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Comparative Evaluation of the Effectiveness of Conventional vs Disposable Dental Unit Waterline in Limiting the Contamination of Microorganisms in the Waterline System

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ABSTRACT

Context: Cross infection from contaminated dental instruments is a matter of great concern. On sudden stoppage of an air-rotor, the airflow is cut off creating negative pressure in the unit leading to suck-back of saliva from the patient's mouth.

Aims: To detect the presence of microorganisms in the waterline of air rotor units with time. The effect of using disposable waterline on the microbial load was also investigated.

Methods and Material: The study was conducted on randomly selected patients having age range of 20 to 30 years, with indication for single or multiple units fixed partial denture fabrication in the Department of Prosthetic Dentistry. In Group A, the existing waterline system after treatment on each patient was examined for determining the influence of time over the degree of microbial contamination. In Group B, the air-rotor handpiece-coupling joint was attached with sterilised disposable plastic tubelines instead of existing tubeline. Water samples collected from air rotor units and watercan of both Group A and B before and during use for 15,30 & 45 minutes were subjected to microbiological study.

Statistical analysis used: Chi-square test was used to assess the effect of time on the microbial load from the data obtained.

Results: After 45 minutes of use, 50% of the samples in group A were positively related to microbial contamination and the result obtained from Group B were negative.

Conclusions: Air-rotor with existing tube line after 45 minutes of use is significantly responsible for the contamination. Air-rotor with disposable tube line on the contrary could be used without risk of contamination on the next patient even after 45 minutes.

Key Words: Air rotor handpiece, Source of bacterial infection, Biofilm, Disinfection

INTRODUCTION

Cross infection from contaminated instruments and equipment used in dental office is a matter of concern. The instrument unless disinfected or sterilised, there remains a possibility of transmission of infection from the patient to the operator or from one patient to the next[1]. On sudden stoppage, the airflow in the air-rotor is cut off creating negative pressure in the unit leading to suck-back of saliva and oral fluids from the patient's mouth. This

contaminates the handpiece, waterline and water reservoirs.[2] The unit if not sterilised in the interval between two patients, there is every possibility of transmission of infection.[3] Dental Unit Waterline has water inlet at its other end which become continuous with the waterline unit through a handpiece coupling socket. Contaminants can backflow through it, ultimately reaching the watercan.[4]

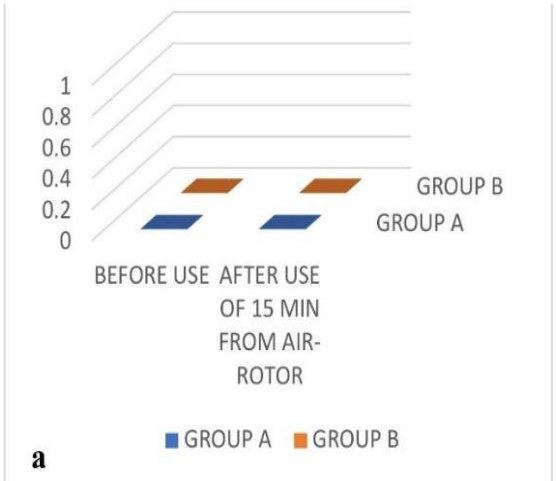
To minimise clogging of the lumen of the water tube line of air rotor handpiece and eliminate contamination from the water supply, purified distilled water was used more often. Microorganism especially bacteria were still found to colonise and form biofilm in the water lines.[5] There is growing concern about the increasing number of patients with diminished resistance to opportunistic infections, attending dental treatment.[6] In addition to this, the complex and delicate nature of the air rotor unit does not permit repeated sterilisation of the unit.[7,8] Introduction of disposable water tubeline, which would permit easy and quick replacement after use on patient, has been investigated in this study.

Aims and Objectives: The purpose of the study was to detect the presence of microorganisms in the waterline of air rotor units and the effect of time on the extent of contamination. Effect of using disposable waterline on the microbial load in the air rotor unit was also investigated.

MATERIALS AND METHOD

Table 1: Inclusion and exclusion criteria of the study

INCLUSION CRITERIA	EXCLUSION CRITERIA
Young patients within age group of 20-30 years.	Infectious diseases like Hep-B, Syphilis, AIDS, Covid-19, TB, Pneumonia, Parotitis, meningitis, mumps or other Respiratory Tract Diseases.
Patients with indication of prosthodontic rehabilitation of Crowns and Bridges.	Water heater or Pre-filter in dental unit (further exacerbates bacterial proliferation and colonisation of dental unit waterline).[30]
Use of treated water which has <500 CFU/ml of heterogenic water bacteria according to CFU guideline.	Any retraction or anti-retraction valve in the air-rotor.[31]



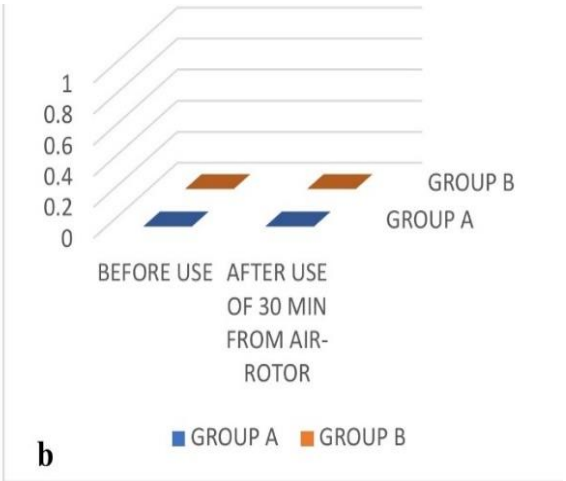


Fig 1a: Chart depicting bacterial growth after 15 minutes of air-rotor use in both Group A and Group B, Fig 1b: Chart depicting bacterial growth after 30 minutes of air-rotor use in both Group A and Group B.

The study was conducted on randomly selected patients within 20 to 30 years age range with indication of single or multiple units fixed partial denture fabrication. Proper history of the patients was taken and examined clinically. None of the selected patients were suffering from any systemic or local infectious diseases. The inclusion and exclusion criteria for the selection of patients has been listed in Table 1. The subjects were explained for the nature and procedure of the study and their consent were taken. Dental Unit Waterline were attached to the air rotor handpieces, presently available in the postgraduate clinic. The units comprised of water

reservoir, water tube lines, adjustable water flow regulating valve, air pressure regulating valve, air pressure gauge, and foot control with accessory tube lines. The water tube line contained a handpiece coupling joint for attachment with air rotor handpiece. This unit was not associated with any retractor valve or anti retraction valve. An autoclave (Midwest 300 SE, manufactured by Dentsply Midwest) was used for sterilizing handpieces, water collection bottles, water containers and flask containing deionised purified water, culture plate, tubes and measuring pipette (supplied by the Department of Microbiology) at 121 degrees centigrade at 15 pounds per square inch pressure for 15 minutes. 2% alkaline glutaraldehyde (CIDEX) was used to sterilise the waterline and reservoir along with their associated coupling for twelve hours (overnight). Water samples were collected following standard aseptic procedures into collection bottles (30 ml McCartney's bottle) from air rotor handpiece-coupling. 20 ml of water was collected and subjected to microbiological study.

In Group A, the existing waterline system of the unit (Image 1a) was used during routine use to determine the influence of time over the degree of microbial contamination. In group B, the water and air tubeline between the watercan and the air rotor handpiece-coupling joint was replaced with sterilised disposable plastic tubelines (Image 1b). Water samples collected before, during and after use from air-rotor units of Group A and Group B were subjected to microbiological analysis. Each of the unit after being filled with CIDEX solution was left overnight before flushing out. Refilling and flushing twice with purified deionised autoclaved water was performed thereafter. The handpiece – coupling joint outlet was then mopped with 70% alcohol and kept covered with disposable sterile plastic packets until use. The tubeline water samples were obtained by holding the handpiece-coupling joint close to the mouth of the sterile collection bottle (30 ml McCartney's bottle) and pressing the foot controller till the bottle was 20 ml full. Collected water samples were cultured within 1 hour. Water from the water reservoir from both the groups was also collected after use for 45 minutes, by withdrawing water using sterile disposable syringe and needle. Samples thus collected were subjected to microbiological investigations. Water samples in measured amount were inoculated in the culture media (Image 2a) for microbiological investigations. Both aerobic and anaerobic culture techniques were employed for each water sample. For aerobic culturing, inoculation of the sample was done in Sheep Blood agar and Brain Heart Infusion broth and agar. These media were then incubated anaerobically in MacIntosh and Filde's jar with hydrogen gas using gas pack system. These jars were incubated at 37 degree for 48 hours and extended for seven days if necessary. The anaerobic isolates were identified by Gram stain, Catalase test, Sugar fermentation test and other tests. Data obtained was tabulated and analysed.

RESULT

The results obtained from Group A and Group B have been tabulated under Table 2. The result of microbial culture report showing collection of water samples from units that were subjected to standard sterilisation protocol before use, were found to be negative. Table 2, Figure 1a, shows the bacterial growth from water samples after 15 minutes of use from handpiece coupling joint of air-rotor units was negative in both the groups. Table 2, Figure 1b reveals that the results from both the group were negative after 30 minutes of use of the air-rotor. Table 2, Figure 2a however shows noticeable difference between the two groups, with Group A showing bacterial growth in fifty percent of the samples after 45 minutes of air-rotor use, while Group B experiences negative results. Table 2, Figure 2b shows no bacterial growth in the water-can of both the groups after 45 minutes of air-rotor use.

Table 2: Chart depicting the difference in bacterial growth in Group A and Group B with respect to time

TIME FACTOR	GROUP A		GROUP B	
	SAMPLE NO.	RESULTS	SAMPLE NO.	RESULTS
After 15 min (Hand-piece coupling joint of air-rotor)	Patient **1**	Negative	Patient **5**	Negative
	Patient **2**	Negative	Patient **6**	Negative
	Patient **3**	Negative	Patient **7**	Negative
	Patient **4**	Negative	Patient **8**	Negative
After 30 min (Hand-piece coupling joint of air-rotor)	Patient **1**	Negative	Patient **5**	Negative
	Patient **2**	Negative	Patient **6**	Negative
	Patient **3**	Negative	Patient **7**	Negative
	Patient **4**	Negative	Patient **8**	Negative
After 45 min (Hand-piece coupling joint of air-rotor)	Patient **1**	Negative	Patient **5**	Negative
	Patient **2**	**Streptococuus sp., Klebsiella sp.**	Patient **6**	Negative
	Patient **3**	Negative	Patient **7**	Negative
	Patient **4**	**Streptococcus viridans**.	Patient **8**	Negative
After 45 min (Watercan of Air-rotor)	Patient **1**	Negative	Patient **5**	Negative
	Patient **2**	Negative	Patient **6**	Negative
	Patient **3**	Negative	Patient **7**	Negative
	Patient **4**	Negative	Patient **8**	Negative
#Patient **1-4**: **Group A**, # Patient **5-8**: **Group B**				

 # Air-rotor was sterilized before use on each patient of Group A & Group B and no bacterial growth was found on culture.

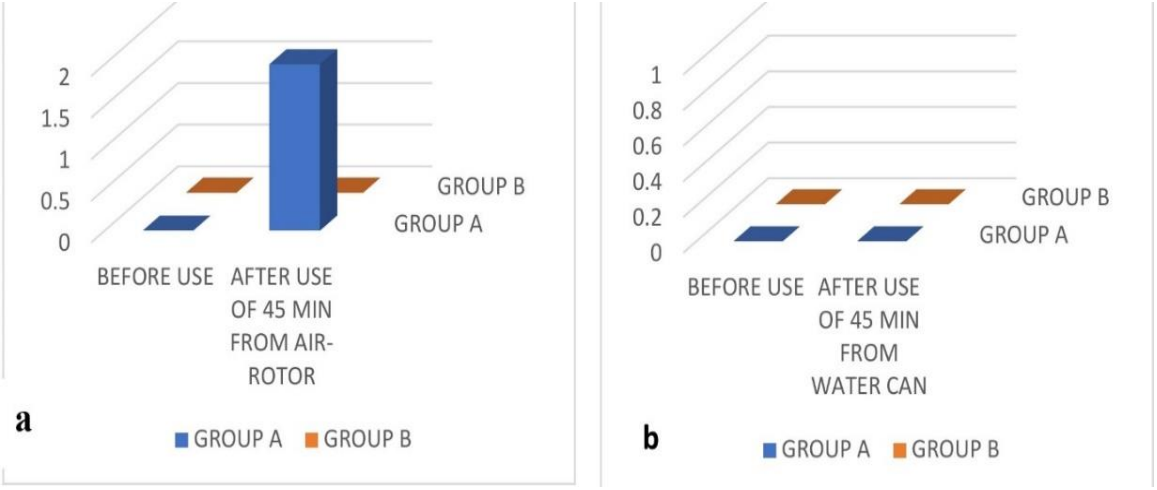


Fig 2a: Chart depicting bacterial growth after 45 minutes of air-rotor use in both Group A and Group B, Fig 2b: Chart depicting bacterial growth from water-can after 45 minutes of air-rotor use in Group A and Group B.

Table 3: Shows the relation of bacterial growth in one patient in Group A and Group B with respect to time through the negativity index.

Percentage of grand total	15 minutes	30 minutes	45 minutes	After use
Group A	13.33%	13.33%	6.67%	13.33%
Group B	13.33%	13.33%	13.33%	13.33%

Table 4: P value and the statistical significance calculated between Group A and Group B.

Test	Chi-square
Chi-square, df	13.39, 3
P value	0.0039
P value summary	**

Negativity index of the subject of a specific group at a time point was calculated as per the given formula.

Negativity index (%) = (No of negative samples/ Total samples) * 100 (Table 3)

Chi-square test was then performed in each set of subjects comparing both groups. p value less than 0.05 was considered as significant (Table 4). All statistical analysis was performed using Graph Pad Prism 8 software. Results of the statistical analysis showed that the bacterial growth in Group A was higher than Group B at 45 minutes of use on one patient, and the p value was statistically significant. ($P < 0.05$). In this study it is important to know, the group of subjects who do not impart infection to the hand-piece coupling joint of air-rotor. Negativity index (percent of subjects who did not introduce infection) is thus more relevant to the study.

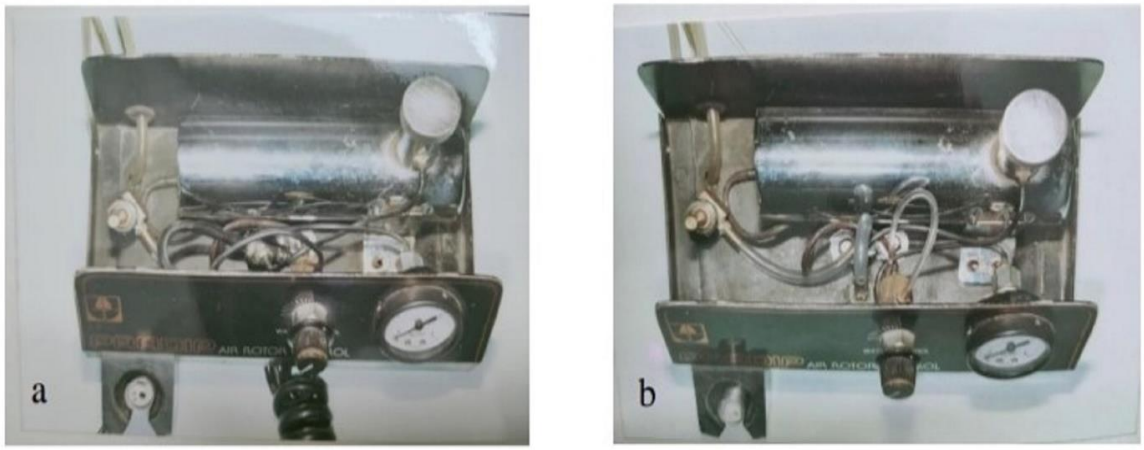


Image 1a: Dental Unit Waterline system with existing tube-line (Group A), Image 1b: Dental unit waterline system with disposable tube-line (Group B)

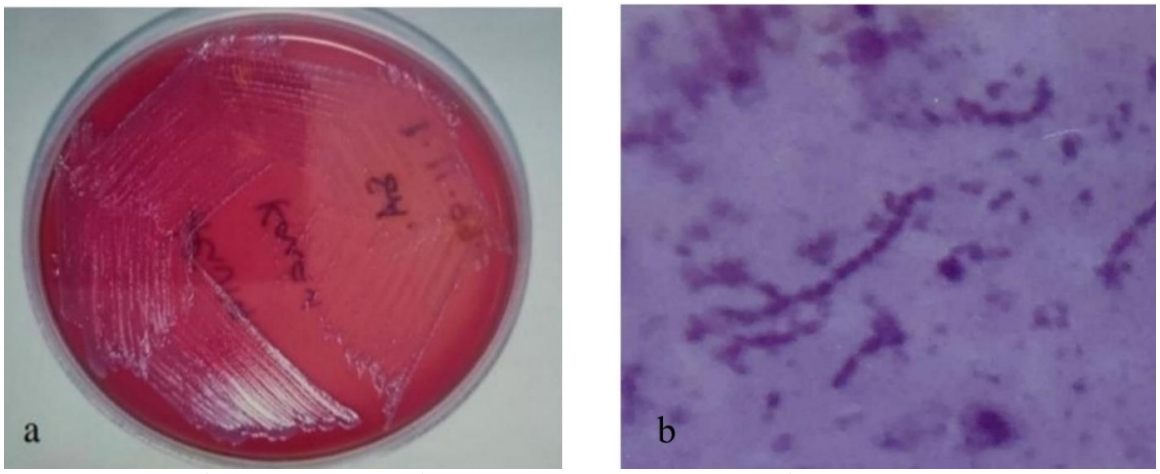


Image 2a: Culture plate for detecting the type of bacterial growth, Image 2b: Growth of Streptococcus viridans as seen under the microscope.

DISCUSSION

The dental unit comprises of an organised water-pipeline network connected to multiple equipment, predisposing the dental working area to risk of microorganism transmission. Use of water by air-rotor (a dental instrument) produces bio-aerosol which acts as a vector in transmitting microbial load to the patients and medical staff during dental treatments. The presence of microbial contamination of the water coming from dental units was first reported by Blake in 1963.[9] Sudden stoppage of the air-rotor leads to negative pressure in the unit as the air-flow is cut off. This could lead to suck back of the oral fluids laden with microorganisms from the water port of the handpiece into the water line of the air rotor unit.[10-12]

The microbial activity in the air-rotor unit waterline and the influence of time on the extent of the same was studied here. The possibility of using disposable water tubeline in controlling the microbial load in air-rotor units was also investigated here. All standard aseptic precautions were observed and purified deionised autoclaved water was used.[13] Samples collected were sent for microbiological investigation. Water samples from units that were subjected to sterilisation procedures before use were found to be negative. This indicates that the sterilisation procedure of filling up the air-rotor unit and the water tubeline with 2% alkaline glutaraldehyde solution for 12 hours (overnight) followed by flushing out with autoclaved deionised purified water was adequate in removing microorganisms in the dental units before

use on patients. This procedure was done under the updated CDC guideline. William H.N et al (1994)[14] also showed the absence of microorganisms following decontamination of air-rotor unit with 100 ml of 1:6 solution of household bleach and tap water left in the unit water line for 10 minutes, followed by flushing with sterile water. Similar observation was made by Kettering et al (2002)[15], Miller TF et al[16] and Lizzadro et al (2019).[17] The microbial culture report following use of the air-rotor unit for 15 minutes on the first patient (Table 2 and Figure 1a) was found to be negative in both the groups A and B. This indicates that microorganisms failed to enter the water tubeline from the handpiece even on suck back.

The microbial culture report of water samples from air-rotor units after use for 30 minutes on the next patient (Table 2 and figure 1b) were found to be negative in both Groups (A and B). The results indicate that microorganisms failed to enter the water tube line, and the extent of suck-back was not sufficient to carry the microbial contaminants from the handpiece to the water tube line. Water samples collected from Group A after 45 minutes of use, revealed that 50% of the samples became contaminated with microorganisms whereas in group B the results were negative with respect to all the samples (Table 2, Figure 2a). The difference in bacterial growth between the two groups were statistically significant, $p<0.05$. Klebsiella species and Streptococcus viridans were the microorganisms identified in Group A. This shows that microbial growth is directly proportional to the time factor (Table 2 and Figure 2a). The existing waterline in Group A were disinfected after each use. Water samples collected from the watercan at the end of 45 minutes of use marked the end of use of the air-rotor for that day. Biofilms represent a complex community of bacteria within an extracellular polysaccharide matrix.[18,19] The presence of biofilms in Dental Unit Waterline is related to several factors, such as water stagnation, which occurs as a result of inactivity during the night, variations in the water supply (tap water, distilled water, or sterile water).[17,20-22] The existing water-line in Group A were found to have microorganisms while the disposable set up in Group B did not show such activities. Presence of rough tubeline wall may have influenced the biofilm formation due to adsorption of macromolecules that have persisted on the inner wall of the tubeline. This promotes rapid attachment of microbes. The unused disposable tubeline having a smooth inner wall, delay colonisation of microorganisms as shown in scanning electron microscopic studies of Mayo J.A et al (1990)[23] and Murdochkinch C. A et al (1997)[24]. The smooth inner wall hampers the attachment of microorganisms which is reflected in the negative result of Group B. Lizzadro et al in 2002, observed that bottle tanks are often composed of polyethylene or polytetrafluoroethylene materials. They are neither autoclavable nor endurable to treatment with high-activity disinfectant.[17,25] They undergo damages like rips and tears, thus promoting bacterial niche formation where the disinfection procedure fails to reach. Water samples from water can of air-rotor before and after use for 45 minutes on one patient have been found to be free of any microbial contamination (Table 2 and Figure 2b). The negative report indicates the microorganisms were not transported to the water can following use for 45 minutes, even though 50% of the tubeline samples in group A under Table 2 at 45 minutes (Figure 2a) were found to be contaminated. The length of the tubeline could be a determining factor in imparting contamination as water from near the handpiece coupling joint would at first have to transverse the tubeline. Collection of 20 ml water samples for analysis from the handpiece coupling joint is bound to withdraw the contaminants away from the water tubeline and water-can thus also giving rise to the sample from the watercan becoming negative. Flushing out the water from handpieces (high-speed drills,

ultrasonic scalers, and air and water syringes) is useful for eliminating the stagnant liquid in the pipes after an inactive period (at the beginning of the work day) because it generates a pressure suitable for removing bacteria that weakly adhere to the biofilm. This process though generates false negative culture reports.[26] Bagga B.S.R et al (1984)[27] detected the presence of microorganisms following use of air rotor unit on the second patient after 45 minutes even after use of anti-retraction valve. William H.N et al (1994)[14] also detected the presence of microbial contamination on second day even after overnight use of bleach solution for decontamination followed by flushing with water. The units were attached to in-house waterlines during use which could have resulted in contamination via the water supplied. Such phenomenon has also been documented by Spagnolo et al.[28,29]

CONCLUSION

Air rotor with existing tubeline (group A) did not show contamination after use for 30 minutes. Contamination after use of air-rotor for 45 minutes showed a significant relationship of time to the bacterial contamination. Air rotor with disposable tube line (group B) can be used without risk of contamination of water-can on 2 consecutive patients. Use of 2% alkaline glutaraldehyde solution for 12 hours (overnight) followed by flushing with autoclaved deionised purified water was effective in keeping the air rotor sterile. Observations performed only at the beginning of the working day with the possibility of testing the water quality at different time points (e.g., during treatment procedures, at the end of the working day, and after a long period of inactivity) may permit effective disinfection. These processes are time consuming and cost-oriented. The use of disposable tubeline thus eliminates the need of constant monitoring and disinfection procedure to a greater extent thus reducing the chances of cross-contamination. The prevailing COVID-19 pandemic situation has resulted in limited attendance of patients who could meet the all the inclusion and exclusion criteria at the OPD. We have received statistically significant results even on limited number of patients in this study. This highlights the gravity of the chances of contamination and the requirement of introduction of modified dental setup as early as possible. This study is thus intended to bring more awareness among dental and medical professionals on this matter of concern.

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