Efficacy of Two Pre-Procedural Rinses at Two Different Temperatures in Reducing Aerosol Contamination Produced During Ultrasonic Scaling in a Dental Set-up - A Microbiological Study

Amruta Arun Joshi, Ashvini Mukul Padhye and Himani Swatantrakumar Gupta

Department of Periodontics, MGM Dental College and Hospital, Navi Mumbai, India

Abstract

Aerosol has been considered one of the main concerns in the dental community because of possible risk of infection transmission. Antiseptics used in the form of pre-procedural rinses can reduce aerosol contamination during dental procedures. The aim of this study is to evaluate and compare the efficacy of 0.05% cetylpyridinium chloride and 0.2% chlorhexidine pre-procedural rinses at 47ºC and 18ºC in reducing aerosol contamination during ultrasonic scaling procedures. Forty subjects were divided randomly and equally into four groups: A1 and A2 to receive cetylpyridinium chloride and B1 and B2 to receive chlorhexidine as a pre-procedural rinses. Aerosol produced during the ultrasonic scaling procedure was collected on blood agar plates at three different locations, which were incubated at 37ºC for 48 hours and analysed for bacterial colony forming units (CFU). Cetylpyridinium chloride (0.05%) as a pre-procedural rinse was found to be equally effective in reducing aerosol contamination when compared with 0.2% chlorhexidine rinse ($p > 0.05$). Also, greater reduction of CFU was found with the use of tempered rinses at 47ºC with a highly statistically significant difference ($p < 0.001$). Cetylpyridinium chloride (0.05%) can be considered as a promising alternative to the gold standard 0.2% chlorhexidine, with tempering the rinse showing the definite edge.

Keywords: Aerosol, pre-procedural mouthrinse, cetylpyridinium chloride, chlorhexidine, ultrasonic scaling, temperature

Introduction

Aerosols emanating from human fluids and medical procedures are solid or liquid particles, ranging in size from sub- to multi-micrometre, that are suspended in a gas (Occupation Safety and Health Administration). Harrel *et al.* (1998), Harrel (2004) and Saini (2015) stated that dental aerosols produced with the use of high speed hand pieces, ultrasonic scalers, air polishing devices and abrasion units, are complex and dynamic, wherein some particles are projected onto surfaces, some settle due to gravity, and others can remain suspended in the air for long periods of time. Elevated levels of these contaminants present in the aerosol have been found during ultrasonic scaling procedures that may get inhaled and transported to alveoli. This process could result in respiratory problems and increase the risk of transmission of tuberculosis, severe acute respiratory syndrome, avian flu and herpetic infections from patients to health care workers (Barbeau 2000; Harrel, 2004).

Worrall *et al.* (1987), Harrel (1996) and Gupta *et al.* (2014) inferred that use of a pre-procedural antimicrobial mouthrinse may decrease the microbial aerosol contamination to a great extent. According to Lyle (2000), chlorhexidine (CHX) is considered the gold standard rinse due to its broad-spectrum antimicrobial activity and high substantivity. However, CHX presents side effects such as temporary loss of taste, staining of teeth, dryness and soreness of mucosa, and bitter taste.

Correspondence to: Amruta Arun Joshi, Department of Periodontics, MGM Dental College and Hospital, Junction of NH4 and Sion-Panvel Highway, Sector 1, Kamothe – 410209, Navi Mumbai, Maharashtra, India. Phone: +919619140326; E-mail: amrutajoshi2611@gmail.com

Therefore, a need arises for evaluation of other equally effective antimicrobial rinses. Albert-Kiszely *et al.* (2007) and Silva *et al.* (2009) found that cetylpyridinium chloride (CPC), a member of the quaternary ammonium compound family, is an effective anti-plaque and anti-gingivitis agent. It is monocationic at oral pH that permits dual retention in the oral environment, as both surfactant chains and cationic charges may adsorb to intraoral surfaces, which are lipophilic and anionic. CPC acts primarily by penetrating the bacterial cell membrane, causing leakage of cell components, disruption of bacterial metabolism, inhibition of cell growth and finally cell death (Quirynen *et al.,* 2005)

Complementarily, Konig (2002) demonstrated that temperature appears to be a key determinant in altering the kinetics of the active ingredient in a mouthrinse. Tempering 0.2% CHX rinse to 47ºC demonstrated significantly more intensive anti-plaque effect as compared to the solution cooled at 18ºC. In a previous study by Reddy *et al.* (2012), tempered 0.2% CHX demonstrated reduced bacterial counts in dental aerosols when compared to that of non-tempered CHX and sterile water. A vista that remains to be explored is modulating the temperature/concentration of CPC for enhancing its antimicrobial activity when used as a pre-procedural rinse. Hence, this study attempts to evaluate and compare the efficacy of two pre-procedural mouth rinses containing 0.05% CPC and 0.2% CHX at two different temperatures of 47ºC and 18ºC in reducing aerosol contamination during ultrasonic scaling procedure.

Materials and methods

This single-centre, double-masked, randomized, prospective, four-group parallel designed study was conducted over a period of 60 days. From September 2015 to October 2015, 40 patients (28 males and 12 females, mean age 32.5 years) were recruited. The patients were included if they had a minimum of 20 natural teeth present, excluding third molars, diagnosed with chronic gingivitis having a sulcus probing depth of ≤ 3 mm, modified gingival index ≥ 1 (Lobene *et al*., 1986) and gingival bleeding index > 30% of the sites examined (Ainamo and Bay, 1975).

Exclusion criteria were history of known allergies to constituents found in conventional mouthrinses, patients with untreated/grossly carious teeth, those who had undergone non-surgical or surgical periodontal therapy and antibiotic and/or anti-inflammatory therapy within the past 6 months. Systemically compromised patients and pregnant and lactating women were also excluded.

The study was carried out in accordance with 'The Code of Ethics of the World Medical Association' (Declaration of Helsinki, 64th WMA General Assembly, Fortaleza, Brazil, October 2013) for experiments involving humans and the protocol was approved by the Institutional Ethics Review Committee of Mahatma Gandhi Mission's Dental College and hospital, Navi Mumbai and a detailed informed consent was obtained from the 40 empirically selected patients.

Two commercially available solutions of 0.05% of CPC mouthwash (Colgate Plax® Colgate Palmolive Ltd, Mumbai, India) and 0.2% CHX mouthwash (Hexidine® ICPA Health Products Ltd, Ankleshwar, India) were procured from manufacturers and were transferred into identical opaque white bottles labelled as A1, B1, A2 and B2 for the purpose of blinding, by an investigator not involved in the study. The identity of the samples was revealed at the completion of the study. To calculate the sample size at 80% power, the level of significance was set at 0.05, to detect a standard deviation (SD) in CFU counts of 18.80 (Konig, 2002) with a ratio of sample sizes in both groups equal to 1. These data, when analyzed by MedCalc Statistical Software version 13.3.1 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2015) yielded a sample size of 10 per group. Computer-generated random numbers were used for randomization, and 40 patients were divided into four groups of 10 each as follows: Group A1 - warm 0.05% CPC (47ºC); Group B1 - warm 0.2% CHX (47ºC); Group A2 - cold 0.05% CPC (18ºC); Group B2 - cold 0.2% CHX (18ºC).

For tempering the rinses to 47ºC, 10 mL of specified mouthwash was heated in a calibrated beaker in a thermostatically regulated water bath. Similarly, the solutions were cooled to 18ºC using a portable cooler (Lab Hosp Corporation, Kalbadevi, Mumbai, India). All patients were asked to rinse with 10 mL of the assigned rinse for 60 seconds, 10 minutes prior to the ultrasonic scaling procedure.

Prior to each appointment, a closed operatory was fumigated using 34% formaldehyde and all surfaces were disinfected with 70% isopropyl alcohol (A.B Enterprises, Mumbai). Only one patient was treated per day by the same right handed operator for the entire span of the study, so as to allow the room to be free of aerosols. At the beginning and end of the treatment, the ultrasonic scaler unit (SUPRASSON® P5 Booster, France) was flushed with 0.5% sodium hypochlorite (Clorox disinfectant cleaner) for 10 minutes to ensure disinfection of water lines. Oral prophylaxis for all of the study participants was carried out in a standardized dental chair using distilled water with controlled frequency (30 KHz) and water pressure (0.3 MPa). No person other than the patient, the operator and the assistant was allowed in the vicinity of the operatory within the diameter of four feet to avoid contamination of the operating field.

Blood agar plates, used to collect airborne microorganisms were prepared as instructed by the manufacturer (Micro Master Labs Pvt Ltd, Thane, Maharashtra, India). Briefly, blood agar base was sterilized at 121ºC for 15 minutes and then cooled to 50ºC in a water bath to which 5% sterile sheep blood was added aseptically.

This was dispensed in sterile petri plates and stored at 2 - 8ºC until further use. The blood agar plates mounted firmly on a board were suspended with strings around the necks of the patient, the assistant and the operator, such that it rested on their chests at a standardized distance of 12 inches from the patient's mouth with the philtrum of the upper lip serving as a fixed reference point, as shown in *Figure 1*. These plates were labelled as P, A and O respectively.

The labelled plates were exposed at the start of the scaling procedure performed for a duration of 30 minutes, and were left uncovered on the operator's stool, assistant's stool and the back rest of the dental chair for additional 30 minutes to collect samples of any aerosolized bacteria. These were then closed with a lid and placed upside down to prevent contamination of blood agar with the moisture on the under surface of the lid. The plates were incubated at 37º C for 48 hours to facilitate the growth of micro-organisms, following which they were analyzed for bacterial colony forming units (CFUs) using a colony counter (Lab Line Stock Centre, Mumbai, India).

Statistical analysis

Data analysis was done using 'MedCalc Statistical Software' version 13.3.1 (MedCalc Software Bvba, Ostend, Belgium). Scores were averaged for age, number of teeth present, MGI and GBI across all groups. All variables were expressed as mean with standard error. The averaged values were tested for normality using the Kolmogorov Smirnov test. The data presented normality and the parametric test of analysis of co-variance (ANCOVA) was used to find which pairs differed significantly with respect to temperature difference. Intergroup comparisons were done using unpaired *t*-tests at $p < 0.05$.

Results

The non-compliance rate for our study was zero and there were no dropouts. *Table 1* represents demographic characteristics of the sampled population. *Figure 2* demonstrates mean ± standard error of the mean (SEM) CFU counts for all groups at three different locations, namely the chest areas of patient (P), assistant (A) and operator (O). Among all the groups, the patients' chest area of group A2 demonstrated maximum mean CFU counts (103.60 ± 4.08) while the assistant's chest area of group B1 showed the lowest counts (38.50 ± 2.20). On applying an unpaired *t*-test to compare groups A2 and B2, a statistically significant difference was found for the assistant's chest area location ($p = 0.02$). However, no statistical difference ($p > 0.05$) was found on intergroup comparison for all other locations, thereby proving that both mouthrinses were equally effective irrespective of the temperature difference (*Table 2*). *Table 3* represents multiple comparisons using ANCOVA performed to find out which pairs differed significantly at the 5% level of significance with respect to temperature. A highly statistically significant difference was found on comparing group A1 with A2 and B1 with B2 ($p < 0.001$).

Table 1: Baseline demographic and clinical characteristics of the four groups.

Subjects	Group A1	Group A2	Group B1	Group B2	*p* value
Age	32.5	32.7	32.64	33.0	NS
Males/females	7/3	6/4	7/3	8/2	NS
Number of teeth	26.52	27.24	27.11	26.83	NS
MGI	2.5	2.7	2.3	2.5	NS
GBI	46%	49%	47%	43%	NS

NS, not statistically significantly different; MGI, modified gingival index; GBI, gingival bleeding index.

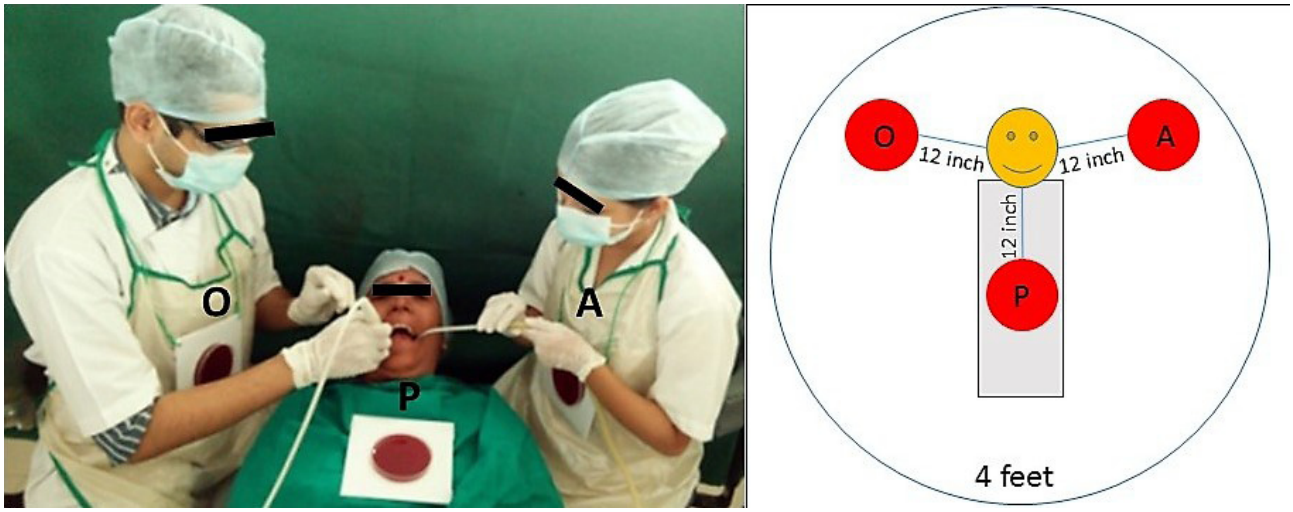

Figure 1. The position of the blood agar plates at three different locations, namely the chest areas of the patient (P), operator (O) and assistant (A).

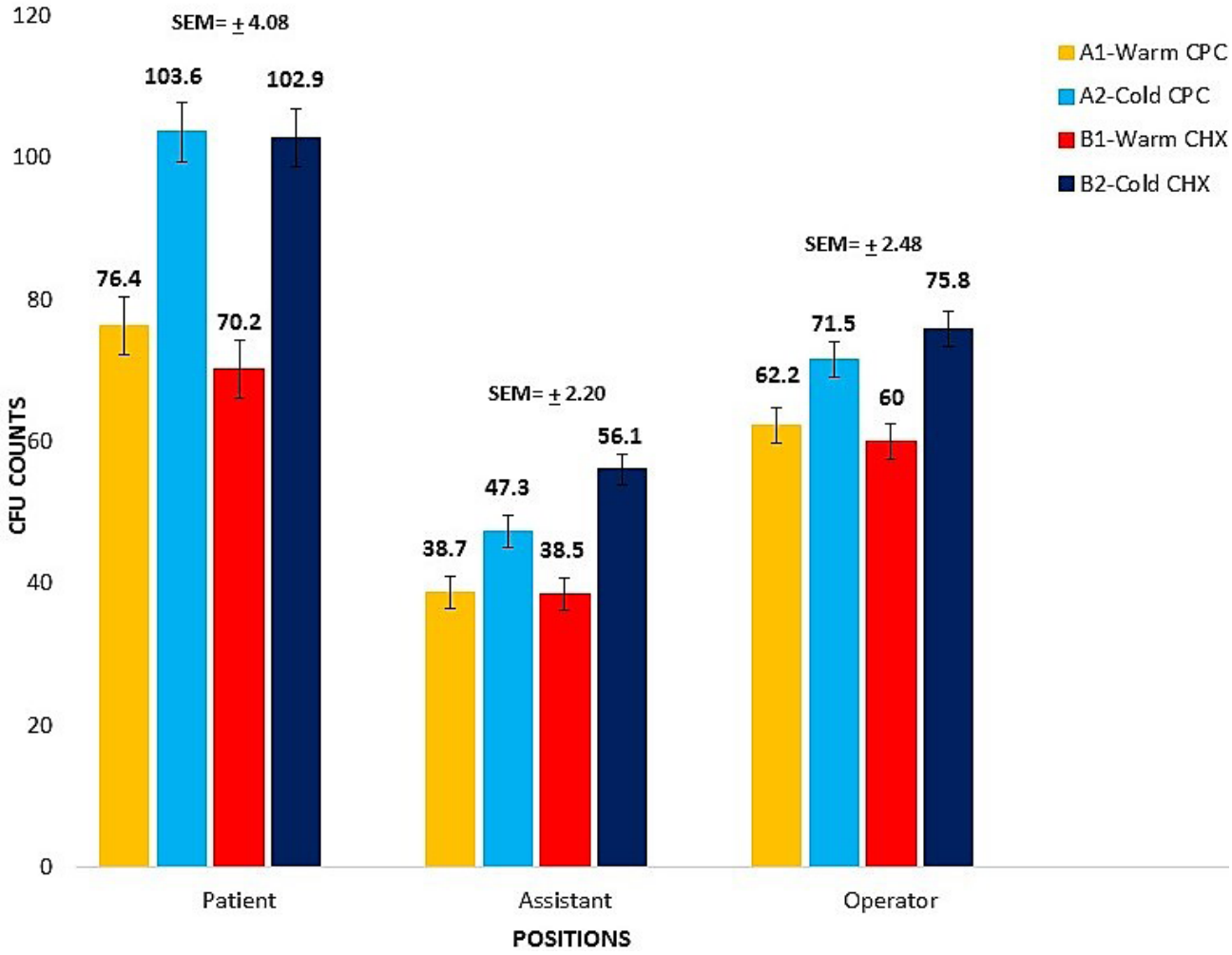

Figure 2. Comparison of mean ± standard error of the mean (SEM) colony forming units (CFU) counts at three different locations in all four groups.

Table 2: Results of unpaired *t*-tests comparing mean colony forming unit (CFU) counts between groups.

Location and rinse	*t*-test	*p* value	Significance
Patient			
Group A1 (Warm CPC) Group B1 (Warm CHX)	1.712	0.104	NS
Group A2 (Cold CPC) Group B2 (Cold CHX)	0.095	0.9249	NS
Assistant			
Group A1 (Warm CPC) Group B1 (Warm CHX)	0.083	0.9348	NS
Group A2 (Cold CPC) Group B2 (Cold CHX)	2.383	0.0284*	S
Operator			
Group A1 (Warm CPC) Group B1 (Warm CHX)	0.655	0.5208	NS
Group A2 (Cold CPC) Group B2 (Cold CHX)	1.177	0.2547	NS

*p value < 0.05; NS, non-significant; S, significant; CPC, 0.05% cetylpyridinium chloride; CHX, 0.2% chlorhexidine;

Table 3: Results of ANCOVA test comparing mean CFU counts within groups with respect to temperature difference

Location and rinse	F value	*p* value	Significance
Patient			
Group A1 (Warm CPC)	53.79	< 0.001*	HS
Group A2 (Cold CPC)			
Group B1 (Warm CHX)		< 0.001*	HS
Group B2 (Cold CHX)			
Assistant			
Group A1 (Warm CPC)	35.31	< 0.001*	HS
Group A2 (Cold CPC)			
Group B1 (Warm CHX)		< 0.001*	HS
Group B2 (Cold CHX)			
Operator			
Group A1 (Warm CPC)	25.56	< 0.001*	HS
Group A2 (Cold CPC)			
Group B1 (Warm CHX)		< 0.001*	HS
Group B2 (Cold CHX)			

*p value < 0.05; HS, highly significant; CPC, 0.05% cetylpyridinium chloride; CHX, 0.2% chlorhexidine

This analysis revealed that the groups A1 and B1 (47ºC) showed the maximum reduction in bacterial counts in all three areas as compared to their cold counterparts A2 and B2 at 18ºC.

Discussion

Under the traditional paradigm, a dental health care worker would be considered to be at high risk for droplet transmission. Legnani *et al.* (1994) and Bennett *et al.* (2000) showed that use of ultrasonic scaling procedures resulted in peak concentrations of microbial aerosols in dental treatment rooms. Harrel and Molinari (2004) enumerated three levels of defence in the reduction of aerosols as personal protective barriers, routine use of pre-procedural rinses and high volume evacuation devices. Personal protective equipment, such as a surgical mask, face shield, or eyewear prevents the projection of microorganism-laden particles onto the mucosal membranes. However, they do not counter an ever present hazard of inhaling particulate aerosols when near an infectious patient.

CHX has already proven its efficacy as a pre-procedural rinse in reducing bacterial aerosol contamination with the use of an air polisher, as studied by Logothetis and Martinez-Welles (1995) and ultrasonic scaler as demonstrated by Sawhney *et al.* (2015). Konig (2002) inferred that increasing the temperature of 0.2% CHX to 47ºC was effective in reducing vital plaque content, as assessed by the vital fluorescence technique. A 25% increase in bacteria kill rate was observed following irrigation with the tempered CHX solution. This effect was not solely due to the physical parameter "temperature," as the combination of heat and water resulted in unchanged vitality rate of micro-organisms cultured from plaque samples. The biologic basis and rationale for this anti-plaque effect could be attributed to enhanced bactericidal activity at an elevated temperature. Also, CHX has been found to have an increase in sporicidal effect when the temperature was increased to 60 - 70ºC (Shaker, 1986).

However, use of CHX is not free of undesired side effects. The potential of CPC as an anti-plaque agent is well documented in the literature (Silva *et al.*, 2009; Garcia *et al.,* 2011). But there exists lacunae regarding the role of tempered 0.05% CPC as a pre-procedural rinse to control aerosol contamination. The Scientific Committee on Consumer Safety (2015) reported that degradation of pure CPC occurs at 130ºC, with complete thermal decomposition at 234ºC. Konig (2002) also reported that tempering rinses to 47ºC exhibits neither painful sensations nor permanent pulpal damage. To the best of our knowledge, this study is the first to simultaneously assess the efficacy of two commercially available mouthrinses, 0.05% CPC and 0.2% CHX, when used as pre-procedural rinses at two different temperatures, in reducing aerosol contamination.

Various investigators (Bentley, 1994; Chiramana, 2013) have studied the spread of aerosols within the range of 2 to 6 feet. In the present study, the perimeter of the operatory was limited to a diameter of 4 feet and the aerosols were collected on blood agar plates, which are considered as a valid non-selective medium for culturing airborne bacteria (Johnston *et al.,* 1978). These plates were positioned at three different locations at a distance of 12 inches from the patient's philtrum as performed previously by Gupta *et al.* (2014). Further exposure of these plates for an additional 30 min after ultrasonic scaling was done so as to allow gravitational settling of airborne bacteria (Larato *et al.,* 1966).

The results of our study suggest that the pre-procedural rinse containing 0.05% CPC is as effective as 0.2% CHX in reducing aerosol contamination, which is in agreement with the study conducted by Feres *et al.* (2010). Our results are also in accordance with the study conducted by Reddy *et al.* (2012), where the tempered solutions heated to 47ºC were found to be significantly more effective in reducing aerosol contamination at the chest areas of patient, operator, and assistant as compared to the cold solutions used at 18ºC. Nevertheless, on comparing mean bacterial counts at the assistant chest area after rinsing with only cold CPC and CHX solutions, a statistically significant difference was noted, indicating CPC was more effective than CHX at 18ºC.

The CFU estimation in our study includes only aerobic bacteria capable of growth on blood agar plates; anaerobic bacteria and viruses that require specialized media were not isolated, which needs to be addressed in further investigations. No attempt was made to differentiate these bacteria based on cultural characteristics. Within the limits of our study, the emerging evidence beckons towards the role of tempered CPC in effectively reducing aerosol contamination. Nonetheless, these data need to be interpreted with caution, as sample size was small. Hence, there is a need for longitudinal studies of a single cohort with a larger sample size demonstrating efficiency of tempered CPC in mitigating aerosol bacteria and viruses.

Conclusion

Overall, the results of our investigation clearly indicate that a pre-procedural rinse containing 0.05% cetylpyridinium chloride can be considered as a promising alternative in reducing aerosol contamination during ultrasonic scaling procedures when compared to the gold standard 0.2% chlorhexidine, with tempering the rinse showing the definite edge. Also, it can be concluded that the amount of viable bacteria in aerosol is maximum at the patient's chest area followed by the operator and assistant in a descending manner, thus reinforcing the use of personal protective barriers to minimize the risk to dental professionals.

Acknowledgments

Department of Microbiology, MGM Medical College and Hospital, Navi Mumbai, India.

References

Albert-Kiszely A, Pjetursson BE, Salvi GE, *et al.* Comparison of the effects of cetylpyridinium chloride with an essential oil mouth rinse on dental plaque and gingivitis: A six-month randomized controlled clinical trial. *Journal of Clinical Periodontology* 2007; **34**:658-667.

Ainamo J and Bay I. Problems and proposals for recording gingivitis and plaque. *International Dental Journal* 1975; **25**:229-235.

Barbeau J. Waterborne biofilms and dentistry: The changing face of infection control. *Journal of the Canadian Dental Association* 2000; **66**:539-541.

Bennett AM, Fulford MR, Walker JT, Bradshaw DJ, Martin MV and Marsh PD. Microbial aerosols in general dental practice. *British Dental Journal* 2000; **189**:664-667.

Bentley CD, Burkhart NW and Crawford JJ. Evaluating spatter and aerosol contamination during dental procedures. *Journal of the American Dental Association* 1994; **125**:579-584.

Chiramana S, Bindu OSH, Kadiyala KK, Prakash M, Prasad TD and Chaitanya SK. Evaluation of minimum required safe distance between two consecutive dental chairs for optimal asepsis. *Journal of Orofacial Research* 2013; **3**:12-15.

Feres M, Figueiredo LC, Faveri M, Stewart B and de Vizio W. The effectiveness of a preprocedural mouthrinse containing cetylpyridinium chloride in reducing bacteria in the dental office. *Journal of the American Dental Association* 2010; **141**:415-442.

García V, Rioboo M, Serrano J, O'Connor A, Herrera D and Sanz M. Plaque inhibitory effect of a 0.05% cetyl-pyridinium chloride mouth-rinse in a 4-day non-brushing model. *International Journal of Dental Hygiene* 2011; **9**:266-273.

Gupta G, Mitra D, Ashok KP, *et al.* Efficacy of preprocedural mouth rinsing in reducing aerosol contamination produced by ultrasonic scaler: A pilot study. *Journal of Periodontology* 2014; **85**:562-568.

Harrel SK, Barnes JB and Rivera-Hidalgo F. Reduction of aerosols produced by ultrasonic scalers. *Journal of Periodontology* 1996; **67**:28-32.

Harrel SK, Barnes JB and Rivera-Hidalgo F. Aerosol and splatter contamination from the operative site during ultrasonic scaling. *Journal of the American Dental Association* 1998; **129**:1241-1249.

Harrel SK and Molinari J. Aerosols and splatter in dentistry: A brief review of the literature and infection control implications. *Journal of the American Dental Association* 2004; **135**:429-437.

Johnston JR, Butchart AM and Kgamphe SJ. A comparison of sampling methods for airborne bacteria. *Environmental Research* 1978; **16**:279-284.

Konig J, Volkerr S, Kocher T, Bossmann K and Plagmann HC. Antiplaque effect of tempered 0.2% chlorhexidine rinse: An *in vivo* study. *Journal of Clinical Periodontology* 2002; **29**: 207-210.

Larato DC, Ruskin PF, Martin A and Delanko R. Effect of a dental air turbine drill on the bacterial counts in air. *The Journal of Prosthetic Dentistry* 1966; **16**:758-765.

Legnani P, Checchi L, Pelliccioni GA and D'Achille C. Atmospheric contamination during dental procedures. *Quintessence International* 1994; **25**:435-439.

Lobene RR, Weatherford T, Ross NM, Lamm RA and Menaker L. A modified gingival index for use in clinical trials. *Clinical Preventive Dentistry* 1986; **8**:3-6.

Logothetis DD and Martinez-Welles JM. Reducing bacterial aerosol contamination with a chlorhexidine gluconate pre-rinse. *Journal of the American Dental Association* 1995; **126**:1634-1639.

Lyle D. The role of pharmacotherapeutics in the reduction of plaque and gingivitis. *Journal of Practical Hygiene* 2000; **Suppl. 9**:46-50.

Occupation Safety and Health Administration. Section 9. IX Summary and explanation of standards. Bloodborne pathogens (December 1991). Regulations (Preamble to final rules).

Quirynen M, Soers C, Desnyder M, Dekeyser C, Pauwels M and van Steenberghe D. A 0.05% cetyl pyridinium chloride/0.05% chlorhexidine mouth rinse during maintenance phase after initial periodontal therapy. *Journal of Clinical Periodontology* 2005; **32**:390-400.

Reddy S, Prasad M, Kaul S, Satish K, Kakarala S and Bhowmik N. Efficacy of 0.2% tempered chlorhexidine as a pre-procedural mouth rinse: A clinical study. *Journal of Indian Society of Periodontology* 2012; **16**:213-217.

Saini R. Efficacy of preprocedural mouth rinse containing chlorine dioxide in reduction of viable bacterial count in dental aerosols during ultrasonic scaling: A double-blind, placebo controlled clinical trial. *Dental Hypotheses* 2015; **6**:65-71.

Sawhney A, Venugopal S, Babu GR, *et al.* Aerosols how dangerous they are in clinical practice. *Journal of Clinical and Diagnostic Research.* 2015; **9**:ZC52-ZC57.

SCCS (Scientific Committee on Consumer Safety), Opinion on cetylpyridinium chloride – submission II (P97), SCCS/1548/15, 25 March 2015, revision of 15 December 2015.

Shaker LA, Russel AD and Furr JR. Aspects of the action of chlorhexidine on bacterial spores. *International Journal of Pharmaceutics* 1986; **34**:51-56.

Silva MF, dos Santos NB, Stewart B, DeVizio W and Proskin HM. A clinical investigation of the efficacy of a commercial mouthrinse containing 0.05 percent cetylpyridinium chloride to control established dental plaque and gingivitis. *Journal of Clinical Dentistry* 2009; **20**:55-61.

Worrall SF, Knibbs PJ and Glenwright HD. Methods of reducing bacterial contamination of the atmosphere arising from use of an air-polisher. *British Dental Journal* 1987; **163**:118-119.