Evaluation Of Efficacy Of 0.2% Chlorohexidine Mouthwash As A Pre-Procedural Rinse In Reduction Of Streptococcal Colonies Following Ultrasonic Scaling: A Pilot Study

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Abstract: Objectives: To evaluate the efficacy of chlorohexidine mouthrinse as a pre-procedural rinse in reducing streptococcal bacterial colonies.Study Design: A single-centre, double-masked, placebo-controlled randomised clinical trial conducted over a period of 15 days. 6 patients were included in the study, patients were first asked to rinse with the placebo (distilled water) for 1 minute before the scaling of control site followed by test site rinsing with chlorhexidine mouthwash for 1 minute. The microbial contamination was checked in the operatory, operator's mouth mask, the patient's chest, the operator's chest and the patient's breath with the help of agar plates. Results: The results revealed that there was statistically significant reduction in the Colony Forming Units (CFU's) formation. Paired sampled t test was used for checking the statistical significance, confidence interval (CI) was 95% and ($p<0.05$).There was statistical reduction in CFU's in breath sample, 30 minutes after scaling and less number of CFU's were formed on the operator's mask after the scaling of the patients with the use of chlorhexidine as the pre-procedural rinse compared to distilled water. Conclusion: The 0.2% chlorohexidine mouth rinse as a pre-procedural rinse has comparatively greater efficacy than distilled water in reduction of streptococcal colony forming units, however this finding was not statistically significant.[Sachit A NJIRM 2016; 7(5):46-52]

Key Words: Chlorohexidine, Aerosols, Pre-procedural mouth rinse

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Introduction: The potential harmful effects produced by aerosols and splatter are a concern in dentistry as they adversely affect the health of patients and dental personnels. Dental health professionals, because of repeated exposures to such aerosol contamination, are at high risk for developing infectious diseases. Transmission of microorganisms may occur directly by contact with contaminated tissues or instruments or by aerosols containing infectious agents.[1,2] Aerosols can be defined as suspensions of liquid and/or solid particles in the air generated by coughing, sneezing, or by any other act that expels oral fluids into the air. Splatter are the aerosols containing particles of size more than 50 µm in diameter, while particles measuring less than 50 µm are called droplet nuclei. The gravitational pull causes splatter aerosols to settle very quickly on surfaces, thus they are less likely to carry microorganisms that induce infection.[3] Droplet nuclei, however, remain suspended in the air for many hours and can infect persons by direct inhalation and penetration deep into the lungs.

The use of air turbines, ultrasonic and sonic scalers, and air polishers results in aerosol production which is well documented in the dental literature.[4]Material produced from ultrasonic scaler consists of both aerosol and splatter which routinely contains bacteria. Harrel and Molinari recommended three levels of defence in the reduction of aerosols.[5] The first recommended layer of defence is personal protective barriers such as mask, gloves, and safety glasses. The second layer being the routine and meticulous use of an antiseptic preprocedural rinse. The final one is the use of high evacuation device. Personal protective barriers and high evacuation devices are commonly used in the dental office to prevent aerosol contamination; however preprocedural rinses are not used routinely. This study was carried out to compare the efficacy of pre-procedural rinsing with chlorhexidine in reducing the viable bacteria in dental aerosol following oral prophylaxis

Aim: To evaluate the efficacy of 0.2% Chlorohexidine as a pre-procedural rinse in reduction of streptococcal bacterial colonies during ultrasonic scaling.

Method: This single-centre, double-masked, placebo-controlled study was conducted over a period of 15 days. The protocol was approved by the ethical committee of the Institutional Review Board (ITS

Dental College, Hospital and Research centre, Greater Noida, India).

6 patients (4 males and 2 females, aged 25 to 55 years; mean age: 40 years) with chronic periodontitis were recruited into the study from the Out Patient Department of the Department of Periodontics at ITS Dental College, Hospital and Research centre, Greater Noida, India.

Inclusion criteria:

1) A minimum of 20 permanent teeth;
2) A mean plaque score of 2.0 to 3.0 on the Plaque Index (PI)[6]
3) Four or more sites with pocket probing depth ≥4 mm; and
4) Systemically healthy patients with no contraindications for ultrasonic scaling

Exclusion criteria:

1) Oral prophylaxis within last months; and
2) Women who were pregnant or lactating.
3) Antibiotic intake in last two months.

Subjects participated in the study after submitting an informed consent. Disinfection of the operatory surfaces using a disinfectant solution by spray-wipe-spray method and formaldehyde fumigation of the operatory room was done 24 hours prior to the procedure so as to make the operatory room free of bacterial aerosols. Efforts were also made so as to minimize the contamination of dental unit waterline by using independent reservoir system and by flushing the same with 100ml of chlorhexidine mixed with 900ml of sterile water. All personal protection equipments as per the standard protocols were followed in the study. A total of 60 coded sheep blood agar plates[£] were used for culturing of the airborne bacteria generated during ultrasonic scaling.

The study outline as depicted in the flowchart (Figure 1) was used. Media taken was blood agar plates and plates were numbered from 1-10 . Each subject underwent ultrasonic scaling in the same operatory room but on a different day. Ten minutes prior to scaling procedure, baseline samples were collected by placing a blood agar plate (plate no 1) in the designated area, for determination of bacterial aerosols present in the operatory before scaling. Maxillary central incisor, lateral incisor, canine and first premolars of first quadrant constituted the control site, where as test site were the complementary teeth from the second quadrant. Prior to the scaling of control site, subjects were asked to rinse with 15ml of water for 1 minute as a pre-procedural rinse. Immediately following the rinse, breath sample was collected on a coded blood agar plate(plate no. 2) which was held at a distance of 3 inches from the mouth and subject was asked to exhale through the mouth, intermittently for a period of one minute to collect samples of aerosolized bacteria.

During the ultrasonic scaling of control site, two coded blood agar plates were attached to the gowns of the subject (plate no. 3) and dental personnel(plate no. 4) in the designated areas and these were left uncovered to collect samples of aerosolized bacteria.

Immediately after completing the scaling, samples were collected from the face mask of the operator by cutting (1 inch x 1 inch) size from the middle part of the face mask, which were then embossed on blood agar plate (plate no. 5). No dental treatment was received by the subject for next 30 minutes,however they continued to remain seated in the dental chair.
To control for background bacterial contamination in the operatory before the scaling of test site, bacterial levels were determined by exposing one more coded culture plate (plate no. 6) to the air for 10 minutes, which was considered as baseline sample prior to scaling of test site.

The operator donned a fresh face mask prior to the scaling of test site. Subjects were subsequently, instructed to rinse with 15ml of 0.2% chlorohexidine gluconate (Hexidine* manufactured by ICPA, a division of ICPA Health Products Ltd.), an antiseptic mouth wash for 1 minute.

Immediately following the chlorohexidine rinse, breath samples were again collected on the coded blood agar plate(plate no. 7). Samples of any aerosolized bacteria during the scaling of test site were inoculated onto Agar plates attached in the same designated areas as in the control group i.e.(plate no.8),(plate no.9) and (plate no. 10).

After the collection of samples, these blood agar plates were incubated at 37 degrees Celsius for 24 hours and colonies were counted using handheld digital colony counter€.The laboratory technician was blinded to the plates carrying samples from the control and test site. Figure II is depicting the colony counting of streptococcal colonies on agar plate. Figure III is showing the CFU presence in breath sample and figure IV is showing CFU'S on operator's chest .

£ = Sheep blood agar plate 90 mm- Pouring India, Delhi
€= HIMEDIA Digital Colony Counter LA663-1NO

Results:

Table I : Comparison Of Colony Forming Units (CFU's) Formation With Chlorhexidine And Placebo.

Confidence interval (CI) is 95% and for significant testing the degree of freedom was 5. Significance of two-tailed test is ($p< 0.05$), so there is statistically significant correlation between the variables.

The results in Table 1 revealed there was statistically significant reduction in the CFU formation in breath sample, 30 minutes after scaling and lesser no. of CFU's were formed on the operator mask after the scaling of the patients with the use of chlorhexidine as the pre-procedural rinse with sterile water .

Table Ii : Paired Samples Correlations

Correlation is significant at 0.01level, N= sample size

Table I : Comparison of Colony forming units (CFU's) formation with Chlorhexidine and Placebo.

CFU'S FORMATION		Paired Differences			T	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean			
Parameter 1	AF(W) - 1AF(CHX)	-.167	.753	.307	-.542	5	.611
Parameter 2	2B(W) - 2B(CHX)	5.333	3.933	1.606	3.322	5	.021
Parameter 3	3M(W) - 3M(CHX)	-6.500	2.429	.992	-6.555	5	.001
Parameter 4	PC(W) - 4PC(CHX)	6.500	9.690	3.956	1.643	5	.161
Parameter 5	OC(W) - 5OC(CHX)	1.333	2.944	1.202	1.109	5	.318
Parameter 6	6OM(W) - 6OM(CHX)	5.667	3.204	1.308	4.332	5	.007

Abbreviations : AF- After fumigation; PC- Patients' chest ; B- Breath sample ;OC-Operator's chest; CHX-chlorhexidine; M- 30 minutes of scaling ; OM- Operator's mask; W-water

Table II : Paired Samples Correlations

	N	Correlation	Sig.
AF(W) &AF(CHX)	6	.000	1.000
B(W) &B(CHX)	6	-.019	.972
M(W) &M(CHX)	6	-.668	.147
PC(W) &PC(CHX)	6	.544	.265
OC(W) &OC(CHX)	6	-.075	.888
OM(W) &OM(CHX)	6	.156	.768

Abbreviations : AF- After fumigation; PC- Patients' chest ; B- Breath sample ;OC-Operator's chest; CHX-chlorhexidine; M- 30 minutes of scaling ; OM- Operator's mask; W-water

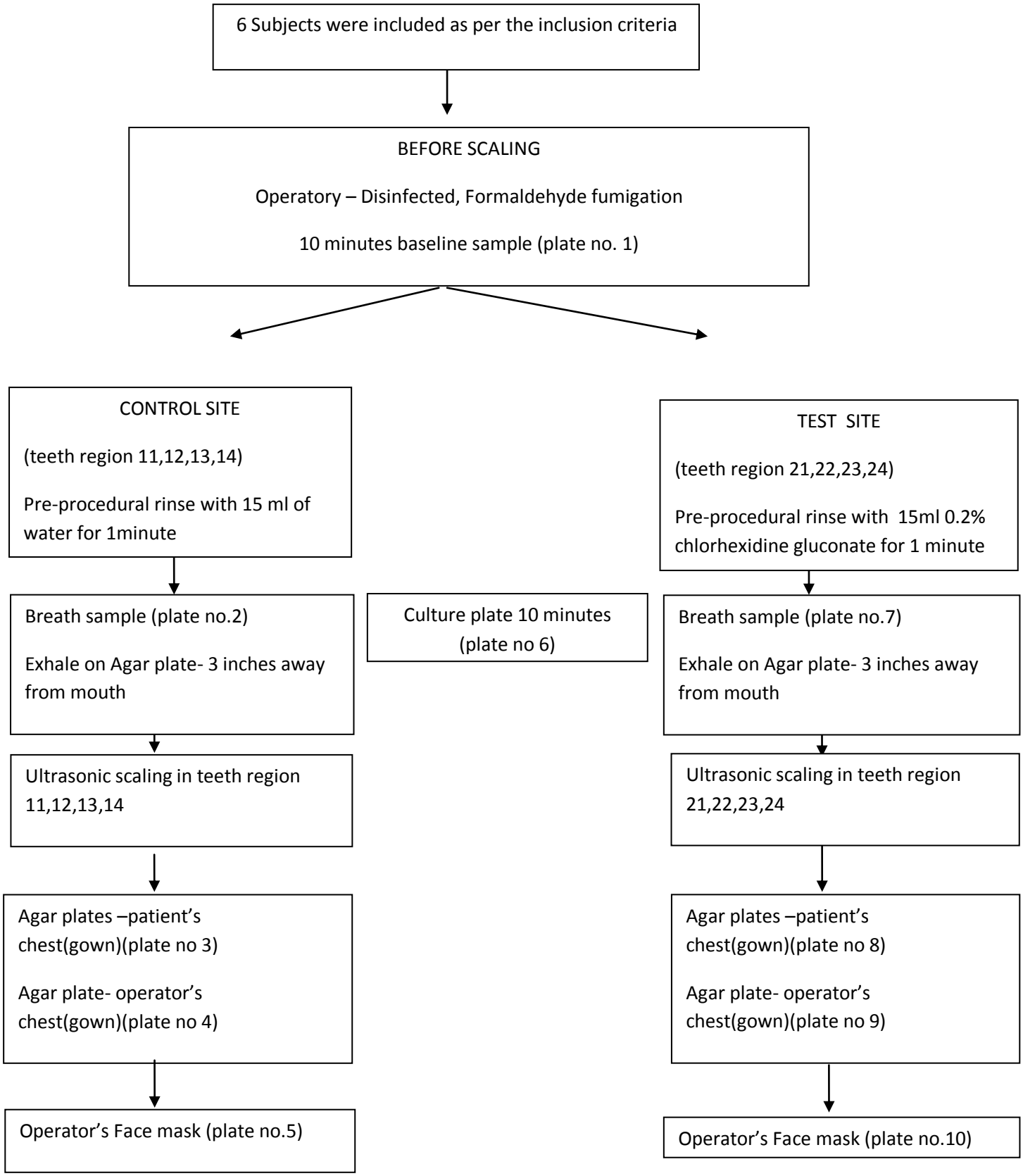
6 Subjects were included as per the inclusion criteria
BEFORE SCALING
Operatory – Disinfected, Formaldehyde fumigation
10 minutes baseline sample (plate no. 1)
CONTROL SITE
(teeth region 11,12,13,14)
Pre-procedural rinse with 15 ml of water for 1minute
TEST SITE
(teeth region 21,22,23,24)
Pre-procedural rinse with 15ml 0.2% chlorhexidine gluconate for 1 minute
Breath sample (plate no.2)
Exhale on Agar plate- 3 inches away from mouth
Culture plate 10 minutes (plate no 6)
Breath sample (plate no.7)
Exhale on Agar plate- 3 inches away from mouth
Ultrasonic scaling in teeth region 11,12,13,14
Ultrasonic scaling in teeth region 21,22,23,24
Agar plates –patient's chest(gown)(plate no 3)
Agar plate- operator's chest(gown)(plate no 4)
Agar plates –patient's chest(gown)(plate no 8)
Agar plate- operator's chest(gown)(plate no 9)
Operator's Face mask (plate no.5)
Operator's Face mask (plate no.10)

Figure I : Flowchart depicting study design

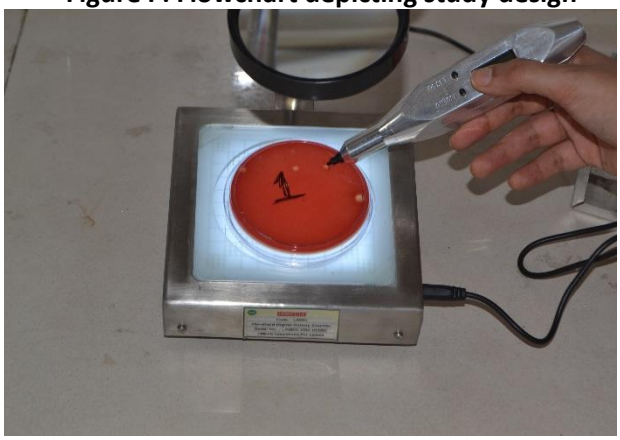

Figure II: Colony counting of streptococcal colonies on agar plate.

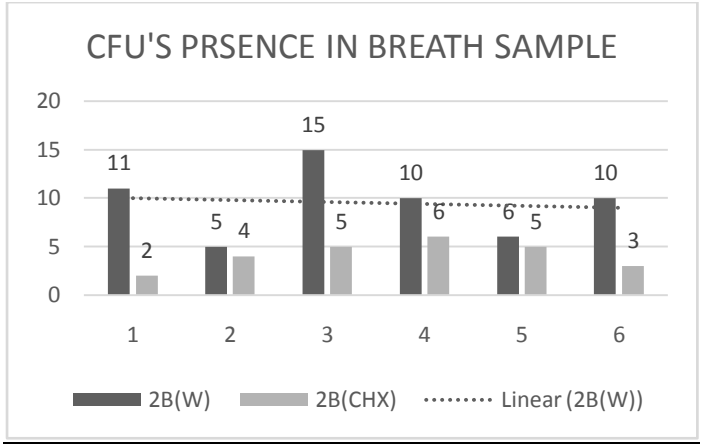

Figure III: CFU Presence in breath sample

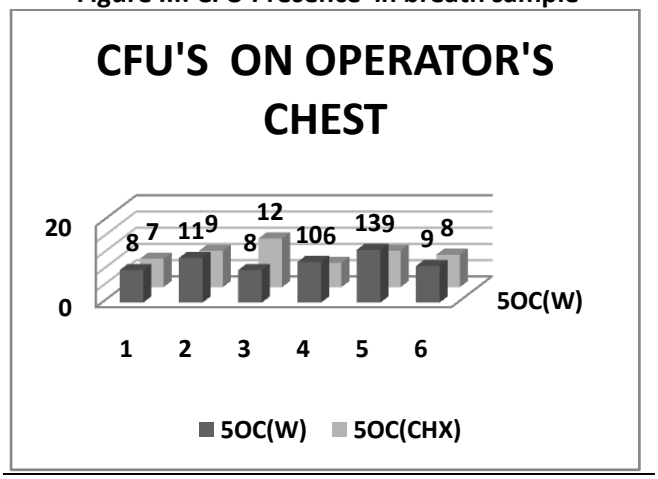

Figure IV: CFU'S on operator's chest (OC= operator's chest)

Discussion: The oral cavity harbours various bacteria and viruses from the oral fluids, dental plaque and respiratory tract. Any dental procedure that has a potential to aerosolize saliva will cause airborne contamination with these organisms.

Dental handpieces, ultrasonic scalers, air abrasion units and air polishing device produce airborne particles by the combined action of compressed air, water sprays, organic particles such as tissue and tooth dust, and organic fluids such as saliva and blood and from the sites where the instrument is used.[7,8]

Miller [7] found that aerosols which are generated from patients' mouths contained nearly a million bacteria per cubic foot of air. Reports have associated these aerosols with respiratory infections, ophthalmic and skin infections, tuberculosis, herpes, hepatitis and HIV infections.[9-16]

Pre-procedural rinse with chlorhexidine was used as an effective method for reducing bacterial aerosols during ultrasonic scaling. [16-18] In a study conducted by Briner and co-workers[18], the antimicrobial effects of chlorhexidine resulted in 65% to 85% reduction in total aerobes and 42% to 80% reduction in total anaerobes. In another study by Nagarale and co-workers[19], it was found that chlorhexidine pre-procedural rinse has a limited efficacy in reducing the level of viable bacteria in aerosols generated by ultrasonic scaling. Environmental aerosols were considerably less when compared to that of aerosols generated during ultrasonic scaling .While conducting our study, we have made an attempt to evaluate the efficacy of 0.2% chlorhexidine as a pre-procedural rinse in reducing the level of viable bacteria in aerosols generated by ultrasonic scaling.

We have used blood agar plates for culturing airborne bacteria as Johnston and colleagues[20]proved it is an enriched, broad spectrum non-selective media which supports the growth of many oral species. Results of our study showed that when 0.2% CHX was used as pre-procedural rinse for 60 seconds, 10 minutes before ultrasonic scaling, similar numbers of colony forming units were formed when compared to water as pre-procedural rinse.

However, our results contradict those documented by Fine [21] and Klyn[22] , where they found 94% reduction in the colony count after rinsing with

chlorhexidine, and most of the bacteria cultured were aerobic ones. The difference in results appears to be due to the inability of 0.2% chlorhexidine to affect bacteria in a biofilm such as established dental plaque, as studies by Pratten et al[23]and Gilbert et al[24]have proved that bacteria in a biofilm is 1000-1500 times more resistant to antimicrobials than bacteria in planktonic form.

In the present study both test and control sites in all the subjects had a thick band of plaque with similar plaque scores which was completely removed by scaling[26]. Position of tooth in the mouth and levels of microorganisms in subjects' mouth, affects the position of the operator relative to the subject. A study by Bently and colleagues[7]showed that maximum aerosol contamination occurs during scaling of maxillary anterior teeth. In the present study, we have included maxillary teeth of first quadrant from central incisors to first premolar in control site and same maxillary teeth of second quadrant in test sites. The position of the operator relative to the subject was same during scaling of both test and control sites. Background bacterial contamination in the operatory was more during scaling of test sites.

Larato and co-workers[25]hypothesized that droplets containing organisms from the mouth, including possible viable pathogenic organisms, remain suspended in the air 30 minutes after a dental procedure is completed. Therefore, for 30 minutes after the treatment, agar plates were left uncovered at sites to collect samples of any aerosolized bacteria.

Chlorhexidine pre-procedural rinse considerably reduced bacterial count from the subjects' breath before scaling of test sites. This finding was consistent with previous studies by Nagarale et al[19] and Weeks et al[26]. We found an extremely variable distribution of bacterially contaminated aerosols and splatter, that may be influenced by many factors as mentioned earlier.

According to the Stephen[27,28] whenever an ultrasonic scaler is used the following steps should be followed:(1) barrier protection (2) high volume evacuation, and (3) pre-procedural rinsing. Each of these adds a layer of protection for the operator and others in the dental office.

The limitations of this study should be considered in interpreting these results. The CFU counts here includes only aerobic bacteria capable of growth on blood agar plates; anaerobic bacteria, and viruses which require specialized media were not cultured in this study. Comprehensive full mouth scaling was not done. Therefore these results may show a lower microbial count than would have been collected if full mouth scaling had been performed.

Conclusions: Based on the results of this study we would like to conclude that 0.2% CHX pre-procedural rinse has a limited efficacy in reducing the level of viable bacteria in aerosols generated by ultrasonic scaling. Environmental aerosols were considerably less when compared to that of aerosols generated during ultrasonic scaling.

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