Original Research Article

Qualitative analysis of bacterial aerosols generated during ultrasonic dental scaling.

ABSTRACT

Aims: To compare the efficacy of two mouth rinses (0.2 % Chlorhexidine and 5 % green tea mouth rinse) in reducing the bacterial load (CFUs) in aerosol samples collected during ultrasonic dental scaling and to do the qualitative analysis of bacterial isolates.

Study design: Quasi experimental study

Place and Duration of Study: Department of Periodontics (Ziauddin college of Dentistry), Ziauddin University, Karachi, between January 2019 to August 2019.

Methodology: This study comprised of seventy patients (43 males and 27 females) within the age group of 18 to 65 years having gingival and plaque score between 1 and 3 and mean probing depth less than 5 mm. All study subjects were divided into 2 equal groups (group 1 and group 2). A split mouth design was used for ultrasonic scaling (oral prophylaxis). Control side was scaled without pre rinsing while the test side was scaled after pre procedural mouth rinsing. Group 1 study subjects were instructed to rinse with 10 ml 0.2 % Chlorhexidine mouth rinse for one minute and Group 2 rinsed with 10 ml 5 % green tea mouth rinse for a minute. Fresh blood agar plates were used for air sampling, which were then transported to Microbiology laboratory(JPMC) for aerobic culturing and microbiological examination.

Results: Greater percentage of Gram positive bacteria were found in aerosol samples generated during ultrasonic scaling. Gram positive cocci (Staph epidermidis and Micrococci species) were in abundance and very few gram negative bacteria were detected.
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Conclusion: Dental health care providers and patients can easily acquire infections because of contaminated aerosols and splatters and so all infection control measures should be taken to minimize these risks. Pre procedural mouth rinsing with effective mouthwashes should be done before dental procedures as it is easiest and much economical way of reducing cross infection.

Keywords: Aerosols, Cross infection, Infection control, Streptococci, Staphylococci, Gram positive bacteria, Ultrasonic scaler, Aerobic culturing.

1. INTRODUCTION

Dentistry is surrounded with many health hazards that can be serious threat for the lives of practitioners and patients (1). Cross infection is one of the serious occupational hazard in

medical and dental profession and it is defined as the transmission of infectious agents between patients and staff within a clinical environment (2).

Because of the nature of their profession, Dental practitioners are more prone to acquire different infections. Performing procedures in close proximity to patient`s mouth, using sharp instruments excessively, performing dental procedures capable of producing light and heavy particles are some of the common reasons of spreading cross infection among dental practitioners. (3).

As documented in studies, body fluid transmission and airborne microorganisms are major vectors of cross contamination in clinical settings. (4) .

Exposure of non-intact skin and mucosal lesion to infectious material (blood or other body fluid) can lead to blood borne contamination. Needle stick or other sharp instrument injuries are found to be the commonest cause of blood borne contamination in health care profession. (5)

Various microorganisms found to cause infections in the dental personnel include Staphylococci and Streptococci groups, Hepatitis C, Hepatitis B, HSV type 1, Mycobacterium tuberculosis, HIV, Influenza, mumps etc. (6).

For the entire dental community, contamination through airborne route has remained a major concern for centuries and aerosols and splatters have remained the center of attention in the discussion of airborne contamination in dental settings. (7)

The terms “aerosol” and “splatter” were used by Micik and colleagues as a result of their pioneering work on Aerobiology. (8).

Aerosols are solid and liquid particles with particle size 50 µm or less and suspended in air by machines, instruments or humans. (9). The generation of aerosols by humans occur as a result of breathing, talking, sneezing or coughing. (10).

Different dental procedures performed by dentists are capable of producing contaminated aerosols and splatters in the dental operatory and ultimately increase cross contamination. (11)

During dental procedures, Aerosol is created when high-powered devices need compressed air and water to work effectively (12). Aerosol may comprise of saliva, blood, calculus, tooth particles or any other dental material (13)

Most pathogenic aerosols are considered to be those having particle size less than 50 µm (9). Studies have reported that these aerosols can contaminate surfaces in range of one meter (3 ft.). Respiratory passages and lungs are easily penetrated by small aerosol particles carrying the greatest pathogenic potential (14)

Studies reported longer duration of presence of aerosols in clinical environment with long time survival of bacteria and viruses in these aerosols for as long as six days. (11)

Ultrasonic scalers, dental hand pieces and air polishers are reported to be the greatest producers of aerosols and splatters in the dental operatory. (15)

With the advancement of dental practice, infection control program has also become an integral part because of widely spread infections which are serious threat to human lives. (5)

For infection control and occupational health, bio aerosols are an important consideration. (16)

Different materials and procedures are recommended for reducing bio aerosol contamination by the center of disease control and prevention (CDC) and American Dental association(ADA) such as use of personal protective equipment, dental staff immunization, surface decontamination, equipment sterilization and dental unit water line treatment. (17)

Pre procedural rinsing with effective mouthwashes (mainly Chlorhexidine in varying concentrations) and high volume evacuation are also recommended for reducing bio aerosol contamination (18)

Studies have shown significant reduction in bacterial count of aerosols and splatters as a result of preprocedural mouth rinsing with effective mouthwashes. (19)

Broad antibacterial spectrum and high substantivity of Chlorhexidine has made it the “Gold standard” among many other mouth rinses. (20)

Significant reduction in bacterial count has been seen when 0.2% Chlorhexidine gluconate was used preprocedurally (21).

Some side effects are associated with long term use of CHX including altered taste sensation, teeth staining, soreness of oral mucosa and tongue. It also tends to stain composite and glass ionomer restorations. (22) In order to reduce the adverse effects of the chemical products, various researches are underway on different herbal products for improving patient’s compliance, minimizing toxic effects and making them more cost effective. Studies are reported on successful treatment of various oral diseases using different herbs including Triphala, Green tea, Neem, Aloe Vera etc. (23)

Hence this study had the aims of comparing efficacy of two mouthwashes (0.2 % Chlorhexidine and 5 % green tea) in reducing Colony forming units(CFUs) on blood agar plates when used as pre procedural rinse before ultrasonic scaling and to do the qualitative analysis of bacterial species grown on the culture plates.

2. METHODOLOGY

The study design was quasi experimental and conducted in the department of Periodontology, Ziauddin college of dentistry, Ziauddin university, Karachi from January 2019 to August 2019. The study protocol was approved by the three research committees of university (Research advisory committee, Ethical review committee and Board of advanced sciences and research)

Non inferiority sample size calculator was used for sample size calculation and calculated sample size was 70. Patients were recruited in study through non probability consecutive sampling and were then divided into two equal groups.

The selected patients were initially screened for their plaque index (silness and loe) and gingival index (loe and silness) and 70 subjects from both sexes within the age group of 18 to 65 years were chosen.

Inclusion Criteria

- Minimum of 20 permanent functional teeth
- Less than 5 mm mean probing depth.
- Patients with plaque index score and gingival index score between 1 -3.

Exclusion Criteria

- History of any systemic disease or respiratory problem.
- Presence of cardiac pacemaker.
- Pregnant and lactating women
- Immunocompromised subjects
- Patients who are taking drugs or need prophylactic antibiotics.
- History of periodontal treatment in previous six months' consumption of tobacco in any form.

70 subjects who met the inclusion criteria, were randomly assigned into two groups, group 1 and group 2 (35 subjects in each group)

Criteria for Group Division

- **Group 1**: Thirty-five patients who rinsed with 10 ml 0.2% Chlorhexidine gluconate for a minute prior to ultrasonic dental scaling.
- **Group 2:** Thirty-five patients who rinsed with 10 ml 5% green tea mouth rinse for one minute before ultrasonic scaling.

  This study used a split-mouth deign for ultrasonic scaling of study participants. One side (maxillary and mandibular) of the subject's mouth was scaled using

piezoelectric ultrasonic scaler without preprocedural rinsing (control side) following which the other side (test side) was scaled using the same ultrasonic scaler with preprocedural rinsing. (24)

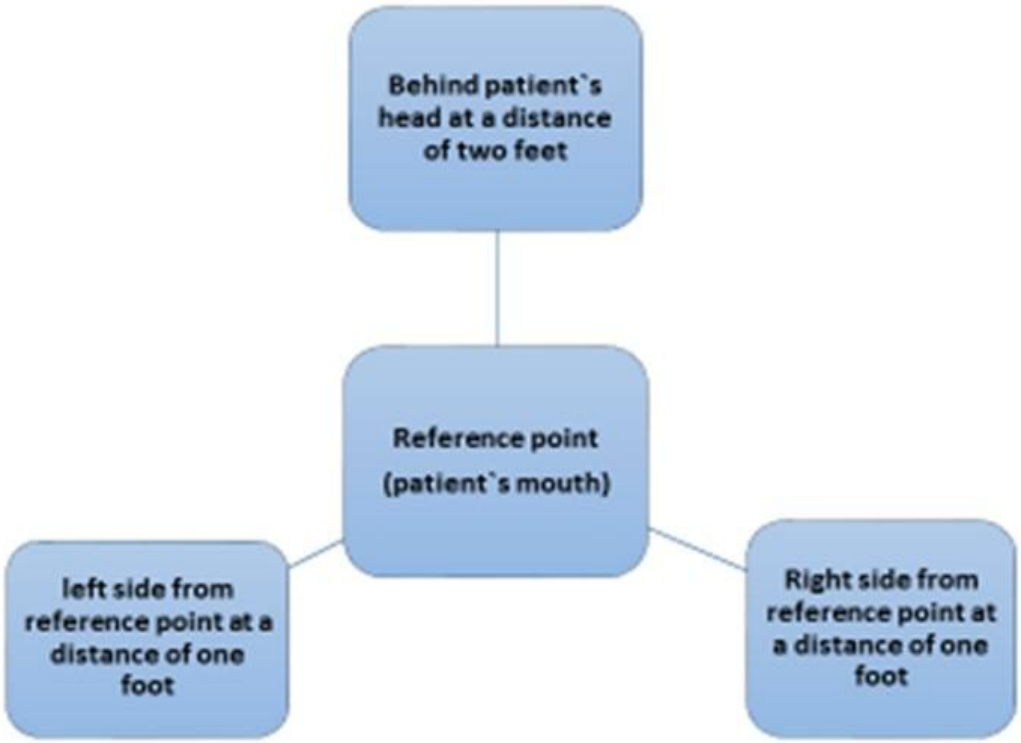
Blood agar plates were used as culture media for gravimetric settling of airborne bacteria as it is a general purpose and non-selective media for bacterial growth.

CULTURE PLATE LOCATIONS

Reference point: Mouth of the patient.

- left side from reference point at a distance of one foot
- right side from reference point at a distance of one foot
- behind the patient's head at a distance of 2 feet

FIGURE 1: BLOOD AGAR PLATE LOCATIONS



Clinical Protocol

Study subjects were asked to give written informed consent.

Ultraviolet radiation was used for sterilizing the dental operatory. All preventive measures were taken by operator and the assistant. For minimizing unnecessary aerosol contamination, only one subject was treated in a day and all study subjects were treated by same dentist. Before each appointment, all the operatory surfaces were cleaned and disinfected using ethyl alcohol 70%. (25). 0.5 % sodium hypochlorite was used for flushing of dental unit water lines.

Sterile ultrasonic inserts were used for ultrasonic scaling. Uniformity was ensured regarding power settings and water pressure on the ultrasonic unit for all study participants. (19)

Air sampling was done through fresh and uncovered blood agar plates on fixed positions from reference point.

Commercial Preparation of 0.2 % Chlorhexidine

In this study, mouth wash corsodyl (0.2 % Chlorhexidine) was used.

Preparation of 5 % Green Tea Mouth Wash

The extract of green tea was prepared in the Pharmacology laboratory of Sir Syed college of medical sciences (Karachi) with the following protocol.

Green tea leaves were powdered using electrical mortar and 100 gram of powder was soaked in ethanol (500ml) for two days. Filtration of this solution was done after two days and sediment was removed. Filtered solution was kept in hot air oven for four days and then green tea extract powder was obtained (21). 5 gm of extract powder was then mixed with 100 ml distilled water for preparing 5 % green tea mouth rinse and were then poured in bottles.

ORAL PROPHYLAXIS OF STUDY SUBJECTS

Group 1:

Group 1 comprised of 35 patients. Ultrasonic scaling was done on one quadrant (control side) for period of 30 minutes. Fresh blood agar plates were kept exposed during this duration for air sampling and were then taken off.

After 10 minutes, fresh agar plates were kept on the same positions from the reference point as shown in Figure 1. 10 ml of 0.2% Chlorhexidine was given to each patient for pre procedural rinsing for one minute. After rinsing, Ultrasonic scaling was performed on the other side (test side) for 30 minutes. Blood agar plates were then taken off after aerosols sampling. (24)

Group 2

Group 2 also consists of 35 patients. Oral prophylaxis was done similarly on one quadrant (control side) for 30 minutes and blood agar plates were kept exposed for

air sampling. After completion of ultrasonic scaling on control side, blood agar plates were taken off.

Similar protocol was followed as in group 1 for air sampling from the test side and patients were asked to rinse with 5 % green tea mouth rinse pre procedurally. (24)

MICROBIOLOGICAL EXAMINATION:

Blood agar plates were then transported to the Microbiology department of BMSI (JPMC) Karachi for Aerobic culturing. The blood agar plates were incubated at 37ºC for 48 hours after which the plates were observed for microbial growth.

Bacterial Species identification:

Bacterial aerosols were also analyzed qualitatively. Morphological analysis was performed for identifying isolated bacterial species. Gram stained preparations and different biochemical tests were applied for identification of bacterial isolates.

Data Analysis

Data collected was statistically analyzed using Statistical Package for Social Sciences (SPSS) version 17. Mean and standard deviation were calculated for numerical variables while for categorical variables, frequency and percentages were calculated. A p value of less than 0.05 was considered as significant.

3.RESULTS

Gram positive bacteria were in greater concentration than the Gram negative bacteria. Among gram positive bacteria, the Gram Positive Cocci constituted around 78% of the sample. Remaining were the gram positive rods. Very few gram negative cocci were detected in the whole sample. Table 1

Table :1 **Qualitative analysis of bacteria in aerosols generated during Ultrasonic scaling**

Bacterial microflora during ultrasonic scaling	Bacterial contamination levels (% of total bacteria)
GRAM POSITIVE GRANULOMATA (84 %)	
Gram Positive Cocci	
Staphylococcus epidermidis	35
Other Staphylococcus species	10
Micrococcus spp.	26
Streptococcus spp.	7
Gram-Positive Rods	6
GRAM-NEGATIVE GRANULOMATA (16%)	
Gram-Negative Cocci (Aerobes)	16

DISCUSSION:

The present study was undertaken to evaluate the efficacy of two different mouth rinses in reducing bacterial load in aerosols samples generated during ultrasonic scaling and also to qualitatively analyze the bacterial species in aerosol samples.

Mirhoseini et al reported hospital air as potential route of transmission of infectious agents (air borne). Mycobacterium tuberculosis, Streptococcus pyogenes, Corynebacterium diphtheriae and Neisseria meningitides are the main pathogens transmitted through air borne route and cause hospital acquired infections. (2

Studies reported the frequent spread of microorganisms in closed spaces like dental surgeries where the procedures performed can easily contaminate the instruments, surfaces and objects in dental operatory and the operative field (27).

Aurangjeb et al reported that dental clinics are frequently exposed with aerosols because of the procedures performed in them which result in aerosol production. Such contaminated aerosols can be serious threat for workers and patient`s lives. (12).

Sethi et al reported in their study that the bacterial count estimated by Miller in 1976 in the aerosol generated during dental procedure was up to a million bacteria per cubic foot of the air. (28).

According to the study by Acharya et al, bio aerosols are produced from the operating site as a result of different dental procedures using mechanical instrumentation including ultrasonic scalers, hand pieces, air abrasion units, air polishing device etc. (29).

Rautemaa et al reported that much concern has been raised in past few decades regarding the extent of spread of these aerosols in dental offices and the level of contamination caused by them. (30)

Increased aerosol production in the dental offices lead to reduce air quality as reported by Sawhney et al. (31).

In our study, when analyzing the bacterial aerosols qualitatively, the highest percentage was of gram positive organisms (Staphylococcus epidermidis and Micrococcus species followed by gram positive rod shaped bacteria and very small amounts of gram negative bacteria were detected. In line with our study, the study by Al Maglouth et al reported that micro flora that dominated in the whole aerosol sample collected during ultrasonic scaling were Micrococcus species, Staph epidermidis and Diphtheroids. (4)

In line with our study, Kobza et al found significant increase in bacterial and fungal concentration in aerosols sample during dental procedures as compared to before procedure. Kobza et al reported the presence of highest percentage of gram positive organisms in air sample and the possible reason for their abundance is human skin and respiratory system as their potential sources. (13)

According to Ramesh et al, infection control is the core component of dental practice and different health agencies have recommended various universal precautions for every single patient. (32). Strict aseptic principles need to be incorporated in the clinical practice in order to reduce microbial cross contamination (33)

For minimizing bio aerosol contamination, American dental association (ADA) and the center of disease control and prevention (CDC) have recommended different materials and procedures such as use of personal protective barriers, decontamination of surfaces, immunization of dental staff, treatment of dental unit water lines and sterilization of instruments .Pre-procedural rinse (mainly Chlorhexidine (CHX) in varying concentrations should be used to reduce airborne contamination during dental procedures(17).

Use of expensive methods such as high-efficiency particulate air and ultraviolet chambers in the ventilation system have also been recommended (17)

Studies reported on significant reduction of bacterial load when pre-procedural rinsing with effective mouthwashes was done before ultrasonic scaling as compared to rinsing with normal saline or water. (11)

4. CONCLUSION

The aim of present study was to evaluate the efficacy of two mouth rinses in reducing CFUs (bacterial load) in aerosol samples and to do the qualitative analysis of the bacterial species cultivated on blood agar plates. The study comprised of seventy patients with plaque index and gingival index score ranging between 1 and 3. Study subjects were then divided into two equal groups (group 1 and group 2) of 35. Split mouth design was used for oral prophylaxis of study subjects. Group 1 rinsed with 0.2% Chlorhexidine gluconate preprocedurally for one minute while the second group was asked to rinse with 5% green tea mouth rinse for a minute before ultrasonic scaling.

It was concluded from the study that pre procedural mouth rinsing before ultrasonic scaling significantly reduces the CFU count in aerosols sample as compared to the non-rinsing side and 0.2% Chlorhexidine was found to be more effective than 5 % green tea mouth rinse in reducing bacterial load in aerosol samples. Qualitative analysis of bacteria in the aerosols sample revealed domination of gram positive cocci over other air microflora. Very few gram negative organisms were detected.

The present study reemphasized the use of pre procedural mouth rinses before dental procedures along with implementation of other infection control measures to minimize the risk of cross infections among all individuals present in dental operatory.

CONSENT (WHERE EVER APPLICABLE)

Written informed consent was taken from every patient.

ETHICAL APPROVAL

Ethical approval was obtained from ethics review committee of Ziauddin University.

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