



Research Article

Phenotypic and Molecular Detection of ESBL-Production in Enterobacterales and *Pseudomonas aeruginosa* isolated from Office Door Handles of a tertiary institution in North-eastern Nigeria

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Door handles are mechanical devices used to open or close doors manually and are among the most common fomites facilitating contamination. This study seeks to identify Gram-negative bacterial species from surfaces of office door handles within the Federal Polytechnic Mubi and to analyze their antibiotic resistance profiles and the presence of Extended-Spectrum β -lactamase (ESBL) encoding genes. Gram-negative bacterial isolates were isolated from swabs of 100 office door handles and screened for antibiotic resistance, multiple antibiotic resistance (MAR) index, and Extended-Spectrum β -lactamase (ESBL) encoding genes using standard protocols. Of the 100 samples analyzed, only 41(41.0%) were contaminated with at least a bacterial isolate. 48 Gram-negative bacteria were isolated with the most preponderance (29.2%) from the Registry department. Also, *Pseudomonas aeruginosa* (47.9%) and *Escherichia coli* (20.8%) were the most detected bacteria isolates. Of the 48 Gram-negative bacterial isolates, 47(97.9%) were resistant to at least one antibiotic. Resistance to all the antibiotics by all the isolates was high and variable. Resistance to cefuroxime was the highest and least to ofloxacin and pefloxacin. Multi-drug resistance (MDR) phenotype was detected in 32(68.1%) bacterial isolates. The isolates' Multiple antibiotic resistance (MAR) index varies from 0.1-1.0. A MAR index greater than 0.2 was observed in 43(91.5%) bacterial isolates. Of the bacterial isolates resistant to either ceftazidime or ceftriaxone or both, only 16(42.1%) isolates were found to be positive for ESBL enzyme by double disc synergy test, mostly among *E. coli* (43.8%), and *P. aeruginosa* (31.3%). Of these, only 6(37.5%) isolates harbour the ESBL-encoding genes: *bla*TEM, *bla*SHV, or both. The *bla*TEM and *bla*SHV ESBL-encoding genes were detected in 4(66.7%) and 3(50.0%) isolates, respectively, whereas 1(16.7%) isolate harboured both *bla*TEM and *bla*SHV. The presence of potential pathogenic, drug-resistant, and ESBL-producing Gram-negative bacteria on office door handles in this study area is of public health importance. This is because organisms carrying ESBL genes exhibit resistance to first-line antibiotics and a broad spectrum of other critical antibiotic classes.

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INTRODUCTION

Human societies have consistently grappled with infectious diseases often caused by numerous and different categories of microbes, including bacteria, fungi, viruses, helminths, and protozoan parasites, which can produce cysts, oocysts, and eggs on surfaces they contact. These pathogens thrive in diverse environments such as soil, air, water, and food. While the environment often acts as a critical intermediary for transmission, direct transmission between infected and susceptible individuals is also possible. Surfaces and objects in the environment serve as reservoirs for the proliferation of microorganisms (Worku *et al.*, 2018).

Fomites are inanimate objects that, although innocuous, can harbour and contribute significantly to the dissemination of pathogens across locations and between individuals (Umeanaeto *et al.*, 2021; Ugwu *et al.*, 2024). Examples of fomites include but are not limited to door handles, tables, books, utensils, currency notes, and mobile phones.

Door handles are mechanical devices used to open or close doors manually (Ayuba *et al.*, 2019; Ugwu *et al.*, 2024). Research indicates that bacterial transfer to hands is most prevalent on non-porous surfaces like door handles. This is often due to insufficient cleaning and disinfection of these surfaces. (Tiku *et al.*, 2019). Bacterial species commonly associated with door handles include *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Citrobacter* spp., *Proteus* spp., *Shigella* spp., and *Salmonella* spp., among others. These organisms are capable of causing a spectrum of diseases ranging from mild to severe (Nwoire *et al.*, 2012; Tiku *et al.*, 2019; Umeanaeto *et al.*, 2021; Ugwu *et al.*, 2024).

The risk of disease transmission from contaminated handles is affected by the concentration of bacteria on the handle, the duration of bacterial survival, the bacterial virulence factors, and the immune status of the person coming into contact with the contaminated handle (Alothaim *et al.*, 2020). The survival and persistence of bacteria on fomites such as door handles are influenced by several factors, including temperature, biofilm formation, humidity, the presence of organic matter, and adherence to infection control practices, among others (Shiferaw *et al.*, 2013;

Alothaim *et al.*, 2020; Umeanaeto *et al.*, 2021). The material composition of door handles, predominantly stainless steel, is conducive to bacterial growth (Ikede *et al.*, 2022). Although previous studies have documented the persistence of both Gram-positive and Gram-negative bacteria on inanimate surfaces for extended periods, Gram-negative bacteria, however, have been found to survive longer than Gram-positive bacteria (Alothaim *et al.*, 2020; Umeanaeto *et al.*, 2021).

Students, staff, and visitors frequently access service offices in the study environment for various purposes. Due to the infrequent disinfection of door handles, there is a heightened risk of transmitting contaminating microorganisms. Doors endure substantial traffic, and users introduce pathogens from other locations, leaving them on the handles upon entering and exiting. Consequently, individuals may come into contact with microbes deposited on surfaces like desks, keyboards, office furniture, toilet handles, and office locks (Umeanaeto *et al.*, 2021).

There is insufficient data on the extent and nature of bacterial species contaminating office door handles in the study area and their antibiotic resistance profiles and underlying mechanisms. Hence, this study seeks to identify the bacterial species isolated from door handle surfaces within the offices of the study area. It aims to analyze their antibiotic resistance profiles, including their multi-drug resistant (MDR) phenotype, multiple antibiotic resistance (MAR) index, and the presence of Extended-Spectrum β -lactamase (ESBL) encoding genes.

MATERIALS AND METHODS

Study Area

The study area is Federal Polytechnic Mubi, Adamawa State, Nigeria. The selection of this higher institution as the study site was based on its large population and the high traffic associated with door handle usage. The aim was to assess the relative safety or potential risks these frequently touched surfaces pose to the general public. The institution is located in the Mubi-north Local Government Area within the coordinates of 10°16'47.8" N 13°17'23.6" (Tula *et al.*, 2022a)

Sampling technique/collection

One hundred (100) samples of office door handles were randomly selected for sampling

within the various departments in the Federal Polytechnic Mubi, Adamawa State. A sterile cotton swab collected samples from each office door handle. The samples were then transported to the Microbiology Laboratory of the Department of Biological Science Technology, Federal Polytechnic Mubi, Adamawa State, for bacteriological analyses.

Isolation of Bacteria from the office's door handles

A sterile cotton swab moistened with sterile physiological saline was used to swab the office door handles. The swabs were introduced into Eosin Methylene blue (EMB) agar, MacConkey agar plates, and Cetrimide agar by streaking to isolate Gram-negative bacteria. The plates were then incubated aerobically at 35-37°C for 24-48 hours for bacterial growth. Pure cultures of the isolates were obtained by streaking a small portion of cells from an isolated colony on the corresponding medium and subsequently on a Nutrient agar slant. The plates were incubated for 24 hrs at 35°C-37°C and refrigerated for further use.

Identification of bacterial isolates

After Gram staining, each bacterial isolate underwent biochemical tests, including Simmon's citrate test, triple sugar iron (TSI) agar reaction, and oxidase test. Oxidase-negative bacterial isolates were identified using the Microgen A kit. This kit consists of a plastic strip containing 12 microwells, each with dehydrated reagents designed to detect 12 biochemical properties: lysine decarboxylation, ornithine decarboxylation, hydrogen sulphide production, glucose fermentation, mannitol fermentation, xylose fermentation, indole production, urease activity, Voges-Proskauer reaction, citrate utilization, tryptophan deaminase (TDA) activity, and ortho-nitrophenol- β -galactoside (ONPG) hydrolysis. After adding the test isolate and 24 hours of incubation, the results from the 12 microwells (GN-A kit) were converted into a 4-digit octal code, which was used to confirm the bacterial identity using Microgen ID computer software version 2.0.8.33. In contrast, *Pseudomonas aeruginosa* was identified based on its growth and reaction on a Cetrimide agar plate (Tula *et al.*, 2022b).

The Antibiotic Susceptibility Testing

The antibiotic susceptibility of the isolated bacterial species was performed on Muller Hinton agar plates by disc diffusion method as

described by the National Committee for Clinical Laboratory Standards (CLSI, 2017). The discs were aseptically placed on the surfaces of the Mueller-Hinton agar plates with sterile forceps and incubated at 37°C for 24h. Zones of inhibition after incubation were observed, and the diameters of the inhibition zones were measured in millimetres. The interpretation of the measurement as sensitive and resistant was made according to the manufacturer's standard zone size interpretative table. The MDR phenotypes were identified when an isolate demonstrated resistance to at least three distinct classes of antibiotics (Tula *et al.*, 2022c).

Screening for Multiple Antibiotic Resistant (MAR) index of the isolates

The isolates' multiple antibiotic resistance (MAR) index was determined using the formula $MAR = x/y$. Where 'x' is the number of antibiotics the test isolate displayed resistance and 'y' is the total number of antibiotics to which the test organisms have been evaluated for sensitivity (Tula *et al.*, 2016).

PHENOTYPIC DETECTION OF ESBL

Presumptive test

In the presumptive test to detect potential ESBL producers, all the isolates were screened for susceptibility to ceftazidime (30 μ g) and ceftriaxone (30 μ g) antibiotics by disc diffusion method. Isolates with inhibition zones of ≤ 22 mm for ceftazidime and ≤ 25 mm for ceftriaxone were considered potential ESBL producers (CLSI, 2017).

Confirmatory Test

Presumptive ESBLs-producing isolates were subjected to confirmatory tests by double disc synergy test (DDST). Bacterial suspension equivalent to McFarland standard resistant to afore-mentioned antibiotics was swabbed onto a Mueller-Hinton agar plate. An antibiotic disc of amoxicillin/clavulanic acid (20/10 μ g) was placed at the centre, together with ceftazidime (30 μ g) and ceftriaxone (30 μ g) placed at a distance of 15 mm apart, and incubated. The organism that showed a straightforward extension of ceftriaxone or ceftazidime inhibition zone towards the disc containing amoxicillin/clavulanate was considered an ESBL producer (CLSI, 2017).

MOLECULAR SCREENING FOR ESBL-ENCODING GENES

Screening for detecting ESBL coding genes: blaTEM and blaSHV.

Specific primer sets (Table 1) were used to detect blaTEM and blaSHV coding genes in selected bacterial isolates in multiplex PCR reactions. The reaction cocktail that was used for all PCR per primer set included (Reagent Volume µl)-5X PCR SYBR green buffer (2.5), MgCl₂ (0.75), 10 pm Dntp (0.25), 10 pM of each forward and backward primer, 8000U of Taq DNA polymerase (0.06) and was made up to 10.5 with sterile distilled water to which 2 µl template was added. PCR profile includes an initial denaturing of 5min at 94°C, then 35 cycles of 94°C for 50, 62°C for 45s, and 72°C for 60s, then terminated at 72°C for 10 minutes. PCR was carried out in a Gene Amp 9700 PCR System Thermocycler (Applied Biosystem Inc., USA) using the appropriate profile designed for each primer pair.

Five microlitres (5µl) of each PCR product was electrophoresed in 2% agarose gel containing 5µl of 10 mg/ml ethidium bromide at 100V for 45m. A 100bp DNA marker was used as a molecular size marker. DNA amplification was examined under an ultraviolet (UV) transilluminator, and results were documented (Zaki & El-Shabrawy, 2015; Adegoke *et al.*, 2020)

Statistical analyses:

A simple percentage was used to calculate the frequency of bacterial isolates about sample size.

RESULTS

The results in Table 2 show the frequency of swabs collected from office door handles in the Federal Polytechnic Mubi. The results show that the Registry unit has the highest number of swabs (29.0%), closely followed by the Rectory (20.0%), and the least was the Applied Physics Department (3/0%). The results also show that only 41(41.0%) of the samples were contaminated with at least a bacterial isolate.

Table 3 shows the frequency distribution of Gram-negative bacteria isolates recovered from office door handles in some selected departments in Federal Polytechnic Mubi. Of the 41 swabs contaminated with at least a bacterial isolate, 48 Gram-negative bacteria were recovered. Of these, 29.2% and 20.8% were recorded from the institution's registry and Chemical Science units. Also, of the 48

Gram-negative bacteria recovered, *Pseudomonas aeruginosa* (47.9%) and *Escherichia coli* (20.8%) were the most detected bacteria isolated, and both were recovered mainly in the registry and chemistry units.

The antibiotic resistance pattern of the bacterial isolates is documented in Table 4. Resistance to all the antibiotics by all the isolates was high and variable. *Pseudomonas aeruginosa* demonstrated notable and variable resistance to all the tested antibiotics. *Citrobacter diversus* and *Escherichia coli* showed variable resistance to all the tested antibiotics except with ofloxacin. *Klebsiella pneumoniae* was 100% resistant to cefuroxime, gentamycin, and ceftazidime. Resistance to cefuroxime was the highest and least to ofloxacin and pefloxacin.

The resistance profile of the bacterial isolates, as presented in Table 5, was highly variable. Of the 48 Gram-negative bacterial isolates, 47(97.9%) were resistant to at least one antibiotic. Of these, 22(97.9%) *P. aeruginosa* were resistant to at least one antibiotic, whereas 100% of other bacterial isolates were also resistant to at least one antibiotic. Multi-drug resistance (MDR) phenotype was detected in 32(68.1%) bacterial isolates. Of these, 2(100.0%), 8(80.0%), 3(75.0%), and 2(66.7%) were *Providencia stuartii*, *E. coli*, *Pantoea agglomerans*, and *Morganella morgani*, respectively. Also, *P. aeruginosa* and *Citrobacter diversus* had an MDR phenotype of 63.6% and 60.0%, respectively. However, this study's only *K. pneumoniae* isolate did not exhibit the MDR phenotype. The isolates' multiple antibiotic resistance (MAR) index varies from 0.1-1.0. Remarkably, all the bacterial isolates show MAR indices more significant than 0.2, except 4(8.5%) *P. aeruginosa* isolates with a MAR index equal to or less than 0.2. A MAR index greater than 0.2 was observed in 43(91.5%) bacterial isolates.

Resistance of the isolates to 3rd generation cephalosporins was documented in Table 6. Whereas 38(79.2%) of the isolates were resistant to ceftazidime, only 34(70.8%) were resistant to ceftriaxone. All the tested isolates of *E. coli* were 100% resistant to ceftazidime and ceftriaxone. Other bacterial isolates were either 100% resistant to ceftazidime, ceftriaxone, or less. However, *K. pneumoniae* was found susceptible to ceftriaxone.

The phenotypic determination of ESBLs was carried out on the bacterial isolates resistant to either ceftazidime or ceftriaxone or both, and thirty-eight (38) bacterial isolates were examined for this purpose. Of these, only 16 isolates were positive for ESBL enzyme by double disc synergy test. The ESBL enzyme was detected more in *E. coli* (43.8%) and *P.*

aeruginosa (31.3%) than in other bacterial isolates (Table 7). Of these, only 6(37.5%) isolates harbour the ESBL-encoding genes: *blaTEM*, *blaSHV*, or both. The *blaTEM* and *blaSHV* ESBL-encoding genes were detected in 4(66.7%) and 3(50.0%) isolates, respectively, whereas 1(16.7%) isolate harboured both *blaTEM* and *blaSHV* (Table 6).

Table 1: Primer sequences and expected size of PCR amplified genes

Targeted gene	Primer sequence 5'-3'	Amplicon size (bp)	References
<i>blaTEM</i>	F- GTCGCCGCATACACTATTCTCA R-CGCTCGTCGTTTGGTATGG	258	Adegoke <i>et al.</i> , 2020
<i>blaSHV</i>	F-GCCTTGACCGCTGGGAAAC R-GGCGTATCCCGCAGATAAAT	319	Adegoke <i>et al.</i> , 2020

Table 2: Frequency and Distributions of swabs from door handles in Federal Polytechnic Mubi

SN	Department	CODE	Frequency (%)	Contaminated (%)
1	Registry	RG	29(29.0)	12(29.3)
2	Rectory	RC	20(20.0)	7(17.1)
3	Works	WK	10(10.0)	4(9.8)
4	Bursary	BR	9(9.0)	6(14.6)
5	Food Science and Technology	FST	7(7.0)	0
6	Applied Physics	AP	3(3.0)	3(7.3)
7	Chemical Science	CS	8(8.0)	6(14.6)
8	Science Laboratory Technology	SLT	8(8.0)	2(4.9)
9	Biological Science Technology	BST	6(6.0)	1(2.4)
TOTAL			100(100.0)	41(41.0)

Table 3: Frequency and Distributions of Gram-negative bacteria from swabs of offices door handle

SN	Bacterial Isolates	RG(%)	RC(%)	BR(%)	WK(%)	SLT(%)	FST(%)	CS(%)	AP(%)	BST(%)	TOTAL (%)
1	<i>P. aeruginosa</i>	7(30.4)	5(21.7)	2(8.6)	3(13.0)			4(17.3)	1(4.3)	1(4.3)	23(47.9)
2	<i>E. coli</i>	5(50.0)	1(10.0)	3(30.0)				1(10.0)			10(20.8)
3	<i>P. agglomerans</i>	1(25.0)		1(25.0)				1(25.0)	1(25.0)		4(8.3)
4	<i>C. diversus</i>	1(20.0)	1(20.0)	1(20.0)	1(20.0)			1(20.0)			5(10.4)
5	<i>K. pneumoniae</i>								1(100)		1(2.1)
6	<i>M. morgani</i>							3(100)			3(6.3)
7	<i>P. stuartii</i>					2(100)					2(4.2)
TOTAL		14(29.2)	7(14.6)	7(14.6)	4(8.3)	2(4.2)		10(20.8)	3(6.3)	1(2.1)	48

Legend: RG= Registry, RC= Rectory, BR= Bursary, WK= Works, SLT= Science Laboratory technology, FST= Food Science Technology, CS= Chemical Science, AP= Applied Physics, BST= Biological Science Technology

Table 4: Antibiotic resistance pattern of Gram-negative bacterial isolates from swabs of office door handle

Organisms	AU(%)	CEF(%)	CPX(%)	CN(%)	CTZ(%)	PEF(%)	OFX(%)	S(%)	TRX(%)
<i>P. aeruginosa</i>	16(69.5)	19(82.6)	7(30.4)	14(60.8)	17(73.9)	8(34.7)	3(13.0)	13(56.5)	16(69.5)
<i>E. coli</i>	10(100)	10(100)	2(20.0)	7(70.0)	10(100)	2(20.0)	-	4(40.0)	10(100)
<i>P. agglomerans</i>	3(75.0)	4(100)	1(25.0)	4(100)	4(100)	-	-	-	3(75.0)
<i>C. diversus</i>	5(100)	5(100)	3(60%)	2(40.0)	4(80.0)	1(20.0)	-	3(60.0)	3(60.0)
<i>K. pneumoniae</i>	-	1(100)	-	1(100)	1(100)	-	-	-	-
<i>M. morgani</i>	2(66.6)	3(100)	-	3(100)	3(100)	-	-	-	1(33.3)
<i>P. stuartii</i>	2(100)	2(100)	1(50.0)	2(100)	-	-	1(50.0)	1(50.0)	1(50.0)

Legend: AU= Augmentin, CEF= Cefuroxime, CPX= ciprofloxacin, CTZ= ceftazidime, PEF= perfloxacin, OFX= ofloxacin, S= streptomycin, TRX= ceftriaxone, - = nil

Table 5: Antibiotic resistance profile and MAR index of bacterial isolates from door handles in Federal Polytechnic Mubi

Isolates	Code	Resistance profile	No. of isolate	No. of antibiotics	MDR status	MAR index
<i>Pseudomonas aeruginosa</i>	W01, R8	au,cef,cn,cpx,ctz,ofx,pef,s,trx	2	9	MDR	1.0
	R23, P01, B02	au,cef,cn,cpx,ctz,pef,s,trx	3	8	MDR	0.89
	R1	au,cef,cn,ctz,pef,s,trx	1	7	MDR	0.78
	W06	au,cef,cn,cpx,ctz,s,trx	1	7	MDR	0.78
	RC07, RC10, W03, B08	au,cef,cn,ctz,s,trx	4	6	MDR	0.67
	CH03	au,cef,cn,cpx,ctz	1	5	MDR	0.56
	CH04	au,cef,cpx,ctz,s	1	5	MDR	0.56
	CH05	au,cef,cn,pef,trx	1	5	MDR	0.56
	R7	cef,ctz,pef,trx	1	4	-	0.44
	RC15	cef,cn,ctz,trx	1	4	-	0.44
	RC01	au,cef,trx	1	3	-	0.33
	CH08	au,cef,ctz	1	3	-	0.33
	R28	cef,trx	1	2	-	0.22
	RC06	cn,ctz	1	2	-	0.22
	BST06	cef,ofx	1	2	-	0.22
	R16	S	1	1	-	0.11
	<i>Escherichia coli</i>	R02, B05	au,cef,cn,ctz,pef,s,trx	2	7	MDR
B02		au,cef,cn,cpx,ctz,s,trx	1	7	MDR	0.78
B07		au,cef,cn,ctz,s,trx	1	6	MDR	0.67
CH06		au,cef,cn,cpx,ctz,trx	1	6	MDR	0.67
R29, RC03		au,cef,cn,ctz,trx	2	5	MDR	0.56
R25		au,cef,ctz,s,trx	1	5	MDR	0.56
R11, R18		au,cef,ctz,trx	2	4	-	0.45
<i>Pantoea agglomerans</i>	CH07	au,cef,cn,cpx,ctz,trx	1	6	MDR	0.67
	R03	au,cef,cn,ctz,trx	1	5	MDR	0.56
	B04	au,cef,cn,ctz	1	4	MDR	0.44
	P02	cef,cn,ctz,trx	1	4	-	0.44
<i>Citrobacter diversus</i>	R09	au,cef,cn,cpx,ctz,pef,s,trx	1	8	MDR	0.89
	W04	au,cef,cn,cpx,ctz,s,trx	1	7	MDR	0.78
	CH08	au,cef,cn,cpx,ctz	1	5	MDR	0.56
	RC12	au,cef,trx,ctz	1	4	-	0.44
<i>Klebsiella pneumoniae</i>	B03	au,cef,s	1	3	-	0.33
	P03	cef,cn,ctz	1	3	-	0.33
<i>Morganella morgani</i>	CH03, CH04	au,cef,cn,ctz	2	4	MDR	0.44
	CH05	cef,cn,ctz,trx	1	4	-	0.44
<i>Providencia stuartii</i>	SLT 06	au,cef,cn,ofx,s,trx	1	6	MDR	0.67
	SLT 04	au,cef,cn,cpx	1	4	MDR	0.44

Legend: au= Augmentin, cef= Cefuroxime, cpx= ciprofloxacin, ctz= ceftazidime, pef= perfloracin, ofx= ofloxacin, s= streptomycin, trx= ceftriaxone, - = nil

Table 6: Resistance to 3rd Generation cephalosporin's

SN	Bacterial Isolate	Cetazidime (%)	Ceftriaxone (%)
1	<i>Pseudomonas aeruginosa</i>	16(69.6)	16(69.6)
2	<i>Escherichia coli</i>	10(100.0)	10(100.0)
3	<i>Pantoea agglomerans</i>	4(100.0)	3(75.0)
4	<i>Citrobacter diversus</i>	4(80.0)	3(60.0)
5	<i>Klebsiella pneumoniae</i>	1(100.0)	0
6	<i>Morganella morgani</i>	3(100.0)	1(33.3)
7	<i>Providencia stuartii</i>	0	1(50.0)
Total		38(79.2)	34(70.8)

Table 7: Prevalence of ESBLs-encoding genes among phenotypic ESBL-producing Enterobacterales and *P. aeruginosa*

SN	Bacterial Isolate	Number tested (%)	Phenotypic ESBLs Positive (%)	No (%) with bla _{TEM}	No (%) with bla _{SHV}	No (%) with bla _{TEM} & bla _{SHV}
1	<i>P. aeruginosa</i>	16(41.0)	5(31.3)	1(25.0)	1(33.3)	0
2	<i>E. coli</i>	10(25.6)	7(43.8)	2(50.0)	1(33.3)	1(100.0)
3	<i>P. agglomerans</i>	4(10.3)	0	-	-	-
4	<i>C. diversus</i>	4(10.3)	1(6.3)	0	0	0
5	<i>K. pneumoniae</i>	1(2.6)	1(6.3)	1(25.0)	0	0
6	<i>M. morgani</i>	3(7.7)	2(12.5)	0	1(33.3)	0
7	<i>P. stuartii</i>	1(2.6)	0	-	-	-
Total		39(81.3)	16(43.6)	4(66.7)	3(50.0)	1(16.7)

Key: No.= number

DISCUSSION

Pathogenic bacteria frequently found on inanimate surfaces and in human surroundings can lead to the colonization and contamination of objects, particularly door handles. Studies reveal that door handles commonly harbour various microorganisms, including bacteria, fungi, and viruses, which can act as reservoirs for cross-infection and contribute to the recontamination of recently washed hands. Some of these microorganisms are highly pathogenic, posing risks of person-to-person transmission or self-inoculation (Ikede *et al.*, 2022).

Of the 100 samples processed, 41(41.0%) showed bacterial contamination. The contamination rate in this study is however lower than the 86.7% (Nworie *et al.*, 2012) indoor handles of public conveniences in Abuja, 64.5% bacterial contamination recorded by Abiose *et al.* (2019) in Adekunle-Ajasin University, Akungba-Akoko, Ondo State, 75.29% reported by Tiku *et al.* (2019) in door handles in University of Calabar community, 58.8% contamination rate (Ugwu *et al.*, 2024) documented in the office door handles and palms of students in a University Faculty Building, Enugu State University of Science and Technology, 43.5% of the bacterial contamination recorded by Hisham

(2015) in office door handles of different Universities in Khartoum State, and 60.0% (Ikede *et al.*, 2022), contamination rate at The Federal School of Medical Laboratory Technology Offices in Jos. However, the 41.0% contamination rate in this study is higher than the 20.4% by Omololu-Aso *et al.* (2011) in door knobs in Obafemi Awolowo University Teaching Hospital complex, Ife, Nigeria.

Door handles in high-traffic areas like the registry, bursary, and rectory offices showed higher contamination rates than those on lecturer office doors in academic departments. This is likely due to increased foot traffic and frequent contact by staff, visitors, and students in these areas, raising the risk of cross-contamination between hands and surfaces. Another potential reason for the high bacterial load on these door handles may be inadequate personal hygiene or improper handwashing practices. When individuals touch door handles without washing or sanitizing their hands—especially after using restrooms or handling contaminated surfaces—bacteria are transferred to these handles. This finding is consistent with previous studies (Nworie *et al.*, 2012; Umeanaeto *et al.*, 2021; Ugwu *et al.*, 2024). However, other studies have reported higher contamination in academic

department offices (Gani *et al.*, 2023; Ugwu *et al.*, 2024). This discrepancy may be due to differences in study design, geographic location, hygiene practices, sampling times, or experimental methods, among other factors.

The dominant bacterial species identified in this study were *Pseudomonas aeruginosa* and *Escherichia coli*. This aligns partially with other studies, which commonly report *E. coli* and species from the genera *Klebsiella* and *Citrobacter* as the predominant bacterial contaminants on surfaces such as door handles (Gani *et al.*, 2023; Itah & Ben, 2004). While the prevalence of *E. coli* in this study is consistent with these findings, the absence of a dominant presence of *Klebsiella* and *Citrobacter* marks a notable difference. A study at the Federal School of Medical Laboratory Technology in Jos identified *E. coli* and *Klebsiella* spp. as prominent Gram-negative bacteria on office door handles (Ikede *et al.*, 2022). While these findings are similar to ours, *Klebsiella* spp. was not dominant in our study.

Furthermore, a study from the Faculty of Sciences, Sokoto State University, documented the dominance of *E. coli* and *P. aeruginosa* on door handles, also aligning with our findings (Gani *et al.*, 2023). Additionally, a study by Mensha *et al.* (2016) found *Pseudomonas* species, *Klebsiella pneumoniae*, *Neisseria* species, and *E. coli* on university door handles in Southwestern Nigeria, supporting the findings here except for the absence of *Neisseria* species. The variations in the dominant bacterial species documented in this study compared to others may stem from differences in environmental and geographical conditions, the types of sampled materials, local endemic factors, personal hygiene practices, seasonal influences, and the methods employed for bacterial detection.

In this study, the presence of Gram-negative rods, including *Escherichia coli* and *Klebsiella* species, indicate possible faecal contamination on door handles (Bashir *et al.*, 2016; Gani *et al.*, 2023). This could result from individuals contaminating their hands with faecal or urinary matter after using restrooms without adequate handwashing. Many people overlook the importance of hygiene practices, such as handwashing, a simple yet effective way to reduce the spread of infectious agents.

The high prevalence of *Pseudomonas aeruginosa* observed in this study is likely due to its significant environmental adaptability and survival mechanisms, enabling it to persist on dry, inanimate surfaces for periods ranging from 6 hours to 6 months (Pachori *et al.*, 2019;

Spagnolo *et al.*, 2021). The predominance of *P. aeruginosa* on office door handles in this tertiary institution has important clinical and public health implications. This bacterium is one of the primary Gram-negative pathogens responsible for nosocomial and healthcare-associated infections, particularly among hospitalized patients. Furthermore, *P. aeruginosa* can cause various infections, including urinary tract infections, septicaemia, burn and wound infections, gastroenteritis in newborns, and meningitis (Spagnolo *et al.*, 2021).

All bacterial isolates in this study showed significant resistance to most tested antibiotics, particularly cephalosporins (β -lactam antibiotics), with the lowest resistance observed against ofloxacin and pefloxacin. The higher resistance to cephalosporins may be attributed to the frequent exposure of these isolates to this class of antibiotics. Global studies confirm that cephalosporins are among the most commonly prescribed and used antibiotics (Yang *et al.*, 2020). Cephalosporins, especially β -lactam agents, are widely used to treat bacterial infections and are a significant factor in developing resistance to β -lactam antibiotics among Gram-negative bacteria. Persistent exposure to various β -lactams has driven bacteria to produce and mutate β -lactamases, expanding their ability to resist even newer β -lactam antibiotics (Sharma *et al.*, 2023).

This study's increased susceptibility of bacterial isolates to pefloxacin and ofloxacin may stem from their reduced exposure to these antibiotics, likely due to lower prescription rates. Supporting this, studies have shown that while fluoroquinolones are a vital class of antibiotics for treating various infections caused by both Gram-positive and Gram-negative bacteria, the U.S. Food and Drug Administration (FDA) has imposed restrictions on their use due to potential adverse effects (USAFDA, 2018; Baggio *et al.*, 2021). Though these side effects are clinically uncommon, they can significantly impact a patient's quality of life. As a result, clinicians are advised to consider alternative antibiotics for uncomplicated infections and reserve fluoroquinolones for cases where no other effective treatment option are available (USAFDA, 2018; Kherroubi *et al.*, 2024).

The selective resistance of bacterial isolates in this study to various antibiotics can be attributed to several factors, including restricted access to the antibiotic target site, reduced absorption of active compounds due to efflux mechanisms, and the overuse or misuse of commonly available antibiotics. This resistance has made many

bacterial infections more challenging and costly to treat, with reduced treatment success rates due to the spread of resistant strains (Tsaku *et al.*, 2019).

The multidrug-resistant (MDR) phenotype observed in the isolates likely results from acquiring resistance traits through various mechanisms (Tula *et al.*, 2022a). The high MDR levels detected in this study suggest that these isolates may have been subjected to significant antibiotic pressure in their environment. Additionally, the presence of distinct resistance profiles, even among the same bacterial species, implies that these strains may originate from different clones of similar or divergent species.

A multiple antibiotic resistance (MAR) index above 0.2 indicates organisms originating from high-risk sources of contamination where antibiotics are heavily used. In contrast, MAR indices ≤ 0.2 suggest origins in environments where antibiotics are rarely or infrequently used (Tula *et al.*, 2016). In this study, 95.1% of the bacterial isolates had a MAR index above 0.2, implying that a large portion of the bacterial population had significant exposure to the tested antibiotics.

Extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria emerged as a global health threat linked to high mortality rates (Famojuro *et al.*, 2023). This study's phenotypic detection of ESBL-encoding enzymes in the bacterial isolates from door handles aligns with findings from previous studies conducted in western Nigeria (Famojuro *et al.*, 2023) and Western Nepal (Sharma *et al.*, 2023).

Among the Gram-negative bacteria analyzed, only six harbour ESBL-encoding genes, representing a prevalence rate of 35.7%. This is comparable to a prior study where 25.0% of Gram-negative isolates carried ESBL genes (Famojuro *et al.*, 2023). In this study, *bla*TEM was the most frequently detected gene, particularly in isolates of *E. coli*, *P. aeruginosa*, and *K. pneumoniae*, corroborating findings from Ogun State (Famojuro *et al.*, 2023). However, another study reported a higher prevalence of the *bla*SHV gene over *bla*TEM among ESBL-encoding bacteria (Tsaku *et al.*, 2019).

Identifying a bacterial isolate (*E. coli*) co-expressing both *bla*TEM and *bla*SHV genes may expand the antibiotic resistance spectrum of the organism, as previously observed (Rawat *et al.*, 2018). This could exacerbate the burden of antibiotic resistance within the community, particularly those caused by ESBL-producing organisms.

The detection and prevalence of ESBL-encoding genes in bacterial isolates from door handles in the study area and the broader region have been minimally documented or not reported at all. Thus, the findings of this study not only provide essential baseline data for epidemiological surveillance but also address a critical knowledge gap in the study area. These findings also hold significant public health implications due to the rapid spread of ESBL genes, which are plasmid-mediated and encoded on transposons and insertion sequences (Mirriagou *et al.*, 2006; Tsaku *et al.*, 2019). This mechanism allows these genes to be easily transmitted to other strains within the same species or across different species. Additionally, the exchange of plasmids among bacterial cells and the incorporation of resistance genes into transferable genetic elements play a critical role in acquiring and disseminating antibiotic resistance genes among bacterial isolates (Jena *et al.*, 2017; Tsaku *et al.*, 2019).

A significant concern is that organisms harbouring ESBL genes resist first-line antibiotics and other important antibiotic classes. This significantly limits treatment options and complicates the management of infections caused by these resistant organisms (Sharma *et al.*, 2023).

This study identified the presence of potentially pathogenic, multidrug-resistant, and extended-spectrum beta-lactamase (ESBL)-producing organisms on office door handles within the institution. Consequently, regular monitoring and evaluation of door handles should be implemented to safeguard staff, students, and visitors. Additionally, frequent handwashing and door handle sanitizers or sprays should be mandated and enforced as crucial measures to reduce the risk posed by these multidrug-resistant organisms (Sharma *et al.*, 2023).

CONCLUSION

The presence of potential pathogenic, drug-resistant, and ESBL-producing Gram-negative bacteria on office door handles in this study area is worrisome. The bacteria found on door handles in this study heightens the infection risk, as students, lecturers, and visitors frequently contact them during working hours. This may significantly contribute to the spread of ESBL-producing organisms within the community. As a result, office door handles could serve as fomites, facilitating the transmission of drug-resistant pathogens with profound public health implications. Therefore, staff, students, and visitors must consistently practice proper hand hygiene before and after the day's work.

Furthermore, regular monitoring and routine disinfection of office door handles should be implemented to prevent cross-contamination and reduce the spread of potentially harmful bacteria.

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REFERENCES

- Abiose, O.F. (2019). Bacterial contamination of selected public toilet door handles within Adekunle Ajasin University Campus, Akungba-Akoko, Ondo State, Nigeria. *International Journal of Sciences: Basic and Applied Research*, **43**(1), 76-86
- Adegoke, A.A., Madu, C.E., Aiyegoro, O.A., Stenström, T.A. and Okoh, A.I. (2020). Antibigram and beta-lactamase genes among cefotaxime resistant *E. coli* from wastewater treatment plant. *Antimicrobial Resistance and Infection Control*. <https://doi.org/10.1186/s13756-020-0702-4>
- Allothaim, R., Almatroudi, A., Ahmed, M.U., Khan, M.A., Joseph, R.J., Alharbi, A. et al. (2020). Public health Risk Assessment of the Door handles of the Community Pharmacies in Qassim Region, Saudi Arabia. *Journal of Pure and Applied Microbiology*, **14**(4), 2649-2654. <https://doi.org/10.22207/JPAM.14.4.41>
- Ayuba, L., Suwange, M.P. and Enefiok, U.O. (2019). Bacterial contamination of door handles/knobs in Gombe State University, Nigeria. *International Journal of Modern Science and Technology*, **4**(8), 204-211.
- Baggio, D. and Ananda-Rajah, M.R. (2021). Fluoroquinolone antibiotics and adverse events. *Australian Prescriber*, **44**, 161–164. <https://doi.org/10.18773/austprescr.2021.035>
- Bashir, S.F., Muhammad, H., Sani, N.M and Kawo, A.H. (2016). Isolation and Identification of Bacterial Contaminants from Door Handles of Public Toilets in Federal University Dutse, Jigawa State, Nigeria. *IOSR Journal of Pharmacy and Biological Sciences*, **11**(5), 53-57.
- CLSI (2017). *Performance Standards for Antimicrobial Susceptibility Testing, 27th ed. CLSI supplement M100*; USA: Wayne PA: Clinical and Laboratory Standards Institute.
- Famojuro, O.B., Adesanya, I.O., Ajewole, J.O. and Famojuro, T.I. (2023). Genotypic characterization of extended spectrum beta-lactamase in gram negative bacterial contaminants of some door handles in Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun State. *African Health Science*, **23**(2), 208-218. <https://dx.doi.org/10.4314/ahs.v23i2.23>
- Gani, M., Usman, U.A., Usman, H.M., Jumare, F.I. and Abdulkadir, N. (2023). Incidence and antibiotic susceptibility profile of bacteria from door handles in Faculty of Science complex, Sokoto State University Sokoto. *Caliphate Journal of Science and Technology*, **1**, 81-87. <https://dx.doi.org/10.4314/cajost.v5i1.12>
- Hisham, A.A.S. (2015). Assessment of Gram-negative bacterial contamination on door handles of service offices in Sudanese Universities, Khartoum State. A Dissertation Submitted In Partial Fulfilment for the Requirements of M. Sc In Medical Laboratory Science (Microbiology). 1-34
- Ikedo, R.E., Iyevhobu, K.O., Barnabas, F.O., Ibrahim, S.M., Abinokhauno, S., Igbuan, E.A. et al. (2022). Bacteriological assessment of door handles and knobs at the Federal School of Medical Laboratory Technology offices in Jos. *American Journal of Biomedical Science and Research*, **7**(5) <https://doi.org/10.34297/AJBSR.2022.17.002387>.
- Itah, A.Y and Ben, A.E. (2004). Incidence of Enteric Bacteria and Staphylococcus aureus in daycare centers in Akwa Ibom State, Nigeria. *The Southern Asian Journal of Tropical Medicine and Public Health*, **4**, 202 - 209. PMID: 15272770.
- Jena, J., Sahoo, R.K., Debata, N.K. and Subudhi, E. (2017). Prevalence of TEM, SHV, and CTX-M genes of extended spectrum b-lactamase-producing *Escherichia coli* strains isolated from urinary tract infections in adults. *Biotechniques* **7**, 2–7. <https://doi.org/10.1007/s13205-017-0879-2>
- Kherroubi, L., Bacon, J. and Rahman, K.M. (2024). Navigating fluoroquinolone resistance in Gram-negative bacteria: a comprehensive evaluation. *JAC Antimicrobial Resistance*. 1-20. <https://doi.org/10.1093/jacamr/dlae127>

- Kraay, A.N.M., Michael, A.L., Hayashi, M.A.L., Hernandez C.N., Spicknall, I.H., Eisenberg, M.C., et al. (2018). Fomite-mediated transmission as a sufficient pathway: a comparative analysis across three viral pathogens. *BMC Infectious Diseases*, **18**: 540. <https://doi.org/10.1186/s12879-018-3425-x>.
- Mensha, O., Okeke, A., Oyewole, O. and Osopale, A. (2016) Isolation of bacterial pathogens from selected door handles in a Southwestern University in Nigeria. *Acta Satech*, **7**(1), 1-5.
- Miriagou, V., Carattoli, A. and Fanning, S. (2006). Antimicrobial resistance islands: resistance gene clusters in Salmonella chromosome and plasmid. *Microbes and Infections*. **8**, 1923–1930. <https://doi.org/10.1016/j.micinf.2005.12.027>
- Nworie, A., Ayeni, A., Eze, U. and Azi, O. (2012). Bacterial contamination of door handles/knobs in selected public conveniences in Abuja metropolis, Nigeria: A public health threat. *Continental Journal of Medical Research*, **6**(1), 7-11.
- Omololu-Aso, J., Kolawole, D.O., Omololu-Aso, O.O. and Ajisebutu, S.O. (2011). Antibiotics Sensitivity Pattern of *Staphylococcus aureus* from fomites in the Obafemi-Awolowo University Teaching Hospital Complex (OAUTHC) Nigeria. *International Journal of Medicine and Medical Sciences*, **3**(2), 32-36.
- Pachori, P., Gothwal, R. and Gandhi, P. (2019). Emergence of antibiotic resistance *Pseudomonas aeruginosa* in intensive care unit; a critical review. *Genes and Diseases*, **6**:109–119. <https://doi.org/10.1016/j.gendis.2019.04.001>.
- Rawat, N., Singh, F., Hirpurkar, S.D., Sannat, C., Gade, N.E. (2018). Detection and characterization of extended-spectrum beta-lactamase genes (bla_{TEM} and bla_{SHV}) among beta-lactam-resistant fecal coliforms of dairy cattle from Chhattisgarh, India. *Turkish Journal of Veterinary and Animal Sciences*, **42**: 503-511. <http://dx.doi.org/doi.org/10.3906/vet-1706-77>
- Sharma, B.K., Sharma, B.P., Kunwar, A., Basnet, N., Magar, P.D. and Adhikari, S. (2023). Prevalence of Extended Spectrum β -Lactamase Producers (ESBLs) with antibiotic resistance pattern of Gram-negative pathogenic bacteria isolated from door handles in hospitals of Pokhara, Western Nepal. *Journal of Genetic Engineering and Biotechnology*, **21**:139. <https://doi.org/10.1186/s43141-023-00616-4>
- Spagnolo, A.M., Sartini, M. and Cristina, M.L. (2021). *Pseudomonas aeruginosa* in the healthcare facility setting. *Reviews in Medical Microbiology*, **32**:169–175. <https://doi.org/10.1097/MRM.0000000000000271>
- Tiku, D.R., Bassey, I.U. and Asikong, E.B.E. (2019). Evaluation of public health hazards of door handles In University of Calabar community. *International Journal of Scientific and Engineering Research*, **10**(6): 39-71.
- Tsaku, P.A., Ngwai, Y.B, Pennap G.R.I., Ishaleku D., Ibrahim, T., Nkene I.H. et al. (2019). Extended-Spectrum Beta-Lactamase–production in *Escherichia coli* isolated from door handles in Nasarawa State University, Keffi, Nigeria. *Heliyon*, **5**, e02177. <https://doi.org/10.1016/j.heliyon.2019.e02177>
- Tula, M.Y., Azih, A.V., Okojie, R.O. and Iyoha, O. (2016). Antimicrobial Susceptibility Pattern and Plasmid-mediated Antibacterial Resistance in *Staphylococcus aureus* and Coagulase-negative Staphylococci (CoNS). *Highland Medical Research Journal*, **16**(2),80-86
- Tula, M.Y., Enabulele, O.I., Ophori, E.A., Aziembemhin, A.S., Iyoha, O. and Filgona, J. (2022c). Phenotypic and molecular detection of multi-drug resistant Enterobacteriaceae species from water sources in Adamawa-North senatorial zone, Nigeria. *Dysona-Life Science*, **3**(2), 57-68. <https://doi.org/10.30493/DLS.2022.351097>
- Tula, M.Y., Filgona, J., Iyoha, O., Wafari, U.U. and Elisha, R. (2022b). Screening of hospital fomites and hands of healthcare workers for Metallo- β -Lactamase producing Gram-negative bacteria in Mubi general hospital Nigeria. *Dysona-Life Science*, **3**(2), 76-81.

- <https://doi.org/10.30493/DLS.2022.3542>
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- Tula, M.Y., Usman, Z.M., Chiroma, Z.M., Elisha, R., Okpalauwaekwe, E.O. and Ogu, M.N. (2022a). Prevalence and Antibiotic Susceptibility Pattern of *Salmonella enterica* Isolated from Apparently Healthy Students Screened for *Salmonella* Agglutinins. *Sokoto Journal of Medical Laboratory Science*; **7**(1), 115–123. <https://dx.doi.org/10.4314/sjmls.v7i1.15>
- Ugwu, C.C., Ezugwu, R.I., Ozochi, C.A. (2024). Assessment of bacterial colonization of office door handles and palms of students in a University Faculty building. *IOSR Journal of Pharmacy and Biological Sciences*, **19**(1), 21-27. <https://doi.org/10.9790/3008-1901022127>
- Umeanaeto, P.U., Okafor, U.C., Unam, M.C., Ilo, C.C., Okoli, C.C., Afulukwe, S.C. et al. (2021). Assessment of Parasites and bacterial contamination of office door handles in Nnamdi Azikiwe University, Awka, Anambra State. *American Journal of Biomedical and Life Sciences*, **9**(2), 120-127. <https://doi.org/10.11648/j.ajbls.20210902.13>
- USA Food and Drug Administration (2018). FDA Drug Safety Communication: FDA updates warnings for oral and injectable fluoroquinolone antibiotics due to disabling side effects. <https://www.fda.gov/drugs/drugsafety-and-availability/fda-drug-safety-communication-fda-updateswarnings-oral-and-injectable-fluoroquinolone-antibiotics>
- Worku, T., Derseh, D. and Kumalo, A. (2018). Bacterial profile and antimicrobial susceptibility pattern of the isolates from Stethoscope, Thermometer, and inanimate surfaces of Mizan-Tepi University Teaching Hospital, Southwest Ethiopia. *International Journal of Microbiology* Volume 2018, Article ID 9824251, 7 pages <https://doi.org/10.1155/2018/9824251>
- Yang, P., Chen, Y., Jiang, S., Shen, P., Lu, X. and Xiao, Y. (2020). Association between the rate of fluoroquinolones-resistant gram-negative bacteria and antibiotic consumption from China based on 145 tertiary hospitals data in 2014. *BMC Infectious Diseases*, **20**:269 <https://doi.org/10.1186/s12879-020-04981-0>
- Zaki, M.E., El-Shabrawy, W.O. (2015). Study of Extended Beta-Lactamase producing Gram-negative bacilli in Mansoura University, Hospital in Egypt. *International Journal of Current Microbiology and Applied Sciences*, **10**: 48-59.