Isolation, characterization and plasmid profiling of multi-drug resistant bacteria isolated from automated teller machines (ATM device) in Enugu State, Nigeria.

ABSTRACT

Background: Due to high frequency of use, many kinds of microbes may contaminate the ATM device resulting in the emergence and spread of infectious agents especially multi-antibiotic resistant (MAR) bacteria.

Aim: This study was conducted to isolate and phenotypically characterize bacteria isolates from ATM devices in Enugu State, Nigeria.

Study Design: This is a prospective, cross-sectional study involving 30 different ATM devices.

Place and Duration: The research was conducted in Enugu State from January 2024 to August 2024

Methodology: The isolation, phenotypic characterization and plasmid profiling of the test bacteria were carried out using standard methods.

Results: A total of one hundred and twelve (112) bacteria were isolated and they include *Klebsiella* spp, *Pseudomonas aeruginosa, Enterobacter* spp, *Serratia marcescens, Escherichia coli, Streptococcus spp, Staphylococcus aureus* and coagulase negative *Staphylococcus spp* (CONS). In the study, CONS had the highest isolation rate of 20.5 % and *Serratia marcescens* had the lowest isolation rate of 1.8 %. A total of 20 plasmids with molecular weight ranging from 2322 – 23130 bp were detected from the bacteria tested. The MAR index of the isolates fell within 0.2 to 0.8 indicating a high risk source of contamination where antibiotics misuse exist by the users in this study area.

Conclusion: Therefore, it is advisable that people start adopting the culture of washing and sanitizing one's hands before and after using the ATM devices as hand transmission is a major source of MAR infectious agents that can contaminate ATM surfaces.

Keyword: Isolation, Plasmid, ATM, Bacteria, Resistance, Enugu State.

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Introduction

Microorganisms are ubiquitous in distribution. They inhabit different environments either as free-living microbes or as parasitic organisms. Reports from studies have shown that many contaminated surfaces played a major role in the spread of infectious diseases (Tekerekoğlu *et al*., 2013; Nagajothi *et al.,* 2015). Some examples of such contaminated surfaces include buttons of automated teller machines popularly known as ATM devices, door handles, sinks, lockers, pens, tables and chairs (Osman *et al*., 2024). The machine is

an electronic device that enables customers of banks to perform financial transactions like cash withdrawals, deposits, funds transfers, balance inquiries, or account information inquiries at any time and without the need for direct interaction with bank staff. Most ATMs allow access to cash for anyone with a credit card or debit card. The card may harbor micro-organisms that can be transferred to the button of the ATM device. The hand has been demonstrated to have a role in the transfer of organisms, and humans have a notable propensity to pick up microbes from the surroundings. Automated Teller machines are the most widely used form of computer driven public technology (Hone *et al.*,2008) with an estimated over 2.4 million units in use. Since there are no restrictions on who can access the facility and no rules to ensure hygienic usage, once infected, the machines become vehicles for the spread of infection, putting users at risk of contracting these infections after using the machine. Just like other surfaces, these metallic keypads are susceptible to microbial colonization, particularly in locations where there are no adequate cleaning procedures in place.

Many bacterial, fungal and viral pathogens can survive on the inanimate objects for several months, and such pathogens could cause epidemic infections as a result of direct or indirect contact. Bacterial infection has recently been estimated to be the leading cause of death by 2050, causing 10 million deaths across the globe (Stanton *et al.*, 2022). To combat the looming upsurge in health challenges due to bacterial infections, the use of antibiotics should have been the major instrument but for antimicrobial resistance. Antibiotics have been used for many years to fight against pathogens in a wide range of actions, in hospitals, homes and industrial premises but inappropriate use of them has resulted in the development of resistant microorganisms (Odeyemi *et al.*, 2018). It is predicted that there will be lesser treatment options to some infections caused by bacteria as these organisms are persistently becoming superbugs. (Aragoneses *et al.*,2021, Nabawanuka, 2021). Previously, we investigated that infections with fluoroquinolone resistant pathogens limit the options available to treat infectious diseases of animals and humans (Adonu *et al*., 2018).

One of the major mediators of antibiotic resistance is the presence of resistant plasmid in bacteria. While bacteria isolated from the surrounding are often regarded as contaminants, the progressive development of antimicrobial resistance ignites the zeal for

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exploring the antibiotic resistant patterns and mediators of resistance like plasmid in the isolates. The action is important because these plasmids could be the potential carriers of antibiotic-resistant genes among bacteria in the environment. The plasmids can be transferred from one bacterium to another within the same or different genera by conjugation and transduction (Rozwandowicz *et al*., 2018).

To understand the contribution of bank ATM in spreading antibiotic resistance in environmental bacteria, we conducted a study in Enugu State of Nigeria. In the study, we isolated and characterized multiple antibiotic-resistant bacteria based on antibiotic susceptibility testing, plasmid profiling and MAR index determination.

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MATERIALS AND METHODS

MATERIALS

Study area

The study was carried out in Enugu State, South-East Nigeria.

Test Sample

Thirty (30) different ATM devices were used. Each local government (out of 17) in the State has at least one ATM stand built either alone without a bank or beside a banking facility.

Culture media

All the culture media (Titan Biotech, India) used were of the analytical grade and include Nutrient Agar, MacConkey agar, Tryptone soya broth (TSB), Mannitol salt agar (MSA),

Eosin-methylene blue (EMB), Blood agar, Chocolate agar, Sabouraud Dextrose Agar (SDA) and Cetrimide agar

Standard antibiotic disk

The standard antibiotic disc used for the study were; Amoxycillin-clavulanic acid 10µ g, Ceftraxone 30 µg, Cefoxitin 30 µg Nitrofurantion 10 µg, Gentamicin 10 µg, Ciprofloxacin 10 µg , Chloramphenical 10 µg, Ofloxacin 10 µg, Meropenem 10 µg, Pefloxacin 10 µg, Amoxicillin 30 µg, Erythromycin 10 µg, Levofloxacin 5 µg Ampicillin 30 µg, Cloxacillin µg, Cephalexin 30 µg , Clindamycin 10 µg. (Oxoid UK).

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Method

Bank approval:

Approval for the collection of samples was obtained from each manager of the banks used for study in Enugu State.

Sample collection

Each sterile swab-stick was aseptically soaked in sterile water and then rubbed on the metallic key pads of different ATM machines, inserted back into its casing, labelled appropriately and then transported to the Microbiology Laboratory of Enugu State University of Science and Technology immediately for microbiological studies.

Cultivation, isolation and characterization of microorganism

The ATM swabs were inoculated into sterile Trypton soya broth for enrichment and incubated at 37 $^{\circ}$C for 16 h. After the enrichment, the broth cultures were sub-cultured on

different biological selective media for presumptive identification of different bacteria according to the artificial medium used. Cetrimide, MacConkey, EMB and MSA were used for the identification of *Pseudomonas aeruginosa*, lactose fermenters, *Escherichia coli* and *Staphylococcus aureus* respectively. Several biochemical tests –Catalase production, Coagulase positivity, citrate utilization, Indole production, Oxidase production, Urease Methyl red and Voges-Proskauer tests- were carried out to further identify the organisms to species level.

Antibiotics susceptibility testing:

Antibiotic sensitivity testing was performed on each of the isolates using the paper disc agar diffusion method as recommended by the Clinical and Laboratory Standards institute (CLSI, 2023) using Muller- Hinton agar (Oxoid, UK)). Each time the test was to be conducted, each isolate was freshened-up and standardized using 0.5 MacFarland opacity standard. The identified and standardized bacteria were tested against many commonly used antibiotics in the study area: Amoxycillin-clavulanic acid 10 µg, Ceftraxone 30 µg, Cefoxitin 30 µg Nitrofurantion 10 µg, Gentamicin 10 µg, Ciprofloxacin 10 µg , Chloramphenical 10 µg, Ofloxacin 10 µg, Meropenem 10 µg, Pefloxacin 10 µg, Amoxicillin 30 µg, Erythromycin 10 µg, Levofloxacin 5 µg Ampicillin 30 µg, Cloxacillin µg, Cephalexin 30 µg, Clindamycin 10 µg.(Oxoid UK). The test bacteria were cultivated on sterile nutrient broth for 16 h and then seeded by streaking on the surface of well dried MHA plates. After placing the drug discs aseptically on the surface of the medium, the plates were incubated overnight at 37 °C. The sensitivity of the isolates to each antibiotic was shown by a clear zone of growth inhibition. The inhibition zone diameter (IZD) was measured using a rule and interpreted using CLSI, 2023.

Plasmid profiling

All the multi-drug resistant isolates were evaluated for the presence of plasmid DNA as described previously (Adonu *et al*., 2020). One milliliter (1ml) of each overnight broth cultures of test organisms in Trypcate Soy Broth (TSB) medium (Merck, Germany) was transferred into 1.5ml sterile Eppendorf micro-fuge tubes and centrifuged at 10.000g for

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10min. The resultant pellets were dissolved in 600µl of lysis buffer (Nacl 1M, Tris – HCL 1M, EDTA 0.5M), 20 µl SDS (25%), 3 µl of proteinase – K (20mg/ml) and incubated at 60^{o}C for 1 h. After the lysis, 620 µl of phenol/chloroform/isoamylalcohol (25:24:1 volume/volume) was added to the above solutions, vortexed and centrifuged at 12.00g for 10min. The supernatants were aseptically transferred to sterile micro-fuge tubes to which 1ml of 95% cold ethanol was added. The micro-fuge tubes were allowed to stand for 1h in refrigeration condition (4^{o}C). Plasmid DNA was precipitated in each tube by centrifugation at 12.00g for 10 mins. The precipitated DNA was dissolved in 50 µl of 10mM Tris EDTA – buffer (TE) containing 10 µl of RNASE. The plasmids were run on 1.5% agarose gel electrophoresis and visualized under UV light transilluminator and photographed as previously described (Adonu *et al.*, 2020).

Multiple antibiotic resistance indexing (MARI)

MAR index values were calculated following the standard equation (Osundiya *et al*. 2013). It was calculated as the ratio of the number of antibiotics to which an organism is resistant to total the number of antibiotics to which the organism is exposed. The multiple antibiotics resistance index was determined for each bacterial isolate. MAR index values greater than 0.2 indicate high risk source of contamination where antibiotics are often used

Statistical Analysis

Results were presented in tabular formats and as figures. The data collected were analysed by SPSS 23 software. Level of significant difference was $P<0.05$.

Result

Isolation rate of bacteria

A total of 120 samples were collected from 30 different ATM devices using a sterile wet swab-stick. Each of the devices yielded more than one bacteria. Ninety two samples (76.7%) yielded one or more bacteria species. There was no significant difference ($P< 0.5$) observed in the number of bacteria isolated among all the contaminated ATM devices in

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all the local governments in the study area. The number and the percentage of each bacterial species isolated from all the machines are shown in Table 1. A total of 112 bacteria comprising 15 *Klebsiella spp*, 10 *Pseudomonas aeruginosa,* 18 *Enterobacter spp.* 5 *Escherichia coli*, 19 *Streptococcus pneumoniae*, 23 *Staphylococcus aureus*, 20 *Coagulase negative Staphylococcus spp* and 2 *Serratia marcescens* were cultured. Therefore, eight (8) different species of bacteria were detected from the positive cultures. The antibiotic susceptibility tests carried out showed that many of these bacteria are multi-antibiotic resistant (Tables 2 and 3). All the strains of *E. coli, Enterbacter spp* and *Serratia marcescens spp* were resistant to Ampicilin, Ceftriaxone and Cefotaxime. This was followed by reduced susceptibility of these bacteria to other drugs tested. More than 50% of *Klebsiella spp* and *Pseudomonas aeruginosa* were resistant to Ampicillin, Nitrofurantoin, Chloramphenicol and Cefoxitin.

Table 3 shows susceptibility of Gram positive bacteria tested against the test antibiotics. All the *Streptococcus spp* tested showed resistance to Ampicillin, Cloxacillin and Cephalexin and more than 50 % of these organisms showed resistance to erythromycin, ceftriaxone, Gentamicin and Clindamycin. More than 50 % of both *Staphylococcus aureus* and CONS showed resistance to Ceftriaxone, Cloxacillin and Cephalexin.

Result of Plasmid profile

A total of 20 plasmids were detected from the bacteria tested (Table 4). A total 15 (75 %) and 5 (25 %) of the plasmids were found in Gram negative and Gram positive bacteria respectively. Some isolates harbored one or more plasmids of different molecular weight ranging from 2322 – 23130 bp. Out of all the plasmids identified, the plasmid with largest molecular size (23130 bp) was present in many of the bacteria (81,25 %) tested except in *Staphylococcus aureus* and coagulase negative *Staphylococcus spp*. The 504 bp plasmid was absent in all the bacteria tested. Figures 1 and 2 show images of gel electrophoresis of the plamid profiles of multi-drug resistant bacteria tested.

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Table 1: Isolation rate of bacteria from the device.

Isolates	Number of bacteria isolated	Frequency of bacteria isolated (%)
Klebsiella spp	15	13.4
Pseudomonas aeruginosa	10	8.9
Enterobacter spp	18	16.1
Escherichia coli	5	4.5
Streptococcus spp	19	17.0
Coagulase negative Staphylococcus spp	23	20.5
Staphylococcus aureus	20	17.9
Serratia marcescens	2	1.8

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Table 2: Antibiotic susceptibility pattern of Gram negative bacteria isolated

Test Bacteria	SUST	AM	N	GN	CPX	CH	OFX	MP	AUG	CEF	CTX
Klebsiella spp	S	1 (8)	6 (46)	12(92)	12(92)	4 (31)	11(85)	12(92)	2 (15)	1 (8)	2 (15)
	I	0 (0)	0 (0)	0 (0)	1 (8)	2 (15)	0 (0)	1 (8)	5 (39)	1 (8)	4 (31
	R	12(92)	7 (54)	1 (8)	0 (0)	7 (54)	2 (15)	0 (0)	6 (46)	11(84)	7 (54)
Pseudomonas aeruginosa	S	2 (40)	2 (40)	3 (60)	2 (40)	2 (40)	3 (60)	3 (60)	0 (0)	2 (40)	2(40)
	I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (60)	1 (20)	0 (0)
	R	3 (60)	3 (60)	2 (40)	3(60)	3 (60)	2 (40)	2 (40)	2 (40)	2 (40)	3 (60)
Enterobacter Spp	S	0 (0)	1 (50)	2 100)	2(100)	2 100)	2(100)	2(100)	1 (50)	0 (0)	0 (0)
	I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)
	R	2(100)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2(100)	2(100)
Serratia marcescens	S	0 (0)	4 (80)	4 (80)	4(80)	5 100)	5(100)	4 (80)	0 (0)	0 (0)	0 (0)

	I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	5 100)	1 (20)	1(20)	1(20)	0 (0)	0 (0)	1 (20)	5 100)	5(100)	5(100)
Escherichia coli	S	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1(25)	2 (50)	0 (0)	0 (0)	0 (0)
	I	0 (0)	0 (0)	0 (0)	1 25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (25)
	R	4(100)	4(100)	4(100)	3(75)	4(100)	3(75)	2(50)	4(100)	4(100)	3 (75)

Key: SUST= Susceptibility**,** R= Resistance, I=intermediate, S =sensitive susceptible, AM=ampicillin, N=nitrofurantoin, GN=gentamcin CPX=ciprofloxacin CH=chloramphenicol, OFX= ofloxacin., MP= meropenem, AUG= Augmentin, CET= ceftriaaxone ,CTX= cefotaxine

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Table 3: **Antibiotics susceptibility pattern of Gram positive bacteria isolated**

Test Bacteria	SUST	OFX	ERY	CEF	AMP	CLO	LEV	CEP	CPX	GN	CLN
Streptococcus Spp	S	5(100)	0 (0)	1 (20)	0 (0)	0 (0)	4 (80)	0 (0)	4 (80)	2 (40)	1(20)
	I	0 (0)	1 20)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)
	R	0 (0)	4(80)	4 (80)	5(100)	5 (100)	1 (20)	5(100)	1 (20)	3 (60)	4(80)
Staphylococcus Aureus	S	6(100)	3 50)	0 (0)	3(50)	2 (16)	6(100)	0 (0)	5 (83)	6(100)	6(100)
	I	0 (0)	3(50)	0 (0)	1(16.7)	1(16.7)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)
	R	0(0)	0(0)	6(100)	2(33.3)	4(66.7)	0 (0)	6(100)	1(16.7)	0 (0)	0(0)
Coagulase negative Staphylococcus Spp	S	3(75)	2(50)	1(25))	1(25)	0(0)	4(100)	2(50)	1(25)	4(100)	2(50)
	I	0(0)	1(25)	0(0)	1(25)	1(25)	0(0)	0(0)	2(50)	0(0)	1(25)
	R	1(25)	1(25)	3(75)	2(50)	3(75)	0(0)	2(50)	1(25)	0(0)	1(25)

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Key: SUST= Susceptibility, R= resistance, I=intermediate, S =sensitive susceptible, OFX= ofloxacin. ERY= erythromycn, CET= ceftriaaxone, AMP=ampicillin, CLO=cloxacillin, LEV=levofloacin CEP= cephalexin CPX= ciprofloxacin GN= gentamicin CLN= clindamycin.

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Multidrug resistant bacteria and plasmid profile

The results of the plasmid profiling conducted on the test multi-drug resistant bacteria showed that many of these bacteria were harbouring one or more plasmid (Table 4). Here, the representative multi-drug resistant bacteria presented as *k1 and k2 (Klebsiella spp), Ps1 and Ps2 (Pseudomonas aeruginosa), Eb1 and Eb2 (Enterobacter spp), etc.*, habouring the resistant plasmids were found to be resistant (nonsusceptibility) to at least one agent in three or more antimicrobial categories (multi-drug resistance). For instance, *k1 and k2 (Klebsiella spp)* showed resistance to AM, N, CH, OFX, AUG, CEF, CTX and GN, AM, N, CH, OFX, AUG, CEF, CTX respectively. Similar resistance profiles were shown with other bacteria tested.

Multiple antibiotic Resistance index (MARI).

Multidrug resistance (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. To confirm the multi-drug resistance properties of these bacteria, the percentage of them that are resistant to test antibiotics were calculated. The range of MARI calculated with the test bacteria was 0.2 – 0.9

depending on the test strain (Table 5). The greatest and lowest ranges of 0.8 – 0.9 and 0.2 -0.3 were calculated with *Escherichia coli* and CONS respectively.

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Table 4: Plasmid and antibiotic resistant profiles of the multi-drug resistant bacteria tested

Strains tested	Lab code	Antibiotic resistant profile	Number of plasmid	Molecular size of plasmid (bp)
K1	P1	AM,N, CH, OFX, AUG, CEF, CTX	3	23130, 9216, 4361
K2	P2	GN, AM,N, CH, OFX, AUG, CEF, CTX	2	23130, 4361
Ps1	P3	CPX, GN, AM,N, CH, OFX, AUG, CEF, CTX.	1	23130
Ps2	P4	CPX, GN, AM,N, CH, OFX, AUG, CEF, CTX	1	23130
Eb1	P5	AM, N, CH, CEF, CTX	1	23130
Eb2	P6	GN, AM ,N, CH, OFX, CEF, CTX	1	23130
Ec1	10	GN, AM, N, CH, OFX, AUG, CEF, CTX	3	23130, 9216, 2322
Ec2	11	GN, AM, N, CH, OFX, AUG, CEF, CTX	1	23130
St 1	12	Ery, CLO, CLN, GN, OFX, AUG, CEF, CTX	1	23130
St2	13	Ery, CLO, CLN, GN, OFX, AUG, CEF, CTX	2	23130, 2322.

CONS 1	14	Ery, CLO, CLN, GN, AUG, CEF, CTX	1	23130
CONS 2	15	Ery, CLO, CLN, OFX, AUG, CEF,	1	23130
Sa1	16	Ery, CLO, GN, CH, AUG, CEF, CTX	0	nil
Sa2	17	Ery, CLO, CLN, GN, AM, N, CH, OFX, AUG, CEF, CTX	0	nil
Sm1	18	GN, AM, N, CH AUG, CEF, CTX	1	23130
Sm2	19	GN, AM, N, CH, OFX, AUG, CEF.	0	nil

Key: 1 and 2 represent strain 1 and strain 2 of each bacteria species tested. *K,Klebsella spp, Ps Pseudomonas aeruginosa, Eb, Enterobacter spp, Ec- Escherichia coli, St, Streptococcus spp, CONS coagulase negative staphylococcus, Sa Staphylococcus aureus. Sm, Serratia marcescens*

OFX= ofloxacin. ERY= erythromycn, CET= ceftriaaxone, AMP=ampicillin, CLO=cloxacillin, LEV=levofloacin CEP= cephalexin CPX= ciprofloxacin GN= gentamicin CLN= clindamycin.

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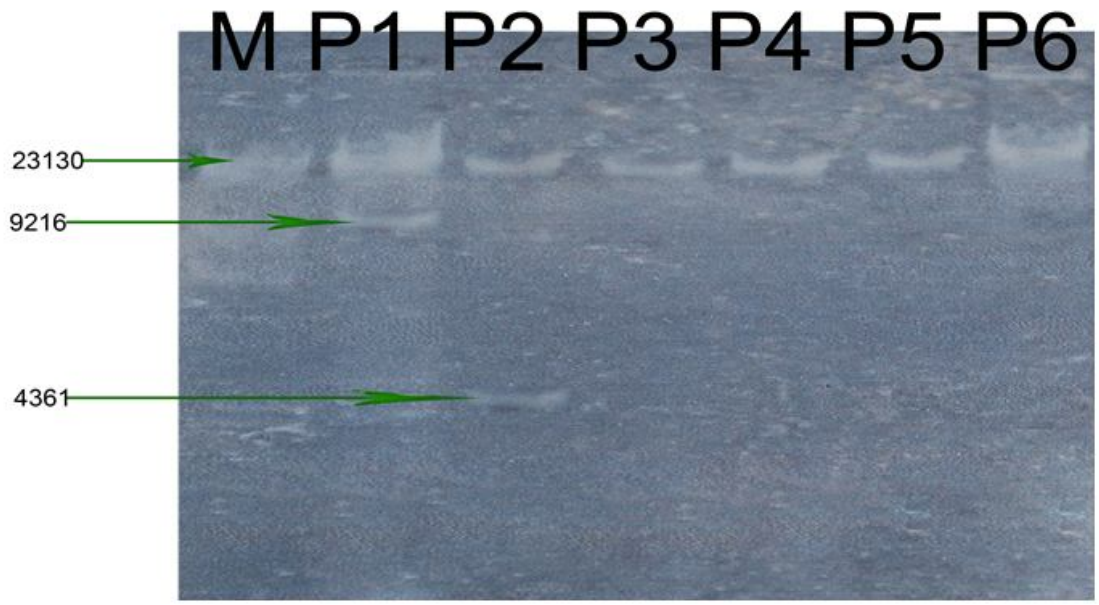

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Figure 1: The Image of gel electrophoresis of Plasmid DNA of *Klebsiella spp* (P1 &P2), *Pseudomonas aeruginosa* (P3 & P4), *Enterobacter spp* (P5 &P6) from ATM machine. Each of the lanes has at least one plasmid band of size 23130 bp.
Key: Lane M = DNA Marker.

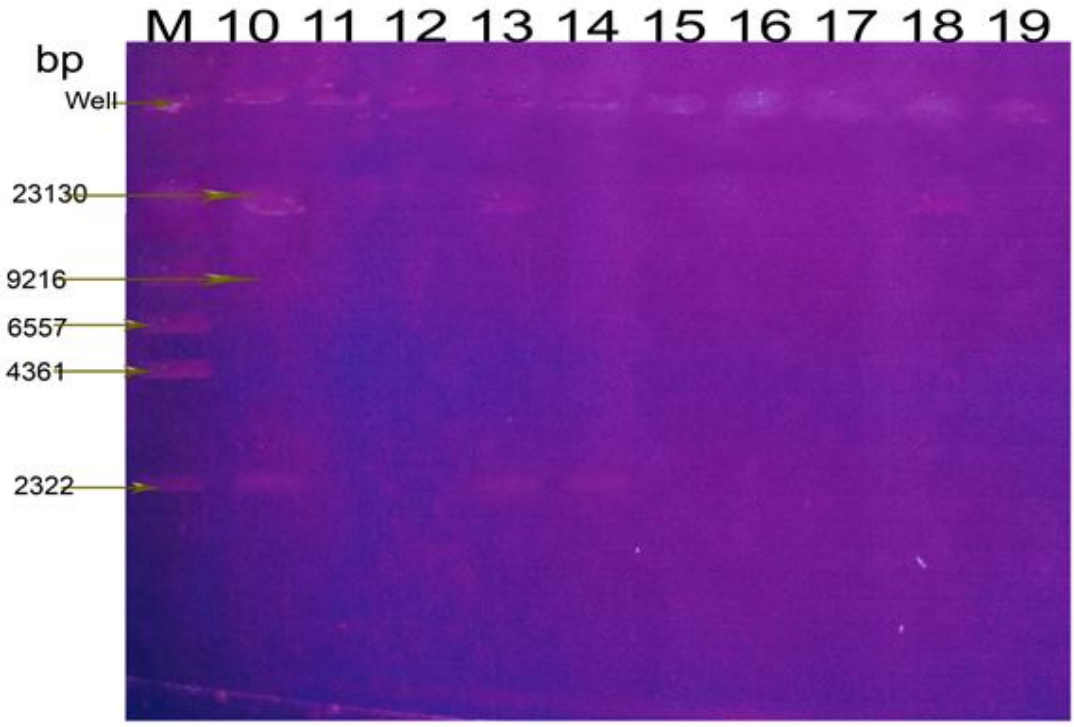

Figure 2: The Image of gel electrophoresis of Plasmid DNA extracted from *E. coli* (10 & 11), *Streptococcus spp* (12 & 13), CONS (14 & 15), *Staphylococcus aureus* (16 &17) and *Serratia marcescens* (18 & 19) from ATM machine. Each of the lanes has at least one plasmid DNA except lane 15 and lane 17.
Key: Lane M = DNA Marker.

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Table 5: Multiple antibiotics resistance index

Isolates (Organisms)	Average	Range
Klebsiella spp	0.44	0.2 - 0.7
Pseudomonas aeruginosa	0.46	0.3 - 0.8
Escherichia coli	0.83	0.8 -0.9
Streptococcus spp	0.83	0. 5- 0.8
Staphylococcus aureus	0.32	0.2 - 0.4
Serratia marcescens	0.48	0.4- 0.6
Enterobacter spp	0.40	0.2 - 0.4
Coagulase negative Staphylococcus spp	0.43	0. 2- 0.3

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Discussion:

Currently, antibiotic resistance is becoming persistently common and spreading globally, always challenging the ability of the clinicians to treat some common bacterial infections with success. Again, there are increasing reports bespeaking the propensity of diverse bacteria to colonize and multiply on inert surfaces that are always in contact with human hands such as door handles, tables, ATM devices (Osman 2024; Kramer and Assadian 2020)

Our study reports a total of 112 bacteria isolated from 120 samples collected from 30 different ATM devices in the study area. Specifically, 13.4 % *Klebsiella spp*, 8.9 % *Pseudomonas aeruginosa,* 16.1 % *Enterobacter spp.* 4.5 % *Escherichia coli,* 17 % *Streptococcus spp,* 17.9 *% Staphylococcus aureus,* 20.5 % *Coagulase negative Staphylococcus spp* and 1.8 % of *Serratia marcescens* were cultured from all the test samples. Similar studies in Iran, Ile Ife (Nigeria) and Ebonyi State (Nigeria) isolated similar species of bacteria from ATM devices (Mahmoudi *et al*. 2017; Oluduro *et al* 2012: Onuoha *et al*., 2024). Most of these bacteria isolated belong to the group of organisms called the ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa and Enterobacter spp*. These pathogens are mostly hospital-acquired pathogens that affect the gut and cause bacteremia, oral infections, wound infections, and urinary tract infections. (Venkateswaran *et al.,* 2023) They share several key biological characteristics, including adaptations for survival in the modern health-care setting, diverse methods for acquiring resistance determinants and the dissemination of successful high-risk clones around the world. They were initially identified as critical multidrug-resistant bacteria for which effective therapies were rapidly needed and despite the introduction of several new

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antibiotics such as novel β-lactamase inhibitors, these organisms continue to represent major therapeutic challenges. (Miller and Arias, 2024).

Generally, our study reported 76.7% bacteria contamination of ATM devices in the study area which was significantly greater than the results of the work done elsewhere (Mahmoudi *et al*. 2017). The results indicate that ATM keyboards should be regarded as a potential source of bacteria, much like other contaminated surfaces seen in public places, like rails of stairs, public bus handles and door handles in hospitals, banks and shopping mall. These devices may be heavily implicated in transmission of both pathogenic and non pathogenic microbes because these microbes can be harbored under the fingertips of the users and as a result they can cause finger-tips to mouth infection which can impair the life of the infected person (Rusin *et al.*, 2002). Therefore hand transmission is a major source of contamination of this device and because the majority of people with varying degrees of hygiene and health standards from different socioeconomic levels use this device (ATM) there is every tendency of hand borne transmission of microorganism. The ATM device, as an inert surface, can also harbor organisms with resistant genes and this poses serious public health problems.

In the present study, the antibiotic susceptibility tests carried out showed that many of the bacteria isolated are multi-antibiotic resistant. The antibiotic resistance crisis has been attributed to the inappropriate use of antibiotics as well as a lack of new drug development by the pharmaceutical industry due to reduced economic incentives and challenging regulatory requirements.(Gould and Bal. 2013; Wright 2014). Most cases of multiple antibiotics resistant bacteria have been observed to be due to transferable, extrachromosomal circular DNA called plasmids (Adonu, *et al.* 2020). In this study, we

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isolated multi-drug resistant bacteria from ATM devices and subjected them to plasmid analysis in order to evaluate their presence, number, and size. The study revealed plasmid sizes ranging from 2322-23130 bp in the MDR resistant strains. Specifically, plasmid profiling of the *Klebsiella spp, Streptococcus spp* and *Escherichia coli* showed that these MDR strains had double to triple plasmids of 2000–23000 bp sizes, similar to the results of work done by other researchers.(Talukder *et al*,2021) All the multi-drug resistant isolates were found to habour at least one plasmid with the exception of *Staphylococcus aureus* strains (Sa1 and Sa2) and *Serratia marcesciens (Sm 2)* (Table 4). Though we did not isolate plasmid in few of the test organisms, multiple antibiotic resistance (MAR) in bacteria is most commonly associated with the presence of plasmids which contain one or more resistance genes, each encoding a single antibiotic resistance phenotype (Daini *et al*., 2005). Many of these isolates tested were resistant to Ampicillin, Cloxacillin, Ceftriaxone, Nitrofurantoin, Chloramphenicol and Cefoxitin. It is one of our findings that the plasmid-encoded antibiotic resistance encompasses many classes of antibiotics commonly used in the study area as the drug of choice for antimicrobial chemotherapy including the amphenicols, penicillins cephalosporins, fluoroquinolones, and aminoglycosides.

The multiple antibiotics index (MAR index) of each of the test bacteria- *Klebsiella spp, Pseudomonas aeruginosa, Enterobacter spp, Serratia marcescens, Escherichia coli, Streptococcus pneumonia, Staphylococcus aureus, Staphylococcus epidermidis* was found to be greater than 0.2. This means that the test bacteria having MAR index ≥ 0.2 originated from a high-risk source of contamination where many antibiotics are frequently used. The multiple antibiotic resistance index (MARI) is a tool used to track bacterial infections and drug resistance, and to help determine the effectiveness of antibiotic chemotherapy. Increased emergence of resistance to antibacterial agents

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constitutes a very serious challenge in medical practice. The spread of pathogenic organisms via inert surfaces such as ATM devices in public places is more daunting and niggling in rural communities of underdeveloped countries like Nigeria where antibiotics can be purchased over the counter. This is worrisome because the emergence of drug resistance by selective pressure is a common phenomenon in such areas. As a result, it is important that one understands how antibiotic resistance develops and spreads in the environment in order to design and develop interventional plans to curb the menace of bacterial infections. Without effective policy and its implementation to limit the spread of pathogenic bacteria in the public places especially through inanimate surfaces and the techniques to limit antibiotic resistance, antibiotic therapy would be a complete waste of time and resources, and this means increased rates of morbidity and mortality from bacterial infection.(Bennett 2008). Secondly, investing in antibiotic drug development would not be gainful if appropriate measures are not put in place to contain the spread of multi-drug resistant bacteria.

Conclusion

ATM devices in use around the banks and outside banking facilities are considerably contaminated with potential pathogens and can therefore serve as vehicles for the transmission of disease-causing multi-drug resistant bacteria among individuals. The results of this work consolidate the importance of routine cleaning and disinfection of ATM devices to limit the danger of bacterial transmission. It also reinforces the importance of public health education on the danger of antibiotic drug misuse and antibiotic resistant gene transfer among bacteria. The varied population of bacteria found

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on ATM devices underscores the need for further investigation to molecular levels to better understand the kinetics of bacterial contamination in diverse surroundings.

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