

Effectiveness of Saltwater Rinse Against Various Periopathogens Identified in the Elderly Population with Prosthetic Needs: A Prospective Clinical Study

SHREYA JAIN¹, NEERJA MAHAJAN², HAREKRISHNA JAYENDRA RAVAL³, RIYA PATEL⁴, KRISHNA DAVE⁵, SANAT GOKHALE⁶, SIDDHARTH GOLECHHA⁷



ABSTRACT

Introduction: Elderly denture wearers face increased oral health risks due to reduced saliva, systemic conditions, and hygiene challenges. Many commercially available mouthwashes contain harmful ingredients, limiting long-term use. The study assesses the effectiveness of a saltwater rinse as a safe, cost-effective alternative to reduce periopathogens and improve periodontal health for elderly denture users.

Aim: To investigate periopathogens in edentulous and partially edentulous patients using Polymerase Chain Reaction (PCR) and to determine the Minimum Inhibitory Concentration (MIC) of sodium chloride for saline mouth rinse.

Materials and Methods: The present prospective clinical study in the Department of Prosthodontics and Crown and Bridge, at KM Shah Dental College and Hospital, Vadodara, Gujarat, India from May 2022 to March 2024 involved 42 healthy participants (24 males, 18 females) with partial (Group A, n=21) and complete edentulism (Group B, n=21). Eight periopathogens were identified using PCR, and the MIC of sodium chloride

evaluated was 3.5 M. Participants rinsed with saltwater twice daily for 14 days, with microbial samples collected before and after to assess Colony-Forming Units (CFUs) and periodontal health. Data were analysed at a 95% confidence level ($p < 0.05$) using paired t-tests and Wilcoxon signed-rank tests.

Results: The 14-day saltwater rinse significantly reduced microbial counts and improved periodontal health. In completely edentulous participants, CFUs decreased from 2.5 to 1.1, while in partially edentulous participants, CFUs dropped from 2.3 to 1.2 ($p < 0.001$). Additionally, the Visible Plaque Index (VPI) improved from 2.4 to 1.8 ($p < 0.001$), Clinical Attachment Level (CAL) from 4.5 to 3.5 ($p < 0.001$), Gingival Bleeding Index (GBI) from 2.01 to 1.2 ($p < 0.001$), and Periodontal Probing Depth (PPD) from 3.5 to 2.7 ($p < 0.001$).

Conclusion: A 3.5 M saltwater rinse effectively reduces oral microbial load and improves periodontal health, suggesting its potential as a simple, safe, and cost-effective home-made adjunct for routine oral hygiene maintenance in both completely and partially edentulous elderly individuals.

Keywords: Microbial load, Oral health, Polymerase chain reaction, Salt rinse, Sodium chloride

INTRODUCTION

Ageing, systemic health conditions, daily oral hygiene practices, and the type of dental prosthesis can strongly influence the oral microbial flora of elderly individuals who wear dentures. The build-up of microorganisms and biofilm formation on dentures and oral tissues can negatively impact oral health, making denture-related microbiology an important area of research [1].

The human oral cavity hosts a diverse microbiota, comprising over 700 bacterial species, including approximately 400 species found in periodontal pockets, as well as organisms that colonise the oral mucosa, tongue, root canals, and carious lesions [2]. Oral microbial balance plays a critical role not only in oral health but also in systemic health, and advances in microbiome research may support the development of personalised dental care [3].

Elderly individuals who are fully or partially edentulous exhibit a heightened vulnerability to microbial accumulation, biofilm formation, and periodontal complications. This susceptibility is primarily attributed to the use of dentures, a reduction in salivary flow, and the inherent challenges associated with maintaining proper oral hygiene.

People who wear dentures have distinct microbial populations influenced by factors such as biofilm development, the materials used in their dentures, and their hygiene practices. Regular professional cleaning, along with personalised hygiene plans, is

crucial for preventing infections associated with dentures and maintaining good oral health [4].

Mouth rinses are frequently utilised in dentistry for the prevention and treatment of oral health conditions. However, a significant number of these mouthwashes contain alcohol, which can lead to various side-effects [5,6]. Consequently, they are generally considered unsuitable for long-term use.

Encouraging daily oral hygiene practices among the elderly is essential, as these habits can provide long-term benefits and reduce undesirable effects. Natural and alternative methods have shown promising potential as antimicrobial mouthwashes and most of them appear to be safe. However, there remains a lack of high-quality research supporting their clinical effectiveness [7].

Therefore, the current study aimed to identify periopathogens in completely and partially edentulous participants and to formulate an effective saltwater rinse based on MIC findings as the primary objective.

The antimicrobial effect of the rinse was assessed by comparing CFUs before and after use, with additional evaluation of periodontal health in partially edentulous participants using the GBI, Visible Plaque Index (VPI), CAL, and PPD as secondary objectives.

The null hypothesis stated that there would be no difference in microbial counts before and after the saltwater rinse, while the alternative hypothesis proposed a significant reduction in CFUs after its use.

MATERIALS AND METHODS

The present study was a prospective clinical study conducted in the Department of Prosthodontics and Crown and Bridge, at KM Shah Dental College and Hospital, Vadodara, Gujarat, India. The study was conducted from May 2022 to March 2024. The research was carried out after obtaining consent from the institutional ethical committee, with the approval number SVIEC/ON/DENT/BNPG21/022065. The study was conducted under the Declaration of Helsinki, which outlines the ethical principles for research involving human subjects. All participants were provided with a participation information sheet, and their informed consent was obtained.

Sample size calculation: The sample size was calculated using the estimates of standard deviation and mean values (VPI-0.43, GBI-0.39, PPD-2.79, and CAL-3.26) from the study conducted by Fernandes CB et al., [8].

The formula used was:

$$n = \frac{2(Z\alpha + Z\beta)^2 (s)^2}{d^2}$$

Where, $Z\alpha$ is the z variate of alpha error, i.e., a constant with a value of 1.96, and $Z\beta$ is the z variate of beta error, i.e., a constant with a value of 0.84. Approximately, a minimum of 21 subjects/patients per group was required for the study. Therefore, the total sample size considered was 42 participants [8].

Twenty-one elderly partially edentulous patients (Group A) and twenty-one completely edentulous patients (Group B), from the Department of Prosthodontics, Crown and Bridge, were selected.

Inclusion criteria: Participants included were completely or partially edentulous elderly individuals who had used dentures for over six months, systemically healthy, and willing to participate in the study. Additionally, partially edentulous individuals with generalised periodontitis who desired Removable Partial Dentures (RPD) were also included.

Exclusion criteria: Patients were excluded from the study if they had surgical or congenital defects in the maxilla or mandible, respiratory tract diseases, tonsillitis, or stomach disorders. Additionally, immunocompromised individuals, pregnant patients, and those with a history of taking antibiotics within the past six months were not included. Patients who smoked were also excluded from the study.

Study Procedure

Detailed dental and medical histories of the subjects were taken.

Identification of Periopathogens

A. Collection of Microorganisms

Microbial samples were collected from the top of the tongue and the left cheek of all participants. A swab, moistened with Ringer's solution, was used to sample an area of approximately one square centimetre. The swab was then placed in a microtube containing one milliliter of Ringer's solution. The same investigator conducted both the sample collection and the clinical measurements [8].

In participants with partial edentulism, subgingival samples were collected. Sterile paper points were inserted into the gum pockets of the maxillary first and second molars, depending on the condition, as well as the maxillary central or lateral incisors (lateral incisors were included only when the central incisors were missing). Before collecting these samples, plaque on the tooth surfaces was carefully removed. If the participants did not have these specific teeth, samples were taken from adjacent teeth instead. The paper points were left in the gum pockets for 60 seconds and then placed into microtubes containing Ringer's solution.

B. PCR Procedure

The PCR is considered the gold standard for identifying microbial organisms. First, the microbial cells in the microtube were mixed

using a vortex mixer at maximum speed for one minute and then stored at -80°C for further laboratory analysis. PCR was employed to detect the presence of several bacteria, including *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Prevotella intermedia*, *Campylobacter rectus*, *Eikenella corrodens*, *Fusobacterium nucleatum*, and *Treponema denticola*.

To begin processing, the bacterial suspensions were thawed and then centrifuged at 12,000 rotations per minute (rpm) for three minutes. Following this, Deoxyribonucleic Acid (DNA) was extracted from the microbial pellet using a commercial DNA isolation kit, and a standardised PCR protocol was employed. The primers [8] used are detailed in [Table/Fig-1].

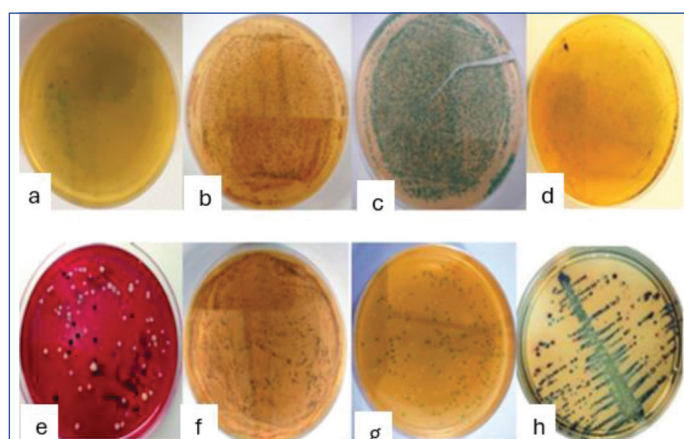
Microorganisms	Primers used
<i>A. actinomycetemcomitans</i>	5'-AAACCCATCTCTGAGTTCTTCTTC3' 5'-ATGCCAACTTGACGTAAAT3'
<i>P. gingivalis</i>	5'-AGGCAGCTTGCCATACTGCGG-3' 5'-ACTGTTAGCAACTACCGATGT-3'
<i>P. intermedia</i>	5'-TTTGTGGGGAGTAAAGCGGG-3' 5'-TCAACATCTCTGTATCTGCGT-3'
<i>T. forsythia</i>	5'-GCGTATGTAACTGCCCGCA-3' 5'-TGCTTCAGTGTGCTATACCT-3'
<i>T. denticola</i>	5'-TAATACCGAATGTGCTCATTTACAT-3' 5'-TCAAGAAGCATTCCCTCTTCTTTA-3'
<i>C. rectus</i>	5'-TTTCGGAGCGTAACTCCTTTT-3' 5'-TTTCTGCAAGCAGACACTCTT-3'
<i>E. corrodens</i>	5'-CTACTAAGCAATCAAGTTGCC-3' 5'-CTAATACCGCATACGCTCTAAG-3'
<i>F. nucleatum</i>	5'-AGA GTT TGA TCC TGG CTC AG-3' 5'-GTC ATC GTG CAC ACA GAA TTG CTG-3'

[Table/Fig-1]: Name of the primers.

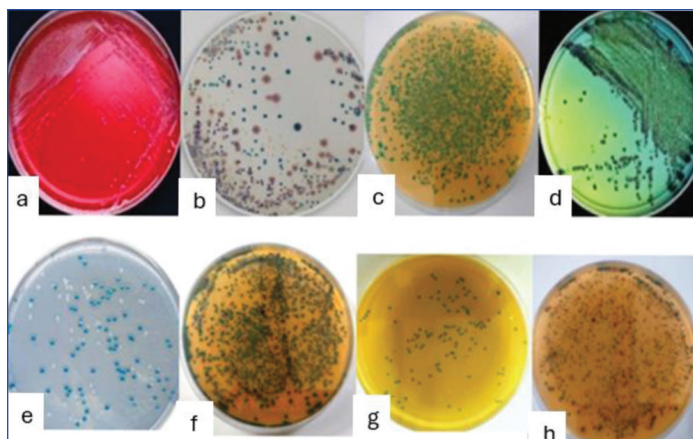
C. Minimum Inhibitory Concentration (MIC)

Identified bacteria via PCR are shown on chrome agar plates for the partially edentulous and edentulous groups in [Table/Fig-2,3], respectively. Later, isolates of the bacteria were grown in Luria broth to conduct the MIC test, illustrated in [Table/Fig-4].

The MIC of the saltwater was determined using the macro broth dilution method. This involved adding 500 microliters of saltwater with concentrations ranging from 0.1 M to 7.0 M into separate labelled test tubes. Next, 500 microliters of the test material, which consisted of broth cultures containing *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Prevotella intermedia*, *Campylobacter rectus*, *Eikenella corrodens*, *Fusobacterium nucleatum*, and *Treponema denticola*, were added to each test tube. The test tubes were then incubated overnight at 37°C . The lowest salt concentration that inhibited visible bacterial growth was considered the MIC [9].

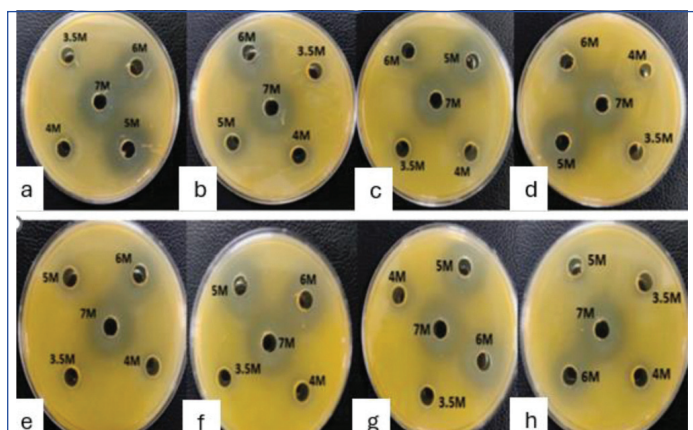


[Table/Fig-2]: Bacteria identified by PCR in the Group A displayed on chrome agar plates.
a) *P. intermedia*; b) *A. actinomycetemcomitans*; c) *P. gingivalis*; d) *T. forsythia* e) *C. rectus*; f) *F. nucleatum*; g) *T. denticola*; h) *E. corrodens*



[Table/Fig-3]: Bacteria identified by PCR in the Group B displayed on chrome agar plates.

a) *Campylobacter rectus*; b) *A. actinomycetemcomitans*; c) *P. gingivalis*; d) *E. corrodens* e) *T. denticola*; f) *T. forsythia*; g) *P. intermedia*; h) *F. nucleatum*



[Table/Fig-4]: Isolates of bacteria grown on Luria broth for MIC.

a) *P. gingivalis*; b) *A. actinomycetemcomitans*; c) *P. intermedia*; d) *T. forsythia* e) *T. denticola*; f) *F. nucleatum*; g) *E. corrodens*; h) *C. rectus*

The MIC of Sodium Chloride (NaCl) against the tested periopathogens was assessed using various concentrations, specifically 100 mM, 500 mM, 1 M, 2 M, 3 M, 3.5 M, 4 M, 5 M, 6 M, 6.5 M, and 7 M. These concentrations were prepared through serial dilution from a stock solution. The results indicated that the MIC for all periopathogens was 3.5 M [Table/Fig-5].

Microorganisms	MIC Molarity (M)
<i>P. gingivalis</i>	3.5
<i>T. forsythia</i>	3.5
<i>P. intermedia</i>	3.5
<i>A. actinomycetemcomitans</i>	3.5
<i>T. denticola</i>	3.5
<i>F. nucleatum</i>	3.5
<i>C. rectus</i>	3.5
<i>E. corrodens</i>	3.5

[Table/Fig-5]: The saltwater's Minimum Inhibitory Concentration (MIC) against different microorganisms.

Formulation of NaCl Rinse: Sodium chloride (Laboratory AaturRasayan, India) was dissolved in 100 millilitres of distilled water in a beaker to create a salt solution.

The formula used to calculate the amount of NaCl required was as follows

Amount of NaCl=(Molecular weight of NaCl/1000)×required molarity×required volume [10].

The molecular weight of NaCl is 58.44.

Procedure for Performing CFU: The CFU procedure was done to quantitatively measure viable oral bacteria before and after the intervention, allowing objective evaluation of the antimicrobial effect

of the saltwater rinse. After thawing at 36°C in a water bath, the test tube was vortexed for 30 to 45 seconds. 100 µL of the undiluted sample and subsequent dilutions were used to isolate and identify microorganisms. In Peptone Yeast (PY) medium, serial dilutions ranging from 10⁻¹ to 10⁻³ were prepared. Each dilution was cultured anaerobically for ten days at 37°C on Brain Heart Infusion (BHI) agar. The resultant colony types were then phenotypically assessed and calculated to know the number of CFUs per milliliter (mL) in the initial sample [11].

Clinical Assessment of Patients for CFUs (Pre-Saltwater Rinse):

For completely edentulous patients, oral health was assessed through a visual examination and a comprehensive case history. In the case of partially edentulous patients, periodontal health was evaluated using a clinical examination that included the following indices: The GBI developed by Muhlemann and Sons S in 1971, [12], The VPI by Silness and Loe from 1964, [12], CAL based on guidelines from the American Academy of Periodontology (AAP) and the European Federation of Periodontology (EFP) from 2018 [13], PPD according to the British Society of Periodontology guidelines from 2011 [14].

Instructions to Patients for Usage: The patients were given a saltwater rinse prepared by the researcher to use for 14 days. They were instructed to use 10 mL of the rinse twice a day: once in the morning after brushing and the other at night before going to bed. The rinsing process was explained verbally and with the help of videos. On the fifteenth day, the participants returned for post-rinse evaluations, and a new microbial sample was taken. Patients were told not to use the mouth rinse on the 15th day.

Clinical Assessment of Patients for CFUs (Post Saltwater Rinse):

The same procedure as above was followed for collecting the sample. Finally, after using a saline mouth rinse for 14 days, pre and post-CFU microbes were compared in completely and partially edentulous patients.

STATISTICAL ANALYSIS

The data were analysed using the Statistical Package for Social Sciences (SPSS) v 26.0, IBM. Statistical tests were performed, representing a p-value of <0.05, indicating statistical significance. Descriptive statistics, including frequencies and percentages for categorical data, and mean and Standard Deviation (SD) for numerical data, were also used. The Shapiro-Wilks test was used to assess normality, indicating that the data for Partially Edentulous Patients (Group A), Completely Edentulous Patients (Group B), PI and CAL followed a normal distribution, allowing for the use of paired t-tests for comparisons. However, the data for GBI and PPD did not follow a normal distribution, needing the use of the Wilcoxon Signed-rank Test for comparisons.

RESULTS

The PCR analysis identified periopathogens in both completely and partially edentulous patients. A total of eight periopathogens-*Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia*, *Prevotella intermedia*, *Campylobacter rectus*, *Eikenella corrodens*, *Fusobacterium nucleatum*, and *Treponema denticola* were detected in both groups.

All 42 participants completed the study and were followed for a duration of 14 days [Table/Fig-6]. In the partially edentulous group

Parameters	Partially edentulous patients (Group A)	Completely edentulous patients (Group B)	Total
Male	11	13	24
Female	10	8	18
Total	21	21	42
Mean age	55.7±7.05 years	57.8±8.69 years	

[Table/Fig-6]: Demographic distribution of the population.

(Group A), there were 10 female patients and 11 male patients. In the completely edentulous group (Group B), there were 8 female patients and 13 male patients.

The CFU counts before and after the saltwater rinse was compared in both groups [Table/Fig-7]. Paired t-tests showed a significant reduction in CFU after 14 days. In the completely edentulous group, CFU decreased from 2.5 ± 0.3 at baseline to 1.1 ± 0.19 at day 14. Similarly, in the partially edentulous group, CFU decreased from $2.3 \pm 0.2 \times 10^3$ CFU/mL at baseline to $1.2 \pm 0.38 \times 10^3$ CFU/mL at day 14 [Table/Fig-7].

Variables	Mean	Std. Deviation	Mean difference	SD of the difference	T value	p-value of paired t-test
Group A Baseline	2.341667	0.2668930	1.1250000	0.3732932	14.764	** p<0.001
Group A Follow-up	1.216667	0.3806935				
Group B Baseline	2.533333	0.3396503	1.3458333	0.2873858	22.942	** p<0.001
Group B Follow-up	1.187500	0.1962972				

[Table/Fig-7]: Comparison of pre vs post values of CFU in partially and completely edentulous groups using paired t-test.

Paired t-test; Group A: Partially edentulous patients; Group B: Completely edentulous patients; **p<0.001 (highly significant)

Periodontal parameters were also assessed in the partially edentulous group. [Table/Fig-8] shows a paired t-test, which presents that the PI reduced from 2.4 ± 0.3 at baseline to 1.8 ± 0.42 after 14 days, and the CAL decreased from 4.5 ± 0.3 to 3.5 ± 0.39 , both showing significant improvements.

Variables	Mean	SD	Mean difference	SD of the difference	T value	p-value of paired t-test
VPI Baseline	2.429167	0.3838922	0.5583333	0.1639636	16.682	p<0.001
VPI Follow-up	1.870833	0.4216728				
CAL Baseline	4.508333	0.3322082	0.9541667	0.3175712	14.764	p<0.001
CAL 14D	3.554167	0.3988889				

[Table/Fig-8]: Comparison of Pre vs post values of Visible Plaque Index (VPI), Clinical Attachment Level (CAL) in the partially edentulous group using paired t-test. Paired t-test; VPI: Visible plaque index; CAL: Clinical attachment level; p<0.05

[Table/Fig-9] of the Wilcoxon Signed-rank Test presents the GBI and PPD results. The GBI decreased from 2.01 ± 0.6 at baseline to 1.2 ± 0.59 after 14 days, and the PPD reduced from 3.5 ± 0.3 to 2.7 ± 0.66 , both showing statistically significant changes. [Table/Fig-10] shows pre and post-CFU in partially and completely edentulous groups inoculated in BHI Broth.

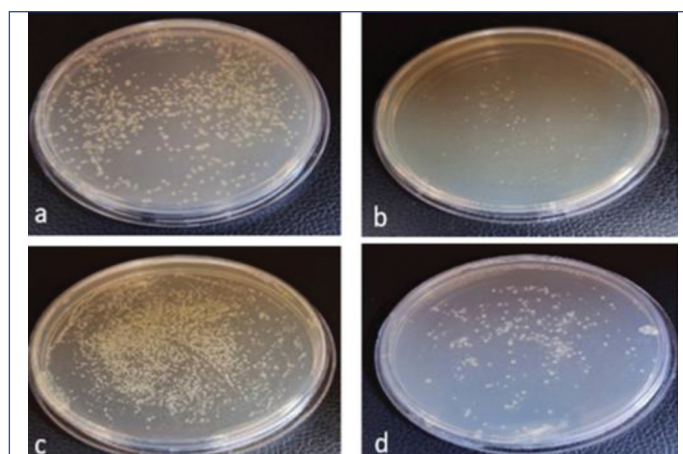
Variables	Mean	SD	Z value	p-value of the Wilcoxon signed-rank test
GBI baseline	2.016667	0.6133774	-4.295	p<0.001
GBI follow-up	1.237083	0.5977893		
PPD baseline	3.595833	0.3928482	-3.501	p<0.001
PPD follow-up	2.775000	0.6634888		

[Table/Fig-9]: Comparison of pre- vs. post-values of Periodontal Probing Depth (PPD) and Gingival Bleeding Index (GBI) in the partially edentulous group using the Wilcoxon Signed-rank Test.

Wilcoxon Signed-rank Test; GBI: Gingival bleeding index; PPD: Periodontal probing depth; p<0.05

DISCUSSION

The study revealed a notable reduction in the number of bacteria in both completely and partially edentulous groups, as well as improvements in the PI, GBI, PPD, and CAL after using saline



[Table/Fig-10]: Pre and post-CFU in partially and completely edentulous groups inoculated in BHI broth: a) Pre-CFU in partially edentulous; b) Post-CFU in partially edentulous; c) Pre-CFU in completely edentulous; d) Post-CFU in completely edentulous.

mouthwash for 14 days. Consequently, the null hypothesis was rejected, showing that rinsing with saltwater is indeed effective against Periopathogens.

At present, there are no published studies evaluating the use of saline mouth rinses specifically in patients with prosthodontic needs, limiting direct comparison of the present findings with existing literature. However, in different research setups and for different microbes, some authors have successfully used a saline mouth rinse. In a separate study by Collins JR et al., it was found that a sodium chloride mouth rinse after periodontal surgery had anti-inflammatory properties like Chlorhexidine (CHX) mouthwash and could be used regularly after such procedures [15]. In another study, Hoover J et al., also stated no difference between the impact of a unique mouthwash containing lysozyme, xylitol, and sea salt on biofilm development and gingival health in young people [16].

The present study used a mouthwash concentration of 3.5 M, as this level effectively targets all tested organisms. Research indicates that increasing the molarity beyond 23% (4 M) often results in participants reporting a strong, pungent, and irritating taste [17]. Irritation may occur due to the hyperstimulation of the ion channels in the taste cells. Since Sodium Ions (Na⁺) are responsible for the perception of a salty taste, a 4M saline solution can lead to an overwhelming stimulus discharge because of the abundant sodium ion receptors present on the tongue [18].

The presence of a salt solution uniquely inhibits the growth of oral microbes. At low concentrations, the environment becomes hypotonic, which allows oral bacteria to absorb ions using a respiratory enzyme in mesosomes powered by adenosine triphosphate. This leads to water entering the cells, allowing the bacteria to thrive and proliferate in a hypotonic environment. However, at high concentrations of salt solution, the solute concentration in the surrounding solution becomes higher than that of the oral bacteria's cytoplasm, causing water to exit the cells. As a result, the oral bacteria start to dehydrate within a minute, ultimately leading to their death [19].

A study was conducted to evaluate the effectiveness of warm saline mouthwash in reducing post-surgery complications after dental extractions. The results showed that rinsing with warm saline mouthwash can effectively prevent alveolar osteitis compared to those who did not rinse [20]. Additionally, another Randomised Controlled Trial (RCT) suggested that using warm saline rinses after dental extractions can lower the risk of developing alveolar osteitis, also known as dry socket, following the procedure [21].

The mechanism of action described involves a significant increase in the movement of human Gingival Fibroblasts (hGFs) when rinsed with a solution containing 0.9-1.8% NaCl. Rinsing with a 1.8% NaCl solution notably enhanced the expression of type I collagen and

fibronectin. The NaCl solution was found to promote the expression of extracellular matrix genes (COL1 and Fn), improve hGF cell motility, and change the arrangement of cytoskeletal components, such as Focal Adhesion Kinase (FAK) and F-actin. These findings provide empirical evidence supporting the use of a salt solution as a mouthwash and in regular dental care to accelerate the healing of oral wounds [22].

The microorganisms identified in this study through PCR analysis are consistent with those commonly reported in previous oral microbiological literature. It has been found that bacteria such as *P. intermedia*, *A. actinomycetemcomitans*, *C. rectus*, *E. corrodens*, *T. forsythia*, *T. denticola*, *P. gingivalis*, and *P. micra* are commonly found in both edentulous and dentulous subjects [8].

After tooth loss, research has shown that the presence of certain bacteria typically found in periodontal pockets, like *P. gingivalis* and *P. intermedia*, tends to decrease in the mouth. However, bacteria that prefer hard surfaces for attachment and growth can still colonise the oral cavity, especially when dentures are in place [23].

After six months following denture insertion, the authors confirmed the presence of several periodontal pathogens, including *A. actinomycetemcomitans*, *F. nucleatum*, *P. intermedia*, *T. denticola*, *T. forsythia*, and *P. gingivalis* [24]. Numerous studies in the literature have shown that the periodontal pathogens *A. actinomycetemcomitans* and *P. gingivalis*, which were previously thought to be eradicated after the removal of all natural teeth, were found in significant quantities among edentulous participants [8,25,26].

Understanding the presence of oral bacteria in elderly patients is essential, especially since implants have become the standard treatment for edentulism. The oral microbiota significantly influences the success of dental implants. A recent systematic review found a connection between peri-implantitis and specific periodontal pathogens, including *P. gingivalis*, *T. forsythia*, *T. denticola*, *F. nucleatum*, and *P. intermedia* [27]. It has also been noted that *P. intermedia*, *P. gingivalis*, *T. forsythia*, *Campylobacter rectus*, *Treponema denticola*, and *A. actinomycetemcomitans* are linked to peri-implantitis [28-30].

Therefore, the significance of maintaining oral health through the utilisation of a salt rinse should not be underestimated, even when addressing advanced treatment modalities. This approach serves as an important adjunct to comprehensive oral hygiene practices, contributing to both the prevention of disease and the facilitation of healing. By integrating a salt rinse into one's oral care regimen, individuals can enhance their overall dental health and support more complex therapeutic procedures.

Limitation(s)

The effects of gender were not analysed in the current study. Furthermore, conducting longer-term studies with extended follow-up periods could offer more comprehensive insights into the lasting effects of saltwater rinsing on oral health care, thereby strengthening the evidence. Another limitation to consider is the compliance of patients in regularly using mouthwash. Future research in this area should include RCTs with a larger and more diverse group of participants, particularly those who are systemically compromised. Comparative studies between saltwater rinsing and standard chemical mouthwashes in a diverse group of compromised patients could provide further insight into the effectiveness of this regimen.

CONCLUSION(S)

Rinsing with 3.5 M saltwater has been demonstrated to effectively reduce the CFUs in both completely and partially edentulous patients. This finding indicates that saltwater rinsing may serve as a promising intervention for the prevention of periodontal disease and the maintenance of oral health in patients requiring both simple and advanced prosthetic solutions.

REFERENCES

- [1] Redfern J, Tosheva L, Malic S, Butcher M, Ramage G, Verran J. The denture microbiome in health and disease: An exploration of a unique community. *Lett Appl Microbiol* [Internet]. 2022;75(2):195-209. Available from: <http://dx.doi.org/10.1111/lam.13751>.
- [2] Paster BJ, Olsen I, Aas JA, Dewhirst FE. The breadth of bacterial diversity in the human periodontal pocket and other oral sites. *Periodontol 2000* [Internet]. 2006;42(1):80-87. Available from: <http://dx.doi.org/10.1111/j.1600-0757.2006.00174.x>.
- [3] Zarco MF, Vess TJ, Ginsburg GS. The oral microbiome in health and disease and the potential impact on personalised dental medicine: The oral microbiome. *Oral Dis* [Internet]. 2012;18(2):109-20. Available from: <http://dx.doi.org/10.1111/j.1601-0825.2011.01851.x>.
- [4] Madhan Kumar S, Natarajan S, Ks S, Sundarajan SK, Natarajan P, Arockiam AS. Dentures and the oral microbiome: Unraveling the hidden impact on edentulous and partially edentulous patients - a systematic review and meta-analysis. *Evid Based Dent* [Internet]. 2025;26(3):150. Available from: <http://dx.doi.org/10.1038/s41432-025-01149-0>.
- [5] Tartaglia GM, Tadakamadla SK, Connelly ST, Sforza C, Martin C. Adverse events associated with home use of mouthrinses: A systematic review. *Therapeutic Advances in Drug Safety*. 2019;10. Available from: <http://dx.doi.org/10.1177/2042098619854881>.
- [6] Alrashdan MS, Leao JC, Doble A, McCullough M, Porter S. The effects of antimicrobial mouthwashes on systemic disease: What is the evidence? *Int Dent J* [Internet]. 2023;73 Suppl2:S82-S88. Available from: <http://dx.doi.org/10.1016/j.identj.2023.08.012>.
- [7] Duane B, Yap T, Neelakantan P, Anthonappa R, Bescos R, McGrath C, et al. Mouthwashes: Alternatives and future directions. *Int Dent J*. 2023;73 Suppl 2(Suppl 2):S89-S97. Doi: 10.1016/j.identj.2023.08.011.
- [8] Fernandes CB, Aquino DR, Franco GC, Cortelli SC, Costa FO, Cortelli JR. Do elderly edentulous patients with a history of periodontitis harbour periodontal pathogens? *Clin Oral Implants Res. Clin Oral Implants Res* [Internet]. 2010;21(6):618-23.
- [9] Shetty S, Thomas B, Shetty V, Bhandary R, Shetty R. An in-vitro evaluation of the efficacy of garlic extract as an antimicrobial agent on periodontal pathogens: A microbiological study. *Ayu* [Internet]. 2013;34(4):445-451. Available from: <http://dx.doi.org/10.4103/0974-8520.127732>.
- [10] Aravindh V, Aswath Narayanan MB, Ramesh Kumar SG, Selvamary A, Sujatha A. Comparative evaluation of salt water rinse with chlorhexidine against oral microbes: A school-based randomized controlled trial. *J Indian Soc Pedod Prev Dent* [Internet]. 2017;35(4):319. Available from: http://dx.doi.org/10.4103/jisppd.jisppd_299_16.
- [11] Bernardi S, Karygianni L, Filippi A, Anderson AC, Zürcher A, Hellwig E, et al. Combining culture and culture-independent methods reveals new microbial composition of halitosis patients' tongue biofilm. *Microbiologopen* [Internet]. 2020;9(2):e958. Available from: <http://dx.doi.org/10.1002/mbo3.958>.
- [12] Peter S. *Essentials of Public Health Dentistry*. Arya Publishing House; 2017.
- [13] Germen M, Baser U, Lacin CC, Firatli E, İşsever H, Yalcin F. Periodontitis prevalence, severity, and risk factors: A comparison of the AAP/CDC case definition and the EFP/AAP classification. *Int J Environ Res Public Health* [Internet]. 2021;18(7):3459. Available from: <http://dx.doi.org/10.3390/ijerph18073459>.
- [14] BPE_Guidelines_2011.pdf. <https://share.google/d00Lg905IHQBcS10m>.
- [15] Collins JR, Veras K, Hernández M, Hou W, Hong H, Romanos GE. Anti-inflammatory effect of salt water and chlorhexidine 0.12% mouthrinse after periodontal surgery: A randomized prospective clinical study. *Clin Oral Investig* [Internet]. 2021;25(7):4349-57. Available from: <http://dx.doi.org/10.1007/s00784-020-03748-w>.
- [16] Hoover J, Tovar E, Zlatnik T, Karunanayake C. Efficacy of a rinse containing sea salt and lysozyme on biofilm and gingival health in a group of young adults: A pilot study. *Int J Dent* [Internet]. 2017;2017:4056708. Available from: <http://dx.doi.org/10.1155/2017/4056708>.
- [17] Nokam Kamdem GSJ, Toukam M, Ntep Ntep DB, Kwedi KGG, Zilefac Brian N, Tamo Fokam S, et al. Comparison of the effect of saline mouthwash versus chlorhexidine on oral flora. *Advances in Oral and Maxillofacial Surgery* [Internet]. 2022;6(100273):100273. Available from: <http://dx.doi.org/10.1016/j.adoms.2022.100273>.
- [18] Caicedo A, Kim KN, Roper SD. Individual mouse taste cells respond to multiple chemical stimuli. *J Physiol* [Internet]. 2002;544(2):501-09. Available from: <http://dx.doi.org/10.1113/jphysiol.2002.027862>.
- [19] Oren A. Microbial life at high salt concentrations: Phylogenetic and metabolic diversity. *Saline Systems* [Internet]. 2008;4(1):2. Available from: <http://dx.doi.org/10.1186/1746-1448-4-2>.
- [20] Osunde OD, Adebola RA, Adeoye JB, Bassey GO. Comparative study of the effect of warm saline mouth rinse on post-extraction complications. *Int J Oral Maxillofac Surg* [Internet]. 2014;43(5):649-53. Available from: <http://dx.doi.org/10.1016/j.ijom.2013.09.016>.
- [21] Stewart M, Levey E, Nayyer N. Salt water mouthwash post extraction reduced post-operative complications: Question: Does the use of saline mouthrinse following dental extractions reduce post-operative complications? *Evid Based Dent* [Internet]. 2015;16(1):27-28. Available from: <http://dx.doi.org/10.1038/sj.ebd.6401084>.
- [22] Huynh NCN, Everts V, Leethanakul C, Pavasant P, Ampornarnveth RS. Rinsing with saline promotes human gingival fibroblast wound healing in vitro. *PLoS One* [Internet]. 2016;11(7):e0159843. Available from: <http://dx.doi.org/10.1371/journal.pone.0159843>.

- [23] Abdul Kareem S. Changes in the oral flora of newly edentulous patients, before and after complete denture insertion. *J Bagh Coll Dent*. 2012;24:65-69.
- [24] Andjelkovic M, Sojic LT, Lemic AM, Nikolic N, Kannosh IY, Milasin J. Does the prevalence of periodontal pathogens change in elderly edentulous patients after complete denture treatment? *J Prosthodont* [Internet]. 2017;26(5):364-69. Available from: <http://dx.doi.org/10.1111/jopr.12402>.
- [25] Sachdeo A, Haffajee AD, Socransky SS. Biofilms in the edentulous oral cavity. *J Prosthodont* [Internet]. 2008;17(5):348-56. Available from: <http://dx.doi.org/10.1111/j.1532-849x.2008.00301.x>.
- [26] Veseli E, Staka G, Tovani-Palone MR. Evaluation of red-complex bacteria loads in complete denture patients: A pilot study. *BDJ Open* [Internet]. 2023;9(1):7. Available from: <http://dx.doi.org/10.1038/s41405-023-00133-z>.
- [27] Carvalho ÉBS, Romandini M, Sadilina S, Sant'Ana ACP, Sanz M. Microbiota associated with peri-implantitis—A systematic review with meta-analyses. *Clin Oral Implants Res* [Internet]. 2023;34(11):1176-87. Available from: <http://dx.doi.org/10.1111/clr.14153>.
- [28] Kocar M, Seme K, Hren NI. Characterization of the normal bacterial flora in periimplant sulci of partially and completely edentulous patients. *Int J Oral Maxillofac Implants*. 2010;25(4):690-98.
- [29] Rajasekar A, Varghese SS. Bacterial profile associated with Peri-implantitis: A systematic review. *J Long Term Eff Med Implants* [Internet]. 2023;33(3):09-20. Available from: <http://dx.doi.org/10.1615/longtermeffmedimplants.2022044320>.
- [30] Persson GR, Renvert S. Cluster of bacteria associated with peri-implantitis. *Clin Implant Dent Relat Res* [Internet]. 2014;16(6):783-93. Available from: <http://dx.doi.org/10.1111/cid.12052>.

PARTICULARS OF CONTRIBUTORS:

1. Private Practitioner, Chandrapur, Maharashtra, India.
2. Professor, Department of Prosthodontics and Crown and Bridge, K. M. Shah Dental College and Hospital, Sumandeep Vidyapeeth University, Vadodara, Gujarat, India.
3. Senior Lecturer, Department of Prosthodontics and Crown and Bridge, AMC Dental College and Hospital, Ahmedabad, Gujarat, India.
4. Ex Postgraduate Student, Department of Prosthodontics and Crown and Bridge, K. M. Shah Dental College and Hospital, Sumandeep Vidyapeeth University, Vadodara, Gujarat, India.
5. Ex Postgraduate Student, Department of Prosthodontics and Crown and Bridge, K. M. Shah Dental College and Hospital, Sumandeep Vidyapeeth University, Vadodara, Gujarat, India.
6. Final Year Postgraduate Student, Department of Prosthodontics and Crown and Bridge, K. M. Shah Dental College and Hospital, Sumandeep Vidyapeeth University, Vadodara, Gujarat, India.
7. Final Year Postgraduate Student, Department of Prosthodontics and Crown and Bridge, K. M. Shah Dental College and Hospital, Sumandeep Vidyapeeth University, Vadodara, Gujarat, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Neerja Mahajan,
Professor, Department of Prosthodontics, Crown and Bridge, K. M. Shah Dental College and Hospital, Sumandeep Vidyapeeth, Piparia, Waghodia, Vadodara-391760, Gujarat, India.
E-mail: drneerjamahajan@gmail.com

PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Jul 20, 2025
- Manual Googling: Mar 14, 2026
- iThenticate Software: Mar 16, 2026 (5%)

ETYMOLOGY: Author Origin**EMENDATIONS:** 7**AUTHOR DECLARATION:**

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Jul 01, 2025**
Date of Peer Review: **Nov 05, 2025**
Date of Acceptance: **Mar 18, 2026**
Date of Publishing: **Jul 01, 2026**