



Phenotypic Detection of Extended Spectrum β -Lactamase and Metallo- β -Lactamase Produced by *Escherichia Coli* on Automated Teller Machines within Sokoto Metropolis, Nigeria

¹UNGOKORE, HY; ¹OMOLE, OA; ¹NUHU, T; ¹OLOWOOKERE, A; ¹JODI, SAADATU, M; ²ALHASSAN, MA; ³OGUNYINKA, IA; ⁴SHUAIBU, AS

¹Department of Pharmaceutics and Pharmaceutical Microbiology, ²Department of Pharmaceutical and Medicinal Chemistry,

³Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria.

⁴Department of Clinical Pharmacy and Pharmacy Practice, Usman Danfodiyo University, Sokoto

*Corresponding Author Email: ungokorehussain@yahoo.com

ABSTRACT: It is no longer a fallacy that environmental objects are grossly contaminated by pathogenic microbes. ATMs especially which is used on daily basis by thousands of people have been reported to be potential habitat for these microbes. The worst-case scenario is the presence and ease of spread of Multi-Drug Resistant (MDR) and Extended Spectrum Beta Lactamase (ESBL) producing pathogens via these machines as a result of their huge patronage. The prevalence and fast spread of these MDR and ESBL producing strains constitute an emerging public health concern. This study was conducted to determine the prevalence of ESBL and MBL producing *E. coli* isolated on ATMs within Sokoto metropolis. A total of 194 isolates were obtained from the culture samples of 100 ATM swabs. The isolated *E. coli* were subjected to antimicrobial susceptibility tests using the modified Kirby Baeur disc diffusion method on six (6) commercial antimicrobial discs (Oxoid, UK): Ceftazidime (CTZ, 30 μ g), Cefotaxime (CTX, 30 μ g), Gentamycin (CN, 10 μ g), Augmentin(AMC, 30 μ g), Ciprofloxacin(CIP, 5 μ g) and Imipenem(IPM, 10 μ g). The isolates were further screened for ESBL production and phenotypic confirmatory test. Confirmation of MBL production was also performed using antibiotic discs containing two Carbapenems (Imipenem IPM, 10 μ g and Meropenem MEM, 10 μ g). The result was interpreted using CLSI guideline 2015. Proteus spp (43%) were the most frequently isolated bacteria, followed by *Shigella spp* (31%) and *E. coli* 31(16%). Drug Resistant (MDR) ESBL producing *E. coli* of 93.3% and 4% MBL producer was recorded. It can be concluded that MDR and ESBL producing *Escherichia coli* (*E. coli*) are the most prevalent species isolated and that the species isolated are more sensitive to Gentamycin, Ciprofloxacin and Imipenem.

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The Automated Teller Machines (ATMs) as fondly referred to is a product of the 20th century technological invention (Ramesh, 2015) which is used by clients of a financial institution to carry out financial services without the need for a Bank teller, Cashier or Human clerk (Ramesh, 2015). ATMs are the most widely used form of computer driven public technology with an estimated over 2.4 million units in use since their invention and use in the late 1960's (Fatokun *et al.*, 2014). A typical usage of the ATM 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. ATMs have over time become an essential part of our social survival (Mehmet *et al.*,

2013). They are used on daily basis for various financial services ranging from cash transactions to stock exchange transaction and even to bill payments (Nagajothi, 2015). As such, they are used by hundreds of people on a daily basis especially as various governments are introducing cashless policy in various countries. Being constantly in use by different individuals of different hygiene and health status, ATM keypads and touch screens have been reported to act as a potential habitat for microbes, which also help in spreading different diseases (Arulazhagan, 2015). *Escherichia coli*, is one of the most commonly found microbes on the ATMs (Ramesh *et al.*, 2015). It is the most common cause of food and water-borne human diarrhea worldwide causing many deaths in children under the age of five

*Corresponding Author Email: ungokorehussain@yahoo.com

years (Kibret, 2011). It is the leading pathogen causing UTIs and is among the most common pathogens causing blood stream infections, wounds, otitis media and other complications in humans (Khan, 2002). Many ATM users are unaware of the presence of these pathogenic bacteria which is capable of spreading different contagious diseases on touch screens and key pads (Arulazhagan, 2015). As such, they pay little or no attention to proper sanitary measures following the use of these machines. This poor hygienic habit aids the spread of these pathogenic microbes. The more worrisome situation is the possibility of existence and easy spread of Multi Drug Resistant (MDR) and Extended Spectrum Beta Lactamase (ESBL) producing strains of these pathogens on these ATMs.

Antimicrobial resistance in *E. coli* has been reported worldwide and increasing rates of resistance among *E. coli* is a growing concern in both developed and developing countries (Bell, 2002); (El Kholy, 2003). As such, rise in bacterial resistance to antibiotics complicates treatment of infections. One of the ways in which bacteria develop resistance is through the production of enzymes. Prominent among them is Beta-lactamases produced by these pathogenic microbes to overcome the activity of beta-lactam group of antibiotics. They pose the greatest resistance ability even to last resort of antibiotics including carbapenems (Ramesh, 2015). Occurrence and susceptibility profiles of *E. coli* show substantial geographic variations as well as significant differences in various populations and environments (Erb, 2007). Our research is aimed at determining the prevalence of *E. coli* contamination, as well as the phenotypic detection of ESBL and MBL producing *E. coli* on ATMs within Sokoto metropolis.

MATERIALS AND METHODS

Study Area: Sokoto metropolis is the capital city of Sokoto State, located in the northwestern part of Nigeria. The state is semi-arid, located in the extreme North-western Nigeria (between Longitudes 4°8 E and 6°54E and latitudes 12°N and 13° 58 N). It is the sixteenth largest state in the country and covered a total land area of about 32,000 square Km, with the estimated population of about 2.4 million (NPC 2008)

Sample Collection and Processing: In this study, samples were collected from 100 ATM points between April and December, 2017 using sterile swab sticks. Prior to the start of the study clearance

was obtained from the management of all included banks. Distinct sterile swab sticks moistened with normal saline was rubbed on the ATM keypads and/or touch screen of the ATM and then returned back into its casing, labelled appropriately and then transported to the Pharmaceutical Microbiology Laboratory in an ice pack laboratory without delay. In the laboratory the swabbed samples were streaked on plates of McConkey's agar media, incubated at 37°C and examined for growth after 24 hours. Mixed growth was further subcultured to obtain pure colonies. The colonies were identified phenotypically by colony characteristics, Gram staining and biochemical tests as per standard protocol (Cheesbrough, 2006).

Antibiotic Susceptibility Testing: Antimicrobial susceptibility was determined using the modified Kirby Baur disc diffusion method. Six (6) commercial antimicrobial discs (Oxoid, UK) used were: Ceftazidime (CTZ, 30 μ g), Cefotaxime (CTX, 30 μ g), Gentamycin (CN, 10 μ g), Augmentin (AMC, 30 μ g), Ciprofloxacin (CIP, 5 μ g) and Imipenem (IPM, 10 μ g). The inhibition zone diameter around the discs were measured and interpreted according to CLSI guideline 2015 interpretation after 24 hours incubation of the plates at 37° C.

Screening and Phenotypic Detection of ESBL: The isolates were screened for ESBL production. Phenotypic confirmatory test was then carried out using CLSI guideline 2015. Confirmation of ESBL phenotype was performed by Double Disc Synergy Test (DDST) using antibiotic discs containing two 3rd generation Cephalosporin (CTZ, 30 μ g and CTX, 30 μ g) and Augmentin (AMC, 30 μ g). Using a sterile needle, CTZ and CTX were placed at a distance of 20mm center to center from AMC on Mueller Hinton Agar. The plates were then incubated at 37°c for 24 hours after which they were examined for an extension of the edge of zone of inhibition of antibiotic disc towards the AMC. Enhancement of zone of inhibition of either or both the CTZ and CTX towards the AMC disc is indicative of ESBL production.

Phenotypic Detection of MBL: Confirmation of MBL was performed using antibiotic discs containing two Carbapenems (Imipenem IPM, 10 μ g and Meropenem MEM, 10 μ g). Using a sterile needle, the discs were placed in pair at 25mm from each other. Using a micropipette, 10 μ l of EDTA was place on one each of the IPM and MEM disc on the same side. The plates were then incubated at 37°C for 24 hours after

which the zone of inhibition was measured and results interpreted according to CLSI guideline 2015.

Data analysis: Sigma plot version 14.0 was used to compute and analyze the data. Descriptive analysis was carried out by calculating frequencies and percentages.

RESULTS AND DISCUSSION

A total of 194 isolates were found to be Gram negative rods of which 31 were *E. coli*. The result of the characterization and identification is as shown in the figure 1 to 4. With this research, an attempt was made to study the prevalence of pathogens at ATM centers within Sokoto metropolis. The study was conducted to create awareness to the public and users of ATM machines on the possibility of contracting diseases from the machines if proper hygienic measures are not taken. The result of this study showed gross bacterial contaminations on the surfaces of the user interface of ATMs within Sokoto metropolis, our results indicate that *Proteus spp* (43%) were the most frequently isolated bacteria on ATMs, followed by *Shigella spp* (31%) and *E. coli* (16%) respectively. This result is in agreement with the results obtained by Okoro *et-al* 2018. The presence of the latter organism on these surfaces indicates the possible existence of fecal contaminations.

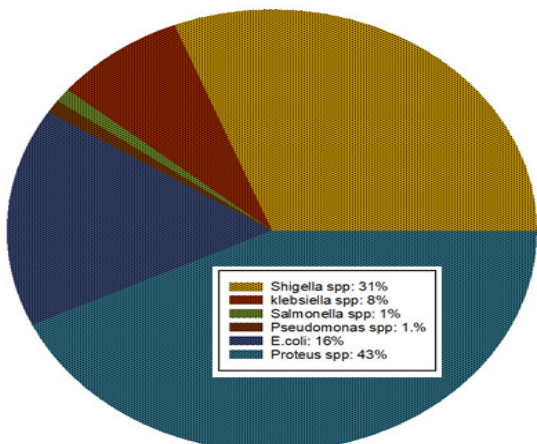


Fig. 1: Distribution of isolates found on ATMs within Sokoto metropolis

The inference of this is that the handling of ATMs could be a probable cause of food poisoning when infected hands are used in eating and food preparation without proper hygiene of hand washing. This study also confirms a high frequency of Multi Drug Resistant (MDR) ESBL producing *E. coli* of 93.3% although only 4% MBL producer was recorded. The isolated *E. coli* species showed gross resistance to Cefotaxime and Ceftazidime and are

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most sensitive to Gentamycin, Ciprofloxacin and Imipenem. Our result is in unison with the result of (Ramesh, 2015) in which Meropenem (a Carbapenem) was found to be the most effective while Cefotaxime and Ceftazidime showed 75% and 61% respectively against *E. coli* isolated from ATMs located in Vellore, Tamil Nadu. It also corresponds with the result of (Fatokun., 2014) in which Gentamicin was found to be the most effective agent against *E. coli* found on Automated Teller Machines (ATMs) in Ebonyi State, Nigeria. The discovery of these MDR ESBL producing *E. coli* and other pathogens at highest percentage reveal the possibility for easy spread of most common diseases. There is especially a great risk of spreading antibiotic resistant bacteria through contact with such highly contaminated public devices like ATM machines.

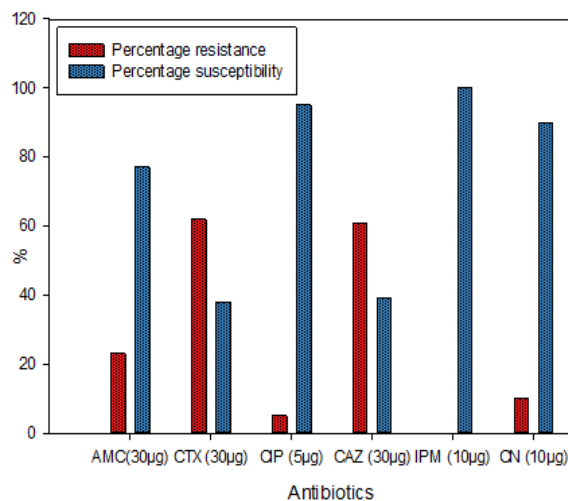


Fig 2: Chart showing the antibiotic susceptibility profile of 30 *E. coli* obtained from ATMs within Sokoto metropolis

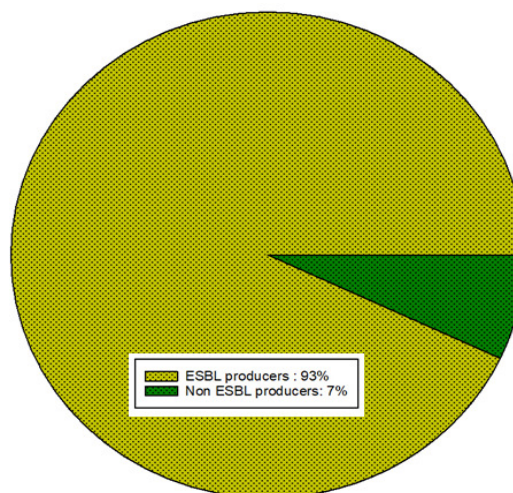


Fig. 3: Percentage distribution of ELBLs producing strains of *E. coli* using Cefotaxime, Augmentin and Ceftazidime

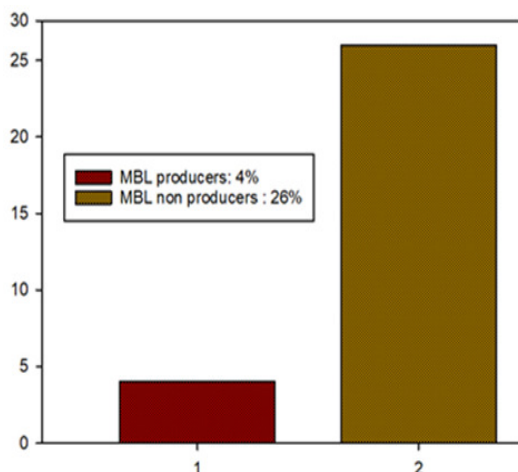


Fig. 4: Distribution of MBLs producing strains of *E. coli* using Imipenem and Meropenem

This can be overcome only when we maintain personal hygiene like washing hands regularly using soap or alcohol. The regular wiping of the screens and keypads of the ATMs using disinfectant may also help in reducing the spread of MDR pathogens. Our study established the existence of *E. coli* and other Gram-negative isolates on the surface of the keypad and touch screen of ATMs within Sokoto metropolis. Very high percentages of the *E. coli* isolated were confirmed to be ESBL producers. Co-production of ESBL and MBL by the *E. coli* was also observed. All isolates (100%) were sensitive to Imipenem.

Conclusion: There is a high frequency of Multi Drug Resistant (MDR) ESBL producing *E. coli* on ATM within Sokoto metropolis and as such proper hygienic precautions should be taken in order to avoid the spread of these MDR and ESBL producing pathogenic microbes. This can be made feasible by proper hand cleaning regimen using appropriate sanitizers. The surfaces of ATM devices should also be regularly cleaned. Washing of hands after the use of ATMs should be promoted.

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