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# Antibiotic Susceptibility Patterns of Bacterial Isolates Recovered from Used Manual Toothbrushes

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# أنماط الحساسية للمضادات الحيوية لدى العزلات البكتيرية المستخلصة من فرش الأسنان اليدوية المُستخدَمة

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**ABSTRACT**

Used manual toothbrushes may harbor bacteria originating from the oral cavity and surrounding environment during routine use. This study aimed to investigate bacterial contamination of used manual toothbrushes after one month of regular use, identify the recovered bacterial isolates, and determine their antibiotic susceptibility patterns. A cross-sectional, laboratory-based study was conducted in Benghazi, Libya, from January 2021 to June 2022. One hundred participants aged 18–55 years used new manual toothbrushes for one month before the toothbrushes were collected and examined microbiologically. Bacterial culture, identification, and antibiotic susceptibility testing were performed using standard microbiological procedures and the Kirby Bauer disk diffusion method. Bacterial growth was detected on 73% of the examined toothbrushes, while 27% showed no bacterial growth. Gram-positive bacteria accounted for 60.3% of all examined toothbrushes, whereas Gram-negative bacteria accounted for 39.7%. Among the recovered bacterial isolates, Staphylococcus epidermidis was the most frequently identified organism, accounting for 20 isolates (27.4%), followed by Streptococcus viridans with 17 isolates (23.3%). Other recovered isolates included Klebsiella spp. (9 isolates, 12.3 %), Escherichia coli (8 isolates, 11 %), Staphylococcus aureus (7 isolates, 9.6 %), Pseudomonas spp. (6 isolates, 8.2 %), Acinetobacter spp. (4 isolates, 5.5 %), and Proteus spp. (2 isolates, 2.7 %). The highest susceptibility rates were observed for ciprofloxacin (63%) and ceftriaxone (53%), while the highest resistance rates were observed for cefoxitin (65%) and ceftazidime (57%). These findings demonstrate the bacterial isolates recovered from used manual toothbrushes exhibit variable antibiotic susceptibility patterns, highlighting the importance of characterizing their susceptibility profiles and promoting appropriate toothbrush hygiene, storage, regular replacement.

**الملخص**

قد تحتوي فرش الأسنان اليدوية المستخدمة على بكتيريا مصدرها تجويف الفم والبيئة المحيطة نتيجة للاستخدام اليومي. هدفت هذه الدراسة إلى فحص التلوث البكتيري في فرش الأسنان اليدوية بعد شهر من الاستخدام المنتظم، وتحديد أنواع البكتيريا الموجودة، وتقييم مدى حساسيتها للمضادات الحيوية. أُجريت دراسة مقطعية مخبرية في مدينة بنغازي بليبيا، في الفترة ما بين يناير 2021 ويونيو 2022. شارك في الدراسة 100 شخص تتراوح أعمارهم بين 18 و55 عاماً، استخدموا فرش أسنان يدوية جديدة لمدة شهر، ثم جُمعت هذه الفرش وخضعت للفحص الميكروبيولوجي. تم إجراء عمليات الاستنبات البكتيري، وتحديد الأنواع، واختبار الحساسية للمضادات الحيوية وفقاً للإجراءات الميكروبيولوجي القياسية، باستخدام طريقة "كيربي-باور **"KIRBY–BAUER** للانتشار القرصي. أظهرت النتائج وجود نمو بكتيري على 73% من فرش الأسنان التي تم فحصها، بينما لم يظهر أي نمو بكتيري على 27% منها. وشكلت البكتيريا موجبة الغرام 60.3% من إجمالي العينات، في حين بلغت نسبة البكتيريا سالبة الغرام 39.7 **%.**

ومن بين البكتيريا التي تم عزلها، كانت بكتيريا "المكورات العنقودية *البشرية* ***STAPHYLOCOCCUS" EPIDERMIDIS*** بـ 20 عزلة (27.4 %), تلتها "المكورات العقدية الخضراء **(*STREPTOCOCCUS VIRIDANS*) "**بـ 17 عزلة (23.3%). كما شملت العزلات الأخرى كلاً من: "الكلبسيلة ***KLEBSIELLA*** "بـ 9 عزلة (12.3%)، و"الإشريكية القولونية **ESCHERICHIA COLI (**"بـ 8 عزلة (11%),و"المكورات العنقودية الذهبية ***STAPHYLOCOCCUS AUREUS*** "بـ7عزلة (9.6%),و"الزائفة ***PSEUDOMONAS SPP.*** بـ 6عزلة (8.2%), و"الراكدة ***ACINETOBACTER SP*P **(**"بـ 4عزلة (5.5%), و"المتقلبة ***PROTEUS SPP*** **(**"بـ عزلتين (2.7%). سُجلت أعلى معدلات الحساسية تجاه مضادي "سيبروفلوكساسين" (63%) و"سيفترياكسون" (53%)، بينما سُجلت أعلى معدلات المقاومة تجاه "سيفوكسيتين" (65%) و"سيفتزيديم" (57%). وتُظهر هذه النتائج أن البكتيريا الموجودة على فرش الأسنان اليدوية المستخدمة تتباين في استجابتها للمضادات الحيوية؛ مما يؤكد أهمية تحديد أنماط حساسيتها، وضرورة تعزيز الممارسات الصحيحة المتعلقة بنظافة فرش الأسنان، وتخزينها، واستبدالها بانتظام.

**الكلمات المفتاحية**: فرش أسنان يدوية مستعملة؛ تلوث بكتيري؛ عزلات بكتيرية؛ حساسية للمضادات الحيوية؛ مقاومة للمضادات الحيوية؛ اختبار كيربي-باور لانتشار الأقراص

## INTRODUCTION

Oral hygiene is an important factor in maintaining oral and general health. Tooth brushing is one of the most common methods used to remove dental plaque and oral debris. Manual toothbrushes are used daily for oral hygiene and are constantly exposed to saliva, oral microorganisms, food particles and the surrounding environment. Although toothbrushes are designed to improve oral hygiene, their frequent use can result in microbial contamination and the persistence of microorganisms on the bristles and head. Previous studies have demonstrated that toothbrushes may become contaminated with microorganisms after use and may serve as reservoir for microbial contamination (Frazelle & Munro, 2012; Blaustein *et al.,* 2021). The mouth houses a complex and diverse microbial community. Microorganisms present in the oral cavity can be transferred directly to the toothbrush during toothbrushing. After using toothbrushes may remain wet and become contaminated with microorganisms from the environment, hands, storage surfaces, and other sources. The survival and persistence of microorganisms on toothbrushes may be affected by factors such as frequency of use, storage location, moisture, ventilation, toothbrush design and use of protective covers. An updated systematic review found that toothbrushes can become contaminated even after their first use, with the degree of contamination potentially increasing with continued use. Environmental and storage factors, such as the increased humidity under certain storage conditions, may also contribute to microbial persistence (Khan *et al*., 2024). The potential microbiological significance of contaminated toothbrushes has received increasing attention because microorganisms retained on the toothbrush may subsequently be reintroduced into the oral cavity during repeated brushing. Although the clinical consequences of toothbrush contamination have not been fully established, previous literature has recognized toothbrushes as potential reservoirs of microorganisms and has emphasized the importance of appropriate toothbrush care and storage. Experimental studies have demonstrated microbial contamination of toothbrushes after use, supporting the need for greater attention to the hygienic management of these frequently handled personal-care devices. In addition to investigating bacterial contamination, determining the antibiotic susceptibility patterns of recovered bacterial isolates are important for characterizing their responses to commonly tested antimicrobial agents. Susceptibility testing helps distinguish susceptible isolates from those exhibiting resistance under the conditions of the test. However, the recovery of bacteria from toothbrushes does not necessarily indicate that these organisms cause infection or that their susceptibility patterns represent those of bacteria circulating in general population.

Investigating the antibiotic susceptibility patterns of bacterial isolates recovered from used toothbrushes may nevertheless provide useful microbiological information about these isolates. Several methods have been studied to reduce microbial contamination of toothbrushes such as chemical disinfection, regular replacement, proper storage. However, their effectiveness may depend on the microorganisms involved, exposure conditions and the concentration of disinfectants used. Recent evidence also supports the effectiveness of toothbrush disinfection strategies but highlights the need for standardized protocols and further high-quality research (Agrawal et al., 2019; Li *et al*., 2026). Although evidence is available from different parts of the world, limited information is available on bacterial contamination of used manual toothbrushes and the antibiotic susceptibility patterns of bacterial isolates in Libya, particularly in Benghazi. Local studies are important because differences may exist in toothbrush-use practices, storage conditions, environmental factors, and patterns of antimicrobial exposure. Thus, the present study was designed to investigate the bacterial contamination of manual toothbrushes after 1 month of regular use by individuals in Benghazi, Libya. Local studies are important because differences may exist in toothbrush-use practices, storage conditions, environmental factors, and patterns of antimicrobial exposure. Thus ,the present study was designed to investigate bacterial contamination of manual toothbrushes after one month of regular use by individuals in Benghazi, Libya .The study also aimed to identify the bacterial isolates recovered from used toothbrush and determine their antibiotic susceptibility patterns .This research provides local evidence on the bacterial isolates from used manual toothbrushes and their susceptibility to selected antimicrobial agents ,contributing to better understanding their microbiological characteristics .

## 2. METHOD

**Study Design, Setting, and Duration**

This laboratory-based cross-sectional study was conducted in Benghazi, Libya, from January 2021 to June 2022 to investigate bacterial contamination of used manual toothbrushes following one month of routine use. A total of 100 toothbrush samples were collected from participants aged 18–55 years.

**Subjects and Use of Toothbrushes**

Participants were provided with new manual toothbrushes and instructed to use them as part of their normal oral hygiene routine including brushing in the morning and at night. Preliminary oral -hygiene instructions were provided before the study. The participants were also instructed not to use antimicrobial mouth rinses during the study period. All participants provided written informed consent before the study began, and ethical approval was obtained.

**Collection and Transport of Toothbrush Specimens**

On the 30$^{th}$ day of use, the participants were asked to place their toothbrushes in sterile transparent zip –lock plastic bags immediately after their morning brushing. The used toothbrushes were then transported to the microbiology laboratory under aseptic conditions and analyzed within 18 hours of the last tooth brushing. The heads of the toothbrushes were removed with sterile gloves and forceps and sent for microbiological analysis.

**Microbiological Culture**

The heads of each toothbrush were placed into a sterile container with tryptic soy broth (TSB) and incubated at 37°C for 24 hours. After incubation, the cultures were plated onto appropriate media for culture including blood agar, MacConkey agar, chocolate agar. The plates were incubated overnight at 37°C under aerobic conditions and examined for microbial growth. Pure cultures were submitted to bacterial identification, while mixed cultures were subcultured to obtain pure isolates.

**Bacterial Identification**

The isolates of bacteria were identified using standard microbiological methods based on colony morphology, Gram staining and biochemical characteristics. The identification protocols included relevant biochemical assays, such as catalase, coagulase and oxidase tests. Selected isolates were further confirmed by the BD Phoenix automated microbiology system.

**Antibiotic Susceptibility Testing**

Antibiotic susceptibility testing was determined using the Kirby-Bauer disk diffusion method. Bacterial suspensions were prepared from pure and inoculated onto Mueller–Hinton agar plates. Antibiotic -impregnated disk representing different antibiotics were placed on the inoculated agar surface. The plates were incubated at 37°C for 16-24 hours. Following incubation, the inhibition zones around the antibiotic disks' diameters were measured to the nearest millimeter, and the isolates were classified as susceptible or resistant according to the applicable interpretive criteria. Representative plates showing antibiotic disks and inhibition zones observed during susceptibility testing are shown in Figure 1.

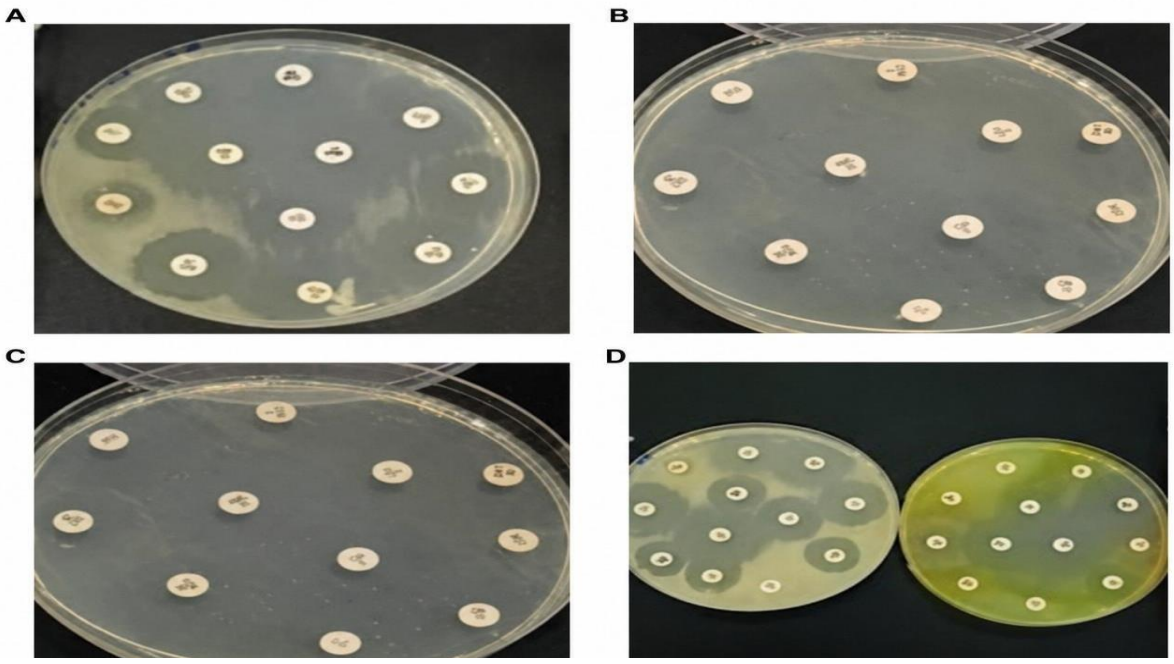



**Fig.1** Representative agar plates showing antibiotic susceptibility testing of bacterial isolates using the Kirby–Bauer disk diffusion method. (A–D) Representative plates showing antibiotic disks and inhibition zones around the tested bacterial isolates.

**Statistical Analysis**

Data were analyzed using SPSS version 23. Descriptive statistics were used to summarize bacterial contamination and the distribution of study variables. The chi-square test was used to assess associations between bacterial growth and selected categorical variables. Statistical significance was considered at $p < 0.05$.

## 3. ETHIC APPROVAL

The study was conducted in accordance with applicable ethical principles. Ethical approval was obtained before the commencement of the study, and written informed consent was obtained from all participants.

## 4. RESULT

**Bacterial Contamination of Used Manual Toothbrushes**

Bacterial growth was detected in 73 (73.0%) of the examined used manual toothbrushes, whereas 27 (27.0%), showed no bacterial growth, as shown in Table 1. Gram-positive bacterial isolates recovered from 44 (60.3%) of the examined toothbrushes, while Gram-negative bacterial isolates were recovered from 29 (39.7%).

**Table 1.** Bacterial growth among examined manual toothbrushes

| Bacterial growth | N | % |
|---|---|---|
| Growth detected | 73 | 73.0 |
| No growth detected | 27 | 27.0 |
| **Total** | 100 | 100.0 |

**Distribution of Bacterial Isolates**

Among the 73 bacterial isolates recovered from the examined toothbrushes. The most frequently isolated bacteria were *Staphylococcus epidermidis* 20 isolates (27.4%), followed by *Streptococcus viridans* with 17 isolates (23.3%) as shown in Table 2. Other recovered bacteria included *Klebsiell*a spp. (9 isolates, 12.3 %), *Escherichia coli* (8 isolates, 11%), *Staphylococcus aureus* (7 isolates, 9.6 %), *Pseudomonas* spp. (6 isolates, 8.2 %), *Acinetobacte*r spp. (4 isolates, 5.5%), and *Proteus* spp. (2 isolates, 2.7 %).

**Table 2.** Distribution of bacterial isolates recovered from used manual toothbrushes

| Bacterial isolate | No. of isolates | Percentage (%) |
|---|---|---|
| *Staphylococcus epidermidis* | 20 | 27.4 |
| *Streptococcus viridans* | 17 | 23.3 |
| *Klebsiella* spp. | 9 | 12.3 |
| *Escherichia coli* | 8 | 11 |
| *Staphylococcus aureus* | 7 | 9.6 |
| *Pseudomonas* spp. | 6 | 8.2 |
| *Acinetobacter* spp. | 4 | 5.5 |
| *Proteus* spp. | 2 | 2.7 |
| **Total** | 73(100%) | |

**4.3Antibiotic Susceptibility Patterns**

The recovered bacterial isolates demonstrated variable antibiotic susceptibility patterns across the tested antimicrobial agents. The highest reported susceptibility was observed with ciprofloxacin (63%), followed by ceftriaxone (53%), doxycycline (49%), and imipenem (48%). In contrast, the highest reported resistance rate was observed with cefoxitin (65%) as shown in Table 3 and Figure 2, followed by ceftazidime (57%), amoxicillin/clavulanic acid (53%), and tobramycin (50%). Resistance rates of 46% were reported for both cefotaxime and gentamicin, while trimethoprim/sulfamethoxazole showed a resistance rate of 45%.

**Table 3.** Reported antibiotic susceptibility and resistance patterns among recovered bacterial isolates recovered from used manual toothbrushes

| Antimicrobial agent | Finding | Percentage |
|---|---|---|
| Ciprofloxacin | Susceptibility | 63% |
| Ceftriaxone | Susceptibility | 53% |
| Doxycycline | Susceptibility | 49% |
| Imipenem | Susceptibility | 48% |
| Cefoxitin | Resistance | 65% |
| Ceftazidime | Resistance | 57% |
| Amoxicillin/clavulanic acid | Resistance | 53% |
| Tobramycin | Resistance | 50% |
| Cefotaxime | Resistance | 46% |
| Gentamicin | Resistance | 46% |
| Trimethoprim/sulfamethoxazole | Resistance | 45% |

Overall, the findings demonstrate considerable variation in the antibiotic susceptibility patterns of the bacterial isolates recovered from the used manual toothbrushes.

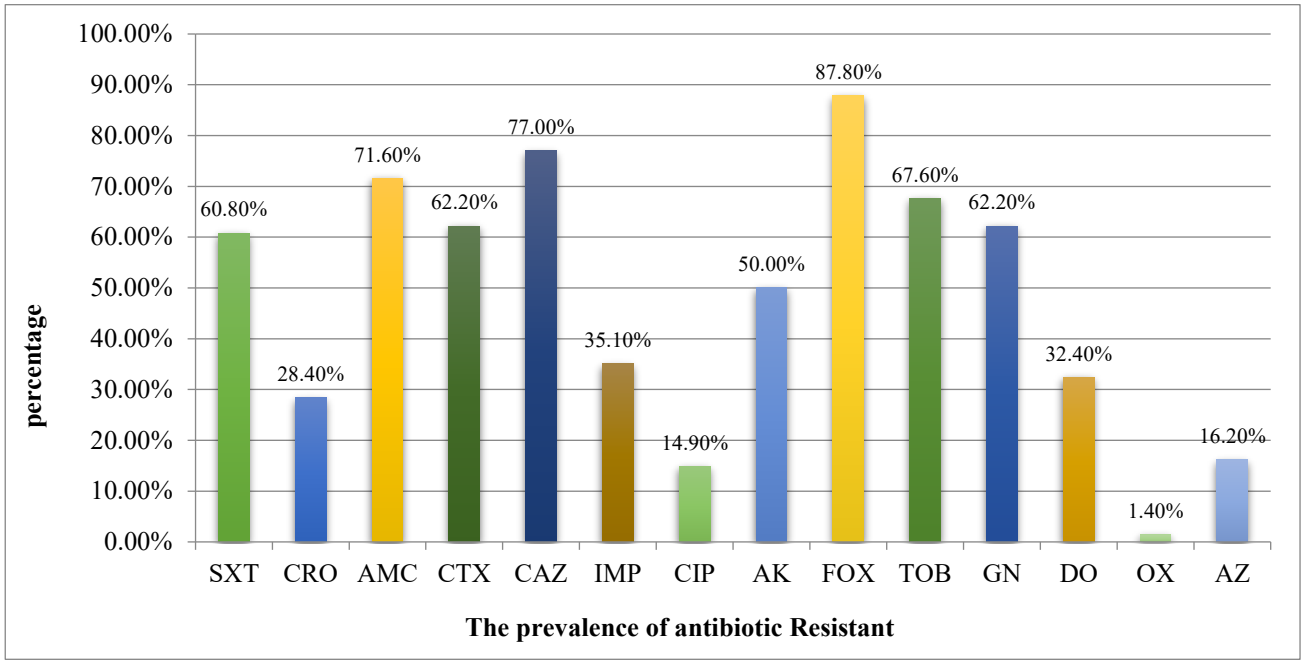



Fig2. Antibiotic susceptibility and resistance pattern of bacterial isolates recovered from used manual toothbrushes

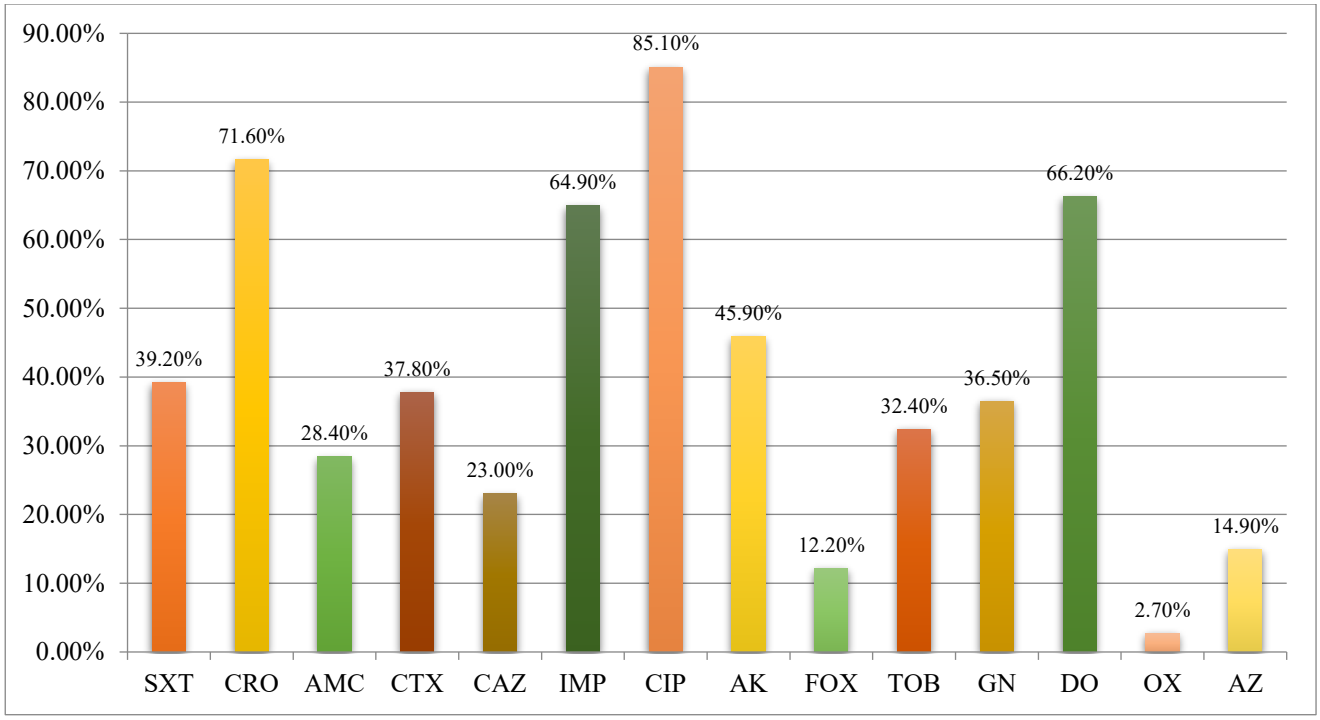



**Fig3**. Antibiotic susceptibility patterns of bacterial isolates recovered from used manual toothbrushes

## 5.DISCUSSION

The present study demonstrated that used manual toothbrushes can harbor bacterial contamination following one month of routine use. Bacterial growth was detected on 73% of the examined toothbrushes, and both Gram-positive and Gram-negative bacterial isolates were recovered. The higher occurrence of Gram-positive isolates suggests that these organisms were more frequently recovered from toothbrushes under conditions examined. These findings are consistent with previous reports showing that toothbrushes may become contaminated with microorganisms during regular use (Frazelle & Munro, 2012; Blaustein *et al*., 2021). In the present study, *Staphylococcus epidermidis* was the most frequently recovered bacterial isolate (27.4%), followed by *Streptococcus viridans* (23.3%). Other bacterial isolates included *Klebsiella* spp.

(12.3 %), *Escherichia coli* (11%), *Staphylococcus aureus* (9.6 %), *Pseudomonas* spp. (8.2 %), *Acinetobacter* spp. (5.5 %), and *Proteus* spp. (2.7 %). The recovery of both Gram-positive and Gram-negative bacteria indicates that used toothbrushes may harbor a diverse range of bacterial organisms. However, the detection of these bacteria on toothbrushes does not, by itself, establish a direct risk of infection.

The antibiotic susceptibility results demonstrated considerable variation among the recovered bacterial isolates. Ciprofloxacin showed the highest reported susceptibility (63%), followed by ceftriaxone (53%), doxycycline (49%), and imipenem (48%). In contrast, cefoxitin showed the highest resistance rate (65%), followed by ceftazidime (57%) and amoxicillin/clavulanic acid (53%). The observed resistance to several tested antimicrobial agents highlights the importance of monitoring antibiotic susceptibility among bacterial isolates recovered from commonly handled personal hygiene devices. Previous research has also investigated antimicrobial resistance bacteria associated with toothbrushes, demonstrating the relevance of examining their microbiological characteristics (Unahalekhaka et al., 2022).

The findings have practical implications for toothbrush hygiene and care. Appropriate rinsing after use, adequate drying, suitable storage, and regular toothbrush replacement may help minimize conditions that favor microbial persistence. Previous studies have investigated various toothbrush contamination methods, although their effectiveness may vary according to the methods and the conditions of application (Agrawal et al., 2019; Li et al., 2026). Nevertheless, the presence of bacterial contamination on toothbrushes does not necessarily establish that they directly cause infection, and further research is required to clarify their clinical significance. Overall, the present study provides local evidence that used manual toothbrushes may harbor bacterial contamination following one month of routine use and may carry bacterial isolates with variable antibiotic susceptibility patterns. The findings highlight the importance of characterizing the antibiotic susceptible profile of bacteria isolates recovered from used toothbrushes and support further research into effective and standardized approaches for reducing microbial contamination.

## 6. CONCLUSION

The present study showed that used manual toothbrushes may serve as reservoirs of bacterial contamination after one month of routine use. Bacterial growth was detected in 73% of the examined toothbrushes, and the recovered bacterial isolates demonstrated variable antibiotic susceptibility patterns. These findings highlight the importance of investigating the susceptibility profile of bacterial isolates recovered from used manual toothbrush and emphasize the value of appropriate toothbrush hygiene, proper storage, and regular toothbrush replacement.

## 7. REFERENCES


Àlvarez, G., Soler-Ollé, A., Isabal, S., León, R., & Blanc, V. (2023). Bacterial decontamination of toothbrushes by immersion in a mouthwash containing 0.05% chlorhexidine and 0.05% cetylpyridinium chloride: A randomized controlled trial. International Journal of Dental Hygiene, 21(2), 357–364. https://doi.org/10.1111/idh.12652

Agrawal, S. K., Dahal, S., Bhumika, T. V., & Nair, N. S. (2019). Evaluating sanitization of toothbrushes using various decontamination methods: A meta-analysis. Journal of Nepal Health Research Council, 16(4), 364–371. https://doi.org/10.33314/jnhrc.v16i4.1865

Assari, A. S., Mohammed Mahrous, M., Ahmad, Y. A., Alotaibi, F., Alshammari, M., AlTurki, F., & AlShammari, T. (2022). Efficacy of different sterilization techniques for toothbrush decontamination: An ex vivo study. Cureus, 14(1), e21117. https://doi.org/10.7759/cureus.21117

Al-Barasi, S. G. M., & Ben-Jweirif, S. (2026). Bacterial Contamination and Antibiotic Susceptibility Profile of Isolates from Shopping Cart Handles in Supermarkets. *Derna Academy Journal for Applied Sciences*, *7*(2), 164-170.

Beneduce, C., Baxter, K. A., Bowman, J., Haines, M., & Andreana, S. (2010). Germicidal activity of antimicrobials and VIOlight® Personal Travel Toothbrush Sanitizer: An in vitro study. Journal of Dentistry, 38(8), 621–625. https://doi.org/10.1016/j.jdent.2009.08.011

Bukhatwa, F. S., Jweiref, S. F. B., ALjahani, A. F., Elzouki, E. M., & ELmoshity, S. F. (2026). Community-Associated Extended-Spectrum Beta-Lactamase-Producing Escherichia coli in a Nephrology Center. *Derna Academy Journal for Applied Sciences*, *7*(1), 110-116.

Blaustein, R. A., Michelitsch, L.-M., Glawe, A. J., Lee, H., Huttelmaier, S., Hellgeth, N., Ben Maamar, S., & Hartmann, E. M. (2021). Toothbrush microbiomes feature a meeting ground for human oral and environmental microbiota. Microbiome, 9, 32. https://doi.org/10.1186/s40168-020-00983-x

Caudry, S. D., Klitorinos, A., & Chan, E. C. S. (1995). Contaminated toothbrushes and their disinfection. Journal of the Canadian Dental Association, 61(6), 511–516.

Dayoub, M. B., Rusilko, D., & Gross, A. (1977). Microbial contamination of toothbrushes. Journal of Dental Research, 56(6), 706. https://doi.org/10.1177/00220345770560063501

Elzouki, E. M., Eljamay, S. M., & Alshilwi, Y. A. (2024). Isolation and identification of Escherichia coli in uropathogenesis. *Derna Academy Journal for Applied Sciences*, *2*(2), 103-110.

Frazelle, M. R., & Munro, C. L. (2012). Toothbrush contamination: A review of the literature. Nursing Research and Practice, 2012, 420630. https://doi.org/10.1155/2012/420630

Khan, S. A., Syed, F. A., Khalid, T., Farheen, N., Javed, F., & Kazmi, S. M. R. (2024). An updated systematic review on toothbrush contamination: An overlooked oral health concern among general population. International Journal of Dental Hygiene, 22(1), 95–105. https://doi.org/10.1111/idh.12740

Kim, J.-H., Kim, D.-A., Kim, H.-S., Baik, J.-Y., Ju, S.-H., & Kim, S.-H. (2018). Analysis of microbial contamination and antibacterial effect associated with toothbrushes. Journal of Dental Hygiene Science, 18(5), 296–304. https://doi.org/10.17135/jdhs.2018.18.5.296

Li, J., Liu, Q., Liang, Z., Li, K. Y., Xing, X., Lin, L., Peng, S., Yiu, C. K. Y., & Wong, H. M. (2026). Effectiveness of disinfection methods for toothbrushes: A systematic review and meta-analysis. Journal of Evidence-Based Dental Practice, 26(2), 102239. https://doi.org/10.1016/j.jebdp.2026.102239

Malta, A. L., Carvalho, J., & Barroso, H. (2019). The effect of toothbrush covers on microbial contamination. Annals of Medicine, 51(Suppl. 1), 112. https://doi.org/10.1080/07853890.2018.1562719

Moura-Filho, A. A. L., Bronzato, J. D., Gomes, B. P. F. A., Silva, C. H. F., & Peralta, S. L. (2026). Knowledge evaluation and assessment of bacterial contamination of toothbrushes. International Journal of Dental Hygiene, 24(1), 45–52. https://doi.org/10.1111/idh.70011

Nascimento, C. do, Trinca, N. N., Pita, M. S., & Pedrazzi, V. (2015). Genomic identification and quantification of microbial species adhering to toothbrush bristles after disinfection: A cross-over study. Archives of Oral Biology, 60(7), 1039–1047. https://doi.org/10.1016/j.archoralbio.2015.03.012

Naik, R., Ahmed Mujib, B. R., Telagi, N., Anil, B. S., & Spoorthi, B. R. (2015). Contaminated tooth brushes—Potential threat to oral and general health. Journal of Family Medicine and Primary Care, 4(3), 444–448. https://doi.org/10.4103/2249-4863.161350

Nelson-Filho, P., Faria, G., da Silva, R. A. B., Rossi, M. A., & Ito, I. Y. (2006). Evaluation of the contamination and disinfection methods of toothbrushes used by 24- to 48-month-old children. Journal of Dentistry for Children, 73(3), 152–158.

Nelson-Filho, P., Macari, S., Faria, G., Assed, S., & Ito, I. Y. (2000). Microbial contamination of toothbrushes and them decontamination. Pediatric Dentistry, 22(5), 381–384.

Saleh, A., Kawafi, W. I., Zafir, Z. A., & Mahmoud, R. (2025). Isolation of Salmonella, Yersinia, and Vibrio bacteria from some seafood sold in retail stores in the Libyan cities of Sousse and Hamama. *Derna Academy Journal for Applied Sciences*, *5*(1), 1-9.

Shembesh, A. R., Jweiref, S. F., Alhadaad, M. A., & Elramli, A. (2026). Incidence of Multidrug-Resistant Klebsiella pneumoniae Related to Nosocomial Infection in Pediatric Wards. *Derna Academy Journal for Applied Sciences*, *7*(2), 197-203.

Sammons, R. L., Kaur, D., & Neal, P. (2004). Bacterial survival and biofilm formation on conventional and antibacterial toothbrushes. Biofilms, 1(2), 123–130. https://doi.org/10.1017/S1479050504001334

Unahalekhaka, A., Butpan, P., Wongsaen, R., Phunpae, P., & Preechasuth, K. (2022). Contamination of antimicrobialresistant bacteria on toothbrushes used with mechanically ventilated patients: A cross-sectional study. Intensive and Critical Care Nursing, 68, 103120. https://doi.org/10.1016/j.iccn.2021.103120

Zinn, M.-K., Schages, L., & Bockmühl, D. (2020). The toothbrush microbiome: Impact of user age, period of use and bristle material on the microbial communities of toothbrushes. Microorganisms, 8(9), 1379. https://doi.org/10.3390/microorganisms8091379