Original Article

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Dentistry Section

Aerosols How Dangerous They Are in Clinical Practice

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ABSTRACT

Background and Objectives: The purpose of the present study was to determine the microbial atmospheric contamination during initial periodontal treatment using a modern piezoelectric scaler and to evaluate the efficacy of two commercially available mouth rinses (0.2% Chlorhexidine mouth rinse and Listerine) in reducing bacterial contamination when used as a pre-procedural rinse, with and without high volume evacuation (Aerosol reduction device).

Materials and Methods: Subjects for the study were selected from the outpatient Department of Periodontics, Sri Siddhartha Dental College and Hospital, Tumkur, India. Total 60 patients were taken for the study and on the basis of inclusion and exclusion criteria's they were divided into three groups. The sampling was carried out in two stages before and after implementing a set protocol. Total duration of study was four months.

Microbiological Evaluation: The samples (blood agar plates) were transported immediately to the Department of Microbiology, Sri Siddhartha Medical College, Tumkur for:

- Identification of microorganisms as per standard procedures (Gram stain, Biochemical Test, Species Identification).
- Counting the number of colonies formed on blood agar plates using colony counter unit.

Results: Out of all the three pre-procedural rinses 0.2% w/v Chlorhexidine is the best in reducing aerobic bacteria (CFU) followed by Listerine and then Water.

Conclusion: The following conclusion was drawn that the use of pre-procedural rinses along with the use of high volume suction apparatus significantly reduced the aerosol contamination and hence chances of cross-infection in the dental units.

Keywords: Piezoelectric scaler, Pre-procedural rinses, Ultrasonic scaling

INTRODUCTION

Professional interest has developed concerning dentally produced aerosols and the potential for disease transmission to clinicians and patients. Aerosol is created when high-powered devices need compressed air and water to work effectively [1]. Most procedures performed by the dental team have the potential for creating contaminated aerosols and splatter. Aerosols are tiny particles or droplets which remain suspended in air [2]. These aerosols represent an infection hazard due to their gross contamination with microorganisms and blood. A fourfold increase of airborne bacteria has been observed in areas where aerosol producing equipment was used. Aerosols can float in air for considerable time before being inhaled by dental staff and other patients [3]. There is some evidence for greater prevalence of respiratory diseases [4] and elevated antibody levels to *Legionella pneumophila* in dental workers. Oral bacteria have been detected two meters from the procedure field, indicating the existence of aerosolized oral bacteria in dental practice [5]. Numerous airborne particles derived from blood, saliva, tooth debris, dental plaque, calculus and restorative material are produced by an ultrasonic scaler when used in combination with water spray [6,7]. Bacterial diseases, viral infections and other skin infections are cause by the microorganisms which were isolated in dental aerosols. Incresed use of ultrasonic scalers and turbine hand pieces is responsible for decreased air quality in the dental office due to increased aerosol contamination [8]. Reducing the aerosol production, microbial load in the water tubing, container will reduce the chances of cross-contamination in the dental surgery [9].

It has been shown that pre-procedural use of an antiseptic mouth rinse significantly reduced the level of viable bacteria in the back spray derived from an air turbine hand-piece. The purpose of the present study was to determine the microbial atmospheric contamination during initial periodontal treatment using a modern and at present widely used piezoelectric scaler and to evaluate the efficacy of two commercially available mouth rinses (0.2% Chlorhexidine mouth rinse and Listerine) in reducing bacterial contamination when used as a pre-procedural rinse, with and without high volume evacuation (Aerosol reduction device).

OBJECTIVE

The purpose of the present study was to determine the microbial atmospheric contamination during initial periodontal treatment using a modern and at present widely used piezoelectric scaler and to evaluate the efficacy of two commercially available mouth rinses (0.2% Chlorhexidine mouth rinse and Listerine) in reducing bacterial contamination when used as a pre-procedural rinse, with and without high volume evacuation (Aerosol reduction device).

MATERIALS AND METHODS

In this study, samples were selected from Outpatient Department of Periodontology, Sri Siddhartha Dental College and Hospital, Tumkur, India. Total 60 Patients (age ranging between 25 to 54 years including both the genders) were taken for the study that were divided equally into 3 groups i.e. 20 subjects in each group. Total duration of study was 4 months. There was no gender criteria in our study. Only systemically healthy patients were included.It is an in vivo randomized microbiological study. A written inform consent was taken from all patients. Their inclusion was purely voluntary.The mean age of the patient was 39 years.

PATIENT SELECTION CRITERIA

Inclusion Criteria

- Patients with mild to moderate gingivitis were selected. Those patients were selected who were systemically healthy. Patients having a minimum number of permanent 20 teeth.

Exclusion Criteria

- Patients suffering from any known systemic diseases or any history of blood dyscrasias, renal or hepatic disease, immunosuppression.

- Patients who had received any antibiotic therapy in the last 6 months.
- Patients who had received any chemotherapeutic mouth rinses and oral irrigation during the past 6 months.
- Presence of cardiac pacemaker or any respiratory infection.
- Patients who had received any surgical or non-surgical therapy, 6 months prior to the start of the study.
- Patients who were smokers.
- Patients who were pregnant or lactating.

The study included 60 patients who were divided into 3 groups randomly. A split mouth method was used–

Group A – 20 patients who rinsed with Water (Control group)

Group B – 20 patients who rinsed with 0.2% Chlorhexidine mouth rinse

Group C – 20 patients who rinse with Listerine mouth rinse

Before the start of study patient was given oral hygiene instructions and demonstrated correct brushing technique on a dentoform model and explained the significance of daily biofilm disruption and removal. Patient was advised not make use of any mouth wash or any other chemical plaque control aid atleast 30 days prior to study. Patient was explained about the local factors causing gingivitis and the objective of doing ultrasonic scaling was explained to remove stains and to disrupt the bacterial matrix. The objective was to create an environment in which the gingival tissues can heal and be maintained in health by the patient. The patient was also explained about high concentration of microorganisms and the particles released during the use of ultrasonic scaling and use of blood agar plates was done to evaluate the aerosol production .A written consent form was signed by each individual who participated in the study after procedure was explained. A Pilot study was done before start of study in the same operatory with and without use of high volume suction apparatus in all three groups. Ethical clearance was taken before start of study from the ethical committee meet held on 22ndNovember 2011

A Closed operatory measuring, 13feet x10 feet x15feet, with the facility to fumigate the room was chosen for all treatment procedures. Only one patient was treated per day and the treatment ended the same day. The patient was the first patient of the day. So the treatment time was 1 hour between 9-10 A.M. Next patient was given appointment for next day. The study was conducted from 1.3.12-29.6.12. Fifteen patients were treated per month during the first fifteen working days. Before each appointment, at the start of study all operatory surfaces were cleaned and disinfected with Ethyl alcohol (70%). Between each treatment and at start of treatment, ultrasonic scaler units were flushed with water for 2 minutes .Use of 0.5% Sodium Hypochlorite (Clorox, A.B enterprises) was done for flushing the tubing of dental chair waterline and the same solution was allowed to stay in tubing for 10 minutes followed by water flushing to remove the unwanted biofilm from the tubing surfaces. This procedure was done at the end of each treatment.

Then operatory was fumigated each day at the end of all procedures to allow the room to be free of aerosol before department was closed and left unused for 15 hours .Dental unit used distilled water in self contained system, for the study. The operator wore autoclaved gloves, face masks, autoclaved surgical drape, head caps and use of protective eye-glasses was also done throughout the procedure. All the activities like conversation, sneezing, coughing were strictly prohibited and the subject were instructed to refrain from actions which generated aerosols. The patient also wore new drape wiped with cotton having 70%ethyl alcohol and use of autoclaved green cloth was done in all the experimental area.

Three standardized locations included were chest of patient, on a tray of dental chair, 6 inches away from subjects mouth. Two plates were used on either side of the chair where the patient was seated [9]. The trays were adjusted so that the base of the support board was at 50° angle to get maximum aerosol during scaling procedure. Blood agar plates were used to collect the aerosol sample during the experimental procedure [Table/Fig-1a,b]. It was chosen because it is a general purpose, non selective and enriched medium that promotes the growth of microorganisms such as those sampled from air, 5% sheep blood agar plates were used. Each subject was treated by the same operator, ultrasonic insert and high volume suction apparatus. The power setting and the water pressure was set on medium during each treatment.

[Table/Fig-1a]: Blood agar base **[Table/Fig-1b]:** Freshly prepared blood agar plates

Group A: Control Group (n=20)

In the first group of patient's use of water as a pre-procedural rinse was done for two consecutive 30 sec periods. After first rinse for 30 sec patient expectorated and again immediately rinsed again for 30 sec. A split mouth method was followed. On one quadrant of patient mouth ultrasonic scaling was done, after the patient had rinsed with water and then blood agar plates were placed in the three standardized locations as described above, without the use of high volume suction tube. To prevent air turbulence that could cause the dispersion of aerosols particles from blood agar plate, both investigator and subject remained stationary for ten minutes after the scaling procedure. This was done for the gravitometric settling of airborne bacteria. Although splatter aerosols settle very quickly on surfaces the droplet nuclei (<50 µm) remain suspended in air for longer time and can infect person by direct inhalation and penetration deep in lungs [10]. The aerosol peaks decreased to the background levels within 10 and 30 min caused by rapid deposition of particles after aerosol generation at patient head height. The plates were covered and sent for microbiological identification test in an airtight vacuum box.

In the second step, after 30 min again ultrasonic scaling was done on other quadrant of same patient after he had rinsed with water and then the blood agar plates were kept in the three standardized locations, but this time use of high volume suction tube as a aerosol reduction device was done [Table/Fig-2a]. The high volume suction apparatus tube was kept as close as possible to the tip of ultrasonic, to prevent aerosol formation and the same protocol was followed and blood agar plates were covered and immediately sent for microbiological identification test in a air-tight vacuum box. Use of 0.5% Sodium Hypochloirite (Clorox, A.B enterprises) was done for flushing the tubing of dental chair waterline and the same solution was allowed to stay in tubing for 10 minutes followed by water flushing to remove the unwanted biofilm from the tubing surfaces. This procedure was done at the end of each treatment.

Group B: Experimental Group 1 (n=20)

In the second group of patients 0.2% Chlorhexidine mouth rinse instead of water as a pre-procedural mouth rinse was used. The patient rinsed with 15 ml of 0.2% Chlorhexidine (1:1dilution) under

the supervision for 30 second periods expectorated immediately, rinsed again for 30 seconds [11]. Chlorhexidine was used in 1:1 dilution with water and same protocol was followed as in Group A patients.

Group C: Experimental Group 2 (n=20)

In the third group of patients Listerine mouth rinse was used.The patient rinsed with 20ml of Listerine (1:1dilution) [11] for 30 seconds expectorated immediately , rinsed again for 30 seconds and same study protocol was followed as in Group A patients.

MICROBIOLOGICAL EVALUATION

The samples were covered and transported immediately for:

- Identification of microorganisms as per standard procedures (Gram stain, Biochemical Test, Species Identification).
- Counting the number of colonies formed on blood agar plates using colony counter unit [Table/Fig-2b].

[Table/Fig-2a]: A High volume suction apparatus **[Table/Fig-2b]:** Colony counter

The blood agar plates were kept in a bacteriological incubator and were incubated aerobically at 37°C for 24 h [Table/Fig-3a]. Blood agar plates showing growth were procured further by standard microbiological procedure like Gram Stain and Biochemical reaction [Table/Fig-3b] and colonies were counted using colony counter unit. Use of Specific microbiological solutions like Crystal Violet, Gram's Iodine, Acetone, Carbon Fuschin was done as a specialized media for identification of specific bacteria. In other studies only main focus was on the colony formed and the concentration of microorganisms at various sites. No attempt was made to culture or identify the bacteria.

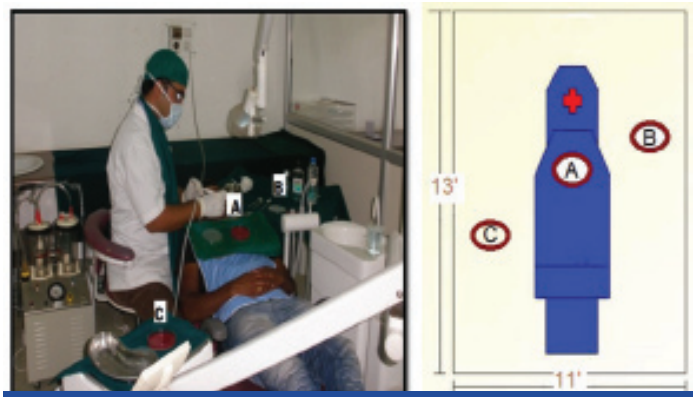
[Table/Fig-3a]: Biological incubator **[Table/Fig-3b]:** Biochemical Medias

CLINICAL PARAMETERS

a. Gingival Index (Loe H And Silliness J 1963) [12].

b. Papillary Bleeding Index (Muhlemann HR, 1977) [13].

c. Simplified Oral Hygiene Index(OHI-S) (John C Greene And Jack R Vermillion 1964) [14].



[Table/Fig-4]: Positioning of blood agar plates with high volume suction

METHOD OF STASTICAL ANALYSIS

a. ANOVA

b. Student s t-Test For The Comparison Of Two Means

RESULTS

Mean Values of Gingival Bleeding Index

Mean Gingival bleeding index for Group A, Group B, Group C was found to 1.385 ± 0.27, 1.40 ± 0.416, 1.40 ± 0.43 respectively. The mean difference between 3 groups was 0.011 and the p-value>0.05, which was statistically not-significant [Table/Fig-5].

Groups	Gingival Index	Papillary Bleeding Index	Oral Hygiene Index Simplified (OHI-S)
Group A (n=20)	1.385 ± 0.27	1.384 ± 0.27	1.425 ± 0.25
Group B (n=20)	1.40 ± 0.416	1.399 ± 0.415	1.41 ± 0.414
GROUP C (n=20)	1.40 ± 0.43	1.403 ± 0.433	1.42 ± 0.42
Anova f-value	0.011	0.013	0.002
p-value	0.988, NS	0.986, NS	0.99, NS

[Table/Fig-5]: Mean of Gingival Index, Papillary Bleeding Index, Oral Hygiene Index Simplified (OHI-S) For Group A , Group B, Group C.
One way ANOVA P>0.05 not significant

Mean Values of Papillary Bleeding Index

Mean Papillary bleeding index for Group A, Group B, Group C was found to be 1.384± 0.27, 1.399 ±0.415, 1.403±0.433 respectively. The mean difference between 3 groups was 0.013 and the p-value>0.05, which was statistically not- significant [Table/Fig-5].

Mean Values of Oral Hygiene Index Simplified (OHI-S)

Mean Oral hygiene index simplified (OHI-S) for Group A, Group B, Group C was found to be 1.425± 0.25, 1.41± 0.414, and 1.42±0.42 respectively. The mean difference between 3 groups was 0.002 and p-value>0.05 which was statistically not- significant [Table/Fig-5].

On Inter-group comparison the difference in microbial growth between Group A and Group B was found to be statistically highly significant (p<0.01), between Group A and Group C the difference was statistically not-significant (p>0.05) and between Group B and Group C the difference was statistically significant (p<0.05) [Table/Fig-6].

DISCUSSION

Aerosols containing microbes from the oral cavity are created when modern high-speed rotating instruments are used. How far these aerosols spread and what level of contamination they cause in the dental surgery has become a matter of growing concern [15-17].

As stated by Bentley et al., [16], there are several factors which influence aerosol distribution and include:

Group	Without suction		With suction		p-value
	Growth	No growth	Growth	No growth	
GROUP A (WATER)	16 (80%)	4 (20%)	8 (40%)	12 (60%)	0.025(S)
GROUP B CHLORHEXIDINE (0.2% w/v)	4(20%)	16(80%)	0	20(100%)	0.04(S)
GROUP C (LISTERINE)	12(60%)	8(40%)	4 (20%)	16(80%)	0.02 (S)

Comparison of microbial growth between different groups		
Groups	**p-value**	**Interpretation**
GroupA (Water)- GroupB(Chlorhexidine0.2%w/v)	0.001	HS
GroupA(Water)-GroupC(Listerine)	0.232	NS
GroupB(Chlorhexidine0.2%w/v)-GroupC (Listerine)	0.025	S

[Table/Fig-6]: Distribution of Microbial Growth without and with use of suction NS: Not significant S: Significant (p-value <0.05) HS: Highly significant (p-value <0.01)

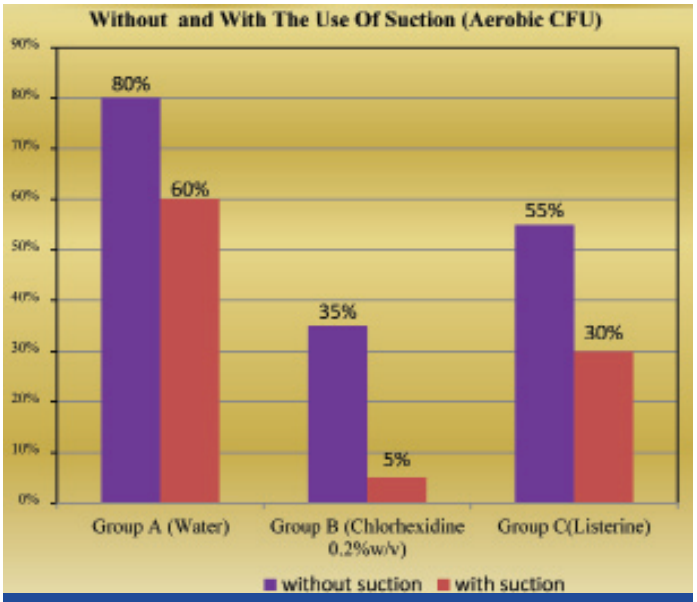
WATER	
SAMPLE 1	Mixed group of microbes predominantly *Streptococci*
SAMPLE 2	Aerobic spore forming bacilli
SAMPLE 3	Mixed group of microbes predominantly *Streptococci*
SAMPLE 4	Aerobic spore forming bacilli
SAMPLE 5	Aerobic spore forming bacilli
SAMPLE 6	Mixed group of microbes predominantly *Streptococci*
SAMPLE 7	Aerobic spore forming bacilli
SAMPLE 8	Mixed group of microbes predominantly *Streptococci*
SAMPLE 9	Mixed group of microbes predominantly *Streptococci*
SAMPLE 10	*Staphylococci* species
SAMPLE 11	Aerobic spore forming bacilli
SAMPLE 12	Mixed group of microbes predominantly *Streptococci*
SAMPLE 13	Mixed group of microbes predominantly *Streptococci*
SAMPLE 14	*Staphylococci* species
SAMPLE 15	*Pseudomonas* species
SAMPLE 16	Mixed group of microbes predominantly *Streptococci*
CHLORHEXIDINE (0.2%w/v)	
SAMPLE 1	Mixed group of microbes predominantly *Streptococci*
SAMPLE 2	Mixed group of microbes predominantly *Streptococci*
SAMPLE 3	Aerobic spore forming bacilli
SAMPLE 4	*Staphylococci* species
LISTERINE	
SAMPLE 1	Mixed group of microbes predominantly *Streptococci*
SAMPLE 2	Aerobic spore forming bacilli
SAMPLE 3	Mixed group of microbes predominantly *Streptococci*
SAMPLE 4	*Staphylococci* species
SAMPLE 5	*Staphylococci* species
SAMPLE 6	Mixed group of microbes predominantly *Streptococci*
SAMPLE 7	Mixed group of microbes predominantly *Streptococci*
SAMPLE 8	*Pseudomonas* species
SAMPLE 9	Mixed group of microbes predominantly *Streptococci*
SAMPLE 10	Aerobic spore forming bacilli
SAMPLE 11	Mixed group of microbes predominantly *Streptococci*
SAMPLE 12	Aerobic spore forming bacilli

[Table/Fig-7]: Microbial Analysis (Without Suction)

- Type of procedure and whether high volume evacuation was used
- The position of the tooth in the mouth, which affects the position of the operator relative to the subject in the dental chair
- Levels of microorganisms in the subject's mouth and other factors.

Harrel and Molinari [18] recommend three levels of defense in the reduction of aerosols:

1. Personal Protective Barriers



[Table/Fig-8]: Mean percentage of colony forming units using pre-procedural mouth rinses without and with the use of suction (Aerobic CFU)

WATER	
SAMPLE 1	Mixed group of microbes predominantly *Streptococci*
SAMPLE 2	Aerobic spore forming bacilli
SAMPLE 3	Mixed group of microbes predominantly *Streptococci*
SAMPLE 4	Mixed group of microbes predominantly *Streptococci*
SAMPLE 5	*Staphylococci* species
SAMPLE 6	Aerobic spore forming bacilli
SAMPLE 7	*Pseudomonas* species
SAMPLE 8	Mixed group of microbes predominantly *Streptococci*
LISTERINE	
SAMPLE 1	Mixed group of microbes predominantly *Streptococci*
SAMPLE 2	Aerobic spore forming bacilli
SAMPLE 3	Mixed group of microbes predominantly *Streptococci*
SAMPLE 4	*Staphylococci* species

[Table/Fig-9]: Microbial Analysis (With Suction)

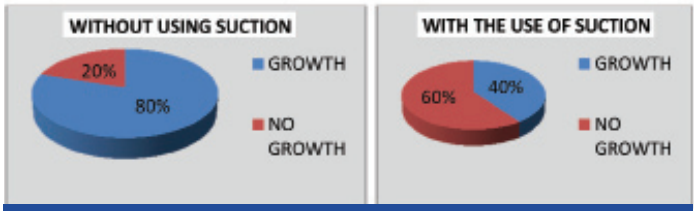
GROUPS	Without Suction	With Suction	% Reduction	p-value
GROUP A (WATER)	80%	60%	20%	0.232
GROUP B (CHLORHEXIDINE 0.2%w/v)	35%	05%	30%	0.04*
GROUP C (LISTERINE)	55%	30%	25%	0.1661

Comparison of Colony Forming Units Between Different Groups		
Groups	**p-value**	**Interpretation**
Group A (Water) - Group B (Chlorhexidine0.2%w/v)	0.012	S
Group A (Water) - Group C (Listerine)	0.1438	NS
Group B (Chlorhexidine 0.2%w/v) - Group C (Listerine)	0.2709	NS

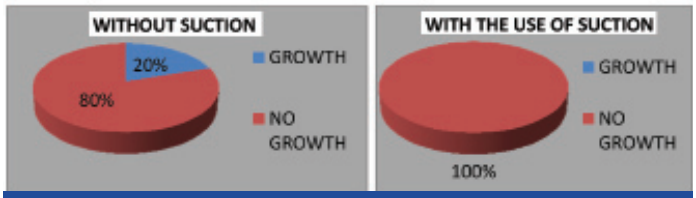
[Table/Fig-10]: Mean Percentage of Colony Forming Units Without And With the Use of Suction (Aerobic CFU).
*p<0.05: significant, p>0.05: not-significant
NS: Not significant S: Significant

2. Routine use of Pre-procedural rinses
3. Use of high evacuation device.

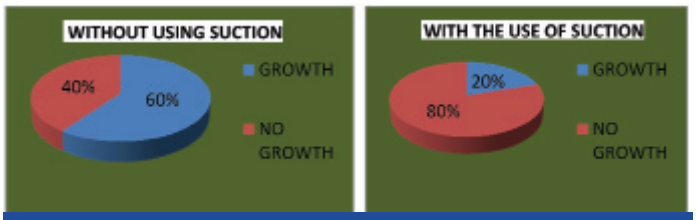
In accordance with our study Harrel et al., has shown high volume evacuators are more effective in minimizing the danger of contaminated aerosols [Table/Fig-7-9]. Out of all the three groups studied 0.2% Chlohexidine group was the most effective as a pre-



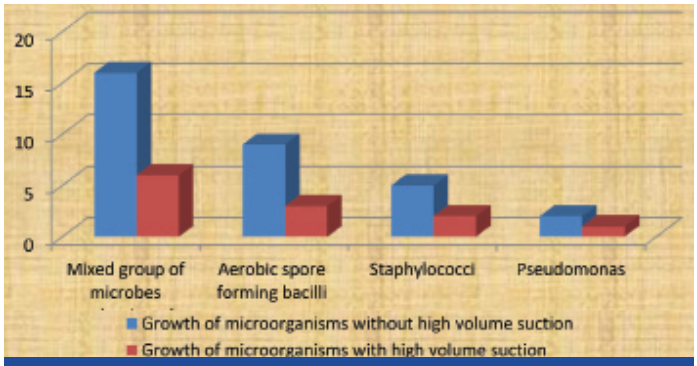
[Table/Fig-11]: Percentage of Microbial Growth Using Water as a Pre-Procedural Rinse



[Table/Fig-12]: Percentage of Microbial Growth Using Chlorhexidine as a Pre-Procedural Rinse

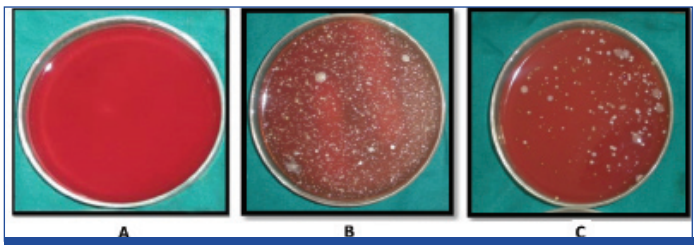


[Table/Fig-13]: Showing Percentage of Microbial Growth Using Listerine as a Pre-Procedural Rinse



[Table/Fig-14]: Comparative growth of microorganisms

procedural rinse followed by Listerine and then Water [Table/Fig-10]. Chlorhexidine showed the maximum amount of reduction in microbial growth and demonstrated maximum reduction of aerobic colonies [Table/Fig-11-13]. Significant reduction was seen with the use of high volume suction apparatus [Table/Fig-14]. In all the three groups use of high volume suction decreased the microbial growth [Table/Fig-15]. Chlorhexidine is a bisbiguanide molecule that binds strongly to the hydroxyapatite, the organic pellicle of the tooth, oral mucosa, salivary proteins and bacteria. Due to this binding Chlorhexidine containing mouth rinses exhibit high substantivity with 30% of drug released after rinsing and slow release for long time. A combination of essential oils (eucalyptol, thymol, methyl salicylate and menthol) in an alcohol base reduce bacterial enzymes and reduce pathogencity of plaque. The results of our study was in accordance with the study done by Suresh S et al., where authors have compared the efficacy of pre-procedural rinsing with Chlorhexidine mouth rinse and essential oil containing mouth rinse. Results showed that that when Chlorhexidine was used as a pre-rinse before ultrasonic scaling, sampling developed consistently fewer colony forming units in the two standard locations than when essential oil mouth rinse was used as a pre-rinse [11]. Studies have shown that that ultrasonic scaling in conjunction with various plaque control agents used as a pre-procedural rinse have been found to



[Table/Fig-15]: A- Freshly prepared blood-agar plates
B – Blood agar plates showing growth without the use of high volume suction
C- Blood agar plates showing growth with the use of high volume suction

be more effective in reducing bacteria loads when compared with distilled water [19] or saline. In agreement to our study, another study [20] compared chlorhexidine gluconate, essential oils, and water; the results indicated higher reduction in the bacterial counts achieved with the chlorhexidine gluconate solution.

Other studies conducted also show the advantages of Chlorhexidine as a pre-procedural rinse:

Logothetis et al., [21] showed that Chlorhexidine gluconate pretreatment rinse was effective in reducing bacterial aerosol contamination with the use of air polisher. Veksler [22] concluded that two consecutive pre-procedural rinsing with 15 ml of 0.12% Chlorhexidine for 30 seconds had up to 97% reduction in salivary bacterial load and have sustained effect on the salivary bacterial load. Muir [23] and others found that a 2 minutes pre-rinse with Chlorhexidine significantly reduced aerosols produced by ultrasonic scalers. On the other hand Toroglu et al., [24] reported that the level of viable microbial bacteria cannot be reduced significantly by pre-procedural rinse of 15 ml of 0.2% CHX for 1 minute. This study demonstrates that a sufficient amount of aerosol and splatter from the patient will be ejected far enough to come into contact with dental personnel performing treatment. Though the results strongly support for mouth rinsing before dental procedure, yet only few dentists use mouth rinsing on a routine basis, either to minimize endogenous spread of infection from patient to dentist or the dental auxillaries. The results of the present study must be used for increasing awareness and quantifying the risk of staff and patient exposure to aerosolized microbial pathogens in general dental office, which must be controlled by efficient preventive measures.

In India there is little awareness regarding the quality of indoor air, mould contamination, potential source for transmission of nosocomial infections in health care facilities [25]. Insufficient awareness of health risk, working habits and economic factors are the reason why dentist do not apply the recommended methods of protection against bioaerosols and splatter [26].

CONCLUSION

Within the limitations of this study it was found that the use of pre-procedural mouth rinses along with the use of high volume suction apparatus significantly reduced the aerosol contamination and hence chances of cross infection in the dental units.Although the results of our study were satisfactory but in future use of a ventilation and air conditioning system in good working order, including air filters in air conditioning devices and purification of air-borne microbial pollutants (air- conditioning, disinfection with physical and chemical means) should be used to reduce contamination of dental surgery environment and prevent circulation of microbiologically contaminated air. Also immunization of dental team against biological hazards in their workplace through specific (vaccines) or non-specific (e.g. gamma globulin) immunization is mandatory. Fungi, virus and anaerobic bacteria were not cultured in this study.Main focus was identification/culturing of aerobic bacteria. Hence, use of pre-procedural mouth rinses and suction should be made mandatory before any procedure is performed so that dentist, assistant and patients are benefitted following the principle “Prevention is better than Cure.”

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