Original Article

Contamination of Dental Scaler Waterlines with Legionella Pneumophila, Pseudomonas Aeruginosa and Gram Positive Cocci

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Abstract

Background and Aim: Dental staff are exposed to aerosols. Water supply of dental units has insignificant bacterial count but the exiting water in the waterlines has over 100,000 microorganisms per milliliter. Various types of microorganisms exist in the waterline of dental units. Legionella pneumophila (L. pneumophila), Pseudomonas aeruginosa (P. aeruginosa) and Gram-positive cocci are among the most important ones. Scaling and root planning is a dental procedure carrying a high risk of bacterial contamination. This study aimed to assess water contamination in private dental offices in Isfahan city.

Materials and Methods: In this descriptive study, water sampling was done in 50 private offices; 10 mL samples of dental unit water were collected from each scaler and a sample from the city tap water as control. We used 3-step polymerase chain reaction (PCR) for detection of L. pneumophila. The extracted DNA was evaluated for presence of mip gene sequence using spectrophotometry. For detection of P. aeruginosa, samples were cultured in Brilliant Green Bile broth. To confirm P. aeruginosa, the grown colonies were cultured in Cetrimide agar medium and presence of P. aeruginosa was re-confirmed with oxidase test. For evaluation of Gram-positive cocci, multiple smears were prepared and after Gram staining, Gram-positive specimens were cultured in blood agar medium. Data were analyzed using SPSS version 20 and reported in tables and diagrams as number and percentage.

Results: None of the control samples were positive for any bacterium. Thirty-two test samples were also negative for the understudy bacteria; but 18 offices tested positive for these bacteria**.**

Conclusion: Our results shows that hazardous bacteria may be present in dental unit biofilm. Special attention must be paid to the cleanliness of water used in dental procedures.

Key Words: Legionella pneumophila, Pseudomonas aeruginosa, Gram-positive cocci, Dental unit waterline

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Introduction

Patients and dental staff are usually exposed to aerosols produced by water spray and handpiece attached to dental unit. Thus, it is of utmost importance to assess possible microbial contamination of this water [1]. Water supply of dental units has insignificant bacterial count (10 to 100 per milliliter). However, the water sprayed by the handpiece, air/water spray and dental scaler contains more than 100,000 microorganisms per milliliter originating from the microbial biofilm present over the internal surface of waterlines.

Different microorganisms are found in the waterlines of dental units; the most important of which being the P. aeruginosa, cocci and L. pneumophila [2]. Dentists have a higher prevalence of L. pneumophila infections compared to other individuals. Aerosols are mainly responsible for this higher prevalence and are produced in large amounts during scaling [3, 4].

P. aeruginosa is a cause of pulmonary infection and dental aerosols are important route of transmission especially in patients with cystic fibrosis and immunodeficiency [5, 6]. Moreover, cocci, which play an important role in gingivitis, have also been isolated from the dental unit water [7]. The origin of all these bacteria is the microbial biofilm over the internal surface of the waterline of dental units. Biofilm is a complex heterogeneous microbial mass that forms on any non-sterile moist surface [8]. Moreover, oral microbial flora of patients can also enter into the waterlines via suctioning of saliva by the head of the hand piece known as backward contamination. Anderson in 1999 reported backward contamination of the head of high-speed turbines by 500,000 colony-forming units per milliliter [9].

Pankhurst in 2004 reported that bacteria and viruses might be aspirated from the oral cavity into dental hand pieces and contaminate the water. Contaminated water can also enter into the waterlines of the dental scalers and expose other patients as well as the dentists [10]. Scaling and root planning is a traumatic dental procedure with high risk of bacterial contamination. Many studies such as the one by Maki et al. reported bacteremia after scaling and root planning [11]. Scaling and prophylaxis produce numerous aerosols exposing both the dentist and patient. The water sprayed is in direct contact with the gingiva. The gingival tissue is often wounded in these patients and bleeds during the procedure. As the result, this procedure may cause infection in the elderly dentists or immunocompromised subjects [8].

This study aimed to assess dental scaler water contamination with L. pneumophila, S. aeruginosa and Gram-positive cocci in private dental offices in Isfahan city.

Materials and Methods

In this descriptive cross-sectional study, based on significant results of Pouralibaba et al [12] at P=0.05 level of significance, 50 dental offices with active dental units equipped with ultrasonic scaler were selected using census sampling and samples were collected from the water of dental units. The office managers consented to participate in this study. To allow the formation of biofilm, sampling was done at least 24 hours after closing the offices. Problem in the flushing system of dental unit, recent repair and recent washing and disinfection of the waterline of units were the exclusion criteria. None of the offices used a separate water source. The Declaration of Helsinki was followed for the office managers [13]. For each dental unit, 10mL water sample was collected from the tip of the scaler. A sample was also obtained from the tap water of offices before supplying the dental units as control. Samples were poured into test tubes. The lids were closed and the samples were stored in an ice container and immediately transferred to a microbiology laboratory.

For assessment of the presence of L. pneumophila, PCR was performed [14]. Samples were transferred to a microbiology lab at standard temperature (2-8°C) and DNA was extracted the same day. Extraction was done using proteinase K protocol and deposition was done with salt (several centrifugation cycles were carried out at 10,000 rpm using PBS and TES buffers and proteinase K). After visualization of DNA threads and irrigation with 70% ethanol, samples were centrifuged again at 10,000 rpm and 20mL of the TE buffer was added to DNA and PCR was initiated. Extracted DNA was subjected to spectrophotometry to assess the presence of mip gene sequence [15]. PCR was carried out using the 3-step protocol. In other words, three steps of denaturation were repeated for 40 times.

Initial denaturation was performed by incubation at 94°C for 5 minutes, annealing at 62°C for 1 minutes and extension at 72°C for 5 minutes. After amplification, the presence of primer sequence forward: 5-GGT GAC TGC GGC TGT TAT GG-3 and reverse: 5-GGC CAA TAG GTCCGC CAA CG- 3 was evaluated.

To assess the presence of P. aeruginosa, water and sewer assessments were done according to the standard protocol [16]. First, water samples were cultured in Brilliant Green Bile broth medium and

the grown colonies were cultured in Cetrimide agar medium to confirm the presence of P. aeruginosa. Formation of green colonies after incubation of media for 24 hours at 44°C indicated the presence of P. aeruginosa and was reconfirmed with the oxidase test.

To assess the presence of Gram positive cocci, several smears were prepared of each specimen, Gram-stained to confirm presence of Gram-positive cocci and were then cultured on blood agar and incubated at 37°C for 48 hours [17].

Data were collected and analyzed using SPSS version 20 and reported in tables and diagrams as number and percentage.

Results

In all 50 dental offices under study, the control samples from tap water were free from microorganisms; 32 of the test specimens were negative for L. pneumophila, Gram-positive cocci and S. aeruginosa (Diagram 1). However, in 18 offices, dental unit water samples tested positive for these three bacteria (Table 1).

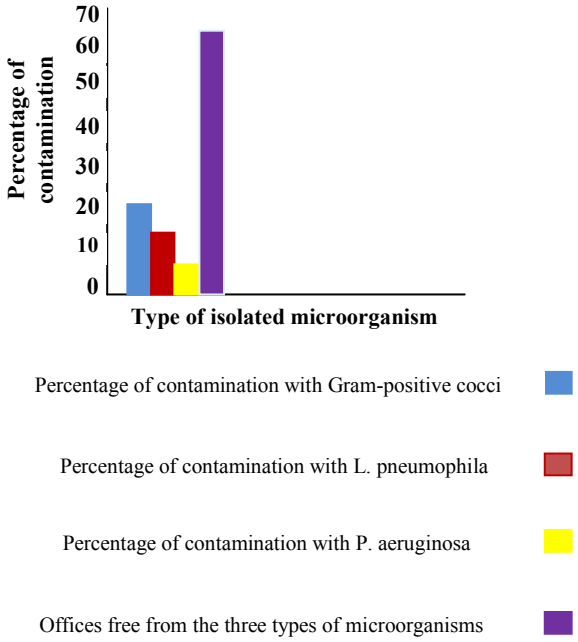

Diagram 1. Percentage of contaminated and contamination-free offices in terms of the three understudy bacteria

Table 1. Number of units of the contaminated or contamination-free offices in terms of the three understudy bacteria

Bacteria	Number of positive test results
Gram positive cocci	8
L. pneumophila	5
P. aeruginosa	3
Gram positive cocci and L. pneumophila	1
P. aeruginosa	1

Discussion

Clinical this study evaluated the contamination of dental unit waterlines with three important bacteria responsible for respiratory infections in patients and dentists; 36% of the offices tested positive for at least one out of the three understudy bacteria. Several studies have measured bacterial counts. Turetgen in 2009 reported that microbial contamination of water in dental units was high for L. pneumophila [18]. Ma'ayeh in 2007 reported that contamination of dental units with L. Pneumophila had a direct correlation with the frequency and duration of service of dental unit [19]. A study in the United States reported 951 cases of infection in pools containing microbial biofilm. The mentioned study indicated the pathogenicity of these bacteria particularly P. aeruginosa in mucocutaneous infections [20].

Ghasempour et al, in their study in Babol city reported the presence of Gram-positive cocci and P. aeruginosa in dental unit waterlines [21]. Araujo in 2002 stated that the work environment of dentists and aerosols are among the routes of transmission of P. aeruginosa to dentists [22].

Abbasi et al, in Shahid Beheshti University reported presence of Gram-positive cocci in dental unit waterlines [17]. In the current study, Gram-positive cocci were the most commonly isolated bacteria indicating the important role of these bacteria in contamination of dental unit waterlines. This finding is in accord with the results of Murphy et al [23]. An important finding in this study was isolation of L. pneumophila from the dental unit waterline of 6 private offices.

However, these bacteria were not found in dental units of other offices. Contamination of dental unit waterlines with this bacterium has not been

evaluated previously in Iran due to problems in culture of this bacterium. In the current study, contamination of the waterlines with this bacterium was evaluated using PCR via the detection of mip gene sequence, which is specific for detection of the pathogenic strain of L. pneumophila [15].

Also, it should be noted that dental staff show a higher degree of positivity for L. pneumophila serum antibodies [12].

A 12% contamination rate of the waterlines of dental units with this bacterium in the city of Isfahan seems concerning. The reason may be backward contamination of the system through the hand pieces from infected patients or personnel [5].

For P. aeruginosa, it should be noted that in addition to respiratory infections, this bacterium is the first cause of septicemia in patients with skin wounds [24].

In the current study, P. aeruginosa was isolated from 6% of dental units. Although this study was conducted in offices that consented to this investigation, positive results in 1/3 of the offices seem alarming in terms of infection control. The bacterial variation found may be due to the duration of usage, method and accuracy of infection control in dental offices and soundness and age of the waterline system. Based on the results of this study, future studies are required to assess the efficacy of addition of different disinfectants, use of waterline system coated with disinfectants, use of a separate water source in dental offices for easier disinfection and designing biofilm removal systems. Also, use of protective measures by dental staff during scaling and prophylaxis must be investigated. Considering the increasing prevalence of resistant, hazardous viruses, presence of these viruses in waterlines of dental offices must also be evaluated.

Conclusion

Based on the results, hazardous bacteria may be present in biofilm of dental units. Special attention must be paid to the cleanliness of water used in dental units, sterilization methods for instruments such as scalers and decontamination of waterline and flushing systems in dental offices.

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References

1. Szymanska J, Wdowiak L, Puacz E, Stojek NM. Microbial quality of water in dental unit reservoirs. Ann Agric Environ Med. 2004 Nov;11(2):355-358.

2. Singh R, Stine OC, Smith DL, Spitznagel JK Jr, Labib ME, Williams HN. Microbial diversity of biofilms in dental unit water systems. Appl Environ Microbiol. 2003 Jun;69(6):3412–3420.

3. Fotos PG, Westfall HN, Synder IS, Miller RW, Mutchler BM. Prevalence of legionella lgM antibody in a dental clinic population. J Dent Res. 1985 Dec; 64(12):1382-1385.

4. Reinthaler FF, Mascher F, Stunzer D. Serological examinations for antibodies against Legionella species in dental personnel. J Dent Res. 1988 Jun; 67(6):942-943.

5. Franco FFS, Spratt D, Leao JC, Porter SR. Biofilm formation and control in dental unit waterlines. Biofilms 2005 Jan;2(1):9-17.

6. Walker JT, Bradshaw DJ, Bennett AM, Fulford MR, Martin MV, Marsh PD. Microbial biofilm formation and contamination of dental-unit water systems in general dental practice. Appl Environ Microbiol. 2000 Aug; 66(8):3363-3367.

7. O'Donnell MJ, Boyle MA, Russell RJ, Coleman DC. Management of dental unit waterline biofilms in the 21st century. Future Microbiol. 2011 Oct;6(10):1209-1226.

8. Miller CH, Palenik CH. Infection control and management of hazardous materials for the dental team. 3th ed. Philadelphia: Mosby; 2005,190-204.

9. Andersen HK, Fiehn NE, Larsen T. Effect of steam sterilization inside the turbine chambers of dental turbine. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 1999 Feb;87(2):184-188.

10. Pankhurst CL, Philpoott-Howard J. Microbiological quality of water in dental chair unit. J Hos Inf. 1993 Mar; 23(3):167-174.

11. Waki MY, Jolkovsky DL, Otomo-Corgel J, Lofthus JE, Nachnani S, Newman MG, et al. Effects of subgingival irrigation on bacteremia following scaling and root planing. J Periodontol. 1990 Jul;61(7):405-411.

12. Pouralibaba F, Balaei E, Kashefimehr A. Evaluation of gram negative bacterial contamination in dental unit water supplies in a university clinic in Tabriz, Iran. J Dent Res Dent Clin Dent Prospect. 2011 Summer;5(3):94-97.
13. World Medical Association. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. Nurs Ethics. 2002 Jan; 9(1):105-109.
14. Den Boer JW, Yzerman EP. Diagnosis of Legionella infection in Legionnaires' disease. Eur J Clin Microbiol Infect Dis. 2004 Dec; 23(12):871-878.
15. Lindsay DS, Abraham WH, Fallon RJ. Detection of mip Gene by PCR for diagnosis of legionnaires' disease. J Clin Microbiol. 1994 Dec; 32(12):3068-3069.
16. American Public Health Association, American Water Works Association and Water Pollution Control Federation. Standard Methods for the Examination of Water and Wastewater. 20st ed. Philadelphia:Mosby; 1999.
17. Abbasi F, Bakhtiari S, Eslami g, Ghaem maghami A. Prevalence of gram positive cocci contamination in the water lines of Shahid Beheshti Dental School units and drinking water supply of local area. J Dent Sch. 2005 Jun; 23(2): 256-263.
18. Türetgen I, Göksay D, Cotuk A. Comparison of the microbial load of incoming and distal outlet waters from dental unit water systems in Istanbul. Environ Monit Assess. 2009 Nov; 158 (1-4):9-14.
19. Ma'ayeh SY, Al-Hiyasat AS, Hindiyeh MY, Khader YS. Legionella pneumophila contamination of a dental unit water line system in a dental teaching centre. Int J Dent Hyg. 2008 Feb; 6(1):48-55.
20. Freije MR. Spas, hot tubs and whirlpool bathtubs: A guide for disease prevention. 1st ed. USA: HC Information Resources Inc; 2000, 1-3.
21. Ghasempour M, Ghobadi Nejad MR, Haji Ahmadi M, Shakki H. Microbiological evaluation of dental unit water at dental offices and dental school in the city of Babol. J of Dent, Mashhad Univ of Med Sci. 2005 Spring-Summer; 29(1-2): 97-104.
22. Araujo MW, Andreana S. Risk and prevention of transmission of infectious diseases in dentistry. Quintessence Int. 2002 May;33(5):376-82.
23. Bagge BS, Murphy RA, Anderson AW, Punwani I. Contamination of dental unit cooling water with oral microorganisms and its prevention. J Am Dent Assoc. 1984 Nov; 109(5):712-6.
24. Brooks GF, Carroll KC, Butel JS, Morse SA, Mietzner TA. Jawetz Melnick & Adelbergs Medical Microbiology. 26th ed. USA: Lange Basic Science Series; 2013,284.