Antibiogram of Fungal and Bacterial Isolates Associated with Toilet Door Handles in The Students' Residential Hall at a Nigerian University

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Abstract

In this study, the prevalence of bacterial and fungi species on toilet door handles in male and female hostels and their antibiotic susceptibility patterns were assessed. A total of 48 swab samples were collected, and 21 isolates were identified through cultural, morphological, and biochemical and comparison with standard organisms. Fifty seven percent isolates (57%) were found on female toilet door handles and 43% on the male hostels. The identified isolates included *Citrobacter freudii, Enterobacter* spp*, Bacillus subtilis, Klebsiella pneumoniae, Bacillus megaterium, Staphylococcus aureus, Pseudomonas aeruginosa,* and *Candida* spp. Antibiotic susceptibility patterns revealed that all isolates were resistant to cefuroxime, cefotaxime, and vancomycin (100% resistance). However, the organisms exhibited high susceptibility to Amikacin (100%), Ciprofloxacin (89%), and Cephalexin (86%).

Article Highlights

- The isolation and characterization of the microorganisms on the door handles of the restrooms of both male and female residential halls at Covenant University.
- The Evaluation of the antimicrobial resistance patterns of the bacterial isolates on selected antibiotics.
- Proper Good Hygiene needs to be practiced often.

Keywords: Toilet, Door handle, Antibiogram, Residential halls, Microorganisms, Bacteria.

Introduction

Microorganisms, especially bacteria are ubiquitous and can be found in the human body and around the environment. While most bacteria are harmless and do not affect humans or animals, a few species can pose a threat and may cause fatal infections (Abiose, 2019). Notably, the transfer rate of bacteria from strong, non-porous surfaces such as door handles to hands is very high (Tiku *et al*., 2019). One's hygiene standards must be raised to prevent the spread of pathogens from fomites such as toilet door handles to humans (Fakhoury and Nawas, 2019).

Some notable organisms which have been isolated from fomites include *Staphylococcus aureus Staphylococcus epidermidis, Bacillus subtilis, Enterococcus species, Citrobacter freundii, Esherichia coli, Klebsiella oxytoca, Pseudomonas* spp. These organisms which are gram-positive and gram- negative bacteria species have been known to cause various ailments.

For instance, boils, impetigo or cellulitis, are commonly caused by *S. aureus* in humans (Kourtis *et al*., 2019). However, more severe infections such as bacteremia, endocarditis and pneumonia may also occur (Kourtis *et al*., 2019). *Staphylococcus aureus* causes nosocomial infections, e.g., those associated with surgery sites and bloodstream infections, and hospital-related cases of pneumonia (Troeman *et al*., 2019). Nausea, vomiting, diarrhea, and stomach cramps are common symptoms of *S. aureus* food poisoning (Kock *et al*., 2019).

S. epidermidis is capable of causing infection when entering the body via a prosthesis; once in the body, many bacteria can travel along the prosthetic device and enter the bloodstream (Lee and Anjum, 2022).

Bacillus subtilis is found frequently on high-contact surfaces such as toilet handles in public places (Faparusi, 2020). These infections can result in various clinical conditions such as infective endocarditis (IE), urinary tract infection (UTI), bacteremia, peritonitis, prosthetic joint infection (PJI), and endophthalmitis, which can be life-threatening if not treated promptly (Goh *et al*., 2017).

Escherichia coli is made up of harmless strains common in the body; pathogenic variants also exist which may cause different diseases in humans. In addition, *Klebsiella* is widely distributed and is commonly found as a commensal organism in the human nasopharynx and intestinal tract. They can transfer resistance genes through plasmids, allowing them to exchange genetic material with other bacteria (Neog *et al*., 2021). Infections caused by *Klebsiella pneumoniae* are commonly associated with hospital-acquired infections and are considered opportunistic pathogens, often causing infections in hospitalized or immunocompromised patients (Martin *et al*., 2018).

P. aeruginosa infections can be severe, especially among patients with underlying medical conditions (Lupo *et al*., 2018). Individuals with weakened immune systems, such as the immunosuppressed or those using mechanical ventilation, are at heightened risk of contracting infections from this bacterium (Lupo *et al*., 2018).

Antimicrobial resistance in bacteria is a worldwide concern and is linked to substantial illness and death (Frieri *et al*., 2017). In numerous healthcare facilities, there is a deficiency in the prompt identification of pathogens and their susceptibility to antimicrobials, resulting in the overuse of broad-spectrum antibiotics that may not be necessary (Frieri *et al*., 2017). Improper utilization of antibiotics, combined with inadequate infection control measures, facilitates the rapid spread of resistant bacteria among patients and the surrounding environment (Frieri *et al*., 2017). Considering the limited progress in developing new antibiotics, there is an urgent requirement to investigate innovative treatment approaches and alternative antimicrobial therapies (Frieri *et al*., 2017). In this study, we assessed the predominant microorganisms associated with door handles in a tertiary institution and evaluated the antimicrobial resistance patterns.

MATERIALS AND METHODS

Reagents, Media, and Chemicals

All the reagents and chemicals used in this study are of analytical grade.

The media used are Mueller Hilton agar, MacConkey agar, Nutrient agar, Eosin methylene blue agar, Cetrimide agar, *Salmonella shigella* agar, and Mannitol salt agar (HiMedia, India).

Antimicrobial Agents Used

The antimicrobial agents used include Cefotaxime 30µg (CTX), Ceftazidime 30 µg (CPZ), Tetracycline 10 µg (TET), Cotrimoxazole 25 µg (COT), Gentamycin 10 µg (GEN), Cefuroxime 30 µg (CRX), Chloramphenicol 10 µg (CHL),

Ceftriaxone 30 µg (CTR), Ciprofloxacin 5 µg (CIP), Meropenem 10 µg (MEM), Vancomycin 30 µg (VAN), Amikacin 30 µg (AMK), Ampicillin 10 µg (AMP), Erythromycin 5 µg (ERY), Tetracycline 30 µg (TET), Cefuroxime 10 µg (CRX), Augmentin 30 µg (AUG), Ceftazidime 10 µg (CPZ), Cephalexin 1.5 µg (CP) (Biomark Laboratories, India)

Sample Collection

Sterile swab sticks dampened with sterile distilled water were used to obtain swab samples from the toilet door handles in Male and Female hostels at the University. The samples collected were promptly transported to the microbiology laboratory of the Department of Biological Sciences, Covenant University, for immediate analysis. Identification of the microorganisms from the samples was carried out using methods such as colony morphology, gram staining, biochemical tests, and antibiotic susceptibility testing.

Sample Culture

Swab samples were inoculated on Mannitol salt agar, MacConkey agar, and Cetrimide agar and incubated for 24 hours at 37ºC. After incubation, several colonies were spotted on the plates. These colonies were sub-cultured on nutrient agar plates until pure cultures were obtained thereafter-, and transferred to agar slants for preservation at 4ºC.

Identification of Bacterial Isolates

Pure bacterial isolates were identified using Cowan and Steel's method of bacteria identification (2003). The methods used include

Macroscopic colonial characteristics: Each bacterium's colonial appearance including size, shape, consistency, color, and elevation, as well as its distinguishing characteristics like lactose fermentation on MacConkey agar and Gram staining, were performed to aid in the further identification of the isolates.

Microscopic examination: Gram staining was performed and the color of the bacteria was identified and their shape. For this, a loopful of the sample of the organism was applied to a glass slide. Two to three drops of crystal violet which is the primary stain was added and left for 1 minute. The slide was then rinsed, and two to three drops of iodine were applied as a mordant for 1 minute. After draining the iodine, one to two drops of alcohol were used as a decolourizer for 15 seconds. The slide was washed with water and two to three drops of safranin was applied as a secondary stain for 1 minute. The slide was dried and examined under an oil immersion lens at a magnification of 100X. Bacteria that appear purple indicated a gram+ve bacterium, while those that appear pink indicated a gram-ve bacterium. This test was done following the methods described by Bartholomew and Mittwer (1952).

BIOCHEMICAL IDENTIFICATION

Further characterization of the isolates was performed through the following biochemical tests, according to the procedures outlined by Cowan and Steel (1993). The tests include catalase , sugar test, test, citrate utilization test, hydrogen sulphide, Indole, Methyl red, Urease test, Oxidase tests and Vogues Proskauer tests and Motility tests.

FUNGI IDENTIFICATION

Macroscopic Examination and sugar fermentation tests were used for the identification of fungi: Gram staining was performed and the shape of the yeast was identified, thereafter a series of sugar fermentation tests were used to determine the sugar reactions of the fungi species. The sugar used includes glucose broth, lactose broth, sucrose broth, and maltose broth. Peptone dextrose broth was prepared and then the medium was kept sterile to prevent contamination. The growth medium was then inoculated with a small amount of yeast culture using a sterile inoculation loop. The inoculated

culture was paced in the incubator for 24 hours. The tubes were then incubated at a temperature of 37℃ for 48 hours, allowing enough time for fermentation to occur. After the incubation period, the tubes were carefully observed for the presence of gas production (such as bubbles or displacement of the media), alterations in color, or the formation of precipitates.

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility tests for the various isolates, including *Staphylococcus aureus, Bacillus spp, Enterococcus spp, Pseudomonas spp,* and Enteric bacteria such as *Escherichia coli, Enterobacter spp, Klebsiella spp, and Proteus spp,* were tested using the Kirby Bauer disk diffusion technique. The isolates were exposed to a range of antibiotics. Sterile Normal saline and Mueller-Hilton agar were prepared according to protocols. The Mueller-Hinton agar was allowed to cool to room temperature. Using a sterile inoculating loop, three to four distinct colonies of the bacterial isolate were picked and suspended in 5ml of sterile saline, creating a homogenous suspension. The turbidity of the suspension was adjusted to 0.5 MacFarland standard before inoculating on the Mueller-Hinton agar. Antibiotic discs were picked up with sterile forceps and firmly placed on the agar, ensuring enough space between each disc to observe the zone of inhibition for each one. After 24 hours of incubation, the plates were examined, and the size of the zone of inhibition was measured. The interpretation of the zones of inhibition followed the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2021).

RESULTS

A total of 48 samples were collected from the toilet door handles of both the male and female residential hall toilets.

Biochemical tests conducted indicated a total of 19 bacterial and 2 fungal isolates of which 8 were Gram-positive organisms,11 were Gram-negative organisms and 2 were yeasts. These include *Citrobacter freudii* 1(4.8%), *Enterobacter spp* 1(4.8%), *Escherichia coli* 2(9.5%), *Klebsiella oxytoca* 1(4.8%), *Enterococcus spp* 1(9.5%), *Bacillus subtilis* 1(4.8%), *Klebsiella pneumoniae* 1(4.8%), *Bacillus megaterium* 1(4.8%), *Staphylococcus aureus* 2(9.5%), *Pseudomonas fluorescens* 2(9.5%), *Proteus mirabilis* 1(4.8%), *Micrococcus luteus* 1(4.8%), *Staphylococcus saprophyticus* 2(9.5%), *Candida* spp 2(9.5%) and *Pseudomonas aeruginosa* 2(9.5%) (Table 4.2, Table 4.5, Table 4.6, Table 4.7).

From the results *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Escherichia coli*, *Candida* spp, *Pseudomonas aeruginosa,* and *Pseudomonas fluorescens* were more prevalent in both female and male toilet door handles (Figure 4.1). According to the antibiotic susceptibility testing (AST), all isolates showed no zone of inhibition to Cefuroxime, Cefotaxime, and Vancomycin (100%) and were highly susceptible to amikacin (100%), ciprofloxacin (89%), and cephalexin (88%).

The Table 4.1 shows the organism isolated from the toilet door handles of both the male and female residential halls.

Table 1: Organisms isolated from the toilet door handles of both male and female hostels

Location	Isolates
FB1	*Citrobacter freudii*
FB2	*Staphylococcus saprophyticus*
FB3	*Escherichia coli*
FB4	*Klebsiella pneumoniae*
FB5	*Pseudomonas fluorescens*
FB6	*Enterococcus spp*
FB7	*Candida spp*
FB8	*Klebsiella oxytoca*
FB9	*Bacillus megaterium*
FB10	*Pseudomonas aeruginosa*
FB11	*Staphylococcus aureus*
FB12	*Enterobacter* spp
MB1	*Pseudomonas aeruginosa*
MB2	*Escherichia coli*
MB3	*Staphylococcus aureus*
MB4	*Bacillus subtilis*
MB5	*Proteus mirabilis*
MB6	*Micrococcus luteus*
MB7	*Pseudomonas fluorescens*
MB8	*Candida spp*
MB9	*Staphylococcus saprophyticus*

Keys: FB stands for female restroom while MB stands for male restroom

The Table 2 shows the biochemical characteristics results of bacterial isolates found on the toilet door handles of female residential halls.

Table 2: Biochemical characteristics of bacterial isolates from toilet door handles of female halls

ISOLATE	Gram Reaction	Motility	Glucose	Lactose	Mannitol	Maltose	Indole	Methyl Red	Voges Proskauer	Citrate	H_2S	Sucrose	Urea	Oxidase	Coagulase	Catalase
Citrobacter freudii	GNB	+	+	+	+	+	-	+	-	+	+	-	-	-	-	+
Staphylococcus aureus	GPC	NA	+	+	+	+	NA	-	+	NA	NA	+	-	-	+	+
Enterobacter spp	GNB	+	+	+	+	+	-	-	-	+	-	+	-	-	-	+
Escherichia coli	GNB	+	+	+	+	+	+	+	-	-	-	NA	-	-	NA	+
Staphylococcus saprophyticus	GPC	NA	+	+	+	+	NA	-	+	NA	NA	+	-	-	-	+
Pseudomonas fluorescens	GNB	+	+	-	+	+	-	+	+	+	+	+	+	+	NA	+
Klebsiella oxytoca	GNB	-	+	+	+	+	-	-	-	+	-	+	+	-	-	+
Klebsiella pneumonia	GNB	-	+	+	+	+	-	+	-	+	-	+	+	-	-	+
Enterococcus specie*	GPC	-	+	+	+	-	NA	+	+	+	-	-	+	-	-	-
Bacillus megaterium	GPB	+	+	+	+	+	-	-	-	-	-	+	-	-	NA	+
Pseudomonas aeruginosa	GNB	+	+	-	+	-	-	+	-	+	-	+	+	+	NA	+

Keys For Sugar Fermentation Test:
**Not Specific*
+ = Positive
- = Negative
NA= Not Applicable
GNB= Gram Negative Bacilli
GPC= Gram Positive Cocci

The Table 3 shows the biochemical characteristics results of bacterial isolates found on the toilet door handles of male residential halls.

Table 3: Biochemical characteristics of bacterial isolates from toilet door handles of male halls

ISOLATE	Gram Reaction	Motility	Glucose	Lactose	Mannitol	Maltose	Indole	Methyl Red	Voges Proskauer	Citrate	H_2S	Sucrose	Urea	Oxidase	Coagulase	Catalase
Micrococcus luteus	GPC	NA	+	+	+	+	NA	-	+	NA	NA	+	-	+	-	+
Staphylococcus aureus	GPC	NA	+	+	+	+	NA	-	+	NA	NA	+	-	-	+	+
Escherichia coli	GNB	+	+	+	+	+	+	+	-	-	-	NA	-	-	NA	+
Staphylococcus saprophyticus	GPC	NA	+	+	+	+	NA	-	+	NA	NA	+	-	-	-	+
Bacillus subtilis	GPB	+	+	+	+	+	NA	-	+	NA	NA	+	-	-	NA	+
Proteus mirabilis	GNB	+	+	-	-	-	-	+	-	+	-	+	+	-	NA	+
Pseudomonas fluorescens	GNB	+	+	-	+	+	-	+	+	+	+	+	+	+	NA	+
Pseudomonas aeruginosa	GNB	+	+	-	+	-	-	+	-	+	-	+	+	+	NA	+

Keys For Sugar Fermentation Test:
+ = Positive
- = Negative
NA= Not Applicable
GNB= Gram Negative Bacilli
GPC= Gram Positive Cocci

The Table 4 shows the sugar fermentation test results of yeast organisms found on the toilet door handles of both male and female residential halls.

Table 4: Sugar fermentation test of yeast organisms from toilet door handles of student hostels

ISOLATES	LOCATION	GALACTOSE		FRUCTOSE		SUCROSE		LACTOSE		GLUCOSE	
		Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas	Acid	Gas
Candida spp	Female Restroom	+	+	+	+	+	+	-	-	+	+
Candida spp	Male Restroom	+	+	+	+	+	+	-	-	+	+

Keys For Sugar Fermentation Test of Yeast organisms:
+ = positive
- = Negative
Spp = species

The Figure 1 shows the occurrence of pathogenic microorganisms isolated from the toilet door handles of both male and female residential halls.

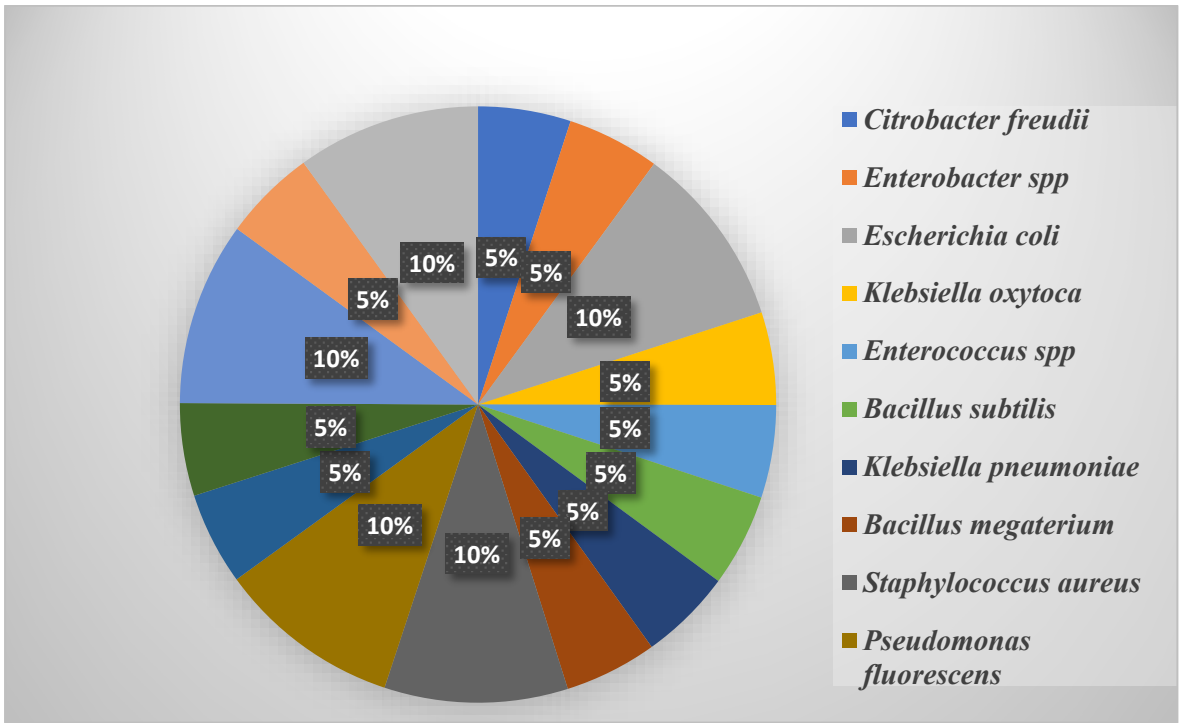

Figure 1: Occurrence of microorganisms isolated from the toilet door handles of student hostels

The Figure 2 shows the antibiotic susceptibility pattern of bacterial isolates from toilet door handles of both male and female student residential halls

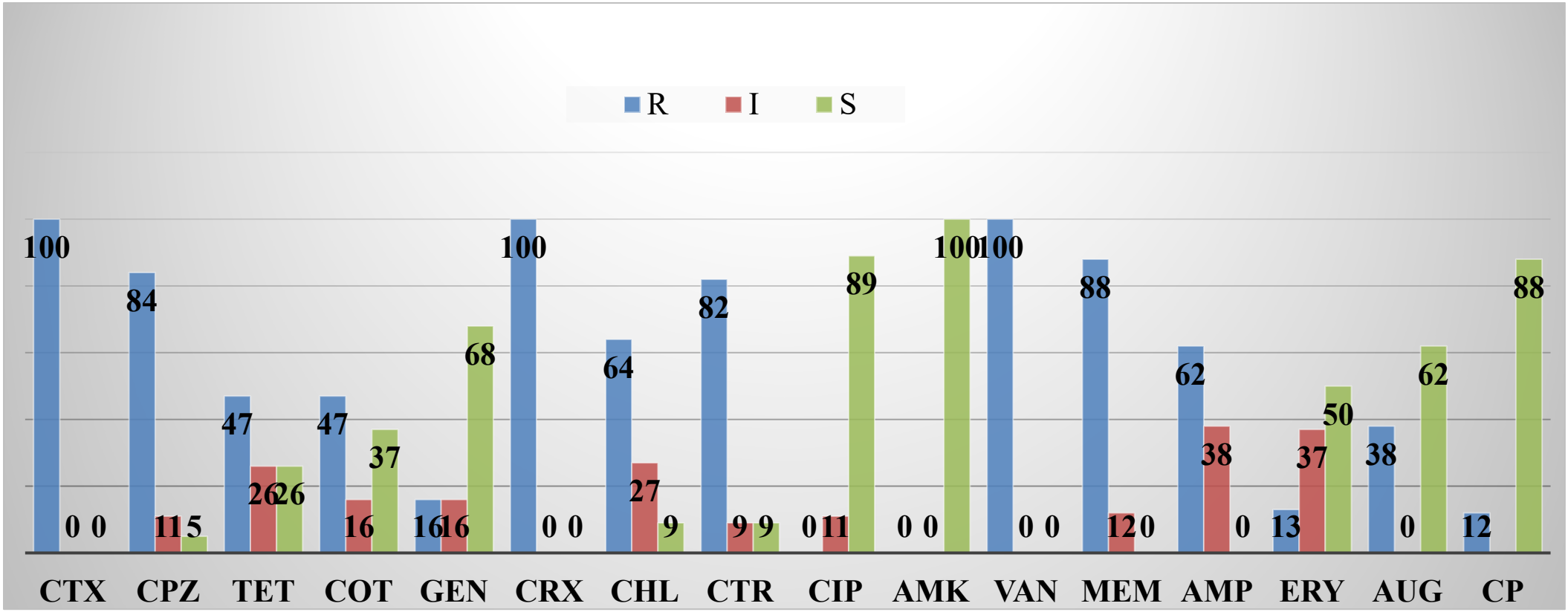

Figure 2: Antimicrobial pattern of bacterial isolates from toilet door handles of students' residential halls

Antibiotics: Tetracycline (TET), Cotrimoxazole (COT), Gentamycin (GEN), Cefuroxime (CRX), Augmentin (AUG), Ciprofloxacin (CIP), Vancomycin (VAN), Ceftazidime (CPZ), Meropenem (MEM), Chloramphenicol (CHL), Ceftriaxone (CTR), Cefotaxime (CTX)Erythromycin (ERY), Ampicillin (AMP), Cephalexin (CP). Meropenem (MEM) and Amikacin (AMK)

DISCUSSION

The toilet door handles of public toilets are inanimate objects which could harbor and transmit infectious agents. As people come in contact with surfaces such as door handles, there are chances of picking up bacteria cells deposited on them (Abiose, 2019).

The isolates obtained in this study include *Citrobacter freudii*, *Enterobacter spp*, *Escherichia coli*, *Klebsiella oxytoca*, *Enterococcus spp*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Bacillus megaterium*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Proteus mirabilis*, *Micrococcus luteus*, *Staphylococcus saprophyticus*, *Candida* spp and *Pseudomonas aeruginosa*. A previous study also reported the presence of bacteria isolates such as *S. aureus*, *Bacillus* spp, *Pseudomonas* spp*, E. coli*, *Klebsiella* spp, and *Enterobacter* spp on both male and female toilet door handles (Faparunsi, 2022). Muhammad *et al* reported the presence of *S. aureus*, *Bacillus* spp, *Micrococcus* spp, *E. coli*, *Klebsiella* spp, and *Salmonella* spp from door handles of public toilets in Federal University, Dutse, Jigawa state, Nigeria (Bashir *et al*., 2016).

In this study, male and female toilet door handles had a relatively high number of pathogenic organisms. However, female hall toilet door handles had more microorganisms (57%) than male toilet door handles (43%) (Table 4.1). This could be due to poor hygiene from female genitalia. Women tend to have different hygiene practices compared to men, which may impact the microbial load on door handles. Factors such as menstrual hygiene products, makeup application, and personal care routines can introduce additional microorganisms onto hands and subsequently to door handles. Women typically have more frequent contact with surfaces such as door handles due to factors like restroom usage patterns or the need to access facilities for personal hygiene reasons. Increased contact can lead to higher microbial transfer onto door handles and restrooms in female residential halls. This study contradicts the reports of Bashir (2016) in the sense that the male restroom had more microbial load than the female restrooms (Bashir *et al*., 2016). The most frequent organisms in this study were *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Escherichia coli*, *Candida* spp, *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* (Figure 4.1). This agrees with the research done by Odigie *et al*. (2017).

Antibiotic susceptibility test was carried out to investigate the antibiotic resistance patterns of the isolated organisms. From (Figure 4.2), it indicated no zone of inhibition by the organisms to Cefuroxime, Cefotaxime and Vancomycin (100%), and highly susceptible to Amikacin with a zone of inhibition of ≥ 17(100%), Ciprofloxacin with a zone of inhibition of ≥ 26(89%), and Cephalexin with a zone of inhibition of ≥ 15 (88%). The

isolates could be resistant to Cefotaxime, Cefuroxime and Vancomycin antibiotics due to the following reasons: some bacterial species naturally possess mechanisms that make them resistant to specific antibiotics. For example, *Enterococcus* spp and *Pseudomonas aeruginosa* are known to exhibit intrinsic resistance to certain antibiotics due to acquired resistance through genetic changes or the transfer of resistance genes (Lupo *et al*., 2018). The lack of a zone of inhibition may indicate the presence of acquired resistance mechanisms, such as the production of specific enzymes (e.g., beta-lactamases) that inactivate the antibiotics genes (Lupo *et al*., 2018). Over time, bacteria can develop resistance to antibiotics through the selection pressure exerted by the use of these drug genes (Frieri *et al*., 2017). Furthermore, the lack of zones of inhibition could indicate the presence of resistance strains within the tested bacterial species. *Candida* species were among the organisms isolated from the toilet door handles. Their occurrence could be due to sheddings from the skin, particularly in areas where moisture is present. Lavatory users may touch toilet door handles after using the restroom without washing their hands properly thus leading to the transfer of *Candida* from their hands to the toilet door handles. Toilet door handles can become contaminated with microbes from various surfaces including fecal matter, urine, and other bodily fluids (Abiose, 2019). Inadequate cleaning and sanitization of public restroom facilities, including toilet door handles can contribute to the persistence of microorganisms on these surfaces (Monica and Louise, 2019). *Candida* thrives in warm and moist environments (Karprinski *et al*., 2021). Bathrooms particularly public restrooms, often have high humidity levels, providing favourable conditions for the growth of *Candida* spp on surfaces.

Conclusion

A significant number of bacteria were found in toilet handles and such may constitute public health concerns, especially with the antibacterial resistance patterns exhibited in this study. In addition, the isolates were resistant to Cefotaxime, Cefuroxime, and Vancomycin antibiotics. Thus there is a need to adopt an efficient strategy against bacterial contamination and minimize the risk of transmission of antibiotic-resistant bacteria (ARB) among the student community, through the installation of toilet doors that are coated with antimicrobial properties or resist biofilm formation and proliferation. Several factors such as cost, effectiveness, durability, regular compliance, environmental impact, maintenance requirements, and user acceptance can affect the feasibility of this approach.

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