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ORIGINAL ARTICLE

An Herbal Alternative to Control Nosocomial Pathogens in Aerosols and Splatter During Ultrasonic Scaling

Yamini Rajachandrasekaran[1], Mohan Valiathan[2], Bagavad Gita Jayaraman[3], Krishnan Mahalakshmi[4], Kesavaram Padmavathy[5]

[1]Department of Periodontology and Implantology, CSI College of Dental Sciences and Research, Madurai, Tamilnadu, India. 0000-0003-0829-1204
[2]Department of Periodontology and Implantology, Sree Balaji Dental College and Hospital, Bharath Institute of Higher Education and Research, Chennai, Tamilnadu, India. 0000-0002-2803-2480
[3]Department of Periodontology and Implantology, Sree Balaji Dental College and Hospital, Bharath Institute of Higher Education and Research, Chennai, Tamilnadu, India. 0000-0002-6755-3369
[4]Department of Microbiology, Research Lab for Oral-Systemic Health, Sree Balaji Dental College and Hospital, Bharath Institute of Higher Education and Research, Chennai, Tamilnadu, India. 0000-0003-2753-9092
[5]Department of Microbiology, Research Lab for Oral-Systemic Health, Sree Balaji Dental College and Hospital, Bharath Institute of Higher Education and Research, Chennai, Tamilnadu, India. 0000-0002-0790-1464

Author to whom correspondence should be addressed: Professor Krishnan Mahalakshmi, Department of Microbiology, Research Lab for Oral-Systemic Health, Sree Balaji Dental College and Hospital, Bharath Institute of Higher Education and Research, Velachery - Tambaram Road, Chennai, 600100, Tamilnadu, India. Phone: +91 9444184403. E-mail: kmagvenkat@gmail.com.

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Abstract

Objective: To evaluate the efficacy of herbal mouthwash (Himalaya Hiora Regular) against methicillin-resistant *Staphylococcus aureus* and *Acinetobacter baumanni* during ultrasonic scaling. **Material and Methods:** Group B (n=25) received herbal mouthwash and Group A (n=25) received 0.12% chlorhexidine mouthwash respectively as a preprocedural rinse. The aerosols produced by the ultrasonic unit were collected on MeReSa and Leeds Acinetobacter Agar plates. The experimental setting included eight different locations covering all areas of the operatory. The plates exposed to aerosols for a period of 30 minutes were incubated aerobically at 37°C for 48hrs and the colony forming units (CFU) were statistically analyzed. **Results:** Herbal mouthwash (Himalaya Hiora Regular) showed a significant reduction in mean CFU of MRSA compared to 0.12% chlorhexidine. While herbal mouthwash was on par with 0.12% chlorhexidine in the reduction of *A. baumannii*. **Conclusion:** Herbal mouthwash was found to be more effective against MRSA than 0.12% Chlorhexidine mouthwash as a pre-procedural rinse. Both herbal mouthwash and chlorhexidine mouthwash was found to be effective against *A. baumannii*. Herbal mouthwash may be a safe alternative to chlorhexidine against nosocomial pathogens like MRSA and *A. baumannii*.

Keywords: Mouthwashes; Chlorhexidine; Plant Preparations; Aerosols.

Introduction

Aerosol and splatter have long been considered as one of the major concerns in the dental setup because of possible transmission of infectious agents and their potentially harmful effects on the health of patients and dental health care workers. Aerosols are suspensions of liquid and solid particles in the air measuring less than 10 microns in diameter which are not visible to the naked eye [1].

Any dental procedure that has the potential to aerosolize saliva will cause airborne contamination with microorganisms. Virulent microorganisms can be transmitted from the patient to oral health care workers (OHCW) or vice versa [2]. In clinical dentistry, the ultrasonic scalers and the air polishers generate aerosols which can infect OHCW and other personnel in the dental setup.

Recently, there has been an increased report of oro-nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii* among the patients who visit the dental clinic [3,4]. MRSA is a gram-positive coccus that is genetically different from other strains of *Staphylococcus aureus*. This bacterium is responsible for several difficult to treat infections in humans. It shows multidrug resistance to ß lactam antibiotics. Oral colonization of MRSA has been reported even in the absence of nasal carriage [5].

A. baumannii is a gram-negative bacterium that has emerged as highly virulent nosocomial bacteria with multidrug-resistant properties. *A. baumannii* is an inhabitant of oral biofilm and colonizes both supragingival and subgingival plaque. Subgingival plaque colonization of *A. baumannii* results in refractory periodontitis [4].

Thus both MRSA and *A. baumannii* have the ability to persist in a dental setup such as dental instruments, dental chairs, and dental unit waterlines (DUWLs). Aerosolization of these bacteria would result in airborne contamination leading to cross-infection to the dental professional, dental assistants and other patients as it persists in the environment. To reduce the aerosol contaminations OHCW uses high vacuum suction, patient positioning, rubber dams, and preprocedural antibacterial mouth rinse [6].

Accumulating evidence has proved the antibacterial efficacy of chlorhexidine as a pre-procedural mouth rinse [7]. However, it has its own side effects which include staining of teeth and mucosa, mucosal desquamation, salivary stones creation, irritation, dryness of mouth and taste alteration, which limits its long term use [1]. The World Health Organization has recommended on the discovery of new natural sources such as the herbal extracts for overcoming the side effects of chemical agents [8].

Hiora-regular mouth rinse, a commercially available herbal product (Himalaya Drug Company, Bengaluru, Karnataka, India) is a biocompatible mouth rinse with antimicrobial, antiplaque, anti-inflammatory properties. Hiora-regular mouth rinse contains herbal extracts such as, *Salvodarapersica* (Meswak) with anti-oxidant, antimicrobial properties, *Terminalia bellerica* (Bibhitaki) with anti-bacterial and anti-inflammatory properties, Piper betel (Nagavalli) with anti-

oxidant, anti-inflammatory, anti-microbial properties, oils of *Gaultheria fragrantissima* (Gandhapura) with anti-inflammatory, antimicrobial and analgesic properties, *Elettaria cardamomum* (Ela) with antiseptic property and agent for malodor, and powders of Mentha spp. (peppermint satva) as a natural mouth freshener, *Yavanisatva (*thymol) has anti-microbial properties. Among this, the active ingredients are *Salvadora persica* (Meswak), *Terminalia bellirica* (Bibhitaki), *Gaultheria fragrantissima* (Gandhapurataila) and *Piper betel* (Nagavalli) that act against common strains of oral bacteria, fungi and prevent gum and tooth disease.

Hence, the present study was chosen to evaluate the efficacy of herbal mouth rinse against the two important nosocomial pathogens (MRSA and *A. baumannii*) during ultrasonic scaling.

Material and Methods

Sample

Fifty patients (24 males and 26 females) were recruited for the study from the outpatients of the Department of Periodontics, Sree Balaji Dental College and Hospital, Chennai, India. A detailed medical and dental history was taken from each volunteer.

The participants were selected with the following inclusion criteria: age between 20 to 50 years, both gender, systemically healthy patients, the presence of 22 permanent teeth and Mean OHI between 3.1 to 6.0 were the inclusion criteria. Pregnant or lactating women, smokers, patients on antibiotic or steroid therapy for the past 3 months, patients with a history of systemic disease and periodontal management were excluded.

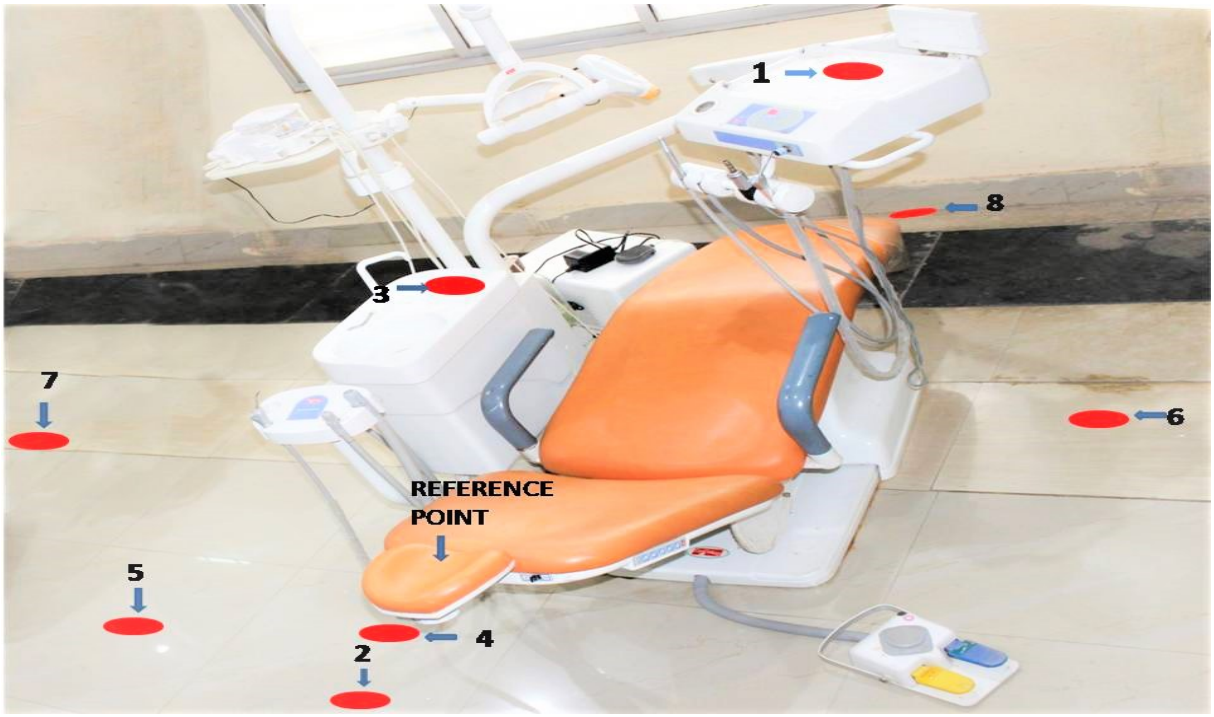
- Group A (Control Group): Subjects were given 15 ml of 0.12% of chlorhexidine mouth rinse as a pre-procedural rinse.
- Group B (Study Group): Subjects were given 15 ml of herbal oral rinse (Hiora Regular) as a pre-procedural rinse.

A closed operatory with the facility to fumigate the room was used for all treatment procedures. Prior to 24 hours, disinfection of the operatory surfaces was performed using Bacillocid® special (Raman & Weil Pvt Ltd, Daman, India in collaboration with Bod Chemie Hamburg, Germany) fumigation for 30 minutes, to make the operatory room free of aerosols. The efficacy of fumigation was checked by an open plate method. Only one subject was treated per day. Prior to the procedure, the ultrasonic unit was switched on and flushed for 2 min, in order to get rid of contaminated water due to overnight stagnation in waterlines.

Selective Isolation of MRSA and *A. baumannii*

Each treatment session consisted of 30 minutes of ultrasonic scaling and 10 minutes prior to the treatment patients were given mouth rinses, 15 ml of Herbal mouthwash (study group) and 15 ml of 0.12% of chlorhexidine mouth wash (control group). Patients were instructed to rinse for 60 seconds. The patient was made to sit in a reclined position with their mouth at a standardized height of 3 feet from the floor of the operatory. Ultrasonic scaling was performed using a Cavitron Bobcat

Pro (Dentsply Sirona, Mumbai, India) ultrasonic scaler with standard ultrasonic tip and motorized suction. Sterile distilled water was used for all the ultrasonic scaling procedures. The amount of water dispensed, the water pressure and power settings on the ultrasonic unit were identical for each subject. Each subject was treated by the same operator. The experimental setting included eight different locations covering all areas of the operatory (Figure 1).



1: Two feet from the reference point to the patient's right. 2: Two feet from behind the reference point. 3. Two feet from the reference point to the patient's left. 4: Three feet from the reference point to the patient's right. 5: Three feet from the reference point to the patient's left. 6: Five feet from the reference point to the patient's right. 7: Five feet from the reference point to the patient's left. 8: Nine feet from the reference point.

Figure 1. Experimental setting showing eight different locations.

MeReSa Agar base (M1594; HiMedia Laboratories, Mumbai, India) plates supplemented with Selective supplement (FD229) and Cefoxitin supplement (FD259) and Leeds Acinetobacter Agar Base (M1839; HiMedia Laboratories, Mumbai, India) plates supplemented with Selective supplement (FD271) were placed at eight pre-designated positions (Figure 1). The settle plate method was performed by exposing the agar plates to aerosols for 30 minutes during the scaling procedure. Once the initiation of scaling, the sixteen selective plates for MRSA and *A. baumannii* were left uncovered at the pre-designated sites to collect samples of aerosolized bacteria. The selective medium was then transported immediately to the microbiology laboratory. The selective medium was incubated at 37°C for 48 hours aerobically. After the incubation period, the plates were observed for microbial growth. Using a colony counter, the Colony Forming Units (CFU) were recorded for each plate.

Statistical Analysis

Chi-square test was done to determine the significance between the study and control group with regard to the demographic details and clinical parameters. The mean, standard deviation and standard error of the colony forming units were calculated using SPSS software 21.0 version. Independent sample t-tests were performed to find the significance between the mean colony counts of MRSA and *A. baumannii* between the two groups. The paired sample t-test was done to find the statistical difference between the CFU of MRSA and *A. baumannii* in the two groups individually. One way ANOVA was used to study the significant difference among eight different positions for MRSA and *A. baumannii* in both study and control group. A p-value of <0.05 was considered significant.

Ethical Aspects

The present study was reviewed and approved by the Institutional Ethics Committee of Sree Balaji Dental College and Hospital. Written informed consent was obtained from all the participants.

Results

A comparison of the two groups with respect to demographic conditions and clinical parameters showed no statistically significant difference at the baseline (Table1). The significant difference was not observed (p= 0.150) in the mean CFU of MRSA between eight different positions in the study group while a significant difference was observed among the control group. Conversely, a significant statistical difference was observed (p= 0.0001) for *A. baumannii* mean CFU between eight different positions in the study group and control group.

Amongst eight different positions, a very high CFU of MRSA and *A. baumannii* was observed at position P2 in both study and control group (Figures 2 and 3). A significant reduction of MRSA colony count was noted in the study group compared to controls. While a significant difference in the reduction of CFU was not observed between the study and control group for *A. baumannii* (Figure 4).

Table 1. Baseline demographic and clinical parameters of study and control group.

Parameters	Groups				p-value
	Hiora Regular		Control		
	Mean	SD	Mean	SD	
Age	45.28	4.55	44.76	4.23	0.99€
Number of Teeth	25.72	1.34	24.96	1.79	0.99€
OHI-S	5.13	0.37	4.96	0.41	1.0€
GBI	1.95	0.34	2.04	0.35	1.0€

€ = Statistically non-significant.

The results of the present study clearly show a very good antibacterial effect of Hiora regular mouth rinse on MRSA and *A. baumannii*. Conversely, chlorhexidine mouthwash has shown a good inhibitory effect on *A. baumannii* compared to MRSA.

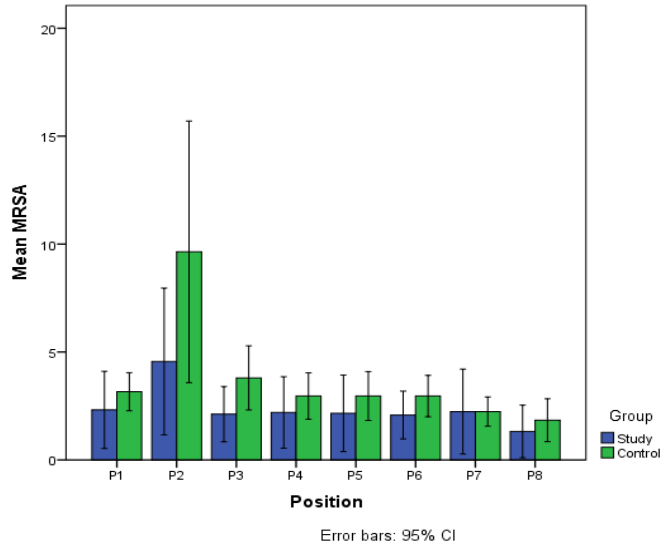


Figure 2. Mean colonies of MRSA at eight different positions of study and control group.

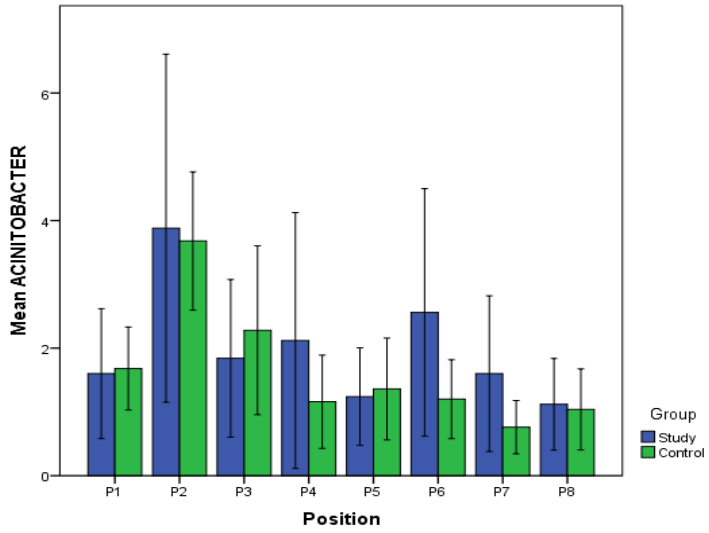


Figure 3. Mean colonies of *A. baumannii* at eight different positions of study and control group.

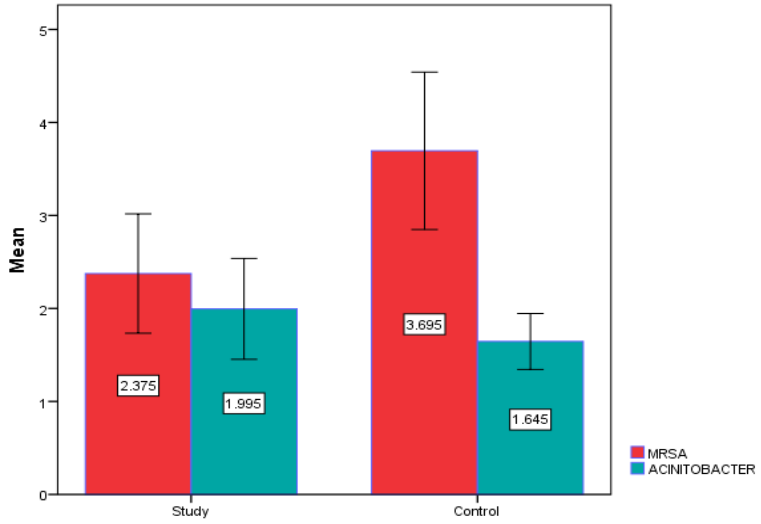


Figure 4. Mean CFU of MRSA and *A. baumannii* of all eight positions in the study and control group.

Discussion

Dental procedures with mechanical instrumentation will generate airborne particles from the site where the instrument is used. Instruments such as dental handpiece, ultrasonic scalers, air polishers, and air abrasion units produce the aerosols which are composed of saliva, nasopharyngeal secretions, plaque, blood, tooth components and any material used in the dental procedure and hence the aerosol generated is highly infectious to the patients and oral health care workers [9].

Recently, aerosol generated in the dental setup has become a growing concern as there is an increased report of oro-nasal carriage of MRSA and *A. baumannii* among the patients who visit the dental clinic [3,4]. Aerosolization of these bacteria would result in airborne contamination leading to cross infections to oral health care workers and other patients, as it persists in a viable state in the environment for a long period. Quite a few studies have reported that ultrasonic unit produces the greatest amount of aerosol and splatter in dentistry [10-12]. Hence, the present study was chosen to assess the aerosol contamination of nosocomial pathogens using an ultrasonic scaler.

It is of primary importance to control and minimize the bacteria-laden aerosol cloud. Many protective measures like good air ventilation, air conditioning filters, laminar air purge, U.V. irradiation, use of face masks and eye shields designed for both dentist and patients, pre-procedural mouth rinses and high volume evacuators have reported being effective in reducing aerosols contamination [13-15]. Among the various methods reported one of the most effective and economical methods of reducing the bacterial count in the aerosol is the use of pre-procedural mouth rinse [9].

Although chlorhexidine mouth rinse is the gold standard in controlling bacterial pathogens, its adverse effects include staining of teeth and mucosa, mucosal desquamation, salivary stone creation, irritation, dryness of mouth, and systemic side effects as the result of swallowing [13-17].

The results of the present study showed a significant reduction in mean colony count of MRSA by herbal mouthwash (Hiora Regular mouthwash) when compared to 0.12% chlorhexidine mouthwash. Conversely, a significant difference was not observed for *A. baumannii* between the study and control groups. To the best of our knowledge, there are hardly any reports regarding the efficacy of herbal mouth rinse against the nosocomial pathogens (MRSA and *A. baumannii*).

Earlier studies that evaluated the efficacy of pre-procedural rinse during ultrasonic scaling have reported on the total aerobic count [7,18]. The present study was focused on the reduction of two nosocomial pathogens (*A. baumannii* and MRSA) colonies by a pre-procedural rinse with the commercially available herbal formulation. The eight different positions selected for the study were as per Logothetis and Martinez-Welles with a few modifications [19].

The mean count in both groups was comparatively high in the P2 position. This increase in mean count may be attributed to the short distance (2 feet) from the reference point (patients head). This finding of the present study is well in line with a previous study [20].

In spite of the distance of P1 and P3 positions being similar to P2 position, the mean count of MRSA and *A. baumannii* were less in P1 and P3 positions. The reason for the reduction in P1

(Doctor's tray) and P3 (Spittoon) position could be due to the elevated level of the positions from the reference point.

The results of the present study with respect to the control group (Chlorhexidine) against *A. baumannii* were well in agreement with two in vitro studies [21,22]. On the contrary, the results with regard to MRSA were not in concurrence with an earlier study [23] who has shown a better efficacy for chlorhexidine against MRSA. The results of our finding have shown a promising antibacterial effect of herbal mouthwash against MRSA and *A. baumannii* compared to the conventional 0.12% chlorhexidine.

The antibacterial effect of herbal mouthwash (Hiora Regular) against MRSA and *A. baumannii* may be attributed to the phytochemical components (trimethylamine, Salvadorin, chlorides, fluoride, silica, sulphur, vitamin C, tannins, saponins, flavonoids, sterols, beta-sitosterol, gallic acid, ellagic acid, ethyl gallate, galloyl glucose, chebulagic acid) of major active ingredients of Hiora-regular mouthwash such as Salvadora persica and Terminalia bellerica [24,25].

Conclusion

Herbal mouthwash was found to be more effective against MRSA than the conventional 0.12% chlorhexidine mouthwash as a pre-procedural rinse. While herbal mouthwash was on par with chlorhexidine mouthwash against *A. baumannii.* Herbal mouthwash may be a safe alternative to chlorhexidine against nosocomial pathogens like MRSA and *A. baumannii.* Further, studies with a larger sample size may help in substantiating the present finding.

Authors' Contributions: YR designed the study, performed the experiment, data analysis and interpretation and wrote the manuscript. MV and BGJ designed the study. KM performed the experiment, data analysis and interpretation and wrote the manuscript. KP performed the experiment, data analysis and interpretation. All authors declare that they contributed to critical review of intellectual content and approval of the final version to be published.

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Conflict of Interest: The authors declare no conflicts of interest.

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