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# Genetic relatedness in extended-spectrum beta-lactamase-producing *Escherichia coli* from clinical isolates using enterobacterial repetitive intergenic consensus polymerase chain reaction

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## ABSTRACT

**Objectives:** The aim of this study is to determine the genetic relatedness of extended-spectrum beta-lactamases (ESBL)-producing *Escherichia coli* using the enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC-PCR) technique.

**Methods:** Suspected Gram-negative bacteria with their identities from the clinical samples were confirmed using Microgen GN-A-ID Kit. The double-disc synergy test was used to confirm for ESBL-producing *E. coli.* The susceptibility of the organisms was tested against eleven antimicrobial agents. A singleplex PCR assay was carried out targeting TEM, SHV, CTX-M, and OXA. ERIC-PCR performed, and band patterns obtained were visually evaluated. A dendrogram of the ERIC-PCR fingerprint pattern was done with the aid of DendroUPGMA using the cluster method.

**Results:** Of the 576 clinical samples collected, 23 isolates were confirmed *E. coli*, and all (100%) are ESBL producers. The highest antibiotic resistance rate was recorded in cefixime (95.6%), and the least was amikacin (17.4%). The predominant ESBL gene is *bla*TEM genes (95.6%). Gel analysis of ERIC-PCR revealed 1–6 bands. The profiles of the ERIC-PCR differentiated the 23 *E. coli* isolates into four ERIC cluster types.

**Conclusion:** More than 80% of the isolates are sensitive to amikacin, with greater than 95% harboring *bla*TEM genes. Overall, ERIC obtained from the clinical specimens indicated some evidence in the genetic relatedness of the ESBL genes among *E. coli* isolates.

**Keywords:** Antimicrobial resistance, *Enterobacteriaceae*, Enterobacterial Repetitive Intergenic Consensus-Polymerase chain reaction, Extended-spectrum beta-lactamases, *Escherichia coli*

## Introduction

One of the main approaches to modern medicine used to combat infections is the use of antibiotics. A defining moment in humanity's history that revolutionized medicine and saved uncountable lives was the discovery of antibiotics. The "golden era" of antibiotics from the 1930s to 1960s gave rise to many new antibiotics.[1] Unfortunately, these "magic bullets" have been accompanied by the emergence of antibiotic-resistant pathogens.[2]

The production of enzymes that hydrolyze antibiotics is one of the ways bacteria circumvent the effect of some antibiotics.[3] An example of these enzymes is beta-lactamases. There are two general classification schemes for beta-lactamases: The Bush-Jacoby-Medeiros functional classification system and the Ambler molecular classification scheme.[4-6] Based on the Ambler molecular classification scheme, beta-lactamases are divided into four major classes (A–D), and the protein homology (amino acid similarity), and not phenotypic characteristics are observed to be the basis of this classification scheme. Beta-lactamases A, C, and D are the serine beta-lactamases in the Ambler classification, and the Class B enzymes are metallo-beta-lactamases. However, the Bush-Jacoby-Medeiros classification scheme used functional similarities (substrate and inhibitor profile) to classify beta-lactamases, with four

major groups and various subgroups in this system. As a result of consideration of beta-lactamase inhibitors and beta-lactam substrates that are clinically relevant, Paterson and Bonomo noted that the classification of Bush-Jacoby-Medeiros scheme is of much more direct importance to the microbiologist or physician in a diagnostic laboratory.[7]

One of the major breakthrough in the battle against beta-lactamase-mediated bacterial resistance to antibiotics is the introduction of the third-generation cephalosporins into clinical practice in the early 1980s.[7,8] In addition to effective activity against most beta-lactamase-producing organisms, the third-generation cephalosporins had significant advantage of lessened nephrotoxic effects compared to polymyxins and aminoglycosides.[7] However, the new generation of antibiotics also gave rise to new strains of antibiotic-resistant bacteria.

Extended-spectrum beta-lactamases (ESBLs) are a collection of plasmid-encoded enzymes that confer resistance to the third-generation cephalosporins.[9] Bush noted that there is no unanimity on the exact definition of ESBLs.[10] A generally used description is that ESBLs are beta-lactamases that have the capacity of conferring bacterial resistance to the penicillins, first-, second-, and third-generation cephalosporins, and aztreonam (but not the cephamycins or carbapenems) by hydrolyzing these antibiotics. They are inhibited by beta-lactamase inhibitors such as clavulanic acid.[10] However, the worldwide exponential increase in beta-lactamase resistance which is attended by a substantial increase in the prevalence of ESBL-producing *Enterobacteriaceae* has been reported over the past two decades.[9]

The first step in the curtailment of infectious disease is the rapid and accurate identification of pathogens involved in the infection.[11,12] Phenotypic methods such as biotyping, phage typing, serotyping, and antibiotic resistance patterns have been used in the past to characterize bacterial strains. The traditional techniques were followed by molecular approaches to evaluate genotypic differences directly in prokaryotes. Restriction fragment investigation of genomic DNA by conventional agarose gel electrophoresis or pulsed-field gel electrophoresis as well as plasmid profiles generation are some of the molecular methods used.[13] Apart from genomic fingerprinting, polymerase chain reaction (PCR) methodologies employing enterobacterial repetitive intergenic consensus (ERIC) sequences as PCR primer binding sites could be used to study the distribution of repetitive sequences in different genomes.[13] It implies that bacteria vary in repeated sequences. Hence, this technique yields diverse configurations of definite primers in electrophoresis. Therefore, the ERIC-PCR technique made it so that primers are designed to attach to repetitive sequences and increase the reserves among those attached primers to repetitive arrangements.[13,14] Despite being defined as intergenic repetitive units, ERIC sequences vary from other bacterial repeats. Such sequences are spread through a wide variety of species and have remained only in intergenic regions, seemingly only inside copied or transcribed regions.[15] The basis for comparison between species and strains as reported is copy number differences, which implies that orthologous intergenic regions may have an ERIC arrangement in one species but not the other.[15]

Globally, ERIC-PCR has also been indicated for typing epidemic organisms and other enteric bacteria for epidemic detection.[16] The sequences have been used as the basis of a method for fingerprinting bacterial genomes.[13] Therefore, the working principle of ERIC-PCR is PCR primers which were made to amplify between duplicates of the ERIC sequence at neighboring positions in the bacterial genome,[15] and the technique was found to give results in a wide range of bacterial species.[13] Bakshi *et al*. observed that the previous studies focused on the ability of ERIC-PCR to distinguish between strains of same or closely related species.[16] Their findings have shown that ERIC-PCR could discriminate between different species members.[17] ERIC-PCR fingerprints are considerably easier to examine, rapid and practical, suited to most modern, well-equipped laboratories. As a result of its accuracy, simplicity, rapidity, and economy, this technology was generally used in strain identification and traceability investigation of disease causing organisms.[18] In addition to strain identification and traceability, the technique has been used for studying other bacterial species and even eukaryotes.[15] ERIC-PCR provides more sensitive typing results than basic phenotyping methods, hence its suitability to its traditional role in distinguishing closely associated members of a genus or typing within species rather than overall identification of bacteria.[17] It has been noted that one of the pitfalls of ERIC-PCR is the inability of dendrogram analysis to provide flawless information on relatedness in some cases.[17]

Although our study is the first reported work examining the genetic relatedness of ESBL-producing *Escherichia coli* using ERIC-PCR in Northwest Nigeria, a related work was done in Nigeria on ERIC-PCR as a tool for genetic classification of bacterial isolates.[17] Therefore, the present study was carried out as an important pioneer of a study to understand the genetic similarity of ESBL-producing *E. coli* from clinical samples using the ERIC-PCR technique.

## Methods

### Study area

Sokoto State is located in extreme Northwest Nigeria, near the Sokoto River's confluence and the Rima River, with an average annual temperature of 38.3°C. It shared border with Niger Republic in the north, to the east with Zamfara State, while it shared borders with Kebbi State in the south and western parts. The majority of the indigenes are farmers and nomads who engaged in animal rearing.

This study was carried out in the Microbiology Department, Usmanu Danfodiyo University Teaching Hospital (UDUTH) Sokoto. UDUTH Sokoto is a tertiary health institution and a teaching hospital for Usmanu Danfodiyo University Sokoto. It has a 500-bed capacity with clinical departments, including pediatrics, general outpatient clinic, medical outpatient clinic, surgical outpatient clinic, radiotherapy, neurosurgery, anesthesiology, dental, and psychiatry, among others. The hospital's laboratories include hematology and blood transfusion, microbiology/parasitology, chemical pathology, and histopathology/morbid anatomy. Post basic training is also conducted in the hospital. UDUTH Sokoto provides tertiary health-care services to neighboring Zamfara and Kebbi States, entire Northwest Nigeria, and neighboring Niger Republic (info@uduth.org.ng).

## Collection of sample, isolation of bacteria, and their identification

The Health Research Ethics Committee (HREC) of UDUTH Sokoto gave approval for this study. The main objective of the study was explained to patients on an individual basis. Participants who indicated intention to participate signed or thumb print an informed consent form before their sample was collected. Patients were equally informed that participating in the research is voluntary, and their anonymity was highly assured.

The total sum of 576 clinical samples from vaginal swab, pus, stool, aspirate, wound swab, and urine was collected from patients at UDUTH, Department of Microbiology, between January and June 2019. Only samples from outpatients were collected, with only suspected Gram-negative bacteria considered. A single isolate is only considered from the same patient. All participants were 18 years and above, and those who declined participation were excluded from the study.

Mueller-Hinton agar (MHA) plates were used for culturing the isolates, while Gram staining was used to screen the isolates, their identities were confirmed using Microgen GN-A ID identification kit UK, as described by the manufacturer. The isolates were stored in Mueller-Hinton broth (MHB) (Oxoid, UK) at −20°C for further analysis.

## Detection of ESBL-positive *E. coli*

All *E. coli* isolates showing reduced sensitivity to ceftazidime and cefotaxime were screened for the production of ESBL. ESBL screening was performed using the two 3$^{rd}$-generation cephalosporins (cefotaxime and ceftazidime) on MHA. The double-disc synergy test (DDST) as described by Tsering *et al*. (2009)[19] was used to confirm ESBL production in *E. coli* isolates. The bacterial inoculum concentration for all potential ESBL producers from the screening test was adjusted to 0.5 McFarland turbidity standard. A sterilized cotton swab was dipped into the suspension of organism and then inoculated over the surface of MHA plates. Cefotaxime (CTX, 30 μg) and ceftazidime (CAZ, 30 μg) discs were placed on the inoculated MHA at a space of 30 mm center to center from a combination disc of ampicillin/sulbactam (SAM, 10/10 μg). The results were interpreted using the European Committee for Antimicrobial Susceptibility Testing (EUCAST) guidelines to detect resistance mechanisms and specific resistances of clinical and epidemiological significance.[20] An extension of the cefotaxime inhibition zone's edge and ceftazidime toward ampicillin/sulbactam disc after incubation for 24 h at 37°C aerobically indicated production of ESBL in *E. coli* isolates.

## Determination of antibiotic susceptibility of *E. coli* isolates

The modified Kirby–Bauer agar disc diffusion method was used to determine the susceptibility of *E. coli* against 11 antimicrobial agents. The antimicrobial agents tested include gentamicin (CN) (10 μg), amikacin (AMK) (30 μg), ampicillin/sulbactam (SAM) (10/10 μg), cefotaxime (CTX) (30 μg), ceftazidime (CAZ) (30 μg), cefixime (CFM) (5 μg), meropenem (MER) (10 μg), imipenem (IPM) (10 μg), ciprofloxacin (CIP) (5 μg), nitrofurantoin (F) (300 μg), and chloramphenicol (CMN) (30 μg). Antibiotic discs were sourced from Oxoid, UK.

A fresh subculture of isolates was prepared on MHA (Oxoid, UK). Two well-discrete colonies of similar appearance were picked and transferred into the tube of sterile 0.9% normal saline with a wire loop aid. The inoculum was emulsified inside the tube. The bacterial concentration was then adjusted to 0.5 McFarland. After 15 min of preparing the adjusted inoculum, a sterilized cotton swab was immersed into the inoculum. The swab was rotated a number of times and pressed gently on the inside of the tube above the fluid level to get rid of surplus inoculums from the swab. The discs were placed 30 mm center to center on the agar surface using a sterilized disc dispenser. *E. coli* ATCC 25922 standard strain was used as a control.

At 37°C following 24 h of incubation, the inhibition zone's diameter was measured and interpreted according to Clinical and Laboratory Standards Institute guidelines.[21] All measurements were made with the unaided eye while viewing the back of the Petri dish.

## Extraction of bacterial genomic DNA

Phenotypically confirmed ESBL-producing *E. coli* isolates were prepared by inoculating two colonies into 5 ml freshly prepared Luria Bertani (LB) broth (Oxoid, UK) and incubated at 37°C in a shaker (200 rpm) for 24 h as described by Nuhu *et al*., 2020.[12] Extraction of DNA was by boiling method as described by Barbosa *et al*., 2015.[22]

## ESBL genes detection by PCR

Amplification of resistant DNA fragments was carried out using Nexpro™ DNA polymerase (Genelabs, Korea). After

thawing, Nexpro PCR master mix (2X) was vortexed and centrifuged for 30 s at 6000 rpm. A singleplex PCR assay targeting four ESBL genes of CTX-M, OXA, SHV, and TEM were carried out. The thermal cycling conditions have initial denaturation at 95°C for 3 minutes, then 35 cycles at 95°C for 30 seconds. The annealing temperature was 53°C for 30 seconds, extension was 72°C for 30 seconds and then 72°C for 5 minutes for final extension. The final hold was 4°C [Table 1].

### Enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR) of ESBL-producing *E. coli*

The extracted DNA of ESBL-producing *E. coli* was used to perform ERIC-PCR. The thermal cycling conditions were modified; 95°C for 7 min (initial denaturation), then 35 cycles at 90°C for 30 s, 52°C for 1 min (annealing), 65°C for 8 min (extension), and 62°C for 16 min (final extension). The primers used were those described by Versalovic *et al*. (1991)[13] [Table 1]. To verify the experiment's reproducibility, the ERIC-PCR reaction was repeated 3 times for each strain of the organism. Amplicons were visualized after electrophoresis at 50 V, 400 mA for 1 h in 1.5% agarose gel prepared in 0.5% TBE using the bioimaging machine. The obtained band patterns were visually evaluated, and the location of each band was identified as described by Codjoe *et al*.[23] A dendrogram of ERIC-PCR fingerprint pattern of ESBL-producing *E. coli* was constructed with the aid of DendroUPGMA (http://genomes.urv.es/UPGMA/) using the unweighted pair group method with arithmetic mean (UPGMA) cluster method.

## Results

### Isolation and phenotypic detection of ESBL production in *E. coli*

During the study period, 576 clinical samples were analyzed. A total of 339 were sourced from male patients accounting for 58.8%, while female patients had 237 with 41.1%. The confirmed *E. coli* isolates from the clinical samples were 23, and their distribution was only from vaginal swab (4%), stool (35%), and urine (61%), as shown in Figure 1.

Out of the 23 *E. coli* isolates, all 23 were ESBL producers by ESBL screening and DDST.

### Antibiotic susceptibility of ESBL-producing *E. coli* isolates

Figure 2 shows the antimicrobial resistance (AMR) in the ESBL-producing *E. coli* isolates. About 95.6% and 91.3% of *E. coli* isolates were resistant to cefixime and cefotaxime, respectively. High resistance to ampicillin/sulbactam, ciprofloxacin, and chloramphenicol with 82.6% each, 73.9% for ceftazidime, 69.6% for gentamicin, 56.5% for nitrofurantoin, 47.8% for imipenem, and 43.5% for meropenem was observed. About 82.6% of the isolates were susceptible to amikacin.

### Molecular detection of ESBL genes

The main ESBL gene in this study was blaTEM, found in 22 isolates (95.6%). The blaSHV gene was found in 9 isolates (39.1%), whereas blaCTX-M and blaOXA were found in 7 isolates each (30.4%). Some isolates (39.1%) showed coexistence of greater than 1 gene, while 26.1% of the ESBL-producing *E. coli* harboring blaTEM, blaSHV, blaCTX-M, and blaOXA. The coexistence of three and two genes was also obtained in 4.3% and 8.7% of the isolates, respectively, while 60.9% of isolates harbored only one ESBL gene.

### ERIC-PCR analysis of *E. coli*-producing ESBL

The ERIC-PCR gel analysis of ESBL-producing *E. coli* revealed 1–6 bands. According to ERIC-PCR fingerprinting,

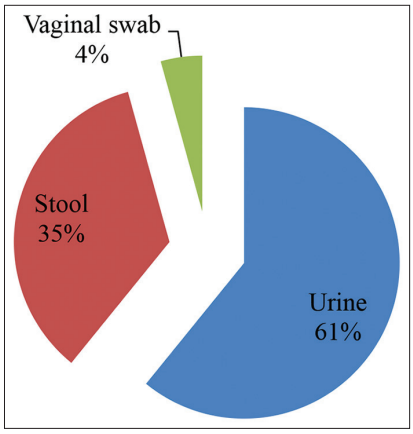


**Figure 1:** Distribution of *E. coli* isolates in clinical samples

**Table 1:** Oligonucleotides sequence for the detection of ESBL genes and ERIC-PCR

| Genes | Nucleotides sequence 5'–3' | Size (bp) | Reference |
|---|---|---|---|
| CTX-M | TGCGATGTGCAGTACCAG-F<br>ATCGTTGGTGGTGCCATA-R | 544 | Adapted from[24] |
| OXA | ACACAATACATATCAACT-F<br>GTGTTTAGAATGGTGATC-R | 813 | Adapted from[24] |
| SHV | TGTCGCTTCTTTACTCGC-F<br>TATGGCGTTACCTTTGAC-R | 490 | Adapted from[24] |
| TEM | ATGAGTATTCAACATTTC-F<br>TTACCAATGCTTAATCAG-R | 840 | Adapted from[24] |
| ERIC-1R | ATGTAAGCTCCTGGGGATTCAC | - | [13] |
| ERIC-2 | AAGTAAGTGACTGGGGTGAGCG | | |

ERIC: Enterobacterial repetitive intergenic consensus

the 23 *E. coli* isolates' genotyping profile of the fingerprint patterns involved is above 200 bp–1500 bp. Predominant bands included 250 bp and 500 bp.

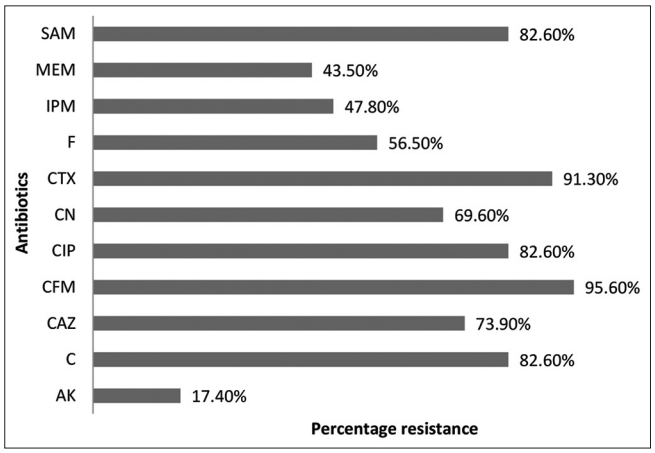


**Figure 2:** Antibiotic susceptibility testing result of the extended-spectrum beta-lactamases-producing *E. coli* using modified Kirby–Bauer disc diffusion method. Key: SAM = Ampicillin/sulbactam, MEM – Meropenem, IPM = Imipenem, F = Nitrofurantoin, CTX = Cefotaxime, CN = Gentamycin, CIP = Ciprofloxacin, CFM = Cefixime, CAZ = Ceftazidime, C = Chloramphenicol, AK = Amikacin

The ERIC-PCR profiles allowed differentiating the 23 *E. coli* isolates into four ERIC types, which are grouped into four clusters (CL1–CL4) based on the dendrogram analysis. A bulk of *E. coli* isolates are found in CL2 (47.8%), which has three isolates that all produced blaCTX-M, blaOXA, blaSHV, and blaTEM, followed by CL3 (26.1%), then CL1 (17.4%), and CL4 (8.7%). The cluster analysis and genetic diversity of these organisms are shown in Table 2 and Figure 3. ERIC data obtained also depicted little connection between the clinical source of the isolates and the fingerprint profile. In the case of CL1, for example, three isolates out of the four are from urine, while in CL3, 50% are from urine and 50% from the stool. The fingerprint profile in CL2 depicted that 36.4% of the isolates are from the stool, while 63.6% from urine.

## Discussion

The study aimed at determining the genetic relatedness of ESBL-producing *E. coli* using the ERIC-PCR technique. There are no reported studies on ERIC-PCR to determine the relationship among ESBL-producing isolates in Nigeria. As such, the study will be the first in Sokoto and Nigeria.

**Table 2:** Distribution of ESBL genes by PCR among *E. coli* isolates with their corresponding resistance pattern using modified Kirby–Bauer disc method and their cluster

| S/N | ID | Source | Antimicrobials resisted | ESBL genes detected | | | | No. ESBL | Cluster |
|---|---|---|---|---|---|---|---|---|---|
| | | | | CTX-M | OXA | TEM | SHV | | |
| 1 | *Ec1* | Stool | MEM, CTX, CFM, CIP, SAM, AK, C, CAZ | + | + | − | + | 3 | CL1 |
| 2 | *Ec2* | Urine | CTX, CFM, F, CIP, CN, SAM, CAZ | − | − | + | + | 2 | CL3 |
| 3 | *Ec3* | Urine | MEM, CTX, CFM, F, CIP, CN, SAM, AK, C, CAZ, IPM | + | + | + | + | 4 | CL4 |
| 4 | *Ec4* | Urine | CTX, CFM, F, CIP, CN, SAM, C, CAZ, IPM | + | + | + | + | 4 | CL1 |
| 5 | *Ec5* | Stool | CTX, CFM, F, CN, SAM, C, CAZ | + | + | + | + | 4 | CL2 |
| 6 | *Ec6* | Urine | MEM, CTX, CFM, CIP, CN, SAM, C, CAZ, IPM | + | + | + | + | 4 | CL2 |
| 7 | *Ec7* | Stool | CFM, F, CN, SAM, C, CAZ | − | − | + | − | 1 | CL3 |
| 8 | *Ec8* | Urine | CTX, CFM, F, CIP, CN, SAM, C, CAZ, IPM | − | − | + | − | 1 | CL3 |
| 9 | *Ec9* | Urine | MEM, CTX, CFM, CIP, SAM, AK, C, IPM | − | − | + | − | 1 | CL2 |
| 10 | *Ec10* | Stool | MEM, CTX, CFM, F, CIP, SAM, C | + | + | + | + | 4 | CL3 |
| 11 | *Ec11* | Urine | CTX, CFM, CIP, SAM, CAZ, IPM | − | − | + | − | 1 | CL1 |
| 12 | *Ec12* | Urine | MEM, CTX, CFM, CIP, CN, C, CAZ | − | − | + | − | 1 | CL1 |
| 13 | *Ec13* | Stool | CTX, CFM, F, CIP, CN, SAM, C, CAZ, IPM | − | − | + | − | 1 | CL3 |
| 14 | *Ec14* | Urine | CTX, CFM, F, CIP, CN, CAZ | − | − | + | − | 1 | CL2 |
| 15 | *Ec15* | Urine | MEM, CTX, CFM, F, CIP, CN, C, CAZ, IPM | − | − | + | − | 1 | CL2 |
| 16 | *Ec16* | Urine | CTX, CFM, CN, SAM, C, CAZ, IPM | − | − | + | − | 1 | CL2 |
| 17 | *Ec17* | Stool | CTX, CFM, CIP, CN, SAM, CAZ, IPM | − | − | + | − | 1 | CL2 |
| 18 | *Ec18* | Urine | CTX, CFM, CIP, SAM, C, CAZ | − | − | + | − | 1 | CL3 |
| 19 | *Ec19* | HVS | MEM, CTX, CFM, F, CIP, CN, SAM | − | − | + | − | 1 | CL4 |
| 20 | *Ec20* | Urine | CTX, CFM, F, C | − | − | + | − | 1 | CL2 |
| 21 | *Ec21* | Stool | CTX, CFM, F, CIP, CN, SAM, C, CAZ | − | − | + | − | 1 | CL2 |
| 22 | *Ec22* | Stool | MEM, CTX, CFM, CIP, SAM, AK, C, IPM | − | − | + | + | 2 | CL2 |
| 23 | *Ec23* | Urine | MEM, CTX, CFM, CIP, SAM, C, CAZ, CN | + | + | + | + | 4 | CL2 |

The results for antimicrobial resisted presented above were part of the results in one of our study Nuhu *et al*., [12] KEY: CL = Cluster, *Ec* = *Escherichia coli*, AK = Amikacin, C = Chloramphenicol CAZ = Ceftazidime, CFM = Cefixime, CIP = Ciprofloxacin, CN = Gentamycin, CTX = Cefotaxime, F = Nitrofurantoin, IPM =Imipenem, MEM = Meropenem, SAM = Ampicillin/sulbactam

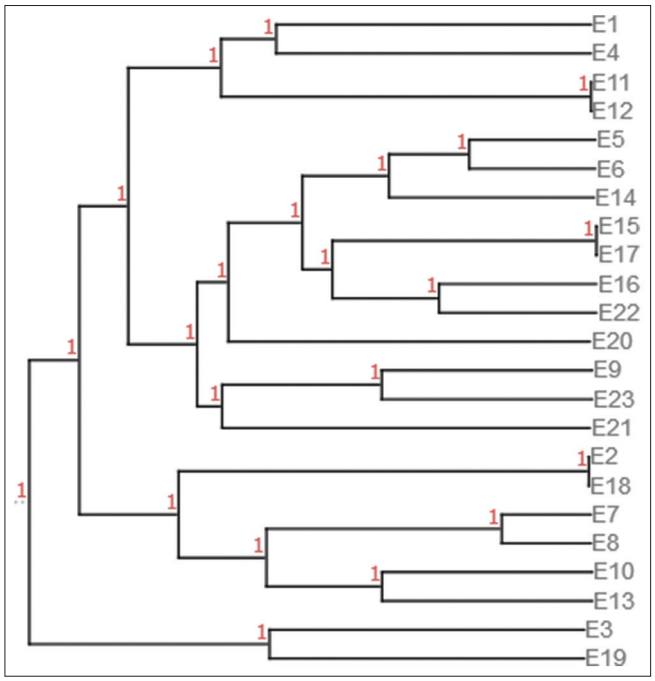


**Figure 3:** Dendrogram of enterobacterial repetitive intergenic consensus sequences using simple match similarity matrix clustered by the UPGMA

*E. coli* belong to the family of *Enterobacteriaceae,* which is a Gram-negative bacterium known to be a general commensal flora in humans as well as in many animal species.[25,26] *E. coli* was considered for this study because it is one of the essential opportunistic bacteria. They are predominantly facultative anaerobe and commensal microbiota,[27] known for many infectious diseases such as abdominal pain, vomiting, diarrhea, hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura.[26] *E. coli* is also a well-studied pathogen among the Gram-negative bacteria that play a vital position or role in the community, and nosocomial infections.[28] Specimens from which *E. coli* isolates were obtained may contain commensal or pathogenic *E. coli*. This study did not consider the pathogenicity of *E. coli*. Our aim was only on ESBL-producing *E. coli*. Additional investigation may be conducted to ascertain the pathogenic and commensal *E. coli* and serve as a basis for comparison.

Recently, there have been significant reports on the prevalence of ESBL-producing Gram-negative bacteria in Nigeria,[12,29] with most studies on *E. coli*[30,31] sourced from the hospital community. The detection of ESBLs using DDST method revealed that 100% of *E. coli* isolates are ESBL producers. Although, several studies have reported the prevalence of ESBL can differ from one geographic setting or another, or even from one health-care location to another, and for a specific place over a period of time.[12,32,33] This finding is the highest so far from studies in Nigeria. However, there are studies in which high rates were recorded in Nigeria. For example, in Oyo State, Southwest Nigeria, a prevalence of 79.6% was recorded[34] using DDST, while a 67.7% prevalence was recorded in a study in Enugu State, Southeast Nigeria, recorded a prevalence of 67.7%.[35] However, a study from Bauchi State, Northeast Nigeria, reported the highest prevalence of 82.3% of the isolates harbored ESBL genes in *E. coli*-producing ESBL.[36] With these data and the absence of newer antibiotics in the pipeline to treat Gram-negative bacteria producing ESBL[37] and the upsurge in ESBL-producing *E. coli* in Nigeria, therefore, a necessity for antibiotic stewardship and control use of antibiotics can never be overemphasized. Thus as noted by Dhillon and Clark, ESBLs are predominantly produced by Gram-negative *Enterobacteriaceae* with prominence in *E. coli* and *Klebsiella pneumoniae,* thereby harboring the ESBL enzymes chromosomes or mobile genetic elements (plasmids) [37]. Therefore, infections as a result of ESBL-producing *E. coli* can be a serious threat due to its inherent failure to manage diseases and ease of spread.

Another implication of these organisms harboring ESBLs genes on the plasmids is the ability of gene transfer from one organism to another, invariably leading to a more significant number of antibiotic-resistant pathogens. ESBL genes are known to carry additional resistant determinants for tetracyclines, aminoglycosides, chloramphenicol, trimethoprim quinolones, and sulfamides.[38]

Studies have shown that the effective usage of an antimicrobial drug is rendered of less use as a result of possible tolerance or development of resistance from the 1st time this antimicrobial is used. From our study, amikacin, an aminoglycoside, is effective on most *E. coli*-producing ESBL isolates. The rate at which these organisms are resistant to amikacin in this study is as low as 17.4%. Many studies have reported a similar rate of resistance both within and outside Nigeria. For example, Iliyasu *et al*. reported ESBL-producing *E. coli* to be resistant to the action of amikacin by 28.3% in Nigeria in 2018.[36] Lower resistance was observed against amikacin in studies from Ethiopia (13.1%),[39] Nigeria (4%),[40] and Burkina Faso (12.3%).[41] Studies from Nigeria[42] and Qatar[9] reported 100% sensitivity to amikacin of the isolates producing ESBL. The low resistance of *E. coli*-producing ESBL to amikacin from our study might be attributed to the low level of usage to manage infections as a result of these organisms. Furthermore, a wide clinical practice with amikacin in the management of infections due to microbes producing ESBL is lacking. If amikacin is to be use, extra caution should be taken to prevent resistance.

Previously, the carbapenems are the preferred drugs in the management of infections as a result of ESBL-producing organisms. Carbapenems are active against ESBL-producing organisms and show tremendous *in vitro* activity on *Enterobacteriaceae* organisms producing ESBLs.[32,39] Interestingly, our study showed that the organisms' resistance to meropenem was 43.5% and imipenem was 47.8%. The emergence of carbapenem-resistant *Enterobacteriaceae* may be due to rise in the use of carbapenems to treat organisms

producing ESBL. It is believed that the level of this resistance might result to a likely treatment failure or impasse.

Beta-lactamase inhibitors (clavulanic acid, sulbactam, or tazobactam) usually inhibit the hydrolyzing effect ESBL-producing organisms. These combinations have been considered for the management of infections for organisms producing ESBL.[43] This study showed that the beta-lactam/beta-lactamase inhibitor was also highly resisted by the microorganisms. The combination of ampicillin/sulbactam used was resisted in 82.6% of the strains. A related study in Ghana, similar to high resistance to ampicillin/sulbactam, was recorded.[44] Based on our findings, there is limited clinical experience using ampicillin/sulbactam in managing severe infections with organisms producing ESBL. As a result, the beta-lactam/beta-lactamase combination should not be generally considered as a first-line option.

Wang *et al*. stated that worldwide antibiotic resistance appears to show no signs of reduction. However, it may perhaps change direction, the etiology of antibiotic resistance is multidimensional, and its after effect has impact globally.[1] In this study, the highest rate of resistance by the ESBL *E. coli* was observed for the following; cefixime 95.6% and cefotaxime 91.3%. Cefixime and cefotaxime have been reported in many studies indicating low sensitivity against ESBL producers.[45,46]

The blaTEM represented the dominant family of the enzyme identified in this study. Several studies have reported varied dominance at different times and locations. As reported in our study, the high prevalence is consistent with other studies in Nigeria.[35,47] However, Egwuatu *et al*. (2019) reported non-production of TEM type ESBL in their study.[48] In Central India, Bajpai *et al*. (2017) reported TEM as the most dominant enzyme family followed by CTX-M and SHV.[49] In contrast to our study, many reports in the past decade found that *Enterobacteriaceae* producing CTX-M-type ESBLs have increasingly become a major reason of MDR UTIs and bloodstream infections.[2,50] Our study recorded SHV as the second most prevalent with 38.9%, while other studies reported CTX-M as the dominant genes.[51,52]

There was an incidence of some isolates harboring greater than 1 gene from our study. The coexistence of ESBL genes in a single isolate further explains the growing complexity of AMR problems, and more so, a reason for further investigation. Our study revealed six isolates possessed four genes (blaCTX-M, blaOXA, blaSHV, and blaTEM) which accounted for 26.1%, one isolate possessed three genes (blaCTX-M, blaOXA, and blaSHV). Two isolates possessed two genes (blaSHV and blaTEM). Our findings with regard to possession of more than 1 beta-lactamase by an isolate are aligned to studies from Egypt,[53] South Ecuador,[54] and Port Sudan.[55] The detection of ESBL producers may be hindered in an isolate if it possesses more than 1 gene and this may complicate treatment strategy for the clinicians. Another challenge in ESBL detection is that many microbiology laboratories of hospitals or private laboratories in Nigeria do not carry out routine test for ESBL production. Hence, the necessity for laboratory testing for the detection of ESBL among bacteria isolates should be emphasized.

Bakhshi *et al*. have reported that ERIC-PCR has been useful for routine epidemiological investigations due to its rapid nature, relative easiness, and demonstrated discriminatory power.[16] There are few molecular typing studies carried out on *E. coli*-producing ESBL in Nigeria. None of these studies used ERIC-PCR on ESBL-producing *E. coli*, thereby making it difficult to compare our study with other studies. Our study is the first to investigate the conserved inverted repeat in *E. coli*-producing ESBL. These repeats were predominantly found in the second cluster (CL2). A possible reason for this is that these isolates may likely originate from the same environment. According to Waturangi *et al*., identical fingerprint profiles may also occur in samples of diverse origins. Therefore, ERIC-PCR has been chosen for intraspecies profiling of some bacteria.[56] From the dendrogram, some isolates showed similar ERIC profiles, two from CL1 (E11 and E12), two from CL2 (E15 and E17), and two from CL3 (E2 and E18). This indicated similarities between the isolates. A further look at the resistant patterns exhibited by these isolates tend to have the same resistant profile; E11 and E12 from CL1 were resistant to cefotaxime, cefixime, ciprofloxacin, and ceftazidime. Likewise, E15 and E17 from CL2 were highly resistant to some antibiotics (cefotaxime, cefixime, ciprofloxacin, gentamicin, ceftazidime, and imipenem). This is also an indicator these isolates might be similar in origin. On the isolates' source showing similar ERIC profile, E11 and E12 are sourced from urine and same with E2 and E18. This shows that isolates from a particular source had similar ERIC profiles and clustered in the same group. This is also reported in a similar study on food products.[57] Bacterial isolates from this study are mainly from urine, stool, and a vaginal swab. Based on the ERIC-PCR, no significant association was found based on the source of the isolates from our study. Another possibility is that these isolates may share similar genetic material since they displayed or have the same resistant mechanism (ESBL producers). This is in line with Waturangi *et al*. that clustering is based on other aspects such as production of toxin, antibiotic resistance, or pathogenesis influence.[56] This can be linked to specific ESBL types produced by these organisms, as seen from the ESBL production. In CL1 and CL2, the isolates with similar ERIC profiles also have the same ESBL type (TEM). This is also a pointer to the earlier assertion that clustering can be based on other factors like antibiotic resistance. However, in CL3, despite the similarity in their ERIC profile, E18 possessed the only TEM, while E2 possessed both TEM and SHV. In CL4, where there are only two isolates, most of the antibiotics they resisted are similar (meropenem, cefotaxime, nitrofurantoin, cefixime, gentamicin, ciprofloxacin, and ampicillin/sulbactam). Therefore, comparing the antibiogram

results with the dendrogram showed that they differ with similar antibiotic-resistant patterns despite E3 and E9 in the same cluster. In addition to that, CL2 has three isolates that all produced blaCTX-M, blaOXA, blaSHV, and blaTEM, and in the same cluster, majority of the isolates produced blaTEM. We observed a correlation of these isolates with the antibiotic-resistant pattern and the ESBL genes they produced. There is inconsistency in the genes' distribution in other clusters (CL1, CL3, and CL4). In a related study on *E. coli* from the urinary tract, there exists difference in the number and position of ERIC sequences between strains of *E. coli* that are not related.The thermal cycling conditions have initial denaturation at 95°C for 3 minutes, then35 cycles at 95°C for 30 seconds. The annealing temperature was 53°C for 30 seconds,extension was 72°C for 30 seconds and then 72°C for 5 minutes for final extension.[58] Based on the banding patterns from the gel picture obtained from the ESBL-producing isolates, there seems to be a little correlation between the organisms and the ERIC print profile, bearing in mind the resistant pattern and the production of ESBL. The horizontal transfer of plasmids, transposons, and other mobile genetic elements might be responsible for this genetic relatedness. However, there was enormous inconsistency in a related study from the configurations obtained in three successive PCR runs carried out using similar conditions.[59] They further noted that the technique is based on the anticipation that complementary oligonucleotides will anneal to ERIC sequences. The DNA between the ERIC sequences may be amplified, so long as the space between ERIC sequences is 15 kilobases.[59] From the results of their study, they concluded a flaw in the ERIC-PCR technique due to its non-reproducibility. However, our study observed that the banding patterns vary slightly from each other in the three consecutive PCR ran under the same conditions. Some bands are found to be brighter than others from the gel pictures. This is aligned with a study earlier that reported bands that are bright are generally consistent. Still, lighter bands are often subject to interexperimental variability and are often ignored by human readers.[59] Computer software was used to account for bands' presence or absence since variations in the bands' intensity. As such, band gain or loss can pose a problem for computer-based analysis.[59] Light bands were removed from analysis by some researchers. This is to eliminate fingerprints' irreproducibility, but light bands were considered in this study. Therefore, selecting which bands will be incorporated in a specific molecular fingerprint presents personal bias and decreases the fingerprint down to only a few bands, thereby considerably decreasing the technique's discriminatory power.[60]

## Conclusion

The majority of the ESBL-producing *E. coli* isolates are sensitive to amikacin and highly resistant to the third-generation cephalosporins. The blaTEM represented the dominant family of enzymes identified genes. Overall, ERIC obtained indicated some evidence in the genetic relatedness of the ESBL genes among *E. coli* isolates.

## Authors' Declaration Statements

### Ethics approval and consent to participate

The HREC of UDUTH Sokoto gave approval for this study (UDUTH/HREC/2019/No.611). All participants who indicate their intention to participate signed or thumb printed before their sample was collected.

## Availability of Data and Material

The data that support the findings of this study are available from the corresponding author, **NT**, on request.

## Competing Interest

The authors declare that there are no conflicts of interest.

## Funding Statement

Nil.

## Authors' Contributions

BOO: Conceptualization, data collection, investigation, methodology, and writing – original draft preparation. NT: Formal analysis, methodology, data collection, writing – original draft preparation, and writing – review and editing. ROB: Conceptualization, supervision, methodology, validation, and writing – review and editing. ATO: Conceptualization, supervision, methodology, validation, and writing – review and editing. EBBO: Supervision, methodology, validation, and writing – review and editing. MY: Conceptualization and writing – review and editing.

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## References

1. Wang W, Arshad MI, Khurshid M, Rasool MH, Nisar MA, Aslam MA, *et al*. Antibiotic resistance: A rundown of a global crisis. Infect Drug Resist 2018;11:1645-58.
2. Pitout JD, Mulvey MR, Simner PJ, Denisuik AJ, Lagace PRS, Tailor F, *et al*. Molecular epidemiology of extended-spectrum beta -lactamase-, AmpC beta -lactamase- and carbapenemase-producing Escherichia coli and Klebsiella pneumoniae isolated from Canadian hospitals over a 5 year period: CANWARD 2007-11. J Antimicrob Chemother 2013;68 Suppl 1:i57-65.
3. Mahamat OO, Kempf M, Lounnas M, Tidjani A, Hide M, Benavides JA, *et al*. Epidemiology and prevalence of extended-spectrum beta-lactamase and carbapenemase-producing *Enterobacteriaceae* in humans, animals and the environment in West and Central Africa. Int J

Antimicrob Agents 2021;57:106203.

4. Bevan ER, Jones AM, Hawkey PM. Global epidemiology of CTX-M β-lactamases: Temporal and geographical shifts in genotype. J Antimicrob Chemother 2017;72:2145-55.
5. Wilson AP, Livermore DM, Otter JA, Warren RE, Jenks P, Enoch DA, *et al*. Prevention and control of multi-drug-resistant Gram-negative bacteria: Recommendations from a Joint Working Party. J Hosp Infect 2016;92:S1-44.
6. Tansarli GS, Poulikakos P, Kapaskelis A, Falagas ME. Proportion of extended-spectrum β-lactamase (ESBL)-producing isolates among enterobacteriaceae in Africa: Evaluation of the evidence-systematic review. J Antimicrob Chemother 2014;69:1177-84.
7. Paterson D, Bonomo R. Extended-spectrum beta-lactamases: A clinical update. Clin Microbiol Rev 2005;18:657-86.
8. Oduro-Mensah D, Obeng-Nkrumah N, Bonney EY, Oduro-Mensah E, Twum-Danso K, Osei YD, *et al*. Genetic characterization of TEM-type ESBL-associated antibacterial resistance in *Enterobacteriaceae* in a tertiary hospital in Ghana. Ann Clin Microbiol Antimicrob 2016;15:29.
9. Eltai NO, Al Thani AA, Al-Ansari K, Deshmukh AS, Wehedy E, Al-Hadidi SH, *et al*. Molecular characterization of extended spectrum β -lactamases *Enterobacteriaceae* causing lower urinary tract infection among pediatric population. Antimicrob Resist Infect Control 2018;7:1-9.
10. Bush K. The ABCD's of beta-lactamase nomenclature. J Infect Chemother 2013;19:549-59.
11. Srinivasan R, Karaoz U, Volegova M, Mackichan J, Kato M. Use of 16S rRNA gene for identification of a broad range of clinically relevant bacterial pathogens. PLoS One 2015;10:e0117617.
12. Nuhu T, Boon EO, Bolaji RO, Olayinka TA, Olayinka BO, Yahaya M. Antimicrobial resistant and virulence genes profiles of some Gram-negative bacteria from clinical isolates at Usmanu Danfodiyo University Teaching Hospital, Sokoto, Nigeria. J Preclin Clin Res 2020;14:52-7.
13. Versalovic J, Koeuth T, Lupski R. Distribution of repetitive DNA sequences in eubacteria and application to finerpriting of bacterial enomes. Nucleic Acids Res 1991;19:6823-31.
14. Ardakani MA, Ranjbar R. Molecular typing of uropathogenic *E. coli* strains by the ERIC-PCR method. Electron Phys 2016;8:2291-6.
15. Wilson LA, Sharp PM. Enterobacterial repetitive intergenic consensus (ERIC) sequences in *Escherichia coli*: Evolution and implications for ERIC-PCR. Mol Biol Evol 2006;23:1156-68.
16. Bakhshi B, Afshari N, Fallah F. Enterobacterial repetitive intergenic consensus (ERIC)-PCR analysis as a reliable evidence for suspected *Shigella* spp. outbreaks. Braz J Microbiol 2018;49:529-33.
17. Otokunefor K, Ogugbue CJ, Fajoyomi BU. Enterobacterial repetitive intergenic consensus (ERIC) as a tool for genetic characterisation of bacterial isolates in Nigeria. Niger J Biotechnol 2020;37:122-8.
18. Liao Z, Chen X, Li Z, Gao Y, Hu S. Molecular detection of virulence and drug resistance genes of pathogenic *Escherichia coli* from calves in Chongqing, China. Pak Vet J 2019;39:423-7.
19. Tsering DC, Das S, Adhiakari L, Pal R, Singh T. Extended spectrum beta-lactamase detection in gram-negative bacilli of nosocomial origin. J Glob Infect Dis 2009;1:87.
20. EUCAST. EUCAST Guidelines for Detection of Resistance Mechanisms and specific Resistances of Clinical and/or Epidemiological Importance. Euroian Committee Antimicrobial Susceptibility Testing; 2013. p. 1-40.
21. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. Wayne, PA: Clinical and Laboratory Standards Institute; 2016. p. 1-256.
22. Barbosa C, Nogueira S, Gadanho M, Chaves S. DNA extraction: Finding the most suitable method. In: Molecular Microbial Diagnostic Methods: Pathways to Implementation for the Food and Water Industries. Amsterdam, Netherlands: Elsevier Inc.; 2015. p. 135-54.
23. Codjoe FS, Brown CA, Smith TJ, Miller K, Donkor ES. Genetic relatedness in carbapenem-resistant isolates from clinical specimens in Ghana using ERIC-PCR technique. PLoS One 2019;14:e0222168.
24. Feizabadi MM, Delfani S, Raji N, Majnooni A, Aligholi M, Shahcheraghi F, *et al*. Distribution of blaTEM, blaSHV, blaCTX-M genes among clinical isolates of klebsiella pneumoniae at Labbafinejad hospital, Tehran, Iran. Microb Drug Resist 2010;16:49-55.
25. Jena J, Debata NK, Sahoo RK, Gaur M, Subudhi E. Genetic diversity study of various β-lactamase-producing multidrug-resistant *Escherichia coli* isolates from a tertiary care hospital using ERIC-PCR. Indian J Med Res 2017;146:S23-9.
26. Zoolkifli NW, Mutalib SA. Molecular typing among beef isolates of *Escherichia coli* using consensus repetitive intergenic enterobacteria-polymerase chain reaction (ERIC-PCR). Molecular typing among beef isolates of *Escherichia coli* using consensus repetitive intergenic enterobacteria-polymerase chain reaction (ERIC-PCR). AIP Conference Proceeding; 2013. p. 654-9.
27. Abimiku RH, Ngwai YB, Nkene IH, Bassey BE, Tsaku PA, Ibrahim T, *et al*. Phenotypic detection of extended spectrum beta-lactamase resistance of *Escherichia coli* from patients attending selected healthcare facilities in Nasarawa State, Nigeria. South Asian J Res Microbiol 2019;4:1-10.
28. Kpoda DS, Guessennd N, Bonkoungou JI, Ouattara MB, Konan F, Ajayi A, *et al*. Prevalence and resistance profile of extended-spectrum beta-lactamases-producing *Enterobacteriaceae* in Ouagadougou, Burkina Faso. African J Microbiol Res 2017;11:1120-6.
29. Nuhu T, Bolaji OR, Olayinka TA, Olayinka BO. A systematic review on the prevalence of extended-spectrum beta lactamase-producing Gram-negative bacteria in Nigeria. J Glob Antimicrob Resist 2020;22:488-96.
30. Deji-Agboola A, Olaosebikan O, Adenipekun E, Osinupebi O, FA O. Extended spectrum beta-lactamases (ESBLs)-producing *Escherichia coli* and *Klebsiella pneumoniae* among asymptomatic out-patients in a university health centre. Ann Health Res 2020;6:133-42.
31. Nwafia IN, Ohanu ME, Ebede SO, Ozumba UC. Molecular detection and antibiotic resistance pattern of extendedspectrum betalactamase producing *Escherichia coli* in a Tertiary Hospital in Enugu, Nigeria. Ann Clin Microbiol Antimicrob 2019;18:1-7.
32. Abike TO, Olufunke OA, Temitope OO. Prevalence of extended spectrum beta-lactamases in multidrug resistant strains of Gram-negative bacteria. Afr J Microbiol Res 2018;12:147-51.
33. Beigverdi R, Jabalameli L, Jabalameli F, Emaneini M. Prevalence of extended-spectrum β -lactamase-producing *Klebsiella pneumoniae*: First systematic review and meta-analysis from Iran. J Glob Antimicrob Resist 2019;18:12-21.
34. Olukemi OA, Adeola F. Extended-spectrum beta-lactamase production among clinical isolates of *Escherichia coli*. Int Res J Microbiol 2012;3:140-3.
35. Chioma OP, Martina CA, Emmanuel AE. ESBL mediated antimicrobial nonsusceptibility of uropathogenic *Escherichia coli* and *Klebsiella pneumoniae* isolates from pregnant women in Nnewi, Nigeria. J Adv Microbiol 2019;18:1-13.
36. Iliyasu MY, Uba A, Agbo EB. Phenotypic detection of multidrug resistant extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* from clinical samples. Afr J Cell Pathol 2018;10:25-32.
37. Dhillon RH, Clark J. ESBLs: A clear and present danger? Crit Care Res Pract 2012;2012:625170.
38. Anago E, Ayi-Fanou L, Akpovi CD, Hounkpe WB, Agassounon-Djikpo Tchibozo M, *et al*. Antibiotic resistance and genotype of beta-

lactamase producing *Escherichia coli* in nosocomial infections in Cotonou, Benin. Ann Clin Microbiol Antimicrob 2015;14:1-6.

39. Teklu DS, Negeri AA, Legese MH, Bedada TL, Woldemariam HK, Tullu KD. Extended-spectrum beta-lactamase production and multi-drug resistance among *Enterobacteriaceae* isolated in Addis Ababa, Ethiopia. Antimicrob Resist Infect Control 2019;8:1-12.
40. Giwa FJ, Ige OT, Haruna DM, Yaqub Y, Lamido TZ US. extendedspectrum betalactamase production and antimicrobial susceptibility pattern of uropathogens in a tertiary hospital in Northwestern Nigeria. Ann Trop Pathol 2018;9:11-6.
41. Kouadio I, Guessennd N, Dadié A, Konaté A, Gbonon V, Tiékoura B, *et al*. Characterization of the genetic supports for betalactam resistance in *Escherichia coli* strains of porcine origin producing extended-spectrum beta-lactamase (ESBL). Microbiol Res J Int 2017;22:1-9.
42. Braide W, Madu LC, Adeleye SA, Korie MC, Akobondu CI. Prevalence of extended spectrum beta lactamase producing *Escherichia coli* and *Pseudomonas aeruginosa* isolated from clinical samples. Int J Sci 2018;4:89-93.
43. Rupp ME, Fey PD. Extended spectrum β-lactamase (ESBL) producing *Enterobacteriaceae* considerations for diagnosis, prevention and drug treatment. Drugs 2003;63:353-65.
44. Solomon W, Frimpong EH, Feglo PK. Molecular characterization of extended-spectrum beta-lactamase producing urinary *Escherichia coli* isolated in Brong-Ahafo Regional Hospital, Ghana. Eur Sci J 2017;13:365-77.
45. Gharrah MM, Mostafa El-Mahdy A, Barwa RF. Association between virulence factors and extended spectrum beta-lactamase producing *Klebsiella pneumoniae* compared to nonproducing isolates. Interdiscip Perspect Infect Dis 2017;2017:251-64.
46. Sangare SA, Rondinaud E, Maataoui N, Maiga AI, Guindo I, Maiga A, *et al*. Very high prevalence of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in bacteriemic patients hospitalized in teaching hospitals in Bamako, Mali. PLoS One 2017;12:1-11.
47. Olowe OA, Oladipo GO, Makanjuola O, Olaitan JO. Prevalance of extended spectrum beta-lactamases (ESBLS) carrying genes in *Klebsiella* Spp from clinical samples at ile-ife, South Western Nigeria. Int J Pharma Med Biol Serv 2012;1:129-38.
48. Egwuatu TO, Orkeh GO, Ifeanyichukwu C, Aladesokun A, Egwuatu TF, Freda C. Extended-spectrum beta lactamase and carbapenemase producing *Klebsialla* spp. in urine and fecal samples obtained from hospitals and communities in Lagos, Nigeria. Suan Sunandha Sci Technol J 2019;6:6-14.
49. Bajpai T, Pandey M, Varma M, Bhatambare GS. Prevalence of TEM, SHV, and CTX-M Beta-lactamase genes in the urinary isolates of a tertiary care hospital. Avicenna J Med 2017;7:12-6.
50. Hijazi SM, Fawzi MA, Ali FM, Abd El Galil KH. Prevalence and characterization of extended-spectrum beta-lactamases producing *Enterobacteriaceae* in healthy children and associated risk factors. Ann Clin Microbiol Antimicrob 2016;15:3.
51. Abrar S, Ain NU, Liaqat H, Hussain S, Rasheed F, Riaz S. Distribution of bla CTX M, bla TEM, bla SHV and bla OXA genes in Extended-spectrum-β-lactamase-producing Clinical isolates: A three-year multi-center study from Lahore, Pakistan. Antimicrob Resist Infect Control 2019;8:1-10.
52. Zeynudin A, Pritsch M, Schubert S, Messerer M, Liegl G, Hoelscher M, *et al*. Prevalence and antibiotic susceptibility pattern of CTX-M type extended-spectrum β-lactamases among clinical isolates of gram-negative bacilli in Jimma, Ethiopia. BMC Infect Dis 2018;18:524.
53. Sana T, Rami K, Fouad D, Marcel A, Hassan M, Sani H, *et al*. Detection of genes TEM, OXA, SHV and CTX-M in 73 clinical isolates of *Escherichia coli* producers of extended spectrum Beta-lactamases and determination of their susceptibility to antibiotics. Int Arab J Antimicrob Agents 2011;1:1-6.
54. Yessenia D, Delgado C, Patricia Z, Barrigas T, Genoveva S, Astutillo O, *et al*. Detection and molecular characterization of lactamase genes in clinical isolates of Gram-negative bacteria in Southern Ecuador. Braz J Infect Dis 2016;20:627-30.
55. Abd Elrahman AM, Osman E, Hashim SO, Musa MA, Tahir OM. Detection of CTX-M, TEM and SHV genes in gram negative bacteria isolated from nosocomial patients at port sudan teaching hospital. Eur J Clin Biomed Sci 2017;3:101-8.
56. Waturangi DE, Joanito I, Yogi Y, Thomas S. Use of REP and ERIC-PCR to reveal genetic heterogeneity of *Vibrio cholerae* from edible ice in Jakarta, Indonesia. Gut Pathog 2012;4:2.
57. Ateba C, Mbewe M. Genotypic characterization of *Escherichia coli* O157: H7 isolates from different sources in the North-West Province, South Africa, using enterobacterial repetitive intergenic consensus PCR analysis. Int J Mol Sci 2014;15:9735-47.
58. Chulain M, Morris D, Cormican M. Enterobacterial repetitive intergenic consensus polymerase chain reaction for typing of uropathogenic *Escherichia coli* is not what it seems. Clin Infect Dis 2006;42:1805-6.
59. Meacham KJ, Zhang L, Foxman B, Bauer RJ, Marrs CF. Evaluation of genotyping large numbers of *Escherichia coli* isolates by enterobacterial repetitive intergenic consensus-PCR. J Clin Microbiol 2003;41:5224-6.
60. Ranjbar R, Tabatabaee A, Behzadi P, Kheiri R. Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) genotyping of *Escherichia coli* strains isolated from different animal stool specimens. Iran J Pathol 2017;12:25-34.