Journal of Cardiovascular Disease Research

ISSN: 0975-3583,0976-2833 VOL11, ISSUE 11, 2020

# Evaluation of tempered 0.2% chlorhexidine mouthwash as a pre-procedural rinse:A clinical study

**Dr. Janardhana Amaranath B.J.**[1]

Professor and Head, Department of Periodontology, Rama Dental College, Hospital & Research Centre, Rama University, Mandhana, Kanpur (UP)- 209217, Email address: dramarbj@gmail.com, Mobile no: 8791079004

## Abstract

### Background:

During ultrasonic scaling, the harboured microorganisms in the oral cavity get aerosolized, which have important impacts on air quality and can cause a serious health threat to the clinician, patients, and the surroundings. Therefore, this study was conducted to evaluate whether preprocedural tempered chlorhexidine mouth rinse has any effect on bacterial load in aerosols generated during ultrasonic scaling.

### Materials and Methods:

A total of 60 subjects with chronic periodontitis were selected and randomly placed into threegroups comprising twenty in each. The groups were based on the use of preprocedural mouth rinse.Group A: consisted of subjects performing mouth rinse with sterile water, Group B: with preprocedural mouth rinse with non-tempered 0.2% Chlorhexidine gluconate and Group C: with tempered 0.2% Chlorhexidine gluconate. The aerosol produced during ultrasonic scaling was collected on blood agar plates positioned at 3’o clock, 6’o clock and 12’o clock positions. Colonies on the blood agar plates were counted after incubating at 37°C for 24 h. Position wise mean microbial colonies were calculated and pairwise comparisons involving mouth rinses on microbial colonies were conducted using independent sample t-test and Tukey's test for post hoc analysis considering 0.05 as the significance level.

### Results:

Microbial colonies were significantly reduced with tempered chlorhexidine gluconate compared to that of others ($P < 0.001$), followed by non-tempered chlorhexidine gluconate and water. Again, microbial colonies were highest at the 12’o clock position and lowest at the 3’o clock position.

### Conclusion:

Tempered 0.2% Chlorhexidine gluconate is more superior in reducing the microbial load in aerosols produced during ultrasonic scaling.

**Keywords:** chlorhexidine, ultrasonic scaling, microbial colony count

ISSN: 0975-3583,0976-2833 VOL11, ISSUE 11, 2020

## Introduction

The oral cavity has uniquely moist and warm environment which harbours millions of bacteria and viruses from the respiratory tract, saliva, and dental plaque. These microorganisms get aerosolized when they come in contact with the dental equipment, particularly the high-speed dental drills and ultrasonic scalers.[1,2] The ultrasonic scaler uses vibrations to remove calculus deposits from the teeth effectively. It oscillates (move forward and backward) a typically blunt metal tip at a high-frequency producing mechanical vibratory, cavitational, and acoustic microstreaming forces in the associated cooling water that remove/disrupt the deposits. However, the water used as a coolant is splattered during the vibration of the tip and becomes contaminated when it is mixed with saliva and plaque. The amount of contamination of dental aerosol depends on the quality of saliva, nasal and throat secretion, blood, dental plaque, and any dental infection including periodontal.[3]

The maximum amount of aerosol is produced by ultrasonic scalers compared to that of the other dental equipment. [4,5] Patients and practitioners are regularly exposed to tens of thousands of bacteria per cubic meter generated during procedures,[6,7] and inhalation of this may cause adverse health effects such as common cold, tuberculosis, severe acute respiratory syndrome (SARS), and even transmission of blood-borne pathogens, namely human immunodeficiency Virus, Hepatitis B and C virus.[8] This disease transmission may be bidirectional, i.e., from patient-to-patient, patient-to-clinician, or clinician-to-patient.[2,9,10,11] Notably, the aerosols are not dispersed evenly in the entire operatory room, and the greatest concentration has been shown within two feet of the patient, radiating more toward the chest of the patient or the face of the operator.[11,12,13,14,15] In addition, the aerosolized microorganisms remain suspended in the air for extended periods with greater potentiality.[11,16] Thus, the risk of health threat by aerosols exists even after the completion of the treatment procedure. Oral health professionals should be aware of these invisible dangers in the operatory and should follow the recommended protocols for the prevention of infection before, during, and after patient care.

Harmful effects of the microbial load in aerosols demand the minimization of microbial quantity in the oral cavity before the generation of aerosol/splatter to reduce the risk of cross-infection in the dental environment[16]. Current research suggests that making a patient rinse with antimicrobial mouthwash before the treatment procedures may reduce the number of microorganisms in aerosols,[11,14,17,18,19] though no antimicrobial agent has been identified as a superior prerinse so far. Commonly used mouthwashes in dental practice are chlorhexidine gluconate and herbal mouthwash.

Chlorhexidine gluconate is a bisbiguanide antiseptic and is widely used chemical plaque control agent. It is effective against an array of microorganisms and also exhibits substantivity up to 12 h. [20] This makes it the “gold standard of chemical plaque control,” though a number of side effects, such as brownish discoloration of teeth, restorative materials and the dorsum of the tongue, taste perturbation, oral mucosal erosion, etc., have been reported.[21]

ISSN: 0975-3583,0976-2833 VOL11, ISSUE 11, 2020

Considering the potential hazard of cross-contamination from aerosols produced during ultrasonic scaling, this prospective, randomized, double-blind study was conducted to assess the effectiveness of tempered 0.2% chlorhexidine gluconate, non-tempered 0.2% chlorhexidine gluconatemouthwash, and water as preprocedural mouth rinse on the bacterial load in aerosols by assessing the number of bacterial colonies formed in blood agar culture plates positioned at various areas of the operating room during ultrasonic scaling.

## Materials and methods

It was a randomized, prospective, double-blind clinical trial, carried out in the Department of Periodontics and Oral Implantology in collaboration with the Department of Microbiology in accordance with the ethical guidelines of the Institutional Research and Ethical Committee. A total of 60 subjects with periodontitis were selected from the outpatient department irrespective of sex, religion, and socioeconomic status. Subjects were explained the entire procedure in detail and written consent was obtained from each of them. The subjects were selected based on the following criteria.

**Inclusion criteria:**

- Subjects of 20–65 years old with chronic periodontitis with not <20 teeth.
- Systemically healthy patients.
- Subjects received no antibiotics and periodontal treatment during the last 6 months.

**Exclusion criteria:**

- Allergic to mouthwash.
- Pregnant and lactating mothers.
- Smokers.
- Patients having any type of uncontrolled systemic illness like cardiovascular diseases, uncontrolled diabetes, chronic respiratory disorders, bleeding disorders, allergy etc.

The subjects were randomly categorized into threegroups by a single investigator using block randomization. The groups were named A, B, and C containing 20 subjects in each. The groups were based on the use of preprocedural mouth rinse. Group A: consisted of subjects performing mouth rinse with sterile water, Group B: with preprocedural mouth rinse with non-tempered 0.2% Chlorhexidine gluconate and Group C: with tempered 0.2% Chlorhexidine gluconate.

All subjects underwent periodontal examination by a single examiner.

Non-selective culture medium (Blood agar) was prepared by boiling 40 g of HIMEDIA Blood agar base in 1000 ml of distilled water, which was then cooled to 45°C–50°C and autoclaved. After that 5% sterile defibrinated sheep blood was added. It was then poured into sterile Petri plates, which were stored in the refrigerator at 2°C–8°C for 5–6 days. The agar plates were coded and positioned at three different places prior or during ultrasonic scaling for the collection of aerosols.The aerosol produced during ultrasonic scaling was collected on blood agar plates positioned at 3’o clock, 6’o clock and 12’o clock positions.

ISSN: 0975-3583,0976-2833 VOL11, ISSUE 11, 2020

Before the treatment procedure, the subjects were instructed to undergo a 30sec preprocedural rinse of 10 ml of tempered chlorhexidine gluconate (0.2%), non-tempered chlorhexidine gluconate (0.2%), or water depending upon the group to which the subjects were assigned. Coded agar plates were placed accordingly and stabilized for aerosol collection. The treatment procedure comprising of scaling was carried out for 30 min using a piezoelectric ultrasonic scaler (DTE-D5), with controlled frequency and the pressure of the water coolant was maintained at constant level. A motorized suction was used during the treatment procedure. Immediately after scaling, agar plates were removed and sealed, which were then incubated at 37°C for 24 h in an increased $CO_2$ chamber and microbial colonies that grew on each plate were counted using a colony counter.

## Statistical analysis

All the data collected wereanalyzed statistically using IBM Statistical Package for Social Sciences (SPSS) version 20 (Armonk, New York, US). Analysis of variance (ANOVA) tests was done to compare the mean of four groups by the variance between and within groups ratio with significant inference at $P \leq 0.05$. In the case of statistical significance, *post hoc* pairwise comparisons were done by Tukey's Honestly Significant Difference test. Pair-wise independent sample *t*-tests were conducted at a 5% level of significance to test the pair-wise difference in the mean values of microbial colonies grown in agar plates position wise. The inferences were drawn with the help of the $P \leq 0.05$.

## Results

In Table 1 at 6’o clock position, the mean number of microbial colonies was 232.50 ± 90.33, 129.70 ± 53.35 and 70.55 ± 31.78 in the groups of preprocedural mouth rinse with sterile water, non-tempered chlorhexidine gluconate, and tempered chlorhexidine gluconate, respectively.

At12’o clock position, the mean number of microbial colonies was 378.80 ± 73.94, 206.60 ± 53.12 and 121.75 ± 32.37 in the groups of preprocedural mouth rinse with sterile water, non-tempered chlorhexidine gluconate, and tempered chlorhexidine gluconate, respectively, as depicted in Table 1.

At3’o clock position, the mean number of microbial colonies was 132.55 ± 61.98,79.00 ± 47.60 and 27.50 ± 21.80 in the groups of preprocedural mouth rinse with sterile water, non-tempered chlorhexidine gluconate, and tempered chlorhexidine gluconate, respectively.

On position-wise comparison, the lowest number of microbial colony was seen at 3’o clock position, while the highest number of the microbial colony was observed in the agar plates placed at 12’o clock position followed by 6’o clock position.

Table 2 presents the pairwise comparison of post-rinse bacterial colony-forming unit counts among three groups. The tempered CHX group showed significantly lesser post-rinse bacterial colony-forming unit counts as compared to the other two groups. Also, there was a significant difference in the post-rinse bacterial colony-forming unit count of the non-tempered CHX group and sterile water group.

ISSN: 0975-3583,0976-2833 VOL11, ISSUE 11, 2020

**Table 1: Position wise mean microbial colonies**

| Position of agar plate | Rinse group | Microbial colony counts (Mean ± S.D) |
|---|---|---|
| 3'o clock position | Group A | 132.55 ± 61.98 |
| | Group B | 79.00 ± 47.60 |
| | Group C | 27.50 ± 21.80 |
| 6'o clock position | Group A | 232.50 ± 90.33 |
| | Group B | 129.70 ± 53.35 |
| | Group C | 70.55 ± 31.78 |
| 12'o clock position | Group A | 378.80 ± 73.94 |
| | Group B | 206.60 ± 53.12 |
| | Group C | 121.75 ± 32.37 |

**Table 2: Pairwise comparison of post-rinse bacterial colony forming unit count among three groups**

| Group | Mean difference in CFU | p-value |
|---|---|---|
| **Sterile water vs Non-tempered CHX** | 85.50 | <0.001* |
| **Sterile water vs Tempered CHX** | 95.50 | <0.001* |
| **Non-tempered CHX vs Tempered CHX** | 10.00 | 0.003* |

Post hoc Tukey test; * indicates a significant difference at $p \leq 0.05$

## Discussion

Aerosols produced during the various dental procedures are the potential to spread the infection to dental personnel and other individuals in the dental operatory room. This has long been considered as one of the main concerns in dentistry. It must be emphasized that "layering of protective procedures" is required in reducing the potential danger from dental aerosols. In this procedure, multiple steps are involved in the reduction of the risk of infection; a single step reduces to a certain extent to which another step is added that further reduces the remaining risk until the risk is minimal. Personal protection barriers constitute the first layer of defense, which is upgraded by antiseptic preprocedural mouth rinse (second layer of defense). This is further elevated by the routine use of a high-volume evacuator (HVE), which is further augmented by high-efficiency particulate air (HEPA) filter. The first two layers of defense are inexpensive and should be followed routinely as a part of infection control practices. Furthermore, the maximum amount of contaminated aerosol is observed to be within two feet of the patient,[12] where the dental health professional is usually positioned. This observation reinforces the importance of personal protective barriers such as eye shields and face masks, head cap, gloves, and gowns.

The maximum amount of aerosol production is reported during the ultrasonic scaling procedure.[22] By following the American Dental Association protocols dental aerosols may be minimized, though complete elimination is difficult. The most basic and feasible methods to reduce bacterial load in the aerosols is preprocedural rinse suggested by a number of investigators.[11,14,15,23]

ISSN: 0975-3583,0976-2833 VOL11, ISSUE 11, 2020

In this study, non-tempered chlorhexidine gluconate, tempered chlorhexidine gluconate and water were used as preprocedural mouth rinses. Chlorhexidine gluconate (0.2%) has a broad-spectrum antimicrobial activity against both Gram-positive and-negative organisms, yeasts, dermatophytes and some lipophilic viruses with a substantivity for 12 hours.[11,20] Higher effectiveness of 0.2% chlorhexidine gluconate may be related to its substantivity on oral tissues and its subsequent slow release in an active form.

Blood agar was used to collect the aerosols[24]. This is a valid medium for culturing airborne bacteria that settle downs in the blood agar culture medium, in which bacteria grows and multiply to form clusters of colonies. In this study, these microbial colonies were counted in the agar plates to evaluate the usefulness of using mouthwash as pre-procedural mouthrinse.

Highest number of microbial colonies was observed in water rinse group (Group A), followed by 0.2% non-tempered chlorhexidine gluconate (Group B), and 0.2% tempered chlorhexidine gluconate (Group C) preprocedural mouth rinse. Maximum reduction of microbial colonies was observed with 0.2% tempered chlorhexidine gluconate (Group C). The pairwise comparison between tempered chlorhexidine gluconate (Group C) with water (Group A), non-tempered chlorhexidine gluconate (Group B), withwater (Group A), non-tempered chlorhexidine gluconate (Group B) and tempered chlorhexidine gluconate (Group C) showed a statistically significant difference ($P < 0.01$). This supports the observations of various studies.[11,14,25,26,27]

Three plates were kept at different positions, namely 3’o clock position, 6’o clock position and 12’o clock position during the scaling procedure to collect aerosols. The highest number of microbial colonies was observed in 12’o clock position, followed by 6’o clock position and 3’o clock position. This indicates that 12’o clock position is closer to the patient's mouth compared to that of 3’o clock position. This finding supports the observation made by Rani et al., (2014).[15] However, a large number of studies observed a greater number of microbial colonies in the agar plates placed over the patient's chest than that of the operator, explaining the fact larger salivary droplets generated during dental procedures settle rapidly from the air and would heavily contaminate the agar plates on a patient's chest. Greatest concentration of the microorganisms in aerosols was observed within 2 feet of the patient and the number of microbial colonies decreases with increase in distances from the operating area. Bentley et al. (1994) suggested that distribution of bacterially contaminated aerosols and splatter is extremely variable and may be influenced by the type of therapeutic procedure, use of HVE, the position of the subject in the dental chair, position of the tooth in the mouth that affects the position of the operator relative to the subject, levels of the microorganisms in the subject's mouth, etc.[8]

This study suggests that tempered 0.2% chlorhexidine gluconate is more effective as a preprocedural mouthwash in reducing microbial load in aerosols produced during ultrasonic scaling. Even preprocedural mouth rinse with water also plays a significant role in reducing microbial load in aerosols. Notably, the observations of this study reinforce the significance of personal protective equipment and validate preprocedural mouth rinsing as an additional barrier to cross-contamination and minimizes the risk of team members and the patients.

The limitation of this study should be considered in interpreting the results. Microbial colonies give a good picture of total airborne bacterial count from a particular procedure, but it does not provide any differentiation between whether the bacteria are relatively benign or a

ISSN: 0975-3583,0976-2833 VOL11, ISSUE 11, 2020

pathogenic species. Any bacteria that require special media or growth conditions, such as mycobacteria or strict anaerobes that are common in periodontal pockets, were not cultured and counted in this study. Furthermore, because they do not grow on the type of media used for bacterial studies, no viral particles such as influenza, rhinoviruses, and SARS coronavirus would be measured. Moreover, the plate count or "fall out" approach used for the collection of the bacteria is subjected to a level of inaccuracy, because bacteria exposed to the air may remain viable, or may lose the ability to form colonies and become nonculturable[27]. Thus, counting only aerobic bacteria gives only a partial picture of the airborne contamination that occurs during dental procedures and underestimates the true extent of bacterial populations in aerosols.[23] Future studies are necessary to investigate the viable pathogenic organisms generated during ultrasonic scaling. To evaluate the levels of airborne bacteria remaining in the operatory room after the ultrasonic scaling procedure, culture plates would have exposed post-therapeutically as well.

**Conclusion**

In the light of the study carried out, we may conclude that preprocedural mouth rinse could eliminate the majority of bacterial aerosols generated by the ultrasonic scalers. Tempered chlorhexidine gluconate (0.2%) is effective in reducing the microbial load in aerosols produced during ultrasonic scaling compared to that of water when used as a preprocedural mouth rinse. Again, more microbial colonies are formed on the agar plates placed on the 12'o clock position than that of the plates placed on the 6'o clock position and the assistants at the 3'o clock position. This states the importance of protection for the dentists, who are the main targets of the microorganisms generated during oral procedures.

## References

1. Grenier D. Quantitative analysis of bacterial aerosols in two different dental clinic environments Appl Environ Microbiol. 1995;61:3165–8

2. Harrel SK, Barnes JB, Rivera-Hidalgo F. Aerosol and splatter contamination from the operative site during ultrasonic scaling J Am Dent Assoc. 1998;129:1241–9

3. Szymańska J. Dental bioaerosol as an occupational hazard in a dentist's workplace Ann Agric Environ Med. 2007;14:203–7

4. Miller RL, Micik RE, Abel C, Ryge G. Studies on dental aerobiology. II. Microbial splatter discharged from the oral cavity of dental patients J Dent Res. 1971;50:621–5

5. Hinds WC Aerosol Technology: Properties, Behavior and Measurement of Airborne Particles. 19992nd New York Wiley

6. Pippin DJ, Verderame RA, Weber KK. Efficacy of face masks in preventing inhalation of airborne contaminants J Oral Maxillofac Surg. 1987;45:319–23

ISSN: 0975-3583,0976-2833 VOL11, ISSUE 11, 2020

7. Kharbuli D, Das SJ, Deka A, Alam ST, Bora GH. Position wise quantification of microbial load in aerosols produced during ultrasonic scaling J Med Dent Sci. 2020;2:175–80

8. Bentley CD, Burkhart NW, Crawford JJ. Evaluating spatter and aerosol contamination during dental procedures J Am Dent Assoc. 1994;125:579–84

9. Smith WH, Davies D, Mason KD, Onions JP. Intraoral and pulmonary tuberculosis following dental treatment Lancet. 1982;1:842–4

10. Legnani P, Checchi L, Pelliccioni GA, D'Achille C. Atmospheric contamination during dental procedures Quintessence Int. 1994;25:435–9

11. Logothetis DD, Martinez-Welles JM. Reducing bacterial aerosol contamination with a Chlorhexidine gluconate pre-rinse J Am Dent Assoc. 1995;126:1634–9

12. Holbrook WP, Muir KF, Macphee IT, Ross PW. Bacteriological investigation of the aerosol from ultrasonic scalers Br Dent J. 1978;144:245–7

13. Cochran MA, Miller CH, Sheldrake MA. The efficacy of the rubber dam as a barrier to the spread of microorganisms during dental treatment J Am Dent Assoc. 1989;119:141–4

14. Gupta G, Mitra D, Ashok KP, Gupta A, Soni S, Ahmed S, et al Efficacy of preprocedural mouth rinsing in reducing aerosol contamination produced by ultrasonic scaler: A pilot study J Periodontol. 2014;85:562–8

15. Rani KR, Ambati M, Prasanna JS, Pinnamaneni I, Reddy PV, Rajashree D. Chemical vs. herbal formulations as preprocedural mouth rinses to combat aerosol production: A randomized controlled study J Oral Res Rev. 2014;6:9–13

16. Reddy S, Prasad MG, Kaul S, Satish K, Kakarala S, Bhowmik N. Efficacy of 0.2% tempered chlorhexidine as a pre-procedural mouth rinse: A clinical study J Indian Soc Periodontol. 2012;16:213–7

17. Fine DH, Mendieta C, Barnett ML, Furgang D, Meyers R, Olshan A, et al Efficacy of preprocedural rinsing with an antiseptic in reducing viable bacteria in dental aerosols J Periodontol. 1992;63:821–4

18. Yamada H, Ishihama K, Yasuda K, Hasumi-Nakayama Y, Shimoji S, Furusawa K. Aerial dispersal of blood-contaminated aerosols during dental procedures Quintessence Int. 2011;42:399–405

19. Sawhney A, Venugopal S, Babu GR, Garg A, Mathew M, Yadav M, et al Aerosols how dangerous they are in clinical practice J Clin Diagn Res. 2015;9:C52–7

20. Bonesvoll P, Lökken P, Rölla G, Paus PN. Retention of chlorhexidine in the human oral cavity after mouthrinses Arch Oral Biol. 1974;19:1209–22

21. Flötra L, Gjermo P, Rölla G, Waerhaug J. Side effects of chlorhexidine mouth washes Scand J Dent Res. 1971;79:119–25

22. Leggat PA, Kedjarune U. Bacterial aerosols in the dental clinic: A review Int Dent J. 2001;51:39–44

ISSN: 0975-3583,0976-2833 VOL11, ISSUE 11, 2020

23. Mamajiwala AS, Sethi KS, Raut CP, Karde PA, Khedkar SU. Comparative evaluation of chlorhexidine and cinnamon extract used in dental unit waterlines to reduce bacterial load in aerosols during ultrasonic scaling Indian J Dent Res. 2018;29:749–54

24. Johnston JR, Butchart AM, Kgamphe SJ. A comparison of sampling methods for airborne bacteria Environ Res. 1978;16:279–84

25. Rao RM, Shenoy N, Shetty V. Determination of efficacy of pre-procedural mouth rinsing in reducing aerosol contamination produced by ultrasonic scalers NUJHS. 2015;5:52–6

26. Yadav S, Kumar S, Srivastava P, Gupta KK, Gupta J, Khan YS. Comparison of efficacy of three different mouthwashes in reducing aerosol contamination produced by ultrasonic scaler: A pilot study Indian J Dent Sci. 2018;10:6–10

27. Sethi G, Kumar K. A comparative evaluation of efficacy of 0.2% chlorhexidine with a herbal mouthwash as pre-procedural mouthrinse in the reduction of aerosol contamination produced by ultrasonic scaler Acta Sci Dent Sci. 2018;2:2–6

**Correspondence address:** Dr. Janardhana Amaranath B.J., [1]Professor & Head, Department of Periodontology, Rama Dental College, Hospital & Research Centre, Rama University, Mandhana, Kanpur (UP)- 209217, Email address: dramarbj@gmail.com, Mobile no: 8791079004.