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ORIGINAL RESEARCH

Comparative Evaluation of Colony Forming Unit Count on Aerobic Culture of Aerosol Collected Following Pre-Procedural Rinses of Either 0.2% Chlorhexidine Gluconate or 1% Stabilized Chlorine Dioxide During Ultrasonic Scaling: A Clinical and Microbiological Study

[1]Sneha V Waghmare, [2]Vineet V Kini, [3]Shatakshi Srivastava

ABSTRACT

Aim: The aim of this study was to evaluate and compare the efficacy of 0.2% aqueous chlorhexidine gluconate (CHG) and 1% aqueous stabilized chlorine dioxide (ClO_2) mouthrinse as pre-procedural mouthrinses on colony forming unit (CFU) count from aerosols generated during ultrasonic scaling.

Materials and methods: This prospective controlled parallel arm clinical and microbiological study done over a period of 2 months included 60 systemically healthy participants with no signs of gingivitis or periodontitis divided into 3 groups (1 to 3) with 20 participants each receiving CHX, ClO2 and normal saline as control respectively as pre-procedural rinses followed by collection of aerosols generated during ultrasonic scaling on agar plates kept at three positions namely patient (P), operator (O) and assistant (A) and incubated for 24 hours at 37°C. The CFUs were then counted and statistically analyzed.

Results: Data for the inclusion criteria of plaque index (PI) for all 60 subjects (mean 0.5088 ± 09001) presented normal distribution (p = 0.200). The mean and standard deviation values of retrievable CFUs for all three treatment groups 1 to 3 at three positions of P, A and O were performed using one way ANOVA test with intergroup comparison between by Tukey HSD test. Highest reduction in CFUs was seen in CHX group followed by ClO_2 followed by normal saline which showed least reduction in CFU counts.

Conclusion: It was concluded that aqueous 0.2% CHX was more efficacious in reducing retrievable CFU counts when compared to aqueous 1% stabilized chlorine dioxide mouthrinse and normal saline when used as pre-procedural mouthrinses.

Limitations: As only aerobic culture was done to assess the CFU counts, anaerobic culture would have added more value to the study as it can influence the retrievable CFU counts. Also, despite of best efforts to standardize the settings in dental operatory, there could be some degree of variability.

[1]Postgraduate Student, [2]Professor, [3]Lecturer

[1,2]Department of Periodontics, Mahatama Gandhi Mission's Dental College and Hospital, Navi Mumbai, Maharashtra, India

[3]Department of Public Health Dentistry, Mahatama Gandhi Mission's Dental College and Hospital, Navi Mumbai, Maharashtra, India

Corresponding Author: Sneha V Waghmare, Department of Periodontics, Mahatama Gandhi Mission's Dental College and Hospital, Navi Mumbai, Maharashtra, India, e-mail : drsnehaw9@gmail.com

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INTRODUCTION

Dental surgeons and allied health professionals in the dental office are constantly exposed to aerosols and splatter which is a source of nosocomial infections for the operator, assistants and the patients. Powered instruments such as ultrasonic scalers, piezoelectric surgical motors, air abrasion polishing units and airotor handpieces produce aerosols during operative procedures.[1,2] Aerosols comprise of suspension of solid or liquid particles containing viruses and bacteria suspended in gas for a few seconds. The particlesize may vary between 0.001 mm to more than 100 µm. Whereas splatter being airborne particles with particle size generally larger than 50 µm which is a mixture of air, water, and solid particles visible to naked eyes.[3]

The smaller particles of an aerosol (0.5–10 µm in diameter) pose the greatest potential risk to invade and infect the respiratory tract of personnel at the operatory.[4] As a pre-emptive measure, pre-procedural rinsing with antiseptic mouthrinses by the patients before ultrasonic scaling reduces the bacterial load of aerosol generated during the procedure.[5]

Chlorhexidine (CHX) is a proven pre-procedural mouthrinse with its broad-spectrum antimicrobial activity attributed to its bi-cationic inhibitory action, bacterial cell protein chelation and high substantivity.[6-8] Purohit et al. conducted a study (2009) in which it was concluded that CHX mouthwash led to reduction of CFU colonies

by 94.1% when compared to plain water.[9] Whereas, 1% stabilized chlorine dioxide(ClO_2) mouthrinse is a chemical agent with known antimicrobial properties attributed to its ability to cause oxidative damage to bacterial cell membranes by releasing nascent oxygen.[3] The oxidative consumption of critical biomolecules by ClO_2 is primarily responsible for its wide range of biocidal activity and can also act as a reactive oxidant toward many electron-donating biomolecules (e.g., methionine, pyruvate, urate, and endogenous thiols, such as cysteine).[10] It has been shown that chlorine dioxide is effective in reducing the viable bacterial count in aerosol samples collected from patients with a statistically significant reduction in CFU colonies attributed to the fact that sodium chlorite (stabilized chlorine dioxide) may act as a strong component to obliterate the microbiota via oxygenation and neutralization of toxins.[11] Both mouthrinses have been shown to have an antimicrobial effect against periodontopathic bacteria within the plaque biofilm.

The aim of this study was to evaluate and compare the efficacy of 0.2% aqueous chlorhexidine gluconate mouthrinse, and 1% aqueous stabilized chlorine dioxide mouthrinse as pre-procedural mouthrinses on the retrievable CFU count from aerosols generated during ultrasonic scaling in a controlled parallel arm clinical and microbiological study.

AIM AND OBJECTIVES

The aim of this study was to evaluate and compare the efficacy of 0.2% aqueous chlorhexidine gluconate mouthrinse against 1% aqueous stabilized chlorine dioxide mouthrinse and control normal saline as pre-procedural mouthrinses on the retrievable colony forming unit (CFU) count collected on blood agar plates from aerosol generated during ultrasonic scaling following 24 hour incubation in a prospective controlled parallel arm clinical and microbiological study in a volunteer cohort.

The objective being to assess and compare the retrievable CFU count upon 24 hour aerobic incubation of aerosol collected on blood agar plates placed on 3 positions in relation to the patient (P), assistant (A) and operator (O) during full-mouth ultrasonic scaling over a time span of 30 minutes following pre-procedural rinsing of 1 minute duration with either 0.2% aqueous chlorhexidine gluconate mouthrinse (Group 1) or 1% aqueous stabilized chlorine dioxide mouthrinse (Group 2) or control normal saline (Group 3).

MATERIALS AND METHODS

The study was carried out in accordance with 'The Code of Ethics of the World Medical Association' Declaration of Helsinki, 64th WMA General Assembly, Fortaleza, Brazil, October 2013 for experiments involving humans and the protocol was approved by the Institutional Ethics Review Committee. A total of 60 volunteer subjects rendering informed consent by written document (24 males and 36 females) with age ranging between 20 to 28 years were selected for this study by convenience sampling meeting the inclusion criteria set for the study.

Inclusion criteria were:

- Subjects who were systemically healthy having 20 permanent teeth with intact periodontium.
- Subjects having Full mouth Plaque Index score < 1
- Subjects who did not have had any dental treatment performed for the past 3 months.

Exclusion criteria were:

- Subjects with plaque-induced gingivitis or periodontal disease.
- Subjects who have been administered any antibiotics or analgesics in the past 3 to 6 months.
- Subjects who have received any surgical and nonsurgical periodontal therapy in past 1 month.
- Subjects who had undergone any oral prophylaxis procedure within the past 3 months.
- Subjects having allergy to assigned mouth rinses.
- Pregnant and lactating mothers.
- Tobacco and alcohol consumption in any form.

The study conducted was a prospective single-blinded controlled parallel arm clinical and microbiological study carried by a single operator. The subjects were randomly assigned by computer-generated randomization method to the three test groups comprising of 20 subjects each, i.e., Group 1 (assigned mouthrinse 0.2% aqueouschlorhexidine gluconate Hexidine® ICPA Healthcare Ltd.), Group 2 (assigned mouthrinse 1% aqueous stabilized chlorine dioxide Freshchlor®, Rowpar Group pharmaceuticals, Bengaluru, India) and control Group 3 (assigned mouthrinse of normal saline). Baseline parameters of full mouth plaque index scores (PI)[12] for all subjects were recorded. All subjects were subjected to pre-procedural rinsing of 15 mL of the assigned undiluted mouthrinse for 1 minute prior to ultrasonic scaling performed over a period of 30 minutes during which aerosols generated were collected on sheep blood agar plates kept at three designated positions – operator (O), patient (P) and assistant (A) and were examined for retrievable CFU counts following aerobic incubation for 24 hours. The flowchart for the study is shown in Figure 1. A confined non-air conditioned dental operatory area measuring 10 feet by 10 feet and pre-fumigated by potassium permanganate 99% in (37–40% Formaldehyde) was used to conduct the study. Three sites at which sheep blood agar plates measuring 10 × 1 cm in diameter placed for aerosol collection were determined for the chair position corresponding to that

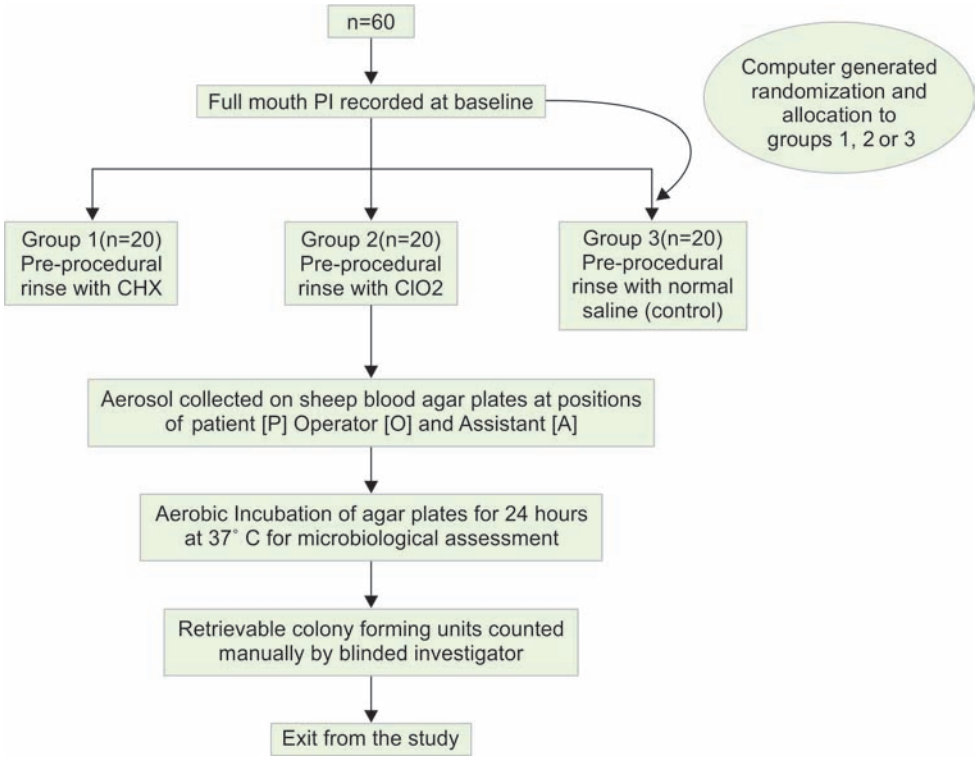

Fig. 1: Flowchart of the study

of a right-handed operator at the locations of position P (plate mounted on a board suspended with string around the neck of patient at 6 'o' clock position from patient's mouth placed on the thorax at a distance of 12 inches, position A (plate mounted on a board suspended by string around the neck of the assistant at 3 'o' clock position at the level of the patient's mouth at a distance of 12 inches and position O (plate mounted on a board suspended by string around the neck of the operator at 9 'o' clock position at the level of the patient's mouth at a distance of 12 inches as shown in Figure 2. Ultrasonic supragingival scaling for all of the study participants was carried out using a piezoelectric scaler unit (Satelec, Acteon®, France) with a triangular tipno. F00247, a preset power settings and lavage setting of frequency (30 KHz) and water pressure (0.3 MPa) respectively. The evacuation was performed for all subjects using the same high volume saliva ejector during the scaling procedures. The aerosols were collected on the agar plates (Micro Master Labs Pvt Ltd, Thane, Maharashtra, India) for 30 minutes of the scaling procedure following which they were closed with a lid and placed upside down to prevent further contamination from moisture condensation within the lid.

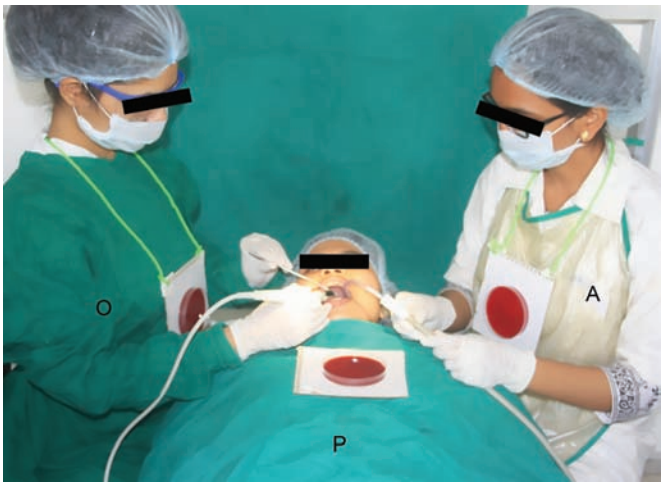

Fig. 2: The position of blood agar plates at three different locations, the chest area of patient (P), the assistant's position (A), the operator's position (O)

The agar plates were then transported immediately for aerobic culture and stored in an Incubator at 37 °C for 24 hours (Fig. 3). After 24 hours incubation period, the cultured agar plates were manually counted using a colony counter by a blinded investigator for retrievable bacterial CFUs considering the entire surface of agar plate for evaluation.

Statistical Analysis

Data were tabulated, and analysis was performed by the statistician using Statistical Package for the Social Sciences (SPSS) software version 16.0. Test for normal distribution of data was performed using the Kolmogorov Smirnov test. The data for the CFU counts for positions of P, A and O for Groups 1 to 3 were found to be parametric and compared by one way ANOVA and Tukey HSD testfor intergroup comparison between the Groups 1 to 3 with the mean difference between parameters significant at the p-value of 0.05.

Fig. 3: Incubator which was used for incubation of blood agar plates for 24 hours.

RESULTS

Kolmogorov–Smirnov and Shapiro–Wilk tests were used for determining the normality. The data of plaque index (PI) for all 60 subjects (mean 0.5088 ± 09001) in all the three groups presented normal distribution (p-value = 0.200). The mean and standard deviation values of retrievable CFUs for all the three treatment Groups 1 to 3 at three different positions of P, A and O (Table 1) were performed by using one way ANOVA test (Table 2). Further, the intergroup comparison between P, O, A positions for all the three groups was performed by using-Tukey HSD test and summarized in Table 3. Comparison of mean postprocedural values of CFUs in group CHX versus ClO_2 versus 0.2% CHX and saline are illustrated in Table 3. Through the analysis, it was found that there was a significant difference in the retrievable CFU counts between all the three groups.

DISCUSSION

Dental personnel is always at high risk of nosocomial infection from aerosols generated during operative procedures such as scaling and restorative procedures. Legnani et al. and Bennett et al. showed that the use of ultrasonic scaling procedures resulted in peak concentrations of microbial aerosols in dental treatment rooms. It has been found from studies of Worrall et al. and Gupta et al.[13] that pre-procedural mouth-rinsing along with universal barrier protection and high power evacuation significantly reduces the infectious risk of aerosols.

The present study aimed to assess and compare the efficacy of preprocedural rinsing with 0.2% aqueous chlorhexidine gluconate or 1% aqueous stabilized Chlorine dioxide or control normal saline on the retrievable bacterial colony forming unit count following aerobic culture incubated for 24 hours, counted on aerosols generated during a 30 minute duration scaling procedure performed using ultrasonic scaler in a clinical study within an healthy volunteer cohort.

Considering the inclusion criteria, the normal distribution of data for full mouth Plaque Index scores for all subjects meant that the plaque levels for all subjects were comparable at baseline and since the study settings were standardized, the retrievable CFU count from aerosol generated at positions P, A and O postintervention for all three Groups 1 to 3 would be directly attributed to the pre-procedural mouth-rinsing before the intervention.

CHX is a proven gold standard as a pre-procedural rinse in reducing bacterial aerosol contamination with the use of ultrasonic scaler as demonstrated by Sawhney et al. (2015). It has also been proven that CHX is efficacious in reducing bacterial CFUs due to its antimicrobial action at the point of generation and even the formation of aerosols. Similarly, enhanced efficacy of ClO_2 in reducing the CFUs could be attributed to the fact that it may act as a strong component in the obliteration of the microbiota via oxygenation and neutralization of toxins produced by the bacteria in the oral cavity. In vitro studies done by Wirthlin MR and Drake DR[14,15] demonstrated stabilized-ClO_2 based oral rinse microbicidal activity againstvarious oral pathogens.

Table 1: Retrievable CFU counts for positions P, O and A for Groups 1 to 3

Group	*Position (n = 20 each)*	*CFU count minimum*	*CFU count maximum*	*Mean CFU count*	*SD*
	P	30	48	38.300	5.07937
1	O	25	42	33.000	4.44854
	A	22	35	27.450	3.45612
	P	39	56	46.800	5.35675
2	O	35	50	41.650	4.82619
	A	25	46	34.450	5.29623
	P	83	109	93.850	7.56915
3	O	80	99	89.200	6.45307
	A	75	91	83.800	5.26758

Group 1 = 0.2% aqueous chlorhexidine gluconate; Group 2 = 1% stabilized chlorine dioxide, Group 3 = Control normal saline; SD = Standard deviation

The data for retrievable CFU count at three positions P, A and O (Table 1) were compared by using one way ANOVA (Table 2). It suggested that upon intergroup comparison there was difference in the retrievable CFU counts at position P ($p < 0.01$) between Group 1 to 3 and at Position A ($p < 0.01$) between Group 1 to and Position O ($p < 0.01$) between Groups 1, 2 and 3. Following post hoc analysis performed using Tukey HSD test (Table 3) for intergroup comparison of the three groups were found to be significantly different from each other with CFU count being maximum in Group 3 ($p < 0.01$) followed by Group 2 ($p < 0.01$) and least in Group 1 ($p < 0.001$) for all patient, operator and assistant's positions respectively (Fig. 4).

Thus, the results of our study suggest that 0.2% CHX preprocedural rinse significantly reduces the CFUs at all the three different positions which are also in agreement with the with data reported by Feres M et al.[16] and Muir et al.[17] Similarly, it was found that Chlorine dioxide was efficacious in reducing the CFUs in all three groups for three different locations which were in accordance with the results of study done by Wirthlin MR in 2006 and Rajiv Saini in 2015.

From the observations of this study we could infer that when used as a pre-procedural mouthrinse 0.2% aqueous chlorhexidine gluconate (Group 1) was more efficacious as compared to 1% aqueous stabilized chlorine dioxide mouthrinse (Group 2) and control group normal saline (Group 3) in reducing the retrievable CFU counts from aerosols at the positions in relation to the P, A and O with a p-value of < 0.001 which is in agreement to the

Table 2: One way ANOVA for intergroup comparison of CFU counts from positions P, O and A

CFU counts from position		*Sum of squares*	*df*	*Mean square*	*F*	*p-value*
P	Between groups	35811.7	2	17905.85	480.536	< 0.001*
O	Between groups	36628.433	2	18314.217	648.492	< 0.001*
A	Between groups	37731.633	2	18865.817	835.484	< 0.001*

P = Patient's position; O = Operator's position; A = Assistant's position
*Statistical significance level

Table 3: Tukey HSD for intergroup comparison of CFU counts in Groups 1 to 3 for positions P, O and A

CFU count at position	*(I) Group*	*(J) Group*	*Mean difference (I-J)*	*Std. error*	*p-value*	*95% confidence interval*	
						Lower bound	*Upper bound*
P	1	2	-8.50000	1.93034	<0.001*	-13.1452	-3.8548
		3	-55.55000	1.93034	<0.001*	-60.1952	-50.9048
	2	1	8.50000	1.93034	<0.001*	3.8548	13.1452
		3	-47.05000	1.93034	<0.001*	-51.6952	-42.4048
	3	1	55.55000	1.93034	<0.001*	50.9048	60.1952
		2	47.05000	1.93034	<0.001*	42.4048	51.6952
O	1	2	-8.65000	1.68051	<0.001*	-12.694	-4.606
		3	-56.20000	1.68051	<0.001*	-60.244	-52.156
	2	1	8.65000	1.68051	<0.001*	4.606	12.694
		3	-47.55000	1.68051	<0.001*	-51.594	-43.506
	3	1	56.20000	1.68051	<0.001*	52.156	60.244
		2	47.55000	1.68051	<0.001*	43.506	51.594
A	1	2	-7.00000	1.50269	<0.001*	-10.6161	-3.3839
		3	-56.35000	1.50269	<0.001*	-59.9661	-52.7339
	2	1	7.00000	1.50269	<0.001*	3.3839	10.6161
		3	-49.35000	1.50269	<0.001*	-52.9661	-45.7339
	3	1	56.35000	1.50269	<0.001*	52.7339	59.9661
		2	49.35000	1.50269	<0.001*	45.7339	52.9661

Group 1 = 0.2% aqueous chlorhexidine gluconate; Group 2 = 1% stabilized chlorine dioxide; Group 3 = Control normal saline
P = Patient's position; O = Operator's position; A = Assistant's position.
* = Statistical significance level

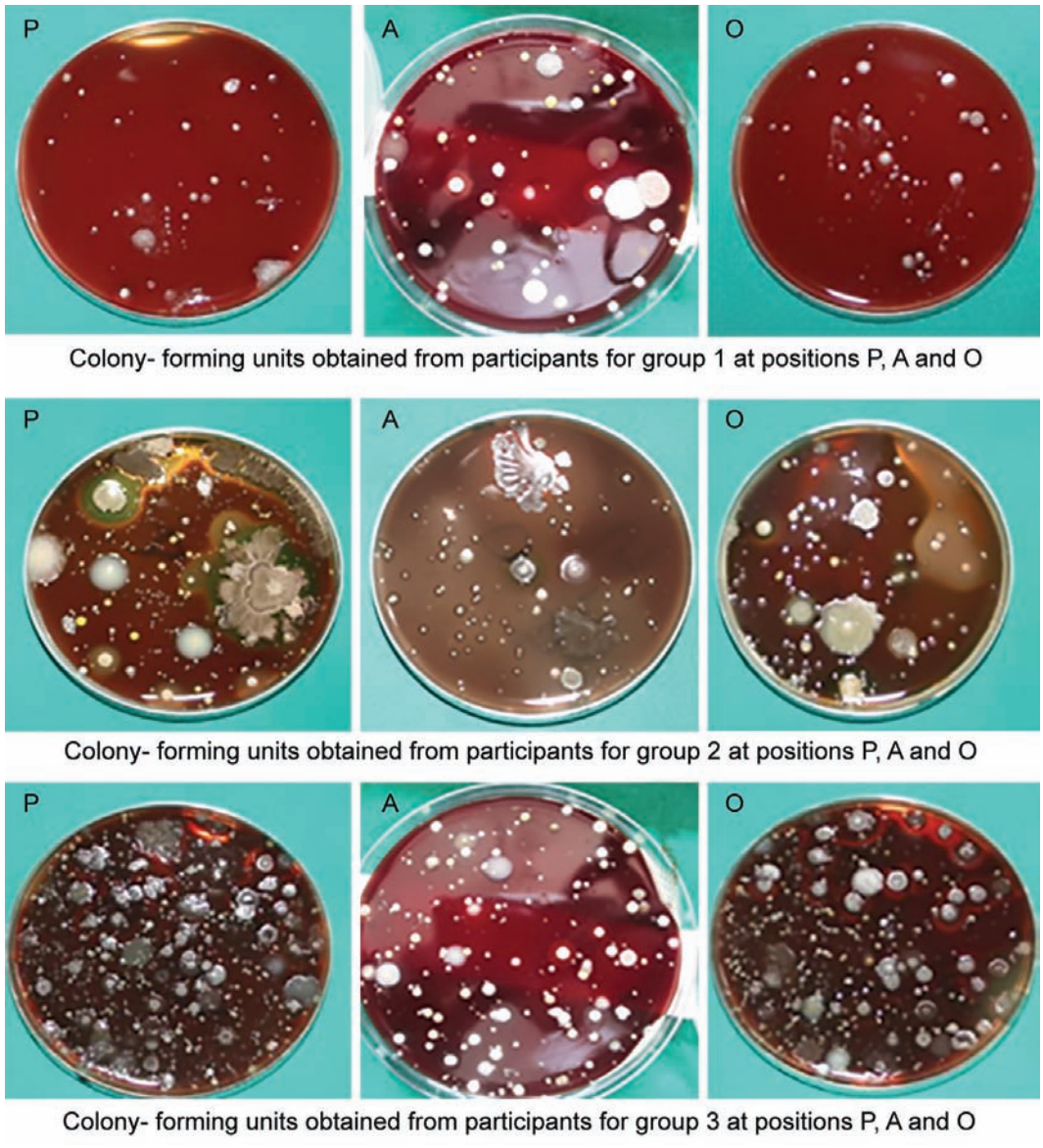

Fig. 4: Retrievable CFU counts on agar plates in Group 1: 0.2% aqueous chlorhexidine gluconate; Group 2: 1% aqueous stabilized chlorine dioxide and Group 3: Normal saline at position P/A/O

findings of Saini et al. (2015)[11] where chlorhexidine gluconate demonstrated the highest reduction in CFU count as compared to chlorine dioxide in a placebo-controlled clinical trial conducted in 120 patients.

However, it was also observed that when used as a preprocedural mouthrinse 1% aqueous stabilized chlorine dioxide mouthrinse (Group 2) was more effective as compared to the control group normal saline (Group 3) in reducing the retrievable CFU counts from aerosols at the positions in relation to P, A and O generated during ultrasonic scaling which was in agreement with study done by Saini (2015) conducted in 80 participants in a clinical interventional study, where chlorine dioxide showed a significant reduction in bacterial CFU counts when compared to sterile water[18] thus concluding that preprocedural mouthrinse using chlorine dioxide significantly reduces the aerosols generated during oral prophylaxis procedures.

CONCLUSION

Within the limitations of the study, it may be permissible to conclude that aqueous 0.2% CHG was more efficacious in reducing retrievable CFU counts and thereby reducing infectious risk from aerosol generated during ultrasonic scaling as compared to aqueous 1% stabilized chlorine dioxide mouthrinse and normal saline when used as preprocedural mouthrinses.

LIMITATIONS

Anaerobic culture would have added more value to the study as it could influence the retrievable CFU counts. The operatory, if equipped with heating, air venting

and cooling systems with air currents could influence the distance of aerosol would spread. Despite the best of efforts to standardize the settings of the ultrasonic scalers, evacuation system and the positions of the patient, operator, and assistant within the scope of human error, there could be some degree of variability in regards to the same.

REFERENCES

1. Bentley CD, Burkhart NW, Crawford JJ. Evaluating splatter and aerosol contamination during dental procedures. J Am Dent Assoc. 1994;125:579-584.
2. Micik RE, Miller RL, Mazzarella MA, Ryge G. Studies on dentalaerobiology. I. Bacterial aerosols generated during dentalprocedures. J Dent Res. 1969;48(1):49-56.
3. Holbrook WP, Muir KF, MacPhee IT, Ross PW. Bacterial investigation of the aerosol from ultrasonic scalers. Br Dent J. 1978;144(8):245-247.
4. Acharya S, Priya H, Purohit B, Bhat M. Aerosol contamination in a Rural University Dental Clinic in South India. Int J Infect Control. 2010;6(1);1-7.
5. Sawhney A, Venugopal S, Babu GRJ, et al. Aerosols How Dangerous They Are in Clinical Practice. Journal of Clinical and Diagnostic Research : JCDR. 2015;9(4):ZC52-ZC57
6. D. Gopalakrishnan, RavichandraJuluri, J. Srihari, Vidya Viswanathan. Comparing the Efficacy of Two Mouth Rinses in Reducing Bacterial Aerosol Contamination. 2017;3(3): 066
7. Purhohit B, Priya H. Efficacy of preprocedural rinsing in reducing aerosol contamination during dental procedures. J Infect Prevent. 2009;10:190-192.
8. Feres M, Figueiredo LC, Faveri M, Stewart B, de Vizio W. The effectiveness of a preprocedural mouthrinse containing cetylpyridinium chloride in reducing bacteria in the dental office. J Am Dent Assoc. 2010;141:415-422.
9. Purhohit B, Priya H. Efficacy of preprocedural rinsing in reducingaerosol contamination during dental procedures. J InfectPrevent. 2009;10:190-192.
10. Paraskevas S, Rosema NA, Versteeg P, Van der Velden U, Van der Weijden GA.Chlorine dioxide and chlorhexidine mouthrinses compared in a 3-day plaque accumulation model. J Periodontal. 2008 Aug;79(8):1395-1400.
11. Saini R. Efficacy of preprocedural mouth rinse containing chlorine dioxide in reduction of viable bacterial count in dental aerosols during ultrasonic scaling: A double-blind, placebo-controlled clinical trial. Dent Hypotheses 2015;6:65-71.
12. Silness J, Loe H. Periodontal Disease in Pregnancy. II. Correlation between Oral Hygiene and Periodontal Condtion. ActaOdontol Scand. 1964 Feb;22:121-135.
13. Harrel SK, Molinari J. Aerosols and splatter in Dentistry: a brief review of the literature and infection control implications, *J Am Dent Assoc.* 2004;135(4):429-437
14. Saini R. Chlorine dioxide: An ideal preproceduralmouthrinse in dental set-up. EurJ Gen Dent. 2015;4:113-116.
15. Feres M, Figueiredo LC, Faveri M, Stewart B, de Vizio W. The effectiveness of a preprocedural mouthrinse containingcetylpyridinium chloride in reducing bacteria in the dentaloffice. J Am Dent Assoc. 2010;141:415-422.
16. Muir KF, Ross PW, MacPhee IT, Holbrook WP, Kowolik MJ. Reduction of microbial contamination from ultrasonic scalers.Br Dent J. 1978;145:76-78.
17. Wirthlin MR, Choi JH, Kye SB. Use of chlorine dioxide-mouthrinses as the ultrasonic scaling lavage reduces theviable bacteria in the general aerosols. J West SocPeriodontol Periodontal Abstr. 2006;54:35-44.
18. Drake DR, Villhauer A, Olson B. Bacterial activity of stabilizedchlorine dioxide against polymicrobial biofilms. J Dent Res. 2009;88:3417.