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Evaluation of the effect of Herbal Mouthwash on the Aerosol Contamination during tooth preparation- An Interventional Study

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Abstract
Background*: This study demonstrates that contamination from spatter and aerosol dissemination remains a significant hazard to dental personnel when high-speed dental equipment is used. These observations support the need for universal barrier precautions and effective infection control, which must be used routinely during the treatment of all patients. However, high-volume dental suction may signifcantly reduce aerosol spread and therefore should be used whenever possible to prevent aerosol-transmitted infections during dental treatments. The maximum number of bacterial aerosols was belonged to prosthodontic treatments.The most intensive dental aerosol is presenting during work with a high-speed handpiece.*
Herbal mouthrinse was found to be effective in reducing the aerosol contamination produced by crown preparation. Also due to its natural ingredients, it does not cause any side effects and can serve as a good alternative to patients who wish to avoid alcohol (e.g., those with xerostomia), sugar (e.g., those with diabetes), and any artificial preservatives and colors in their mouthrinses. In this study, we were comparing to evaluate the effect of herbal mouthwash on aerosol contamination during tooth preparation.
Materials and Methods*: In this study two main group were present. So descriptive quantitative data will be expressed in mean and standard deviation respectively. Data normality will be checked by Data normality will be checked by Shapiro-Wilktest. In this study two group are measured so F Test- ANOVA Repeated Measures is used. A total of 15sampleswill be required for measuring the amount of aerosol spread on agar plates 15 at baseline and 15 after use of mouthwash.A total of 15 sample will be prepared.Group 1 : before using mouthwash (n=15) & Group 2 : after using mouthwash (n=15), To evaluate bacterial aerosol contamination during tooth preparation before and after using mouthwash. Compare bacterial contamination before and after using mouthwash.*
Results*: In the present study, 15 samples were considered.This 15 samples were divided into 2 groups**, Group 1** : before using mouthwash,**Group 2** : after using mouthwash.Each group had 3 samples. The purpose of this comparative study was to evaluate the effect of herbal mouthwash on aerosol contamination during tooth preparation.Compare bacterial contamination before and after using mouthwash.The Data was collected and subjected to statistical analysis. Mean, Median, Standard Deviation, Standard Error values were calculated.Independent t test and Paired t test is used.Group 1 that is before using mouthwash samples were having highest number of microbial colonies as compared to group 2 that is after using herbal mouthwash of microbial colonies on them.In mean comparison of colony count for efficacy of herebal mouthwash for patient's, doctors and assistant's group before using mouthwash has higher number of microbial count than mean comparison of after using mouthwash of paitent's, doctors and assistant's colony count.*
Conclusion: *This study demonstrates that contamination from spatter and aerosol dissemination remains a significant hazard to dental personnel when high-speed dental equipment is used. However, high-volume dental suction may signifcantly reduce aerosol spread and therefore should be used whenever possible to prevent aerosol-transmitted infections during dental treatments. Herbal mouthrinse was found to be effective in reducing the aerosol contamination produced by crown preparation. The above results show strong evidence for mouth rinsing before any dental procedures is must, yet very few clinicians follow this protocol. The implication of this procedure depends on the professional understanding and realizing the protective benefits in reducing the spread of microorganisms from their patients' mouths.*
Key Word*: Aerosol, high-speed dental equipment, Herbal mouthrinse*

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I. Introduction

The production of aerosols in dentistry is a major health concern as aerosols generated during dental procedures are contaminated with micro-organisms which can lead to spread of infections among dental professionals and their patients[1]. The dissemination of oral microbes following various dental procedures has been a concern in clinical practice. Equipment such as high-speed handpieces, ultrasonic scalers or three-way syringes might cause the spread of aerosol in the environment[1]. The aerosol from dental procedures is composed of water, microbes, tissue, tooth dust, and fluids such as saliva and blood. The spread of aerosol might relate to cross-infection in the dental clinic, resulting in impairment of the health status of patients, dental professionals, and dental assistants. Within a general dental practice, numerous procedures are performed on a daily basis that results in the production of aerosols and splatter[2]. The human mouth is a highly contaminated environment, the dentist and dental hygienist are exposed to a variety of bacteria, viruses, fungi and protozoan. The spread of infection through aerosol and splatter has long been considered one of the main concerns in the dental community because of possible transmission of infectious agents and their potential harmful effects on the health of patients and dental personnel[3]. Aerosol is a suspension of solid or liquid particles containing bacteria or viruses, suspended (for at least a few seconds) in a gas. Particle size may vary from 0.001 to >100 mm.2 The smaller particles of an aerosol (0.5 to 10 mm in diameter) have the potential to penetrate and lodge in the smaller passages of the lungs and are thought to carry the greatest potential for transmitting infections. Infection control is one of the main concerns of the dental community. These microorganisms may cause cross-infections in the dental office, jeopardizing the health of patients and dental professionals[3].

Microorganism present in the mouth and upper respiratory tract can be transported in the aerosol produced during dental procedures such as restorations or scaling, leading to infection of the respiratory system, skin infections and other systemic diseases in immuno-compromised patient[4].

The oral cavity harbors numerous bacteria and viruses from the respiratory tract, dental plaque, and oral fluids. Any dental procedure that has a potential to aerosolize saliva will cause airborne contamination with organisms[3].

High speed dental handpieces, ultrasonic scalers and air abrasion units produce airborne particles by the combined action of water sprays, compressed air, organic particles such as tissue and tooth dust, and organic fluids such as blood and saliva from the site where the instrument is used. Miller found that aerosols generated from patients' mouths contained up to a million bacteria per cubic foot of air. Other studies have reported association of these aerosols with respiratory infections, ophthalmic and skin infections, tuberculosis, and hepatitis B[3].

Current research suggests that having patients use an antimicrobial rinse before treatment may decrease microbial aerosols. Many studies have been conducted on chlorhexidine mouthrinses; however, little research has been conducted to determine the efficacy of herbal mouthrinses.

In the emerging era of pharmaceuticals, herbal medicines with their naturally occurring active ingredients offer a gentle and enduring way for restoration of health by the most trustworthy and least harmful method. Herbal medicine is both promotive and preventive in its spread. The herbal mouthwash (HRB) used in this study is made from natural herb extracts Azadirachta indica (neem) 21gm, Ocimum sanctum (tulsi) 21gm, citrus limon (lemon) 11gm, curcuma longa (haldi) 9gmsyzgium aromaticum (lavang oil) 0.1ml. It has active herbal ingredients that exhibit excellent antimicrobial activity against oral pathogens owing to the presence of anionic components . Azadirachta indica (A. Juss), commonly known as the neem tree various parts of the neem tree have been used for millennia in traditional Indian medicine for their claimed antipyretic, antacid, antiparasitic, antibacterial, antiviral, antidiabetic, contraceptive, antidermatitic, anticancer, antiinflammatory, antioxidant, antifungal, dental, and other healing and protective properties[5]. Traditional uses of Tulsi have been claimed to have numerous useful properties, including as expectorants, analgesics, anti-emetics, and antipyretics; stress reducers and inflammation relievers[6].To evaluate the antimicrobial activity of methanolic extract from the peel of the fruit of Citrus Limon (Family-Rutaceae) in conjugation with phytochemical analysis[7].

Different procedures, materials and antimicrobial agents have been proposed to minimize microbial cross-contamination in the dental office, such as decontamination of surfaces, sterilization of instruments, use of personal protective barriers.

In this study, we were comparing to evaluate the effect of herbal mouthwash on aerosol contamination during tooth preparation.

II. Aims & objectives

Aims of the study

- The aim of the study is to evaluate the effect of herbal mouthwash on aerosol contamination during tooth preparation.

Objectives

- To evaluate bacterial aerosol contamination during tooth preparation before using mouthwash.
- To evaluate bacterial aerosol contamination during tooth preparation after using mouthwash.
- Compare bacterial contamination before and after using mouthwash.

III. Materials and methodology

This study was carried out on patients of Department of prosthodontics, crown and bridge, of college of dental science and hospital, amargadh, Bhavnagar, Gujarat during 45days.In this study two main group were present. In this study two group are measured so F Test- ANOVA Repeated Measures is used. A total of 15sampleswill be required for measuring the amount of aerosol spread on agar plates 15 at baseline and 15 after use of mouthwash.

Study Design:

Study Location: Department of prosthodontics, crown and bridge, of college of dental science and hospital, amargadh, Bhavnagar, Gujarat

Study Duration: 45 days

Sample size: 15 patients.

Sample size calculation: In this study two main group were present. So descriptive quantitative data will be expressed in mean and standard deviation respectively. Data normality will be checked by Data normality will be checked by Shapiro-Wilktest. If the data is parametric, intergroup comparison of means between two groups will be done using Paired t-test. Confidence interval is set at 95% and probability of alpha error set at 5%andbetaerrorat20%.Powerofthestudy setat80%.

In this study two group are measured so F Test- ANOVA Repeated Measures is used. A total of 15sampleswill be required for measuring the amount of aerosol spread on agar plates 15 at baseline and 15 after use of mouthwash.

- A total of 15 sample will be prepared.
- Group 1 : before using mouthwash (n=15)
- Group 2 : after using mouthwash (n=15)

Subjects & selection method: First the data sample would be collected and then after the collection of sample (paitents) oral prophylaxis was done.The procedure was done in same dental chair unit, double-masked, two-group parallel design was conducted over a period of 45 days.

The protocol was approved by the ethics committee of the institutional review board (College of dental science and Hospital, amargadh) , 15 patients of crown preparation were recruited into the study from the Out Patient Department of the Department of Prosthodontics, crown and bridge & oral implantology, College of dental science and hospital, amargadh, Gujarat, India.

Inclusion criteria :

- Missing teeth or single missing tooth in mandibular arch
- Root canal treated teeth
- Vital teeth (abutment)

Exclusion criteria :

- Grossly decayed teeth
- Infectious condition-Gingivitis, periodontitis, periapical abscess
- AIDS
- Hepatitis Diseases
- Candidiasis, angular chelitis
- Antibiotics medication taken within last six months

Armamentarium

- Diagnostic instruments set (Mouth mirror, Probe, Twizzer) (API)

- Tooth preparation burs for tooth cutting, finishing and polishing (Mani and Sofu)
- High speed airotor (NSK PanaAir FX)
- Suction tip and chamber
- Needle and syringe (Unlok 2.5ml)
- Fumigator
- Water distiller (Runyes)
- Agar plate with petri dish (SCDA Plate)
- Incubator (Globe instruments)
- Colony forming unit
- Autoclave (Runyes)
- Ultraviolet light chamber

Materials
- Herbal mouthwash (Bioayurveda)
- Agar media (SDCA Nutrient agar media)
- Distilled water
- Isopropyl alcohol (I.P.A)

Methods
When the patient was coming for the treatment first to take history of patient, diagnosis of patient and then decide the treatment plan. After decide the treatment plan first we took diagnostic impression of patient and then proceed with treatment.Then first oral prophylaxis procedure was done in all the patient.

Preparation of Nutrient agar plate
Nutrient agar plates used to collect airborne microorganisms and area where procedure was going to be carried out would be fumigated first. It was proven that Nutrient agar plates are valid medium for culturing airborne microorganism.
First preparation of agar plate was done in petridish. In that first suspended 28gm of nutrient agar powder in 1lit of distilled water. Then heat this mixture while stirring to fully dissolve all components. Then Autoclave the dissolved mixture at 121degrees Celsius for 15 minutes.
Once the nutrient agar has been autoclaved, allow it to cool but not solidify. After that pour nutrient agar into each plate(petridish) and leave plates on the sterile surface until the agar has solidified. After Forming of agar plate, all the agar plate was replaced the lid of each petri dish and store the plates in a refrigerator.

Procedure
Three standardized locations of the operatory were chosen to be evaluated for each treatment group. One was positioned at the patient's chest area, another at the doctor's chest area, and a third at the assistant's chest area. The average distance was 12 inches from the patient's mouth to the agar plate.

Before every appointment, the staff person cleaned and disinfected all surfaces using isopropyl alcohol.

All treatment procedure would be carried out in a standardized dental chair with same close operatory.Patients who met the minimal criteria for entry were selected. All the patients would be treated by the same dental personal and only one patient would be treated per day to allow the room to be free of aerosol.

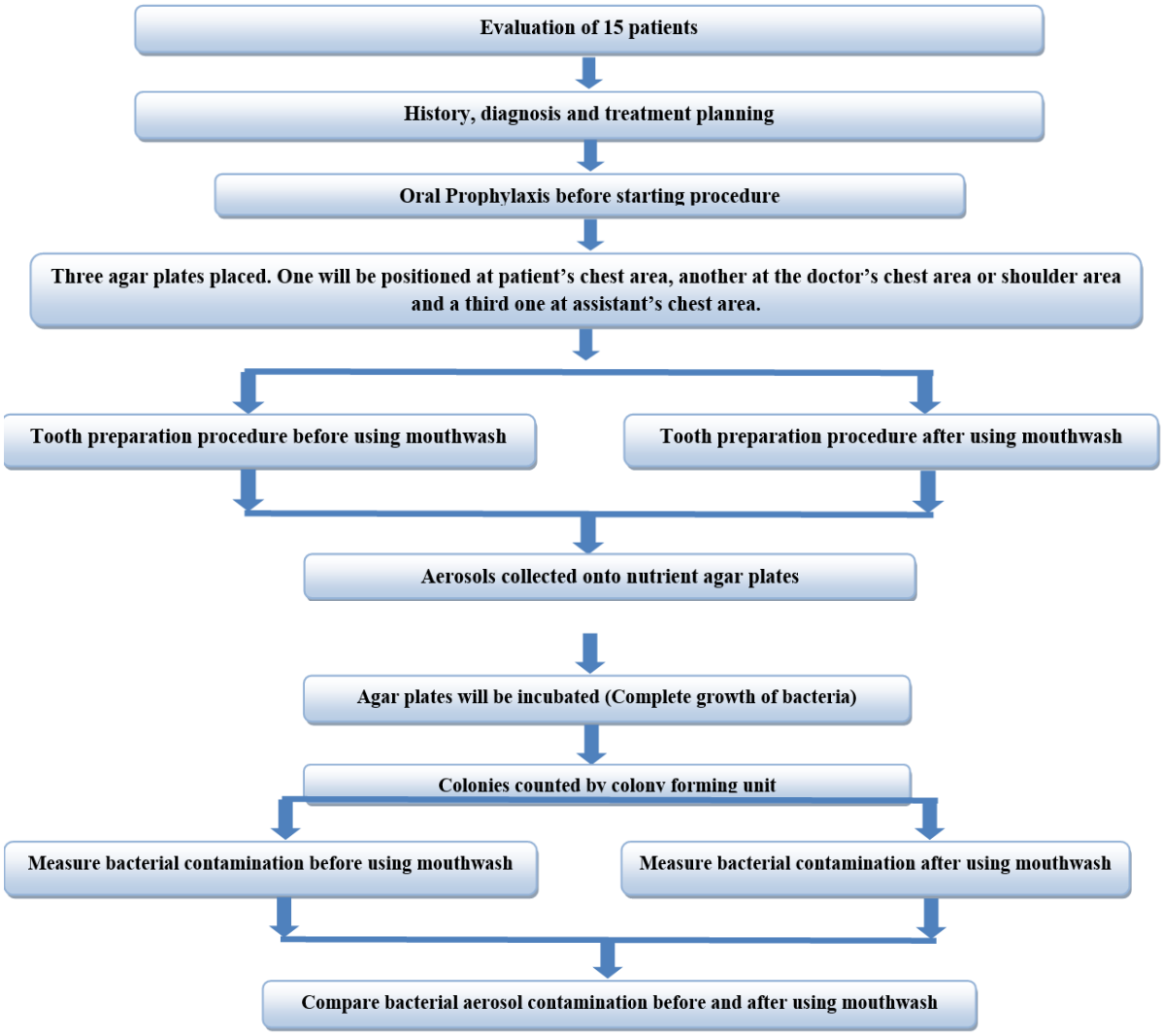
The nature of the procedure and possible discomforts and risks were fully explained, and written informed consent was obtained from each patient.

During the crown preparation we could collect the aerosol on the nutrient agar plate.Patient were recruited according to inclusion and exclusion criteria. After the selection of patient start the treatment.

Before start the treatment nutrient agar plates were attached as per the place.First abutment preparation had been done before using mouthwash. After that patient was guided to use mouthwash. Patients rinsed for 1 minute with 10 mL of bioayurveda, anti bacterial herbal mouthwash. After rinsing mouth with mouthwash wait for 30min.

Wait for 30 min after mouthwash because we were settle down the aerosol on agar plate. Now every agar plates had been change with new one.After 30min of using mouthwash remaining abutment preparation had been done.During the treatment and for 30 minutes after the treatment, three coded blood agar plates were left uncovered at predesignated sites to collect samples of any aerosolized bacteria.

Bacterial contamination on agar plates had measured before and after using mouthwash. After collection the samples, the blood agar plates were incubated at 37C for 24 hours. After incubation colonies would be counted by colony forming unit. Comparison of bacterial contamination before and after using mouthwash will be done.



IV. Result

In the present study, 15 samples were considered. This 15 samples were divided into 2 groups**, Group 1** : before using mouthwash, **Group 2** : after using mouthwash.

Each group had 3 samples. The purpose of this comparative study was to evaluate the effect of herbal mouthwash on aerosol contamination during tooth preparation. Compare bacterial contamination before and after using mouthwash.

The Data was collected and subjected to statistical analysis. Mean, Median, Standard Deviation, Standard Error values were calculated.

Independent t test and Paired t test is used.

Table 1 : Before and after use of mouth wash in Group 1 and Group 2

Sample size	Before using mouthwash Bacterial colonies Group 1	After using mouthwash Bacterial colonies Group 2
Sample 1	57	23
Sample 2	52	27
Sample 3	59	24
Sample 4	50	25
Sample 5	54	29
Sample 6	58	22
Sample 7	53	23
Sample 8	51	25
Sample 9	52	28

Sample 10	55	24
Sample 11	60	21
Sample 12	53	26
Sample 13	59	23
Sample 14	56	25
Sample 15	57	24

	VAR00002	N	Mean	Std. Deviation	F-Value	p-Value
VAR00001	Group 1	15	55.0667	3.19523	4.10	0.05*
	Group 2	15	24.6000	2.19740		

Test applied Independent t test
Level of significance p≤0.05*

Graph 1

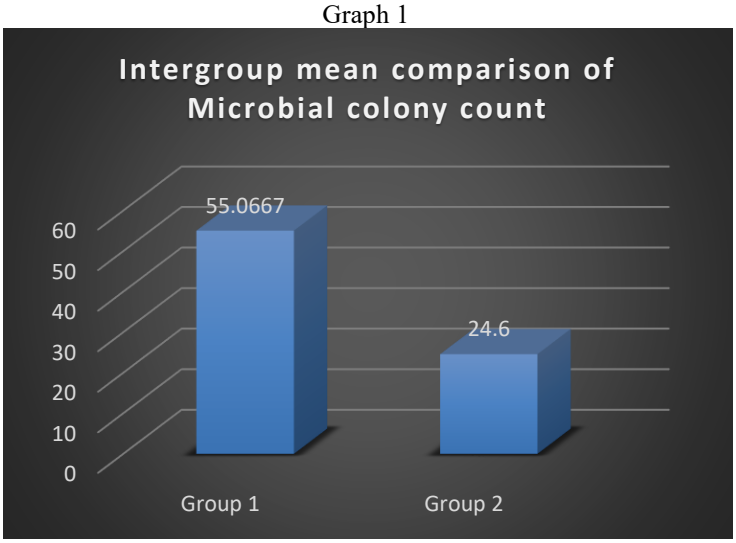


Table 2 : Mean comparison of colony count for efficacy of herbal mouth wash for Patient's group

Sample	Patient's agarplate before using mouthwash	Patient's agarplate after using mouthwash
Sample 1	23	10
Sample 2	21	12
Sample 3	24	9
Sample 4	20	9
Sample 5	25	11
Sample 6	22	8
Sample 7	26	10
Sample 8	19	9
Sample 9	20	12
Sample 10	21	11
Sample 11	25	8
Sample 12	23	10
Sample 13	24	8
Sample 14	22	9
Sample 15	23	11

		Mean	N	Std. Deviation	p-Value
Pair 1	Patient's B	22.5333	15	2.06559	0.01*
	Patient's A	9.8000	15	1.37321	

Test applied Paired t test
Level of significance p≤0.05*

Graph 2

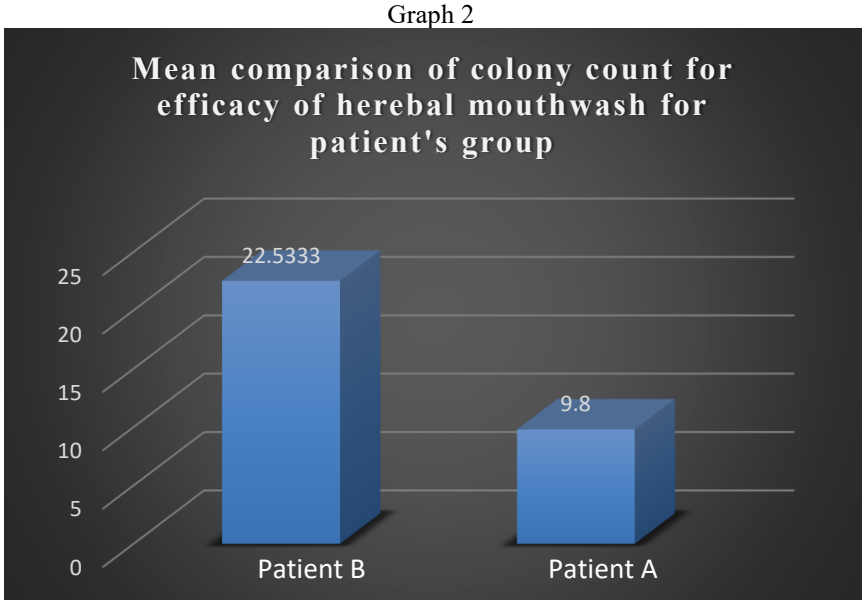


Table 3 : Mean comparison of colony count for efficacy of herbal mouth wash for Doctors group

Sample	Doctors agarplate before using mouthwash	Doctors agarplate after using mouthwash
Sample 1	18	7
Sample 2	17	8
Sample 3	19	8
Sample 4	16	10
Sample 5	15	10
Sample 6	19	7
Sample 7	14	7
Sample 8	17	8
Sample 9	16	9
Sample 10	17	7
Sample 11	19	7
Sample 12	16	9
Sample 13	18	8
Sample 14	19	8
Sample 15	17	7

		Mean	N	Std. Deviation	p-Value
Pair 2	Doctors B	17.1333	15	1.55226	0.01*
	Doctors A	8.0000	15	1.06904	

Test applied Paired t test
Level of significance p≤0.05*

Graph 3

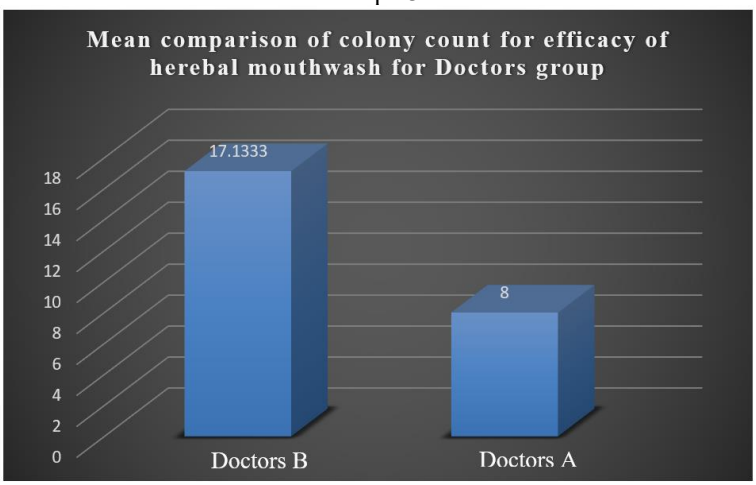


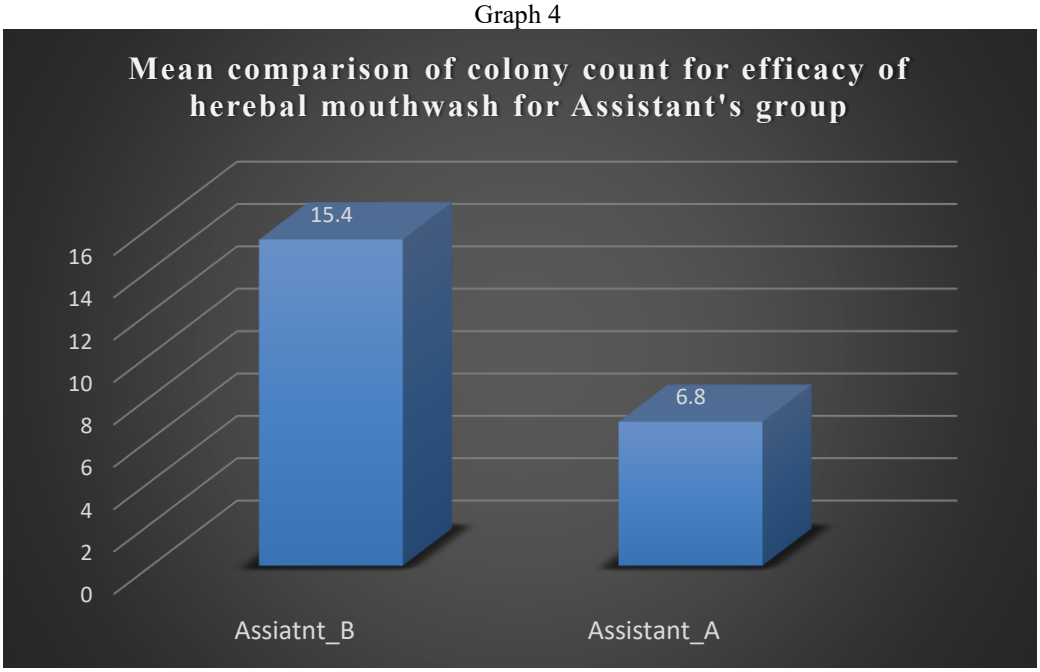
Table 4 : Mean comparison of colony count for efficacy of herbal mouth wash for Assistant group

Sample	Assistant's agarplate before using mouthwash	Assistant's agarplate after using mouthwash
Sample 1	16	6
Sample 2	14	7
Sample 3	16	7
Sample 4	14	6
Sample 5	14	8
Sample 6	17	7
Sample 7	13	6
Sample 8	15	8
Sample 9	16	7
Sample 10	17	6
Sample 11	16	6
Sample 12	14	7
Sample 13	17	7
Sample 14	15	8
Sample 15	17	6

		Mean	N	Std. Deviation	p-Value
Pair 3	Assiatnt_B	15.4000	15	1.35225	0.01*
	Assistant_A	6.8000	15	.77460	

Test applied Paired t test
Level of significance p≤0.05*

Graph 4



As per the data analysis, results came out was the:

- Group 1 that is before using mouthwash samples were having highest number of microbial colonies as compared to group 2 that is after using herbal mouthwash of microbial colonies on them.
- In mean comparison of colony count for efficacy of herebal mouthwash for patient's, doctors and assistant's group before using mouthwash has higher number of microbial count than mean comparison of after using mouthwash of paitent's, doctors and assistant's colony count.

V. Discussion

The control and minimization of microorganisms contained in aerosol are of great importance to the health of dental personnel[3]. Reports have associated these aerosols with respiratory infections, ophthalmic and skin infections, tuberculosis, and hepatitis B[8].

Dental office personnel working in the patient's respiratory tract are constantly exposed to potentially infectious bioaerosols[9]. Working high and low-speed handpieces pose a special risk, increasing transmissions of potentially dangerous pathogens (bacteria, viruses, and fungus) in a mixture of bioaerosol with a water spray.

The results of the study showed that caries removal with a high-speed handpiece and saliva ejector generated the highest amount of spray particles at each measured site. The measurement of aerosol at the manikin mouth showed the highest particle amount during caries removal with the low and high-speed handpiece[9].

We found an extremely variable distribution of bacterially contaminated aerosols and spatter that may be influenced by many factors. In all of the restorative procedures, and the first ultrasonic scaling, the highest counts were detected on plates positioned on the subject's chest. This finding agrees with Cochran and others who concluded that the larger salivary droplets generated during dental procedures settle rapidly from the air with heavy contamination of a patient's chest.High-volume evacuation was used during all the restorative procedures, which may explain the negligible bacterial counts reaching plates more than 12 inches from the mouth[10].

When high-speed rotating instruments are used, the air is momentarily contaminated; as the bacteria settle, the air quality increases. It has been claimed that settle plates have no role in monitoring operating theatre counts, and that air samplers collecting, for example, 1 m3 of air

over a 15-min sampling period would be more appropriate. These results show that a dental procedure is a potential hospital infection risk if the extent and nature of microbial aerosols created by high-speed rotating instruments is underestimated.[11].

That research shows that both the professional and the patient are exposed to high amounts of bacteria. Thus, the present study is designed to compare the efficacy of before and after using mouth rinsing with bioayurveda herebal mouthwash to lower microbial counts before the use of aerosol-producing instruments. Results of this study show that when bioayurveda antibacterial was used as a mouth rinse before crown preparation of one abutment teeth, consistently fewer CFUs developed than when mouthwash used during crown preparation of other abutment teeth or water was used.

Pistorius et al.showed that mouthwash containing a mixture of herbal extracts was found to be an effective regimen for reducing aerosol contamination. Kaim et al.[12] investigated the antimicrobial activity of herbal mouthrinse compared with another mouthrinse alone. Herbal mouthrinse was found to produce the largest zones of microbial inhibition compared with the other mouthrinse, alone and with other mouthwash, against all three bacteria tested[12].

This study is in accordance with these authors, because greater reduction was achieved in the average number of CFUs after rinsing with herebal mouthwash, showing the effectiveness of its use as a preprocedural mouthrinse in reducing bacterial contamination[3].

The decrease in microbial activity after using herebal mouthwash may result from its ability to inhibit microbial growth. Although both mouthwashes reduced growth of CFUs, these data suggest that a herebal mouth rinse is superior in reducing aerosolized bacteria.

Larato et al.[13]hypothesized that droplets containing organisms from the mouth, including possible viable pathogenic organisms, remain suspended in the air 30 minutes after a dental procedure is completed. Therefore, for 30 minutes after the treatment, agar plates were left uncovered at sites to collect samples of any aerosolized bacteria.

It was originally planned to use the sample size of 15 patients per group as a study, but after statistical analysis, the results indicated a significant difference among groups even with the small sample size. Therefore, it can be determined that the results of this study indicate an adequate statistical sample. The limitations of this study should be considered in interpreting these results. The CFUs counted here are values that represent only aerobic bacteria capable of growth on nutrient agar plates; anaerobic bacteria, and organisms requiring specialized media were not cultured in this study.

This study shows that the patient's chest area receives a greater number of microorganisms than that of the dental professional, followed by that of the assistant. This reinforces the importance of using personal protective equipment such as eye and face shields, head cap, mask, gloves, and gowns/white coats and validates the use of after rinsing in the form of mouthwash as an additional barrier to cross-contamination, minimizing the risk of team members and the patient.

However, these results clearly suggest that after using mouthrinse is a more effective primary measure reducing aerosol cross-contamination during use of crown preparation in the practice of dentistry.

VI. Conclusion

This study demonstrates that contamination from spatter and aerosol dissemination remains a significant hazard to dental personnel when high-speed dental equipment is used. These observations support the need for universal barrier precautions and effective infection control, which must be used routinely during the treatment of all patients[10].

However, high-volume dental suction may signifcantly reduce aerosol spread and therefore should be used whenever possible to prevent aerosol-transmitted infections during dental treatments[14].The maximum

number of bacterial aerosols was belonged to prosthodontic treatments[15].The most intensive dental aerosol is presenting during work with a high-speed handpiece[16].

Herbal mouthrinse was found to be effective in reducing the aerosol contamination produced by crown preparation. Also due to its natural ingredients, it does not cause any side effects and can serve as a good alternative to patients who wish to avoid alcohol (e.g., those with xerostomia), sugar (e.g., those with diabetes), and any artificial preservatives and colors in their mouthrinses[3].

The above results show strong evidence for mouth rinsing before any dental procedures is must, yet very few clinicians follow this protocol. The implication of this procedure depends on the professional understanding and realizing the protective benefits in reducing the spread of microorganisms from their patients' mouths[3].

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