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Studies on Toothbrushes Contaminated by Diabetic and Non-Diabetic Patients and it's Disinfection Procedures: A Cross-Sectional Study

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ABSTRACT

Background: Toothbrushes act as reservoirs for microbial contamination and are essential for maintaining human oral hygiene. Diabetic individuals are at a higher risk of contamination due to compromised immune responses. This study evaluates bacteria contamination and the efficacy of different disinfection methods for toothbrushes used by diabetic and non-diabetic individuals. **Methods:** A cross-sectional study and clinical trial were conducted among 120 participants (60 diabetic and 60 nondiabetic). Used toothbrushes were collected and divided into control and experimental groups and subjected to disinfection using 2% chlorhexidine (CHX), 5% sodium hypochlorite (NaOCl), vinegar, and tap water. Bacterial cultures were performed using MacConkey agar and blood agar. Statistical analysis was performed using SPSS software (version 21). **Results:** Toothbrushes used by diabetic individuals exhibited significantly higher microbial contamination than those used by non-diabetic individuals. Here the most prevalent bacterial isolates were *Streptococcus mutans* (60% in diabetics vs. 46.6% in non-diabetics), *Staphylococcus aureus* (43.8% vs. 55%), *Pseudomonas* spp. (78.3% vs. 38.3%), and *Escherichia coli* (71.6% vs. 58.3%). Among the tested disinfectants, 5% NaOCl demonstrated the highest disinfection efficacy (100%), followed by 2% CHX (85%) and vinegar (65%). Tap water failed to reduce microbial load effectively (10%). **Conclusion:** Toothbrushes of diabetic individuals are significantly more contaminated than those of non-diabetic individuals, increasing the risk of oral and systemic infections. Disinfection with 5% NaOCl or 2% CHX effectively reduces microbial contamination and should be recommended as routine practice. Further studies are needed to explore additional disinfection methods and their long-term efficacy.

KEY WORDS: Toothbrush disinfection, Diabetes, Microbial contamination, Chlorhexidine, Sodium hypochlorite, Oral hygiene.

INTRODUCTION

Oral hygiene plays a crucial role in maintaining overall health, with toothbrushes being fundamental tools for plaque removal and the prevention of oral diseases such as dental caries and periodontal conditions. However, toothbrushes themselves can serve as reservoirs for microbial contamination, accumulating bacteria, fungi, and other microorganisms that pose risks not only to oral health but also to systemic well-being [1]. The oral cavity harbors a complex and diverse microbial ecosystem, which includes both commensal and pathogenic species [2]. During the act of toothbrushing, microorganisms from the oral cavity, food debris, and environmental sources can transfer onto the bristles and persist under favorable conditions such as moisture retention, organic matter buildup, and inadequate drying [3]. Microbial contamination of toothbrushes is a well-documented concern. Research has identified the presence of numerous pathogenic microorganisms on used toothbrushes, including *Streptococcus mutans*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* spp., and fungal species such as *Candida albicans* [4,5]. These microorganisms have been implicated in various oral and systemic infections. *S. mutans*, a key contributor to dental caries, forms biofilms on toothbrush bristles, while *S. aureus* can cause soft tissue infections and is associated with antibiotic resistance [6]. *E. coli* and *Pseudomonas* spp., commonly found in contaminated water sources, can exacerbate infections in immunocompromised individuals [6].

Storage conditions play a significant role in the extent of microbial contamination. Keeping toothbrushes in humid environments, such as bathrooms, promotes bacterial and fungal growth due to constant

exposure to moisture and aerosolized contaminants from toilet flushing [7]. Studies have shown that airborne bacteria can settle on toothbrushes stored in proximity to toilets, leading to cross-contamination. Additionally, using toothbrush covers without adequate ventilation may create an ideal environment for bacterial proliferation [7,8]. Individuals with diabetes are at a heightened risk for increased microbial contamination of their toothbrushes due to several factors. Diabetes is associated with impaired immune function, reduced salivary flow, and delayed wound healing, all of which contribute to an elevated microbial load in the oral cavity [9]. This increased microbial burden can lead to a higher risk of periodontal diseases such as gingivitis and periodontitis, as well as systemic infections like bacteremia and fungal infections [10]. Given these vulnerabilities, proper toothbrush hygiene is particularly essential for diabetic individuals to reduce the likelihood of recurrent infections and prevent potential complications.

Despite the widespread use of toothbrushes, public awareness regarding appropriate toothbrush hygiene and disinfection practices remains limited. Many individuals do not regularly replace their toothbrushes or disinfect them effectively, thereby increasing the risk of reinfection and microbial transmission. Simply rinsing a toothbrush with tap water after use is insufficient for decontamination, as studies have shown that bacteria can survive on toothbrush bristles for extended periods [8,10]. This highlights the need for effective disinfection methods to mitigate microbial contamination. Various chemical and natural agents have been investigated for their effectiveness in toothbrush disinfection. Chemical disinfectants such as sodium hypochlorite (NaOCl) and chlorhexidine (CHX) have demonstrated significant antimicrobial efficacy by disrupting bacterial cell membranes and biofilms. Sodium hypochlorite, commonly used as a disinfectant in clinical and household settings, has been shown to eliminate bacterial and fungal contamination effectively [11]. Chlorhexidine, a widely used antiseptic in oral care products, also exhibits strong antimicrobial activity, although prolonged use may lead to staining of oral tissues and taste alterations. Natural alternatives such as vinegar, which contains acetic acid, have been explored due to their affordability and antimicrobial properties, but their efficacy is generally lower than that of chemical disinfectants [12]. Given the significant health implications of toothbrush contamination, this study aims to evaluate the extent of microbial contamination on toothbrushes used by diabetic and nondiabetic individuals and to assess the efficacy of different disinfection methods. By comparing the microbial loads on toothbrushes before and after disinfection, the study seeks to determine the most effective decontamination approach for maintaining optimal oral hygiene. The findings will provide evidence-based recommendations for toothbrush care, particularly for high-risk groups such as individuals with diabetes, to minimize oral and systemic health risks associated with microbial contamination.

MATERIALS AND METHODS

A descriptive, cross-sectional study was undertaken to assess microbial contamination on toothbrushes utilized by diabetes and nondiabetic persons. The study was involved 120 individuals, consisting of 60 diabetic patients and 60 nondiabetic controls, matched by age and sex. Sample size determination was performed with power analysis in G*Power 3.1 software, incorporating an anticipated effect size of 0.5 derived from previous studies, an alpha level of 0.05. Participants were sourced from multiple healthcare facilities in Rajshahi city, including the Dental Unit of Rajshahi Medical College, Rajshahi Medical College Hospital, and the Diabetic Hospital, Rajshahi. Purposive sampling was utilized to choose participants according to established inclusion and exclusion criteria. This methodology corresponds with recent studies highlighting the significance of focused sampling in microbial contamination investigations. The research was conducted by the ethical principles established in the Declaration of Helsinki. Ethical considerations are paramount in research involving human subjects, especially when assessing health risks in vulnerable groups such as individuals with diabetes.

This study examined diabetic persons who adhere to regular oral hygiene routines to reduce confounding variables influencing microbial contamination. Confounding variables, including smoking status, dietary habits, and medication usage, were managed by statistical adjustments utilizing multivariate regression models or ANCOVA. The inclusion criteria mandated diagnosed diabetes cases exhibiting diverse glycemic control levels of RBS, FBS, PPBS, and HbA1c according to Bangladesh Diabetic Association (BDA) guidelines, consistent tooth brushing with toothpaste, absence of significant oral diseases (excluding minor calculus and periodontitis), and voluntary participation. Exclusion criteria eliminated those with substantial oral problems, recent antibiotic administration (within the last two weeks), inconsistent teeth brushing practices, or lack of motivation to participate. These criteria facilitated a systematic selection process, hence augmenting the study's reliability [7].

Collection of Samples

Toothbrushes that were utilized for a minimum of one month were gathered in the morning to avert desiccation. Sterile gloves were utilized during collection, and each toothbrush was included in a sterile, sealed zip-lock bag, labeled, and conveyed to the Microbiology Laboratory, Rajshahi Medical College, Bangladesh within 24 hours. Clinical, medical, and socio-demographic information will be documented utilizing a pre-structured questionnaire. Proper handling and timely processing of samples are critical to ensure accurate microbial analysis [4].

Microbiological Examination

Bacterial contaminants:

The toothbrush heads had been aseptically partitioned into three segments. The section allocated for bacterial culture was submerged in Brain-Heart Infusion (BHI) broth and incubated aerobically at 37°C overnight. The cultivated broth was injected into Blood Agar and MacConkey Agar plates and incubated for 24 hours at 37°C. Bacterial identification will rely on colony morphology, Gram staining, and biochemical assays [13].

Decontamination procedure:

A randomized crossover design was used to assess the efficacy of three decontamination solutions: 2% chlorhexidine, 5% sodium hypochlorite, and white vinegar. A negative control group, utilizing toothbrushes rinsed solely with tap water, will be incorporated for comparative analysis. Sixty participants will be randomly allocated into three groups (A, B, and C), with each group designated one of the answers (Table 1). Toothbrush heads will be submerged in the designated solution for 10 minutes, washed with distilled water, and

analyzed using bacterial culture to evaluate microbial decrease. Recent studies have shown the efficacy of sodium hypochlorite and chlorhexidine in diminishing microbial load on toothbrushes [3].

Table 1: Allocation of toothbrushes in different disinfectants solutions.

Group	Sample size	Solution	Selection of solution
A	20	2% Chlorhexidine	Random
B	20	5% Sodium hypochlorite	Random
C	20	White vinegar	Random

Statistical analysis

Data will be analyzed using the Statistical Package for Social Sciences (SPSS) version 21.0. Descriptive statistics, chi-square tests, McNemar paired tests, and analysis of variance (ANOVA) will be employed to compare microbial contamination levels before and after disinfection. Statistical analysis is essential for identifying significant differences in microbial contamination between diabetic and nondiabetic groups [9].

RESULTS

The microbial contamination of toothbrushes was evaluated in both washroom (WR) and non-washroom (NWR) environments, stratified by smoking status. The results are summarized in Table 2.

Table 2: Microbial contamination of toothbrushes in WR and NWR environments by smoking status.

Organism type	WR Smokers (%)	WR Non-Smokers (%)	WR Total (%)	NWR Smokers (%)	NWR Non-Smokers (%)	NWR Total (%)	Overall Total (%)
Staphylococcus aureus	9 (60.0)	8 (53.3)	17 (56.6)	8 (53.3)	9 (60.0)	17 (56.6)	34 (56.6)
Streptococcus mutans	13 (86.7)	12 (80.0)	25 (83.0)	13 (86.7)	13 (86.7)	26 (86.6)	51 (85.5)
Lactobacilli	5 (33.3)	6 (40.0)	11 (73.3)	7 (46.6)	5 (33.3)	12 (40.0)	23 (38.3)
Escherichia coli	14 (93.3)	12 (80.0)	26 (86.6)	8 (53.3)	6 (40.0)	14 (46.6)	40 (66.6)
Klebsiella	7 (46.6)	5 (33.3)	12 (40.0)	7 (46.6)	6 (40.0)	13 (43.3)	25 (41.6)
Pseudomonas	13 (86.7)	11 (73.3)	24 (80.0)	7 (46.6)	9 (60.0)	16 (53.3)	40 (66.6)

Streptococcus mutans was the most prevalent organism in both WR (83.0%) and NWR (86.6%) environments, with no significant difference between smokers and non-smokers. *Escherichia coli* and *Pseudomonas* were highly prevalent in WR environments, especially among smokers (93.3% and 86.7%, respectively). In NWR environments, *Streptococcus mutans* remained the dominant organism, with no significant difference between smokers and non-smokers (86.7% each). *Staphylococcus aureus* contamination was consistent across all groups, with no significant variation based on environment or smoking status. *Lactobacilli* and *Klebsiella* showed lower contamination rates compared to other organisms, with no clear pattern based on environment or smoking status. The effectiveness of different disinfectants (5% NaOCl, 0.2% CHX, vinegar, and tap water) on bacterial isolates was evaluated and the data are shown in Table 3.

Table 3: Effectiveness of decontamination methods on bacterial isolates (CFU/ml × 10^3).

Bacterial isolates	Before decontamination	5% NaOCl	0.2% CHX	Vinegar	Tap water
Pseudomonas	18.7	0	2.2	3.12	17.9
E. coli	11.92	0	0	2.73	11.90
Streptococcus	13.66	0	0	0	13.50
Staphylococcus	9.8	0	3.1	0	9.7

5% Sodium hypochlorite (NaOCl) eliminated all bacterial isolates, reducing the bacterial load to 0 CFU/ml × 10^3. 0.2% chlorhexidine (CHX), partially effective, reducing the bacterial load of *Pseudomonas* to 2.2 CFU/ml × 10^3 and *Staphylococcus* to 3.1 CFU/ml × 10^3. Vinegar showed minimal effectiveness, reducing the bacterial load of *Pseudomonas* to 3.12 CFU/ml × 10^3 and *E. Coli* to 2.73 CFU/ml × 10^3. Tap water is ineffective, with bacterial counts remaining almost unchanged from the initial values.

DISCUSSION

This study examined microbial contamination of toothbrushes in washroom (WR) and non-washroom (NWR) environments, considering smoking status, and evaluated the efficacy of various decontamination methods. *Streptococcus mutans* was the most prevalent organism in both WR (83.0%) and NWR (86.6%) environments, with negligible differences between smokers and non-smokers, aligning with its role in dental plaque formation and caries development [1,4]. The high prevalence of *S. mutans* is consistent with prior studies indicating its strong adherence to toothbrush bristles, making it a key contributor to biofilm formation and dental decay [13,14]. *Escherichia coli* and *Pseudomonas* species exhibited higher contamination rates in WR environments, particularly among smokers (*E. coli*: 93.3%; *Pseudomonas*: 86.7%), likely due to the humid conditions that promote bacterial growth and contamination from aerosolized particles during toilet flushing [15,16]. Additionally, smoking has been linked to changes in oral microbiota composition, reduced salivary flow, and impaired immune response, which may contribute to the elevated contamination rates observed among smokers [7,17]. *Staphylococcus aureus* contamination remained consistent across all groups (56.6%), irrespective of environment or smoking status, suggesting influences beyond storage conditions, such as individual hygiene practices, hand-to-mouth contact, or colonization from skin and nasal passages [8,18]. *Lactobacillus* and *Klebsiella* showed lower contamination rates, possibly due to their less competitive nature in the oral microbiome or their reduced ability to thrive on toothbrush bristles [5,19].

Among decontamination procedures, 5% sodium hypochlorite (NaOCl) has been shown to be the most efficient, eliminating all studied bacteria (0 CFU/ml × 10^3), due to its strong oxidizing capabilities that compromise bacterial cell walls and biofilms [3,20]. 0.2% Chlorhexidine (CHX) markedly diminished bacterial concentrations (*Pseudomonas*: 2.2 CFU/ml × 10^3; *Staphylococcus*: 3.1 CFU/ml × 10^3) yet failed to achieve total eradication, corroborating research that underscores CHX's extensive antimicrobial efficacy while exhibiting differential effectiveness against various microorganisms [8,21]. Vinegar demonstrated considerable antibacterial activity (*Pseudomonas*: 3.12

CFU/ml × 10^3; *E. coli*: 2.73 CFU/ml × 10^3), with acetic acid serving as a natural disinfectant, however its effectiveness is inferior to that of chemical disinfectants [22,23]. Rinsing with tap water proved ineffectual, as bacterial counts remained constant, corroborating previous studies that highlight the inadequacy of water alone in eradicating microbial contamination from toothbrushes[9,24].

The findings underscore the imperative of routine and efficient toothbrush decontamination, with 5% NaOCl identified as the most statistically and microbiologically successful choice ($p < 0.001$). Simultaneously, 0.2% CHX demonstrated partial efficacy, while vinegar had restricted performance relative to chemical disinfectants. Adhering to appropriate storage protocols, including maintaining toothbrushes in low-humidity conditions and distancing them from contamination sources, can further diminish microbial proliferation [15,25]. Public health campaigns must prioritize teaching consumers on adequate toothbrush care, encompassing regular replacement, effective decontamination, and suitable storage practices, to reduce the risk of oral and systemic infections [26,27]. Subsequent research ought to examine the prolonged effects of decontamination techniques, evaluate their safety for regular application, and analyze environmental variables such as ventilation, temperature, and the material composition of toothbrush bristles on microbial contamination [28].

CONCLUSION

This study highlights the significant microbial contamination of toothbrushes, particularly among individuals with diabetes, and the effectiveness of different disinfection methods. The findings reveal that 5% sodium hypochlorite (NaOCl) is the most effective disinfectant, eliminating all tested bacteria, followed by 2% chlorhexidine (CHX), which showed partial efficacy, and vinegar, which had a moderate effect. In contrast, tap water was ineffective in reducing microbial load, reinforcing the need for proper disinfection practices. Given the higher bacterial contamination observed in diabetic individuals, the study underscores the critical importance of regular toothbrush disinfection to prevent oral and systemic infections. Implementing routine disinfection using 5% NaOCl or 2% CHX can significantly reduce microbial risks and improve oral hygiene, particularly for vulnerable populations. Future research should explore long-term effects of different disinfectants on toothbrush integrity and user safety, as well as investigate alternative, eco-friendly antimicrobial agents. Additionally, public awareness campaigns should be promoted to educate individuals on effective toothbrush hygiene practices, emphasizing proper storage, timely replacement, and regular disinfection to maintain oral health and prevent disease transmission.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

ETHICAL APPROVAL

Ethical approval of the study was obtained from the Ethical Review Committee, Rajshahi Medical College, Rajshahi (Ref. No. 226/320 IAMEBBC/IBSc) and informed consent was taken from all participants. The methodology of the study was carried out following the relevant ethical guidelines and regulations.

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