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TO DETERMINE THE EFFICACY OF 0.12%CHLORHEXIDINE MOUTHRINSES IN REDUCING VIABLE BACTERIAL COUNT IN DENTAL AEROSOLS WHEN USED AS A PREPROCEDURAL RINSE DURING THE PANDEMIC ERA -A PROSPECTIVE CLINICAL PILOT STUDY

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: In dentistry , aerosols being the major concerns that brings about transmission of infectious agents and harmful to patients and dentists . Thus the vigilant use of barriers, appropriate immunisation procedures all could safeguard the dental fraternity from the ill effects of aerosols.

Aim: To Determine The Efficacy of 0.12% chlorhexidine mouth rinse as preprocedural rinse in reduction of bacterial aerosol contamination generated during Ultrasonic scaling in a closed operatory and compared with1% Povidone iodine rinse and without preprocedural rinse.

Materials and Methods: Thirty patients were selected from Department of Periodontics, Sree Balaji Dental College and Hospital, Chennai. The subjects were grouped into A -10 patients each receive 1% POVIDINE IODINE mouth rinse and Group B of 10 patients received 0.12% CHLORHEXIDINE mouth rinse respectively as a pre-procedural rinse. Group C 10 patients without preprocedural rinse. The aerosols produced by the ultrasonic unit were collected on blood agar plates placed at 8 standard positions around the dental chair. These plates were sent for microbiologic analysis for the assessment of bacterial Colony Forming Units (CFUs) was evaluated and statistically analysed.

Results: The significance of the study was both the mouthrinses reduced the bacterial colony forming units (CFUs) in aerosol samples. 0.12%Chlorhexidine mouth rinses were found to be superior to 1%Povidone iodine in reducing aerosolized bacteria when used preprocedurally.

Conclusion: The study highlights the efficacy of preprocedural mouth rinses , during any dental treatment which generates aerosols, reduces the risk of cross- contamination within the dental operatory.

Keywords: Dental aerosols; bacterial colony forming unit; preprocedural mouth rinses.

1. INTRODUCTION

In dental practise we use high speed airotors , ultrasonics, and air water syringes . They all work under high pressure and generate spatter and aerosols that contaminate the surroundings and carry infectious agents that are hazardous to health . Aerosol is defined as a droplet nuclei that usually less than 5μM

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in diameter, they remain suspended in air for relatively longer period of time. The size is smaller and they have the potential to penetrate and lodge into passage of lungs and are implicated to be the primary mode of transmission of air borne infectious agents [1]. The term aerosols and splatter was defined in aerobiology [2].

Chlorhexidine gluconate has been proved to be effective against broad spectrum bacteria and long-time substantivity in the oral , they release certain active molecules whose concentratios can be detected in saliva even after 24 hrs and has been effectively proved [3]. The 0.02% of chlorhexidine gluconate is commonly used in mouth rinses. 0.12% chlorhexidine commercially called as periogard is also used for its antibacterial activity . The long-time use of chlorhexidine brings tooth staining [4].

Iodine is commonly utilised in medicine for topical treatments because of its antiseptic and antibacterial characteristics.

Whereas the povidone iodine mouth rinses they are basically diatomic iodine , prevent bacteraemia post extraction and reduces the local inflammation [5]. Irrigation of periodontal pockets with Povidone iodine also reduces bacteraemia after scaling and root planing [6].

And in the current scenario in this pandemic era we are in the urge to reduce the infectivity where the COVID 19 virus transmits predominately through aerosols and splatters. In dental practice we are working in close proximity with the patients and get exposed to blood , saliva and other body fluids. [16] The infection control plays a vital role and the significance 1% Povidone Iodine when used as preprocedural rinse reduces the viral load . Therefore its efficacy in reduction bacteria in dental aerosols will also help us during this time [7,8].

Hence, the aim of the present study is to evaluate the efficacy of PI(PVP-I) against nosocomial pathogens by settle plate method using selective media placed systematically around dental units during ultrasonic scaling.

2. MATERIALS AND METHODS

The present study was reviewed and it was approved by the Institutional Ethical Committee, Sree Balaji Dental College and Hospital, Bharath University (Ref No. SBDCH/IEC/09/2016/4).The subjects enrolled in this study were selected from the Department of Periodontology, Sree Balaji Dental College and Hospital.

Total of 30 study participants included both male and female were informed about the procedures and a written consent was obtained.

The operatory room was fumigated. Disinfection of operatory surfaces was done using a baccilloid disinfectant solution and fumigation was done for 30 min, 24 hrs prior to the procedure so as to make the operatory room free from aerosols.

To avoid cross contamination, just one patient was treated per day, and the treatment was completed on the same day. Prior to the treatment, the ultrasonic device was turned on and flushed for 2 minutes, as advised by the manufacturer, to remove contaminated water that had accumulated in the waterlines overnight. Efforts were also made so as to minimize the contamination by usage of autoclaved mouth mask, head cap and disposable patient apron.

Eight different locations were used in the experiment, encompassing every aspect of the operatory. Trypticase Soya Agar plates supplemented 5% sheep blood were placed at eight pre-designated positions as depicted in the Fig. 1. The settle plate method was performed by placing the blood agar plates open once the scaling procedure was started to collect samples of aerosolized bacteria and closed after 30 minutes. The preparation of stellate plates includes , Four grams of TSA was suspended in 100ml of distilled water. The medium was dissolved completely by boiling. The medium was autoclaved at 121º C, 15 lbs pressure for 15 minutes. The medium was cooled to 45 - 50º C and 5 ml of defribinated sheep blood was added aseptically. 20 ml of the medium was poured into sterile disposable petriplates (Vijayplast, India). Sterility check was performed for each lot by incubating a representative plate at 37ºC. The plates were stored at 4º C until use.

Group A :The study participants were instructed to rinse 15 ml of 1%Povidone Iodine (BETADINE GARGLE) preprocedurally for 30 seconds.

Group B: The similar instructions was followed with 15 ml of 0.12% OF CHLORHEXIDINE (PERIOGARD) mouthrinse

Group C : ultrasonic scaling alone was performed without any preprocedural rinse. Total duration was around 30 minutes .

THE CAVITRON BOBCAT pro ultrasonic scaler with standard scaler tip and motorized suction along with distilled water was used for oral prophylaxis .The amount of water dispensed, the water pressure and power settings on the ultrasonic unit were identical for each participant. Each study participant

Plate 1	two feet distance of the right side of the patient chair from the reference point
Plate 2	Two feet behind the patient's chair
Plate 3	Left side of patient's chair
Plate 4	Three feet to the patient's right
Plate 5	Three feet to the patient's left
Plate 6	Five feet to the patient's right
Plate 7	Five feet to the patient's left
Plate 8	Nine feet from the reference point

Fig. 1. Position of plates based on the reference point from the patient chair [3]

was treated by the same operator. During the treatment and after the treatment, the sixteen selective plates (8 for control group I, 8 for *control groupII,*8 for control group III) were kept open at the specified position from the reference point to collect samples of any aerosolized bacteria (Fig. 1). The agar plates were then transported immediately to the microbiology laboratory for microbial assessment of colony forming units. The selective medium were placed in an incubator at 37°C for 48 hours. The plates were then examined for microbiological growth. The generated CFUs for each plate were measured using a colony counter.

2.1 Statistical Analysis

On statistical analysis (Mean, & standardisation error) of the colony counts of all the samples at 8 different positions were calculated using SPSS software 18.0 version. Paired T tests were performed to analyse the 2 tailed significance of the difference in the mean values of the colony counts from Positions 1 – position 8. WILCOXON analysis was done to calculate the significance of the results group A, B and C was done. The criteria for statistical significance was a P value < 0.05.

Bacterial aerosol contamination level in the closed operatory was assessed at baseline before and after fumigation. The number of colonies forming units (CFU) at baseline in all positions P1 –P8 are shown in Table 1. Paired t-test performed to compare the mean difference in colony forming units at the eight predesignated positions before and after fumigation (at baseline) showed a t -test value of 14.675 and a statistical significant difference of 0.005($P < 0.05$).

2.2 Colony Forming units at Baseline in Positions P1-P2 (before & after fumigation)

After fumigation, contamination was practically non-existent (mean 17.25 *cfu*) in all positions P1–P8 at baseline. Hence, fumigation using 2% HOSPAL^{TM} – OT for 30 minutes was performed before each appointment and the dental operatory was closed for 19 hours to avoid air circulation. Also, scaling was performed for one patient per day so as to avoid cross contamination.

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The wilcoxson comparative analysis that compares the data between the two test groups and the percentiles of ranks was less in group B.

Table 1. Colony forming units at positions P1 – P8 at baseline (before & after the fumigation)

Position	Before	After	Difference
P1	48	14	34
P2	57	22	35
P3	46	15	31
P4	45	12	33
P5	50	22	28
P6	56	21	35
P7	43	19	24
P8	59	13	46
Mean	50.50	17.25	33.25
Paired t-test t-test value: 14.675 Sig.: 0 .005			

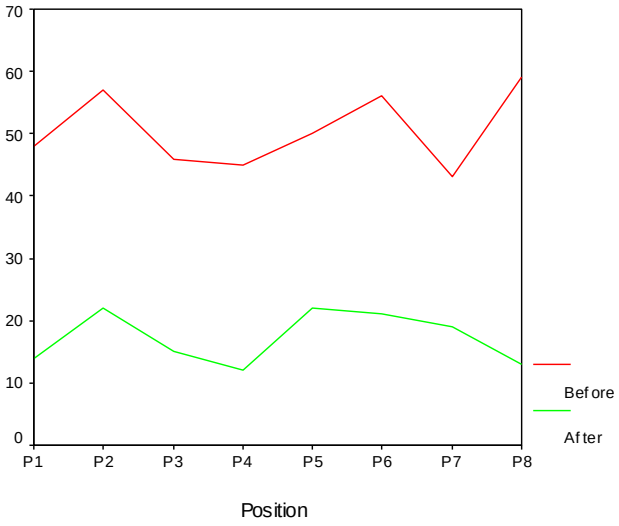

Fig. 2.

Table 2. Wilcoxson signed rank test with 1%pi group and 0.12% CHX

	Descriptive Statistics					
	N	**Minimum**	**Maximum**	**Percentiles**		
				25th	**50th (Median)**	**75th**
GroupA	8	43	59	45.25	49.00	56.75
Group B	8	12	22	13.25	17.00	21.75

Table 3. Mean and standard deviation of control group and pi preprocedural rinse with 0.12% CHX

Ranks			
group	N	Mean	SD
CONTROL GROUP	8	11.81	94.50
PI	8	5.19	41.50
CHX	8	4.50	36.00
Total	16		

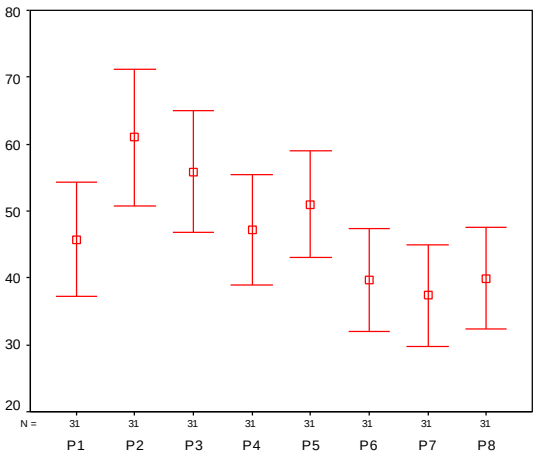

Fig. 3. Difference in mean colony forming units at positions P1 – P8 before and after periogard rinse at 95% Confidence Interval

Table 4. Post hoc tests multiple comparisons dependent variable: Difference

	(I) Position	(J) Position	Mean Difference (I-J)	Std. Error	Sig.
Tukey HSD	P1	P2	-15.26	5.825	.154
		P3	-10.10	5.825	.665
		P4	-1.42	5.825	1.000
		P5	-5.26	5.825	.986
		P6	6.06	5.825	.968
		P7	8.39	5.825	.838
		P8	5.81	5.825	.975
	P2	P3	5.16	5.825	.987
		P4	13.84	5.825	.258
		P5	10.00	5.825	.676
		P6	21.32	5.825	.007
		P7	23.65	5.825	.002
		P8	21.06	5.825	.009
	P3	P4	8.68	5.825	.812
		P5	4.84	5.825	.991
		P6	16.16	5.825	.106
		P7	18.48	5.825	.036
		P8	15.90	5.825	.119
	P4	P5	-3.84	5.825	.998
		P6	7.48	5.825	.904
		P7	9.81	5.825	.698
		P8	7.23	5.825	.919
	P5	P6	11.32	5.825	.522
		P7	13.65	5.825	.275
		P8	11.06	5.825	.553
	P6	P7	2.32	5.825	1.000
		P8	-.26	5.825	1.000
	P7	P8	-2.58	5.825	1.000

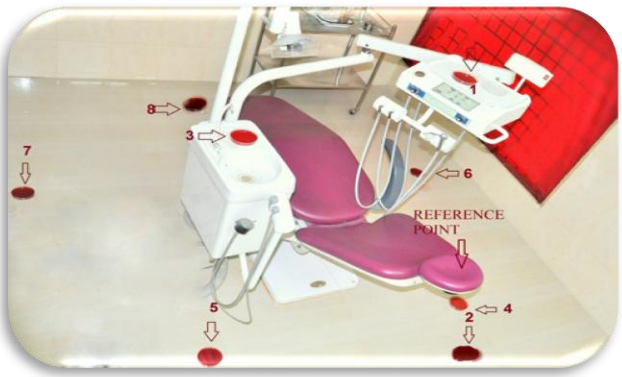

Fig. 4. Position of plates

The mean reduction of microorganisms in group A was much lesser when compared to other two group

Multiple comparisons done by Post Hoc tests showed that the difference in mean colony-forming units between P2 and P6 (61.00 *vs* 39.68, p= 0.000), P2 and P7 (61.00 *vs* 37.35, p= 0.000), P2 and P8 (61.00 *vs* 39.94, p= 0.000), P3and P7 were found to be statistically significant. Hence, it is clear that the bacterial aerosol contamination levels decrease with increase in distance from the reference point Table 4.

3. DISCUSSION

A Cavitron BOBCAT Pro ultrasonic scaler with a standard ultrasonic tip and motorised suction was

utilised for ultrasonic scaling. Ultrasonic scaling techniques were performed using distilled water. For each individual, the amount of water discharged, the water pressure, and the power settings on the ultrasonic device were similar. The same operator worked on all of the subjects. During the treatment and after the treatment, the sixteen selective plates (8 for control group I ,8 for *control groupII*,8 for control group III) were left uncovered at their pre-designated sites to collect samples of any aerosolized bacteria. The selective medium was then immediately delivered to the microbiology lab for microbiological testing. The selective medium was cultured for 48 hours at 37°C in an incubator. The plates were examined for microbial growth after the incubation time. The generated CFUs were counted using a colony counter for each plate. Accumulating evidences suggests that aerosols are generated at a very high rate when ultrasonic scalers are used during dental procedures. Both the public and working dentists inhale aerosols generated by dental equipment on a routine basis and hence they are at a risk of acquiring tuberculosis, respiratory infection, ophthalmic and skin infection [9-11]. Hence, the present study used ultrasonic scaler for the dental procedure to assay the bacterial load in the aerosols generated. In addition, the efficacy of an oral rinse in the reduction of the aerosol contamination was evaluated by standard microbiological assay [12,13].

In our study the Bacterial aerosol contamination level in the closed operatory was assessed at baseline before and after fumigation. The numbers of colony forming units (CFU) at baseline in all positions P1 – P8 were evaluated. The study has used 0.12% chlorhexidine gluconate (Periogard) and 1% Povidone Iodine pre-rinse in evaluating the reduction of aerosol contamination [14]. The study also has a control group where the patients underwent Ultrasonic Scaling without any Preprocedural rinse and the number of colonies forming units were counted with the Stellate Agar Plates method.

The agar plates that were arranged in different position from the patient's chair was in accordance to the study done by logethics [15]. It was observed that a Very high bacterial load was observed on plates kept in position P2, P3 and P4, which is inversely proportional to the distance. The bacterial load was almost similar on the plates kept at P6 and P9 in spite of 4 feet difference in the distance from the reference point.

This difference in the load was noticed since the aerosol contamination would have dropped on the dentist working. The bacterial count was low at P_7 compared to P_8 as the spittoon of the dental chair was a barrier for P_7 position even though the distance from the reference point was less compared to P_8 position.

In other study that evaluated efficacy of 1% Povidone Iodine when given prior to the procedure had a significant bactericidal effect [16]. This finding was in par to our present study. The effect 1% Povidone iodone (betadine gargle) when given as preprocedural rinse and Ultrasonic scaling was done with the agar plates placed from positions P1-P8 and colony forming units were evaluated and statistical analysis was done the great reduction of CFU was seen in the PI (POVIDONE IODINE) group when compared with the control group.

The Chlorhexidine(Periogard) pre-rinse resulted in highly significant reduction in the colony forming units at all the selected locations [8]. From our present study it is clearly evident that the colony forming units of bacteria due to aerosols after ultrasonic scaling without preprocedural rinse was higher than when compared with the patients who underwent ultrasonic scaling after the preprocedural rinse of both 1% povidone group and 0.12% Chlorhexidine (Periogard) rinse group.

The mean reduction of CFU in PI group and CHX group was compared and the results showed significantly greater reduction in CHX group.

4. CONCLUSION

The preprocedural mouthrinses significantly reduced the bacterial colony forming units in aerosol samples. When utilised pre-procedurally, Chlorhexidine rinses were found to be superior to Povidone iodine in decreasing aerosol bacteria. Pre-procedural rinsing with an efficient antimicrobial mouth rinse (Periogard) during any dental procedure that generates aerosols minimises the risk of infectious agent cross-contamination in the dental operatory, according to this study.

5. LIMITATIONS OF THE STUDY

However, the present study did not focus on the viruses, obligate anaerobes and very highly fastidious organisms, as the media (blood agar) and method of incubation used supports only the non-fastidious, facultative anaerobes and fastidious aerobic organisms. Further investigations may be carried out in this direction to substantiate the conclusions drawn from this study.

The numbers presented as CFUs are relative values representing only aerobic bacteria capable of growth on blood agar media plates. It is likely that the actual

bacterial content in the specified areas was much higher than that reported here, as the culture medium and growth conditions used did not allow the identification of all types of organisms including viruses, anaerobic bacteria, and organisms requiring specialized medium.

Any dental procedure that has the potential to aerosolise saliva will cause airborne contamination with organisms from some or all of these sources. The most serious potential threat present in aerosols is Mycobacterium tuberculosis, ,the organisms causes tuberculosis [9]. therefore this study carries clinical significance that use of any antiseptic mouthwashes is essential in reducing microbial load.

CLINICAL SIGNIFICANCE

The results of the study are important, because a reduction in the number of aerosolized bacteria may reduce the risk of cross-contamination in the dental office, thus helping protect dentists, dental office personnel, and patients. Although the use of personal protective equipment and the other infection -control measures are common practice among dentists, these present limitations.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

The subjects who agreed to participate in the study were informed about the procedure and a written consent was obtained.

ETHICAL APPROVAL

The present study was reviewed and it was approved by the Institutional Ethical Committee, Sree Balaji Dental College and Hospital,Bharath University (Ref No. SBDCH/IEC/09/2016/4).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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