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Assessment of Oral Hygiene Practices and Microbial Contamination of Toothbrushes among Medical, Physiotherapy, and Nursing Students

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ABSTRACT
Background: Toothbrushes, essential tools for maintaining oral hygiene, can harbor pathogenic microorganisms, including *Streptococcus mutans*, *Candida albicans*, *Escherichia coli*, and *Staphylococcus aureus*. These pathogens may lead not only to oral diseases like gingivitis and periodontitis but also to systemic infections, particularly in immunocompromised individuals. Healthcare students, despite being educated about hygiene, may still exhibit unsafe practices due to a lack of awareness regarding the proper use of toothbrushes, including replacement, storage, and disinfection. **Objective:** To evaluate oral hygiene practices and assess microbial contamination of toothbrushes among medical, physiotherapy, and nursing students. **Methods:** A cross-sectional study was conducted among 120 hostel students (40 from each of the MBBS, BPT, and Nursing programs). Participants completed a structured questionnaire, and the used toothbrushes were analyzed microbiologically. **Results:** Over 79 () students showed positive for bacterial contamination out of 120. The major microbial load was found in the nurses group 34 (97.1%), followed by BPT 15 (75%) and M.B.B.S 30 (43%). Among organisms isolated, the most common organism isolated was Staphylococcus 19(24%), followed by E.coli 913%), Klebsiella (12%), and Pseudomonas (11%). Higher cfu was associated with damp storage, no cover use, and less frequent replacement. **Conclusion:** A significant proportion of healthcare students had suboptimal toothbrush hygiene and high microbial contamination. Awareness and institutional interventions are needed to reduce the risk of infection.

INTRODUCTION:

Oral health is an essential component of overall health, directly and indirectly reflecting an individual's general well-being. Good oral hygiene plays a vital role in preventing dental and periodontal diseases by reducing the microbial load in the oral cavity[1]. Beyond the mouth, poor oral health has been associated with systemic conditions such as cardiovascular disease, diabetes, and respiratory infections. Therefore, maintaining optimal oral hygiene is not only fundamental for a healthy smile but also for promoting overall health and quality of life. Proper oral hygiene maintenance plays a key role in reducing microbial accumulation and achieving optimal oral health. Among the available methods, the toothbrush is the most widely used and effective mechanical aid for plaque removal. However, despite its essential role in maintaining oral hygiene, toothbrushes are prone to contamination during use, handling, and improper storage. Such contamination may contribute to reinfection of the oral cavity and compromise the effectiveness of daily oral hygiene practices [2]. Common bacteria such as

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Streptococcus, Staphylococcus, and Lactobacillus species are frequently transferred onto toothbrush bristles during brushing, where they may survive and proliferate. This microbial colonization poses a potential risk for reinfection of the oral cavity or even transmission of pathogenic organisms.(3) In addition to the normal oral microorganisms, toothbrushes have also been found to harbor organisms not typically associated with the oral cavity, such as *Enterobacteriaceae* and *Pseudomonas* species. Their presence suggests that toothbrush contamination may occur not only from the oral cavity during brushing but also from environmental exposure and improper storage practices.[4]. These infectious microorganisms can reinfect the mouth and spread to other parts of the body, potentially causing serious health problems such as heart disease, stroke, arthritis, bacteremia, and chronic infections. Toothbrushes, while essential for oral hygiene, can become vectors for microbial colonization. Improper storage and infrequent replacement can harbor bacteria and fungi, which may re-enter the oral cavity and cause infections.Although regular replacement of toothbrushes is recommended to improve plaque removal efficiency, it does not completely eliminate the issue of microbial contamination. Studies have shown that newly introduced toothbrushes may harbor significantly higher levels of *Streptococcus mutans* compared to worn toothbrushes,reason might be due to increased bristle surface area, lack of conditioning, or manufacturing/storage contamination suggesting that replacement alone is insufficient to control bacterial colonization."[5] Toothbrushes can serve as breeding grounds for billions of bacteria, facilitated by various environmental factors. Aerosols generated during toilet flushing, coupled with storage in damp or poorly ventilated environments, provide favorable conditions for microbial survival and proliferation on toothbrush bristles."[6,7]. Microorganisms may originate not only from the oral cavity but also from the environment where toothbrushes are stored. studies have shown that toothbrushes stored in open air environments harbor fewer bacteria compared to those kept in closed containers, with microbial growth reported to be up to 70% higher in moist and enclosed conditions. Bacteria that attach, accumulate, and multiply on toothbrush bristles can subsequently be reintroduced into the oral cavity, potentially contributing to various oral and systemic diseases. Therefore, the establishment of standardized oral health guidelines for toothbrush use, storage, and disinfection is essential to minimize the risk of contamination and safeguard overall health[8,9]

This study aimed to assess the oral hygiene practices and microbial contamination of toothbrushes among medical, physiotherapy, and nursing students at a rural tertiary teaching institution. The general objective is to isolate and identify these bacteria to understand the extent of contamination. To achieve this, the study will collect toothbrush samples from students at GEMS Medical College and Hospital.Subsequently, the bacterial profile of the collected toothbrush samples will be identified and analyzed. Based on these findings, evidence-based recommendations will be formulated to raise awareness regarding proper toothbrush maintenance and the potential risks of contamination. Such measures aim to promote better oral hygiene practices and minimize the transmission of pathogenic microorganisms.

MATERIALS AND METHODS:

Study Design and Participants A cross-sectional observational study was conducted among 120 undergraduate hostel students from Great Eastern Medical School, Srikakulam.

Data Collection:

A validated questionnaire about toothbrush technique, frequency, storage, replacement behaviors, and hygiene awareness was filled out by the participant

Microbiological Analysis:

A total of 120 undergraduate students of both sexes were included in the study. The participants had a clinically healthy periodontium, with no signs of gingival inflammation, no history of systemic diseases, and had not received antibiotic therapy for at least three months prior to and during the study period. For sample collection, toothbrush heads were carefully removed using sterile gloves and sterile forceps to minimize the risk of external contamination."

Each toothbrush was gathered in sterile coverings and transported to the microbiology laboratory. In the laboratory, test tubes measuring 25X125 were utilized, containing 5 ml of brain heart infusion agar (BHA) broth. The openings of these test tubes were sealed with cotton. The toothbrushes were submerged in the test tubes filled with BHA broth and vortexed for 3 minutes at a speed of 2000 rpm. Following the vortexing, 100 µL of BHA broth was diluted with 900 µL of BHA broth. Duplicates of this dilution were prepared. The resulting solution was streaked onto blood agar, MacConkey agar, and SDA agar. Colony-forming units were enumerated after 24 hours of incubation at 37°C. The average of the duplicates was recorded as the final colony count, and the morphology of the colonies was examined. Further confirmation was achieved through biochemical reactions (indole, citrate, and urease). For fungal isolation, SDA

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plates were incubated for up to 3 days before analysis. Suspected fungal colonies were subjected to confirmatory tests, including the germ tube test for *Candida* species."

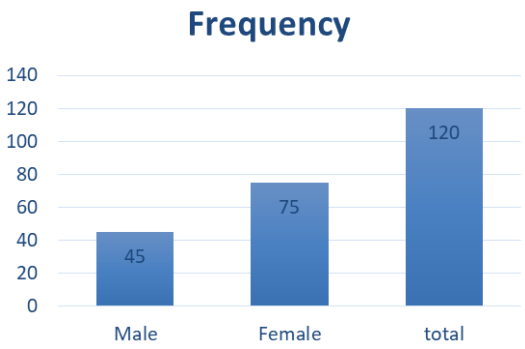
Data were analyzed using SPSS v25. ANOVA and Chi-square tests were used for group comparisons. Correlation tests were used to assess hygiene habits and microbial load.

Results:

Table 1: Demographic Data of the study population.

sex	Frequency	percentage
Male	45	37.5
Female	75	62.5
total	120	100%

Females were dominant in this study (62.5%), with males representing 37.5% of the participants.



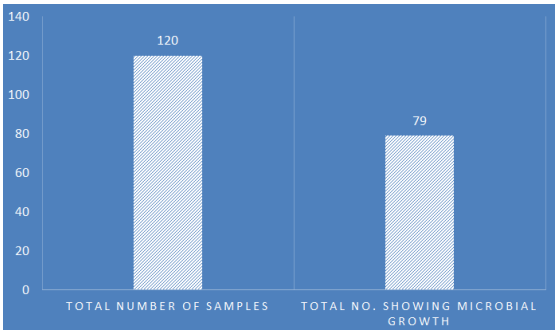
Graph 1. Distribution of study participants by sex

Table2: Habit patterns in the use of the manual toothbrush

frequency	**Brushing frequency**
55 (45.8%)	Once
65 (54.16%)	Twice
120 (100%)	total
Frequency	**Toothbrush replacement frequency**
43 (35.8%)	One month
45 (37.5%)	Three months
32 (26.6%)	more than three months
120 (100%)	total
Frequency	**Place of Toothbrush Storage**
55 (45.8%)	Inside the bathroom
65 (54.1%)	Outside the bathroom
120 (100%)	total
frequency	**practice of covering the toothbrush with a cap after usage.**
12 (10%)	Yes
108 (90%)	No
120 (100%)	total

Hygiene Practices:

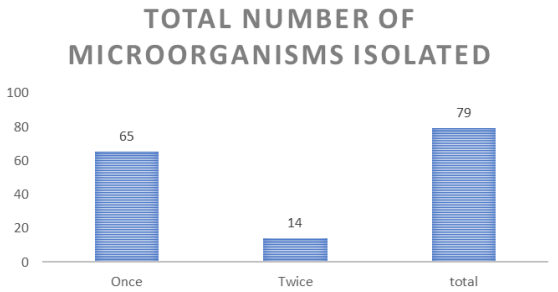
- 45.8% brushed once daily
- 90% did not cover the toothbrush head
- 45.8% stored the brush in damp environments
- 26,6% failed to replace the brush within 3 months



Graph2 - As shown in Graph 2, 79 out of 120 samples (65.83%) demonstrated the presence of microorganisms.

Table 3 presents the microbial load with respect to various oral hygiene habits.

percentage	**frequency**	**Brushing frequency**	**Total number of microorganisms isolated**
45.8	55	Once	65 (82.%)
54.16	65	Twice	14(18%)
100%	120	total	79 (100%)
percentage	**frequency**	**Toothbrush replacement frequency**	
35.8	43	One month	32(26.5%)
37.5	45	Three months	(36.7%)
26.6	32	more than three months	30 (37.9%)
100	120	total	79 (100%)
percentage	**frequency**	**Place of Toothbrush Storage**	
45.8	55	Inside the bathroom	45 (57%)
54.1	65	Outside the bathroom	34(43%)
100%	120	total	79(100%)
percentage	**frequency**	**The practice of covering the toothbrush with a cap after usage.**	
10	12	yes	2 (2.53%)
90	108	No	77(97.47%)
100%	120	total	79 (100%)



Graph 3 showed that students who brushed twice had a lower microbial load [14 (18%)] compared to those who brushed once [65 (82%)].

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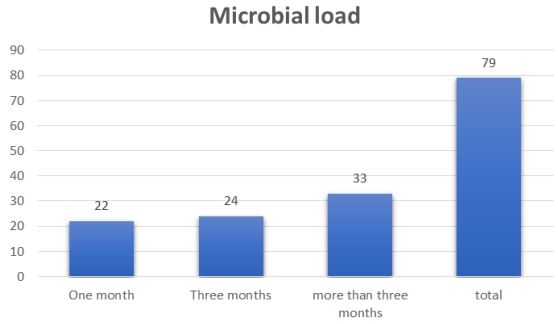
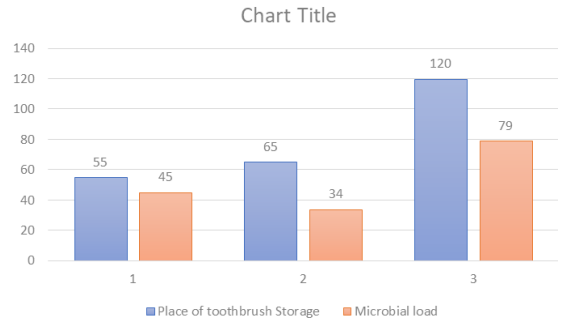
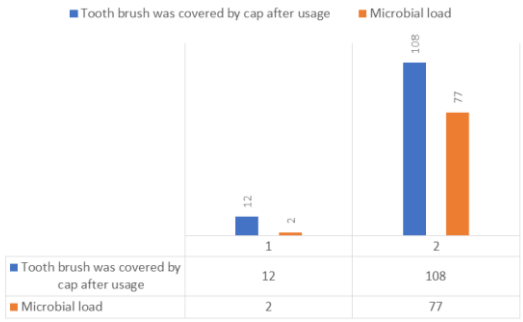


Graph 4 illustrates that students who replaced their toothbrushes monthly or every three months exhibited lower microbial contamination.



Graph 5 showed that a higher microbial load was observed in toothbrushes kept inside the bathroom (n = 55), of which 45 (57%) showed microbial growth, compared to those kept outside the bathroom.



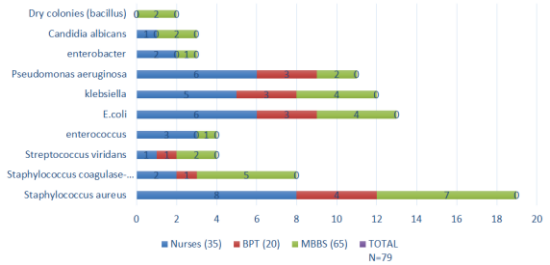
Graph 6 showed that individuals who cover their toothbrush with a cap had a lower microbial load.

Table 4 presents the bacterial prevalence on toothbrushes following one month of daily use in 79 culture-positive samples."

Organisms isolated	Nurses (35)	BPT (20)	MBBS (65)	TOTAL N=79
Staphylococcus aureus	8	4	7	19 (24)
Staphylococcus coagulase-negative	2	1	5	8 (10.1)
Streptococcus viridans	1	1	2	4(5.1)
enterococcus	3	0	1	4(5.1)
E.coli	6	3	4	13(16.4)
klebsiella	5	3	4	12(15.2)
Pseudomonas aeruginosa	6	3	2	11(13.9)
enterobacter	2	0	1	3(3.8)
Candidia albicans	1	0	2	3(3.8)
Dry colonies (bacillus)	0	0	2	2(2.6)
total	34 (97.1%)	15 (75%)	30 (43%)	79 (100)

From the above table, the most commonly isolated organisms were *Staphylococcus aureus* 19 (24%), *Escherichia coli* 13 (16.4%), *Klebsiella* 12 (15.2%), and *Pseudomonas* 11 (13.9%)."

CFU load among student groups: highest in Nursing students (97.1%), followed by BPT (75%) and MBBS (43%)."



Graph 7 showed bacterial prevalence on toothbrushes after one month of daily use in 79 culture-positive samples and CFU was highest in Nursing students (97.1%), followed by BPT (75%) and MBBS (43%)."

DISCUSSION:

This study provides critical insights into oral hygiene practices and the bacterial contamination of toothbrushes. Our findings reveal significant trends and correlations, identifying key factors that influence microbial colonization and toothbrush hygiene. These results have important implications for improving oral health practices and reducing the risk of infection associated with contaminated toothbrushes.

"In the present study, females were the majority, accounting for 62.5% of participants, while males represented 37.5%. This contrasts with studies by Snezana Pesevska, Kiro Ivanovski et al.^10 and Naveed Mansoori, Imran Bakar et al.^11, which reported a male predominance."

Tables 2 and 3 illustrate the habit patterns in the use of manual toothbrushes and the corresponding microbial load across different groups. The results indicate that poor storage and infrequent replacement were significantly associated with higher microbial load ($p < 0.05$), demonstrating that over time, a used toothbrush can become a breeding ground for microbes, thereby increasing the risk of dental problems and reinfection."

Regular replacement of toothbrushes, at least every three months, helps prevent reinfection, particularly after illnesses such as colds or flu. Worn bristles are less effective at removing plaque and bacteria and can harbor harmful microorganisms, increasing the risk of dental problems. They may also become sharp, potentially irritating the gums and causing

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bleeding or inflammation. Using a cap to cover the toothbrush after use promotes hygiene by reducing bacterial growth on the bristles. Proper storage of toothbrushes is another key factor in maintaining oral health.

Our study found that individuals who store their toothbrushes outside the bathroom have a lower microbial load compared to those who store them inside. Warm and moist bathroom conditions create an ideal environment for bacterial growth on toothbrushes. Therefore, thoroughly rinsing and air-drying toothbrushes after use can help minimize bacterial proliferation."

Our study showed that a lower microbial load was observed in toothbrushes covered with a cap, 2 (2.53%), compared to those left uncovered, 77 (97.47%). This finding is consistent with the study conducted by Rajkumar Manohar, Keerthi Venkatesan et al.,^23 but contrasts with the study by Eman Abdulrahman ALSHEHRI, Tasneem Sakinatul AIN et al.,^24 which reported that microorganisms were more prevalent on covered toothbrushes than on uncovered ones. but ideally, a covered toothbrush with a dry environment is helpful; otherwise covered toothbrush carries a higher chance of microbial growth as plastic covers trap moisture, and a damp, dark environment promotes the multiplication of bacteria. Instead, it is crucial to prioritize maintaining excellent oral hygiene practices, and your toothbrush is thoroughly cleaned and dried after each use.

In the present study, microbial contamination was detected in 79 of 120 tested toothbrushes (65.8%). These results are consistent with previous findings by Mehta et al.12 and Ferreira et al.13, who reported that approximately 70% of toothbrushes were heavily colonized by diverse pathogenic microorganisms.

In our study, the microorganisms isolated were Staphylococcus aureus 19 (24%), followed by E. coli 13 (16.4%), klebsiella 12(15.2%), and Pseudomonas 11(13.9%) similar to study conducted by Ji-Hyang Kim, Da-Ae Kim et al [14]where staphylococcus were predominant isolate but in contrast to a survey conducted by TRMD RalephenyaI; J Molepo et al[15], where CONS were the predominant isolate and also study conducted by Snezana Pesevska, Kiro Ivanovski et al [16]where coliform bacilli were predominant, E.coli followed by klebsiella.

Staphylococcus aureus, a common component of human skin flora, can readily transfer to the brush, and the humid environment of a bathroom promotes bacterial growth. S. aureus can accumulate from the user's own skin and be reintroduced to the mouth. The presence of Staphylococcus aureus in toothbrushes is a serious concern because it can cause oral infections such as periodontal disease, and cause bloodstream infections through breaks in the skin or mucous membranes, leading to severe illness like sepsis, pneumonia, and endocarditis [17],[18] In addition, *Staphylococcus aureus* has been reported to develop resistance to multiple antibiotics, including methicillin (MRSA), penicillin, and cephalosporins. Sharing toothbrushes can facilitate the transmission of MRSA from infected to uninfected individuals, emphasizing the need for regular toothbrush disinfection."

The second most important isolate was Escherichia coli (E. coli), Coliforms, which are organisms not normally associated with oral flora, were isolated in 13 (16.4%) of the tested toothbrushes. "The practice of storing toothbrushes in bathrooms may have facilitated the high contamination rates observed with fecal bacteria.". The presence of coliforms in toothbrushes in the current study shows faecal contamination.In addition, these bacteria may have contaminated the toothbrushes through the rinsing water, as coliforms are commonly present in water. Beyond gastrointestinal tract infections, coliforms have also been reported to cause respiratory tract infections.[20,21,22]

Though isolated in low numbers, C. albicans is clinically significant, causing serious morbidity and mortality in patients with AIDS, bone marrow transplants, or intensive anti-cancer therapy.".[23]

The absence of growth in 22 toothbrushes from this study could have been due to participants submitting new, purchased toothbrushes instead of the ones they used at home, or they could have disinfected the toothbrushes before submission. In addition, the media used for culture were specific for the growth of enterobacteria, mutans streptococci, and yeasts; therefore, these 22 toothbrushes could be harboring other pathogens not tested in this study.

Our study reinforces the importance of maintaining proper toothbrush hygiene practices to minimize bacterial contamination. Regular replacement of toothbrushes every three months, and brushing twice daily as recommended, remains crucial in reducing microbial colonization (ADA)

CONCLUSION:

Improving awareness and practice among healthcare students can prevent potential oral and systemic infections. Bridging the gap between

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knowledge and personal hygiene behavior is essential to fostering a culture of comprehensive infection prevention.

DECLARATION:
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Informed Consent: Taken from all participants
Funding: Nil
Conflict of Interest: None

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