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ANTIBIOTIC SUSCEPTIBILITY AND DETECTION OF EXTENDED-SPECTRUM B-LACTAMASE GENES IN GRAM-NEGATIVE BACTERIA ISOLATED FROM AUTOMATED TELLER MACHINES

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ABSTRACT

Antibiotic-resistant (AR) bacteria especially from commonly shared surfaces in the environment, pose a serious threat to global public health. The increasing demand for electronic banking and the associated risk of the spread of pathogenic bacteria makes it crucial to assess the antibiotic susceptibility and the presence of extended-spectrum beta-lactamase genes in bacteria isolated from automated teller machines (ATMs). Following standard procedures, a total of 22 samples were collected randomly from different ATMs using sterile cotton swabs soaked in physiologic saline and cultured on selective media. Isolates were characterized biochemically. Antibiotics susceptibility test was carried out on isolates using the Kirby-Bauer disc diffusion method. All Gram-negative isolates were subjected to polymerase chain reaction (PCR) to evaluate the common extended-spectrum beta-lactamase –encoding (ESBL) genes. Bacterial isolates were characterized as *Klebsiella pneumoniae* (33.33%), *Pseudomonas aeruginosa* (19.61%), *Escherichia coli* (15.69%), *Staphylococcus aureus* (9.80%), *Acinetobacter* spp. (7.84%), *Enterobacter* spp. and *Bacillus subtilis* (5.88%), and *Enterococcus faecalis* (1.96%). The highest antibiotic resistance pattern was displayed against ceftazidime (45.1%), followed by tetracycline (43.1%), cefixime (41.2%), ciprofloxacin and chloramphenicol (29.4%) and meropenem (9.8%). The *TEM* gene (54.8%) was recovered most, followed by *CTX-M* (28.6%) and *SHV* (19.0%). This study reveals that ATMs could harbour pathogenic bacteria with antibiotic resistance (AR) genes especially ESBL genes (*bla*TEM) which could be responsible for the widespread resistance to antibiotics. Therefore, adequate personal hygiene by users, proper cleaning regimen to sanitize these facilities regularly and public enlightenment are recommended to reduce the accompanying risks of spreading AR genes within the environment.

Keywords: Antibiotic resistance pattern, Automated Teller Machine, Beta-lactamase, Extended-spectrum

INTRODUCTION

The objects and surfaces in the environment which often come in contact with the human skin could be reservoirs for the transmission of antibiotic resistant (AR) bacteria within the environment (Akinola *et al*., 2022). Previous studies have reported the presence of viable pathogenic bacteria on inanimate objects such as money, door handles, plastics, keyboards, faucets, phones, fabrics, plastics and other surfaces (Kramer and Assadian, 2014; Dorost *et al*., 2018; Raad *et al*., 2019; Obajuluwa *et al.*, 2023) play an important role in the spread of various bacterial infections (Wißmann *et al*., 2021). Also, it has been reported that pathogenic bacteria can survive a few hours to several months on dry inanimate surfaces (Russotto *et al.*, 2015) and such environmental surfaces, especially those in close contact with humans and frequently touched, threaten human health and is a cause for public health concern.

The wide acceptance of electronic banking technology has promoted human dermal contact with the keypads of automated teller machines (ATMs) (Okoro *et al.*, 2018). This has led to regular and wide sharing of interfaces among users; with the harbouring of microorganisms acquired from the human microflora or from the environment, and cross contamination of microorganisms (Dawodu and Akanbi, 2021). Many factors influence the bacteria transfers between surfaces, including the source and destination surface features, bacterial species involved moisture levels, touch force (Zhao *et al*., 2019). Consequently, ATMs could be a point for the spread infections to humans (Okoro *et al.*, 2018). The increasing spread of multidrug resistance and extended-spectrum β-lactamase producing bacteria in the environment poses a serious threat to global public health as (Dougnon *et al*., 2021). The ESBL-producing bacteria are capable of hydrolyzing penicillins, broad-spectrum cephalosporins, and monobactams. In addition, microorganisms frequently exhibit resistance to other antimicrobial classes such as fluoroquinolones, aminoglycosides, and trimethoprim/sulfamethoxazole due to associated resistance mechanisms (Abayneh *et al*., 2018). Temoneira (TEM), Sulfhydryl variable (SHV), and Cefotaximases (CTX-M) are the three main types of ESBLs. CTX-M, includes a rapidly expanding family which has spread among a wide range of clinically important bacteria and over wide geographic areas (Pishtiwan and Khadija, 2019).

The dearth of information in the public space about the potential public health impact of ATM- borne infections may be responsible for the poor hygiene which sees users not bothering to ensure that their hands are properly sanitized before and after use. Considering the fact that hand contact is an important route for the spread of infections in the environment (Tekerekoğlu *et al*., 2013). Therefore, the increasing incidence of antimicrobial resistance by many pathogenic microorganisms, it is important that the level of danger posed by the use of ATMs to our society health by bacteria is evaluated. This study aims to investigate the antibiotic resistance pattern and the presence of extended-spectrum beta-lactamase genes in bacteria, particularly Gram negative bacteria, on keypads of ATMs of commercial banks in Lokoja Metropolis, North-Central Nigeria.

MATERIALS AND METHODS

Study area

Twenty two automated teller machines (ATMs) located within Lokoja metropolis, Kogi State, North-Central Nigeria were used for this study. These ATMs were sampled randomly from various locations within the city. These locations included the ATM at the banking areas of Lokongoma, Adankolo, Post office and Paparanda within Lokoja Local Government in Kogi State, North-Central, Nigeria.

Sample collection and processing

Following approval by the management of 10 different banks, a total of 22 samples were collected from the keypads and screens of the ATM devices with sterile cotton swabs soaked in physiologic saline. The swab sticks were immediately transported under ice pack to the Biological Sciences Laboratory, Federal University, Lokoja and inoculated into media within 1 hour of sample collection to prevent drying of the samples for microbiological analysis.

Isolation and biochemical characterisation of isolates from the samples

The physiologic saline-soaked swab sticks from the sampling points were streaked on the sterile nutrient agar (Oxoid Ltd., Basingstoke, Hampshire, UK), MacConkey agar (Oxoid Ltd., Basingstoke, Hampshire, UK), Bile esculin agar (Oxoid Ltd., Basingstoke, Hampshire, UK) and Eosin methylene blue (Oxoid Ltd., Basingstoke, Hampshire, UK) agar plates and incubated for 24 hours at 37^0C.

Distinct colonies were observed and further subcultured by streaking on freshly prepared media to obtain pure cultures. The pure strains were characterized through Gram's reaction, morphology and biochemical tests such as, catalase, oxidase, methyl red, indole, citrate, lactose fermentation test, hydrogen sulfide production test; Voges-Proskauer test, nitrate, urease, motility test and starch hydrolysis (Holt *et al.*, 1994; Olutiola *et al*., 2000). Gram negative bacteria belonging to the Enterobactiaceae were also confirmed using API 20E test kit (bioMerieux, Hazelwood, MO, US).

Antibiotic susceptibility testing of bacterial isolates

Antibiotic susceptibilities were determined by the Kirby-Bauer Disc Diffusion method in accordance to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2018) was used. The following antibiotic discs (Oxoid Ltd, Basingstoke, Hampshire, UK): cefixime (CFM - 5 µg), ceftazidime (CTZ, 30 µg), cefotaxime (CTX, 30 µg), meropenem (MEM, 10 µg) chloramphenicol (C - 30 µg), erythromycin (E - 15 µg), sulphamethoxazole/ trimethoprim (SXT 25 µg), ciprofloxacin (CIP - 5 µg), tetracycline (TET - 30 µg), and levofloxacin (LEV - 5 µg) were used. All plates were incubated at 37^0C for 24 hours. The diameters of zones of inhibition were measured to the nearest millimeter using a metre rule. Control strain *Escherichia coli* ATCC 25922 was used in the testing to validate the results of the antibiotic discs. Approved CLSI (2018) susceptibility zone diameter interpretative standard was used.

Detection of ESBL genes among Gram negative bacterial isolates

The DNA molecules were extracted by boiling method and used to prepare the PCR reaction mixture. All Gram negative isolates were analysed for the presence of some ESBL genes; bla_{TEM} (*TEM-H F*: CCCCGAAGAACGTTTTC, *TEM-H R:*ATCAGCAATAAACCAGC), bla_{SHV} (*SHV -1 F*:AGGATTGACTGCCTTTTTG, *SHV -1 R:* ATTTGCTGATTTCGCTCG), bla_{CTX-M} (*CTX-M F*: CGATGTGCAGTACCAGTAA, *CTX-M R*: TTAGTGACCAGAACAGCGG). At the completion of the amplification, PCR products were resolved on l.2% agarose gel stained with 0.5 µl of ethidium bromide. The DNA bands were visualized and photographed using a gel bio-imaging system (UVP Imaging System, Upland, CA, USA). The type-specific PCR products were recognized clearly by their distinct band sizes (Mentasti *et al.*, 2019).

Statistical analysis of data

Descriptive statistics were carried out on bacterial counts using SPSS software (SPSS version 18) at $P < 0.05$. Data were presented as frequencies and percentages where the main outcome was presence or absence of bacteria. The diameters of the zones of inhibition (mm) of the organism to the antibiotics tested were interpreted as signifying susceptibility, intermediate or resistant according to the approved (CLSI, 2018) susceptibility zone diameter interpretative standard.

RESULTS AND DISCUSSION

Quantification of the bacterial isolates from the ATMs

All the ATMs were found to be contaminated with bacteria. The mean bacterial counts varied with ATM sampling locations. The ATM at bank A had the highest mean bacterial counts of 4.28 x 10^5 cfu/mL, while the least bacterial counts was observed at bank H with mean bacterial counts of 4.9 x 10^4 cfu/mL. The heterotrophic plate counts of bacteria is shown in Table 1.

Table 1: **Heterotrophic plate counts from the ATMs**

Bank Source	Mean Bacterial Count cfu/mL
A	4.28×10^5
B	2.25×10^5
C	2×10^5
D	2.34×10^5
E	5.9×10^4
F	9.8×10^4
G	4.14×10^5
H	4.9×10^4
I	7.7×10^4
J	6.8×10^4

cfu/mL = colony forming unit per mililitre

Biochemical characterisation of isolates

Fifty-one bacterial isolates were recovered from the ATMs. The most dominant bacterial isolate was *Klebsiella pneumoniae* (33.33%), while the least dominant was *Enterococcus faecalis* (1.96%). The isolates obtained from the ATMs are shown in Table 2.

Table 2: Occurrence of bacterial isolates on the ATMs

Bacterial isolates	Frequency (n)	% (n)
Klebsiella pneumoniae	17	33.33
Pseudomonas aeruginosa	10	19.61
Enterobacter spp.	3	5.88
Acinetobacter spp.	4	7.84
Escherichia coli	8	15.69
Staphylococcus aureus	5	9.80
Bacillus subtilis	3	5.88
Enterococcus faecalis	1	1.96
Total	51	100

Antibiotic susceptibility pattern of bacterial isolates

Bacterial isolates displayed their most frequent resistance against ceftazidime (45.1%), while bacterial isolates were least frequently resistant to meropenem (9.8%) as shown in Table 3.

Table 3: Antibiotic resistance pattern of bacteria isolates from ATMs

Isolates	CFM	C	E	SXT	CIP	TET	CTZ	LEV	MEM	CTX
***K. pneumoninae* (17)**	8(47)	3(17.6)	4(23.5)	4(23.5)	3(17.6)	6(35.3)	6(35.3)	2(11.8)	1(5.9)	3(17.6)
***P. aeruginosa* (10)**	3(30)	4(40)	2(20)	4(40)	2(20)	7(70)	4(40)	2(20)	2(20)	2(20)
***E. coil* (9)**	2(25)	4(50)	3(37.5)	2(25)	3(37.5)	3(37.5)	4(50)	1(12.5)	0.00	3(37.5)
***S. aureus* (5)**	2(40)	1(20)	1(20)	1(20)	3(60)	2(40)	4(80)	0.00	0.00	1(20)
***Acinetobacter* spp. (4)**	4(100)	0.00	0.00	2(50)	2(50)	3(75)	2(50)	1(25)	0.00	1(25)
***Enterobacter* spp (3)**	1(33.3)	1(33.3)	0.00	0.00	1(33.3)	0.00	2(66.7)	0.00	1(33.3)	1(33.3)
***Bacillus subtilis* (3)**	1(33.3)	2(66.7)	2(66.7)	1(33.3)	1(33.3)	1(33.3)	1(33.3)	0.00	1(33.3)	2(66.7)
***E. faecalis* (1)**	0.00	0.00	1(100)	0.00	0.00	1(100)	1(100)	0.00	0.00	0.00
Total (51)	21(41.2)	15(29.4)	13(25.5)	14(27.5)	15(29.4)	22(43.1)	23(45.1)	6(11.8)	5(9.8)	13(25.5)

CFM-Cefixime, C- Chloramphenicol, E- Erythromycin , SXT- Sulphamethoxazole/ trimethoprim, CIP- Ciprofloxacin, TET- Tetracycline, CTZ- Ceftazidime, LEV- Levofloxacin, MEM- Meropenem, CTX- Cefotaxime

Distribution of the ESBL genes produced by the Gram negative bacterial isolates

Table 4 depicts the occurrence of ESBL-associated genes among the Gram negative bacterial isolates. The *TEM* gene (54.8%) occurred most frequently, while *SHV* gene (19.0%) occurred least frequently. Among *K. pneumoniae* isolates, 10(58.8%) carried *blaTEM* gene, 60% of *P. aeruginosa* showed the presence of *blaTEM* gene, while 5(62.5%) of *E. coli* carried the same gene. Extended spectrum beta-lactamase genes were not detected in *Enterobacter* spp. The electrophoresis pictures of the ESBL-associated genes are shown in Figures 1 – 3.

Table 4: Distribution of the ESBL genes produced by the Gram negative bacterial isolates

Isolates	*TEM*	*SHV*	*CTX-M*
***Klebsiella pneumoniae* (17)**	10(58.8)	3(17.6)	4(23.5)
***Pseudomonas aeruginosa* (10)**	6(60.0)	3(30.0)	2(20.0)
***Enterobacter* spp. (3)**	0	0	0
***Acinetobacter* spp. (4)**	2(50.0)	1(25.0)	2(50.0)
***Escherichia coli* (8)**	5(62.5)	1(12.5)	4(50.0)
Total	23(54.8)	8(19.0)	12(28.6)

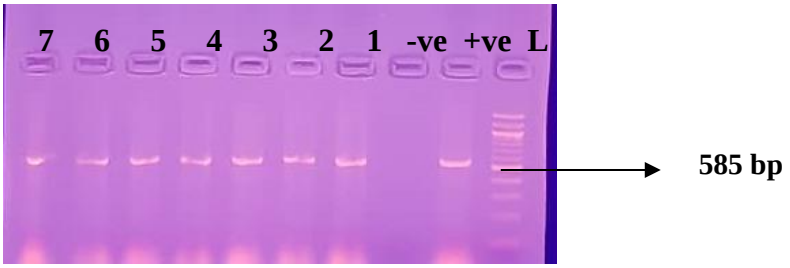

Figure 1: Band Pattern of *bla*CTX-M gene through gel electrophoresis
Lane L = 100 bp ladder, +ve = *CTX-M* positive control, -ve = *CTX-M* negative control, Lane 1 - 7 = Sample of *bla*CTX-M positive isolates.

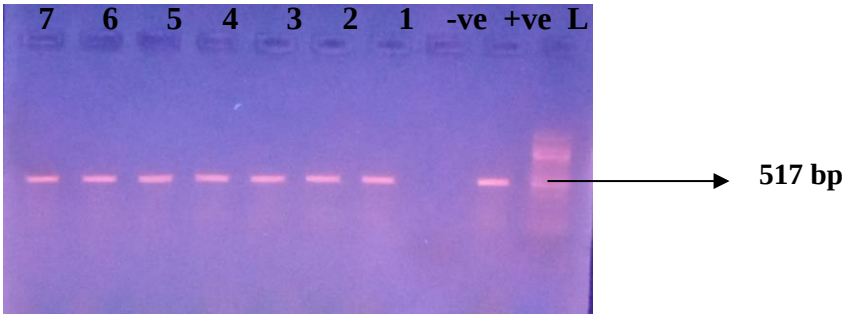

Figure 1: Band pattern of *bla*TEM gene through gel electrophoresis
Lane L = 100 bp ladder, +ve = *TEM* positive control, -ve = *TEM* negative control, Lane 1 - 7 = Sample of *bla*TEM positive isolates.

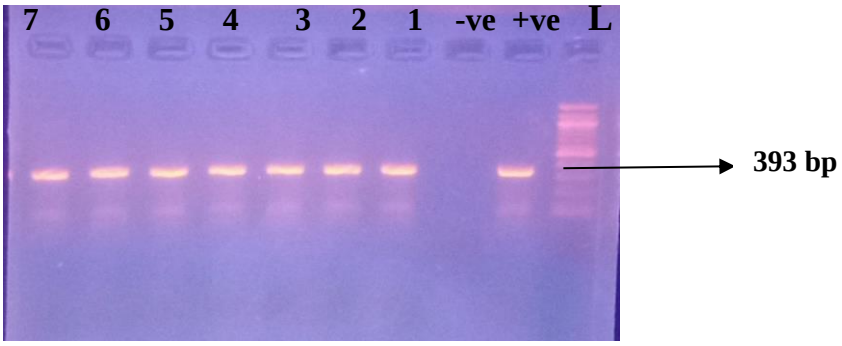

Figure 3: Band pattern of *bla*SHV gene through gel electrophoresis
Lane L = 100 bp ladder, +ve = *SHV* positive control, -ve = *SHV* negative control, Lane 1 - 7 = Sample of *bla*SHV positive isolates.

Discussion

Epidemiological studies have reported the role of contaminated surfaces such as ATMs in the spread of infectious diseases (Nagajothi *et al.*, 2015; Adedoyin, 2019). This study reveals the surfaces of Automated Teller Machines (ATMs) were largely colonized with bacteria as shown by the heterotrophic plate counts across the sampled ATMs. This could be explained in three ways; one, the poor hygienic practices of users such as hand washing techniques. Two, poor cleaning practices by the banks operating these machines may be responsible for the bacterial load on ATM surfaces. The proximity of the machines to local markets and commercial areas of Lokoja may also account for the bacterial counts observed across the ATMs. For example, traders and commercials having several dealings with different people daily visit ATMs for financial transactions. This study detected higher mean bacterial counts across the ATMs sampled in comparison with previous total bacterial counts from ATM keypads (Rufa'I and Kowa, 2019; Ya'aba *et al*., 2020). The variation in study locations and hygienic practices by users and bank operators may be responsible for the higher bacterial counts observed.

Among the bacterial isolates, *Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* were dominant. The high presence of *Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* suggests contamination of ATMs by bacteria associated with the health care environment leading to the spread of hospital-borne bacteria in the environment. Similarly, Okoro *et al.* (2018) reported *K. pneumoniae* was the most dominant bacteria present on the ATMs. Our findings, however, is in contrast with the works of Anderson and Palombo (2009); Dawodu and Akanbi (2021) who reported *Staphylococcus aureus* as the most frequently occurring bacterial contaminant on ATMs. This contrast could be as a result of different locations of study and proximity of the ATMs to healthcare facilities within Lokoja town; as the ATM users are engaged in various activities that may result in the variation of bacteria observed. The occurrence and potential spread of *Pseudomonas aeruginosa* in the environment could pose a serious public health risk especially in immune-compromised individuals (Wu and Li, 2015).

In addition, the presence of *Escherichia coli* on ATMs is suggestive of recent faecal contamination which can cause serious infections in the environment. *Escherichia coli* has

been linked to being the most frequent cause of many common bacterial infections such as cholecystitis, bacteremia, neonatal meningitis, cholangitis, urinary tract infection (UTI), traveler's diarrhoea (Tarun, 2014). Isolation of *Staphylococcus aureus* from ATMs can be ascribed to regular dermal contacts of humans with the ATMs as *Staphylococcus aureus* are well known colonizing bacteria of the human skin microbiome (Otto, 2010; Bryd *et al*., 2018). The isolation of *S. aureus* in our study is in similarity with the findings of Nwankwo and Afuruobi (2015).

Furthermore, the bacterial isolates exhibited varying resistance to the antibiotics tested; with *P. aeruginosa* and *K. pneumoniae* exhibiting multidrug resistance (MDR) against most antibiotics tested. Due to their resistance to multiple antibiotics, the presence of *Pseudomonas* aeruginosa and *Klebsiella pneumoniae* on ATMs may pose a challenge of the spread of multidrug resistance in the environment. Therefore, these multidrug resistant bacteria could be responsible for making treatment difficult for the infections caused by these bacteria. *Pseudomonas* and various Enterobacteriaceae including *Klebsiella* spp have been categorized as most critical group of bacteria urgently requiring new antibiotics (WHO, 2017). Also, the antibiotics resistance displayed by the bacterial isolates revealed that environmental bacteria are increasingly acquiring resistance to antibiotics and this is of public health concern. This finding is in line with the work of Barbosa *et al*. (2019).

Bacterial isolates demonstrated their most frequent resistance against ceftazidime; with predominantly *Enterobacter* spp., *Staphylococcus aureus, Escherichia coli*, *Acinetobacter* spp. and *Pseudomonas aeruginosa* displaying resistance of over 40% each to ceftazidime. This suggests a growing resistance of bacteria to ceftazidime. Moreover, resistance of bacteria to third-generation cephalosporins has been linked to the production of enzymes such as the extended-spectrum β-lactamases (Abayneh *et al*., 2018). Also, low resistance rates were observed against meropenem. This suggests that bacterial isolates are susceptible to meropenem and could still be an effective drug of choice against drug-resistant bacterial infections (Armstrong *et al*., 2021). Although this study observed high susceptibility rates of bacterial isolates to meropenem, the use of carbapenems (meropenem) due to their effectiveness may result in an increasing multidrug resistance. Hence, our study observed a higher meropenem-resistance in comparison with the earlier report of 4.8% resistance to meropenem (Gargoum *et al*., 2021).

The genotypic detection of extended-spectrum β-lactamase genes in Gram negative bacterial isolates reveals the presence of one or more genes in *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Acinetobacter* spp. The most frequent gene detected was the *bla*TEM which was detected predominantly in *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*. The prevalence of *bla*TEM in these bacterial isolates may be largely responsible for the multidrug resistance displayed by these bacteria. This finding is in line with the reports of (Akinbami *et al.* 2018; Pishtiwan and Khadija, 2019). In contrast with the report of Okogeri *et al.* (2020), this study detected *bla*SHV gene least frequently. This contrast could be as a result of the different study locations as these machines are sometimes left in open spaces. The occurrence of the *bla*CTX-M gene among bacterial isolates may be responsible for the resistance observed against third-generation cephalosporins (cefotaxime) as opined by (Tian *et al*., 2018). In fact, cefotaxime-resistance has been linked to the presence of extended-spectrum β-lactamase genes (Adegoke *et al*., 2020; Samreen *et al*., 2021).This findings of the occurrence of gene *bla*CTX-M among Gram negative bacterial isolates is in consonance with the work of Olowo-okere *et al.* (2020).

CONCLUSION

Pathogenic bacteria such as *Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Escherichia coli, Staphylococcus aureus, Acinetobacter* spp., and *Enterococcus faecalis* on the ATMs were isolated from the Automated Teller Machines (ATMs) located within Lokoja metropolis. The ATMs had microbial contaminants and have a role in the transmission of pathogenic microorganisms. This study revealed the presence multidrug resistant (MDR) bacteria and ESBL genes particularly *bla*TEM genes in Gram negative bacteria isolated from ATMs. Regular disinfection of ATM keyboards, screen and other parts with antibacterial covers for the contact surfaces or using alcohol wipes before and after use may help in limiting bacterial transmission.

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