microbiol. 2014;69(2):135-142.