

Antibiograms of *Staphylococcus aureus* as microbial carriage via Shuttle Door Handles at University of Jos, Nigeria

ABSTRACT

Shuttle door handles as fomites are contributing to the spread of infections, by harboring and retaining pathogens for extended periods. The microbial transmission of infection via shuttle door handles constitutes a major threat to public health especially in the developing countries. This study was carried out to investigate the Antibiogram and microbial carriage on shuttle door handles of University of Jos Teaching Hospital. A total of 50 swab-samples were collected from the handles of university of Jos shuttle buses. Samples were collected by swabbing the handles of the shuttle buses with sterile cotton swab sticks moistened with sterile physiological saline water. The cotton swab-sticks were then immediately transferred to the laboratory within 15 minutes of collection for microbial analysis. Enumeration of the bacterial counts were carried out using pour plating technique while the bacteria isolates were identified using cultural, morphological and biochemical characteristics. The antibiotics pattern of the bacteria indicated that all the bacteria isolated were sensitive to Cpx(93.3), Au(100), Gn(100), Pet(100), Lev(100), Ofx(76.9), Spx(33.9) Apx(8.3) while some are resistant to Sep(100), Apx(91.7), Spx(66.7), Ofx(31.3), and Cpx(6.7). Findings concluded that the campus shuttles door handles used in public transportation could also be serving as a means of transmission of both pathogenic and non-pathogenic microorganisms which pose public health risks. Personal hygiene and sanitation such as hand washing and the use of antimicrobial wipe to clean the hands could serve as a means of reducing the incidence of microbial transmission.

KEY WORDS: Antibiograms, Microbial carriage and door handles, *Staphylococcus aureus*, hand hygiene

1.0 INTRODUCTION

“Microorganisms are ubiquitous organisms that can cause microbial contamination in both indoor and outdoor settings, with frequently touched surfaces acting as fomites that increase the ability of pathogens to be transferred from host to host” (Osuntokun *et al.*, 2024). “Fomites are inanimate porous and non-porous surfaces that can become contaminated with microorganisms and serve as reservoirs of microbial pathogens and vectors for cross-transmission in the domestic environment and in community settings” (Stephens *at al.*, 2019). “The spread of infectious disease through hand contact has been an area of major public health concern because of the frequent contact of the hand with fomites which are potential carriers of pathogenic organisms. This may lead to an alarming rate of outbreaks of infections transmitted by the fomites. Worldwide annually there are 1.7 million deaths from diarrhoeal diseases and 1.5 million deaths from respiratory infections” (Pruss-Ustun and Covahan, 2006). “These are examples of diseases which could be contracted by humans via fomites.

Studies have shown that hard, non-porous surfaces such as shuttle door handles have the highest microbial transfer rates to hands. In recent past, a lot of effort has been invested in emphasized

hand hygiene through hand wipes and hand sanitizers. Even though people are commonly aware of such practices, the possibility of inaccessibility or lack of use of these practices do exist” (Adirimo et al., 2023; Abdelgader et al., 2024). “Up to 60% Of adults do not wash their hands when appropriate. People believe that microbes are only present in research laboratories, hospitals or clinics and thus they have a misleading feeling of security in other places or while touching other surfaces such as shuttle door handles”. (Vijayalaxmi *et al.*, 2021). “Lack of knowledge of the roles of micro-habitat such as shuttle door handles in dissemination of microorganisms is a threat to public health. In fact 80% of infections are spread through hand contact with hands or other objects” (Reynold and Hurst, 2010). “Worldwide, infections with drug-resistant pathogens significantly affect not only the public health but also the economic stability of societies. At least 25% of the 60 million year-based deaths in the world is due to microbial diseases. Despite significant advances in infection control practices, clinical infections with drug-resistant pathogens remain significant causes of morbidity and mortality among hospitalized patients and in the community settings, affecting developed countries, middle-income countries and sub-Saharan Africa” (Stone *et al.*, 2017).

“The discovery and development of antimicrobial agents has significantly changed the negative narrative of how infectious diseases has plagued the human race over history” (O'Hara *et al.*, 2013). “However, due to slow pace at which these agents are developed and the emergence of resistance to antimicrobials, treating common pathogens has become a challenge. Different types of resistance have developed over time resulting in their classification as multidrug resistance (MDR), extended resistance (XDR) and Pandrug resistance (PDR)” (CDC, 2019). “The Gram-positive *Staphylococcus aureus*, and Gram-negative bacteria such as *Escherichia coli*, *Klebsiella* species, *Pseudomonas* species, are common causes of a wide variety of infections involving diverse anatomic sites in both healthy and compromised hosts” (Itah and Ben, 2004). “In general, among adults, the incidence of infection due to these agents increases with age. Thus, as the mean age of the population increases, so will the number of these infections. Drug resistance is a serious medical problem. Progressive increase in resistance to commonly used antibiotics with many gram-negative bacilli being multidrug-resistant has been noticed. The emergence of antibiotic resistance in the management of infections is a serious public health issue, particularly in the developing world where apart from high level of poverty and ignorance, there is also high prevalence of fake and spurious drugs of questionable quality in circulation. This has led to a significant increase in morbidity” (Vijayalaxmi *et al.*, 2021). “The World Health Organization (WHO) emphasizes the key role of the microbiology laboratory in antimicrobial stewardship (AMS) by informing the appropriate use of antibiotics through development of Antibigrams” (Pakyz, 2007).

“Antibiograms assist clinicians in making initial antibiotic treatment selections for a patient before their individual susceptibility results is available” (CDC, 2019; Francois *et al.*, 2010). “Routine antibiogram techniques are based on a phenotypic study in which microbial growth is observed in the presence of different antibiotics. These techniques include agar dilution (the gold standard for the antibiogram), broth microdilution and microdilution, and strips with an antibiotic gradient. They yield results in around 17hrs. To evaluate reliability, according to the US Food and Drug Administration (FDA), the results of a rapid antibiogram are classified, compared to the antibiogram obtained through the gold standard, as agreements (concordance), minor errors (erroneous intermediate sensitivity result), major errors (false resistance) and very major errors

(false sensitivity)” (FDA, 2011). “Comprehensive data on the antibiogram of microbial pathogens isolated from different body sites of infections is needed for surveillance systems which aid in monitoring antimicrobial use and resistance thus improving decision making and assessing the effect of interventions at the local, national and international level. There is scarcity of such data in developing countries including Nigeria” (Gelband *et al.*, 2015). “Networking between laboratories may increase infection surveillance within a huge geographical region. The WHO supports the establishment of national networks for the regular exchange of information and proper support for the laboratory. However, only a few countries have local, national and international laboratory networks. The establishment of an integrated laboratory system is very important to combat many infectious diseases. In Low- Middle- Income- Countries (LMICs), there are few or no documented data on the microbial isolates and antibiogram profiles in healthcare facilities even at local level and integrated systems are financially neglected in developing countries”. (Masanza *et al.*, 2014).

“Microbial transmission via several surfaces such as automated teller machines, mobile phones, door handles” (Ikede *et al.*, 2022) and currency (Iyevhobu *et al.*, 2023) has been extensively studied but the role of shuttle door handles in the dissemination of pathogenic microorganisms have not been properly investigated, hence this research to determine the role of shuttle door handle in the transmission of pathogenic microorganism to human via hand contact and the antibiotics susceptibility pattern of the potential isolates. The objectives of the study include to isolate and identify microorganisms from shuttle door handles of the University of Jos campus and to identify the antibiogram pattern of the isolated organisms.

2.0 MATERIALS AND METHODS

Study Area: This study was carried out on shuttle door handles of the University of Jos campus, Jos North area of Plateau state, Nigeria between June to August 2024. The temperature in Jos, Nigeria from June to August is around 81°F (27°C).

Sample Collection: A total of 50 swab-samples were collected from the handles of university of Jos shuttle buses. Samples were collected by swabbing the handles of the shuttle buses with sterile cotton swab sticks moistened with sterile physiological saline water. The cotton swab-sticks were then immediately transferred to the laboratory within 15 minutes of collection for microbial analysis.

Procedures for Culturing: With the aid of a sterile cool wireloop, the sample was picked and inoculated into a culture media (Nutrient agar, MacConkey agar and Blood agar), the inoculum was smeared thoroughly over area (A- D). The wire loop was sterilized and then drawn from the three (3) parallel lines and was incubated for 18 - 24hrs.

Procedures for Gram's Staining Technique: A loop full of the overnight culture was place on a glass slide and a drop of normal saline was placed and the suspension were emulsified with the aid of an applicator stick, and passed through a burnsen burner flame three (3) times before leaving to air dry on the staining rack. The smear was flooded with crystal violet (primary stain) for 60 seconds and was rinsed with water. It was then flooded with the Lugol's iodine (Mordant) for 60 seconds and was rinsed in water. Then the smear was flooded with acetone (decolorizer)

briefly and raised in water. It was then flooded with Safranin (counter stain) briefly, rinsed in water and allowed to air dry. They were examined microscopically using x100 objective lens.

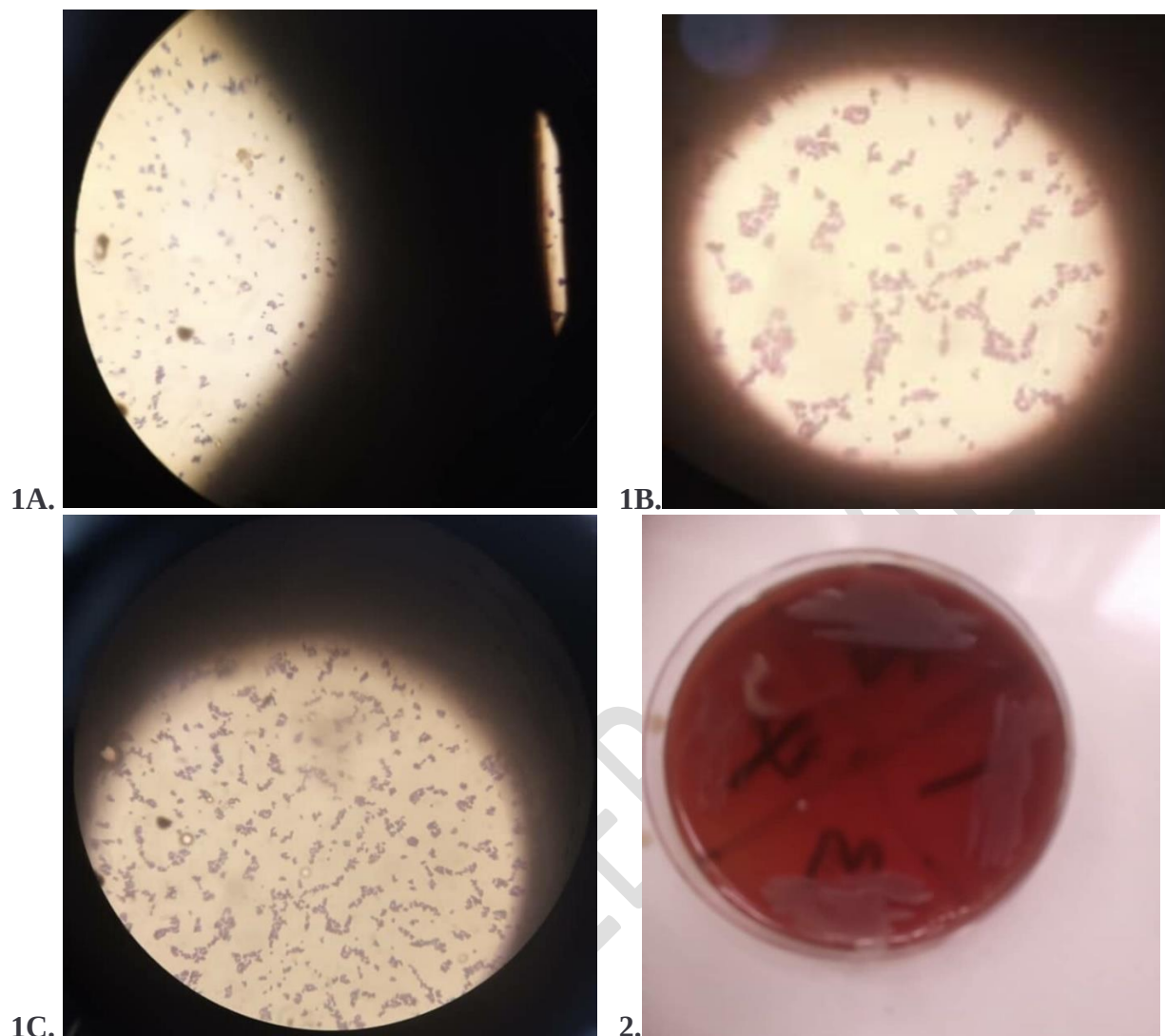
Antibiotic Susceptibility Test

Nutrient agar was prepared from a commercially available dehydrated base according to the manufacturer's instructions. Immediately after autoclaving it was allowed to cool in a water bath, before being poured into plastic flat bottomed Petri dishes on a level horizontal surface. The agar medium was then allowed to cool to room temperature and stored in a refrigerator. At least three to five well isolated colonies of the same morphological type were selected from an agar plate culture. The growth was then transferred into a tube containing 4-5 ml of nutrient broth. The nutrient broth was then incubated until it achieves or exceeds the turbidity of the McFarland standard solution.

Inoculation of Test Plates: Swab stick was dipped into the inoculum suspension and then streaked on the dried surface of the nutrient agar plate. The surface was streaked two more times rotating the plate approximately 60° each time to ensure an even distribution of the inoculum.

Drug impregnated disks were then placed on the inoculated nutrient agar plates after being exposed for about 3 minutes. Each disc was pressed down to ensure complete contact with the agar surface. The plates were inverted and placed in an incubator set to 35°C (Iyevhobu *et al.*, 2024).

Modified Kirby-Bauer disk diffusion method (Cheesbrough, 2006) was used to test the susceptibility of the bacteria isolates to different antimicrobial agents (obtained from BDH London, UK): ampiclox (10 µg), pefloxacin (30 µg), ciprofloxacin (30 µg), augmentin (30 µg), sparfloxacin (30 µg), ofloxacin (30 µg), gentamicin (10 µg), and septrin (10 µg). The inocula were prepared by growing the various organisms on separate agar plates and colonies from the plate were transferred with inoculating loop into 3 ml of normal saline in a test tube. The density of these suspensions was adjusted to 0.5 McFarland standards. The surface of Nutrient agar (Oxoid Cambridge, UK) plate was evenly inoculated with the organisms using a sterile swab. The swab was dipped into the suspension and pressed against the side of the test tube to remove excess fluid. The wet swab was then used to inoculate the Nutrient agar by evenly streaking across the surface. By means of Disc Dispenser (Oxoid Cambridge, UK), the antibiotic discs were applied to the surface of the inoculated agar and the plates were incubated overnight at 37 °C. The diameter of zone of growth-inhibition observed was measured and compared to the chart provided by National Committee for Clinical Laboratory Standards (NCCLS).



Plates 1(A-C) Gram positive cocci, plate 2 growth on a blood agar plate

4.0 RESULTS

Table 1 shows the Antibiotics Sensitivity pattern of *Staphylococcus* specie isolated from the cultured samples. 15 *Staphylococcus* specie isolate from the cultured samples gotten from university of Jos campus shuttle door handles with antibiotic sensitivity and antibiotic resistance. 35 samples cultured showed no growth. When the drugs were tested against the isolated *Staphylococcus aureus*, Ciprofloxacin showed 93.3% sensitivity and 6.7% resistance, Augmentin, Petfloxacin, Gentamicin and Levofloxacin showed 100% sensitivity and no resistance, Ofloxacin showed 76.9% sensitivity and 23.1% resistance, Ampiclox showed 8.3% sensitivity and 91.7% resistance, Sperfloxacin showed 33.3% sensitivity and 66.7% resistance while Seprtin showed no sensitivity and 100% resistance.

Table 1: Antibiotics Sensitivity pattern of *Staphylococcus* specie isolated from the cultured samples

S/N	Organisms isolated	Antibiotics Sensitivity/Resistance								
		Cpx (30 µg)	Au (30 µg)	Pet (30 µg)	Ofx (30 µg)	Apx (10 µg)	Spx (30 µg)	Sep (10 µg)	Gn (10 µg)	Lev (10 µg)
001	Staphylococcus specie	+	+	+	+	-	-	-	NIL	NIL

002	Staphylococcus specie	+	+	-	+	-	NIL	NIL	+	NIL
003	Staphylococcus specie	+	+	-	+	NIL	-	-	+	NIL
004	Staphylococcus specie	+	+	+	-	NIL	-	-	+	NIL
005	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
006	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
007	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
008	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
009	Staphylococcus specie	+	+	NIL	NIL	NIL	NIL	NIL	+	+
010	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
011	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
012	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
013	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
014	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
015	Staphylococcus specie	+	+	NIL	NIL	-	-	-	+	NIL
016	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
017	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
018	Staphylococcus specie	+	+	NIL	-	-	+	-	+	+
019	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
020	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
021	Staphylococcus specie	+	NIL	-	+	-	+	-	+	+
022	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
023	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
024	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
025	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
026	Staphylococcus specie	+	+	-	+	-	NIL	-	+	+
027	Staphylococcus specie	+	+	-	+	-	NIL	-	+	+
028	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
029	Staphylococcus specie	+	+	-	+	-	NIL	-	+	+
030	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
031	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
032	Staphylococcus specie	+	+	-	-	+	NIL	-	+	+
033	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
034	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
035	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
036	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
037	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
038	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
039	Staphylococcus specie	+	+	-	+	-	NIL	-	+	+
040	Staphylococcus specie	+	+	-	+	-	NIL	-	+	+
041	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
042	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
043	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
044	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
045	Staphylococcus specie	-	+	NIL	+	-	NIL	-	+	+
046	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
047	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
048	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
049	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
050	No growth	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL
No (%) of Sensitivity		14 (93.3)	14 (100)	9 (100)	10 (76.9)	1 (8.3)	2 (33.3)	0 (0)	14 (100)	10 (100)
No (%) of Resistance		1 (6.7)	0 (0)	0 (0)	3 (23.1)	11 (91.7)	4 (66.7)	13 (100)	0 (0)	0 (0)

KEY: + = Sensitive; - = Resistance

Apx – ampicolox; Pet – petfloxacin; Cpx – ciprofloxacin; Au – augmentin;
 Spx – sperfloxacin; Ofx – ofloxacin; Gn – gentamicin; Sep – septrin; Lev - levofloxacin

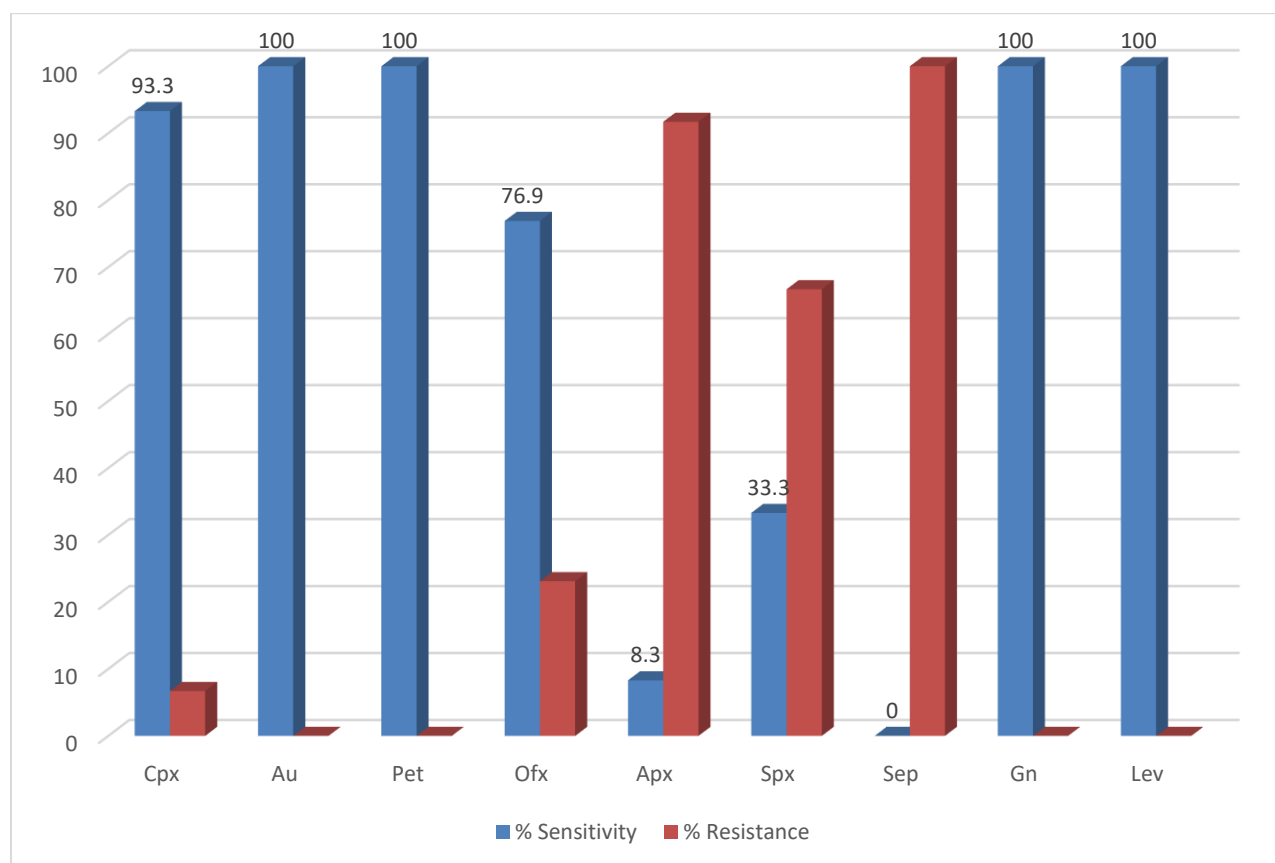


Figure 1: Sensitivity and Resistant pattern of isolated *Staphylococcus aureus*

Key:

Apx – ampicolox; Pet – petfloxacin; Cpx – ciprofloxacin; Au – augmentin;
 Spx – sperfloxacin; Ofx – ofloxacin; Gn – gentamicin; Sep – septrin; Lev - levofloxacin

Table 2: Results on the morphological and biochemical tests from the isolate of the studied.

Colony morphology	Cell character	Gram staining	Catalase test	Coagulase test	Oxidase test	Probable identity
Yellow, small and irregular	Cocci	+	+	+	-	<i>Staphylococcus aureus</i>

5.1 DISCUSSION

“Over the years, different researches have been conducted to examine the role of various surfaces, such as tables, computer key boards, door handles” (Ikede *et al.*, 2022) and mobile phones on the carriage and dissemination of pathogenic infection, but the role of shuttle door handles as a route of microbial transmission has not been reported. Hence the need to assess the carriage and transmission of microorganism by shuttle door handles. The knowledge of this is

expected to broaden our understanding of the microbial carriage of the shuttle door handles and their antibiotics pattern. This will help to implement public health preventive and control measure to forestall future outbreak of infection that may result from these pathogenic microorganisms associated with the shuttle door handles.

“In the past 60 years, antibiotics have been critical in achieving a dramatic rise in life expectancy and significant improvements in public health. However, the viability of Gram positive and Gram-negative organism under various environment conditions have been described” (Mazel and Monastery, 2012; Noskin *et al.*, 2020). “Disease-causing microbes have become increasingly resistant to the antibiotics commonly in use. It has been clearly shown that the use of antimicrobials leads to selection of resistant strains both in the individual and in the community, and overuse or inappropriate use only increases this risk. History suggests that microbes will never run out of ways of developing resistance, but we may run out of effective antimicrobials” (Reynold and Hurst, 2010; Ikede *et al.*, 2022).

This study found 15 *Staphylococcus* specie isolated from the cultured samples collected from university of Jos campus shuttle door handles with antibiotic sensitivity and antibiotic resistance. The *Staphylococcus* specie were sensitive to ciprofloxacin, Augmentin, petfloxacin, ofloxacin, Ampiclox, levofloxacin and were resistant to Ampiclox, septrin, sperfloxacin, ofloxacin, petfloxacin, ciprofloxacin. *Staphylococcus* specie demonstrated the highest resistance to Ampiclox, septrin, sperfloxacin, ofloxacin and ciprofloxacin. A research study carried out by Ansari *et al.*, 2014 shows a similar antibiotic resistance. “The viability of Gram positive and Gram-negative organism under various environment conditions have been described” (Reynold and Hurst, 2010). “Studies also found Gram positive *Staphylococcus aureus*, and Gram-negative bacteria such as *Escherichia coli*, *Klebsiella* species, *Pseudomonas* species contaminated various contact surfaces including chairs, tables, windows, shuttle door handles and many other common household fixtures. The presence of these pathogenic bacteria on environmental surfaces poses a potential risk” (Pruss-Ustun and Covahan, 2006; Masanza *et al.*, 2014; Ikede *et al.*, 2022). Ikede *et al.*, (2022) isolated *Staphylococcus aureus*, *Bacillus* spp., *Escherichia coli* and *Klebsiella* spp., in the analyzed door handle samples in their study which is in agreement with this study.

5.2 CONCLUSION

Antimicrobial resistance is one of the major global threats in the spectrum of infectious diseases. Worldwide, studies has revealed the declining of the effectiveness of antibiotics in the stock and the rising of bacterial resistance to all first-line and last-resort antibiotics. Thus, the impact of antibiotic resistance is clinical, economical and societal. Most of the staphylococcus specie isolated in this study were resistant to ampiclox, septrin, sperfloxacin, augmentin, ofloxacin, ciprofloxacin. At the same time, the rate of multiple drug-resistant isolates are alarmingly high. Therefore, it is recommended to have strict antibiotics utilization policies within standard laboratories or hospitals to support clinicians on rational choice of antibiotics therapy and regularly update the list and reliable sources of drugs.

Disclaimer (Artificial intelligence)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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