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Research Article

Detection of Extended-Spectrum Beta-Lactamase (ESBL) producing *Escherichia coli* Associated with Urinary Tract Infections in Ado Ekiti, Nigeria

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Abstract

The growing prevalence of multidrug-resistant *Escherichia coli*, particularly strains producing Extended-Spectrum Beta-Lactamases (ESBLs), complicates the treatment of Urinary Tract Infections (UTIs) and warrants urgent attention. This study investigated the occurrence of ESBL-producing *Escherichia coli* associated with UTIs in Ado Ekiti, Nigeria. One hundred and forty-one non-duplicate *Escherichia coli* isolates associated with UTIs obtained from Medical Microbiology Laboratory at Ekiti State University Teaching Hospital were Sub-cultured on MacConkey agar and Eosin Methylene Blue agar and re-characterized using biochemical assays and Polymerase Chain Reaction. Disc diffusion method was used to determine antimicrobial susceptibility patterns. ESBL brilliance agar and the double disc diffusion synergism test were used to identify the phenotypic expressions of ESBLs. Polymerase chain reaction was used to characterize the *bla* TEM, CTX-M and SHV genotypes. Results showed high resistance rates to Ampicillin (73.9%), Amoxicillin (72.5%), Augmentin (55.8%), Cefotaxime (76.1%), Cefuroxime (68.8%) and Cotrimoxazole (73.9%) but extreme susceptibility to Imipenem (100%). Of the 141 *E. coli* isolates, 56 (39.7%) were multi antibiotic resistant, 44 (78.6%) isolates were identified as ESBL producers using the double-disk synergy test and 46 (82.1%) on ESBL Brilliance agar. *Bla* TEM genes 35 (62.5%) and CTX-M genes 23 (41%) only were the genotypes identified. This study highlights the prevalence of ESBL production among *Escherichia coli* associated with UTIs in Ado Ekiti, Nigeria. This underscores the urgent need for effective monitoring and management strategies to combat antibiotic resistance in this region. Immediate action is required at both clinical and policy levels to prevent the spread of these pathogens and preserve the efficacy of last-resort antibiotics such as carbapenems.

Keywords: *Escherichia coli*, Urinary tract infections, Extended-Spectrum Beta-Lactamases Bla CTX, Bla TEM

Introduction

The rise of multidrug-resistant bacteria, particularly Extended-Spectrum Beta-Lactamase (ESBL)-producing *Escherichia coli*, poses a significant threat to public health, complicating the treatment of common infections like Urinary Tract Infections (UTIs) 1,2]. The production of ESBLs is the primary mechanism of resistance to third-generation cephalosporins among *Enterobacteriaceae*, including *Klebsiella pneumoniae* and *E. coli* [3,4]. UTIs are a major public health problem, with an estimated 150 million cases diagnosed globally each year [5]. Gram-negative bacilli, particularly *E. coli*, are the predominant pathogens, responsible for 75-90% of cases [6]. UTIs caused by ESBL-producing organisms are associated with unpredictable treatment outcomes and prolonged hospital stays due to their multidrug-resistant nature. Once primarily confined to healthcare facilities, ESBL-producing organisms are now increasingly reported in community-acquired infections [3,7]. This trend has significant implications for treatment and public health, as ESBL organisms often exhibit co-resistance to multiple antimicrobial classes [3,7,8]. Hence, ESBL-producing organisms remain of enormous clinical and microbiological concern, warranting close surveillance to prevent outbreaks [9]. This study investigates the prevalence and characteristics of ESBL-producing *E. coli* associated with UTIs in Ado Ekiti, Nigeria.

Materials And Methods

Study Area

This cross-sectional study was carried out in Ado Ekiti, Ekiti State, Nigeria, located on latitude 7°35′-7°47′ north of the equator and longitude 5°11′-5°16′ east of the Greenwich meridian.

Duration of Study

The study lasted six months, from September 2023 to March 2024.

Sample Collection and Processing

One hundred and forty-one non-duplicate *E. coli* isolates were obtained from the Medical Microbiology Laboratory of a tertiary hospital in Ado Ekiti. Colonies were inoculated on Mueller-Hinton agar slopes, incubated at 37°C for 24 hours, and stored in a refrigerator for further analysis.

Media Preparation

Media were prepared according to manufacturer's instructions, sterilized by autoclaving at 121°C for 15 minutes, cooled, and poured into Petri dishes.

Characterization and Identification of Isolates

Samples were streaked onto MacConkey agar and Eosin Methylene Blue (EMB) agar, incubated for 24 hours at 37°C, and examined for lactose fermentation and metallic green sheen formation. Identification was confirmed using standard microbiological and biochemical tests [10]. On MacConkey agar, *E. coli* ferments lactose, producing pink colonies, while on EMB agar it produces a characteristic green metallic sheen [11].

Biochemical Identification

Biochemical characterization followed standard procedures [10].

Antibiotic Susceptibility Testing

Antimicrobial susceptibility of pure isolates was assessed using the disc diffusion method following Clinical Laboratory Standards Institute (CLSI) guidelines [12]. Results were interpreted as susceptible, intermediate, or resistant based on zone diameters [12]. Control strains included *E. coli* ATCC 25922 and ESBL-positive *E. coli* ATCC 35218.

Inoculum Preparation and Standardization

Fresh colonies were inoculated in Mueller-Hinton broth, incubated for 3 hours at 37°C, and adjusted to a turbidity equivalent to 0.5 McFarland standard (1.5 × 10^6 CFU/ml).

Disk Diffusion Procedure

Inoculated Mueller-Hinton agar plates were streaked evenly and antibiotic discs were placed at appropriate distances. Tested antibiotics included: Ampicillin (10 µg), Amoxicillin (25 µg), Augmentin (30 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Cefuroxime (30 µg), Ofloxacin (5 µg), Ciprofloxacin (10 µg), Gentamicin (10 µg), Nitrofurantoin, and Imipenem. Plates were incubated at 37°C for 18 hours.

Phenotypic Detection of ESBLs

ESBL production was assessed by the Double Disk Synergy Test (DDST) [13]. Positive (*E. coli* ATCC 35218) and negative (*E. coli* ATCC 25922) controls were included. ESBL production was inferred when the inhibition zone of ceftazidime or cefotaxime was expanded ≥5 mm by clavulanate [14]. ESBL Brilliance Agar (Oxoid) was also used for chromogenic detection, enabling presumptive identification within 24 hours [15].

Molecular Detection

DNA extraction was performed using the QIAGEN DNA mini kit with slight modifications. PCR amplification targeted *E. coli*-specific *usp*A genes [16] and ESBL-associated genes (*blaTEM, blaCTX-M, blaSHV*) using established primers [10]. PCR products were visualized by agarose gel electrophoresis.

Statistical Analysis

Sensitivity and specificity were calculated, and chi-square tests determined significance ($p < 0.05$). Analyses were conducted using SPSS version 21 (Table 1).

Table1: Primers used for the amplification of ESBL genes.

Forward primer	Sequence (5´to3´)	Reverse primer	Sequence (5´to3´)	Annealing temp. (oC)	Product size (bp)
CTXM	CGATGTGCAGTAC-CAGTAA	CTXM	TTAGTGAC-CAGAATAAGCGG	60	485
SHV1	AGGATT-GACTGCCTTTTTG	SHV1	ATTTGCTGAT-TTCGCTCG	56	393
TEM	CCCCGAAGAACGT-TTTC	TEM	ATCAGCAATAAAC-CAGC	51	517
USPA	CCGATACGCTGC-CAATCAGT	USPA	ACGCAGACCG-TAGGCCAGAT	55	884

Results

Identification using Standard Laboratory Methods

Following recharacterization and genomic identification of the organisms only 138 isolates were characterized and identified *Escherichia coli*. *Escherichia coli* grew with a green metallic sheen EMB agar as shown on Figure 1.

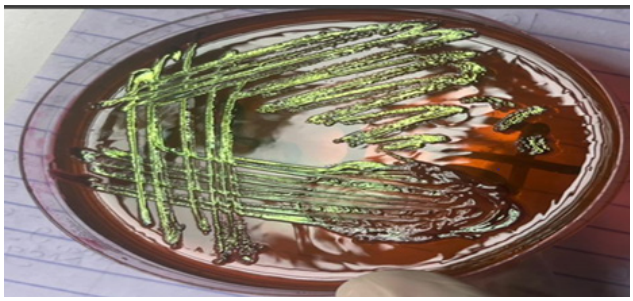


Figure 1: Pictograph of Escherichia coli on Eosin Methylene Blue agar with metallic green sheen.

Antibiotic Susceptibility Pattern of the Isolated Organism

All the 138 *E. coli* isolates were subjected to antimicrobial testing. Based on the antibiotic susceptibility tests, the overall resistance of the isolates to antibiotics showed that resistance to Ampicillin (AMC) (73.9%), Amoxicillin (AMX) (72.5%), Augmentin (AUG) (55.8%), Cefotaxime (CTX) (76.1%), Cefuroxime (CXM) (68.8%) and Cotrimoxazole (STX) (73.9%) as presented in Table 2. The highest resistance was observed in Ampicillin (AMC) (73.9%) and Chloramphenicol (CHL) (84.1%) while Imipenem was observed as the most sensitive antibiotic tested (100%). Fifty- six (56) isolates were identified as Multidrug Resistant (MDR) isolates and were selected for possible ESBL production testing.

Table 2: Antimicrobial susceptibility patterns of *Escherichia coli* isolates.

Class/Antibiotics	No of resistant isolates (%)	No of intermediate isolates (%)	No of sensitive isolates (%)
β-lactams			
Amoxicillin	100 (72.5%)	4 (2.9%)	34 (24.6%)
Ampicillin	102 (73.9%)	12 (8.7%)	24 (17.4%)
Augmentin	77 (55.8%)	04 (2.9 %)	57 (41.3%)
Ceftazidime	80 (58%)	7 (5.1%)	51(36.9%))
Cefotaxime	99 (71.8%)	9 (6.5%)	30 (21.7%)
Cefuroxime	95 (68.8%)	10 (7.2%)	33 (24.0%)
TETRACYCLINS			
Tetracycline	100 (72.5%)	9 (6.5%)	29 (21%)
MACROLIDE			
Erythromycin	94 (68.1%)	17 (12.3%)	27 (19.6%)

AMINOGLYCOSIDE			
Gentamicin	56 (40.6%)	27 (19.6%)	55 (39.8%)
QUINOLONES			
Ciprofloxacin	6 (4.3%)	30 (21.7%)	102 (74%)
Ofloxacine	8 (5.8%)	27 (19.6%)	103 (74.6%)
SULFONAMIDE			
Cotrimoxazole	102 (73.9%)	15 (10.9%)	21(15.2%)
Nitrofurantoin	12 (8.7%)	24 (17.4%)	102 (73.9%)
Chloramphenicol	116 (84.1%)	22 (15.9%)	-
CARBAPENEMS			
Imipenem	-	-	138 (100%)

Phenotypic Detection of Extended Spectrum β-Lactamase (ESBLS)

The Double-Disk Synergy Test (DDST) using cefotaxime/ceftazidime with clavulanate as a substrate produced positive findings in 44 out of 56 isolates (78.6%), showing a synergistic impact indicative of Extended-Spectrum β-Lactamase (ESBL) synthesis. Screening with the ESBL Brilliance agar identified ESBL-producing *Escherichia coli* in 46 of the 56 isolates (82.1%), characterized by the appearance of deep blue colonies as shown in Figure 2.

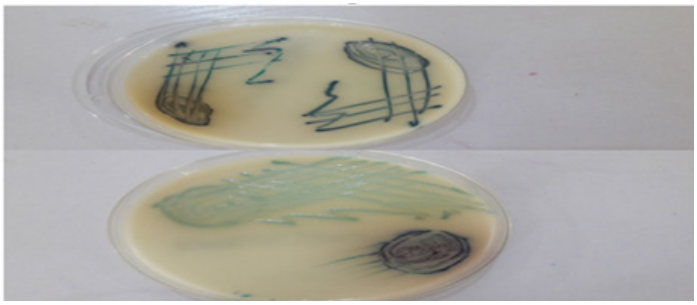
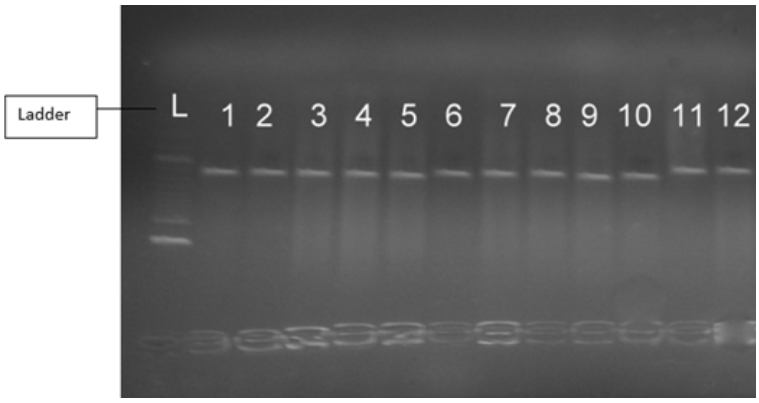


Figure 2: Green colonies representing a positive ESBL Producing Klebsiella spp and blue colonies representing ESBL producing Escherichia coli on the ESBL brilliance agar.

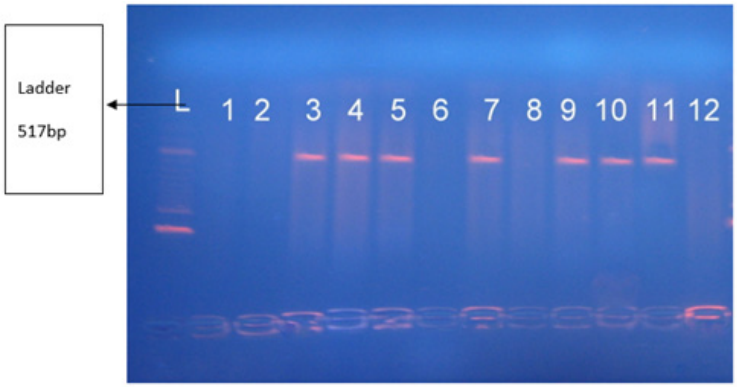
Detection of ESBL Genes

Molecular characterization of resistant genes of the *Escherichia coli* isolates, the result of the genomic DNA polymerization by PCR is shown on Figure 3a and 3b for *bla* CTX-M, *bla* TEM genes and *bla* SHV genes respectively. A total of 35 (62.5%) isolates showed detectable genes. *Bla* TEM genes 35 (62.5%) isolates, CTX-M genes 23 (41%) of the *Escherichia coli* isolates while *bla* SHV gene was not detected.



***Note:** Lanes 2-12: tests; Lane 1: positive control; Lane L: DNA ladder

Figure 3a: Positive CTX-M genes.



***Note:** Lanes 2-12: tests; Lane1: negative control; Lane L: DNA ladder

Figure 3b: Positive TEM genes with 517bp.

Discussion

Urinary Tract Infections (UTIs) are among the most common bacterial diseases worldwide, affecting millions each year, especially women and the elderly. *Escherichia coli* has been identified as the predominant causative agent of both community-acquired and hospital-acquired urinary tract infections. However, the emergence of antibiotic-resistant bacteria, especially those that produce Extended-Spectrum β-Lactamases (ESBLs), is compromising the efficacy of traditional antibiotic regimens [17]. Cephalosporin is a commonly used antibiotic for treatment of Urinary tract infections. However, resistance to these antibiotics is increasing in the last decades. This has been attributed to emergence of strains that can be producing Extended-Spectrum β-Lactamases (ESBLs). Generally, antibiotic resistance has continued to constitute serious problems in human medicine [18]. ESBLs have emerged gradually during the last decades in species of *Enterobacteriaceae* and their prevalence reach alarming rates [19]. Infections caused by such pathogens often limit therapeutic options and cause treatment failures [20] Figure 4.

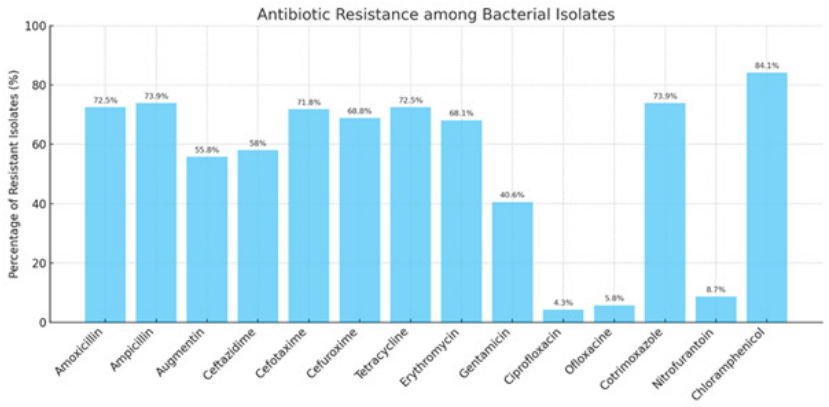


Figure 4: APPENDIX

Moreover, ESBL genes are often co-transferred with plasmid-mediated fluoroquinolone and aminoglycoside resistance genes, thus contributing to the dissemination of multidrug resistance mechanisms [21]. Therefore, an accurate method for the phenotypic detection of ESBLs among *Enterobacteriaceae* irrespective of the presence of other beta-lactamases is essential in order to successfully address surveillance studies as well as for infection control issues [20]. Although many international studies have addressed the emergence of ESBL-producing *K. pneumoniae* and *E. coli*, there are few local reports on this issue. The current study presents alarming levels of antimicrobial resistance among *E. coli* isolates. Resistance was particularly high against β-lactams including amoxicillin (72.5%), ampicillin (73.9%), cefotaxime (76.1%), cefuroxime (68.8%), and cotrimoxazole (73.9%). This observation which has been widely reported might be due to its extensive misuse 0f first line drug of choice in the treatment of uncomplicated UTIs but the usefulness has now been significantly reduced due to high level of bacterial resistance [22-25]. Interestingly, fluoroquinolones which were among the most effective agents of choice in the empiric treatment of most bacterial infections in the last one decade were observed in this study to be largely effective on these *Escherichia coli* strains. This trend was also reported by *Iqbal, et al.,* [26] while *Kader and Kumar* [27] reported that the isolates were highly resistant to cefepime, ciprofloxacin and gentamicin. The ESBLs are encoded by plasmids which also carry resistant genes for other antibiotics. A co-resistance to the quinolones and the amino-

glycosides is common. In this study, an associated resistance with co-trimoxazole (73.9%), and gentamicin (40.6%) was observed. *Gupta, et al.,* [28] had earlier reported 91.17%, 100% and 94.91% resistances respectively to gentamicin, cotrimoxazole and ciprofloxacin in the ESBL producers.

In this study, the carbapenem (Imipenem) was highly effective against the ESBL-producing isolates. In Ibadan, *Okesola, et al.,* [29] have reported a similar occurrence from carbapenems (Meropenem and Imipenem) 100% and 80% respectively; followed by Gentamycin (60%) and Amikacin (50%) were found to be quite active against the ESBL producing isolates. Other studies have also shown similar trends with the Carbapenems; *Kader and Kumar,* [27] reported that carbapenems had a good activity against the ESBL-producing isolates tested with over 92% of isolates being susceptible. This report was also corroborated by *Goosens and Grabein,* [30] who published that meropenem and imipenem had greatest activity against ESBL-producing *E. coli* and *Klebsiella spp* in both Europe (96.9-100.0%) and the United States (100.0%). Of all the tested antibiotic agents, Imepenems were the most sensitive and reliable treatment options for infections caused by ESBL producing isolates. However, over use of carbapenems may lead to resistance of other Gram-negative organisms.

The correct detection of ESBL producing microorganisms is a challenge for most laboratories, requiring not only phenotypic tests, but also genotypic characterization for all genes associated with beta-lactamase production. Phenotypic methods like DDST and chromogenic agar remain vital for ESBL screening especially in resource limited setting but often require molecular confirmation. It is therefore important that combined phenotypic-genotypic approach is used to ensure accurate detection and effective infection control, as recommended by the Health Protection Agency [31] and Livermore [20]. This study demonstrated that 44 of the 56 Multidrug-Resistant (MDR) *E. coli* isolates (78.6%) tested positive for ESBL production via the Double-Disk Synergy Test (DDST), and 46 (82.1%) were identified on ESBL Brilliance agar. This high prevalence of ESBL producers is consistent with other reports across Nigeria and sub-Saharan Africa. In Benin City, Nigeria, *Ogefere and collegues* [32] reported a 44.4% ESBL detection, while in Togo, 93.4% prevalence was reported by *Muhammad and Swedan* [33]. The choice of detection method, geographical variation, antibiotic prescribing practices and compliance with infection control measures may be responsible for the variations in the prevalence rates.

Infections caused by ESBL-producing strains are challenging to treat due to limited therapeutic options. ESBL genes are frequently located on plasmids that carry other resistance determinants, including genes conferring resistance to fluoroquinolones, aminoglycosides, and sulfonamides [34]. This may explain the co-resistance patterns observed in this study, with 40.6% of isolates resistant to gentamicin and 73.9% to cotrimoxazole. The high prevalence of ESBLs producing *Escherichia coli* associated with UTIs is a global problem and varies across regions and it is significantly associated with the uncontrolled use of broad-spectrum antibiotics especially cephalosporins as observed in this study. In this study, the genotypic variants of the ESBL genes among the *Escherichia coli* isolates showed 35 (62.5%) and 23 (41%) isolates had detectable *Bla* TEM and CTX-M genes respectively which was higher than the result obtained from Ile-Ife [35] which reported *bla* SHV, *bla* TEM and *bla* CTX 32%, 32% and 36% respectively while Olowe et al [10] on *Escherichia coli* obtained from faecal samples showed 42.1% for CTX, 44.7% for TEM and 21.1% for SHV. The molecular detection of ESBL resistance genes among the isolates in this study revealed that the *bla*TEM was the most predominant beta lactamase gene. This finding is consistent with reports from other studies [33,36]. This phenomenon may be due to the presence of *bla* TEM on the highly mobile genetic elements which favours its spread among bacteria globally [37]. This study's prevalence rate of *bla* CTX (41%), which was the next most predominant beta lactamases genes among the isolates, confirms its increasing prevalence among *Escherichia coli* strains associated with UTIs as reported in many studies [33,38]. The detection of resistant species capable of producing of extended spectrum beta-lactamase enzyme from clinical isolates is worrisome and requires attention during diagnosis in health sectors.

Conclusion

The significant detection of ESBL-producing *Escherichia coli* associated UTI infections in this study highlights a critical challenge to effective antimicrobial treatment in the study area and Nigeria at large. Immediate action is essential at both the clinical and policy levels to limit the spread of resistant pathogens and maintain the effectiveness of the last resort medications such as carbapenems.

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Conflict of Interest

The authors declare that they have no financial or non-financial conflicts of interest related to this study.

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