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Evaluation of Antibiotic Resistance Pattern of Bacteria Obtained from Surfaces of Automated Teller Machines in Calabar South, Cross River State, Nigeria

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Abstract: Money is an essential commodity in the life of humans and it is required to meet daily needs; as such, there is always a consistent usage of automated teller machines (ATM) to withdraw money to meet these needs. In this process, however, there is exchange of pathogenic and non-pathogenic microbes resulting from direct contact from the numerous users of these piece of equipment, especially with hands which are even known to be a 'house' of microorganisms. The study was carried out to evaluate the antibiotic susceptibility patterns of bacteria present on Automated Teller Machines (ATM) in Calabar Communities in Calabar, Cross River State, Nigeria. One hundred and fifty (150) samples were collected and analysed using standard microbiological procedures. Isolates were identified by microscopic, biochemical and carbohydrate fermentation characterizations. The susceptibility of the isolates to antibiotics was determined by the modified Kirby-Bauer disc diffusion method. Results revealed that all ATMs harbored seven different species of bacteria namely *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhimurium, Klebsiella* spp., and *Shigella* spp. However, *Staphylococcus aureus* was the most frequently occurring bacterial isolate (28.3%) followed by *Escherichia coli* (18.6%), *Bacillus* spp. (17.7%), *Pseudomonas aeruginosa* (14.2%), *Salmonella typhimurium*. (9.7%), *Klebsiella* spp. (6.2%) and *Shigella* spp. (5.2%) respectively. Antibiotic susceptibility results showed a zone of inhibition ranging from 8mm to 45mm. The widest zone of inhibition (45mm) was recorded with Augmentin for *Salmonella typhimurium* while the least zone of inhibition (8mm) was recorded in Ampicillin for *Pseudomonas aeruginosa*. All bacterial isolates showed a high level of resistance (6mm) to Nalidixic acid except *Klebsiella* spp. (10mm) and *Salmonella typhimurium*. Overall, Ceproflox and Quinolone showed the highest efficacy against the bacteria isolates followed by Augmentin and Tarivid. Heavy bacterial presence on ATM surfaces is evident from this study. Frequent disinfection of ATMs surfaces and their accessories along with periodic microbiological surveillance is thereby recommended.

Keywords: Bacteria, Automated Teller Machine, Antibiotic Resistance

1. Introduction

The ATM is likely to be contaminated with many different kinds of microorganisms both pathogenic and non-pathogenic due to their vast usage and dermal contact by many people in a day especially in an overcrowded

environment [1]. Once an ATM is contaminated, the machine then serves as the vehicle for the transmission of infections as well as pathogenic microorganisms. Many factors, both environmental and biological have been shown to influence bacterial transfers between surfaces. Some of these includes the source and destination surface features, bacterial species involved, moisture levels within the environment, pressure and friction and movement between the contact surfaces, and inoculum size on the surface [2]. Human beings have a marked tendency to pick up microorganisms from environmental objects and the hand has been shown to play a role in the transmission of organisms. Furthermore, microorganism found to contaminate fomites has also been shown to persist on environmental surfaces in varying periods ranging from hours to months [3].

It is also reported that some hard and nonporous surfaces including ATMs have the highest rate of bacteria transfer to hands [4]. Disease prevention, therefore, becomes the focus of public health management. Microbes especially those that are drug-resistant pose public health risks [5]. To this end, it has become imperative that the level of contamination of ATMs by drug-resistant microbes to our health be evaluated [3].

2. Sample Collection and Methodology

The study area included all ATMs found within 5 Communities as listed below:

Group A	Yellow Duke
Group B	Atakpa
Group C	Anantigha
Group D	Mbukpa
Group E	Duke Town

A total of 150 samples were used for the study. The collection of samples was done in two phases morning and evening. Sterile swab sticks moistened with sterile saline solution were used to clean the surfaces of the automated teller machines within the study area. The swab sticks were transferred immediately to their tubes to prevent drying of the samples before microbiological analysis in the Microbiology laboratory, Cross River University of Technology, Calabar, Cross River State. In the laboratory, the swabs were washed off in 2 mL of sterile saline in test tubes to serve as stock solutions for analysis.

The method of isolation of bacteria used in this study was the pour plate method described by [6]. At the termination of the incubation period, the plates were examined for growth, and the morphological appearance of the microorganisms on the agar was observed. Each observed culture was transferred onto solidified nutrient agar in slants and incubated. Identification of bacterial isolates was conducted using standard microbiological methods [7, 8].

Kirby-Bauer-NCCLS [9] modified single-disc diffusion technique was employed for the antibiotic susceptibility test. Approximately 20mL of Mueller-Hinton agar was poured into separate plates and allowed to solidify. Cotton swabs were dipped in the standardized inoculum and spread uniformly over the solidified Mueller-Hinton agar. The spread inoculum was allowed to dry for 15 minutes. Five (5) commercial antibiotic discs with different concentrations of the antibiotics were evenly placed on the dried agar surface. The plates were incubated at 37°C for 24 hours. Zones of inhibition were properly measured and recorded.

3. Results

The total heterotrophic bacteria count (THBC) from the different groups of ATM is shown in Table 1; in the morning sample, the highest THBC of 18.8×10^6 CFU/mL was recovered from group E ATMs (Duke Town), while the least THBC of 9.2×10^6 CFU/mL was obtained from group A ATM (Yellow Duke). For the evening round of sampling, a THBC of 355.3×10^6 CFU/mL was recorded in group E ATMs while the least THBC of 29.4×10^6 CFU/mL was obtained from A.

Seven different species of bacteria were identified based on the different morphological, biochemical and sugar fermentation characteristics, namely *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella* spp., *Staphylococcus aureus* and *Bacillus subtilis* (Table 2). Table 3 shows the distribution of the isolated bacteria. Of all the 113 bacteria isolated, 32 (28.3%) were identified as *Klebsiella* spp, 21 (18.6%) were identified as *E. coli*, 20 (17.7%) as *Pseudomonas aeruginosa*, 16 (14.2% as *Salmonella typhimurium*, 11 (9.7%) as *Shigella* spp., 7 (6.2%) as *Staphylococcus aureus*, while 6 (5.3%) were identified as *Bacillus subtilis*. The distribution of each species of bacteria is presented in Table 3.

The antibiotics susceptibility profile of the Gram-negative isolates is shown in Table 4; the *Escherichia coli* isolates were resistant to Nalidixic acid but susceptible to other antibiotics they were tested against. The *Klebsiella* spp. isolates were only resistant to Streptomycin but susceptible to other antibiotics. The *Pseudomonas aeruginosa* were resistant to Rifampicin, Gentamycin, Streptomycin and Nalidixic acid but were susceptible to Ciprofloxacin, Augmentin, Ampicillin, etc., meanwhile the *Salmonella* spp. and *Shigella* spp. did not show resistance to any of the test antibiotics. Table 5 shows the antibiotics susceptibility profile of the Gram-positive bacteria; the *Staphylococcus* spp. isolates were resistant to chloramphenicol but susceptible to the remaining antibiotics. Meanwhile, the *Bacillus* spp. isolates were susceptible to Ciprofloxacin, Streptomycin, Erythromycin, Chloramphenicol and Levofloxacin.

Table 1. *Total heterotrophic bacteria count from ATMs sample.*

ATM Group	Total Heterotrophic Bacteria Count (CFU/mL)	
	Morning Sample	Evening Sample
Group A	9.2 x 10^6	29.4 x 10^6
Group B	13.6 x 10^6	36.6 x 10^6
Group C	16.4 x 10^6	73.9 x 10^6
Group D	17.8 x 10^6	228.5 x 10^6
Group E	18.8 x 10^6	355.3 x 10^6

Table 2. *Morphological and Biochemical Characteristics of the bacterial isolates.*

Test	Gram reaction	MR	VP	Indole	Catalase	Citrate	Oxidase	Coagulase	Motility
Escherichia coli	-	+	-	+	+	-	-	+	+
Klebsiella spp	+	-	+	-	+	+	-	-	-
Pseudomonas aeruginosa	-	+	-	-	+	+	+	-	+
Salmonella typhimurium	-	+	-	-	+	-	-	-	+
Shigella spp	+	+	-	+	+	-	-	-	-
Staphylococcus aureus	+	+	-	-	+	+	-	+	-
Bacillus subtilis	-	-	+	-	+	+	-	-	+

+=Positive, -=Negative

Table 3. *Total Percentage occurrence of bacterial Isolates.*

Isolates	Group A ATM at Yellow Duke Frequency (%)	Group B ATM at Atakpa Frequency (%)	Group C ATM at Anantigha Frequency (%)	Group D ATM at Mbukpa Frequency (%)	Group E ATM at Duke Town Frequency (%)	Total	% occurrence
Escherichia coli	7 (19.4%)	6 (20.0%)	2 (9.5%)	3 (20.0%)	3 (27.3%)	21	18.6%
Klebsiella spp.	99 (25.0%	8 (26.7%)	5 (23.9%)	6 (40.0%)	4 (36.4%)	32	28.3%
Pseudomonas aeruginosa	8 (22.2%)	6 (20.0%)	3 (14.3%)	2 (13.3%)	1 (9.1%)	20	17.7%
Salmonella typhimurium	5 (13.9%)	4 (132.3%)	4 (19.0%)	3 (20.0%)	-	16	14.2%
Shigella spp	4 (11.1%)	3 (10.0%)	2 (9.5%)	1 (6.7%)	1 (9.1)%	11	9.7%
Staphylococcus aureus	2 (5.6%)	1 (3.3%)	2 (9.5%)	-	2 (18.1%)	7	6.2%
Bacillus subtilis	1 (2.8%)	2 (6.7%)	3 (14.3%)	-	-	6	5.3%
Total no. of isolates	36 (31.9%)	30 (26.5%)	21 (18.6%)	15 (13.3%)	11 (9.7%)	113	100%

Table 4. *Antibiotic susceptibility of the gram-negative isolates.*

Zones of inhibition in millimeter (mm)										
Isolates	OFX (10μg)	PEF (10μg)	CPX (10μg)	AU (30μg)	CN (10μg)	S (30μg)	CEP (10μg)	NA (30μg)	SXT (30μg)	PN (30μg)
Escherichia coli	25mm (S)	12mm (S)	38mm (S)	39mm (S)	11mm (S)	16mm (S)	20mm (S)	6mm (R)	21mm (S)	14mm (S)
Klebsiella spp	15mm (S)	6mm (S)	23mm (S)	42mm (S)	19mm (S)	6mm (R)	12mm (S)	10mm (S)	26mm (S)	12mm (S)
Pseudomonas spp	9mm (I)	6mm (R)	17mm (S)	37mm (S)	6mm (R)	6mm (R)	19mm (S)	6mm (R)	16mm (S)	8mm (S)
Salmonella spp	16mm (S)	12mm (I)	32mm (S)	45mm (S)	13mm (S)	17mm (S)	21mm (S)	13mm (S)	30mm (S)	21mm (S)
Shigella spp	12mm (S)	11mm (I)	27mm (S)	20mm (S)	8mm (I)	20 mm (S)	25mm (S)	6mm (S)	22mm (S)	33mm (S)

OFX=Tarivid, PEF=Rifampicin, CPX=Ciprofloxacin, AU=Augmentin, CN=Gentamycin, S=Streptomycin, CEP=Ceprorex, NA=Nalidixic acid, SXT=Septrin, PN=Ampicilin, S=Sensitive, R=Resistance, I=Intermediate.

Table 5. *Antibiotic susceptibility of gram-positive bacteria isolates.*

Zones of inhibition in millimeter (mm)										
Isolate	CPX (10μg)	NB (10μg)	CN (10μg)	AMI (20μg)	S (30μg)	RD (20μg)	E (30μg)	CH (30μg)	APX (30μg)	LEV (30μg)
Staphylococcus spp.	38mm (S)	6mm (R)	12mm (S)	39mm (S)	15mm (S)	27mm (S)	10mm (S)	25mm (R)	18mm (S)	17mm (S)
Bacillus spp.	29mm (S)	6mm (R)	15mm (S)	6mm (R)	21mm (S)	6mm (R)	27mm (S)	6mm (S)	23mm (S)	19mm (S)

AMI=Amikacin, RD=Rifampicin, E=Erythromycin, CH=Chloramphenicol, APX=Ampiclox, LEV=Levofloxaci

4. Discussion

This study has demonstrated the microbial contamination of ATM keypads located in five (5) communities in Calabar, Cross River state, Nigeria. Group A ATM had the highest bacterial abundance (31.9%), this could be because of the high number of individual that uses the ATM. The area usually is densely populated and close to the Calabar main market with poor sanitation level while Group E ATM is only used by fewer individuals who spend few hours within the environment and with probable good sanitation level had fewer bacteria (9.7%). The high level of microbial load seen in this study, which includes *Escherichia coli, Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhimurium*. *Klebsiella* spp*, and*

Shigella spp. is in line with the study of [10], who reported that keyboards of ATM harboured more bacteria than computer keyboards and this may be because they are exposed to many users.

The percentage distribution of the bacterial isolates showed *Staphylococcus aureus* was the most prevalent isolate with a percentage occurrence of 28.3%. This was followed by *Escherichia coli* (18.6%) *Bacillus subtilis* (17.7%), *Salmonella typhimurium* (9.7%), *Klebsiella* spp. (6.2%), and *Shigella* spp. (5.1%). A similar study in Ebonyi State by [11] revealed *S. aureus* to be the most abundant (28.57%) bacterial isolate followed by *E. coli* (21.43%). The study is also in conformity with [12] who reported that *Staphylococcus aureus* was prevalent.

Staphylococcus aureus is a major component of the normal flora of the skin and nostrils, which probable explain its high prevalence as contaminants, and it can easily be discharged by several human activities like sneezing, talking, and contact with moist skin [13]. The abundance of *E. coli*, an enteric bacterium is indicative of possible fecal contamination. This could also be a pointer to poor hygiene practices by ATM users. *E. coli* can cause diseases such as gastroenteritis, urinary tract infections, septicemia, dysentery, vomiting, stomach cramps, and flatulence. *Proteus* species is known to cause infections like urinary tract infections, cystitis, pyelonephritis, and prostatitis [14]. *P. aerugonisa* has emerged as an important pathogen during the past two decades. It causes 10% and 20% of infections in most hospitals. Patients who spend so much time in healthcare facilities like hospitals are usually at a higher risk of being infected by these organisms, thus leading to an infection.

The presence of *Klebsiella* spp. can expose the users of ATMs in the study area to pneumonia if they do not adhere to good and personal hygiene practices. The ingestion of *S. typhimurium* from contaminated hands causes Salmonellosis [15]. Most infections are spread to people through the consumption of contaminated food. Salmonella infection affects the intestines and causes vomiting, fever, cramping which usually clear up without medical treatment.

It can be revealed from previous researches that, antibiotic susceptibility of bacteria is not constant but dynamic and differs with time and environment [16]. There is the need for periodic screening of common bacterial pathogens for their antibiotic susceptibility profiles in different communities [17]. From Tables 4 and 5, the trend of antimicrobial resistance for Penicillin seems to be on the increase [17, 18]. Generally, most of the bacterial isolates showed a high level of resistance to Nalidixic acid except *Klebsiella* spp. and *Salmonella typhimurium*. Overall, Ciprofloxacin and Quinolone showed the highest efficacy against bacterial isolates followed by Augmentin and Tarivid (Table 4). This corroborates with the reports of [5]. However, Norfloxacin, Amoxil, Rifampicin, and Chloramphenicol seem to be losing the battle against *Bacillus subtilis* as high resistance was recorded in this study (Table 5). This is worrisome because the antimicrobials are known to have broad-spectrum antimicrobial activities [5, 19]. Generally, resistance to known common antimicrobial has been revealed to be on the increase and the need for novel antimicrobials with novel mechanisms of action cannot be overemphasized [20].

5. Conclusion

It is evident from this study that ATM surface is an active vehicle of transmission of pathogenic bacteria and this is a cause for public health concern as some of the bacteria species isolated during the study showed resistance to some antibiotics usually known to be active against these bacteria species in clinical medicine. It is therefore recommended that periodic microbiological surveillance of these ATMs and their accessories should be considered alongside frequent disinfection of these surfaces in order to prevent an outbreak of diseases and other health complications to the users of ATMs within the study area.

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