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Comparative antimicrobial efficacy of four pre-procedural mouthwashes in reducing salivary microbial load

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Abstract:
Routine dental procedures generate microbe-laden aerosols, yet standardized recommendations for pre-procedural mouth rinsing to reduce salivary microbial load remain limited in many dental settings. Therefore, it is of interest to compare the immediate antimicrobial efficacy of 0.12% chlorhexidine gluconate, 2% povidone-iodine, essential oil-based mouthwash and plain water in 88 adults randomly allocated into four groups of 22 participants each. Mean reductions in salivary colony-forming units were 69.58 ± 8.82%, 42.58 ± 15.28%, 31.69 ± 11.06% and 7.92 ± 12.19%, respectively, with significant intergroup differences ($F = 98.866$; $p < 0.001$) and a positive correlation between baseline oral hygiene and microbial counts ($r = 0.320$; $p = 0.002$). 0.12% chlorhexidine gluconate showed significantly greater antimicrobial efficacy than all other rinses ($p < 0.001$), while 2% povidone-iodine and the essential oil-based mouthwash showed moderate reductions and plain water produced minimal effect. Thus, data shows that 0.12% chlorhexidine gluconate as the preferred pre-procedural mouth rinse for reducing salivary microbial load and strengthening dental infection control practices.

Keywords: Dental aerosols, pre-procedural mouth rinse, chlorhexidine gluconate, povidone-iodine, salivary microbial load, colony-forming units, randomized controlled trial

Background:
Salivary microorganisms are released into the clinical environment during routine dental procedures, creating aerosols and splatter. Fine particles (<5 µm) stay in the air for a long time and can enter the respiratory system of dental staff and patients in the vicinity **[1, 2]**. In 1969, Micik *et al.* thoroughly characterized the dental aerobiology problem by showing that high-speed instrumentation generates bacterial aerosols that can stay in the air for long periods of time **[3]**. The COVID-19 pandemic brought this hazard into the spotlight as the oral cavity was determined as a major reservoir for SARS-CoV-2 **[4]** and the need for a standardisation of pre-procedural infection control measures in dentistry has become more urgent. Oral microbial reduction prior to the start of aerosol generating instrumentation is one of the most practical and cost-effective measures to reduce the oral microbial burden before the procedure **[5]**. A contemporary review concluded that preprocedural mouthwashes containing CHX, cetylpyridinium chloride or essential oils can reduce bacterial contamination of dental aerosols, although evidence for viral reduction remains limited **[6].** Chlorhexidine gluconate (CHX) is regarded as the gold standard antiseptic rinse due to its broad-spectrum activity and substantivity to oral tissues. Povidone-iodine (PVP-I) has rapid bactericidal and virucidal activity **[7]** and essential oil-based products like Listerine® are readily available, well tolerated and provide an alternative **[5]**. The use of plain water as a rinse in resource-limited areas is a mechanical control that does not have antimicrobial activity. Although there is strong international evidence in support of pre-procedural rinsing there are no standardized guidelines in most dental institutions in India **[5, 8]**. Therefore, it is of interest to compare the immediate antimicrobial activity of these four agents in reducing the salivary CFU count in adults receiving routine dental care.

Materials and Methods:
Design and setting of the study:
The study was a triple blind; four arm parallel group randomized controlled trial with 1:1:1:1 allocation conducted at the Department of Public Health Dentistry, Buddha Institute of Dental Sciences and Hospital, Patna, Bihar. The microbiological investigations were carried out in the Microbiology Department of the institution (September 2025). The trial followed the Declaration of Helsinki (2013) and ICMR guidelines for biomedical research. The study was approved by the Institutional Ethics Committee (IEC 237/BIDSH/IEC/2025-26 (2023-24)). All participants gave written informed consent in their native language. The trial is reported as per CONSORT 2010 guidelines **(Figure 1).**

Sample size:
The sample size was pre-determined using G*Power (version 3.1.9.2) for one-way ANOVA (k=4 groups), with a large effect size (Cohen's f=0.40), α=0.05 and power=0.80. The minimum number of participants per group was 19 and 10% attrition resulted in 22 participants per group (N=88). A pilot study (n=8) was used to validate the protocol and is not included in the main analysis.

Participants:
Patients aged 18-40 years who attended for routine dental examination and oral prophylaxis were screened. Inclusion criteria were: ≥20 natural teeth (excluding third molars), OHI-S ≤3.0 and no use of antimicrobials in the last 2 weeks. Key exclusion criteria were moderate-to-severe periodontal disease, orthodontic treatment or removable prostheses, significant oral pathology, pregnancy/lactation, thyroid disease (PVP-I arm), allergy to any study agent ingredient, recent prophylaxis within four weeks and inability to fast from food/drink for four hours before the visit.

Randomization, allocation concealment and blinding:

Allocation concealment was achieved using a computer-generated random sequence generated with the Redcap Randomization Module, with permuted blocks (4 and 8, randomly alternated) and stratified by gender and baseline OHI-S, opened by an independent staff member not involved in recruitment or analysis after T0 sample collection. The participants were divided into four groups: Group A — Listerine® essential oil mouthwash; Group B — 2% PVP-I (Betadine®); Group C — plain water (control); Group D — 0.12% CHX. The rinses were all given in the same 30-ml opaque coded bottles. Throughout, the biostatistician was provided with only coded data.

Intervention protocol:
A structured questionnaire was used to collect demographic data, oral hygiene practices and 24-hour dietary data at baseline. OHI-S was recorded. After the aborning from food, drink and oral hygiene for specified intervals, participants collected a 2-ml unstimulated saliva sample (T0) by the drool method. Then they rinsed and gargled with 15ml of their assigned solution for exactly 1 minute under timed supervision and expectorated without rinsing with water. The second sample (T1) was taken within 5 minutes of the expectoration. Adverse events were observed for 15 minutes.

Microbiological analysis:
Samples were shipped on ice and processed under sterile conditions. Blood agar plates (40 g/L) were prepared by autoclave sterilization (121°C, 15 lb, 15 min) and poured under laminar airflow. Overnight pre-incubation was used to confirm sterility. Samples were cultured on a sterile swab and incubated aerobically for 24–48 hours at 37°C. Colonies were counted using a digital colony counter and expressed as CFU/ml. Positive and negative control plates were included in every batch.

Statistical analysis:
The data were processed in MS Excel and analyzed in IBM SPSS version 16. Data for continuous variables are presented as mean ± standard deviation (SD). Gender distribution was compared using chi-square tests. Intergroup differences in age, OHI-S and CFU counts at T0 and T1 were assessed by one-way ANOVA with Tukey's HSD post hoc test. Absolute (T0-T1) and percentage change were used to express within-group reduction. Pearson correlation analyzed the OHI-S–CFU relationship. Statistical significance: $p<0.05$.

Results:
Of 100 individuals screened, 88 met eligibility criteria and were randomized (excluded: 10 did not meet criteria, 2 declined). All 88 participants completed the study with no losses to follow-up **(Figure 1).** All 88 participants completed the study without loss to follow-up (Figure 1). Each group comprised 22 participants. The proportion of males ranged from 45.5% to 59.1%, with no significant difference in gender distribution ($\chi^2 = 0.911$, df = 3, $p = 0.823$). Mean age ranged from 27.77 ± 6.29 to 29.91 ± 5.60 years, with no significant intergroup difference ($F = 0.656$, $p = 0.582$). Baseline OHI-S scores were comparable across groups (1.95 ± 0.45 to 2.11 ± 0.52; $F = 0.508$, $p = 0.678$), as were baseline CFU counts (22.27 ± 6.71 to 25.77 ± 6.46 CFU/ml; $F = 1.391$, $p = 0.251$) **(Table 1).** Post-rinse CFU counts differed significantly across groups ($F=21.161$, $p<0.001$). Group D (CHX) achieved the lowest post-intervention count (7.41±1.89 CFU/ml), followed by Group B (PVP-I; 14.82±5.65), Group A (Listerine; 17.91±7.47) and Group C (water; 20.27±6.23). Tukey's HSD confirmed that Group D was significantly lower than all other groups (all $p<0.001$). Group B was significantly lower than Group C ($p=0.011$). No significant difference was detected between Groups A and B ($p=0.282$) or A and C ($p=0.519$). Mean absolute CFU reduction from T0 to T1 was greatest in Group D (17.55±4.54 CFU/ml), followed by Group B (10.95±5.11), Group A (7.77±2.60) and Group C (2.00±3.32). ANOVA for mean difference showed $F=57.255$, $p<0.001$. Percentage reductions established a clear hierarchy: CHX (69.58±8.82%) > PVP-I (42.58±15.28%) > Listerine (31.69±11.06%) > water (7.92±12.19%) ($F=98.866$, $p<0.001$). All pairwise percentage comparisons were statistically significant ($p<0.05$). Full data are presented in **(Table 2).** A significant positive Pearson correlation was found between baseline OHI-S and baseline CFU/ml across the full cohort ($r=0.320$, $p=0.002$), indicating that poorer oral hygiene was associated with higher salivary bacterial loads **(Figure 2).** Group-level analysis revealed this association was strongest in Group B (PVP-I) at both T0 ($r=0.743$, $p<0.001$) and T1 ($r=0.510$, $p=0.015$). In Group D (CHX), the post-intervention correlation was negligible and non-significant ($r=-0.036$, $p=0.873$), suggesting CHX effectively attenuated the microbial disparity arising from differential baseline oral hygiene. OHI-S–CFU correlations in Groups A and C were weak and non-significant at both time-points.

Table 1: Baseline demographic and clinical characteristics across intervention groups

Variable	Group A Listerine (n=22)	Group B PVP-I (n=22)	Group C Water (n=22)	Group D CHX (n=22)
Male, n (%)	13 (59.1)	12 (54.5)	10 (45.5)	11 (50.0)
Female, n (%)	9 (40.9)	10 (45.5)	12 (54.5)	11 (50.0)
Mean age (years)	28.27±4.36	27.77±6.29	27.82±6.72	29.91±5.60
OHI-S (Mean ±SD)	1.95±0.45	2.09±0.59	1.97±0.53	2.11±0.52
Baseline CFU/ml (T0)	25.68±7.94	25.77±6.46	22.27±6.71	24.96±4.52
p-value (intergroup)	p=0.823 (NS)	p=0.582 (NS)	p=0.678 (NS)	p=0.251 (NS)

CHX = chlorhexidine gluconate; PVP-I = povidone-iodine; OHI-S = Oral Hygiene Index-Simplified; CFU = colony-forming units/ml; SD = standard deviation; NS = not significant.

Table 2: Post-intervention CFU/ml counts, absolute reduction and percentage reduction across groups

Group	T0 Mean±SD	T1 Mean±SD	Mean diff. (T0−T1)	% Reduction
Group A [Listerine]	25.68±7.94	17.91±7.47	7.77±2.60	31.69±11.06
Group B [PVP-I]	25.77±6.46	14.82±5.65	10.95±5.11	42.58±15.28

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Group C [Water]	22.27±6.71	20.27±6.23	2.00±3.32	7.92±12.19
Group D [CHX]	24.96±4.52	7.41±1.89	17.55±4.54	69.58±8.82
ANOVA F; p	F=1.391 p=0.251 NS	F=21.161 p<0.001*	F=57.255 p<0.001*	F=98.866 p<0.001*

**Statistically significant (p<0.05). CHX = chlorhexidine gluconate; PVP-I = povidone-iodine; NS = not significant; SD = standard deviation. Post-hoc Tukey HSD: CHX significantly lower than all groups (p<0.001); PVP-I significantly lower than water (p=0.011); Listerine vs PVP-I (p=0.282); Listerine vs water (p=0.519).*

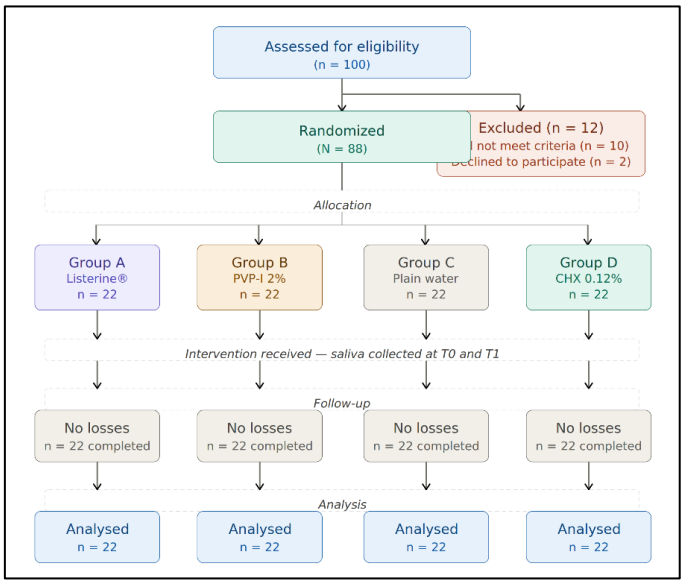


Figure 1: CONSORT flow diagram

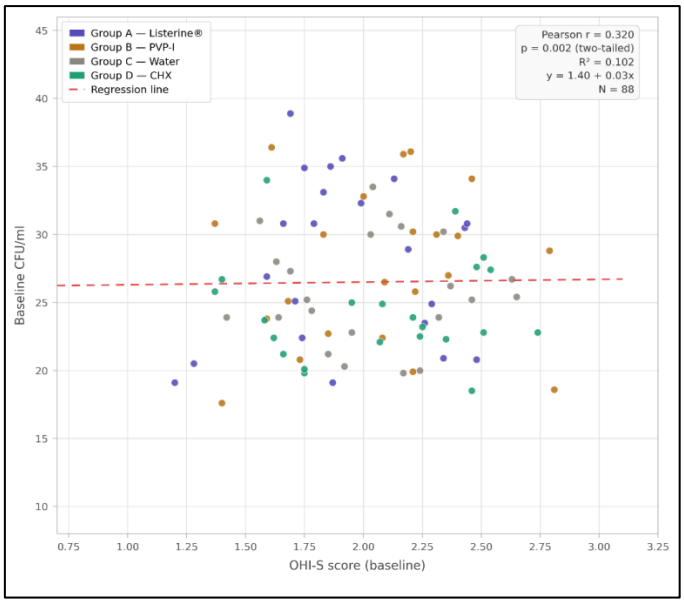


Figure 2: Scatter plot of baseline OHI-S scores vs baseline CFU/ml counts (N=88). Positive correlation: r=0.320, p=0.002. Regression: y = 1.40 + 0.03x; R^2 = 0.102. Groups coded by colour

Discussion:

The results of this triple-blind RCT clearly demonstrate the superior pre-procedural antimicrobial efficacy of CHX 0.12% followed by PVP-I 2%, Listerine® and plain water. This hierarchy was found to be valid for all outcome measures (absolute CFU reduction, percentage suppression and post-intervention residual count) and across all inferential tests used. These findings are consistent with a recent network meta-analysis of 25 RCTs involving 1,111 participants, in which CHX showed the largest reduction in microbial aerosols, followed by herbal mouthwash and PVP-I, although the overall certainty of evidence was low **[9].** The superiority of CHX is mechanistically based. It is a cationic bisbiguanide that disrupts the integrity of bacterial membranes and has high substantivity, adhering to the oral mucosal surfaces and releasing slowly for several hours after rinsing **[10].** Addy and Wright showed that PVP-I causes an immediate reduction in the number of bacteria in saliva, but the counts quickly return to normal levels within 1 hour, while CHX maintains the suppression for up to 7 hours**.** Both agents were measured at near peak activity in our T1 measurement, however CHX already demonstrated significantly higher residual counts **[10]**. The mean reduction of 69.6% CFU observed here is consistent with the range of 60–75% CFU reduction reported in pre-procedural RCTs by Marui *et al.* **[5]** and in support of the results of Retamal-Valdes *et al.* **[11]** and Ashokkumar *et al.* **[12].** PVP-I had a mean CFU reduction of 42.6% which was clinically significant but not as high as CHX. It has a broad bactericidal and virucidal spectrum, including against SARS-CoV-2, as shown by Seneviratne *et al.* **[8]** making it a valuable alternative in the event of an infectious disease outbreak or when CHX is contraindicated. The lower substantivity and organic matter inactivation of this agent may explain the lower absolute reduction compared to CHX**.** Listerine® essential oil mouthwash yielded a 31.7% reduction, which was statistically better than water, but not as good as the antiseptics. The components (thymol, eucalyptol, menthol, methyl salicylate) are active against microbial cell membranes and have broad-spectrum activity but do not have the substantivity of CHX. Similarly, an Indian randomized clinical trial by Bhandare *et al.* found the lowest aerosol microbial counts following 0.2% CHX rinsing, with herbal mouthwash showing intermediate effectiveness and water producing the least reduction **[13].** Comparable findings were reported by Nayak *et al.*, who observed marked aerosol CFU reduction with 0.2% CHX, followed by an herbal mouthwash, whereas water produced no reduction **[14].** Importantly, there was no significant difference between the absolute post-intervention CFU counts for Listerine and PVP-I (p=0.282), indicating a broadly similar immediate effect. The minimal change in the control arm was confirmed by providing plain water, which resulted in a 7.9% reduction. The strong OHI-S–CFU correlation (r=0.320, p=0.002) is in agreement with the findings of Gupta *et al.* **[15]** and Takeshita *et al.* **[16]** who observed that the oral hygiene of the patients is a good determinant of the ecological niche of the salivary bacteria. Interestingly, this correlation was lost after intervention in the CHX group (r=−0.036, p=0.873), indicating that CHX is a good 'microbial equalizer' regardless of baseline hygiene, a clinically

relevant finding for high-risk or low-compliance populations. Baseline oral hygiene assessment is important as OHI-S correlation remained significant in the PVP-I group after the rinse (r=0.510, p=0.015). Routine pre-procedural rinsing is a simple, low-cost supplement to the basic aerosol control measures (high-volume evacuation, PPE) from a public health perspective **[17]**. In COVID-19 positive people, Farmaha *et al.* found that CHX and Listerine decreased salivary viral load for up to 2 hours **[18].** The superiority of CHX was confirmed in the Hoseyni *et al.* RCT, which also employed blood agar CFU results, during dental scaling **[19].** In India, where the volume of dental care is high and resources are limited, the benefit-to-cost ratio of a 15-ml, 1-minute pre-rinse is extremely favourable. The strengths of this study are the triple-blind RCT design, computer-generated block randomization with SNOSE concealment, stratification by gender and OHI-S, the water control to separate the pharmacological from the mechanical rinsing effects and the strict baseline comparability of all four groups. The limitations are: (i) outcome only in the immediate post-rinse period and no longitudinal follow-up; (ii) culture performed on non-selective aerobic blood agar, which excludes strict anaerobes, fungi and viruses; (iii) single-institution design with small per-arm sample sizes; and (iv) no clinical infection endpoints, which is common to all published trials in this field**.**

Conclusion:

CHX 0.12% showed the greatest immediate antimicrobial activity, with PVP-I 2% and Listerine® also producing significant microbial reductions compared with plain water. All antiseptic rinses were more effective than water, supporting their role in reducing oral microbial burden before dental procedures. CHX 0.12% may be considered for routine pre-procedural rinsing, with PVP-I 2% and essential oil-based formulations as potential alternatives.

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