

**Original Article** 

# Detection of antibiotic resistance genes in bacterial isolates from most touched surfaces of public transports in Sagamu, Ogun state, Nigeria

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# ABSTRACT

Background and Aims: The role of fomites in the transmission of infectious diseases is becoming more important because of the possibility that contaminated surfaces act as reservoirs of diseases. The aim of this study was to ascertain the level of bacterial contamination and the prevalence of antibiotic-resistant genes on the most touched surfaces on public transport.

Methods: One hundred samples were collected from door handles and armrests of buses and tricycles, respectively at Isale-Oko motor garage, Sagamu. Bacteria were isolated from the samples and identified following standard microbiological techniques. Antibiotic susceptibility testing was done with the Kirby-Bauer disc diffusion method. The presence of antibiotic-resistant genesvanA, drfA, dfrG genes, and extended-spectrum betta-lactamase (ESBL) genes in Gram-negatives were screened by polymerase chain reaction (PCR) method.

Results: Out of the samples tested, 91% were positive for bacterial contamination. Among the 91 positive samples, 126 bacteria were identified, comprising 98 Gram-positive and 28 Gram-negative bacterial isolates. Staphylococcus aureus had the highest overall frequency of occurrence with 62 (49.2%) isolates. Among the Gram-positives, azithromycin resistance was present in 35(56.5%) S. aureus and 19(52.8%) Staphylococcus epidermidis. Salmonella species was the most resistant to ciprofloxacin (100%). dfrG was the most detected among trimethoprim-resistant genes occurring in 11(55%) of multidrug-resistant S. aureus and 6(54.6%) of S. epidermidis. vanA gene was present in S. aureus (20%). dfrA was present in only Klebsiella pneumoniae and Esherichia coli. E. coli and Shigella species carried bla<sub>TEM</sub> while bla<sub>SHV</sub> was found in Pseudomonas aeruginosa.

Conclusion: The most-touched surfaces of public transportation can serve as a substantial source of spread for potentially harmful bacteria.

Keywords: Antibiotic resistance, Extended-spectrum beta-lactamase, Bacterial isolates, Public transportation

# **INTRODUCTION**

Any environment including soil, air, water, food, and other organisms, as well as environmental surfaces or items, might include pathogenic organisms (Nwankwo, Okey-kalu, & Eze, 2023). The transportation networking system is constantly increasing to fulfill the demand of carrying heavy loads of products and the movement of people from one location to another. There have been countless reports of how the development and extension of global transportation networks have promoted widespread outbreaks of communicable illnesses (Guimera, Mossa, Turtschi, & Amara, 2005; Tatem, Rogers, & Hay, 2006; Rodrigue, 2020). The effectiveness and accessibility of contemporary transportation networks put people in danger of novel strains of pathogenic microbes (Tatem et al., 2006). The World

Health Organization has issued a warning about the pandemic status of several diseases resulting from the global dissemination of pathogenic microbes (WHO, 2020).

Handles, chairs, anchors, floors, and windows of public buses are all potential breeding grounds for infectious germs (Rusin, Maxwell, & Gerba, 2002; Chowdhury et al., 2016). Buses in use are hardly ever cleaned, and when they are, the technique is limited to the removal of visible dirt or stains (Yeh, Simon, Millar, Alexander, & Franklin, 2011). The unsanitary and humid conditions of buses contribute to the accumulation of microbes on contact surfaces and human contact with microbes from these reservoirs can cause mild to serious infection that can be harmful to humans (Rusin et al., 2002; Chowdhury et al., 2016). Passengers who do not cover their mouths when

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coughing or sneezing can significantly increase the number of microorganisms in the air (Birteksöz Tan, & Erdoğdu, 2017). People sitting closely together in a confined setting and breathing the same air pose the greatest risk for respiratory-related infectious diseases in these vehicles (Furuya, 2007). A serious worry is the spread of infectious diseases by contact with hands. Hand washing, which is widely recognized as the first step in stopping the transmission of infections, has been neglected and must be strongly promoted by individuals, families, schools, and medical practitioners (Nwankwo *et al.*, 2023).

Much research had been done on microbial contamination on public hand-touched surfaces such as those on buses, trains, cellphones, hand knobs, ATMs, hospitals, shopping carts, and other surfaces (Choudhary et al., 2016; Bhatta et al., 2018; Kahsay, Asgedom, & Weldetinsaa, 2019). Escherichia coli, multidrug-resistant Staphylococcus aureus, coagulase-negative staphylococci, and Enterococcus species are among the commonly reported pathogens that have been isolated from handtouch surfaces (Ashgar and El-Said, 2012; Chowdhury et al., 2016; Birteksöz Tan, and Erdoğdu, 2017; Kahsay et al., 2019). Antimicrobial resistance (AMR) has become a public health problem (Allcock et al., 2017). The dangers presented by AMR to people's health are especially worrying in developing countries due to limited access to healthcare services, high prevalence of transmissible diseases in the general population, and increased risk of community-acquired resistant infections resulting in greater mortality, extended hospital stays, and more expensive healthcare (WHO, 2021). Although several studies have reported the presence of pathogenic bacteria in public transport, information on antibiotic susceptibility patterns and genes associated with antibiotic resistance is scanty.

It has been established that resistant bacteria can spread to people in a variety of environments, most especially, crowded locations in densely populated urban areas such as public transportation, sporting venues, and schools (Cave, Cole, & Mkrtchyan, 2021). Bacteria from the hospital can also be spread to the community through the public transport system. The Isale-Oko motor garage was chosen as our sample location due to its proximity to the second gate of Olabisi Onabanjo University Teaching Hospital, where most patients and their guests normally board. This study investigated the bacterial burden of buses and tricycles in the Isale-Oko motor garage to determine the role of commuter transport in the transmission of bacteria that could cause serious infections in people and to detect antibiotic resistance genes in bacterial isolates from public transports.

# MATERIALS AND METHODS

#### Sampling site

Samples were collected from randomly selected inter-state public buses and armrests of tricycles in Isale-Oko Motor Garage, Sagamu. Isale-Oko Car Park in Sagamu is one of the motor garages in Sagamu for intra- and inter-state transportation, and it is quite close to the second gate of Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun state, Nigeria.

#### **Collection of samples**

One hundred (100) samples were taken from door handles of public buses and armrests of tricycles. Samples were taken from each area by swabbing a 4 cm<sup>2</sup> section with a sterile cotton swab stick that had been dipped in sterile normal saline and transported to Pharmaceutical Microbiology Laboratory, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu, Ogun State within 15 minutes of collection for culturing.

### Isolation and identification of bacterial isolates

The swab was inoculated into nutrient broth and incubated at 37°C for 24 hours. A loopful from a 24-hour-old culture was used to inoculate the surface of Mannitol salt agar, MacConkey agar, cetrimide agar, and Salmonella-Shigella agar. The culture plates were incubated at 37°C for 24 hours and observed for growth and colony appearance. Lactose-fermenters were streaked on the surface of Eosin methylene blue agar. Biochemical characteristics of the isolates were determined according to standard protocol (Cheesbrough, 2006).

### Antibiotic susceptibility testing

Antibiotic susceptibility tests for selected isolates were performed according to the method of Bauer, Kirby, Sherris, & Turck, (1966). The antibiotic sensitivity of enteric bacterial isolates was evaluated using commonly available standard antibiotic discs of imipenem (10 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), cefepime (30 µg), cephalexin (30 µg), azithromycin (15 µg), meropenem (10 µg), Trimethoprim (5 μg), cefoxitin (30 μg), amoxicillin-clavulanic acid (20/10 μg), amikacin (30  $\mu$ g), ceftazidime (30  $\mu$ g) and aztreonam (30  $\mu$ g) [Mast Group, United Kingdom]. The required bacterial suspension was made in nutrient broth by inoculating 5 ml sterile nutrient broth with 3-4 colonies from 18 hours old culture of the isolate and the turbidity of the culture was adjusted to 0.5 McFarland standard. A cotton swab was dipped in the culture suspension and streaked over the solidified Mueller-Hinton agar medium's surface to create a homogenous inocula. Using a sterile pair of forceps, the antibiotic discs were then arranged appropriately on the surface of the seeded plates and left on the bench at 25°C for 30 minutes to diffuse the antibiotic in the media. Following a 24-hour incubation period at 37°C, the diameter of the clear zone growth of inhibition was measured and interpreted in accordance with the standard provided by the Clinical and Laboratory Standards Institute (CLSI, 2022).

# Amplification of antibiotic resistance genes in Gram-positive isolates

DNA was extracted using the previously known quick alkaline lysis procedure (Dutka-Malen, Evers, & Courvalin, 1990). PCR was performed on a DNA thermal cycler (Applied Biosystem, USA) in a final volume of 25  $\mu$ L containing 1  $\mu$ L of DNA template; 1  $\mu$ L each of forward and reverse primer (0.2 pmol/ $\mu$ L), 12.5  $\mu$ L of master mix (Wizbiosolutions, South Korea) and 9.5  $\mu$ L of sterile filtered water. The primer sequence used in this study is presented in Table 1.

The PCR condition comprised initial denaturation at  $95^{\circ}$ C for 10 minutes, followed by 30 cycles of 45 seconds at  $94^{\circ}$ C, 45 seconds at  $54^{\circ}$ C (*vanA*), 55oC (*dfrA* and *dfrG*), and 1 minute at 72oC, with a final extension for 8 minutes at 72oC.

The PCR condition for ESBL genes involves initial denaturation at 94°C for 10 minutes and 30 cycles of denaturation at 94°C for 40 seconds, annealing at 50°C ( $bla_{TEM}$  and  $bla_{SHV}$ ), 56°C ( $bla_{CTX-M}$ ) for 40 seconds and elongation 72°C for 1 minute with a final elongation step at 72°C for 7 minutes.

Electrophoresis of PCR products was performed on a 1.5% agarose gel containing 0.5 mg/ml of ethidium bromide at 100 volts for 1 hour using a 100 bp marker as standard. The varied bands were seen after migration while illuminated by UV light.

#### Statistical analysis

The descriptive data were presented in terms of relative frequency.

# RESULTS

# Collection and culturing of samples and identification of bacterial isolates from public transport door handles

Out of the 100 public transport door handles swabbed, 91 samples were positive for bacterial growth. Table 1 shows the number of isolates and the types of bacterial species found on the door handles of the vehicles swabbed. One hundred and twentysix (126) bacterial isolates were cultured from the 91 positive samples, including 98 Gram-positive- and 28 Gram-negative bacterial isolates. The bacteria species found were *S. aureus*, *S. epidermidis*, *P. aeruginosa*, *E. coli*, *K. pneumoniae*, *K. oxytoca*, *Shigella* species, and *Salmonella* species. *S. aureus* had the highest overall frequency of occurrence with a total of 62 (49.2%) isolates followed by *S. epidermidis* with 36 (28.6%) isolates out of the total 126 bacterial isolates while *K. oxytoca* and *Salmonella species* were the least having the same frequency of 1 (0.8%) isolate.

# Antibiotic resistance patterns of bacteria isolated from the door handle

The susceptibility patterns of the bacterial isolates to antibiotics are presented in Table 2. Resistance to cefoxitin was found in 35 (56.5%) of S. aureus and 15 (44.4%) of S. epidermidis. Also, 21 (58.3%) and 43 (69.4) of S. epidermidis and S. aureus, respectively were resistant to trimethoprim. Only S. aureus and E. coli were resistant to imipenem with E. coli being the most resistant isolate (14.3%). The highest resistance to cefotaxime (55.6%) was observed in S. epidermidis while K. oxytoca and Salmonella species were totally sensitive to cefotaxime. Salmonella species was the most resistant to ciprofloxacin (100%) while only S. aureus was resistant to meropenem (1.6%). Salmonella species was totally resistant to ciprofloxacin, cefepime, and aztreonam (100%). Table 3 shows the antibiotic resistance profile of multidrug-resistant isolates. Among the Gram-positive isolates, 31(31.6%) out of 98 isolates were resistant to three or more classes of antibiotics, 20(64.5%) of which were S. aureus isolates the remaining 11 (35.5%) were S. epidermidis. Only 4(14.3%) out of 28 Gram-negative bacterial isolates were multidrug resistant.

# Detection of antibiotic resistance genes in MDR Gram-positive and Gram-negative isolates from public transport

Twenty multidrug-resistant *S. aureus* and 11 multidrugresistant *S. epidermidis* were screened for the occurrence of trimethoprim resistance genes (*dfrA*, *dfrD*, and *dfrG*) and vancomycin resistance gene vanA, out of which 3 (15%) and 11 (55%) of the multidrug-resistant *S. aureus* carried *dfrA* and *dfrG*, respectively. The *dfrA* and *dfrG* genes were found in 1 (9.1%) and 6 (54.6%) *S. epidermidis*, respectively (Table 4). vanA gene was present in 4 (20%) of *S. aureus* but not found in *S. epidermidis* (Figure 1). The coexistence of *dfrA* and *dfrG* was discovered in one *S. aureus*. Among the Gram-negative bacteria, *dfrA* was present in only *K. pneumoniae* and *E. coli* (Figures 2-3)

Three (75.0%) of multidrug resistant Gram-negative isolates (*E. coli*, *P. aeruginosa* and *Shigella* species) had ESBL genes. *E. coli* and *Shigella* species carried  $bla_{TEM}$  while  $bla_{SHV}$  was found in only *P. aeruginosa*. None of the four MDR isolates had  $bla_{CTX-M}$  (Figure 4).

| Genes                     | Primer sequence                    | Amplicon sizes | Reference                  |  |  |
|---------------------------|------------------------------------|----------------|----------------------------|--|--|
|                           |                                    | ( <b>bp</b> )  |                            |  |  |
| dfrA                      | F: 5'-AGCTACTCTTTAAAGCCTTGACGTA-3' | 341            | Grape et al. (2007)        |  |  |
|                           | R: 5'-GTGTTGCTCAAAAACAACTTCG-3'    |                |                            |  |  |
| dfrG                      | F: 5'-TGCTGCGATAAGAA-3'            | 405            | Argudin et.al. (2011)      |  |  |
|                           | R: 5'-TGGGCAAATACCTCATTCC-3'       |                |                            |  |  |
| vanA                      | F-5'GGGAAAACGACAATTGC-3'           | 732            | Dutka-Maleen et.al. (1995) |  |  |
|                           | R-5'GTACAATGCGGCCGTTA-3'           |                |                            |  |  |
| bla <sub>SHV</sub>        | F-5'TCGCCTGTGTATTATCTCCC-3'        | 768            | Maynard et al. (2003)      |  |  |
|                           | R-5' CGCAGATAAATCACCACAATG-3'      |                |                            |  |  |
| <i>bla</i> <sub>TEM</sub> | F-5'GAGTATTCAACATTTTCGT-3'         | 857            | Maynard et al. (2003)      |  |  |
|                           | R-5' ACCAATGCTTAATCAGTGA-3'        |                |                            |  |  |
| bla <sub>CTX-M</sub>      | F-5'TTTGCGATGTGCAGTACCAGTAA-3'     | 544            | Edelstein et al. (2003)    |  |  |
|                           | R-5'CGATACGTTGGTGGTGCCATA-3'       |                |                            |  |  |
|                           |                                    |                |                            |  |  |

Table 1. Primer sequences of antibiotic resistance genes

Table 2. Frequency of isolated bacterial species

| Isolated bacterial species | No of isolated bacteria | Isolated bacteria (%) |  |  |  |  |
|----------------------------|-------------------------|-----------------------|--|--|--|--|
| S. aureus                  | 62                      | 49.2                  |  |  |  |  |
| S. epidermidis             | 36                      | 28.6                  |  |  |  |  |
| P. aeruginosa              | 11                      | 8.7                   |  |  |  |  |
| E. coli                    | 7                       | 5.6                   |  |  |  |  |
| K. pneumoniae              | 4                       | 3.2                   |  |  |  |  |
| K. oxytoca                 | 1                       | 0.8                   |  |  |  |  |
| Shigella species           | 4                       | 3.2                   |  |  |  |  |
| Salmonella species         | 1                       | 0.8                   |  |  |  |  |
| Total                      | 126                     | 100                   |  |  |  |  |

| Isolate (Number)       | Number of bacterial isolates (%) |          |          |          |          |          |          |        |          |         |        |          |         |
|------------------------|----------------------------------|----------|----------|----------|----------|----------|----------|--------|----------|---------|--------|----------|---------|
|                        | IMI                              | СТХ      | СІР      | CFP      | CLX      | AZM      | TM       | MEM    | FOX      | AUG     | AK     | CAZ      | ATM     |
| S. aureus (62)         | 2(3.2)                           | 31(50)   | 11(17.7) | 19(30.6) | 10(16.1) | 35(56.5) | 43(69.4) | 1(1.6) | 35(56.5) | 5(8.1)  | 4(6.5) | 52(83.9) | -       |
| CoNS (36)              | 0(0.0)                           | 20(55.6) | 7(19.4)  | 10(27.8) | 3(8.3)   | 19(52.8) | 21(58.3) | 0(0.0) | 15(44.4) | 0(0.0)  | 1(2.8) | 31(86.1) | -       |
| P. aeruginosa (11)     | 0(0.0)                           | 2(18.2)  | 1(9.1)   | 1(9.1)   | 9(81.8)  | -        | -        | 0(0.0) | 3(27.3)  | 1(9.1)  | 0(0.0) | 1(9.1)   | 2(18.2) |
| E. coli (7)            | 1(14.3)                          | 1(14.3)  | 2(28.6)  | 2(28.6)  | 5(71.4)  | -        | -        | 0(0.0) | 3(42.9)  | 1(14.3) | 0(0.0) | 1(14.3)  | 1(14.3) |
| K. pneumoniae (4)      | 0(0.0)                           | 1(25)    | 0(0.0)   | 0(0.0)   | 2(50.0)  | -        | -        | 0(0.0) | 1(25)    | 0(0.0)  | 0(0.0) | 1(25)    | 1(25)   |
| K. oxytoca (1)         | 0(0.0)                           | 0(0.0)   | 0(0.0)   | 0(0.0)   | 0(0.0)   | -        | -        | 0(0.0) | 1(100)   | 0(0.0)  | 0(0.0) | 0(0.0)   | 0(0.0)  |
| Shigella species (4)   | 0(0.0)                           | 1(25)    | 0(0.0)   | 0(0.0)   | 1(25.0)  | -        | -        | 0(0.0) | 2(50)    | 1(25)   | 0(0.0) | 1(25.0)  | 1(25)   |
| Salmonella species (1) | 0(0.0)                           | 0(0.0)   | 1(100)   | 1(100)   | 1(25.0)  | -        | -        | 0(0.0) | 0(0.0)   | 0(0.0)  | 0(0.0) | 0(0.0)   | 1(100)  |

## Table 3. Antibiotic resistance profile of bacterial isolates

 $\textbf{Note: IMI = imipenem; CTX = cefotaxime; CIP = ciprofloxacin; CFP = cefepime; CLX = cephalexin; AZM = azithromycin; TM = trimethoprim; MEM = meropenem; FOX = cefoxitin; CIP = ciprofloxacin; CFP = cefepime; CLX = cephalexin; AZM = azithromycin; TM = trimethoprim; MEM = meropenem; FOX = cefoxitin; CIP = ciprofloxacin; CFP = cefepime; CLX = cephalexin; AZM = azithromycin; TM = trimethoprim; MEM = meropenem; FOX = cefoxitin; CIP = ciprofloxacin; CFP = cefepime; CLX = cephalexin; AZM = azithromycin; TM = trimethoprim; MEM = meropenem; FOX = cefoxitin; CIP = cefepime; CLX = cephalexin; AZM = azithromycin; TM = trimethoprim; MEM = meropenem; FOX = cefoxitin; CIP = cefepime; CLX = cephalexin; AZM = azithromycin; TM = trimethoprim; MEM = meropenem; FOX = cefoxitin; CIP = cefepime; CLX = cephalexin; AZM = azithromycin; TM = trimethoprim; MEM = meropenem; FOX = cefoxitin; CIP = cefepime; CLX = cephalexin; AZM = azithromycin; TM = trimethoprim; MEM = meropenem; FOX = cefoxitin; CIP = cefepime; CLX = cephalexin; AZM = azithromycin; TM = trimethoprim; MEM = meropenem; FOX = cefoxitin; CIP = cefepime; CLX = cephalexin; CLX = cephalexin; CIP = cefepime; CLX = cefepime;$ 

 $AUG = amoxicillin\ clavulanic\ acid;\ AK = amikacin;\ CAZ = ceftazidime;\ ATM = aztreonam$ 

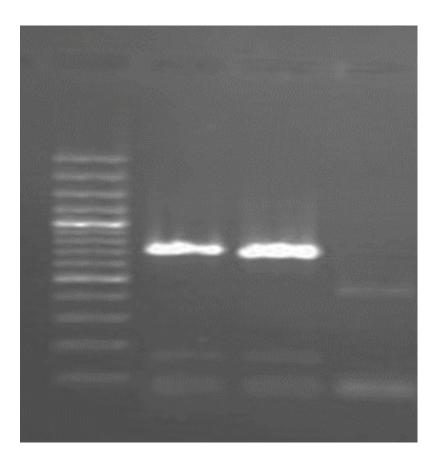


Figure 1. Gel representation of amplified vanA (732 bp). (L = 100bp ladder; lanes 2-4 = S. aureus)

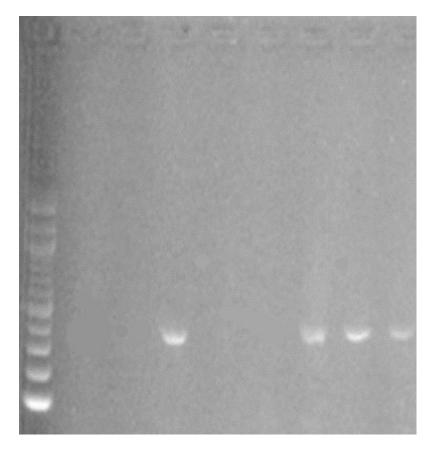


Figure 2. Gel representation of amplified dfrA5 (341 bp). (L = 100bp ladder; lanes 2-10 = S. aureus)

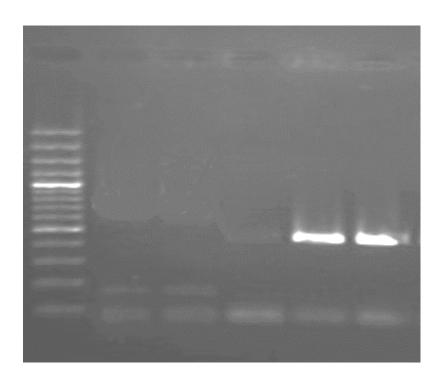
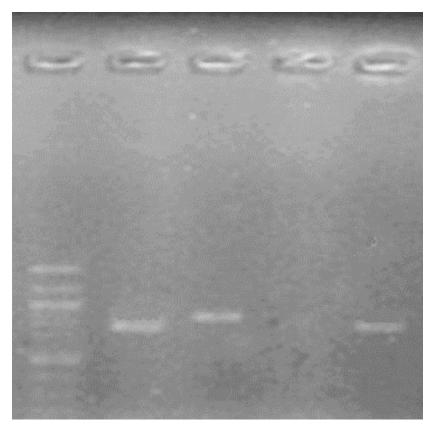


Figure 3. Gel representation of amplified dfrG (405 bp). (L = 100bp ladder; lanes 2-5 = 85, 94a, 97, 100 (S. aureus); Lane 6 = 22a (S. epidermidis))



**Figure 4.** Gel representation of amplified  $bla_{SHV}$  (768 bp) and  $bla_{TEM}$  (857 bp). (L = 100bp ladder; 89b = P. aeruginosa, 80b = Shigella species, 37b = K. pneumoniae, 76b = E. coli)

# DISCUSSION

This study found bacterial contaminants in 91% of samples obtained from public transportation door handles and armrests, which was consistent with the findings of Otter & French (2009), who found bacterial isolates in 95% of samples taken. The increase in bacterial contamination seen in this study could be related to frequent contact between passengers' palm skin and the most frequently touched surfaces of public transit. Despite these surfaces being non-porous and unsuitable for microbial growth, a considerable number of microbes have been found on hand-touch surfaces in buses, trains, mobile phones, door handles, and computer keyboards (Yeh et al., 2011; Choudhary et al., 2016). Several factors contribute to the high number of bacteria on public transport, including frequent skin contact brought about by frequent use, crowdedness, lack of routine bus cleaning, lax public cleanliness standards, and passengers' lack of information. Thus, transportation through exposed surfaces represents a means by which passengers may become infected with the germs besides other routes such as food consumption (Choudhary et al., 2016).

This study found that *S. aureus*, *S. epidermidis*, *E. coli*, *Klebsiella* species, and *P. aeruginosa* were present on the door handles and armrests of public transport. Nwankwo *et al.* (2023) also reported similar results including the presence of *Strep*-

*tococcus faecalis, Enterobacter* species, *Micrococcus* species, and *Bacillus* species which were not isolated in this study. *S. aureus* was the most predominant isolate in this study with a prevalence of 49.2% which was higher than the 8% reported by Otter and French (2009) whereas *Bacillus* species was the most often isolated species (20.5%) reported by Nwankwo *et al.* (2023). Hand-touch sites could get contaminated with staphylococci and may be sources of bacterial transmission between humans, potentially serving as a reservoir for community-associated methicillin-resistant *S. aureus* (CA-MRSA) in high-incidence areas (Otter and French, 2009).

The proportion of Gram-negative isolates (22.2%) found in this study was lower than that of Gram-positives (77.8%), which is consistent with the work of Kahsay *et al.* (2019), who found a prevalence of 18.2% in Gram-positives but differs from a report from Nigeria, which found the highest prevalence (57.5%)of Gram-negative bacteria (Nwankwo *et al.*, 2023). This study also isolated *Shigella* and *Salmonella* species in agreement with the report by Choudhary *et al.* (2016). Kahsay *et al.* (2019) isolated *E. coli* on hand-touch surfaces of public buses, however, they were unable to identify common enteric pathogens such as *Salmonella* and *Shigella* species. The presence of enteric organisms such as *E. coli, Klebsiella* species, *Shigella*, and *Salmonella* species on touch surfaces of public transport in this study is worrisome because these isolates are the most prevalent agents of gastrointestinal illnesses in people. The presence of enteric pathogens on touch surfaces for a significant amount of time indicates fecal-oral transmission and indicates a lack of sufficient hygiene management (Choudhary *et al.*, 2016).

Antibiotic resistance has become a rising concern around the world (Olaniran, Adeleke, Donia, Shahid, & Bokhari, 2021; WHO, 2021). It was observed in this study that 11 (39.3%) and 20 (20.4%) of Gram-negative and Gram-positive isolates, respectively were multidrug resistant. Multidrug resistance was defined as resistance to at least one agent in three or more classes of antibiotics (Magiorakos et al., 2012). The isolates' patterns of resistance to test antibiotics foretell the establishment of serious illnesses once the individuals become infected (Choudhary et al., 2016). This study found that a significant percentage of S. aureus (56.5%) was resistant to cefoxitin, but Kahsay et al. (2019) observed a lower resistance rate of 31.5% to cefoxitin, which necessarily means resistant to methicillin (Fernandes, Fernandes, & Collignon, 2005). Choudhary et al. (2016) also documented methicillin-resistant S. aureus in samples taken from public transport. Co-trimoxazole (trimethoprim and sulfamethoxazole combination) is an effective antibacterial for the treatment of skin and soft tissue infections caused by community-associated methicillin-resistant S. aureus (Montravers & Eckmann, 2021). This study revealed a 28.6% ciprofloxacin resistance rate against E. coli from touch surfaces of public transport, but Kahsay et al. (2019) reported a 37.5% ciprofloxacin resistance rate. Imipenem, meropenem, ciprofloxacin, and amikacin were effective against Gram-negative isolates in this study.

The presence of dfrG gene in S. aureus has been documented (Reeve et al., 2016). The highest frequency of dfrG genes (55%) responsible for trimethoprim resistance in Staphylococcus species was also observed in this study. Nurjadi et al. (2014) demonstrated that the dfrG gene is widespread and the frequently observed genetic source of trimethoprim resistance in MSSA and MRSA from sub-Saharan Africa. The presence of dfrG and dfrA genes was found in 100% and 13.3% MRSA, respectively (Rosato et al., 2020). Moreover, Coelho et al. (2017) reported a higher prevalence of dfrG gene (78%) compared with the dfrA gene (19%) in S. aureus isolates from African countries. Shittu et al. (2011) documented the absence of the dfrA gene in trimethoprim-resistant S. aureus isolates and suggested that mutation of the dihydrofolate reductase is responsible for resistance. In another study, trimethoprim resistance was linked to the presence of dfrG and dfrA genes in S. aureus (Rosato et al., 2020).

The presence of dfr genes in Gram-negative bacteria has been linked with class 1 integrons (Grape, Motakefi, Pavuluri, & Kahlmeter, 2007). Class 1 integrons were discovered to be a major genetic basis of trimethoprim resistance in Gram-negative bacteria, and the presence of dfrA has been attributable to the horizontal transfer of class 1 integrons via conjugative plasmids (Yu, Lee, Kang, Yeong, 2004; Domínguez *et al.*, 2019). The relationship between dfr genes and mobile genetic elements like plasmids and integrons is crucial to understanding why trimethoprim resistance is increasingly evolving, and spreading (Domínguez *et al.*, 2019).

Even though this study found a low incidence of MDR Gramnegative isolates, 75% of them have ESBL genes. ESBL genes have been found in Gram-negative isolates from a variety of sources, including pathological specimens, hospital devices, and surroundings (Atata *et al.*, 2010; Olaniran, Adeleke, Donia, Shahid, & Bokhari, 2021). Furthermore, the proximity of the Isale-Oko motor park to the teaching hospital could contribute to the spread of multidrug-resistant strains, as most patients visiting the hospital will, in some way, get to the garage to board a vehicle. This could lead to the spread of communityacquired infection.

## CONCLUSION

The findings reveal that public tricycles and buses can serve as a reservoir for the propagation of potentially harmful bacteria. The movement of people from the hospital environment to the motor park may be the cause of the higher levels of antibiotic resistance seen in this study when compared to previous studies. The detection of multidrug-resistant strains carrying antibioticresistant genes is worrisome. Therefore, frequent handwashing after exiting public transport and regular disinfection of public vehicles is highly recommended to curtail the spread of infectious pathogens.

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