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Qualitative Analysis of Bacterial Aerosols Generated during Ultrasonic Dental Scaling

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Authors' contributions

This work was carried out in collaboration among all authors. Author AA designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors MH and SAB managed the analyses of the study and the literature searches. Authors Fakhuruddin and YS performed the statistical analysis and also managed the literature searches. All authors read and approved the final manuscript.

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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 20 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

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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.

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].

Splatter is usually described as a mixture of air, water, and/or solid substances, such as carious tissues, dental fillings fragments, sandblasting powder, etc [11]. The size of water droplets in splatter range from 50 μm to several millimeters and can be easily seen by naked eye [12]. Due to increased mass and kinetic energy of splatter particles, they move ballistically and settle quickly on objects due to gravitation forces [11]. Because of their bigger size, they remain air borne very briefly and hence rarely enter the respiratory passages [13].

As documented in studies, the greatest infection causing potential is carried by aerosols as they can stay air borne for longer time and can easily enter the respiratory passages [14].

During dental treatment, airborne contamination commonly occurs through three sources: operative site, dental instrumentation and saliva and respiratory sources [15].

Contamination from dental instrumentation is the result of organisms on instruments and in dental unit water lines DUWLs [13].

Air borne particles are produced as a result of mechanical instrumentation during many dental procedures from the operative site [16]. Ultrasonic scalers, Dental hand pieces, air polishers and air abrasion units are the greatest aerosols producing devices [17]. Each of these instruments removes material from the operative site that becomes aerosolized by the action of the rotary instrument, ultrasonic vibrations or the combined action of water sprays and compressed air [18].

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 20 to 65 years were chosen.

2.1 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.

2.2 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.
- Adolescents.
- Patients with generalized gingival infections of viral, fungal and bacterial origin.

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

2.3 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 design 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.

2.4 Culture Plate Locations

2.4.1 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.

2.4.2 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.

2.4.3 Commercial preparation of 0.2% chlorhexidine

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

2.4.4 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 (500 ml) 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.

2.5 Oral Prophylaxis of Study Subjects

2.5.1 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 Fig. 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].

2.5.2 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].

2.6 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.

2.6.1 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.

2.7 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.

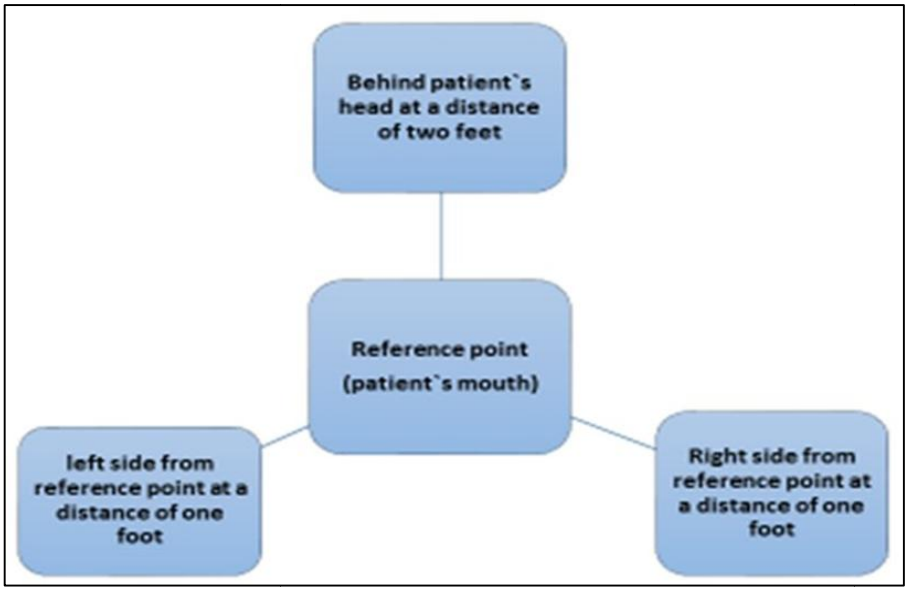

Fig. 1. Blood agar plate locations

3. RESULTS

Gram positive bacteria were in greater concentration than the Gram negative bacteria. Among gram positive bacteria, the Gram Positive Cocci constituted greater proportion of sample. Remaining were the gram positive rods. Very few gram negative bacteria were detected in the whole sample.

Table 1: Table 1a, 1b and 1c are showing the comparison of mean, standard deviation and p values among control sides (right, left and behind from the reference point respectively) between group 1 and group 2.

Table 2: Table 2a, 2b and 2c are showing the comparison of mean, standard deviation and p values among test sides (right, left and behind from the reference point respectively) between group 1 and group 2.

Table 3: Table 3(a, b and c) are showing comparison of Isolated species on control sides (right, left and behind respectively) between group 1 and group 2.

Table 1a. Comparison of mean, standard deviation and p values among control sides (Plate placed at right side from reference point) between group 1 and group 2

Variables (n=70)	Groups	N	Mean	St. deviation	p- value
Plate right control side Gram positive Bacteria	Group 1	35	25.09	7.555	0.60
	Group 2	35	24.26	5.517	
Plate right control side Gram Negative bacteria	Group 1	35	1.91	.742	0.001*
	Group 2	35	2.77	1.239	

Results are presented as mean ± SD and p-value
**Significant p-value*

Table 1b. Comparison of mean, standard deviation and p values among control sides (Plate placed at left side from reference point) between group 1 and group 2

Variables (n=70)	Groups	N	Mean	St. deviation	p- value
Plate left control side Gram Positive Bacteria	Group1	35	20.97	6.732	0.46
	Group 2	35	22.03	5.061	
Plate Left control side Gram Negative Bacteria	Group 1	35	2.66	1.110	0.002*
	Group 2	35	4.00	2.236	

Results are presented as mean ± SD and p-value
**Significant p-value*

Table 1c. Comparison of mean, standard deviation and p values among control sides (Plate placed behind) between group 1 and group 2

Variables (n=70)	Groups	N	Mean	St. deviation	p- value
Plate behind control side Gram Positive Bacteria	Group 1	35	21.31	4.794	0.97
	Group 2	35	21.29	4.436	
Plate behind control side Gram Negative Bacteria	Group 1	35	2.26	1.146	0.70
	Group 2	35	2.37	1.374	

Results are presented as mean ± SD and p-value
**Significant p-value*

Table 2a. Comparison of mean, standard deviation and p values among test sides (Plate placed at right side from reference point) between group 1 and group 2

Variables (n=70)	Groups	N	Mean	St. deviation	p- value
Plate right test side Gram Positive Bacteria	Group1	35	21.77	5.668	0.74
	Group 2	35	21.40	3.720	
Plate right test side Gram Negative Bacteria	Group 1	35	2.00	.907	0.35
	Group 2	35	2.23	1.114	

Results are presented as mean ± SD and p-value
**Significant p-value*

Table 2b. Comparison of mean, standard deviation and p values among test sides (Plate placed at left side from reference point) between group 1 and group 2

Variables (n=70)	Groups	N	Mean	St. deviation	p- value
Plate left test side Gram Positive Bacteria	Group 1	35	18.77	4.899	0.33
	Group 2	35	17.74	3.799	
Plate Left test side Gram Negative Bacteria	Group 1	35	2.23	1.374	0.07
	Group 2	35	2.86	1.498	

Results are presented as mean ± SD and p-value
**Significant p-value*

Table 2c. Comparison of mean, standard deviation and p values among test sides (Plate placed behind) between group 1 and group 2

Variables (n=70)	Groups	N	Mean	St. deviation	p- value
Plate behind test side Gram Positive Bacteria	Group 1	35	17.89	4.676	0.11
	Group 2	35	16.29	3.553	
Plate behind test side Gram Negative Bacteria	Group 1	35	20.11	4.398	0.31
	Group 2	35	19.14	3.557	

Results are presented as mean ± SD and p-value
**Significant p-value*

Table 3a. Comparison of Isolated species on control sides (Right from reference point) between group 1 and group 2

Variables (n=70)	Groups	N	Mean	St. deviation	p- value
Staphylococci on plate right control side	Group1	35	1.31	.471	0.22
	Group 2	35	1.46	.505	
Micrococci plate right control side	Group1	35	1.23	.426	0.36
	Group 2	35	1.14	.355	
Streptococcii plate right control side	Group1	35	1.31	.471	0.054*
	Group 2	35	1.54	.505	
Gram positive rods right control side	Group1	35	1.54	.505	0.33
	Group 2	35	1.66	.482	
Gram negative Cocci right control side	Group1	35	4.63	.490	0.62
	Group 2	35	4.69	.471	

Results are presented as mean ± SD and p-value.
**Significant p-value*

Table 3b. Comparison of Isolated species on control sides (Left from reference point) between group 1 and group 2

Variables (n=70)	Groups	N	Mean	St. deviation	p- value
Staphylococci plate left control side	Group 1	35	1.23	.426	0.12
	Group 2	35	1.40	.497	
Micrococci plate left control side	Group 1	35	1.31	.471	0.80
	Group 2	35	1.34	.482	
Streptococcii plate left control side	Group 1	35	1.20	.406	0.41
	Group 2	35	1.29	.458	
Gram positive rods left control side	Group 1	35	1.54	.505	0.33
	Group 2	35	1.66	.482	
Gram negative cocci left control side	Group 1	35	4.51	.507	0.81

Results are presented as mean ± SD and p-value.
**Significant p-value*

Table 3c. Comparison of Isolated species on control sides (behind) between group 1 and group 2

Variables (n=70)	Groups	N	Mean	St. deviation	p- value
Staphylococci plate behind control side	Group 1	35	1.37	.490	0.63
	Group 2	35	1.43	.502	
Micrococci plate behind control side	Group 1	35	1.31	.471	0.80
	Group 2	35	1.34	.482	
Streptococci plate behind control side	Group 1	35	1.20	.406	0.41
	Group 2	35	1.29	.458	
Gram positive rods behind control side	Croup 1	35	1.54	.505	0.33
	Group 2	35	1.66	.482	

Results are presented as mean ± SD and p-value.
**Significant p-value*

Table 4a. Comparison of Isolated species on test sides (right) between group 1 and group 2

Variables (n=70)	Groups	N	Mean	St. deviation	p- value
Staphylococci on plate right test side	Group1	35	1.29	.458	0.14
	Group 2	35	1.46	.505	
Micrococci plate right test side	Group 1	35	1.40	.497	0.46
	Group 2	35	1.31	.471	
Streptococcii plate right test side	Group 1	35	1.29	.458	0.79
	Group 2	35	1.26	.443	
Gram positive rods right test side	Group 1	35	1.49	.507	0.15
	Group 2	35	1.66	.482	
Gram negative cocci right test side	Group 1	35	4.63	.490	0.62
	Group 2	35	4.69	.471	

Results are presented as mean ± SD and p-value.
**Significant p-value*

Table 4b. Comparison of Isolated species on test sides (left) between group 1 and group 2

Variables (n=70)	Groups	N	Mean	St. deviation	p- value
Staphylococci plate left test side	Group 1	35	1.29	.458	0.14
	Group 2	35	1.46	.505	
Micrococci plate left test side	Group 1	35	1.40	.497	0.46
	Group 2	35	1.31	.471	
Streptococcii plate left test side	Group 1	35	1.29	.458	0.79
	Group 2	35	1.26	.443	
Gram positive rods left test side	Group 1	35	1.49	.507	0.15
	Group 2	35	1.66	.482	
Gram negative cocci left test side	Group 1	35	2.23	1.374	0.07
	Group 2	35	2.86	1.498	

Results are presented as mean ± SD and p-value.
**Significant p-value*

Table 4c. Comparison of Isolated species on test sides (behind) between group 1 and group 2

Variables(n=70)	Groups	N	Mean	St. Deviation	p- value
Staphylococci plate behind test side	Group 1	35	1.29	.458	0.14
	Group 2	35	1.46	.505	
Micrococci plate behind control side	Group 1	35	1.40	.497	0.46
	Group 2	35	1.31	.471	
Streptococci plate behind test side	Group 1	35	1.29	.458	0.79
	Group 2	35	1.26	.443	
Gram positive rods behind test side	Group 1	35	1.49	.507	0.15
	Group 2	35	1.66	.482	

Results are presented as mean ± SD and p-value
**Significant p-value*

Table 4: Table 4 (a, b and c) are showing comparison of Isolated species on test sides (right, left and behind respectively) between group 1 and group 2.

4. 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 [26].

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 [17,34].

Use of expensive methods such as high-efficiency particulate air and ultraviolet chambers in the ventilation system have also been recommended [17,35].

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 [11,36].

5. CONCLUSION

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

Written informed consent was taken from every patient.

ETHICAL APPROVAL

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

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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