

Bacteriological Contamination of User Interface of Automated Teller Machines (ATM) of Banks in Ekiti State University, Ado-Ekiti

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Abstract: As helpful as ATM machine is, recently id has been identify to have a detrimental effect on the health of its users, as it is now a source of infection to the users. Bacteriological examinations were carried out on cash dispensing machine (ATM). Samples (dusts) were taken from the ATM key-pads of some selected banks at Ado-Ekiti, Nigeria. The samples were collected with sterile cotton swab and then placed on culture media using streak-plating techniques. The isolates were identified using biochemical and molecular characterization techniques. Antibiotic susceptibility tests were done using agar diffusion method. Also plasmid analysis was carried out. Biochemically, percentage distribution of the isolates revealed high population of Pseudomonas aeruginosa (50%), Escherichia coli to be (30%) and Proteus vulg two strains possessed two plasmids each with molecular weight of 1.710kbp and 1.415kbp and were characterized molecularly to be strains of Eubacterium siraeum and Enterobacter tabaci. The health implication of the isolated strains on the environment and ATM users is discussed.

Keywords: Automated teller machine, bacteriological, antibiotic susceptibi aris (20%). All microbes were resistant to the antibiotics used during the course of this study. The selected lity, plasmid

1. INTRODUCTION

An Automated Teller Machine (ATM) is a computerized telecommunications device that enables the clients of a financial institution to perform financial transactions without the need of cashier, human clerk or bank teller [1]. A typical usage of the machine involves slotting a card into a recipient hole and following on screen instructions, by punching the keys of the metallic keypads to enter secret codes and commands; thus instructing the machine as to kind of service one requires [2]. The dispensing machine is likely to be contaminated with various normal flora due to their vast dermal contact by multiple users [3].

The presence of viable pathogenic bacteria on inanimate objects has been reported by earlier investigators [4]. Furthermore, microorganisms found to contaminate fomites has also been shown to persist on environmental surfaces in varying periods of time ranging from hours to months [5]. Bacteria that can cause severe gastroenteritis have been found on ATM keypad and cross infection of microorganisms between environmental surfaces and a host has equally been established [6]. Chain of infection occurs when the agent leaves its reservoir or host through a portal of exit, conveyed by some mode of transmission and enters through an appropriate portal of entry to infect a susceptible host.

Antimicrobial resistance to antibiotics has over the year attributed to the presence of plasmid which has been directly implicated in the acquisition of resistance to many antibiotics [7]. Usually the emerging of single or multiple antibiotic resistances are closely associated with various antimicrobial used [8]. Antibiotic Resistance is becoming a worldwide problem, borne from the adaptive response of bacteria to the widespread use of antibiotics [9]. Bacterial infections resistant to many antibiotics are becoming increasingly common and present a worldwide threat.

This study was aimed to assess Automated Teller Machines (ATM) keypads, cards and customers fingers and examined them and machine as a potential source of bacterial infections and means of spreading infections.

2. MATERIALS AND METHODS

2.1. Collection of Samples

The samples (dust) were collected randomly and aseptically from various ATM buttons, cards and user's fingers using sterile swab sticks. The banks visited on Ekiti State University campus are ECO Bank, United Bank of Africa, First Bank, WEMA Bank, Heritage Bank, Access Bank, Zenith Bank. The samples were then transported in ice bag to the Microbiology Laboratory of Ekiti State University and analysed within 2 hours of sample collection.

2.2. Microbiological Analysis

2.2.1. Isolation and Biochemical Characterization

A peptone soaked swab sticks from the sampling point were streaked on the sterile nutrient agar and incubated invertedly at 37°C for 24 hours. Distinct colonies were observed and further subcultured by streaking on freshly prepared nutrient agar to obtain pure strains according to Fawole and Oso [10]. The pure strains were kept in Bijou bottles and preserved at 4°C in a refrigerator for further analyses. The pure strains were subjected to Gram reaction and biochemical characterization tests such as, catalase, oxidase, coagulase, methyl red, indole, citrate and sugar fermentation [11].

2.3. Antibiotic Susceptibility

The identified strains were subjected to antibiotic sensitivity test using agar diffusion method. Mueller-Hinton agar, peptone water, swab stick, antibiotic disc dispenser and sterile petri dishes were used for this procedure. Sterile wire loop was used to pick 3-5 well isolated colonies from the stock culture and emulsifying into 5 ml of sterile normal saline, the turbidity of the suspension was compared to match a 0.5 Mcfarland turbidity standard. Sterile swab sticks were placed into the broth cultures in the normal saline and streaked on the Muller-Hinton agar plate to form a bacterial lawn. The plates were then allowed to dry for approximately 5 minutes. Antibiotics disc dispensers (sterilized forceps) were then used to dispense the impregnated antibiotic discs containing specific antibiotics (augmentin, ceftriazone, nitrofuranton, gentamycin, cotrimoxazole, ofloxacin, amoxycillin, ciprofloxacin, tetracycline, pefloxacin) on the microbial seeded plates. Sterilized forceps were used to press the discs gently to the agar ensuring discs were attached firmly to the agar. The plates were then incubated at 37°C for 18 to 24 hours. After which zones of inhibition were measured and interpreted according to CLSI standard [12].

2.4. Plasmid Profiling

Plasmid DNA of the isolates (with multiple antibiotic resistance) were extracted by modification of the technique described by Dutta et al. [13]. These techniques are used for isolation of plasmids for both Gram-negative and Gram-positive bacteria. The technique used majorly for isolation of Gramnegative negative bacterial plasmids is known as TENS. The TENS composition comprises of Tris 25mM, EDTA 10mM, NaOH 0.1M and SDS 0.5%. The solution contain Tris buffer that maintains the optimum pH; EDTA act as chelating agent; NaOH helps by increasing osmotic pressure, and SDS (Sodium Dodecyl Sulphate) act as a detergent which actually breaks the cell wall to release its content. Plasmids were isolated using the QIAGEN Plasmid Purification mini kit and the integrity of the extracted plasmid was checked on a 1% Agarose gel ran to confirm amplification. The 1XTAE buffer (40 mM Tris-acetate and 1 mM EDTA, pH 8.3) was prepared and subsequently used to prepare 1% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60°C and stained with 3µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2µl) of 10X blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 10µl of each PCR product and loaded into the wells after the 100-3000bp DNA ladder was loaded. The gel was electrophoresed at 120V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of the molecular weight ladder that was ran alongside experimental samples in the gel.

2.5. Molecular Characterization of Bacterial Isolates

Further analysis (Molecular characterization) was done on the isolates that possess plasmid.

2.5.1. Extraction of DNA

To extract bacterial genomic DNA, 1 ml of fresh bacterial culture was dispensed into a labelled microfuge tube and centrifuged at 13,500 rpm for 3 min. The supernatant was decanted and the pellet was resuspended in 200µl of STET buffer (0.1M NaCl, 10mM Tris-Cl (pH 8.0), 1mM EDTA (pH 8.0). and 5% (v/v) Tween 20). Subsequently, 50µl of lysozyme (10mg/ml, pH 8.0) was added to the suspension, vortexed for 5 seconds and incubated at room temperature for one minute. To the suspension, 600μ l of Lysis buffer (68% Guanidium thiocyanate) was added, vortexed for 1 min and incubated at room temperature for 15 min. The supernatant was decanted; 800µl of isopropanol was added to the solution, vortexed for 5 seconds and centrifuged at 13,500 rpm for 15 min. After decanting the supernatant, 1ml of 70% ethanol was added to the pellet. The suspension vortexed for 5 seconds and centrifuged at 13,500 rpm for 5 min. Finally the pellet was resuspended in 200µl of 10mM Tris-Cl (pH 8.0), 1mM EDTA (TE) buffer and 5 µl of the extract was run on 1% Agarose gel to detect DNA if present.

2.5.2. Polymerase Chain Reaction

PCR sequencing preparation cocktail consisted of 10 μ l of 5x GoTaq colourless reaction, 3 μ l of 25mM MgCl₂, 1 μ l of 10 mM of dNTPs mix, 1 μ l of 10 pmol each 27F 5'-AGAGTTTGATCMTG GCTCAG-3' and 1525R 5'- AAGGAGGTGATCCAGCC-3' primers and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 μ l with sterile distilled water 8 μ l DNA template. About 2.5 g of fungal genomic DNA was added to a 50 μ l PCR mix which contained 1 X Hot start reaction buffer, 0.25 mM dNTPs, 0.01 M (each), and 2.5 U Hot start polymerase (Jenabioscience). PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) with a PCR profile consisting of an initial denaturation at 94°C for 5 min; followed by a 30 cycles consisting of 94°C for 30s, 50°C for 60s and 72°C for 1 minute 30 seconds; and a final termination at 72°C for 10 mins and chilled at 4°C.

The integrity of the amplified about 1.5Mb gene fragment was checked on a 1.5% agarose gel. The 1XTAE buffer (40 mM Tris-acetate and 1 mM EDTA, pH 8.3) was prepared as previous and subsequently used to prepare 1.5% agarose gel.

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 μ l of Na acetate 3M and 240 μ l of 95% ethanol were added to each about 40 μ l PCR amplified product in a new sterile 1.5 μ l tube eppendorf, mixed thoroughly by vortexing and keep at -20°C for at least 30 min. Centrifugation for 10 min at 13000 g and 4°C followed by removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 μ l of 70% ethanol and mixed. Then centrifuged for 15 min at 7500 g and 4°C. All supernatant were removed (invert tube on trash) and tubes were inverted on paper tissue and dried in the fume hood at room temperature for 10-15 min. Then suspended with 20 μ l of sterile distilled water and kept in -20°C prior to sequencing. The purified fragment was checked on a 1.5% agarose gel ran on a voltage of 110V for about 1hr confirming the presence of the purified product and quantified using a nano drop of model 2000 from thermo scientific.

2.5.3. Sequencing

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Bio systems using manufacturers' manual while the sequencing kit used was Big Dye terminator v3.1 cycle. Bio- Edit software and MEGA 6 were used for all genetic analyses.

3. RESULTS

The percentage distribution of the bacteria isolates after biochemical identification revealed *Pseudomonas aeruginosa* with the highest frequency of 50%; followed by *Escherichia coli* with 30% and *Proteus vulgaris* having 20% (Table1)

S/N	Isolates	Number of Isolates	Percentage Distribution
1	Pseudomonas aeruginosa	5	50
2	Escherichia coli	3	30
3	Proteus vulgaris	2	20
TOTAL		10	100

Table1. Percentage distribution of bacterial isolates

All Gram negatives and Gram positive isolates showed multiple resistances towards varied antibiotics used (Table 2 and 3). Figure1 and Table 4 showed the plasmid profile of the selected bacteria isolates (with multiple antibiotic resistance). It was revealed that only bacteria isolates from Heritage bank and United Bank of Africa possess two plasmids each. Those microbes with dual plasmids were subjected to molecular identification which revealed the former biochemical characterized *Proteus vulgaris* from Heritage bank to be *Eubacterium siraeum* strain ATCC 29066 16S ribosomal RNA gene and former biochemical characterized *Pseudomonas aeruginosa* from United bank of Africa as *Enterobacter tabaci* strain YIM Hb-3 16S ribosomal RNA gene (Chart 1 and 2).

Table2. Antibiotic susceptibility of isolated Gram negative bacteria

						Antib	oiotics				
Isolates	AUG	CRO	NIT	GEN	COT	OFL	AMX	CPX	TEF	PFX	Phenotype of resistance
											pattern
FB-(E.coli)	R	R	R	R	R	R	R	R	R	R	AUG,CRO,NIT,GEN,COT,
											OFL,AMX,CPX,TEF,PFX
HB-(Proteus	R	R	R	R	R	R	R	R	R	R	AUG,CRO,NIT,GEN,COT,
vulgaris)											OFL,AMX,CPX,TEF,PFX
UB-(Proteus	R	R	R	R	R	R	R	R	R	R	AUG,CRO,NIT,GEN,COT,
vulgaris)											OFL,AMX,CPX,TEF,PFX
WB-(E.coli)	R	R	R	R	R	R	R	R	R	R	AUG,CRO,NIT,GEN,COT,
											OFL,AMX,CPX,TEF,PFX
ZB-(E.coli)	R	R	R	R	R	R	R	R	R	R	AUG,CRO,NIT,GEN,COT,
											OFL,AMX,CPX,TEF,PFX
%resistance of	100%	100%	100%	100%	100%	100%	100%	100%	100%	100%	
antibiotic											

Keys: AUG = Augmentin, CRO = Ceftriazone, NIT = Nitrofuranton, GEN = Gentamycin, COT = Cotrimoxazone OFL = Ofloxacin, AMX = Amoxycillin, CPX = Ciprofloxacin, TEF = Tetracycline, PFX = Pefloxacin FB = First Bank, HB = Heritage Bank, UB = UBA Bank, WB = Wema Bank, ZB = Zenith Bank.

Table3. Antibiotic susceptibility of isolated Gram negative bacteria

Isolate	Antil	biotics		Phenotype of resistance							
	AM	OFL	STR	CHL	CRO	GEN	PEF	COT	CPX	ERY	pattern
	Х										
AB-	R	R	R	R	R	R	R	R	R	R	AMX,OFL,STR,CHL,CRO,
(Pseudomonas)											GEN,PEF,COT,CPX,ERY
UB-	R	R	R	R	R	R	R	R	R	R	AMX,OFL,STR,CHL,CRO,
(Pseudomonas)											GEN,PEF,COT,CPX,ERY
WB-	R	R	R	R	R	R	R	R	R	R	AMX,OFL,STR,CHL,CRO,
(Pseudomonas)											GEN,PEF,COT,CPX,ERY
WB-	R	R	R	R	R	R	R	R	R	R	AMX,OFL,STR,CHL,CRO,
(Pseudomonas)											GEN,PEF,COT,CPX,ERY
ZB-	R	R	R	R	R	R	R	R	R	R	AMX,OFL,STR,CHL,CRO,
(Pseudomonas)											GEN,PEF,COT,CPX,ERY
%resistance of	100	100%	100%	100%	100%	100%	100%	100%	100%	100%	
antibiotic	%										

Key: AMX = Amoxycillin, OFL = Ofloxacin, STR = Streptomycin, CHL = Chloramphenicol, CRO = Ceftriazone, GEN = Gentamycin, PEF = Pefloxacin, COT = Cotrimoxazole, CPX = Ciprofloxacin, ERY = Erythromycin, AB=Access Bank, UB = UBA Bank, WB = Wema Bank

Bacteriological Contamination of User Interface of Automated Teller Machines (ATM) of Banks in Ekiti State University, Ado-Ekiti

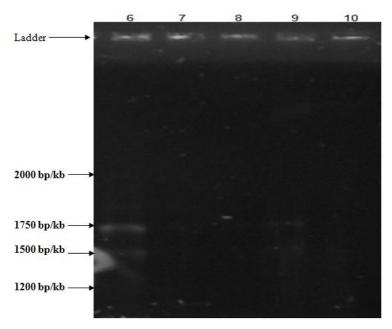


Figure1. Plasmid profile of bacterial isolates showing multiple antibiotics resistance

Isolates	Number of	Molecular	Antibiotic	s to which isolates were resistant		
	Plasmids	weight of	Number	Combination		
		plasmid (bp)				
HB	2	1710, 1415	10	AUG,CRO,NIT,GEN,COT,OFL,AMX,CPX,TEF,PFX		
WB 1	Nil	-	10	AMX,OFL,STR,CHL,CRO,GEN,PEF,COT,CPX,ERY.		
WB 2	Nil	-	10	AUG,CRO,NIT,GEN,COT,OFL,AMX,CPX,TEF,PFX		
UB	2	1710,1415	10	AMX,OFL,STR,CHL,CRO,GEN,PEF,COT,CPX,ERY.		
ZB	Nil	-	10	AUG,CRO,NIT,GEN,COT,OFL,AMX,CPX,TEF,PFX		

Keys: AMX = Amoxycillin, OFL = Ofloxacin, STR = Streptomycin, CHL = Chloramphenicol, CRO = Ceftriazone, GEN = Gentamycin, PEF = Pefloxacin, COT = Cotrimoxazole, CPX = Ciprofloxacin, ERY = Erythromycin, AUG = Augmentin, CRO = Ceftriazone, NIT = Nitrofuranton, GEN = Gentamycin, COT = Cotrimoxazone, OFL = Ofloxacin, CPX = Ciprofloxacin, TEF = Tetracycline, PFX = Pefloxacin, HB = Heritage Bank, WB = Wema Bank (1 and 2: Name of the location), UB = United Bank of Africa, ZB = Zenith Bank.

CAAAGAGGTAGATCCTGGCTCA

ACAAAGAGTTTGATCCTGGCTCA

4. DISCUSSION

After the molecular characterization, the organisms discovered were different from the organisms suspected by the biochemical reaction showing that biochemical characterization is not enough microbiological technique to identify microorganisms. Considerable effort has been invested in the application of molecular techniques such as PCR and hybridization for the identification of microbial samples. However, the extraction, purification, and amplification of nucleic acids by PCR from ATM samples are often selective and limited. This has shown that it is important to carry out molecular characterization on organisms. Molecular identification revealed the former biochemical identified *Proteus vulgaris* from Heritage Bank to be *Eubacterium siraeum* strain ATCC 29066 16S ribosomal RNA gene and former biochemical characterized *Pseudomonas aeruginosa* from United Bank of Africa as *Enterobacter tabaci* strain YIM Hb-3 16S ribosomal RNA gene.

The results of this study showed high level of bacterial contaminations of the surfaces of the metallic keypads of ATMs with Enterobacter tabaci strain YIM Hb-3 and Escherichia coli and Eubacterium siraeum strain ATCC 29066. Apart from the quantity of bacteria, the type and quality of microorganism present on a surface is also an important determinant of whether an infection will occur or not [14]. The high level of bacterial contamination determined through the course of this study is in line with the study of Oluduro et al. [4], who reported that keypads of ATMs harboured more bacteria than computer keyboards and this may be due to the fact that ATMs are usually located at the open place, exposed to wind and rain and to many people which might be sources and carrier of bacterial agents. This study is also in agreement with Abban and Tano-Debrah [15] who reported the presence of Enterobacter tabaci strain YIM Hb-3, Escherichia coli and Eubacterium siraeum strain ATCC 29066 on the keypads of money discharge machines (ATMs). Similarly, Anastasiades et al. [16] reported Enterobacter tabaci strain YIM Hb-3 as prevalent bacteria on computer keyboards and mouse. The result of this study is of public health concern especially for expatriates and students patronizing money discharge machine to withdraw on campus and those residing in Ado-Ekiti metropolis. Rusin et al. [17] reported that even through this, low levels of Salmonella spp. and some Escherichia coli strains can easily be transferred from the fingers to food surfaces.

It has been observed that antibiotic susceptibility of bacterial isolates is not constant but dynamic and varies with time and environment [18]. This therefore demands the need for periodic screening of common bacterial pathogens for their antibiotic susceptibility profiles in different communities [19]. The antibiogram result of this study showed that *Enterobacter tabaci* strain YIM Hb-3 and strains of other bacteria isolated were 100% resistant to ceftriazone, gentamycin, cotrimoxazole, ofloxacin, amoxycillin, ciprofloxacin, tetracycline, pefloxacin, ciprofloxacin, erythromycin and augmentin. This is an indication that the *Enterobacter tabaci* strain YIM Hb-3, *and Eubacterium siraeum* strain ATCC 29066 isolated were multiple drug resistance, which might be as a result of possession of plasmid as indicated in figure 1.

Bacteriological Contamination of User Interface of Automated Teller Machines (ATM) of Banks in Ekiti State University, Ado-Ekiti

Several strains of Enterobacteriaceae are pathogenic and cause opportunistic infections in immunocompromised (usually hospitalized) hosts and in those who are on mechanical ventilation [20]. The urinary and respiratory tracts are the most common sites of infection. The genus Enterobacter is a member of the coliform group of bacteria. Enterobacter tabaci is well adapted to cause nosocomial infections, as it is ubiquitous in the environment and can survive on skin and dry surfaces as well as replicate in contaminated fluids. Enterobacter species cause a wide variety of nosocomial infections, including those affecting the lungs, urinary tract causes neonatal sepsis with meningitis [21]. Therefore, the likely source of the organism might be nosocomial transfer of pathogen to ATM through a patient from the hospital or potential reservoir. *Eubacterium siraeum* is a genus of Gram positive bacteria in the family Eubacteriaceae, characterized by a rigid cell wall. Eubacterium siraeum has been isolated from human feces and is a member of genera of common human gut bacteria. In one comprehensive 16S rDNA sequence-based enumeration of the colonic microbiota of three healthy adult humans it represented, on average, 0.011% of all 16S rDNA sequences and 0.217% of the sequences in its division [22], indicating that the likely source of the microbe might be through a faecal contaminated personality or a nocturnal feacally contaminated flying agent that have visited the automated teller machine.

5. CONCLUSION

The result of this has shown that Automated Teller Machines (ATMs) and automated teller card can be contaminated with bacteria that are capable of causing disease and infection. The organisms isolated were majorly Enterobacter tabaci and Eubacterium siraeum which were found to be resistance to some commonly used antibiotics. This study confirms previous reports of a relatively high prevalence of bacterial contamination of bank Automated Teller Machine keypads. These bacteria can be transported from the contaminated surface to the humans by direct contact other people and items. Depending on environmental conditions, pathogens may remain active on surfaces for weeks after contamination. Furthermore, formation of biofilm by one bacterial agent can affect the survival of other pathogens on the same surface. The greater the concentration of the microbe, the longer it survive, also the longer the survival of a bacterium on surface like the ATM keypads, then the tendency of being picked by someone. People who do not pay attention to hygiene are an important factor as well as the atmospheric movements and other carriers in the spread of bacteria. Specifically, this study has revealed that people living in Ado-Ekiti metropolis and students on campuses who live in an unhygienic condition are at risk of contacting the infections traceable to the Automated Teller Machines and accomplice. In addition there is no indication that most bank owners' observe any form of guidelines or rule in regular cleaning of their ATM.

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