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RESEARCH ARTICLE

EVALUATION OF MICROBIAL CONTAMINATION OF TOOTHBRUSHES AND THEIR DECONTAMINATION USING VARIOUS DISINFECTANTS: AN INVITRO STUDY

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ABSTRACT

Background: Toothbrushes frequently become contaminated both during usage and storage. Despite the fact that previous research has mentioned a number of toothbrush disinfection techniques. There isn't quite a consensus on the subject as of yet. **Aims:** The objective of this study was to assess toothbrush bacterial contamination and disinfect it using different disinfectants. **Subjects and Methods:** In an in vitro trial, 100 participants with a gingival index score of two or three (Loe and Silness, 1963) were given toothbrushes, paste, and comprehensive instructions on oral hygiene. After a month, toothbrushes were gathered and subjected to aerobic culture testing for microbial contamination. Four groups (n = 20) of toothbrushes were given different treatments for one hour: Group A received 0.2% chlorhexidine gluconate, Group B received Listerine, Group C received Dettol, and Group D received tap water. The brushes were further cultured after an hour in order to assess the effectiveness of each disinfectant. The Statistical Package for the Social Sciences (SPSS) 16 program was used to carry out statistical operations. Chi-square analysis was used to perform inferential statistics. We defined statistical significance as $P < 0.05$. **Results:** After one month of use, all of the studied toothbrushes showed considerable ($P < 0.001$) bacterial growth. 17.1% of the brushes had Escherichia coli contamination on them. Dettol was the most efficient disinfection, showing a maximum of 95.3%, although all the tested disinfectants significantly ($P < 0.001$) reduced bacterial growth. **Conclusions:** Following use, toothbrush contamination increases dramatically; however, this is lessened following brush disinfection. All of the disinfectants, while not entirely successful, greatly decreased the amount of bacteria, with Dettol being found to be considerably more efficient than the other agents.

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INTRODUCTION

Systemic health and an individual's general well-being are inextricably linked to oral health. The many bacteria, viruses, and fungi that make up the oral microbiome are accountable for being the source of several mouth illnesses. Maintaining good dental hygiene can significantly lower these microorganisms and promote dental health. The toothbrush is the most common instrument for maintaining dental health. However, misuse and incorrect storage can lead to toothbrush contamination frequently. Since toothbrushes are typically kept in a container in the restroom, bacteria may get transferred easily to it. Furthermore, damp and muggy surroundings of a restroom are favorable to microbial development and can spread to others via skin commensals, contaminated fingers, and aerosols from flushing the toilet. The microbial burden rises with each further use and acts as a reservoir for the reintroduction of harmful microorganisms, resulting in recurrent illnesses. Tissues contaminated with germs have the potential to spread, endangering both oral and general health. Decontaminating toothbrushes is therefore essential for preventing both oral and systemic illnesses. Unfortunately, the

general public lacks knowledge and understanding about how to properly maintain and care for toothbrushes. Despite the fact that many techniques have been previously reported for decontaminating toothbrushes, this subject has received little attention, and there is still disagreement over the most efficient and economical approach. Therefore, the goal of this study was to assess the efficacy of various popular chemical agents as toothbrush disinfectants and to ascertain the degree of microbiological contamination on toothbrushes after a month of use.

SUBJECTS AND METHODS

The patients who came to Department of Periodontics between March 2024 and May 2024 were included in the study. The subjects provided written informed permission and the institutional ethical committee. The obtained 100 participants were selected using the formula:

INCLUSION AND EXCLUSION CRITERIA

Participants having at least 20 natural teeth and a gingival index score of 2 or 3(Loe and Silness, 1963) who were between the ages of 18 and 45 were included in the study. Individuals using antibiotics or antimicrobials, those receiving continuous dental care, smokers, people with systemic disorders, and those with limited dexterity who havetrouble brushing were not allowed to participate in the study. Each of the selected individuals received a medium-tufted toothbrush (made by ICPA Health Products Ltd., Gujarat, India) and toothpaste (made by Colgate Palmolive India Ltd., Himachal Pradesh, India) in addition to thorough oral hygiene instructions. After a month, the study participants were invited back to collect their used toothbrushes. Five brand-new, unused toothbrushes were added to sterile cartons containing their toothbrush (negative control), which was sent right awayfor microbiological examination.

MICROBIAL ANALYSIS

Based on disinfectant used, the toothbrushes were split into four equal groups, with 20 brushes in one group.

Group A: 0.2% Chlorhexidine gluconate (control group),
Group B: Listerine,
Group C: Dettol,
Group D: Tap water.

Five new, unused toothbrushes served as the negative control.

The brushes were first kept for an hour inside test tubes with 5 ml of normal saline, after taking aseptic precautions. Aerobic incubation was performed for 24 hours at 37°C using Hinton-based blood agar and MacConkey agar. The isolates were identified using normal microbiological procedure and Gram staining. Additionally, using a disposable syringe, 1 ml of the sample was removed from the same test tube and added to Robertson's cooked meat (RCM) medium. This was then incubated for 24 hours at 37°C. Mitis Salvarius agar was used to further cultivate the RCM sample. After that, each toothbrush was immersed for one-hour in one of the four disinfectants following which the toothbrushes were submerged in 5 milliliters of neutralizer broth for an hour, and finally the samples from the neutralizer broth were gathered and cultured in order to assess each of the four disinfectants' level of effectiveness. The statistical package for social science (SPSS) version 23 program (SPSS 23.0, IBM, Armonk, NY, USA) was used to analyze all of the data. Chi-square analysis was used to perform inferential statistics. $P < 0.05$ was regarded as statistically significant for all analyses.

RESULTS

After a month of use, the toothbrushes in all four test groups were contaminated, as evidenced by the distribution of aerobic bacterial growth among them prior to disinfection.

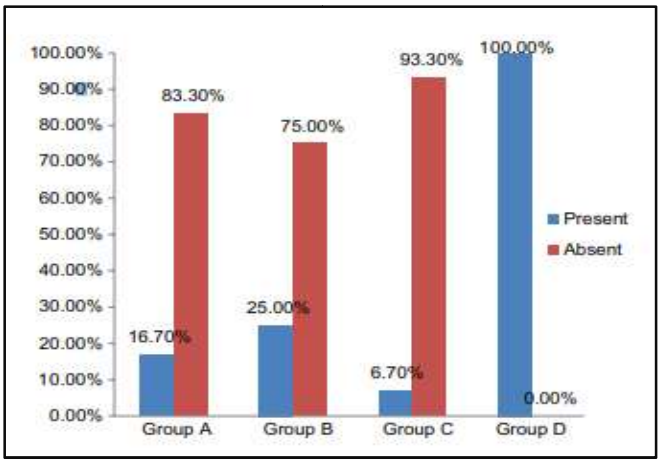
Table 1. Distribution of aerobic bacterial growth among the four test groups before disinfection

Group	Aerobic bacteria before disinfection		Total	P
	Present	Absent		
Group a	48	12	60	0.000
Group b	51	9	60	
Group s	60	0	60	
Group d	43	13	60	

*Statistically significant. Test applied – Chisquare test

Group	Aerobic bacteria before disinfection		Total	P
	Present	Absent		
Group a	15	45	60	0.000
Group b	4	56	60	
Group s	10	50	60	
Group d	60	0	60	

*Statistically significant. Test applied – Chisquare test



Graph 1. Distribution of aerobic bacterial growth among the four test groups after disinfection

Each of the 60 investigated agar plates showed a significant amount of ($P < 0.001$) aerobic bacterial growth: 51 plates (85%) in Group A, 48 plates (80%) in Group B, 43 plates (71.7%) in Group C, and 60 plates (100%) in Group D. Negative growth was seen in the five unused brushes. With the exception of Group D (tap water), all groups showed a notable ($P < 0.001$) decrease in microbial growth following toothbrush cleaning. Out of the sixty cultivated agar plates in each group, Group A had ten plates, Group B had fifteen plates, and only four of Group C's (Dettol) plate, showed bacterial growth. Of all the disinfectants, Dettol had the highest efficacy (93.3%) in preventing the growth of bacteria. A variety of microorganisms, including beta-hemolytic streptococci, Streptococcus, Klebsiella, and Escherichia coli, micrococci, Bacillus species, Viridans streptococci, and coagulase-negative

staphylococci.were identified from the culture prior and subsequent to disinfection. The microorganisms could be found alone or as a combination of several species. Before disinfection, E. Coli (17.1%) was the most common microbe presenting alone in culture; following disinfection, Klebsiella species (7.1%) was the most common microorganism.

DISCUSSION

Contaminated toothbrush is an inherent result of use, and it can cause numerous oral and systemic illnesses when stored improperly. The bristles frequently become worn after frequent use. For this reason, the American Dental Association advises changing toothbrushes every three to four months. However, it is unclear from this statement if changing the toothbrush might not be enough to get rid of the bacteria. Therefore, it is essential to have a cheap and readily available material for disinfecting toothbrushes. In the current study, we examined the effectiveness of a few widely accessible antimicrobial agents as toothbrush disinfectants, comparing them to plain tap water. These agents included 0.2% chlorhexidine gluconate, Listerine, and Dettol. A cationic bisbiguanide that works well against a variety of microbes is chlorhexidine gluconate. It functions as both a bactericidal and bacteriostatic agent, depending on the concentration. Listerine is a phenol precipitate that includes benzoic acid, boric acid, methyl salicylate, thymol, and eucalyptol. The majority of phenols have a broad antibacterial effect that depends on the medication's capacity to pierce germs' lipid-containing cell walls and cause structural damage when it is in its nonionized state. Chloroxylenol is the active component in Dettol. Isopropyl alcohol, soap, caramel, pine oil, castor oil, and water are among the other ingredients. Dettol is effective against Gram-positive and Gram-negative because of these components. In this investigation, we discovered that all of the contaminated toothbrushes had multiple bacterial species, whereas theunused toothbrushes that were negative control showed no bacterial contamination. The mouth cavity, storage containers, storagesettings, and the rinsing water may have all contributed to the bacterial contamination of the used toothbrushes. The toothbrushes in the study were shown to have isolates of E. Coli, Klebsiella, Pseudomonas, beta-hemolytic streptococci, S. mitis, and V. streptococci Micrococci, Bacillus species, and coagulase-negative staphylococci after one month of use. This outcome shares some resemblance to the research conducted by Sogi *et al*, where they reported finding bacteria on used toothbrushes that included beta-hemolytic Streptococcus, E. coli, Klebsiella, Proteus species, and Staphylococcus pyogenes. Grewal and Kaur conducted a study in which they isolated Klebsiella, E. coli, and Streptococcus faecalis. They observed that after a month, the growth of microbes on the toothbrush remained at 100% for a maximum of three months.Sammons *et al*., reported finding presumed Staphylococci, Pseudomonas and coliforms in the toothbrushes they examined. Osho *et al*., separated E. Coli, Staphylococcus aureus, Enterobacter, Pseudomonas aeruginosa and Staphylococcus saprophyticus for used toothbrushes. Moreover, Malmberg and colleagues, separated Staphylococcus epidermidis and Streptococci from toothbrushes after usage, but toothbrushes from patients with oral illness and healthy individuals included potentially harmful bacteria such Pseudomonas species, E. coli, and Staphylococcus species.

In the current investigation, E. Col accounted for 17.1% of the species of bacterial isolates detected, with Klebsiella and Pseudomonas coming in second at 8%. Coliform E. coli belongs to the Enterobacteriaceae family. In large quantities, they are pathogenic to humans. Pyogenic infections, pneumonia, septicemia, urinary tract infections and diarrhea are all caused by Klebsiella. The bacteria may have gotten into the toothbrushes through the rinsing water. Given that enteric rods are a component of the oral flora, oral commensals may also have played a role in the toothbrush contamination.Pathogenic bacterias such as S. aureus, Klebsiella, pseudomonas may undoubtedly cause major risk to both oral and general health if toothbrushes are The toothbrushes had enteric rods on them, which suggested a potential fecal contamination. The used toothbrushes have to have been kept in unclean spaces such sinks in bathrooms and toilets. The damp atmosphere in the bathroom promotes the growth of microorganisms. Pseudomonas aeruginosa is a common bacterium found in water and other natural environments, therefore it's contaminated. Most people consider disinfectants to be chemically prepared liquids that are used to eradicate microorganisms. Numerous research have emphasized the value and efficiency of toothbrush disinfection. The effectiveness of different disinfectants in lowering toothbrush contamination was evaluated in the current investigation. 0.2% chlorhexidine gluconate, Dettol and Listerine were the disinfectants utilized since they were readily available, nontoxic, affordable, and efficient. According to our investigation, soaking toothbrushes in 0.2% chlorhexidine resulted in an 83.3% reduction in bacterial growth. According to Saleh, there was an 87.5% decrease in microbiological growth following disinfection of toothbrush with chlorhexidine. Nanjunda Swamy &Co. , following a 20-hour immersion in chlorhexidine,obtained a 100% reduction in bacteria; their findings are somewhat consistent with ours. The explanation for this divergence from our research may be attributed to the differing chlorhexidine concentrations and immersion times. A study by Konidala *et al*.demonstarted that Dettol was only 40% effective in reducing bacteria, while Hexidine and Listerine demonstrated a 100% reduction in lowering the amount of microorganisms on toothbrushes. Interestingly, in our analysis, Dettol showed greatest efficacy (93.3%) in disinfection contrast to the reported 83.3% and 75% for Listerine and chlorhexidine respectively. For disinfectants to be 100% effective, different immersion times have been suggested by several research .In the current investigation, we discovered that every antimicrobial tested was successful in reducing microbial growth after a one-hour immersion period. Surprisingly, yet, none of the disinfectants demonstrated perfect effectiveness. In order to determine the precise immersion period and most appropriate antimicrobial for toothbrush disinfection, this result justifies the necessity for additional randomized research with bigger sample sizes. There are certain restrictions on the current investigation. Because only aerobic bacterial culture was used during the one-month study period and more pathogenic species, such as anaerobes,were not given any thought. Additionally, the impact of brushing frequency and storage location was overlooked, which could have affected the results. In contrast, our study used 100 participants and examined variety of microbes that could contaminate toothbrushes, therefore helping with the planning of preventive measures. The current study also included suggestions for sterilizing toothbrushes with basic, readily accessible disinfectants.

CONCLUSIONS

Used toothbrushes acts as harbors for microbes and could be a significant factor in the spread of disease among people. It was discovered that every toothbrush used in the trial was infected with various bacteria following a month of use. After being applied for one hour, it was discovered that 0.2% chlorhexidine gluconate (83.3%), Dettol (93.3%) and Listerine (75%), were efficient in decontaminating the toothbrushes, whereas tap water proved to be ineffective. To maximize oral hygiene and overall health, it is advised that every person disinfect their toothbrushes routinely after daily use and store them individually in a clean and dry location. Future randomized studies are advised to assess the impact of varying toothbrush usage durations among various age groups in both sick and systemically healthy people to evaluate the potential effects of toothbrush contamination. To address the constraints of the current study, future research should also concentrate on various agents, such as natural and home remedies.

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