MicroMedicine

ISSN 2449-8947

RESEARCH ARTICLE

Isolation of Enterobacteriaceae and non-fermenting Gram-negative bacilli (NFGNB) from Dental Unit Water Lines (DUWL) in a tertiary care institutional setup

Harshitha J. Naik[1], A. Ebinesh[2]*, Shobha[3], K. Vishwas Saralaya[4]

[1] Department of Microbiology, Shridevi Institute of Medical Sciences and Research Hospital, Tumkur - 572106, Karnataka, India

[2] Intern, Shridevi Institute of Medical Sciences and Research Hospital, Tumkur - 572106, Karnataka, India

[3] Manipal College of Dental Sciences, Mangalore, India

[4] Department of Microbiology, Kasturba Medical College, Mangalore, India

*Corresponding authors: Harshitha J. Naik, harshitha.naik@gmail.com; A. Ebinesh; E-mail: ebineshjezreelsurgctvs@gmail.com

ABSTRACT

Background: The quality of dental unit water lines (DUWL) is of considerable importance since patients and dental staff are regularly exposed to water and aerosols generated from dental units which thereby influence the individual patient outcome and health-care associated morbidity. The aim of the present study was to determine the microbiological quality of water used, presence of biofilms and also the potential of isolated bacterial species in producing biofilms within DUWL.

Methods: Thirty DUWL samples were collected from various departments of Manipal College of Dental Sciences, Mangalore. Bacteriological analysis was done for the presence of various bacterial contaminants. Presence of biofilms on DUWLs and potential of bacterial isolates to form biofilm were also determined.

Results: Seven of 30 samples (23.3%), were found to be of unsatisfactory quality (coliform count > 200 CFU/ml), most frequently from air/water syringes. A total of 45 strains were isolated from 14 water samples. Genera isolated were *Escherichia* spp., *Enterobacter* spp., *Klebsiella* spp., *Pseudomonas* spp. and *Acinetobacter* spp. Four of 10 samples from DUWL tubing showed presence of biofilms (40%), formed mostly by *Acinetobacter* spp. and *Pseudomonas* spp. Out of 45 strains that were isolated, 19 strains displayed ability to form biofilms. Maximum number (10) isolates formed biofilms with 48 hours.

Conclusion: Exposure to contaminated water from DUWL poses threat to the well-being of the patient and the health care personnel as well. Hence, measures should be initiated to ensure the optimum quality of DUWL water.

Keywords: Enterobacteriaceae; Non-fermenting Gram-negative bacilli (NFGNB); Dental Unit Water Lines (DUWL).

OPEN ACCESS

Citation: Harshitha NJ, Ebinesh A, Shobha, Saralaya KV. Isolation of Enterobacteriaceae and non-fermenting Gram-negative bacilli (NFGNB) from Dental Unit Water Lines (DUWL) in a tertiary care institutional setup. MicroMed. 2018; 6(2): 78-84.

DOI: http://dx.doi.org/10.5281/zenodo.1319772

Received: April 27, 2018

Revised: July 12, 2018

Accepted: July 23, 2018

Conflict of interest: The authors declare that they have no conflicts of interest.

Source of funding: The authors did not receive grant or exogenous funding to support the preparation of manuscript. The authors did not receive payment or any benefits from commercial entities.

Ethical considerations: not applicable.

INTRODUCTION

Dental unit water lines (DUWL) plastic tubes which deliver water to hand-held instruments that are routinely used in dental procedures. The significance of the quality of these DUWLs cannot be over-emphasised since the health-state of the patient and the dental staff are considerably influenced [1]. These DUWLs are known to be colonised by multiple micro-organisms. Another significant challenge about these microbes is their ability to rapidly form biofilms following colonization [1]. Biofilm formation is an important survival strategy of these organisms that enable prolonged persistence which in turn is associated with multiple health hazards. Consequently, the bacteriological quality of dental unit water lines is usually unacceptable with high coliform count, sometimes as high as >10^6 CFU/ml [2]. Though available evidences suggest that these microbes are non-pathogenic to healthy individuals, these cause considerable morbidity in immuno-compromised patients and those with generalised severe illnesses [3]. Other than immuno-compromised patients, these microbes cause opportunistic infections in pregnant women, transplant-recipients, aged, alcoholics and smokers. Several studies have reported the isolation of various bacteria such as *Streptococci* spp., *Staphylococci* spp., *Pseudomonas* spp., *Legionella, Escherichia* spp. and few other Gram-negative bacilli [4-7]. According to the Centre for Disease Control (CDC), the recommended coliform count of dental water should be < 500 CFU/ml of aerobic heterotrophic bacteria. But the American Dental Association (ADA) has further reduced the standard cut-off coliform count to < 200 CFU/ml of aerobic heterotrophic bacteria [3]. In this study, ADA guidelines were followed to interpret the bacteriological quality. In this study, the bacteriological quality of DUWL is assessed and characterised on the basis of various parameters like isolation rate, isolated genera/ species, presence of biofilms and the ability of isolated bacteria to form biofilms.

MATERIALS AND METHODS

The study was conducted in the department of microbiology, KMC, Mangalore in collaboration with the clinical department of Manipal College of Dental Sciences, Mangalore. The samples were collected from a total of 10 dental units. From each unit, the water samples were samples were obtained from:

- Air/water syringe - 3 in 1 syringe designed to deliver air, water or air/water into mouth during dental treatment.
- Mouth-wash water-water
- Air rotor water sample.

Hence, it is three water samples from each unit making a total of thirty (n=30) samples.

Sampling of DUWL

30 DUWL samples were collected randomly from 10 dental units at MCODS Mangalore. All the units were supplied with containers for the collection of water samples.

Bacteriological analysis of DUWL samples

- 50 ml volumes of samples collected from air/water syringe, mouth-wash water and air rotor after disinfecting the tip with 70% alcohol.
- Then inoculated into multiple tubes of MacConkeys broth (double/single strength).
- Incubated at 37^{O} C for 48 hours.
- Coliform count per 100 ml was estimated from number of tubes showing acid/gas production.

Isolation and identification of bacterial isolates from DUWL samples

The collected water sample was filtered using membrane filters. Then the organisms were washed by vortexing the membrane in a container containing 10 ml of sterile PBS for 1 min. These samples were inoculated into BHI broth for observation of bacterial growth. The sample showing growth after suitable incubation period was processed further for the identification of the isolate by standard microbiological methods [8].

Detection of biofilm formation on the DUWL

External DUWS tubing surface was wiped with a sterile alcohol wipe. The tubing was sectioned to obtain a specimen representing 1 cm^2. The surface was rinsed with sterile PBS to remove planktonic cells. Using sterile dental probes, the surface of the biofilm was scraped into 1 ml of sterile PBS. These biofilm samples were then inoculated into BHI broth to observe bacterial growth. Any sample showing growth was processed further for the identification of isolate by standard microbiological methods [8].

Determination of the capacity of bacterial isolates to form biofilm

Bacterial strains isolated from DUWL samples were used to determine their capacity to form biofilms using microtitre plate method [9]. Aliquots of 200 µl of the standardized test bacterial suspension in Lauria broth was transferred into pre sterilized 96-well polystyrene microtitre plates. Incubate at 37°C for 6 hours. 25 µl of 1% crystal violet added to each well, shaking the plates three times to help the colorant to get the bottom of the well. After 15min at room temperature, each well is washed with 200 µl sterile PBS to remove the planktonic cells. Washing was repeated for 3 times. The adhered bacteria forming biofilm was remained on the surface of the well. Crystal violet bound to the biofilm was extracted later with 2 washings with 200 µl of ethyl alcohol. The alcohol was then transferred into a glass tube containing 1.2 ml of alcohol and agitated. The degree of biofilm formation was determined by spectrophotometer at 540 nm. The obtained data is used to classify strains.

Data analysis

Results obtained were analysed using Microsoft Excel.

RESULTS

In this study, we analysed the bacteriological quality and collectively studied various bacteriological characteristics like isolation of different bacteria, identification of isolates, detection of the presence of biofilms and assessment of the capacity to form biofilms, in a total of thirty (n=30) water samples collected from 10 random dental units. Out of the 30 samples, the presumptive coliform count of seven (n=7, n/N=7/30, 23.3%) samples were found to be higher than the acceptable limits (i.e. > 200 CFU/ml) with reference to the ADA recommendation (Table 1). Out of the seven (n=7) samples with unacceptable bacteriological quality, five (n=5) samples were collected from 3 in 1 air/water syringe and two (n=2) samples were collected from air rotor.

All the thirty (n=30) water samples were passed through membrane filters and were further cultured in BHI broth. Isolation rate was 46.7%. Several genera of bacteria were grown from fourteen (n=14, n/N=14/30, 46.7%) samples. From fourteen (n=14) samples, 45 genera of bacteria were isolated (Table 2). Most of the samples yielded multiple isolates. Out of the 45 isolates obtained from 14 samples, the isolation rates for *Escherichia* spp., *Enterobacter* spp., *Pseudomonas* spp., *Klebsiella* spp. and *Acinetobacter* spp. were 28.9% (n=13, n/N=13/45), 22.2% (n=10, n/N=10/45), 22.2% (n=10, n/N=10/45), 15.6% (n=7, n/N=7/45) and 11.1%

(n=5, n/N=5/45) respectively. *Escherichia* spp., *Enterobacter* spp. and *Pseudomonas* spp. were the most commonly isolated bacterial genera. Samples collected from air rotor yielded a maximum of twenty one (n=21, n/N=21/45, 46.7%) isolates while those from air/water syringe and mouth-wash water were thirteen (n=13, n/N=13/45, 28.9%) and eleven (n=11, n/N=11/45, 24.4%) isolates respectively.

Table 1. The microbiological quality of collected samples.

Sample	No. of samples with acceptable coliform count	No. of samples with unsatisfactory quality
Air/ water syringe	5	5
Mouth-wash water	10	0
Air rotor	8	2
Total	23	7

Table 2. The spectrum of bacteria isolated from the collected samples.

Sample	*Escherichia spp.*	*Enterobacter spp.*	*Pseudomonas spp.*	*Klebsiella spp.*	*Acinetobacter spp.*	Total
Air/water syringe	4	1	2	3	3	13
Mouth-wash water	3	4	3	0	1	11
Air rotor	6	5	5	4	1	21
Total	13	10	10	7	5	45

A total of ten (n=10) DUWL tubings were collected to detect the presence of biofilms over the surface (Table 3). Out of the collected ten (n=10) tubings, four (n=4, n/N=4/10, 40%) showed the presence of formed biofilms on their surface. Among the four (n=4) detected biofilms, one (n=1) biofilm was formed combinedly by *Klebsiella* spp., *Pseudomonas* spp. and *Acinetobacter* spp., two (n=2) biofilms were formed by *Pseudomonas* spp. and *Acinetobacter* spp. while one (n=1) was purely formed by *Enterobacter* spp. In total, four (n=4) biofilms yielded eight isolates (n=8).

Table 3. The frequency of biofilms formed by various organisms in the collected DUWL tubing samples.

Sample	*Escherichia spp.*	*Enterobacter spp.*	*Klebsiella spp.*	*Pseudomonas spp.*	*Acinetobacter spp.*
No. of samples	0	1	1	3	3

A total of forty-five (n=45) different strains were isolated from fourteen (n=14) water samples. Out of forty-five (n=45) isolates that were isolated, nineteen (n=19, 42.2%) isolated possessed the ability to form biofilms (Table 4). Three (n=3, n/N=3/13, 23%), three (n=3, n/N=3/10, 30%), seven (n=7, n/N= 7/10, 70%), two (n=2, n/N=2/7, 28.6%) and four (n=4, n/N=4/5, 80%) isolates of *Escherichia* spp., *Enterobacter* spp., *Pseudomonas* spp., *Klebsiella* spp. and *Acinetobacter* spp. respectively possessed the capability to form biofilms. The potential to form biofilms was observed maximum with *Pseudomonas* spp. (70%) and *Acinetobacter* spp. (80%) isolates. It is noticeable that four (n=4, n/N=4/5, 80%) out of five (n=5) isolates of *Acinetobacter* spp. possessed the ability to form biofilm.

Table 4. Table representing the potential of various isolates obtained from the collected samples to form biofilms.

Sample	Air/ water syringe		Mouth-wash water		Air rotor		Total	
	No. of isolates	No. of isolates with the ability to form biofilms	No. of isolates	No. of isolates with the ability to form biofilms	No. of isolates	No. of isolates with the ability to form biofilms	No. of isolates	No. of isolates with the ability to form biofilms
Escherichia spp.	4	1	3	0	6	2	13	3
Enterobacter spp.	1	1	4	1	5	1	10	3
Pseudomonas spp	2	2	3	2	5	3	10	7
Klebsiella spp.	3	1	0	0	4	1	7	2
Acinetobacter spp.	3	2	1	1	1	1	5	4

Out of nineteen (n=19) isolates that exhibited the ability to form biofilms, four (n=4, n/N=4/19, 21%) isolates formed biofilms within 24 hours, ten (n=10, n/N=10/19, 52.6%) isolates formed biofilms within 48 hours and five (n=5, n/N=5/19, 26.3%) isolates formed biofilms within 72 hours (Table 5). Majority of *Escherichia spp.* isolates (n=2, n/N=2/3, 66.7%) formed biofilm only between 48 to 72 hours while majority of isolates of the other genera formed biofilms within 24 to 48 hours.

Table 5. The ability of various isolates to form biofilms with respect to the duration of incubation.

Sample	24 hrs	48 hrs	72 hrs	Total
Escherichia spp.	0	1	2	3
Enterobacter spp.	1	1	1	3
Pseudomonas spp.	2	4	1	7
Klebsiella spp.	0	1	1	2
Acinetobacter spp.	1	3	0	4
Total	4	10	5	19

DISCUSSION

The current study is a qualitative assessment and characterization of the microbial contamination of DUWL. In the present study, the bacteriological quality of seven samples was unacceptable according to ADA definition [6]. Previous studies have reported a contamination rate of as high as 96% [10]. In the present study, the frequency of contamination was higher in the samples collected from air/water syringe followed by air rotor. Few studies have recorded an inverse frequency [11, 12] while one more study has reported no significant difference [10]. A total of forty-five isolates were obtained from fourteen water samples which signify contamination of water with multiple bacterial genera. *Escherichia* spp. were the commonest isolates followed by *Enterobacter* spp. and *Pseudomonas* spp. Fotedar et al. [7] have recorded the isolation of Coagulase negative *Staphylococci.* Siang et al. [13] have documented the isolation of *Pseudomonas aeruginosa* and *Legionella pneumophila.* The death of an 81-year female patient who contracted Legionnaire's pneumonia from contaminated dental unit water line has been reported in Italy [14]. Another study undertaken by Smith et al. [15] reported the isolation of oral *Streptococci*, *Pseudomonas* spp. and *Staphylococcus aureus.* There is a

great variation in the microbiological quality and the frequency of isolation different organisms in the existing literature. These wide variations can be attributed to the loco-regional variations in the quality of water supplied, the source of water, the variations in the oral microbial flora and the effectiveness of periodic decontamination.

Biofilms were detected over four out of ten tubings that were sampled. Ten isolates were isolated from these biofilms. A maximum frequency was observed with *Pseudomonas* spp. and *Acinetobacter* spp. Owing to the stagnation of water within the tubings, the microbes settle over the inner surface of the tubings that initiates a sequence of physiological alterations resulting in colonization, micro colony formation and eventually biofilm development [16]. Out of the forty-five isolates, nineteen isolates possessed the ability to form biofilms. The maximum ability to form biofilms was observed with *Pseudomonas* spp. and *Acinetobacter* spp. However, isolates belonging to other genera (*Escherichia* spp., *Enterobacter* spp. and *Klebsiella* spp.) also possessed a moderate ability to form biofilms. Ten of the isolates formed biofilms within 24 to 48 hours while four strains formed biofilms within 24 hours. This poses a significant threat since stagnation of water within the tubings for just 24 to 48 hours might result in colonization and biofilm formation that throws a potential risk to patients and dental care workers.

Currently, there is no available evidence that demonstrates a public health issue due to DUWL exposure. However, minimizing the risk of pathogen exposure will ensure a safe working ecosystem both for the health care workers and the patients. Especially, the immunocompromised patients are at a high risk of developing opportunistic infections following exposure to contaminated DUWL. Dental health care workers are also constantly exposed to aerosols from the dental equipment every day. Unsatisfactory quality of DUWL predisposes the dental personnel to the risk of developing respiratory tract infections especially, if colonised by *Legionella pneumophilia*. Hence, it is essential to ensure the optimum microbiological quality of DUWL by periodic surveillance and regular decontamination measures. As per the recent evidences, the usage of continuous water stay systems with chemical action such as IGN EVO Calbenium and Sterispray would be a superior modality [17].

CONCLUSIONS

The dental unit water lines favor rapid development of biofilms on DUWLs, combined with generation of potentially contaminated aerosols. Contaminated water from DUWL might be consumed, inhaled as aerosols or might contaminate operating site. Exposure to water/aerosols containing bacteria (especially nosocomial pathogens with higher intrinsic antimicrobial resistance such as *Pseudomonas* &*Acinetobacter*) in debilitated patients may lead to life-threatening infections. Therefore it is important to not only maintain a supply of good quality water but also to keep regular quality control checks and regular sterilization/disinfection of dental units.

AUTHOR CONTRIBUTIONS

HJN, VSK and SH conceptualized the study. HJN, VSK and EA drafted the protocol for carrying out the study. HJN, VSK and SH collected samples and carried out the bench work. EA and HJN analysed the results and prepared the manuscript. All the authors read the final draft of the manuscript and approved.

ACKNOWLEDGEMENT

The authors acknowledge the contributions of Mrs. Maya Jose for collecting and processing the samples. The authors also acknowledge the management of Shridevi Institute of Medical Sciences and Research Hospital, Tumkur and Kasturba Medical College, Manipal for their support.

REFERENCES

1. Nandwani SS, Paul JL. Biofilm and dental water lines. J Clin Infect Dis. 2000; 47: 30-33.
2. Barbeau J, Gauthier C, Payment P. Biofilms, infectious agents and dental unit waterlines. Can J Microbiol. 1988; 44: 1019-1028.
3. Szymanska J. Control methods of microbial water quality in dental unit waterlines. Ann Agric Environ Med. 2003; 10: 1-4.
4. Mills SE. The dental unit waterlines controversy: defusing the myths, defining the solutions. J Am Dent Assoc. 2000; 131: 5-10.
5. Ajami B, Ghazvini K, Movahhed T, Ariaee N, Shakeri MT, Makarem S. Contamination of a dental unit water line system by *Legionella pneumophila* in the Mashhad School of Dentistry in 2009. Iran Red Crescent Med J. 2012; 14: 376-378.
6. Oleiwi RS. Bacterial contamination of dental unity water lines (DUWL) in Baghdad city. IOSR J Dent Med Sci. 2017; 16: 47-50.
7. Fotedar S, Ganju S. Microbial contamination of dental unit water lines in H. P. Government dental college, Shimla. Saudi J Dent Res. 2015; 6: 129-132.
8. Collee JG, Marr W. Tests for identification of bacteria. In: Practical medical microbiology. Collee JG, Duguid JP, Fraser AG, Marmion BP, eds. Chruchill Livingstone. 13th edn. 1990: 204-239.
9. Maldonado NC, Silva de Ruiz C, Cecilia M, Nader-Macias ME. A simple technique to detect *Klebsiella* biofilm-forming-strains. Inhibitory potential of *Lactobacillus fermentum* CRL 1058 whole cells and products. Commun Curr Res Educ Topics Trends Appl Microbiol. 2007: 52-58.
10. Nikaeen M, Hatamzadeh M, Sabzevari Z, Zareh O. Microbial quality of water in dental unit waterlines. J Res Med Sci. 2009; 14: 297-300.
11. Barbeau J, Tanguay R, Faucher E, Avezard C, Trudel L, Côté L, Prévost AP. Multiparametric analysis of waterline contamination in dental units. Appl Environ Microbiol. 1996; 62: 3954-3959.
12. Noce L, Di Giovanni D, Putnins EE. An evaluation of sampling and laboratory procedues for determination of heterotrophic plate counts in dental unit waterlines. J Can Dent Assoc. 2000; 66: 262.
13. Siang MM, Yunus Z, Yunus ARM, Ahmad Z, Toosab H. The microbiological quality of water from dental unit waterlines in Malayasian armed forces dental centres. Arch Orofacial Sci. 2012; 7: 14-20.
14. Ricci ML, Fontana S, Pinci F, Fiumana E, Pedna MF, Farolfi P, et al. Pneumonia associated with a dental unit water line. Lancet. 2012; 379: 648.
15. Smith S, Smith A. Microbial contamination of used dental waterlines. Am J Infect Dis. 2014; 42: 1019-1021.
16. Szymanska J. Biofilm and dental unit waterlines. Ann Agric Environ Med. 2003; 10: 151-157.
17. Offner D, Fioretti F, Musset A-M. Contamination of dental unit water lines: Assessment of three continuous water disinfection systems. BDJ Open. 2016; 2: 16007.