

PREVALENCE OF GRAM POSITIVE AND GRAM-NEGATIVE BACTERIA ISOLATED FROM SMARTPHONES OF UNIVERSITY STUDENTS

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ABSTRACT

Background: Smartphones have become indispensable personal devices among university students, yet their role as potential reservoirs and vectors for bacterial pathogens remains an emerging public health concern. Frequent handling, prolonged use, and infrequent disinfection create favorable conditions for microbial colonization. This study aimed to determine the prevalence of Gram-positive and Gram-negative bacteria isolated from smartphones of university students and to identify the bacterial species present on these devices.

Methods: A cross-sectional study was conducted among 200 university students selected through convenient sampling. Sterile cotton swabs moistened with normal saline were used to sample frequently touched areas of smartphones, including screens, back surfaces, and edges. Samples were cultured on Blood Agar and MacConkey Agar, followed by Gram staining and biochemical testing (catalase, coagulase, indole, citrate utilization, and Voges-Proskauer tests) for bacterial identification. Data were analyzed using descriptive statistics, with frequencies and percentages calculated.

Results: Bacterial growth was observed in 176 (88%) of the 200 smartphone samples, while 24 (12%) showed no growth. Gram-positive bacteria predominated, accounting for 163 (92.6%) of the 176 isolates, whereas Gram-negative bacteria constituted 13 (7.4%). Species-wise distribution revealed *Staphylococcus epidermidis* as the most frequently isolated organism (119; 59.5%), followed by *Staphylococcus aureus* (36; 18.0%), *Klebsiella pneumoniae* (11; 5.5%), *Bacillus* spp. (8; 4.0%), and *Escherichia coli* (2; 1.0%). After excluding *S. epidermidis*, *S. aureus* remained the dominant Gram-positive isolate (81.8%), while *K. pneumoniae* was the most prevalent Gram-negative organism (84.6%).

Conclusion: Smartphones of university students are significantly contaminated with both commensal and potentially pathogenic bacteria. The predominance of Gram-positive organisms, particularly *Staphylococcus* species, reflects contamination from normal skin flora, while the presence of Gram-negative bacteria such as *K. pneumoniae* and *E. coli* suggests environmental and fecal contamination due to inadequate hand hygiene. These findings highlight the potential role of smartphones in the transmission of bacterial infections within university settings. Implementing

awareness programs on hand hygiene and regular smartphone disinfection is strongly recommended to reduce microbial contamination and associated health risks.

Keywords: Smartphones, bacterial contamination, Gram-positive bacteria, Gram-negative bacteria, university students, hygiene practices, microbial prevalence, public health

INTRODUCTION

Smartphones have become indispensable tools in modern society, transforming from simple communication devices into multifunctional platforms used for education, social networking, financial transactions, entertainment, and healthcare access (Statista, 2024). Global smartphone penetration continues to rise, with university students representing one of the largest and most active user groups (Montgomery et al., 2021). In educational environments, students rely extensively on smartphones for academic activities, communication, and daily life management, leading to frequent and prolonged handling of these devices throughout the day (Tekerekoglu et al., 2011). While smartphones offer numerous benefits, their constant contact with human hands, faces, clothing, and diverse environmental surfaces has raised concerns regarding their potential role as reservoirs and vectors for microbial contamination (Brady et al., 2009).

The concept of fomites—inanimate objects capable of carrying and transmitting infectious microorganisms—is central to understanding the public health significance of smartphone contamination. Smartphones possess several characteristics that make them highly efficient fomites: they are handled hundreds of times daily, kept in close proximity to the face and hands, rarely cleaned or disinfected, and exposed to varied environments including classrooms, libraries, cafeterias, restrooms, and public transportation (Duhon et al., 2020). Unlike many personal belongings, smartphones accompany users throughout most daily activities, including during meals, restroom visits, and sleeping periods, creating continuous opportunities for microbial acquisition and transfer (Bhoonderowa et al., 2014). The warm surface temperature generated during device operation, combined with residues of sweat, skin oils, and environmental dust, provides favorable conditions for bacterial

adherence, survival, and proliferation (Koscova et al., 2018).

Bacteria are among the most commonly isolated microorganisms from contaminated mobile phones and are broadly classified as Gram-positive or Gram-negative based on cell wall structure and staining characteristics (Madigan et al., 2021). Gram-positive bacteria possess thick peptidoglycan layers that retain crystal violet stain, appearing purple under microscopic examination, while Gram-negative bacteria have thinner peptidoglycan layers and an outer membrane containing lipopolysaccharides, appearing pink after counterstaining (Tortora et al., 2021). Gram-positive organisms frequently isolated from smartphones include *Staphylococcus aureus*, coagulase-negative staphylococci, *Micrococcus* species, *Bacillus* species, and *Streptococcus* species (Mehta et al., 2014). Many of these are normal components of human skin flora but can cause opportunistic infections when introduced into susceptible hosts or sterile body sites (Grice et al., 2009).

Staphylococcus aureus is particularly significant due to its ability to cause a wide range of infections, including skin and soft tissue infections, pneumonia, osteomyelitis, septicemia, and infective endocarditis (Otter et al., 2013). The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) has created substantial challenges for healthcare systems worldwide, and detection of resistant strains on smartphone surfaces indicates the potential for dissemination of clinically important pathogens within community settings (Brady et al., 2011). Gram-negative bacteria isolated from smartphones include *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter* species, *Acinetobacter* species, and *Proteus* species (Ulger et al., 2009). The presence of these organisms often reflects environmental contamination, inadequate hand hygiene, or exposure to fecal microorganisms (Al-Abdallah, 2010). *Escherichia coli* serves as an important

indicator of fecal contamination and poor hygiene practices, and its recovery from smartphone surfaces suggests inadequate handwashing following restroom use (Akinyemi et al., 2009).

University students constitute a unique population for investigating smartphone contamination because of their extensive use of mobile devices and frequent interaction within academic environments (Araya et al., 2021). Students regularly use smartphones in classrooms, libraries, laboratories, cafeterias, hostels, and social settings, exposing their devices to various microorganisms and increasing opportunities for bacterial transfer (Tekerekoglu et al., 2011). Additionally, many students use their phones while eating, during laboratory activities, or after contact with potentially contaminated surfaces without performing adequate hand hygiene (Pal et al., 2015). Several studies conducted worldwide have reported high levels of bacterial contamination on smartphones used by students and healthcare workers. A study by Akinyemi et al. (2009) reported that more than 60% of examined mobile phones were contaminated with bacteria. Similarly, Ulger et al. (2009) found that 94.5% of mobile phones used by healthcare workers harbored bacterial contamination, including potentially pathogenic organisms. Research among university students in different countries has also revealed substantial contamination rates, indicating that mobile phones may serve as important fomites for microbial transmission (Bodena et al., 2019; Hussein et al., 2020).

The emergence of antimicrobial resistance (AMR) has further emphasized the importance of monitoring bacterial contamination on frequently used personal devices. The World Health Organization recognizes AMR as one of the most serious global public health threats of the twenty-first century (WHO, 2023). Antibiotic-resistant bacteria contribute to prolonged illness, increased mortality, higher healthcare costs, and reduced treatment effectiveness (Moura et al., 2021). Several investigations have identified multidrug-resistant organisms on mobile phones, including MRSA and resistant Gram-negative bacilli (Jeske et al., 2007; Bodena et al., 2019). The detection of resistant organisms on smartphones suggests that

these devices may contribute to the dissemination of antimicrobial-resistant pathogens within communities and educational institutions (Araya et al., 2021). The COVID-19 pandemic further highlighted the significance of personal electronic devices as potential reservoirs of microorganisms, emphasizing the importance of high-touch surfaces in the spread of infectious agents and reinforcing recommendations regarding routine device disinfection (Kampf et al., 2020; Santos et al., 2022).

Despite growing awareness regarding smartphone-associated contamination, routine cleaning of mobile devices remains uncommon among university students. Surveys have shown that many students clean their phones infrequently or only when visible dirt accumulates on the device surface (Lee et al., 2023). This lack of routine disinfection may contribute to sustained bacterial colonization and increase opportunities for microbial transmission (Olsen et al., 2020). Monitoring the prevalence of bacterial contamination on smartphones among university students is important for several reasons: it provides valuable information regarding the potential role of personal devices in the transmission of infectious agents, helps identify common bacterial species circulating within educational environments, contributes to awareness regarding personal hygiene and device sanitation practices, and supports the development of preventive strategies aimed at reducing microbial contamination and minimizing infection risks (Bhoonderowa et al., 2014; Koscova et al., 2018).

The investigation of bacterial contamination on smartphones is particularly relevant in developing countries where overcrowding, limited hygiene awareness, and inadequate infection control practices may contribute to increased microbial transmission (Saxena et al., 2021). In university settings, students represent an active and highly mobile population that can facilitate the spread of microorganisms across diverse environments (Rana et al., 2017). Understanding the prevalence and distribution of Gram-positive and Gram-negative bacteria associated with smartphones can therefore provide valuable information for

designing effective infection prevention strategies (Murgier et al., 2016). Given the widespread use of smartphones among university students and the growing concerns regarding microbial contamination and antimicrobial resistance, the present study was conducted to determine the prevalence of Gram-positive and Gram-negative bacteria isolated from smartphones of university students and to evaluate the potential public health implications associated with bacterial contamination of these commonly used devices.

MATERIALS AND METHODS

Study Design and Setting

A cross-sectional study was conducted at Riphah International University, Faisalabad Campus, Pakistan, over a period of two months following approval of the research synopsis. The study was carried out in the Department of Medical Laboratory Technology, Riphah College of Rehabilitation Sciences, in collaboration with the Departmental Microbiology Laboratory. Sample collection was performed within the university premises, while all microbiological analyses were conducted in the microbiology laboratory under standard laboratory conditions.

Ethical Approval and Consent

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Research Ethics Committee of Riphah International University prior to commencement. Written informed consent was obtained from all participants after explaining the objectives, procedures, and potential implications of the study. Participation was entirely voluntary, and participants were assured of confidentiality and their right to withdraw at any stage without consequences. Personal identifiers were coded, and all data were used exclusively for research purposes.

Study Population and Sample Size

The study population comprised students enrolled in different academic departments of the university who owned and regularly used smartphones. A total of 200 students were recruited using a convenient sampling technique.

The sample size was considered adequate to determine the prevalence and distribution of bacterial contamination on smartphone surfaces within the study population.

Inclusion Criteria

Students who own and regularly use smartphones. Students who willingly agreed to participate and provide who informed consent.

Exclusion Criteria

Students who had disinfected or cleaned their smartphones within 2–3 hours before sample collection. Students who declined to participate or did not provide informed consent.

Sample Collection Procedure

A total of 200 smartphone samples were collected using sterile cotton swabs moistened with sterile normal saline to ensure effective recovery of microorganisms. Each smartphone was carefully swabbed using aseptic techniques to prevent external contamination. Frequently touched areas, including the screen, back surface, and edges of the smartphone, were thoroughly swabbed. After collection, each swab was labelled with a unique identification code corresponding to the participant. The collected swabs were immediately transported to the microbiology laboratory under appropriate conditions to preserve microbial viability and prevent contamination. All samples were processed promptly upon arrival.

Culture Media Preparation

Blood Agar

Blood Agar was prepared according to the manufacturer's instructions. The required quantity of Blood Agar base powder was weighed and dissolved in distilled water. The medium was heated until completely dissolved and sterilized by autoclaving at 121°C for 15 minutes. After sterilization, the medium was allowed to cool to approximately 45–50°C. Sterile defibrinated sheep blood (5%) was added aseptically and mixed gently to avoid air bubble formation. The prepared medium was poured into sterile Petri plates and allowed to solidify. Plates were stored under appropriate conditions until use.

MacConkey Agar

MacConkey Agar was prepared according to the manufacturer's instructions to ensure standardized and reliable culture conditions. The required amount of dehydrated MacConkey Agar powder was accurately weighed and suspended in a measured volume of distilled water in a conical flask. The mixture was gently heated with continuous stirring to ensure complete dissolution and to avoid clumping or precipitation. After complete dissolution, the medium was sterilized by autoclaving at 121°C for 15 minutes under standard pressure conditions. Following sterilization, the medium was allowed to cool to approximately 45–50°C to prevent heat damage to selective and differential components. The cooled medium was then aseptically poured into sterile Petri dishes inside a laminar airflow cabinet. Plates were left undisturbed on a level surface to allow uniform solidification, inverted to prevent condensation, and stored at appropriate temperature until use. MacConkey Agar was specifically used for selective isolation of Gram-negative bacteria and differentiation based on lactose fermentation.

Culture Techniques

The collected swab samples were inoculated onto Blood Agar and MacConkey Agar plates using the streak plate method. The inoculated plates were incubated aerobically at 37°C for 24–48 hours. Following incubation, culture plates were examined for bacterial growth. Colony characteristics such as size, shape, color, texture, margin, elevation, and hemolytic reactions on Blood Agar were observed and recorded.

Gram Staining

Gram staining was performed to differentiate bacterial isolates into Gram-positive and Gram-negative organisms. A thin smear of bacterial growth was prepared on a clean glass slide and allowed to air dry. The smear was heat-fixed by gently passing the slide through a flame. Crystal violet was applied as the primary stain for one minute and rinsed with water. Gram's iodine was added as a mordant for one minute and subsequently washed off. The smear was then decolorized using alcohol for approximately 10–20

seconds and immediately rinsed with water. Safranin was applied as a counterstain for one minute and then washed off. The slide was air dried and examined under an oil immersion lens (100×) using a light microscope. Gram-positive bacteria appeared purple, whereas Gram-negative bacteria appeared pink or red.

Biochemical Tests for Bacterial Identification

Catalase Test

The catalase test was performed to detect the presence of the catalase enzyme in bacterial isolates. A clean glass slide was used, and a small portion of a bacterial colony was transferred onto the slide using a sterile applicator stick. One drop of 3% hydrogen peroxide solution was added to the bacterial colony. The formation of immediate bubbles or effervescence indicated a positive catalase reaction. The absence of bubble formation indicated a negative catalase reaction. This test was used primarily to differentiate catalase-positive organisms, such as staphylococci, from catalase-negative organisms, such as streptococci.

Coagulase Test

The coagulase test was performed to detect the presence of the coagulase enzyme in bacterial isolates. A clean glass slide was used, and a small portion of a bacterial colony was transferred onto the slide using a sterile applicator stick. One drop of sterile normal saline was added to prepare a smooth bacterial suspension, followed by one drop of plasma. The slide was gently mixed and observed for immediate clumping or agglutination. The formation of visible clumps indicated a positive coagulase reaction. The absence of clumping indicated a negative coagulase reaction. This test was used primarily to differentiate coagulase-positive organisms, such as *Staphylococcus aureus*, from coagulase-negative staphylococci (CoNS).

Indole Test

The indole test was performed to detect the ability of bacterial isolates to produce the enzyme tryptophanase, which breaks down tryptophan into indole. A pure bacterial colony was

inoculated into a tube containing tryptone broth and incubated at 37°C for 24 hours. After incubation, a few drops of Kovac's reagent were added to the broth. The formation of a red ring at the surface of the medium indicated a positive indole reaction. The absence of a red ring or the appearance of a yellow ring indicated a negative indole reaction. This test was used primarily to differentiate indole-positive organisms from indole-negative organisms.

Citrate Utilization Test

The citrate utilization test was performed to determine the ability of bacterial isolates to use citrate as the sole carbon source. A pure bacterial colony was inoculated onto Simmons citrate agar slants and incubated at 37°C for 24–48 hours. A change in the color of the medium from green to blue indicated a positive citrate reaction, demonstrating citrate utilization by the organism. No color change and retention of the green color indicated a negative reaction. This test was used primarily to differentiate citrate-utilizing Gram-negative bacteria.

Voges-Proskauer (VP) Test

Biochemical identification of *Bacillus* species was further confirmed using the Voges-Proskauer (VP) test. Out of 44 Gram-positive isolates, 8 (18.2%) isolates showed a positive VP reaction, indicating acetoin production and supporting their identification as *Bacillus* species, while the remaining 36 (81.8%) isolates showed a negative VP reaction.

Identification of Bacteria

Bacterial isolates were identified based on colony morphology, Gram staining characteristics, and biochemical test results according to standard microbiological identification schemes (Forbes et al., 2016; Cheesbrough, 2018).

Statistical Analysis

The collected data was entered into Microsoft Excel and analyzed using descriptive statistical methods. Frequencies and percentages were calculated to determine the prevalence of bacterial contamination on smartphones. The distribution of Gram-positive and Gram-negative bacterial isolates was also determined. Results were presented in the form of tables and graphs to provide a clear summary of the findings. No inferential statistical tests were performed because the study focused primarily on the prevalence, isolation, and identification of bacterial contaminants on smartphone surfaces among university students.

RESULTS

Prevalence of Bacterial Contamination on Smartphones

A total of 200 smartphone samples were collected from university students and analyzed for bacterial contamination. Out of these, 176 (88%) samples showed bacterial growth, while 24 (12%) samples showed no bacterial growth (Figure 1). The findings indicated a high prevalence of bacterial contamination among smartphones used by university students.

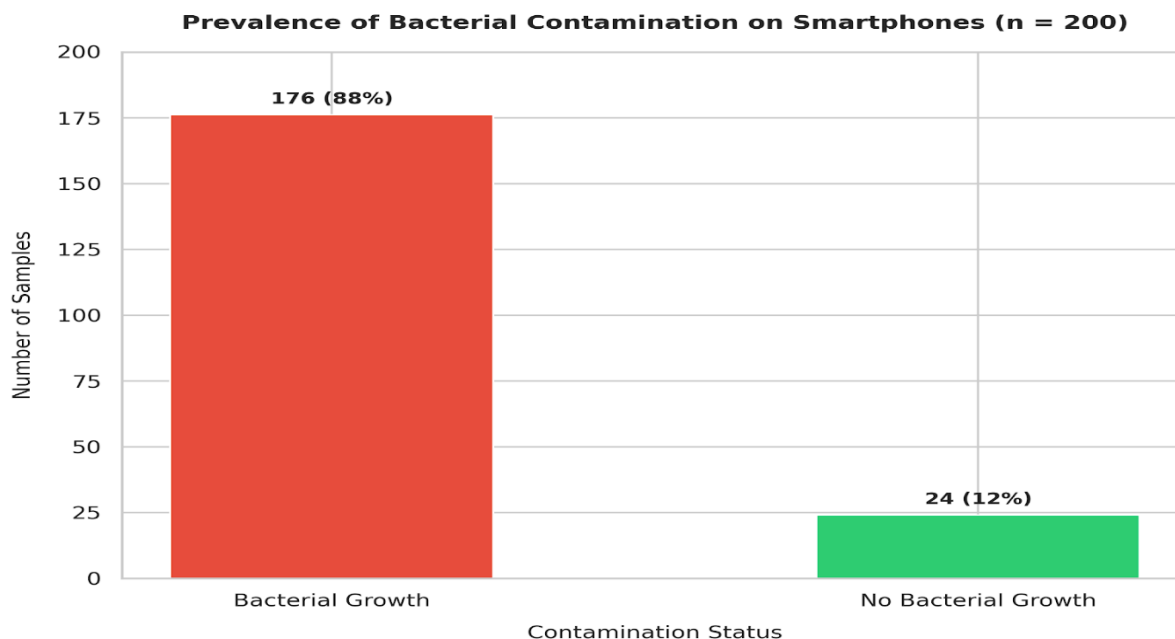


Figure 1. Overall prevalence of bacterial contamination on smartphones (n = 200)

Bacterial Growth on Culture Media

The samples obtained from 200 smartphones were cultured on Blood Agar and MacConkey Agar plates. Out of the 176 culture-positive samples, bacterial growth was observed on Blood Agar in 163 (81%) samples, indicating the presence of a

wide range of bacterial species. MacConkey Agar showed growth in 13 (6.5%) samples, which confirmed the presence of Gram-negative bacteria, while the remaining isolates did not show growth on selective media, suggesting Gram-positive or fastidious organisms (Figure 2).

Distribution of Bacterial Growth Across Culture Media (n = 200)

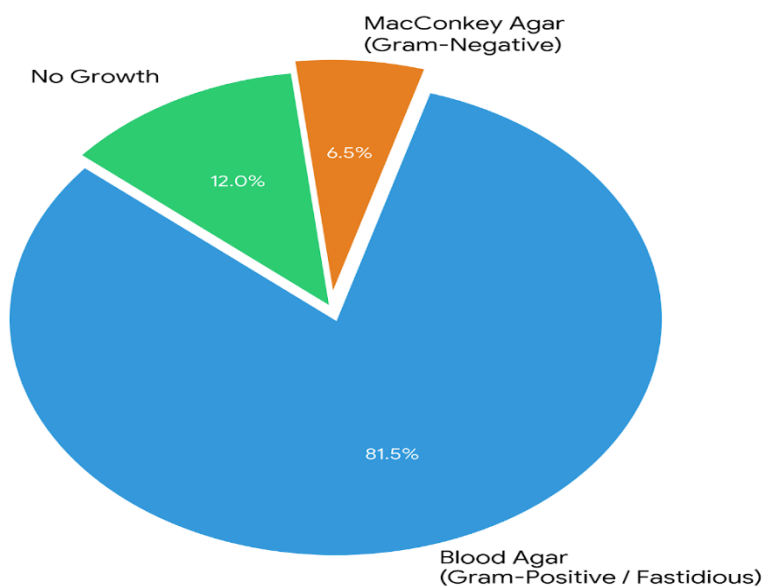


Figure 2. Bacterial growth in culture media

The growth patterns on culture media demonstrated polymicrobial contamination of smartphone surfaces, with Blood Agar supporting the growth of both Gram-positive and Gram-negative bacteria, while MacConkey Agar selectively isolated Gram-negative organisms.

Gram Staining Results of Isolates

Gram staining was performed on all cultured isolates to differentiate bacterial species based on cell wall characteristics. Out of 176 bacterial

isolates, 163 (92.6%) were identified as Gram-positive bacteria, while 13 (7.4%) were identified as Gram-negative bacteria. Gram-positive organisms appeared purple under oil immersion microscopy due to retention of crystal violet stain, whereas Gram-negative organisms appeared pink/red after counterstaining with safranin (Figure 3). The predominance of Gram-positive bacteria indicated that skin flora was the major contaminant of smartphone surfaces.

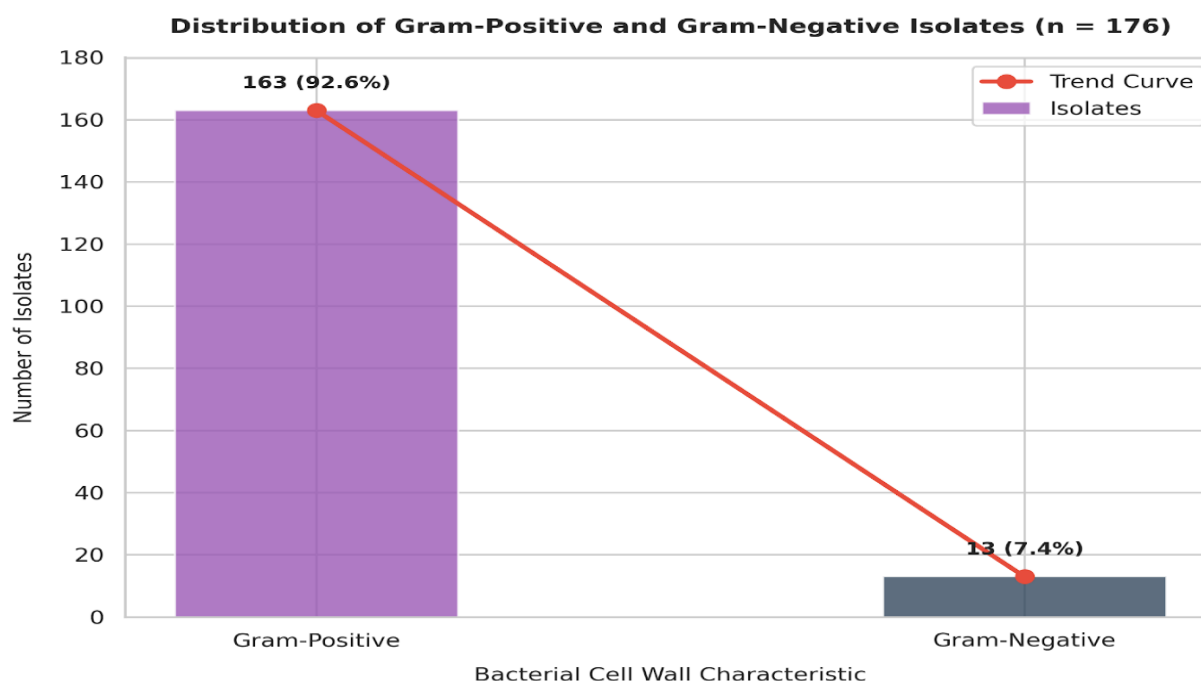


Figure 3. Distribution of Gram-positive and Gram-negative bacteria

Distribution of Gram-Positive and Gram-Negative Isolates

Gram staining of isolates showed that Gram-positive bacteria were predominant, accounting

for 163 (81.5%) of the total isolates, whereas Gram-negative bacteria constituted 13 (6.5%). In addition, 24 (12%) samples showed no bacterial growth (Figure 4).

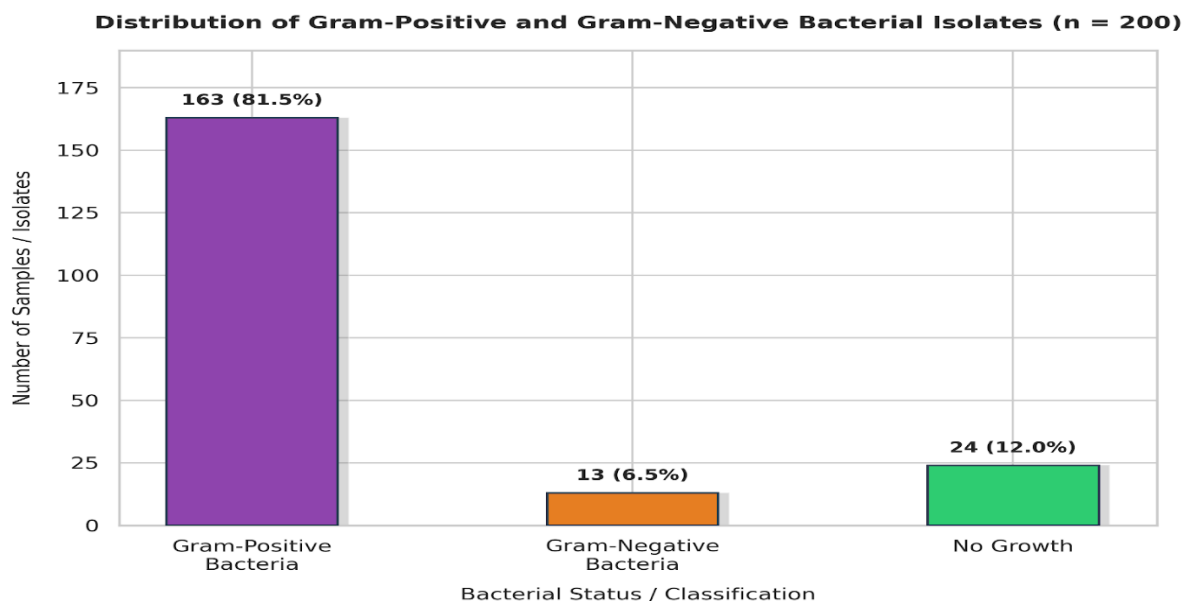


Figure 4. Distribution of Gram-positive and Gram-negative bacterial isolates recovered from smartphones
Biochemical Identification

Catalase Test

Biochemical identification of Gram-positive isolates was performed using the catalase test. Out of 176 Gram-positive isolates, 163 (92.6%) isolates showed strong classical positive catalase reaction, while 13 (7.4%) isolates showed weak positive reactions consistent with coagulase-negative staphylococci and other catalase-variable organisms. The catalase-positive organisms were mainly associated with *Staphylococcus aureus* and related species, while catalase-negative or weak reactions were associated with *Escherichia coli*, *Klebsiella pneumoniae*, and *Bacillus* species.

Coagulase Test

The coagulase test was performed on all staphylococcal isolates recovered from smartphone samples to differentiate coagulase-positive and coagulase-negative species. A total of 163 bacterial isolates were subjected to coagulase testing. Among these isolates, 36 (22.1%) were identified as *Staphylococcus aureus* and exhibited a positive coagulase reaction. The remaining 127 (77.9%) isolates were identified as *Staphylococcus epidermidis* and showed a negative coagulase reaction, classifying them as coagulase-negative staphylococci (CoNS). The predominance of

coagulase-negative staphylococci indicates that *Staphylococcus epidermidis* was the most frequently isolated bacterial species from smartphone surfaces. However, the presence of coagulase-positive *Staphylococcus aureus* demonstrates the potential for smartphones to serve as reservoirs for opportunistic and potentially pathogenic microorganisms.

Indole Test

The indole test was performed on Gram-negative bacterial isolates to differentiate indole-producing organisms from non-indole-producing organisms. A total of 13 isolates were subjected to indole testing. Among the isolates, 2 (15.4%) were identified as *Escherichia coli* and produced a positive indole reaction, indicated by the development of a red ring following the addition of Kovac's reagent. In contrast, 11 (84.6%) isolates were identified as *Klebsiella pneumoniae* and exhibited a negative indole reaction, showing no color change after reagent addition. The results demonstrated that *Klebsiella pneumoniae* was the predominant Gram-negative isolate recovered from smartphone samples, while *Escherichia coli* was detected at a lower frequency.

Citrate Utilization Test

The citrate utilization test was performed on Gram-negative bacterial isolates to determine their ability to utilize citrate as the sole carbon source. A total of 13 isolates were tested. Among the isolates, 11 (84.6%) were identified as *Klebsiella pneumoniae* and showed a positive citrate utilization test, indicated by a color change of the medium from green to blue. In contrast, 2 (15.4%) isolates were identified as *Escherichia coli* and exhibited a negative citrate utilization test, with no significant color change observed in the medium. The results demonstrated that *Klebsiella pneumoniae* was the predominant citrate-positive organism isolated from smartphone samples, whereas *Escherichia coli* showed an inability to utilize citrate under the test conditions.

Voges-Proskauer (VP) Test

The Voges-Proskauer (VP) test was performed on Gram-positive bacterial isolates to detect the production of acetoin as an end product of glucose metabolism via the butylene glycol pathway. A total of 44 isolates were tested. Among the isolates, 36 (81.8%) were identified as *Staphylococcus aureus* and showed a positive VP reaction, demonstrated by the development of a red coloration after the addition of VP reagents. The remaining 8 (18.2%) isolates were identified as *Bacillus* spp. and also exhibited a positive VP reaction. The identification of *Bacillus* spp. was further supported by their characteristic morphological and biochemical features, including Gram-positive rod-shaped appearance, ability to form endospores, and positive catalase reaction. These features, along with the VP test results, confirmed the presumptive identification of *Bacillus* species among the isolates.

Summary of Biochemical Identification Tests for Smartphone Bacterial Isolates

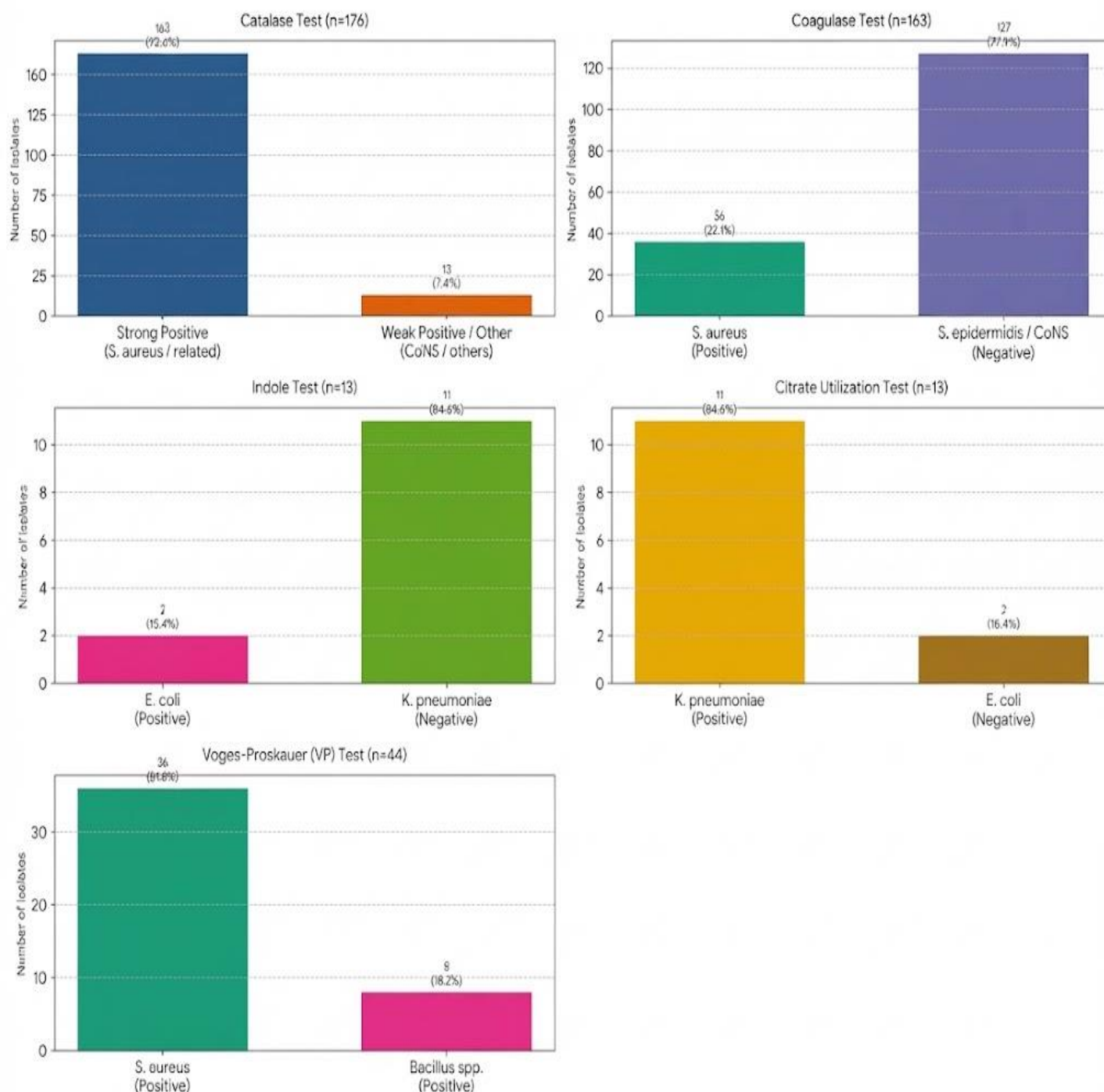


Figure 5: Summary of biochemical identification results for smartphone bacterial isolates across the catalase, coagulase, indole, citrate utilization, and Voges-Proskauer tests.

Species-wise Distribution of Bacterial Isolates

The present study demonstrated a diverse bacterial profile on smartphone surfaces. *Staphylococcus epidermidis* was the most frequently isolated organism 119 (59.5%), followed by *Staphylococcus*

aureus 36 (18.0%), *Klebsiella pneumoniae* 11 (5.5%), *Bacillus* spp. 8 (4.0%), and *Escherichia coli* 2 (1.0%), while 24 (12.0%) samples showed no growth (Table 1).

Table 1. Species-wise frequency and percentage distribution of bacterial isolates (n = 200)

Bacterial Isolate	Frequency	Percentage (%)
<i>Staphylococcus epidermidis</i>	119	59.5
<i>Staphylococcus aureus</i>	36	18.0
<i>Klebsiella pneumoniae</i>	11	5.5
<i>Bacillus</i> spp.	8	4.0
<i>Escherichia coli</i>	2	1.0
No Growth	24	12.0
Total	200	100

Distribution of Gram-Positive Bacteria (Excluding *Staphylococcus epidermidis*)

After exclusion of *Staphylococcus epidermidis*, the Gram-positive bacterial isolates were reanalyzed to better understand the distribution of potentially

clinically significant organisms. The results showed that *Staphylococcus aureus* remained the most prevalent isolate 36 (81.8%), followed by *Bacillus* spp. 8 (18.2%), with a total of 44 Gram-positive isolates (Table 2).

Table 2. Gram-positive isolates excluding *Staphylococcus epidermidis*

Isolate	Frequency	Percentage (%)
<i>Staphylococcus aureus</i>	36	81.8
<i>Bacillus</i> spp.	8	18.2
Total	44	100

Distribution of Gram-Negative Bacterial Isolates

Among the Gram-negative bacterial isolates recovered in the present study, *Klebsiella pneumoniae* was the most frequently isolated

organism 11 (84.6%), followed by *Escherichia coli* 2 (15.4%), with a total of 13 Gram-negative isolates (Table 3).

Table 3. Gram-negative bacterial isolates from smartphones

Isolate	Frequency	Percentage (%)
<i>Klebsiella pneumoniae</i>	11	84.6
<i>Escherichia coli</i>	2	15.4
Total	13	100

DISCUSSION

The present study was conducted to determine the prevalence of Gram-positive and Gram-negative bacteria isolated from smartphones of university students and to identify the bacterial species present on these devices. The findings revealed a high prevalence of bacterial contamination, with 176 (88%) of 200 smartphone samples showing bacterial growth. This high contamination rate is consistent with previous studies conducted globally. Ulger et al. (2009) reported that 94.5% of mobile phones used by healthcare workers harbored bacterial contamination, while Akinoyemi et al. (2009) found that more than 60% of examined mobile phones were contaminated with bacteria. Similarly, Famurewa and David (2019) demonstrated a high rate of bacterial contamination on mobile phones among university students. The high contamination rate observed in the present study confirms that smartphones serve as significant reservoirs for microorganisms and may contribute to the transmission of infectious agents in community settings.

The predominance of Gram-positive bacteria (92.6% of isolates) over Gram-negative bacteria (7.4%) observed in this study is consistent with several previous investigations. Ulger et al. (2009) reported that Gram-positive organisms such as *Staphylococcus aureus* and *Staphylococcus epidermidis* were the most common contaminants on mobile phones of healthcare workers. Similarly, Famurewa and David (2019) reported a higher prevalence of Gram-positive bacteria on mobile phones among university students. The predominance of Gram-positive organisms can be attributed to their natural association with human skin flora, their ability to survive on dry surfaces

for extended periods, and their strong resistance to environmental stress (Grice et al., 2009). In contrast, Tagoe et al. (2011) reported a relatively higher proportion of Gram-negative bacteria on mobile phones, particularly in environments with poor sanitation and higher exposure to fecal contamination. This variation may be due to differences in hygiene practices, environmental conditions, and study populations.

Staphylococcus epidermidis was the most frequently isolated organism in the present study, accounting for 119 (59.5%) of isolates. This finding is consistent with Rana et al. (2017), who reported that coagulase-negative staphylococci were the most frequently isolated organisms from mobile phones of university students. The predominance of *S. epidermidis* is likely associated with its role as a dominant commensal organism of human skin, emphasizing continuous contamination through direct hand contact (Madigan et al., 2021). *Staphylococcus aureus* was the second most common isolate, accounting for 36 (18.0%) of isolates. The presence of *S. aureus* indicates possible colonization by potentially pathogenic organisms and aligns with the study of Tekerekoglu et al. (2011), who found significant contamination of mobile phones with *S. aureus*, suggesting that mobile devices may act as reservoirs for community-associated infections. *Staphylococcus aureus* is a well-known opportunistic pathogen associated with skin infections, respiratory tract infections, and hospital-acquired infections (Otter et al., 2013). The detection of this organism on smartphone surfaces raises concerns regarding the potential dissemination of clinically important pathogens within community settings.

The detection of Gram-negative organisms such as *Klebsiella pneumoniae* (5.5%) and *Escherichia coli* (1.0%) suggest environmental contamination and poor hygiene practices. Comparable findings were reported by Ustun and Cihangiroglu (2012), who observed Gram-negative enteric bacteria on mobile phones and linked their presence to inadequate handwashing and environmental exposure. *Klebsiella pneumoniae* is commonly associated with the gastrointestinal tract and hospital environments, and its presence on smartphones may indicate fecal or environmental contamination (Al-Abdallah, 2010). Similarly, the isolation of *Escherichia coli* further supports the possibility of fecal contamination, likely resulting from inadequate handwashing practices after restroom use (Akinyemi et al., 2009). These findings highlight the importance of proper hand hygiene in reducing bacterial contamination of personal electronic devices.

The isolation of *Bacillus* spp. (4.0%) indicates possible contamination from dust and environmental surfaces, as these organisms are known to survive in harsh environmental conditions. This is supported by Tambekar et al. (2008), who reported *Bacillus* species on frequently used personal devices and attributed their presence to airborne contamination. Kilic et al. (2009) also identified *Bacillus* spp. on frequently handled personal items and linked their occurrence to environmental exposure and poor handling hygiene. The presence of *Bacillus* species, although often considered non-pathogenic, may pose risks to immunocompromised individuals and indicates the extent of environmental contamination on smartphone surfaces.

The distribution of Gram-positive bacteria excluding *S. epidermidis* showed that *S. aureus* remained the most prevalent isolate (81.8%), followed by *Bacillus* spp. (18.2%). This finding is consistent with Al-Ghamdi et al. (2011), who reported a high frequency of *S. aureus* contamination on personal mobile devices and emphasized its role in community-associated infections. The predominance of *S. aureus* among clinically significant Gram-positive isolates highlights the potential public health concern associated with smartphone contamination, as this

organism is capable of causing a wide range of infections, including skin and soft tissue infections, pneumonia, and bloodstream infections (Brady et al., 2011).

Among Gram-negative isolates, *K. pneumoniae* was the most frequently isolated organism (84.6%), followed by *E. coli* (15.4%). This finding is in agreement with Al-Abdallah (2010), who reported a notable presence of *Klebsiella* species on mobile phones of healthcare workers and suggested that improper hand hygiene significantly contributes to its transmission. The predominance of *K. pneumoniae* among Gram-negative isolates may be attributed to its ability to survive on dry surfaces and its association with gastrointestinal and environmental sources (Kramer et al., 2006). The presence of *E. coli* further supports the possibility of fecal contamination and highlights the role of personal devices in transmitting enteric pathogens in community settings (Akinyemi et al., 2009).

The high contamination rate observed in this study has significant public health implications. Smartphones are handled hundreds of times daily and are rarely disinfected, creating favorable conditions for microbial accumulation and transmission (Montgomery et al., 2021). The presence of both commensal and potentially pathogenic bacteria on smartphone surfaces indicates that these devices may serve as vehicles for the dissemination of microorganisms among users and within communities. Furthermore, the detection of Gram-negative organisms such as *K. pneumoniae* and *E. coli* suggests that smartphones may contribute to the spread of antimicrobial-resistant bacteria, as these organisms are known to possess intrinsic and acquired resistance mechanisms (Moura et al., 2021). Several studies have reported the isolation of multidrug-resistant bacterial strains from mobile phones, suggesting that these devices may facilitate the dissemination of resistant microorganisms within communities (Bodena et al., 2019; Araya et al., 2021).

The findings of this study are particularly relevant in university settings, where students frequently interact in shared environments such as classrooms, libraries, laboratories, cafeterias, and hostels. These diverse environments expose smartphones to various microorganisms and

increase opportunities for bacterial transfer (Tekerekoglu et al., 2011). Additionally, many students use their phones while eating, during laboratory activities, or after contact with potentially contaminated surfaces without performing adequate hand hygiene (Pal et al., 2015). The lack of routine disinfection practices among students further contributes to sustained bacterial colonization and increases opportunities for microbial transmission (Lee et al., 2023).

The COVID-19 pandemic highlighted the significance of personal electronic devices as potential reservoirs of microorganisms and reinforced recommendations regarding routine device disinfection (Kampf et al., 2020; Santos et al., 2022). Although the pandemic primarily focused attention on viral transmission, bacterial contamination remains an ongoing concern due to the ability of bacteria to survive on surfaces for extended periods and contribute to community-acquired infections (Olsen et al., 2020). The findings of the present study support the need for increased awareness regarding smartphone hygiene and the implementation of regular disinfection practices among university students.

Based on the findings of this study, several recommendations are proposed. First, awareness programs should be conducted in universities to educate students about the potential microbial contamination of smartphones and the importance of hand hygiene. Second, regular cleaning and disinfection of mobile phones using appropriate disinfectants such as alcohol-based wipes should be encouraged to reduce bacterial load on device surfaces. Third, users should be advised to avoid using smartphones in high-risk environments such as washrooms and during eating to minimize contamination. Fourth, further studies should be conducted with larger sample sizes and advanced molecular techniques to identify antibiotic resistance patterns among isolated bacteria. Fifth, future research should explore the role of smartphones in the transmission of clinically significant and multidrug-resistant organisms in both community and healthcare settings. Finally, infection control policies should incorporate recommendations

regarding mobile phone hygiene, particularly in healthcare and educational settings.

CONCLUSION

The present study demonstrated a high prevalence of bacterial contamination on smartphones of university students, with 176 (88%) of 200 samples showing bacterial growth. Gram-positive bacteria were predominant (92.6%), while Gram-negative bacteria constituted 7.4% of isolates. *Staphylococcus epidermidis* was the most frequently isolated organism (59.5%), followed by *Staphylococcus aureus* (18.0%), *Klebsiella pneumoniae* (5.5%), *Bacillus* spp. (4.0%), and *Escherichia coli* (1.0%). The predominance of Gram-positive bacteria, particularly *Staphylococcus* species, reflects contamination from normal skin flora due to frequent handling of devices. The presence of Gram-negative organisms such as *K. pneumoniae* and *E. coli* indicate environmental and fecal contamination, likely resulting from inadequate hand hygiene practices. It is concluded that smartphones serve as significant reservoirs for both commensal and potentially pathogenic bacteria and may contribute to the transmission of infections in community settings. Regular hand hygiene and smartphone disinfection are strongly recommended to reduce microbial contamination and associated health risks.

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