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ORIGINAL ARTICLE

Microbial contamination of dental unit water lines in H.P. Government Dental College, Shimla

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KEYWORDS

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Abstract *Aim*: To determine the microbial quality of water from DUWLs and also to determine the efficacy of flushing on reducing its microbial count in Himachal Pradesh Government Dental College, Shimla.

Method: Samples were collected from all the dental chairs in the department of public health dentistry. Ten millilitres of water was collected in a sterile container from air water syringe in the mid afternoon, once before flushing and once after flushing for two minutes. One control sample was also taken from tap in the department. The samples were then sent to department of Microbiology, Indira Gandhi Medical College, Shimla where they were investigated for gram positive and gram negative cocci; gram positive and gram negative bacilli.

Results: No organism was detected in the control sample from tap water. The only organism that was detected was staphylococcus coagulase negative. The mean of staphylococci coagulase negative colony forming units in pre flushing sample was 1460.89 and in post flushing sample was 1380. The difference between pre flushing and post flushing sample was not statistically significant.

Conclusion: Though the organism present in water was only staphylococci coagulase negative, the level was higher than as recommended by CDC, so appropriate disinfection methods should be used, and the source of staphylococci coagulase negative should be investigated. The flushing did not show any significant difference before and after flushing in the present study.

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1. Introduction

During the past two decades, it has been established that water used in dental treatment has high microbial counts, typically ranging from 104 to $>10^6$ CFU/ml.[1] Previous studies addressing dental unit water supply (DUWS) contamination have confirmed that the high bacterial count is due to the shedding of biofilm bacteria from the lumen surface of dental waterline tubing into treatment water.[1–4]

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Most of the microorganisms in dental unit water lines are not pathogenic in healthy individuals but, may be of great importance in patients with systemic disease.[5] Contamination of DUWL can be of great importance since the patients and dental personnel are in intimate contact with water and aerosols produced in the environment.[6] Although the results of some epidemiologic studies show that contamination of DUWL can be dangerous in patients with immune-deficiency or other immune system problems, it can be true for pregnant women, elderly, graft recipients or even smokers. The various microorganisms isolated from DUWL are potential opportunistic pathogens such as Streptococci spp., staphylococci spp., Enterococci spp., *Pseudomonas aeruginosa*, Legionella, and other gram negative rods.[7–9] These organisms can cause pneumonia, other respiratory infections, or wound infections in immunocompromised people. Dental personnel have been shown to have altered nasal flora, with colonization of *Pseudomonas* spp. consistent with those found in their dental units.[10,11] Cross infections between patients; chronic infection of dental personnel with long term exposure to oral fluids, splatter, and aerosols; and direct infections of open surgical wounds should be a concern for any therapist. The microorganisms capable of forming biofilms on surfaces of DUWLs may also form biofilms on heart valves, creating endocarditis.[12]

The current guidelines by Center for Disease Control and Prevention (CDC) for infection control in dental healthcare settings recommend that dental unit output water should amount to 500 CFU/ml of the aerobic heterotrophic bacteria. The American Dental Association has set a standard for dental unit output water which is equal to 200 CFU/ml of aerobic heterotrophic bacteria.[13,5] In 1993 the U.S. Centers for Disease Control and Prevention recommended that water lines should be flushed to reduce the microbial load in dental unit water.

The present study was conducted with the aim to determine the microbial quality of water from DUWLs and also to determine the efficacy of flushing on reducing its microbial count in Himachal Pradesh Government Dental College, Shimla.

2. Materials and methods

The present study was conducted in the department of Public Health Dentistry, H.P Govt. Dental College, Shimla. Prior permission to conduct the study was taken from concerned authorities. Samples were taken from all the nine chairs in the department. All the chairs had self contained water systems. Samples of water were collected at mid afternoon after 2–3 patients were done, from air water three way spray twice, once before and once after flushing for two minutes from each dental unit. A control sample was also taken from the tap in the department. Ten millilitres of water from the air water three way spray of each chair was collected in a sterile container once before flushing and another sample of 10 ml was collected in another sterile container after flushing from the same dental unit. The samples were labelled with details of the dental unit along with date and time and then immediately transferred to the Department of Microbiology IGMC where these samples were processed. The Department of Microbiology is at about two minutes away from the Dental College. Three procedures were adopted for each sample.

1. Aerobic culture by the spread plate method on MacConkey agar.
2. Aerobic culture by the spread plate method on blood agar containing 5–10% of sheep blood. They were confirmed on the basis of colony morphology, gram staining, catalase and coagulase test.
3. Presumptive Coliform count. The multiple tube method was used for estimation of the probable number of coliform bacilli. Double and single strength MacConkey broth in bottles containing Durhams' tube were used. The tubes were incubated at 37 °C for 48 h and an estimate of the coliform count per 100/ml was made from the tubes showing acid/gas production.

A control water sample directly from the tap water in the department of Public Health Dentistry was processed along with the study samples.

The data were analysed by the SPSS package version −15. The statistical test used was paired *t* test. A *p* value of 0.05 was considered to be statistically significant (see Table 1).

3. Results

On the MacConkey Agar no growth was obtained in 7 samples (both the initial and the flushed sample). One paired sample showed growth of contaminants and one had growth of gram positive cocci in the unflushed sample.

In all the Sheep blood agar samples ($N = 9$) growth of coagulase negative staphylococci was obtained in both the initial as well as the flushed sample of all the nine chairs. The number of colonies ranged from 10 to 18 in unflushed samples and from 7 to 18 in flushed samples. The number of colony forming units (cfu) ranged from 1040 to 1876 in unflushed samples and from 931 to 1863 in flushed samples. The mean of number of colony forming units in unflushed sample and flushed samples was 1460.89 and 1380 respectively and the difference between the samples was not statistically significant (p value = 0.295 9). The coliform count from the control sample of tap water was zero.

Table 1 Level of contamination of Dental unit water lines before and after flushing.

Sample no.	Before flushing CFU/ml	After flushing CFU/ml
1	1230	1103
2	1460	1370
3	1040	931
4	1557	1311
5	1738	1731
6	1876	1863
7	1043	1002
8	1532	1478
9	1672	1631
Mean ± SD	1460.89 ± 298.55	1380 ± 326.9

p value – 0.295; one tailed paired *t* test.

4. Discussion

The present study was conducted in H.P. Government Dental College to assess the qualitative and quantitative contamination of dental unit water lines. The only species that could be detected in the dental unit water lines was coagulase negative staphylococci in all the samples. The presence of staphylococci coagulase negative in the dental unit waterlines has also been reported by Venkatash,[14] Lachachi[15] and by Messano[16] in the dental environment. The source of microorganisms in DUWLs may be either municipal water piped into the dental unit or suck back of patient's saliva into the line due to the lack of preventive valves.[17–20] But in the present study, the municipal water from the tap in the department was also free from microorganisms, so this possibility is excluded. The next possibility is suck back of patients saliva into the line due to lack of preventive valves but the chairs installed in the department are fitted with anti retraction valves. So, either the anti retraction valves are not very effective or the only hypothesis that can
be drawn from here is that the investigator can be a carrier of coagulase negative staphylococci as they are normal flora of human skin. As coagulase-negative staphylococci are frequently associated with opportunistic human and can co-colonize mucosal surfaces along with *Staphylococcus aureus* which may pose a risk for infection in immune compromised states. So, further studies are required to identify the actual source of staphylococci coagulase negative and to take appropriate measures to prevent it in order to avoid any kind of cross infections.

The mean of cfu before flushing and after flushing was 1460.89 ± 298.5 and 1380 ± 326.9 respectively and the difference between pre flushing and post flushing was not statistically significant which has also been reported by Santiago,[21] Rice[22] but in contrast to Mansourian.[23]

The water sample taken from the tap in the department was free from microorganisms. Also, the dental unit water lines in the present study did not reveal any microorganism other than coagulase negative staphylococcus. This may be because the individual reservoir of each dental unit was drained, dried and placed back at the end of each working day. The reservoir was filled in the morning with the water from the municipal tap in the department. Since there was no stagnant water in the reservoir there may be no microbial growth other than staphylococci coagulase negative. Further the climatic conditions such as temperature and humidity, here in Shimla are such that which may not favour the microbial growth in reservoir.

But the presence of staphylococci in the samples suggests that suitable disinfection procedures should be followed to avoid any cross infections. The recommendations by Centers of Disease Control about flushing the dental unit water lines, alone is not reliable procedure for improving water quality used in dental treatment. A reasonable protocol for disinfecting is required so that water used for dental patient treatment satisfies accepted safe public health standards.

The limitation of this study is that the anaerobic culture methods, special methods for legionella species and free living amoeba were not studied. So, further studies with above mentioned methods and to find out the source of staphylococcus coagulase negative are recommended.

5. Conclusion

No microorganism other than staphylococcus coagulase negative was found in the present study, but the levels being higher than that recommended by CDC shows the quality of water in dental unit water lines is not acceptable. The flushing did not play any significant role in reducing the microbial load. It is recommended to use proper disinfection methods to have acceptable level of water and to conduct more studies to know the source of staphylococci coagulase negative.

Conflict of interest

We have no conflict of interest to declare.

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