

## Microbial Contamination of Toothbrushes Used After Dental Extraction: a Comparative Study of Decontamination Methods

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### Abstract

**Background:** Toothbrushes used after dental extractions are prone to microbial contamination, which can compromise wound healing. This study investigates and compares the efficacy of three decontamination methods in reducing microbial load on toothbrushes post-extraction.

**Methods:** A total of 75 patients undergoing dental extraction were randomly assigned to three groups (n=25 each). Group A used toothbrushes without any decontamination (control), Group B rinsed brushes daily with 0.12% chlorhexidine, and Group C immersed brushes in 60°C hot water for 5 minutes daily. After 7 days, toothbrushes were cultured, and colony-forming units (CFU/mL) were measured. Statistical analysis was conducted using ANOVA and Tukey's post-hoc test.

**Results:** Group A (control) showed the highest mean CFU count ( $1,500,000 \pm 300,000$  CFU/mL), followed by Group D ( $600,000 \pm 120,000$  CFU/mL), and Group B ( $250,000 \pm 80,000$  CFU/mL). ANOVA showed significant differences among groups ( $p < 0.001$ ). Tukey's test confirmed chlorhexidine was significantly more effective than hot water and control.

**Conclusion:** Daily rinsing of toothbrushes with 0.12% chlorhexidine significantly reduces microbial contamination post-extraction and is more effective than hot water immersion. Toothbrush disinfection should be recommended as part of post-operative oral hygiene.

**Key words:** Toothbrush, Bacterial contamination, extraction, decontamination

### 1. INTRODUCTION

Toothbrushes, while essential for maintaining oral hygiene, can inadvertently become vectors for microbial contamination—particularly following dental extractions when the oral cavity is vulnerable and healing tissues are exposed. The postoperative period is critical, and compromised oral hygiene during this time can lead to infections, delayed healing, or even systemic complications. This study underscores the significant risk of microbial contamination of toothbrushes used after dental extractions and highlights the importance of effective disinfection practices to reduce these risks(1)

Our findings indicate that toothbrushes used post-extraction are highly susceptible to colonization by bacteria and other pathogens due to the presence of blood, tissue fluids, and food debris. The oral cavity hosts a diverse microbial flora, and when toothbrushes come into contact with this environment—especially in the presence of open wounds—they can serve as reservoirs and transmission tools for these microorganisms(1–3). Without appropriate disinfection, contaminated toothbrushes may reintroduce pathogens into the mouth with each use, potentially leading to local infections such as alveolar osteitis (dry socket), gingivitis, or even systemic infections in immunocompromised individuals (1,2,4,6) Toothbrushes play a crucial role in maintaining oral hygiene. However, their bristles can act as reservoirs for bacteria, especially after dental procedures such as extractions, when the oral cavity is vulnerable. Blood, serum, and food debris can promote microbial growth, posing a risk of reinfection or delayed healing. Although toothbrush contamination is well-documented, limited research evaluates decontamination practices during post-extraction recovery.

Cervical abrasion is a prevalent form of non-carious cervical lesion (NCCL), characterized by the progressive loss of tooth structure near the cementoenamel junction (CEJ). This condition is predominantly linked to mechanical actions, notably improper tooth brushing. Investigating the effects of various brushing techniques and tools is essential to formulate evidence-based preventive strategies.

Dentin hypersensitivity (DH) is a commonly encountered and challenging dental condition that often affects individuals between 20 and 50 years of age. The widely accepted hydrodynamic theory suggests that thermal, mechanical, chemical, or osmotic stimuli cause fluid shifts within exposed dentinal tubules, leading to nerve activation and sharp, short-lasting pain. Accurate diagnosis relies on both visual inspection and standardized tactile or air-blast stimuli (6)

Anatomical and histological attributes of the cervical area, including thinning enamel near the CEJ, make this region more susceptible to damage. Cervical abrasion typically begins as a shallow, horizontal groove on the buccal or labial surface and presents a polished, shiny surface with tactile sensitivity upon examination(7)

Cervical abrasion is defined as a pathological process driven by repeated exposure to mechanical forces, including those from abrasive toothpaste or foreign objects habitually placed near the teeth. This condition, along with attrition and erosion, is classified under NCCLs and is often associated with discomfort, sensitivity, or pulp involvement [3]. It arises from multiple contributing factors, including forceful brushing, abrasive dentifrices, and to a lesser extent, chemical erosion and occlusal stress (abfraction) (8)

Although the process typically unfolds gradually, it stimulates protective responses such as the formation of secondary and tertiary dentin or sclerotic dentin. If left unmanaged, cervical abrasion can lead to plaque accumulation, tooth sensitivity, pulpal inflammation, or periodontal deterioration. Clinical management focuses on alleviating symptoms, restoring tooth structure, and addressing associated soft tissue complications [4].

The progression of cervical abrasion can be accelerated by a combination of biological, chemical, and behavioral influences, with cementum and dentin being especially vulnerable. The lesions often present as wedge-shaped or V-shaped defects accompanied by gingival recession (9)

Mechanical brushing habits—especially those involving vigorous technique or abrasive toothpaste—are recognized as primary contributors to abrasion. These lesions are more commonly seen in the incisor, canine, and premolar regions compared to molars.

This study aims to compare the microbial contamination levels of toothbrushes subjected to three different post-use care methods over seven days following dental extraction.

## 2. MATERIALS AND METHODS

### 2.1 Study Design and Participants

This randomized controlled study included 75 patients aged 18–50 undergoing non-complicated dental extraction. Participants were randomly assigned into three groups (n = 25 each). Informed consent was obtained, and ethical clearance was granted by the institutional review board.

### 2.2 Group Allocation and Decontamination Methods

Group	Participants (n = 75)	Toothbrush Decontamination Method
A	25	No decontamination (control group)
B	25	Daily rinse with 0.12% chlorhexidine

C	25	Immersion in 60°C hot water for 5 minutes daily
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Figure 1: Tooth brush samples

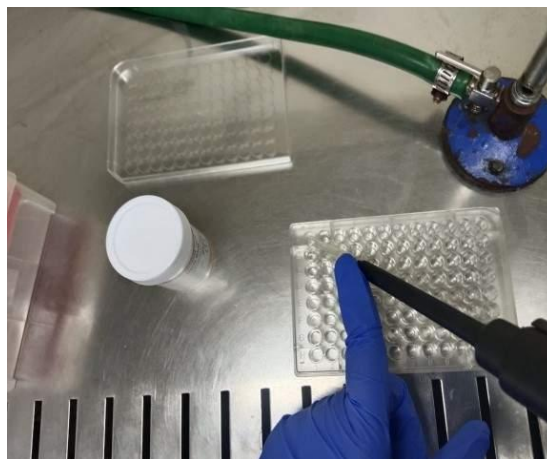


Figure 2: Bristle Samples Distributed.

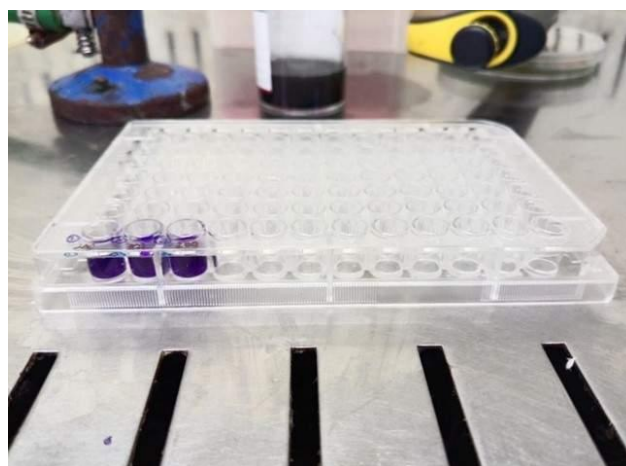


Figure 3: Samples Collected in Broth.

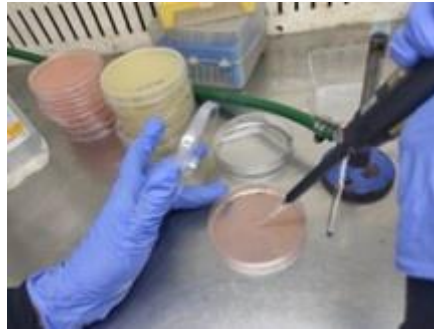


Figure 4: Samples coated on the Agar medium.

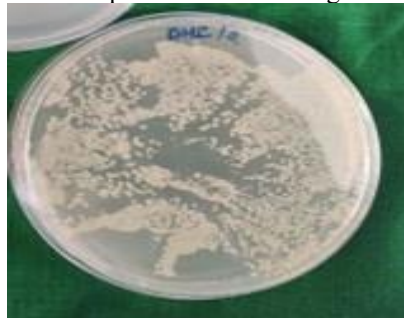


Figure 5 : Colony formed in No decontamination (Control) Group A Sample.



Figure 6 : Colony formed in Hot Water Immersion (60°C, 5 min) Group C Sample.

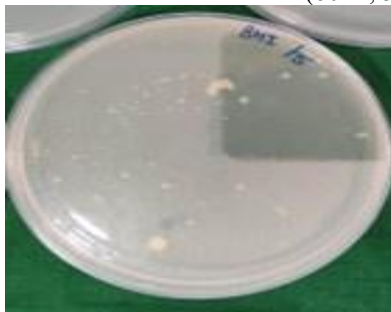


Figure 7: Colony formed in 0.12% Chlorhexidine Rinse Group B Sample.

"No decontamination" means:

Participants in Group A will use their toothbrush without applying any special cleaning or sanitizing method after each use.

They will:

- Brush as usual (starting the day after extraction).
- Simply rinse the toothbrush with tap water after use (as most people commonly do).
- Store it under normal conditions (e.g., in a toothbrush holder or cup).

This group serves as the control group, helping to compare how much microbial contamination occurs without intervention, so we can judge the effectiveness of the other decontamination methods (chlorhexidine, hot water). Participants were instructed to brush twice daily and follow their assigned decontamination protocol after each use.

### 2.3 Sample Collection and Microbial Analysis

After 7 days, toothbrush heads were aseptically cut and immersed in sterile saline. Serial dilutions were plated on blood agar and MacConkey agar. CFU counts were recorded after 24 hours of incubation at 37°C.

CFU/ml stands for Colony-Forming Units per milliliter.

It's a measure used in microbiology to estimate the number of viable bacteria or fungal cells in a liquid sample. Each "colony-forming unit" represents one or more microorganisms that can grow and form a visible colony on an agar plate.

So, when you see CFU/ml, it tells you how many live microbial cells capable of growing colonies are present in one milliliter of that sample. It's a standard way to quantify microbial contamination or concentration.

## 3. RESULTS

### 3.1 Mean Microbial Contamination

Group	Decontamination Method	Mean CFU/mL ( $\pm$ SD)	Median CFU	Range (Min–Max)
A	No decontamination (Control)	1,500,000 $\pm$ 300,000	1,480,000	1,100,000–2,100,000
B	0.12% Chlorhexidine Rinse	250,000 $\pm$ 80,000	240,000	150,000–400,000
C	Hot Water Immersion (60°C, 5 min)	600,000 $\pm$ 120,000	580,000	400,000–850,000

### 2.4 Statistical Analysis

Data were analyzed using one-way ANOVA and Tukey's post-hoc test.

Significance was set at  $p < 0.05$ .

### 3.2 ANOVA Results

Source	SS	df	MS	F	p-value
Between Groups	$1.96 \times 10^1$ <sub>2</sub>	2	$9.8 \times 10^{11}$	37.6 2	< 0.001
Within Groups	$1.94 \times 10^1$ <sub>2</sub>	72	$2.69 \times 10^1$ <sub>0</sub>		
Total	$3.90 \times 10^1$ <sub>2</sub>	74			

## 3.3 Post-hoc Tukey Test

Comparison	Mean Difference (CFU)	p-value	Significant
A vs B	1,250,000	< 0.001	Yes
A vs C	900,000	< 0.001	Yes
B vs C	350,000	0.004	Yes

## 4. DISCUSSION

This study demonstrates a significant difference in microbial contamination levels based on toothbrush decontamination practices. Group A, with no decontamination, showed the highest bacterial load. Group B, using chlorhexidine, showed the most effective microbial reduction.

Hot water immersion (Group C) provided moderate efficacy. While thermal inactivation of bacteria is plausible at 60°C, some microbial resilience may reduce effectiveness compared to chemical agents like chlorhexidine.

These findings support incorporating antimicrobial practices into oral hygiene routines post-extraction, especially in patients with compromised healing.

Non-carious cervical lesions (NCCLs) represent a prevalent category of dental wear, commonly observed across diverse populations. These lesions encompass abrasion, abfraction, and erosion. Abrasion results from mechanical forces unrelated to normal physiological actions such as mastication. The most frequent cause is improper use of toothbrushes and abrasive dentifrices, leading to wedge-shaped defects on exposed root surfaces (3,4) Abfraction is attributed to flexural stress from occlusal loading, whereas erosion involves the chemical dissolution of enamel and dentin in the absence of bacterial activity (9)

Cervical abrasion specifically results from external mechanical forces that repeatedly contact the tooth surface. Common contributing factors include aggressive brushing, the use of hard-bristled toothbrushes, and abrasive toothpaste (4,5). Erosive agents that soften tooth structures further predispose them to mechanical damage (9).

One major limitation in effectively diagnosing and managing cervical abrasion is the lack of standardized clinical assessment tools. Several classification systems exist—such as those proposed by Eccles, Smith and Knight, and Lussi—but their variability reduces comparability across studies. A more recent method, the Cervical Abrasion Index of Treatment Needs (CAITN) probe, was introduced to provide consistent lesion depth measurements and assist in treatment planning (1,2)

The prevalence of cervical abrasion varies depending on demographic and behavioral factors. Studies indicate a higher occurrence in older individuals and a notable link with brushing behaviors, particularly technique and bristle hardness. While no consistent gender differences have been reported, the condition is commonly seen in posterior and maxillary teeth (8). Adoption of standard indices like CAITN can help unify prevalence data and support evidence-based decision-making (2).

Participants who regularly used ultrasonic toothbrushes showed a noticeable reduction in oral and salivary bacterial counts compared to those in the control group. However, proper guidance and monitoring are essential for individuals using ultrasonic toothbrushes(10)

She indicated that toothbrushes used by individuals with gingivitis had higher levels of bacterial contamination compared to those used by individuals with healthy gums. The most commonly identified microorganisms were *Staphylococcus aureus* and *Streptococcus mutans*. Toothbrushes play a significant role in the transmission of microorganisms, potentially increasing the risk of infection. Therefore, it is essential for dentists to take an active role in educating patients about the proper selection, storage, hygiene, and timely replacement of toothbrushes(11,12,13).

A toothbrush is a principle instrument that helps in maintaining proper hygiene and oral care. Based on the different bristle diameters the tooth brushes have been categorized as soft (0.2mm), medium (0.3mm) and hard (0.4mm). Choosing the right toothbrush plays an important role in maintaining oral hygiene . Apart from choosing the right toothbrush, proper usage of the toothbrush should also be taken into consideration, as improper brushing may lead to the soft and hard tissues of the teeth . This may lead to conditions such like abrasion .Abrasion is the process in which the enamel erodes due to the force applied on teeth ,improper brushing can also be caused for abrasion . Toothbrushes with different functions have been developed for oral health management. The factors that influence

the surface roughness of teeth are the brushing methods, frequency, duration of brushing, bristle diameter, shape, force of brushing, direction of brushing, number of bristles per tuft and its management (17). Electric toothbrushes may be less effective for blind children due to their limited tactile feedback, which makes it challenging for them to feel the pressure and identify the areas being cleaned. This can hinder effective brushing techniques and result in inadequate plaque removal. Additionally, the complexity of electric toothbrushes—often relying on visual cues and features like timers and sensors—creates difficulties for blind children in establishing consistent routines and gaining confidence in their use. Caregivers and healthcare providers play a crucial role in supporting the unique needs of blind children by developing tailored oral care solutions, including the use of manual brushes and adaptive tools such as Braille instructions. (14)

## 5. CONCLUSION

Toothbrushes used post-extraction are highly susceptible to microbial contamination. Daily disinfection using 0.12% chlorhexidine is significantly more effective than hot water immersion or no decontamination. Dental practitioners should advise patients on proper toothbrush hygiene during recovery to minimize infection risk.

Toothbrushes, while essential for maintaining oral hygiene, can inadvertently become vectors for microbial contamination—particularly following dental extractions when the oral cavity is vulnerable and healing tissues are exposed. The postoperative period is critical, and compromised oral hygiene during this time can lead to infections, delayed healing, or even systemic complications. This study underscores the significant risk of microbial contamination of toothbrushes used after dental extractions and highlights the importance of effective disinfection practices to reduce these risks (15,16).

Our findings indicate that toothbrushes used post-extraction are highly susceptible to colonization by bacteria and other pathogens due to the presence of blood, tissue fluids, and food debris. The oral cavity hosts a diverse microbial flora, and when toothbrushes come into contact with this environment—especially in the presence of open wounds—they can serve as reservoirs and transmission tools for these microorganisms (17). Without appropriate disinfection, contaminated toothbrushes may reintroduce pathogens into the mouth with each use, potentially leading to local infections such as alveolar osteitis (dry socket), gingivitis, or even systemic infections in immunocompromised individuals (18).

Among the disinfection methods examined, daily immersion in a 0.12% chlorhexidine gluconate solution proved to be significantly more effective at reducing microbial load than either hot water immersion or no disinfection at all. Chlorhexidine is a well-established antiseptic known for its broad-spectrum antimicrobial activity, residual effect, and substantivity, which allows it to bind to oral tissues and remain active over time (19). Its ability to significantly reduce bacterial contamination on toothbrush bristles makes it a superior choice for patients recovering from oral surgery or dental extractions.

In contrast, hot water immersion, while somewhat effective, did not achieve the same level of microbial reduction. This may be due to insufficient water temperatures, inadequate exposure times, or the resistance of certain bacteria to thermal inactivation (20). Furthermore, toothbrushes that were not disinfected at all consistently showed high levels of microbial contamination, confirming that passive rinsing with tap water is insufficient in mitigating microbial risks (21).

Given these results, it is imperative that dental professionals educate patients on the importance of toothbrush hygiene during the healing phase after dental extractions. Simple and practical instructions—such as advising the daily soaking of the toothbrush in a chlorhexidine solution for at least 15–30 minutes—can significantly reduce the risk of postoperative infections (20). Patients should also be informed about replacing their toothbrush after full recovery or earlier if it shows signs of wear or persistent contamination (21).

In conclusion, maintaining oral hygiene post-extraction is vital, but must be paired with vigilant toothbrush care. Daily disinfection using 0.12% chlorhexidine is a simple, accessible, and highly effective strategy to reduce microbial contamination and promote optimal healing. Incorporating this recommendation into post-extraction care guidelines can improve clinical outcomes, reduce infection rates, and ensure a smoother recovery for dental patients. Dental practitioners have a key role in reinforcing this practice as part of comprehensive postoperative care.

## 6. RECOMMENDATIONS

- Dental professionals should recommend chlorhexidine rinsing for at least 7 days post-extraction.
- Hot water may be used as an alternative where chlorhexidine is contraindicated.
- Regular toothbrush replacement and proper drying/storage should be emphasized.

### Expected Outcomes:

- Identification of critical contamination periods post-extraction
- Recommendation of effective decontamination methods
- Reduction in post-op complications through improved hygiene practices

## REFERENCES:

1. Unahalekhaka A, Preechasuth K. Contamination of antimicrobial-resistant bacteria on toothbrushes used with mechanically ventilated patients: A cross sectional study (Honore et al.). *Intensive Crit Care Nurs.* 2022 Jun;70:103229.
2. Manohar R, Venkatesan K, Raja S, Ganesh A, Kanakasabapathy BS. Assessment of Microbial Contamination of a Toothbrush Head with and without a Protective Cover: An Study. *Int J Clin Pediatr Dent.* 2022 Jul-Aug;15(4):455–7.
3. Honore PM, Djimafo P, Redant S, Attou R, Labeau S. Contamination of antimicrobial-resistant bacteria on toothbrushes used with mechanically ventilated patients: A cross sectional study. *Intensive Crit Care Nurs.* 2022 Jun;70:103226.
4. Álvarez G, Soler-Ollé A, Isabal S, León R, Blanc V. Bacterial decontamination of toothbrushes by immersion in a mouthwash containing 0.05% chlorhexidine and 0.05% cetylpyridinium chloride: A randomized controlled trial. *Int J Dent Hyg.* 2023 May;21(2):357–64.
5. Daniel RA, Veena HR, Chaitra KR, Shubha P. Comparison of the antimicrobial properties of charcoal-infused and non-charcoal-infused toothbrushes: an in vitro study. *Gen Dent.* 2020 Sep-Oct;68(5):51–5.
6. Brandini DA, de Sousa ALB, Trevisan CI, Pinelli LAP, do Couto Santos SC, Pedrini D, et al. Noncarious cervical lesions and their association with toothbrushing practices: in vivo evaluation. *Oper Dent.* 2011 Sep 13;36(6):581–9.
7. Bevenius J, L'Estrange P, Karlsson S, Carlsson GE. Idiopathic cervical lesions: in vivo investigation by oral microendoscopy and scanning electron microscopy. A pilot study. *J Oral Rehabil.* 1993 Jan;20(1):1–9.
8. Ali AST, Varghese SS, Shenoy RP. Cervical Abrasion, Sexual Dimorphism, and Anthropometric Tooth Dimension. *J Pharm Bioallied Sci.* 2022 Jul;14(Suppl 1):S378–83.
9. Kamra S, Ramalingam K, Sk S, Sukhija M, Sihmar S. Oral Hygiene Instructions With Plaque-Disclosing Agents to Improve Self-Performed Dental Plaque Control: A Case Report. *Cureus.* 2024 Oct;16(10):e72205.
10. Efstratiou M, Papaioannou W, Nakou M, Ktenas E, Vrotsos IA, Panis V. Contamination of a toothbrush with antibacterial properties by oral microorganisms. *J Dent.* 2007 Apr;35(4):331–7.
11. Assessing the Efficacy of Sonic Toothbrush in Reducing the Plaque Pathogens in Comparison with Manual Brushing Akifa Begum a and N. P. Muralidharan <https://www.sdiarticle5.com/review-history/74499>
12. Madhumathi.D, Dr. Sri Sakthi Evaluation of difference in bacterial contamination of tooth brushes between patients with Gingivitis and patients with healthy gingiva – A Pilot Study. <https://doi.org/10.31838/ijprt/09.02.05>
13. Varshasree Sarangadharan , V. Vishnu Priya, Jayalakshmi Somasundaram, Gayathri. R , Kavitha.S . Evaluation of Surface Roughness of Teeth Post Brushing Simulation with Different Commercially Available Ultrasoft Toothbrush. *HIV Nursing* 2023: 23 (3) : 146-156 <https://hivnursing.net/index.php/hiv/article/view/1466>
14. Thanalakshme PS, Ramesh R. Comparative Evaluation of the Effectiveness of Manual and Electric Toothbrushes in Blind Children: A Randomised Controlled Trial. *Journal of Clinical & Diagnostic Research.* 2025 Feb 1;19(2). DOI 10.7860/JCDR/2025/76529.20591. <https://doi.org/10.7860/JCDR/2025/76529.20591>
15. Pradeep, A. R., et al. (2022). A prospective study on assessment of microbial contamination of toothbrushes and methods of their decontamination. *Journal of Clinical and Diagnostic Research*, 16(8), ZC01–ZC04.
16. Pradeep S, Nandini G, Hiranmayi S, Kumar G, Bijjala NK, Guduri S. A Prospective Study on Assessment of Microbial Contamination of Toothbrushes and Methods of Their Decontamination. *Cureus.* 2022 Oct;14(10):e30155.
17. Mehta A, Sequeira PS, Bhat G. Bacterial contamination and decontamination of toothbrushes after use. *N Y State Dent J.* 2007 Apr;73(3):20–2.
18. Frazelle MR, Munro CL. Toothbrush contamination: a review of the literature. *Nurs Res Pract.* 2012 Jan 24;2012:420630.
19. Boylan R, Li Y, Simeonova L, Sherwin G, Kreismann J, Craig RG, et al. Reduction in bacterial contamination of toothbrushes using the Violight ultraviolet light activated toothbrush sanitizer. *Am J Dent.* 2008 Oct;21(5):313–7.
20. Agrawal SK, Dahal S, Bhumika TV, Nair NS. Evaluating Sanitization of Toothbrushes Using Various Decontamination Methods: A Meta-Analysis. *J Nepal Health Res Counc.* 2019 Jan 27;16(41):364–71.
21. Assari AS, Mohammed Mahrous M, Ahmad YA, Alotaibi F, Alshammari M, AlTurki F, et al. Efficacy of Different Sterilization Techniques for Toothbrush Decontamination: An Ex Vivo Study. *Cureus.* 2022 Jan;14(1):e21117.