# Chlorhexidine gluconate pre-procedural rinse and bacterial aerosol contamination during ultrasonic scaling

**Running title: Chlorhexidine rinse and aerosol contamination**

**Dr. Girish Nagarale, MDS (Corresponding Author), Department of Periodontics, S.D.M College of Dental Sciences and Hospital, Dharwad, Karnataka, INDIA.**

**Dr. Renuka G Nagarale, MDS (Co-Author:1), Department of Community Dentistry, S.D.M College of Dental Sciences and Hospital, Dharwad, Karnataka, INDIA.**

**Abstract**

***Background:*** *Chlorhexidine rinse is used as an effective method for reducing bacterial aerosols during ultrasonic scaling. Although chlorhexidine rinse is an effective antiseptic for free floating oral bacteria, it does not affect bacteria in a biofilm such as in established dental plaque, which needs to be removed by scaling. The purpose of this study was to evaluate the efficacy of 0.2% chlorhexidine gluconate (CHX) pre-procedural rinse in reducing the level of viable bacteria in aerosols generated by ultrasonic scaling, and to compare oral and environmental aerosols.*

***Methods:*** *Three male subjects with no known contraindications for ultrasonic scaling were selected for this study. Efforts were made to maintain bacteria free air in the operatory. Fifteen ml of water was used as pre-procedural rinse before scaling of control site and 15ml of 0.2% CHX was used as pre-procedural rinse before scaling of test site. Ultrasonic scaling was done for 10 minutes duration in both control and test sites by using sterile saline as a coolant. A total of 69 sheep blood agar settle plates were used for bacterial identification from oral and environmental aerosols during the study.*

***Results:*** *Considerable amount of bacterial aerosols were generated during the scaling of both control and test sites.*

***Conclusions:*** *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.*

**Key words:** ultrasonic scaling, bacterial aerosol, chlorhexidine, pre-procedural rinse.

**Introduction**

Potential transmission of disease to personnel during dental procedures has become a source of increased concern to the dental professional and the public.[1, 2] Many dental procedures produce aerosol and splatter composed of various combinations of water; organic particles such as dental plaque and tooth dust; and organic fluids such as blood and saliva. Many studies have demonstrated increase in the number of airborne bacteria during various aerosol producing dental procedures.[3-5] A report by Legnani and colleagues [6] indicates that the ultrasonic scaler is the greatest producer of contaminated aerosol and splatter, as the mean airborne microbial load increased by over 300% during working hours. Micik and colleagues [7] defined dental aerosol as any particles smaller than 50µm, and any particles larger than 50µm as splatter. A true aerosol or splatter that becomes airborne as droplet nuclei are more likely to remain airborne for a longer period [8] and has the potential to enter the respiratory tract. [9, 10] Studies have indicated that there is potential danger from both aerosol and splatter as they may contain blood, saliva and dental plaque elements[11-13]. Aerosols and splatter generated during ultrasonic scaling can transport microorganisms from the patients' mouth and respiratory tract to contaminate the skin and mucous membranes of the mouth, respiratory passages and eyes of dental personnel. [14-16] Bacteria which spreads by aerosols are Mycobacterium tuberculosis (tuberculosis), Bordetella pertussis (whooping cough), Streptococcus pneumoniae (bacterial pneumonia), Streptococcus pyogenes (streptococcus throat), N. meningitides (bacterial meningitis), and others. The Centers for Disease Control and Prevention (CDC) [17, 18] recommends universal barrier techniques for all dental procedures that produce aerosol containing pathogens, and also states that by appropriate use of rubber dams, high-velocity air evacuation and proper patient positioning will minimize the aerosol contamination. Unfortunately, methods to minimize the bacterial aerosol and splatter during ultrasonic scaling are limited. Recent studies have shown that use of chlorhexidine pre-procedural rinse reduces the microbial content of aerosols by 94%.[19-21] Although chlorhexidine gluconate rinse is an effective antiseptic for free floating oral bacteria such as those found in the saliva [22, 23] and those loosely adhering to mucus membranes, but as a pre-procedural rinse does not affect bacteria in a biofilm such as established dental plaque, [24-26] does not penetrate subgingivally,[27] will not affect blood coming directly from the operative site and is unlikely to affect bacteria and viruses harbored in the nasopharynx. The purpose of this study was to evaluate the efficacy of 0.2% chlorhexidine gluconate (CHX) pre-procedural rinse in reducing the level of viable bacteria in aerosols generated by ultrasonic scaling and to compare oral and environmental aerosols.

**Materials and Methods**

Three male subjects in the 35 to 50 years age group meeting the following criteria were selected for the study: systemically healthy with no known contraindications for ultrasonic scaling (tuberculosis, HIV, herpes, or any other infective or respiratory disease), no history of antibiotics in the last two months and a minimum of twenty natural teeth present; a modified Quigley Hein Plaque Index score >2; and a Gingival Index (Loe and Silness) score >2. Subjects completed an informed consent form prior to entry into the study. Ethical committee clearance was obtained from the institutional review board.

The study was carried out in a closed operatory room measuring 12 feet x 12 feet x 10 feet. To allow the operatory room to be free of bacterial aerosols before scaling of each subject, a dental assistant cleaned, disinfected all operatory surfaces using a disinfectant solution by spray-wipe-spray method and formaldehyde fumigation of the operatory room was also done. Efforts were also made to minimize the dental unit waterline contamination[28] by using independent reservoir system and by flushing it with 100ml of chlorhexidine mixed with 900ml of sterile water. Sterile saline was used as a coolant during ultrasonic scaling. All the dental personnel (operator, chair side assistant, circulating assistant) wore sterile gloves, double layered face masks, sterile gowns and head caps. Only the operator and chair side assistant wore face shields. Study subject wore sterile protective clothing, head cap and protective eyewear during the procedure. A total of 63 coded sheep blood agar settle plates were used for culturing airborne bacteria during the study, [29] and 6 coded sheep blood agar settle plates were used for culturing hospital and non-hospital environmental aerosols [3 blood agar settle plates were kept in the different sections of hospital where ultrasonic scaling is not done and 3 blood agar settle plates were kept in non-hospital environment].

Each subject underwent ultrasonic scaling in the same operatory room but on a different day. Before scaling of each subject, 10 minutes baseline sample was collected by placing a blood agar plate in the designated area, to determine if there were any bacterial aerosols present in the operatory room before scaling. A total of eight maxillary teeth were selected for scaling as maximum aerosol contamination occurs during scaling of maxillary anterior teeth, [3] which include central incisors, lateral incisors, canines and first premolars. Control site included four teeth i.e. both maxillary lateral incisors and first premolars, where as test site included four teeth i.e. both maxillary central incisors and canines, so that the pattern of distribution of aerosols/splatter should not vary during the scaling of control and test site.

Prior to the scaling of control site, subject rinsed with 15ml of water for 1 minute as pre-procedural rinse. Immediately following the rinse, breath sample was collected by asking the subject to exhale the breath intermittently for a period of one minute on a coded blood agar plate which was held at a distance of 3 inches from the mouth to collect samples of any aerosolized bacteria. Six coded blood agar plates which were attached to the gowns of the dental personnel and subject in the designated areas were left uncovered to collect samples of any aerosolized bacteria during the ultrasonic scaling of control site. Immediately after completing the scaling, samples were collected from the face mask of the operator and chair side assistant by cutting (1 inch x 1 inch) size from the middle part of the face mask, which were then embossed on culture plates to see if any aerosolized bacteria were present. After completing the scaling of control site, subject was seated in the dental chair for 30 minutes in normal conversation but received no dental treatment. 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 to the air for 10 minutes. Operator and chair side assistant wore new face masks. Prior to the scaling of test site, subject rinsed for 1 minute with 15ml of 0.2% chlorhexidine gluconate (Rexidin* manufactured by Warren, a division of INDOCO Remedies Ltd.), an antiseptic mouth wash. Immediately following the chlorhexidine rinse, breath sample was similarly collected once again. Six more coded blood agar plates attached to the gowns of the dental personnel and subject (in the same designated areas as in the control group) were left uncovered to collect samples of any aerosolized bacteria during the scaling of test site. Samples from the face mask of the operator and chair side assistant were also collected as mentioned earlier. Thirty minutes after the scaling of test site one more blood agar plate was exposed for 10 minutes to check for bacterial contamination in the operatory. After collecting all the samples, blood agar plates were incubated at 37 degrees Celsius for 48 hours. The laboratory technician was not aware which plates have been exposed during the scaling of test and control site.

Ultrasonic scaling in both test and control site was done by using (Dentsply® Cavitron® Bobcat® with 25K TFI®-3 insert, manufactured by Dentsply International, New York, USA). The power setting (medium), flow of coolant liquid (17.5 ml per minute) and duration of scaling (10 minutes) was same during the scaling of test and control site.

[Rexidin*****: Indicates Trade Mark Registration pending]

**Results**

The background bacterial contamination was evaluated in the operatory as shown in Table 1 and we found that contamination in the operatory progressively increased. When breath samples were compared for contamination as shown in Table 2 we found 9 to 13 Colony Forming Units (CFU) after rinsing with water and 0 to 4 CFU after rinsing with chlorhexidine. This suggests that chlorhexidine rinse has reduced bacteria from the subjects' breath. Surprisingly no noteworthy difference was observed in CFU during the scaling of control and test sites in all the three subjects. CFU for the control and test sites at standardized locations are shown in Table 3. This analysis revealed that highest amount of contamination occurred on the culture plates kept on the subjects' chest and the operator's thigh. When hospital and non-hospital environmental contamination was compared as shown in Table 4 we found less than 1 CFU in non-hospital environment and up to 9 CFU in hospital environment. When oral and environmental aerosols were compared as shown in Table 5 we found considerable difference in the types of organisms.

**Discussion**

Even though the most dangerous oral bacteria associated with periodontal disease are facultative and obligate anaerobes, but the majority of bacteria which spread by aerosols (Mycobacterium tuberculosis, Bordetella pertussis, Streptococcus pneumoniae, Streptococcus pyogenes, N. meningitides) and bacteria isolated from hospital acquired infections (Pseudomonas aeruginosa, Staphylococcus aureus and Enterococcus species) are obligate aerobes and facultative anaerobes. Several researchers have documented the importance of controlling and minimizing bacteria laden aerosols produced by the ultrasonic scalers.[1-8] Reports have associated these aerosols with respiratory infections, ophthalmic and skin infections, tuberculosis, herpes, hepatitis and HIV infections.[9-16]

Chlorhexidine rinse was used as an effective method for reducing bacterial aerosols during ultrasonic scaling.[19-21] In a study conducted by Briner and colleagues,[30] they found that the antimicrobial effects of CHX resulted in 65% to 85% reduction in total aerobes and 42% to 80% reduction in total anaerobes. While conducting this study, we attempted to evaluate the efficacy of 0.2% CHX pre-procedural rinse in reducing the level of viable bacteria in aerosols generated by ultrasonic scaling. In the present study blood agar settle plates were used for culturing airborne bacteria as it is an enriched, broad spectrum nonselective media which supports the growth of many oral species. Johnston and colleagues [29] proved that blood agar settle plates are a valid medium for culturing airborne bacteria.

Results of this 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 Holbrook, [16] Fine, [19] Klyn, [20] Logothetis [21] and colleagues, 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 chlorhexidine to affect bacteria in a biofilm such as established dental plaque, as studies by Pratten, Xu, Gilbert [24-26] and colleagues 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 three subjects had a thick band of plaque with similar plaque scores which was completely removed by scaling. 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[3] showed that maximum aerosol contamination occurs during scaling of maxillary anterior teeth, and pattern of distribution of aerosols/splatter varies during the scaling of right and left side of subjects' mouth. In the present study, we have included both maxillary central incisors and canines in test sites and both maxillary lateral incisors and first premolars in control 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.

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 Weeks, Veksler and colleagues.[22, 23] We found an extremely variable distribution of bacterially contaminated aerosols and splatter, that may be influenced by many factors as mentioned earlier.

In the present study, the highest colony forming units were detected on plates positioned on the subjects' chest and operator's thigh during scaling of both test and control sites. These findings were consistent with previous studies by Bently and colleagues.[3] When hospital and non-hospital environmental aerosol contamination were compared, we found less than 1 CFU in non-hospital environment and up to 9 CFU in hospital environment, which were considerably less compared to aerosol generated during ultrasonic scaling. When oral and environmental aerosols were compared we found considerable difference in the types and quantity of organisms. Similar findings were observed in a review published by Leggat and Kedjarune.[31]

According to the Szymańska,[32] air contained in the dental operatory space is the air breathed by both dentist and patient, its composition is extremely important as a potential threat to the dentist's health. Insufficient awareness of health risk, working habits, and economic factors are the reasons why dentists do not apply the available and recommended methods of protection against the influence of bioaerosol and splatter. Behavior protecting a dentist and an assistant from the threat resulting from the influence of dental aerosol cannot be limited to isolated actions.

According to the Stephen,[33] Whenever an ultrasonic scalar 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. All three steps must be followed for adequate protection. The use of only one or two of the steps will not yield the necessary level of protection adequate for safety.

It must be emphasized that no single approach or device can minimize the risk of infection to dental personnel and patient completely. These findings support the use of standard barrier techniques, high volume evacuators in addition to the routine use of 0.2% CHX pre-procedural rinse. These recommendations were in accordance to the review article published by Stephen and John. [34]

The limitations of this study should be considered in interpreting these results. The CFU counted 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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**List of Tables:**

**Table 1: Background bacterial contamination in the operatory**

| **Time of sampling** | | **Subject No. 1** CFU/cm$^2$ | **Subject No. 2** CFU/cm$^2$ | **Subject No. 3** CFU/cm$^2$ |
|---|---|---|---|---|
| 1. | After fumigation [before scaling of control site] | 1 | 2 | 1 |
| 2. | 30 minutes after scaling of control site [before scaling of test site] | 7 | 5 | 6 |
| 3. | 30 minutes after scaling of test site | 12 | 9 | 10 |

**Table 2: Breath contamination before ultrasonic scaling of control and test sites**

| **Breath sampling** | | **Subject No. 1** CFU/cm$^2$ | **Subject No. 2** CFU/cm$^2$ | **Subject No. 3** CFU/cm$^2$ |
|---|---|---|---|---|
| 1. | After rinsing with water [before scaling of control site] | 11 | 9 | 13 |
| 2. | After rinsing with chlorhexidine [before scaling of test site] | 0 | 3 | 4 |

**Table 3: Bacterial contamination of dental personnel during the ultrasonic scaling of control and test sites**

| **Location of sampling** | | **Subject No. 1** | | **Subject No. 2** | | **Subject No. 3** | |
|---|---|---|---|---|---|---|---|
| | | Scaling of control site CFU/cm$^2$ | Scaling of test site CFU/cm$^2$ | Scaling of control site CFU/cm$^2$ | Scaling of test site CFU/cm$^2$ | Scaling of control site CFU/cm$^2$ | Scaling of test site CFU/cm$^2$ |
| 1. | Operator chest | 7 | 6 | 8 | 5 | 10 | 8 |
| 2. | Operator thigh | 83 | 85 | 90 | 77 | 72 | 66 |
| 3. | Assistant chest | 23 | 18 | 17 | 14 | 16 | 15 |
| 4. | Assistant thigh | 19 | 20 | 27 | 22 | 17 | 17 |
| 5. | Subject chest | 130 | 135 | 123 | 118 | 110 | 99 |
| 6. | Circulating assistant | 8 | 4 | 6 | 6 | 4 | 3 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | chest | | | | | | |
| 7. | Operator face mask | 9 | 9 | 5 | 4 | 6 | 5 |
| 8. | Assistant face mask | 4 | 3 | 3 | 3 | 3 | 2 |

**Table 4: Hospital and Non-hospital environmental contamination**

| | | **Coded blood agar plate: 1** CFU/$cm^2$ | **Coded blood agar plate: 2** CFU/$cm^2$ | **Coded blood agar plate: 3** CFU/$cm^2$ |
|---|---|---|---|---|
| 1. | Hospital [where ultrasonic scaling is not done] | 8 | 6 | 9 |
| 2. | Non-hospital environment | 0 | less than 1 | 1 |

**Table 5: Organisms isolated from various locations during the study**

| **Location of sampling** | | **Organisms isolated** |
|---|---|---|
| 1. | Subjects' breath | Staphylococcus, Streptococcus |
| 2. | Background contamination in the operatory | Staphylococcus, Klebsiella, Micrococcus, Esherichia coli |
| 3. | Dental personnel and subjects' clothing during scaling | Enterococcus, Staphylococcus, Streptococcus, Micrococcus, non-fermenting Gram negative bacilli, Staphylococcus citreus |
| 4. | Hospital environment [where ultrasonic scaling is not done] | Enterococcus, Staphylococcus, non-fermenting Gram negative bacilli, aerobic spore bearer |
| 5. | Non-hospital environment | Staphylococcus citreus |

**Name of the department(s) and institution(s) to which the work should be attributed:**

This case report was prepared in the Department of Periodontics, S.D.M College of Dental Sciences and Hospital, Dharwad, Karnataka, INDIA.