Original Article

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Dentistry Section

Comparative Evaluation of the Disinfection Efficacy of Herbal Formulation, 2% Glutaraldehyde, and Electrolysed Oxidising Water on Clinically Derived Polyvinyl Siloxane Impressions: An In-vivo Experimental Study

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ABSTRACT

Introduction: Dental impressions are frequently contaminated with saliva, blood and microorganisms, posing a risk of cross-infection. Polyvinyl Siloxane (PVS) impressions require effective disinfection without compromising material properties. Although glutaraldehyde is widely used, concerns regarding toxicity and environmental impact have encouraged the exploration of safer alternatives such as Electrolysed Oxidising Water (EOW) and herbal formulations. However, limited comparative evidence exists regarding their antimicrobial efficacy on clinically derived PVS impressions.

Aim: To compare the antimicrobial efficacy of herbal formulation (HiOra™), 2% glutaraldehyde, and EOW on clinically derived PVS impressions.

Materials and Methods: This in-vivo experimental study was conducted in the Department of Prosthodontics, Ranjeet Deshmukh Dental College and Research Centre, Nagpur, Maharashtra, India, from December 2023 to April 2025. Eleven dentate subjects were included, yielding a total of 44 samples. The inclusion criteria were healthy dentulous subjects aged 18-45 years with no active oral lesions. Four samples from each impression were allocated to Group A (control), Group B (HiOra™), Group C (2% glutaraldehyde), and Group D (EOW). Samples were immersed for 10 minutes. Aerobic and anaerobic Colony Forming Units (CFUs) were quantified. Demographic parameters such as age and gender of the participants were recorded. Statistical analysis was performed using the Kruskal-Wallis test followed by post-hoc Bonferroni test, with significance set at $p<0.05$.

Results: Significant intergroup differences were observed (aerobic: $p=0.003$; anaerobic: $p<0.001$). Mean aerobic log CFU/mL values were 1.02±0.73 (control), 0.80±0.81 (HiOra™), 0.36±0.51 (glutaraldehyde), and 0.00±0.00 (EOW). EOW showed significantly lower aerobic counts compared to control ($p=0.003$) and HiOra™ ($p=0.046$), and was comparable to glutaraldehyde ($p=1.000$). Mean anaerobic log CFU/mL values were 2.67±1.09, 1.26±1.27, 0.72±1.40, and 0.09±0.30, respectively, with EOW demonstrating near-complete reduction.

Conclusion: EOW demonstrated the highest antimicrobial efficacy, followed by 2% glutaraldehyde, while the herbal formulation showed the least effectiveness. EOW may serve as a promising eco-friendly alternative for routine prosthodontic disinfection.

Keywords: Anti-infective agents, Dental impression materials, Infection control, Plant extracts

INTRODUCTION

Dental impressions are an essential step in prosthodontic treatment, providing an accurate reproduction of oral structures required for the fabrication of fixed and removable dental prostheses. However, due to direct contact with saliva, blood, and oral tissues, impressions are frequently contaminated with microorganisms, posing a significant risk of cross-infection to dental personnel and laboratory technicians [1]. Hence, immediate and effective disinfection of impressions is a mandatory component of standard infection control protocols [1].

The PVS is widely used in clinical practice because of its excellent dimensional stability, elastic recovery, tear resistance, and surface detail reproduction [1]. Additionally, PVS is compatible with various disinfectants without significant alteration of its physical properties. Conventional chemical disinfectants such as glutaraldehyde, sodium hypochlorite, iodophors, and chlorhexidine are commonly employed; however, their use is often associated with cytotoxicity, occupational hazards, and environmental concerns [2,3]. Among these, 2% glutaraldehyde remains a benchmark disinfectant due to its broad-spectrum antimicrobial efficacy and established clinical use.

In recent years, attention has shifted toward safer and environmentally friendly alternatives. EOW, generated by electrolysis of dilute saline, contains Hypochlorous Acid (HOCl) and reactive oxygen species that exert antimicrobial action through oxidative damage to microbial cell walls, proteins, and nucleic acids [2,3]. EOW is non toxic, biodegradable, and minimally irritating, making it a promising alternative to conventional disinfectants, despite its limited shelf life [4].

Similarly, herbal disinfectants have gained interest due to their biocompatibility and antimicrobial properties [5]. HiOra®, an herbal mouthwash containing Meswak (Salvadora persica), Betel leaf (Piper betle), and Belleric Myrobalan (Terminalia bellirica), possesses documented antimicrobial, anti-inflammatory, and antioxidant

activity. The antimicrobial effect of these plant constituents is attributed to bioactive compounds such as flavonoids, tannins, and essential oils, which disrupt microbial cell membranes and inhibit enzymatic activity. Although HiOra® is formulated for oral use at concentrations safe for soft-tissues its broad antimicrobial spectrum provides a rationale for evaluating its potential application as a surface disinfectant for impression materials. However, its efficacy for disinfection of dental impressions has not been adequately investigated.

The selection of these three disinfectants was based on their clinical relevance: glutaraldehyde as the conventional gold standard, EOW as an emerging eco-friendly alternative, and HiOra® as a biocompatible herbal formulation with established intraoral antimicrobial use. Clinical studies directly comparing their antimicrobial efficacy on patient-derived PVS impressions remain limited. The novelty of the present study lies in the direct comparative evaluation of a conventional chemical disinfectant, an eco-friendly oxidising agent, and a standardised herbal formulation on clinically derived PVS impressions under in-vivo conditions.

Therefore the current study aimed to evaluate and compare the antimicrobial efficacy of 2% glutaraldehyde, electrolysed oxidising water, and HiOra® on clinically derived PVS impressions.

Objectives are: To assess the reduction in aerobic microbial load following disinfection with the three agents; To assess the reduction in anaerobic microbial load following disinfection; and To compare the antimicrobial effectiveness among the tested disinfectants.

Null Hypothesis (H_0) states that there is no statistically significant difference in the antimicrobial efficacy of 2% glutaraldehyde, electrolysed oxidising water, and HiOra® when used for disinfection of clinically derived PVS impressions.

Alternate Hypothesis (H_1) states that there is a statistically significant difference in the antimicrobial efficacy of at least one of the tested disinfectants.

MATERIALS AND METHODS

This in-vivo experimental study was conducted in the Department of Prosthodontics, Ranjeet Deshmukh Dental College and Research Centre, Nagpur, India, after obtaining approval from the Institutional Ethics Committee (IEC No. IEC/VSPMDCRC/2/2023). The study was carried out from December 2023 to April 2025.

Sample size calculation: The sample size was determined based on a previous similar in-vivo study evaluating the antimicrobial efficacy of disinfectants on clinically derived poly(vinyl siloxane) impressions [1]. The effect size for the present study was estimated from the mean log CFU/mL values reported in the previous study for the four experimental groups. Based on the reported group means and standard deviations, the estimated standardized effect size (Cohen's) was 5.29. Considering four independent groups, a significance level of 5% and a statistical power of 80%, the estimated minimum sample size was 10 samples per group. To ensure an adequate number of specimens, 11 samples were included in each group, resulting in a total sample size of 44 samples.

ANOVA effect-size:

$$f = \sqrt{\frac{\sum_{i=1}^{k} p_i(\mu_i - \mu)^2}{\sigma^2}}$$

where is Cohen's effect size, is the proportion in each group, is the group mean, is the overall mean, and is the within-group standard deviation.

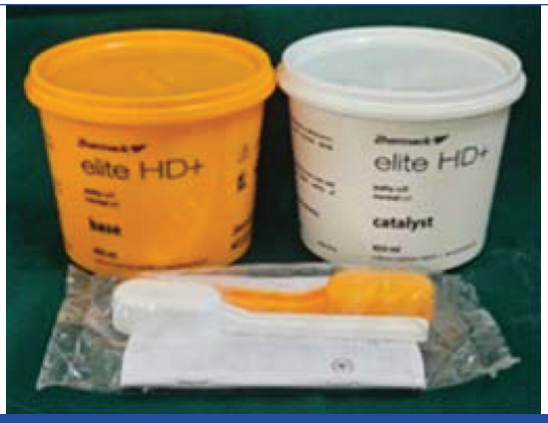
Sampling technique: A convenience sampling technique was employed, wherein eligible subjects reporting to the Department of Prosthodontics during the study period who satisfied the inclusion criteria were recruited until the required sample size was achieved.

Rationale for selection of completely dentulous subjects: Completely dentulous subjects were selected to ensure uniformity in impression surface area and microbial exposure, thereby minimising variability in microbial load. Such subjects reported to the Department of Prosthodontics for diagnostic impressions, pre-prosthetic evaluation, or treatment planning procedures.

Inclusion criteria: Healthy dentulous subjects, aged 18-45 years, absence of active oral lesions.

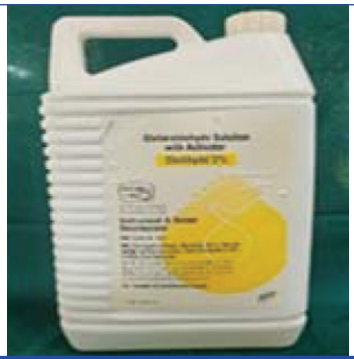
Exclusion criteria: Medically compromised patients, partially or completely edentulous subjects, and patients undergoing antimicrobial therapy.

Materials and Armamentarium: The materials used in the study are PVS impression material (Zhermack Elite HD+; Zhermack) [Table/Fig-1], HiOra™ mouthwash (Himalaya Wellness) [Table/Fig-2], 2% glutaraldehyde (Raman and Weil Pvt., Ltd.,) [Table/Fig-3], EOW prepared using a Kangen™ water electrolyser, and tray adhesive (Xtreme adhesive; Dentsply Sirona) [Table/Fig-4].



[Table/Fig-1]: Polyvinyl Siloxane (PVS) impression material.

[Table/Fig-2]: HiOra™ mouthwash.



[Table/Fig-3]: 2% glutaraldehyde.

Study Procedure

All instruments and armamentarium were sterilised in surgical metal drums using an autoclave at 121°C under 15 lbs pressure for 15 minutes prior to the procedure [6].

[Table/Fig-4]: Kangen™ water electrolyser.

The study included 11 subjects, both sexes, aged 18-45 years. A PVS impression was obtained from each subject. From each impression, four samples were obtained, resulting in a total of 44 samples. The samples were then allocated into four groups (n=11 per group) according to the disinfectant used. The study protocol was explained to all participants, and written informed consent was obtained before the study commenced.

Preparation of Electrolysed Oxidising Water (EOW): The EOW was freshly prepared using a Kangen™ water electrolysis unit. Acidic EOW was used in this study, and its potency was confirmed by measuring pH and chlorine concentration [3]. The pH was maintained between 2.5 and 3.0, as verified using a digital pH meter [Table/Fig-5], and chlorine content was maintained between 20-50 ppm using chlorine test strips [Table/Fig-6]. The solution was stored in a light-protected container and used within two hours of preparation.

[Table/Fig-5]: Digital pH meter.

[Table/Fig-6]: Chlorine test strips.

Preparation of operatory and barrier technique: The operatory and dental chair were prepared using barrier techniques, with working areas covered using autoclaved green cloths. The subject and operator preparation followed standard infection control protocols as recommended by established dental infection control guidelines [7], including the use of sterile drapes, gowns, gloves, head caps, and face masks.

Impression procedure: Maxillary impressions were made using putty and light-body PVS impression material with a single-stage technique. Tray adhesive was applied to metal perforated stock trays and allowed to dry as per the manufacturer's instructions. The tray was seated centrally over the maxillary arch, stabilised until setting, and removed after polymerisation.

Sample preparation and grouping: Each impression was rinsed under running tap water to remove saliva and debris [1]. Using a sterile cork borer of 13 mm diameter, four standardised samples were obtained from each impression [Table/Fig-7] [8]. The samples were divided into four groups:

- Group A: Control
- Group B: HiOra™
- Group C: 2% glutaraldehyde
- Group D: Electrolysed Oxidising Water (EOW)

[Table/Fig-7]: Maxillary impression with four samples removed.

Disinfection procedure: The samples were immersed in their respective disinfectant solutions for 10 minutes [Table/Fig-8] [1,8,9]. Control samples were kept in closed sterile containers for the same duration [Table/Fig-8]. The 10-minute holding period for the control group was maintained to standardise the time interval across all groups and to eliminate time-related variability in microbial survival. This ensured that differences in colony counts were attributable to the disinfectant action rather than variations in processing time. Following disinfection, all samples were rinsed with tap water and transferred to sterile containers containing saline for microbiological analysis [8],

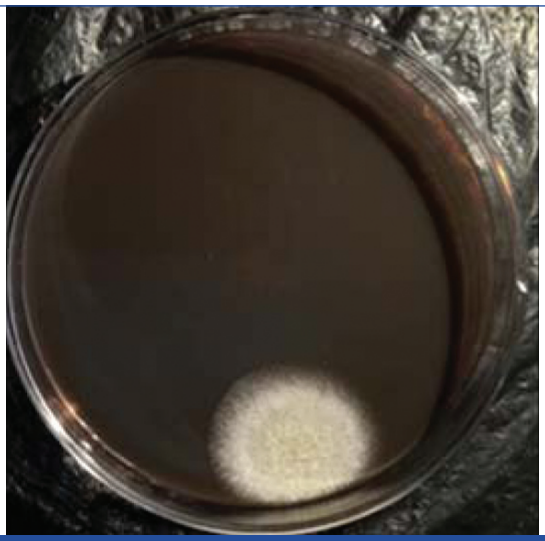
[Table/Fig-8]: Sample testing with sterile containers labelled.

Microbiological analysis: Each impression sample was subjected to the respective disinfection procedure within five minutes of impression removal to minimise variations in microbial load due to

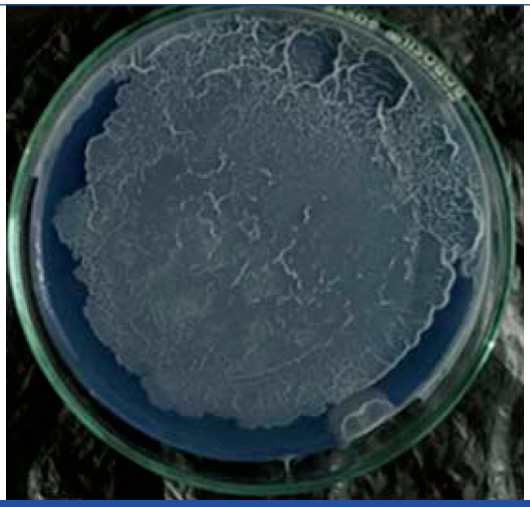
time delay. The samples were then emulsified in 10 mL of sterile saline and agitated for five minutes. A calibrated micropipette was used to inoculate 0.1 mL of the saline suspension onto Mitis salivarius agar for anaerobic microorganisms and Eosin Methylene Blue (EMB) agar for aerobic microorganisms. The plates were incubated at 37°C for 24 hours under anaerobic and aerobic conditions, respectively.

Identification and Colony Counting

Anaerobic organisms, including *Streptococcus* species and *Enterococcus faecalis*, were presumptively identified based on colony morphology on Mitis salivarius agar [Table/Fig-9]. Aerobic organisms such as *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Aspergillus niger* were presumptively identified on EMB agar. EMB based on characteristic colony morphology and growth pattern [Table/Fig-10]. CFU were counted after 24 hours of incubation and expressed as log CFU/mL.



[Table/Fig-9]: Mitis salivarius agar plate after innoculation.



[Table/Fig-10]: EMB agar plate after inoculation.

The CFU assessment was performed using the standard spread plate technique. Following incubation, visible colonies were manually counted using a digital colony counter under adequate illumination. Plates showing 30-300 colonies were considered for reliable quantification.

All microbiological procedures and CFU counting were carried out by a single experienced microbiologist to ensure consistency in interpretation. The examiner was blinded to the group allocation during colony counting to minimise observer bias.

STATISTICAL ANALYSIS

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software version 23.0 (IBM Corp., Armonk, NY, USA). The level of significance was set at 5% ($p<0.05$). Data were assessed for normality using the Shapiro-Wilk test. As the CFU data were not normally distributed, non parametric tests were applied. Quantitative variables were expressed as mean±standard deviation and median with Interquartile Range (IQR). Inter-group comparison of aerobic and anaerobic CFU counts (Log CFU/mL) among the four independent groups was performed using the Kruskal–Wallis test. For pairwise comparison between groups, post-hoc analysis was conducted using Bonferroni correction. As the study design involved independent groups without repeated measurements, intra-group comparison was not applicable.

RESULTS

The present comparative study evaluated the disinfectant efficacy of HiOra™, 2% glutaraldehyde, and EOW on clinically derived PVS impressions obtained from 11 subjects. All participants were within the age group of 18-45 years. The demographic profile of the study participants, including age (mean±SD) and gender distribution {n (%)}, is presented in [Table/Fig-11]. A total of 44 samples were analysed, with 11 samples in each group: group A (control), group B (HiOra™), group C (2% glutaraldehyde), and group D (EOW). Aerobic and anaerobic microbial contamination was quantified and expressed as log CFUs per millilitre (CFU/mL).Species-level quantitative differentiation was not performed, as the primary outcome measure was overall microbial reduction. The effect size could not be calculated as the original dataset was not available for secondary statistical analysis. This is acknowledged as a limitation of the study.

Variables	Value
Male	6 (54.5%)
Female	5 (45.5%)
Age (years)	33.0±7.35

[Table/Fig-11]: Demographic characteristics of the study participants.

Aerobic microbial growth: Aerobic bacterial colony growth was observed in all four groups. The mean aerobic CFU counts in group A (control), group B (HiOra™), group C (2% glutaraldehyde), and group D (EOW) were 1.02±0.73, 0.80±0.81, 0.36±0.51, and 0, respectively [Table/Fig-12]. The median CFU values also showed a similar trend, with the highest values in the control group and lowest in the EOW group [Table/Fig-12].

Group	Mean±SD	Median (IQR)	Mean rank	p-value
Group A	1.02±0.73	1 (1.48)	30.23	0.003*
Group B	0.80±0.81	1 (1.48)	26.41	
Group C	0.36±0.51	0 (1.00)	19.86	
Group D	0	0	13.50	

[Table/Fig-12]: Comparison of CFU count (Log CFU/ml) for aerobic microorganisms. Kruskal–Wallis test; showed a statistically significant difference among the groups (H=13.97, p=0.003) IQR: Interquartile range;
*indicates statistically significant difference at p≤0.05.

Intergroup comparison revealed a statistically significant difference in aerobic CFU counts among the four groups ($p<0.05$). Pairwise comparison demonstrated that group D (EOW) showed significantly lower aerobic CFU counts compared to group A (control) and group B (HiOra™), and was comparable to group C (2% glutaraldehyde). No statistically significant difference was observed among group A, group B, and group C [Table/Fig-13].

Anaerobic microbial growth: Anaerobic bacterial growth was assessed in all groups. The mean anaerobic CFU counts in group A (control), group B (HiOra™), group C (2% glutaraldehyde), and group D (EOW) were 2.67±1.09, 1.26±1.27, 0.72±1.40, and 0.09±0.30, respectively [Table/Fig-14]. Median CFU values followed a similar decreasing trend from the control group to the EOW group [Table/Fig-14].

Comparison	Difference in CFU	p-value
Group A vs Group B	0.22	0.431
Group A vs Group C	0.66	0.195
Group A vs Group D	1.02	0.003*
Group B vs Group C	0.44	1.000
Group B vs Group D	0.80	0.046*
Group C vs Group D	0.36	1.000

[Table/Fig-13]: Pairwise comparison of CFU count (Log CFU/ml) for aerobic microorganisms.
Post-hoc Dunn–Bonferroni test; Z=standardised test statistic; *indicates statistically significant difference at p≤0.05.

Group	Mean±SD	Median (IQR)	Mean rank	p-value
Group A	2.67±1.09	3.24 (IQR=1.08)	34.59	<0.001*
Group B	1.26±1.27	1 (IQR=2.17)	27.05	
Group C	0.72±1.40	0 (IQR=1.00)	17.27	
Group D	0.09±0.30	0 (IQR=0)	11.09	

[Table/Fig-14]: Comparison of CFU count (Log CFU/mL) for anaerobic microorganisms.
Kruskal-Wallis test showed a statistically significant difference among the groups (H=21.63, p<0.001); IQR: Interquartile Range; * indicates statistically significant difference at p≤0.05.

Statistical analysis revealed a significant difference in anaerobic CFU counts among the four groups (p<0.05). Pairwise comparison showed that group D (EOW) exhibited significantly lower anaerobic CFU counts compared to group A (control) and group B (HiOra™), and results comparable to group C (2% glutaraldehyde). Additionally, group C demonstrated significantly lower CFU counts than the control group [Table/Fig-15].

Comparison	Difference in CFU	p-value
Group A vs Group B	1.41	0.940
Group A vs Group C	1.95	0.007*
Group A vs Group D	2.58	<0.001*
Group B vs Group C	0.54	0.399
Group B vs Group D	1.17	0.016*
Group C vs Group D	0.63	1.000

[Table/Fig-15]: Pairwise comparison of CFU count (Log CFU/mL) for anaerobic microorganisms.
Post-hoc Dunn-Bonferroni test; * indicates statistically significant difference at p ≤ 0.05.

Overall, the null hypothesis was rejected, as a statistically significant difference was observed in the antimicrobial efficacy of the three disinfectants tested (p<0.05). EOW demonstrated the highest disinfection efficacy against both aerobic and anaerobic microorganisms, followed by 2% glutaraldehyde, while HiOra™ showed the least antimicrobial effectiveness.

DISCUSSION

Infection control is fundamental in dental practice, as impressions are frequently contaminated with saliva and blood containing pathogenic microorganisms capable of cross-infection [10-12]. Effective disinfection protocols are therefore essential to prevent transmission between patients, clinicians, and laboratory personnel [13].

The PVS was selected due to its dimensional stability and compatibility with immersion disinfectants [1,14]. Unlike in-vitro studies using artificial contamination, this study evaluated patient-derived impressions, better reflecting real clinical microbial variability [1,15].

The results demonstrated a statistically significant difference in antimicrobial efficacy among the tested disinfectants (p<0.05). EOW showed the highest reduction in both aerobic and anaerobic microbial counts, followed by 2% glutaraldehyde, while HiOra™ showed the least efficacy. The superiority of immersion disinfection supports previous findings that spray methods may be inadequate [16].

Although glutaraldehyde remains a well-established high-level disinfectant with broad-spectrum activity [10,13,17], its slightly lower efficacy in the present study may be influenced by interference from organic matter and required contact time. Moreover, concerns regarding toxicity, respiratory irritation, and environmental hazards limit its long-term occupational safety.

The marked efficacy of EOW may be attributed to HOCl and reactive oxygen species, which rapidly disrupt microbial membranes and intracellular enzymes [1,3]. Its high oxidation-reduction potential and small molecular size allow better penetration even in the presence of organic load. The current study findings are consistent with Jeyapalan V et al., and Iram A et al., who reported significant microbial reduction with EOW [1,3]. Unlike aldehyde-based disinfectants, EOW is biodegradable, produces minimal residue, and is less cytotoxic [18].

The lower efficacy observed with HiOra™ requires critical consideration. Herbal agents often exert bacteriostatic effects and may be concentration- or time-dependent. The commercially available formulation and immersion duration used in this study may not have been sufficient to achieve high-level disinfection. Although herbal formulations are valued for biocompatibility and safety [9], they may not provide adequate antimicrobial potency for impression disinfection without modification.

Variability in microbial counts among samples can be attributed to differences in patients' oral hygiene status, salivary composition, and intraoral microbial diversity. The relatively lower bacterial counts observed in the control group may be explained by several factors. Many patients had acceptable oral hygiene levels, which could reduce baseline microbial load. In addition, pre-procedural rinsing and natural salivary cleansing may have reduced the number of loosely adherent microorganisms present at the time of impression making. The short intraoral exposure time of the impression material and prompt transfer of the samples for microbiological processing may have further limited bacterial proliferation. Similar observations have been reported in clinical studies evaluating microbial contamination of dental impressions, where microbial counts may vary depending on patient-related and procedural factors.

While the statistical differences were significant, clinical significance depends on achieving sufficient microbial reduction to prevent cross-infection. The near-complete elimination observed with EOW is clinically meaningful, whereas residual growth with HiOra™ raises concerns regarding its standalone use.

From a practical perspective, glutaraldehyde is economical but associated with occupational hazards. EOW offers advantages such as safety and environmental compatibility; however, its short shelf life requires fresh preparation and standardisation of parameters such as pH and available chlorine concentration, which may affect large-scale implementation.

A major strength of the present study is the evaluation of both aerobic and anaerobic microorganisms, providing a comprehensive assessment of disinfection efficacy. Within the limitations of the study, EOW demonstrated antimicrobial efficacy comparable to or greater than 2% glutaraldehyde with fewer safety concerns. Herbal disinfectants may have adjunctive roles but require further investigation regarding concentration and exposure time. Future studies may evaluate different disinfectants at varying concentrations and assess their effects on multiple impression materials from different manufacturers.

Limitation(s)

The present study did not assess the effect of disinfectants on dimensional stability or surface detail reproduction of the impression material. Lack of blinding and randomisation may introduce bias. Antimicrobial efficacy was evaluated at a single immersion time

(10 minutes) without dose-response assessment. Only quantitative bacterial load was measured; specific microbial species were not identified. Additionally, evaluation of a single impression material limits the generalisability of the findings. The relatively small sample size of 11 specimens per group may limit the generalisability of the findings. Larger, adequately powered clinical studies are warranted to validate the present findings.

CONCLUSION(S)

Cross-infection control in dental clinics and laboratories remains essential, as impressions are commonly contaminated with saliva and blood. Within the limitations of this study, 2% glutaraldehyde and EOW showed superior disinfection efficacy compared to HiOra®, with EOW demonstrating the greatest reduction in both aerobic and anaerobic microorganisms. Clinically, immersion disinfection for at least 10 minutes using either 2% glutaraldehyde or EOW is recommended for PVS impressions. EOW may be preferred where reduced toxicity and environmental safety are priorities. Herbal disinfectants should not be relied upon as sole agents for high-level impression disinfection. Further studies are required to assess long-term material compatibility and broader clinical applicability.

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