

# A Prospective Study on Assessment of Microbial Contamination of Toothbrushes and Methods of Their Decontamination

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## Abstract

**Background:** Toothbrushes may get contaminated by the oral cavity, environment, hands, storage containers, or aerosol contamination. The present study was conducted to assess the microbial contamination of toothbrushes and methods of their decontamination.

**Materials and methods:** The current study included 160 subjects of both genders. All the subjects were provided with a toothbrush and paste with complete hygiene instructions for the oral cavity. After one month, all the brushes were collected. The samples were categorized into four groups of 40 each. Group I was treated with 0.2% chlorhexidine gluconate, group II with Listerine, group III with Dettol, and group IV with tap water. Finally, these toothbrushes were placed in 5 mL of neutralizer broth and then evaluated to study the efficacy of four disinfectants. All the data were analyzed using the statistical package for social science (SPSS) version 23 software (IBM, Armonk, NY, USA). For all analyses,  $p < 0.05$  was considered to be statistically significant

**Results:** Aerobic bacterial growth before disinfection in Groups I, II, III, and IV was 91.6%, 75.84%, 75%, 81.67%, respectively ( $p = 0.01$ ). After disinfecting the brushes aerobic bacterial growth was reduced to 34.17%, 30.84%, 24.17% & 74.17% in Groups I, II, III, and IV, respectively ( $p = 0.002$ ). Klebsiella, Micrococci and Escherichia coli survived the most even after disinfection was done.

**Conclusion:** Most effective agent for the disinfection of toothbrushes was Dettol followed by Listerine and 0.2% chlorhexidine gluconate. Tap water was found to be ineffective in the decontamination of toothbrushes.

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**Categories:** Dentistry

**Keywords:** dettol, listerine, chlorhexidine, decontamination, toothbrushes, microbial contamination

## Introduction

Oral health is the gateway to systemic health and the overall well-being of people. The oral microbiome consists of various bacteria, viruses, and fungi that are responsible for causing a number of oral diseases [1]. Proper oral hygiene maintenance can greatly reduce these microbes and help in achieving oral health. The toothbrush is the most widely used mechanical means of maintaining good oral hygiene. However, toothbrushes often get contaminated with use and improper storage [2].

Toothbrushes are generally stored in a common container in the bathroom, which can introduce microbes to the toothbrush. Toothbrush infected from the unhygienic oral cavity, surroundings, hands, or storage site [3]. Bacteria that attach, accumulate & multiply on toothbrushes may be transferred to the oral cavity leading to different diseases. Thus, it is essential to frame uniform oral health guidelines to prevent toothbrush contamination [4,5]. Contaminated toothbrushes may broadcast microorganisms, which could be detrimental to oral and systemic health [6].

In the general public, a lack of awareness was observed regarding the maintenance and care of toothbrushes. An extensive literature search revealed many studies regarding the cleaning of toothbrushes, but no clear method of disinfection was observed. Therefore, a uniform and ideal cost-effective method is required for proper care of toothbrushes that can culminate in the risk of microbial transmission in the oral cavity. The present study was conducted to assess the microbial contamination of toothbrushes and methods of their decontamination.

### How to cite this article

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## Materials And Methods

The current study included 160 subjects of both genders. The subjects were enrolled only after taking written consent. The study was carried out in Sai Krishna Dental Clinic and Hospital, Khaleelwadi, Nizamabad, Telangana, during the period January 2022 to June 2022. Ethical clearance was obtained (ethical number-SKDC/NZM/Exp/2021-22). The demographic profile of subjects was recorded such as name, age, gender, etc.

The study includes subjects of age 18-45 years with a gingival index score of two or three with at least 20 natural, unbroken teeth. The subjects on any type of treatment like antibiotics, anti-fungal, antimicrobials, any history of dental surgery or patients visiting the clinic and not taking treatment, smoking, and systemic diseases were excluded from the study.

The samples were categorized into four groups of 40 each. Group I was treated with 0.2% chlorhexidine gluconate, group II with Listerine, group III with Dettol, and group IV with tap water. All the subjects were provided with toothbrushes and paste with complete hygiene instructions for the oral cavities. Five unused new toothbrushes (negative control) were also kept along with them. After a period of one month, all the brushes were collected. They were kept in sterile boxes. The samples were transported for microbial analysis.

Firstly, the bristle part of toothbrushes was immersed in test tubes containing 5 milliliters of normal saline for one hour. After that, they were cultured in Mueller-Hinton-based blood agar and MacConkey agar. They were incubated at 37°C for 24 h, aerobically. Identification of isolates was done by Gram staining. Further, 1 mL of the sample was also injected into Robertson's cooked meat (RCM) medium (temperature 37°C for 24 h). Following this, it was further cultured in Mitis Salivarius agar.

Each toothbrush was then disinfected by immersing it in one of the four disinfectants for one hour. After one hour, these toothbrushes were placed in 5 mL of neutralizer broth, and then the samples from the neutralizer broth were collected and cultured to evaluate the efficacy of the four disinfectants used in the study. The evaluation was done by observing the growth of microorganisms after the treatment of disinfectant.

## Statistical analysis

A total of 160 participants were selected, which is derived after taking 5% Type I error and standard normal variate for power as 90%. The mean proportion of reduction is considered 70% which includes 40 patients in each group. All the data were analyzed using the statistical package for social science (SPSS) version 23 software (IBM, Armonk, NY, USA). Inferential statistics were performed using Chi-square analysis. For all analyses,  $p < 0.05$  was considered to be statistically significant.

## Results

Table 1 depicts the aerobic bacterial growth distribution in the four test groups before disinfection after using it for a month. Out of 120 agar plates in each group, there was statistically significant aerobic bacterial growth noted in groups I, II, III, and IV at 91.6%, 75.84%, 75%, and 81.67%, respectively ( $p = 0.01$ ). All the five new brushes included as control showed negative growth.

Groups	Percentage of aerobic bacteria		P-value
	Present	Absent	
Group I	110 (91.6)	10 (8.4)	0.01*
Group II	91 (75.84)	29 (24.16)	
Group III	90 (75)	30 (25)	
Group IV	98 (81.67)	22 (18.33)	

**TABLE 1: Assessment of aerobic bacterial growth before disinfection**

\*Statistically significant. Data presented as Number (Percentage)

After immersing the toothbrushes in disinfectants for one hour, there was a significant reduction ( $p = 0.002$ ) in bacterial growth. After disinfection, the aerobic bacterial growth was observed as 34.17%, 30.84%, and 24.17% in groups I, II, and III, respectively. However, a significant change in bacterial growth was observed after disinfection in the tap water group ( $p=0.002$ ) (Table 2).

Groups	Percentage of aerobic bacteria		P-value
	Present	Absent	
Group I	41 (34.17)	79 (65.83)	0.002*
Group II	37 (30.84)	83 (69.16)	
Group III	29 (24.17)	91 (75.83)	
Group IV	89 (74.17)	31 (25.83)	

**TABLE 2: Distribution of aerobic bacterial growth among the four test groups after disinfection**

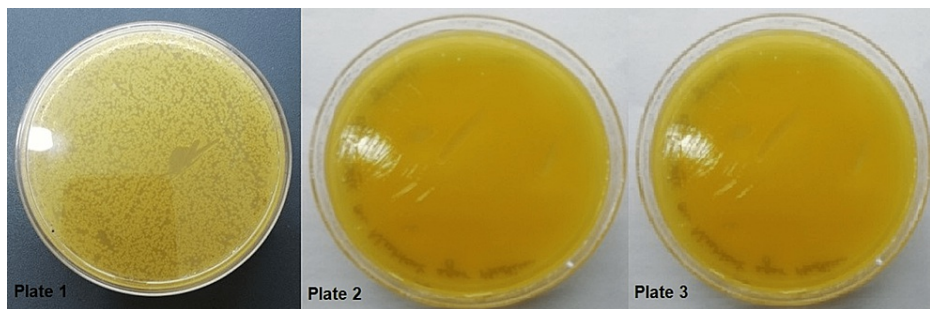
\*Statistically significant. Data presented as Number (Percentage)

Table 3 depicts that Micrococci and Escherichia coli comprised 25.83% and 20% of the organisms isolated from Group I. In groups II and III, the maximum colonies were observed of E. coli. However, 24.16% and 25% of culture plates had no growth in groups I and II, respectively. In group IV, 22.5% of the organism isolated from the culture media plates was Klebsiella while 18.33% of culture media showed no growth (Table 3). Figure 1 depicts culture plates showing the growth of micro-organisms.

Microorganisms	Group I	Group II	Group III	Group IV	Total
Beta-hemolytic streptococci	8 (6.66)	7 (5.83)	7 (5.83)	8 (6.66)	30 (6.25)
Bacillus species	11 (9.16)	8 (6.66)	10 (8.33)	6 (5)	35 (7.29)
Pseudomonas	2 (1.66)	1 (0.83)	0 (0)	0 (0)	3 (0.62)
Escherichia coli	24 (20)	21 (17.5)	18 (15)	15 (12.5)	78 (16.25)
Micrococci	31 (25.83)	18 (15)	15 (12.5)	20 (16.66)	84 (17.5)
Klebsiella	13 (10.83)	11 (9.16)	21 (17.5)	27 (22.5)	72 (15)
Streptococcus mitis	14 (11.66)	16 (13.33)	11 (9.16)	9 (7.5)	50 (10.41)
Viridans Streptococci	7 (5.83)	10 (8.33)	8 (6.66)	13 (10.83)	38 (7.91)
No growth	10 (8.33)	29 (24.16)	30 (25)	22 (18.33)	91 (18.95)
Total	120 (100)	120 (100)	120 (100)	120 (100)	480 (100)

**TABLE 3: Distribution of different microorganisms before disinfection**

Data presented as Number (Percentage)



**FIGURE 1: Plate 1 (Growth of Streptococcus); plate 2 (Growth of Klebsiella); plate 3 (Growth of Klebsiella)**

Table 4 depicts the percentage of microorganisms found in different groups after applying disinfectants for one hour. The micro-organisms observed in the culture after disinfection included *E. coli*, *Bacillus*, *Klebsiella*, Beta-hemolytic streptococci, Viridans streptococci, *Streptococcus mitis*, and micrococci (Table 4). Out of all microorganisms, 9.58% of them were *Klebsiella* and more than 8% were *E. coli* and micrococci (Table 4).

Microorganisms	Group I	Group II	Group III	Group IV	Total
Beta-hemolytic streptococci	2 (1.66)	0 (0)	0 (0)	5 (4.16)	7 (1.45)
Bacillus species	4 (3.33)	3 (2.5)	2 (1.66)	5 (4.16)	14 (2.91)
Pseudomonas	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Escherichia coli	11 (9.16)	10 (8.33)	7 (5.83)	14 (11.66)	42 (8.75)
Micrococci	10 (8.33)	5 (4.16)	6 (5)	18 (15)	39 (8.12)
Klebsiella	7 (5.83)	7 (5.83)	8 (6.66)	24 (20)	46 (9.58)
Streptococcus mitis	6 (5)	9 (7.5)	5 (4.16)	11 (9.16)	31 (6.45)
Viridans Streptococci	1 (0.83)	3 (2.5)	1 (0.83)	12 (10)	17 (3.54)
No growth	79 (65.83)	83 (69.16)	91 (75.83)	31 (25.83)	284 (59.16)
Total	120 (100)	120 (100)	120 (100)	120 (100)	480 (100)

**TABLE 4: Distribution of different microorganisms after disinfection**

Data presented as Number (Percentage)

## Discussion

Toothbrush contamination is an unavoidable outcome of use and improper storage resulting in many systemic and oral infections [7]. With regular use, the bristles often get worn out. Hence, the American Dental Association recommends replacing toothbrushes at an interval every three to four months [8]. But this recommendation does not clearly mention if the replacement of the toothbrush could also help avoid microbial contamination [9]. Secondly, more frequent changes in toothbrushes could pose an economic burden. Routine household procedures such as rinsing and drying seem to be a good method but might not be sufficient to reduce the microbes [10,11]. The present study was conducted to assess the microbial contamination of toothbrushes and methods of their decontamination.

We found that aerobic bacterial growth before disinfection was 91.6%, 75.84%, 75%, and 81.67% in groups I, II, III, and IV, respectively. Joy et al. assessed the microbial toothbrush contamination and their decontamination using different disinfectants by involving 80 subjects with two or three gingival index scores [12]. Toothbrushes were divided into four groups of 20 subjects each. Group A was treated with 0.2% chlorhexidine gluconate, group B with Listerine, group C with Dettol, and group D with tap water for 1 h. All the toothbrushes, which were sampled had significant bacterial growth after one month of use. Similar to our study Joy et al. found a maximum of the toothbrushes were contaminated with *E. coli* (22.7%). All the tested disinfectants significantly reduced bacterial growth and Dettol showed maximum effectiveness. We found that aerobic bacterial growth after disinfection was present in 34.17%, 30.84%, 24.17%, and 74.17% in groups I, II, III, and IV, respectively. Caudry et al. in their study also found that toothbrushes are profoundly contaminated with normal use. Moreover, they observed that disinfection with tap water had not given appreciable results [13].

Mehta et al. found that 70% of the toothbrushes in their study got greatly contaminated with pathogenic microorganisms after their use [14]. According to them, Beta-hemolytic streptococci/*Bacillus* species were seen in 20% of samples, *Pseudomonas*/*Bacillus* in 15%, and coagulase-negative staphylococci, Micrococci/*E. coli* in 10% of samples. Caudry et al. Viridans Streptococci/*E. coli* in 17% of group I, 14% of group II, 10% of group III, and 15% of group IV [13]. Taji and Glass in their studies found widespread toothbrush contamination after its use except in cases where an oral antiseptic, such as mouthwash, was used instantly prior to brushing [15,16].

Warren et al. found that regular and triclosan-containing toothpaste use leads to lower toothbrush contamination than no toothpaste use [17]. Sato et al. found that rinsing toothbrushes just with tap water leads to continued elevated levels of contamination and biofilm [18]. Malmberg et al. isolated

Staphylococcus epidermidis and Streptococci from toothbrushes after use while toothbrushes from both healthy patients and patients with oral disease developed potentially pathogenic bacteria such as Staphylococcus species, Pseudomonas, and E. coli species [19].

One of the limitations of the study is that further studies need to be conducted among different age groups and the results to be observed at different month intervals to have more understanding of contamination. Another limitation of the study was that we did not make a count on the number of colonies, if that had been done it would have provided the most strength to our study findings.

## Conclusions

In the present study, all toothbrushes were contaminated with different microorganisms when used for one month. The effectiveness of 0.2% chlorhexidine (34.17%), Listerine (30.84%), and Dettol (24.17%) were found to have a promising role but tap water (74.17%) was found to be ineffective. However, daily disinfection of toothbrushes and keeping it in a dry place better optimizes oral hygiene and systemic health.

## Additional Information

### Disclosures

**Human subjects:** Consent was obtained or waived by all participants in this study. Sai Krishna Dental Clinic and Hospital, Khaleelwadi, Telangana issued approval SKDC/NZM/Exp/2021-22. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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