Original Article

Influence Of Glycemic Control and Storage Conditions on Toothbrush Microbial Contamination Among Diabetic Patients

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Abstract

Introduction: Toothbrushes can become contaminated with oral and environmental pathogens, posing infection risks. This study evaluates how glycemic control and toothbrush storage location jointly affect microbial contamination in diabetic patients.

Methods: In this cross-sectional study (Rajshahi Medical College), 60 participants (30 diabetics, 30 non-diabetic controls) provided used toothbrushes (≥2 months old) and clinical data via questionnaire. Diabetic subjects were categorized by HbA1c: good (<7%), moderate (7–9%), or poor (>9%) glycemic control (10 per category). Each subject reported toothbrush storage (washroom [WR] vs. non-washroom [NWR]). Toothbrush heads were aseptically processed in sterile saline; aliquots were cultured on selective media (Blood Agar, MacConkey Agar, Sabouraud Dextrose Agar) to isolate bacteria and yeasts (e.g., *Streptococcus mutans*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas* spp., *Candida*). Statistical analysis (SPSS) used chi-square tests and Pearson correlation to assess associations, with $p \leq 0.05$ considered significant. **Results:** All toothbrushes (100%) were culture-positive, with *S. mutans* the most prevalent isolate. Contamination rates and species diversity were significantly higher in diabetics, especially with poor glycemic control . Poor-control diabetics showed elevated prevalence of *S. aureus* (~80%, $p<0.01$), *Candida* spp. (~76.7%, $p<0.01$), and *E. coli* (~83.3%, $p<0.05$) on their brushes. Separately, washroom-stored brushes (all subjects) had significantly more enteric bacteria: *E. coli* contamination was 86.6% (WR) vs. 46.6% (NWR), and *Pseudomonas* 80.0% vs. 53.3% ($p<0.05$). This confirms that bathroom aerosols and moisture enhance toothbrush contamination. The highest contamination occurred in brushes from poorly controlled diabetics stored in washrooms. Notably, poor HbA1c correlated strongly with greater microbial diversity ($r=0.72$, $p<0.001$). **Conclusion:** Both poor glycemic control and unhygienic storage significantly increase toothbrush microbial burden. Diabetic patients, especially with high HbA1c, may harbor more oral pathogens on their brushes, and washroom storage further elevates enteric contamination. These findings underscore the need for patient education on glycemic management and proper toothbrush hygiene (e.g., storing brushes away from toilets and changing them regularly) to reduce infection risk.

Keywords: Toothbrush; Glycemic control; Microbial contamination; Diabetes; Storage practices
Abbreviations: WR: Washroom; NWR: Non-washroom

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Introduction

Toothbrush bristles can harbor bacteria, fungi, and other microorganisms, potentially acting as vectors for oral and systemic infections. Studies report that toothbrushes become contaminated shortly after initial use, with microbial loads increasing over time [1]. Bristles retain oral biofilm and saliva, providing a nutrient-rich environment favorable for microbial growth. Common pathogens isolated from toothbrushes include *Streptococcus mutans*, *Staphylococcus aureus*, enteric bacteria (*Escherichia coli*, *Klebsiella* spp.), *Pseudomonas* spp., and *Candida albicans* [2]. High bacterial loads have been linked to dental caries, gingivitis, stomatitis, and systemic conditions such as infective endocarditis. Environmental factors—such as hand contamination, air exposure, and bathroom storage—further contribute to microbial colonization [3,4]. Storing toothbrushes capped or enclosed can inadvertently trap moisture, intensifying microbial proliferation.

Diabetes mellitus further complicates this situation due to chronic hyperglycemia altering oral microbial ecology and weakening host defenses [5,6]. With over 500 million diabetics globally, and approximately 13 million in Bangladesh alone, diabetes presents a significant public health challenge [7]. Diabetic individuals frequently exhibit more severe periodontal diseases, increased dental caries, and candidiasis compared to non-diabetics [6,8]. Elevated glucose levels in saliva and gingival crevicular fluid foster acidogenic and pathogenic microbes such as *S. mutans*, *Lactobacilli*, and *Candida*, promoting enamel demineralization and gingival invasion [9]. Concurrently, diabetes impairs immune responses, weakening neutrophil function and complement pathways. Thus, hyperglycemia disrupts oral microbial balance, increases pathogen prevalence, and reduces immune defenses [10,11].

These interactions have major public health implications, especially in regions like South Asia, including Bangladesh, where diabetes prevalence is rapidly increasing and dental care infrastructure remains limited [12]. Although current preventive guidelines emphasize glycemic control and basic oral hygiene, toothbrush hygiene is often overlooked. Since contaminated brushes can reintroduce pathogens to the oral cavity, diabetic and immunocompromised individuals may be particularly vulnerable. Clarifying how glycemic status and toothbrush storage conditions influence microbial load is therefore essential. This study places toothbrush contamination and diabetes-related oral microbiology within the broader context of infection control and public health policy.

Materials And Methods

A cross-sectional design was used at Rajshahi Medical College. We recruited 30 known diabetic patients (type 1 or 2) and 30 age- and gender-matched non-diabetic controls (all adults, 18–70 years). Diabetic subjects were stratified by glycemic control: good (HbA1c <7%), moderate (7–9%), or poor (>9%), with 10 participants in each category. Within each group, participants were also classified by primary toothbrush storage location: in the washroom (WR) or outside it (NWR), yielding equal subgroups (15 WR and 15 NWR subjects per group).

Inclusion criteria were: diagnosis of diabetes (for patient group), routine twice-daily brushing, and use of the same manual toothbrush for at least two months prior to the study. Exclusion criteria included: current systemic antibiotic therapy (within 2 weeks), active oral disease (beyond mild gingivitis), or irregular brushing habits. After informed consent, each subject completed a questionnaire on oral hygiene habits and had basic clinical data recorded.

Each participant provided their used toothbrush in exchange for a new sterile brush. Collected toothbrushes (with >2 months of use) were immediately transferred to the microbiology laboratory in sterile containers. In processing, each toothbrush head was aseptically cut off with sterile scissors and immersed in 10 mL sterile saline. The container was vortexed to dislodge microbes. Aliquots were inoculated onto Blood Agar (general bacteria), MacConkey Agar (Gram-negative bacteria),

and Sabouraud Dextrose Agar (fungal pathogens). Bacterial cultures were incubated at 37°C and examined at 24–48 hours; fungal cultures were incubated at room temperature up to 7 days [13]. Colonies were identified by morphology, Gram stain, and standard biochemical tests (e.g., catalase, coagulase, sugar fermentation), confirming species such as *S. mutans*, *S. aureus*, *E. coli*, *Pseudomonas* spp., *Klebsiella* spp., and *Candida* spp [14].

Statistical analyses were performed with SPSS. Descriptive statistics (frequencies, percentages) summarized microbial prevalence. The chi-square test (or Fisher's exact test) compared categorical contamination rates between groups (diabetic vs. control, glycemic categories, storage locations). Pearson correlation assessed associations between HbA1c levels and the number of microbial species per brush. A p-value of ≤0.05 was considered statistically significant.

Results

All 60 toothbrushes yielded microbial growth (100% contamination), confirming heavy colonization of bristles. The most frequently recovered species was *Streptococcus mutans*, followed by *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida* spp. and *Klebsiella* spp. (Table 1). Notably, every organism was found more often on brushes from diabetic patients than from non-diabetic controls. For example, *E. coli* was isolated from 83.3% of diabetic brushes versus only 46.6% of controls, and *Candida* spp. from 76.7% vs. 30.0% (Table 1). These differences were statistically significant, indicating that diabetic status is associated with a higher burden of toothbrush contamination.

Table 1: Prevalence of microbial contaminants on toothbrushes by group

Organism	Diabetics (%)	Non-diabetics (%)
Streptococcus mutans	90.0	80.0
Staphylococcus aureus	85.0	65.0
Escherichia coli	83.3	46.6
Pseudomonas aeruginosa	80.0	53.3
Klebsiella spp.	41.6	20.0
Candida spp.	76.7	30.0

Among the 30 diabetic subjects, contamination prevalence rose with worsening glycemic control (Table 2). Brushes from poorly controlled diabetics (HbA1c >9%) showed the highest rates of contamination: for instance, *E. coli* was present on 83.3% of these brushes compared to 50.0% of brushes from well-controlled diabetics (HbA1c <7%). Similarly, *S. aureus* contamination was 80.0% in the poor-control group versus 60.0% in the good-control group, and *Candida* spp. were found on 76.7% vs. 30.0%, respectively. The increases in *S. aureus* and *Candida* rates with poor glycemic control were highly significant (both $p<0.01$), and the rise in *E. coli* was significant ($p<0.05$). Overall, the number of different species recovered from each brush grew steadily from good to poor control, consistent with a strong positive correlation between HbA1c and microbial diversity ($r = 0.72$, $p<0.001$).

Table 2: Microbial contamination by glycemic control (diabetic patients only, n=30)

Organism	Good (%)	Moderate (%)	Poor (%)
Streptococcus mutans	70.0	80.0	90.0
Staphylococcus aureus	60.0	70.0	80.0
Escherichia coli	50.0	70.0	83.3
Candida spp.	30.0	50.0	76.7

Storage location also had a major impact on contamination (Table 3). Brushes kept in the washroom harbored significantly more enteric and opportunistic bacteria than those stored elsewhere. For example, 86.6% of washroom-stored brushes yielded *E. coli* versus only 46.6% of non-washroom brushes ($p<0.01$). *Pseudomonas* spp. were detected in 80.0% of washroom brushes compared to 53.3% of non-washroom ($p<0.05$). Even *S. aureus* and *S. mutans* showed higher prevalence in washroom storage (90.0% and 85.0%, respectively) than outside (65.0% and 70.0%), reflecting the moist, aerosol-rich environment of bathrooms. These findings confirm that washroom storage significantly elevates toothbrush contamination.

Table 3: Effect of storage environment on microbial contamination (all participants)

Organism	Washroom (%)	Non-washroom (%)
Escherichia coli	86.6	46.6
Pseudomonas aeruginosa	80.0	53.3
Staphylococcus aureus	90.0	65.0
Streptococcus mutans	85.0	70.0

Diabetic individuals have significantly higher toothbrush microbial contamination than non-diabetics, especially with pathogens like *E. coli* and *Candida*. This microbial burden escalates with worsening glycemic control, underscoring the importance of proper diabetes management to mitigate infection risks. Additionally, toothbrushes stored in washrooms exhibited notably greater contamination, highlighting environmental conditions as critical factors in microbial proliferation. Thus, effective preventive strategies should emphasize optimal glycemic management and hygienic toothbrush storage practices, particularly for diabetic patients (Figure-1).

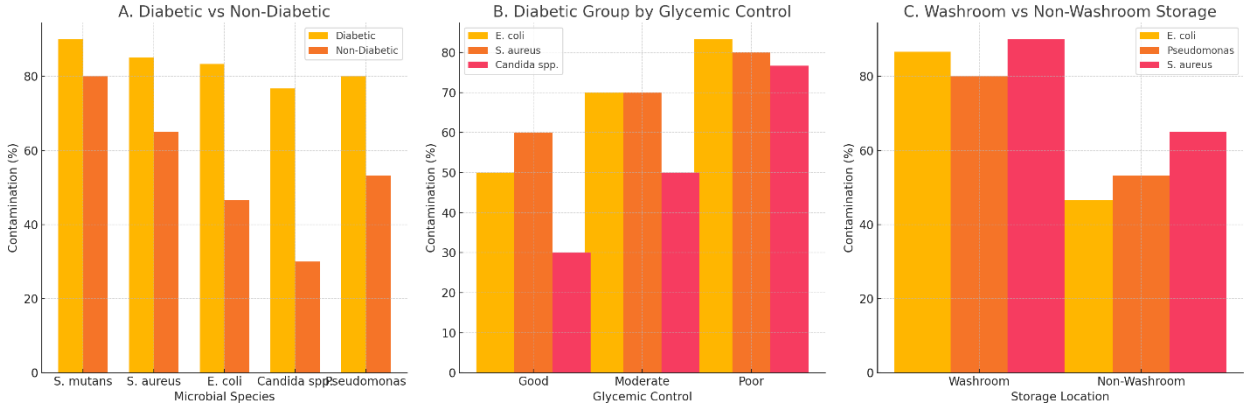

Figure 1. Bar graphs summarizing toothbrush contamination under different conditions.

The combination of these factors produced the highest contamination levels. As shown in Table 4, brushes from poorly controlled diabetics stored in washrooms had the greatest pathogen prevalence: for example, *S. aureus* was found on 93.3% of these brushes versus 66.7% of brushes from poorly controlled diabetics stored outside the washroom. *E. coli* contamination was similarly higher (86.7% vs. 60.0%). These subgroup differences underline that poor glycemic control and unhygienic storage act synergistically to increase microbial load. In summary, diabetic status, higher HbA1c, and washroom storage each independently elevate toothbrush contamination, and their combination produces the heaviest microbial burden.

Table 4: Combined effect of glycemic control and storage in diabetic patients

Subgroup	Staph (%)	Strep (%)	E. coli (%)
Poor, Washroom	93.3	90.0	86.7
Poor, Non-washroom	66.7	86.7	60.0
Good, Washroom	70.0	80.0	50.0
Good, Non-washroom	50.0	70.0	40.0

Discussion

The elevated microbial loads observed on toothbrushes of diabetic individuals, particularly those with poor glycemic control, align with established pathophysiological mechanisms [15]. Chronic hyperglycemia alters salivary composition, providing a nutrient-rich environment for cariogenic and opportunistic pathogens like Streptococcus mutans and Candida albicans [16]. These microbial shifts create a feedback loop: dysbiosis exacerbates oral

inflammation, which in turn worsens insulin resistance. Impaired neutrophil function and excessive NETosis in diabetic hosts further reduce capacity to clear pathogens, allowing biofilm proliferation on oral surfaces-including toothbrushes.

Globally, studies correlate rising HbA1c levels with periodontal disease severity, with each 1% increase linked to 18% higher periodontitis odds [17]. Regional data from Bangladesh highlight urgency, as ~10% of adults have diabetes amid limited dental infrastructure. Contaminated toothbrushes in this context may act as reservoirs for pathogens like Pseudomonas and E. coli, which were disproportionately prevalent in poorly controlled diabetics. These findings mirror observations by Ahamed et al., where diabetic brushes harbored more proteobacteria and antibiotic-resistant strains [18].

Clinical implications are twofold. First, glycemic control remains paramount to reduce oral microbial overgrowth. Second, patient education must emphasize toothbrush hygiene-thorough rinsing, air-drying upright away from toilets, and replacement every 3 months. Simple disinfection methods (5% sodium hypochlorite soaks) proved highly effective in trials, yet guidelines rarely address this. Integrating these practices into diabetes care could mitigate secondary infections, particularly in resource-limited settings where oral-systemic health links are underprioritized.

Storage conditions significantly influenced contamination. Brushes kept in bathrooms showed doubled E. coli and Pseudomonas rates due to toilet aerosols and moisture [19]. For diabetics with HbA1c >9%, this environment compounds risks by introducing enteric pathogens to immunocompromised hosts. Such synergies underscore the need for environmental modifications alongside metabolic management [20, 21].

These results advocate for interdisciplinary collaboration. The WHO's recent integration of oral health into non-communicable disease strategies should catalyze policy changes, particularly in high-burden countries. Dental professionals, endocrinologists, and public health teams must jointly promote toothbrush hygiene as a low-cost, high-impact intervention within holistic diabetes care frameworks. Future research should evaluate antimicrobial brush materials and community education programs to disrupt contamination cycles.

Conclusion

Poor glycemic control and unsanitary storage conditions significantly increase the microbial load on toothbrushes of diabetic patients. Our findings demonstrate that diabetic individuals with high HbA1c harbor more pathogenic organisms on their brushes, and bathroom storage further elevates contamination with enteric bacteria. Preventive measures – including optimal metabolic control, hygienic toothbrush handling, and regular replacement – are essential to reduce the burden of oral pathogens in diabetes.

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Declarations

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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, IBSc, RU, Rajshahi (Ref. No. 226/320 IAMEBBC/IBSc) and informed consent was taken from all participants. The methodology of the study was conducted following the relevant ethical guidelines and regulations.

Consent for publication

Taken

References

1. Joy T, Venugopal S, Sadanandan S, Mathew M. Evaluation of microbial contamination of toothbrushes and their decontamination using various disinfectants: an *in vitro* study. J Indian Assoc Public Health Dent. 2022;20(2):200-205. http://dx.doi.org/10.4103/jiaphd.jiaphd_113_21.
2. Patel M. Oral cavity and *Candida albicans*: colonization to the development of infection. Pathogens. 2022;11(3):335. https://doi.org/10.3390/pathogens11030335.

3. Nissar I, Gupta B, Gupta R, Sharma A, Raina K, Kotia P. A study to compare the efficacy of three different chemical agents as toothbrush disinfectant: a triple-blind study. J Indian Assoc Public Health Dent. 2019;17(4):275-278. http://dx.doi.org/10.4103/jiaphd.jiaphd_95_18.

4. Murukesan S, Varadarajan S, Jagannathan R, Rajendran S, Rajendiran D, Balaji TM. Microbial contamination of toothbrush and methods to overcome: a review. Texila Int J Public Health. 2023;11(3): Art030. https://doi.org/10.21522/TIJPH.2013.11.03.Art030.

5. Li X, Kolltveit KM, Tronstad L, Olsen I. Systemic diseases caused by oral infection. Clin Microbiol Rev. 2000;13(4):547-558. https://doi.org/10.1128/cmr.13.4.547.

6. Lee SY, Lee SY. Assessment of bacterial contamination of toothbrushes using Illumina MiSeq. Oral Biol Res. 2019;43(3):180-188. https://doi.org/10.21851/obr.43.03.201909.180.

7. Yu S-Y, Lee S-K, Yang B, Lee H, Jeon HJ, Lee DH. Glycemic control and oral health outcomes in patients with diabetes: insights from a nationwide Korean survey. J Korean Med Sci. 2024;39(24): e209. https://doi.org/10.3346/jkms.2024.39.e209

8. Pérez-Portilla T, Ortíz-Benitez DL, Lucas-Rincón SE, Canseco-Prado G, Delgado-Pérez VJ, Scougall-Vilchis RJ, Robles-Bermeo NL, Alonso-Sánchez CC, Veras-Hernández MA, & Medina-Solís CE. The importance of toothbrushing and oral hygiene in maintaining oral health. Preprints. Published online September 11, 2023. https://doi.org/10.20944/preprints202309.0596.v1.

9. Rajasekaran JJ, Krishnamurthy HK, Bosco J, Jayaraman V, Krishna K, Wang T, Bei K. Oral microbiome: a review of its impact on oral and systemic health. Microorganisms. 2024;12(9):1797. https://doi.org/10.3390/microorganisms12091797.

10. Saleh FR. The effect of using Dettol, salt and hot tap water in elimination of toothbrush contamination. Indian J Microbiol Res. 2015;2(4):227-230. https://doi.org/10.5958/2394-5478.2015.00019.9.

11. Mavani V, Mahabala KY, Suman E, Natarajan S, Nayak A, Srikrishna SB, Rao A.

Evaluation of effectiveness of home remedies for toothbrush decontamination using vinegar and vinegar with common salt. World J Dent. 2018;9(1):19-23. https://doi.org/10.5005/jp-journals-10015-1500.

12. Jia YJ, Liao Y, He YQ, Zheng MQ, Tong XT, Xue WQ, Zhang JB, Yuan LL, Zhang WL, Jia WH. Association between oral microbiota and cigarette smoking in the Chinese population. Front Cell Infect Microbiol. 2021;28(11)658203. https://doi.org/10.3389/fcimb.2021.658203.

13. Cheesbrough M. District laboratory practice in tropical countries. 2nd ed. Cambridge, UK: Cambridge University Press; 2006.

14. Mavani V, Mahabala KY, Suman E, Okawa R, Nakano K. Inhibitory effect of toothbrush monofilament containing surface pre-reacted glass-ionomer (S-PRG) filler on *Streptococcus mutans*. Sci Rep. 2021; 11:211. https://doi.org/10.1038/s41598-020-80646-x.

15. Axe A, Mueller WD, Rafferty H, Lang T, Gaengler P. Impact of manual toothbrush design on plaque removal efficacy. BMC Oral Health 23, 796 (2023). https://doi.org/10.1186/s12903-023-03518-6.

16. Elizabeth NP, Oluwasola OD, Afolabi MS. Toilet plume bioaerosols in health care and hospitality settings: A systematic review. American Journal of Infection Control. 2023;51(3):324-333. https://doi.org/10.1016/j.ajic.2022.07.006.

17. Dogana SSA, Ugurb SG, Doganc O, Turedid OK, Sekerd E, Ulkerc G. Evaluation of bacterial contamination of miswak toothbrushes in different storage environments: an experimental study. Ann Med Res. 2024;31(3):190-198. https://doi.org/10.5455/annalsmedres.2023.10.292.

18. Jia YJ, Liao Y, He YQ, Zheng MQ, Tong XT, Xue WQ, Zhang JB, Yuan LL, Zhang WL, Jia WH. Association between oral microbiota and cigarette smoking in the Chinese population. Front Cell Infect Microbiol. 2021; 11:658203. https://doi.org/10.3389/fcimb.2021.658203.

19. Otto M. *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. Clin Microbiol Rev. 2015;28(3):603-661. https://doi.org/10.1128/cmr.00134-14.

20. Peker I, Akca G, Sarikir C, Alkurt TM, Celik I. Effectiveness of alternative methods for toothbrush disinfection: an *in vitro* study. Sci World J. 2014; 2014:726190. doi:10.1155/2014/726190.

21. Eriksson S, van der Plas MJA, Mörgelin M, Sonesson A. Antibacterial and antibiofilm effects of sodium hypochlorite against *Staphylococcus aureus* isolates derived from patients with atopic dermatitis. Br J Dermatol. 20177(2):513-521. https://doi.org/10.1111/bjd.15410.