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Genetic Profiling of Extended-Spectrum Beta-Lactamase Genes from Uropathogenic E. coli Isolates in Ekiti State, Nigeria

C. O. Anidiobu[1*]

[1]Department of Microbiology, The Federal Polytechnic Ado-Ekiti, Ekiti State, Nigeria.
*Corresponding author (Email: chyanidiobu@gmail.com, Phone: +2348035802899)

ABSTRACT

The high levels of antimicrobial resistance linked to Extended-Spectrum Beta-Lactamase (ESBL)-producing Enterobacteriaceae hinder effective patient management and significantly contribute to the rising global rates of diseases and death. Therefore, investigating the genetic profiles of ESBL-producing uropathogenic Escherichia coli (UPEC) in Ekiti State, Nigeria, is critical. Methods: Seventy-five E. coli strains were randomly selected from four locations within Ekiti State. Isolates were tested for resistance to 15 antibiotics using the Kirby-Bauer disk diffusion method, and multiplex PCR assays were employed to detect ESBL gene variants. Data were analyzed using Chi-square tests and Poisson regression ($p < 0.05$). Results: The E. coli isolates exhibited high resistance to amoxicillin-clavulanate (59.0%), ampiclox (55.7%), nitrofurantoin (52.5%), tetracycline (41.0%), and cotrimoxazole (41.0%). Conversely, they were most susceptible to meropenem (43.3%), imipenem (41.8%), levofloxacin (39.3%), and ceftazidime (33.6%). The isolates demonstrated significant multidrug resistance with a mean MAR index of 0.5. Molecular screening revealed that 24% (18 isolates) harbored blaTEM genes, while blaCTX-M and blaSHV were not detected. Conclusion: Continuous updates in rapid diagnostic methods and treatment protocols are essential for effectively managing ESBL-producing UPEC, to support antimicrobial stewardship.

***Keywords*— Antibiotic resistance, blaTEM gene, E. coli, Molecular epidemiology, Urinary Tract Infection**

I. INTRODUCTION

Urinary tract infections (UTIs), ranked among the most common infectious diseases (approx. 32.5%) [1], pose a significant challenge to global healthcare systems. These infections originate within the urinary system, which includes the kidneys, ureters, bladder, and urethra all of which play vital roles in waste elimination from the body [2]. Escherichia coli is the primary multidrug-resistant pathogen responsible for both hospital- and community-acquired UTIs. Some strains produce extended-spectrum beta-lactamases (ESBLs) and carbapenemases, enzymes that limit available antimicrobial treatment options [3].

Beta-lactamase enzymes inactivate β-lactam antibiotics by hydrolyzing their core beta-lactam rings, conferring resistance. Bacteria that produce these enzymes often exhibit resistance to other antimicrobial agents, complicating therapeutic interventions [4]. ESBLs are a group of enzymes capable of deactivating the beta-lactam rings of penicillins, aztreonam, and first- through third-generation cephalosporins, but remain susceptible to inhibition by clavulanic acid [4]. ESBLs encompass multiple molecular variants, most notably the TEM, SHV, CTX-M, OXA, and PER groups, with CTX-M being the most prevalent [5].

The genetic determinants of these enzymes can be situated on either the bacterial chromosome or within plasmids. Plasmid-borne ESBL genes are typically located on large extrachromosomal elements that simultaneously harbor resistance factors for alternative antimicrobial classes, including aminoglycosides, tetracyclines, sulfonamides, trimethoprim, and chloramphenicol. This genetic clustering frequently yields multidrug-resistant (MDR) phenotypes, leaving clinicians with restricted therapeutic strategies and escalating the risk of severe or fatal outcomes [6], [7]. In Ekiti State, prior investigations into uropathogenic Escherichia coli (UPEC) resistance have concentrated heavily on documenting the epidemiology and molecular profiles of ESBL-producing strains. For instance, an assessment of clinical UPEC isolates by [8] demonstrated a substantial burden of ESBL-positive phenotypes, pointing to an intensifying multidrug resistance crisis locally. The genotypic landscape was further mapped by [9], who detected blaTEM, blaSHV, and blaCTX-M variants, thereby highlighting the heterogeneous nature and spread of these resistance determinants within the region. More recently, high-resolution molecular approaches have been deployed to map precise gene distributions. [10] confirmed that blaCTX-M alleles specifically blaCTX-M-15 predominated among local E. coli isolates, a finding consistent with global epidemiological patterns. Additionally, the mechanisms driving this dissemination were explored by [11], whose work on plasmid-mediated transmission emphasized the high capacity for horizontal gene transfer among native bacterial populations.

Collectively, these findings underscore an upward trajectory of ESBL-mediated resistance among regional UPEC isolates, emphasizing the critical need for robust epidemiological surveillance and strict antimicrobial stewardship. Because these resistance determinants are primarily plasmid-encoded, they readily propagate via horizontal gene transfer, drastically accelerating the dissemination of resistance. Consequently, elucidating the molecular pathways that drive the coexistence of ESBL-producing Escherichia coli among UTI patients in Ado-Ekiti, Nigeria, offers essential data regarding local resistance dynamics and transmission vectors, directly confronting a severe regional public health threat.

II. MATERIALS AND METHODS

A. Strain Collection, Characterization, and Identification of Isolates

For this analysis, 75 non-repetitive Escherichia coli isolates identified as phenotypic ESBL producers were drawn from a larger cohort of 122 Gram-negative strains, which had been previously isolated from the urine of patients at two tertiary medical institutions. These include Ekiti State University Teaching Hospital and the Federal Teaching Hospital, Ido, Ekiti. The isolates were sub-cultured via streak-plate inoculation onto MacConkey and Eosin Methylene Blue (EMB) agar media. Following a 24-hour incubation period at 37°C, the plates were evaluated for distinct phenotypic markers: the development of pink colonies on MacConkey agar signified lactose fermentation, while the appearance of a vibrant, metallic green sheen was monitored on the EMB agar [8]. Ultimate verification of the E. coli strains was established using standard biochemical and microbiological characterization techniques [12] and subsequently refrigerated for subsequent experimental procedures [13].

B. Antibiotic susceptibility test

To evaluate the phenotypic resistance profiles of the isolates, the Kirby-Bauer disk diffusion method was executed in compliance with Clinical and Laboratory Standards Institute guidelines [12], [14]. Inocula were prepared from overnight bacterial cultures grown on sterile Mueller-Hinton agar (MHA). Specifically, distinct colonies were suspended in sterile normal saline, and the suspension's density was equilibrated to a 0.5 McFarland turbidity standard. Using a sterile swab, this standardized bacterial suspension was inoculated uniformly across the surface of fresh MHA plates to generate a confluent lawn. Commercially prepared antimicrobial disks (Oxoid, England) were subsequently applied aseptically to the agar surface, ensuring uniform contact. The cultures were incubated at 37°C for a 24-hour duration. The specific panel of 14 antibiotics evaluated included: cefexime (5 µg), ceftazidime (30 µg), cefotaxime (25 µg), imipenem (10 µg), ofloxacin (5 µg), tetracycline (30 µg), amikacin (30 µg), levofloxacin (5 µg), ciprofloxacin (5 µg), amoxicillin-clavulanate (20/10 µg), nitrofurantoin (300 µg), meropenem (10 µg), cotrimoxazole (unspecified µg), and gentamicin (10 µg). Multidrug resistance (MDR) is characterized by a pathogen's non-susceptibility to at least one agent in three or more distinct therapeutic classes of antimicrobials (such as penicillins, cephalosporins, carbapenems, fluoroquinolones, aminoglycosides, tetracyclines) [15].

C. Determination of Multiple Antibiotic Resistance Index

Based on the approach of [16], the MAR index was calculated by dividing the number of antibiotics to which a given isolate showed resistance (a) by the overall number of antibiotics tested (a/b).

D. Bacterial DNA extraction

A commercial DNA extraction kit (NIMR BIOTECH, Nigeria) was employed to extract genomic DNA from the multidrug-resistant E. coli isolates. The resulting extracts were maintained at -20°C [17] until further laboratory processing.

E. Multiplex-PCR assay for ESBL genes

All isolates were subjected to a multiplex PCR assay in a total reaction volume of 20 µL per tube. Each reaction mixture contained 50 ng of genomic DNA template, 1X Taq reaction buffer, 1.25 U of Taq DNA polymerase, 0.2 µM of each specific primer, and 0.2 mM of each dNTP. Amplification was carried out using a Bio-Rad thermal cycler (UK) programmed with the following parameters: initial denaturation at 94°C for 3 minutes, followed by 35 cycles of 94°C for 45 seconds (denaturation), 60°C for 40 seconds (annealing), and 72°C for 1 minute (extension). A final 3-minute extension phase at 72°C completed the cycling program. To visualize the amplicons, the PCR products were resolved via electrophoresis on a 2% agarose gel stained with ethidium bromide, following the protocol outlined by [18].

F. Statistical analysis

Qualitative parameters were presented as baseline frequencies and percentages. Distributional differences among the resistance determinants were evaluated using Chi-square and Poisson regression models, with significance different defined as $p < 0.05$.

III. RESULTS

Phenotypic recharacterization of 75 E. coli isolates confirmed their identity through visual and biochemical traits: pink colonies on MacConkey agar indicating lactose fermentation, and a metallic green sheen on EMB agar characteristic of E. coli. These features help differentiate E. coli from other bacteria.

This study examines the antimicrobial susceptibility patterns of E. coli isolates across various antibiotic classes, revealing notable resistance trends. Carbapenems, such as Meropenem and Imipenem, show low resistance (around 13%), maintaining high effectiveness. Cephalosporins exhibit moderate resistance (18-24%), underscoring the need for susceptibility testing before use. Fluoroquinolones have resistance rates of 14-29%, with Levofloxacin showing relatively higher susceptibility. Aminoglycosides like Amikacin and Gentamicin retain considerable efficacy, though resistance is emerging. Penicillins demonstrate high resistance (55-59%), limiting their clinical utility. Older antibiotics such as Tetracycline, Nitrofurantoin, and Cotrimoxazole also face substantial resistance (over 40%), reducing their usefulness as shown in Table 2.

The distribution of multiple antibiotic resistance index (MARI) among the bacterial isolates in this study is summarized in Table 3. Out of a total of 75 isolates, the number of antibiotics to which each isolate exhibited resistance ranged from two to twelve. A notable proportion of isolates (22.7%) were resistant to seven antibiotics, while 24.0% exhibited resistance to eight antibiotics. The most common resistance pattern was resistance to seven or eight antibiotics, accounting for approximately 22.7% and 24.0% of the isolates, respectively, indicating a high level of multidrug resistance within the bacterial population studied. The MARI values, representing the extent of antimicrobial resistance, ranged from 0.1 in isolates resistant to only two antibiotics to 0.8 in those resistant to twelve antibiotics. Specifically, isolates resistant to twelve antibiotics had the highest MARI value of 0.8, reflecting extensive multidrug resistance.

The results obtained from gel electrophoresis analyses designed to detect the presence of the blaTEM gene, a common beta-lactamase gene associated with antibiotic resistance. The figures illustrate the amplification patterns observed in the samples, providing insights into the distribution of blaTEM positive isolates within the tested population. The gel image (Figure 1a) displays the PCR amplification products for the blaTEM gene. Lane M contains the 100 bp DNA ladder, which serves as a molecular size marker. Lane –ve is the negative control, confirming the absence of contamination. Lane 9 exhibits a clear band at 409 bp, indicating a positive detection of the blaTEM gene in that sample. The lanes numbered 1-8 and 10-14 show no such bands, signifying negative results for blaTEM. The presence of a distinct band at the expected size in Lane 9 confirms the successful amplification and the presence of blaTEM carrying bacteria in that particular sample. Similarly, Figure 1b demonstrates the amplification pattern across additional samples. Lane M again contains the 100 bp DNA ladder. The negative control (Lane –ve) shows no amplification, validating the assay's specificity. Lanes 2-3 and 5-9 display bands at approximately 409 bp, confirming the presence of blaTEM gene in these samples. Conversely, lanes 1, 4, and 10-14 lack such bands, indicating negative results. The consistent detection of the 409 bp band in multiple lanes affirms the distribution of blaTEM positive isolates within the sample set. The prevalence of ESBL genes was 24% (18/75), with only the blaTEM gene detected (409 bp). No blaCTX-M, or blaSHV genes were identified.

Table 1: The primer of ESBL genes

S/N	GENE	PRIMERS	ANN.	SIZE	REF
1.	*blaTEM*	Forward: 5-TTTCGTGTCGCCCTTATTCC-3	60	409bp	*[18]*
		Reverse:5- ATCGTTGTCAGAAGTAAGTTGG-3			
2.	*blaSHV*	Forward: 5-CGCCTGTGTATTATCTCCCT-3	60	293bp	*[18]*
		Reverse: 5- CGAGTACTCCACGAGATCCT-3			
3.	*blaCTX-M*	Forward: 5- CGCTGTTGTTAGGAGTG TG-3	60	874bp	*[18]*
		Reverse: 5- GGCTGGGTGAAGTAAGTGAC -3			

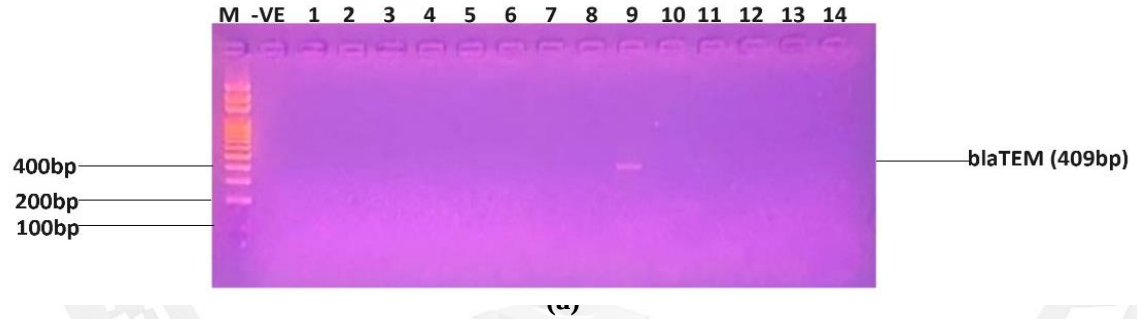
Table 2: Antibiotic resistant and susceptibility profile of the *Escherichia coli* isolates

Classes of Antibiotic	Types of Antibiotic	No of R (%)	No of I (%)	No of S (%)	Total
Carbapenem	Meropenem	17(13.9)	7(5.7)	51(41.8)	
	Imipenem	16(13.1)	6(4.9)	53(43.3)	
Cephalosporin	Cefixime	29(23.8)	10(8.2)	36(29.5)	
	Ceftazidime	26(21.3)	8(6.6)	41(33.6)	
	Cefotaxime	22(18.0)	20(16.4)	33(27.0)	
Fluoroquinolones	Ofloxacin	36(29.5)	14(11.5)	25(20.5)	
	Ciprofloxacin	28(23.0)	21(17.2)	26(21.3)	
	Levofloxacin	18(14.8)	9(7.4)	48(39.3)	75
Aminoglycoside	Amikacin	47(38.5)	6(4.9)	22(18.0)	
	Gentamicin	29(24.6)	15(12.3)	31(25.4)	
Penicillin	Amoxicillin clavulanate	72(59.0)	0(0)	3(2.5)	
	Ampiclox	68(55.7)	5(4.1)	2(1.6)	
Tetracycline	Tetracycline	58(47.5)	10(8.2)	7(5.7)	
Nitrofuran	Nitrofurantoin	64(52.5)	0(0)	11(9.0)	
Cotrimoxazole	Cotrimoxazole	50(41.0)	14(11.5)	11(12.3)	

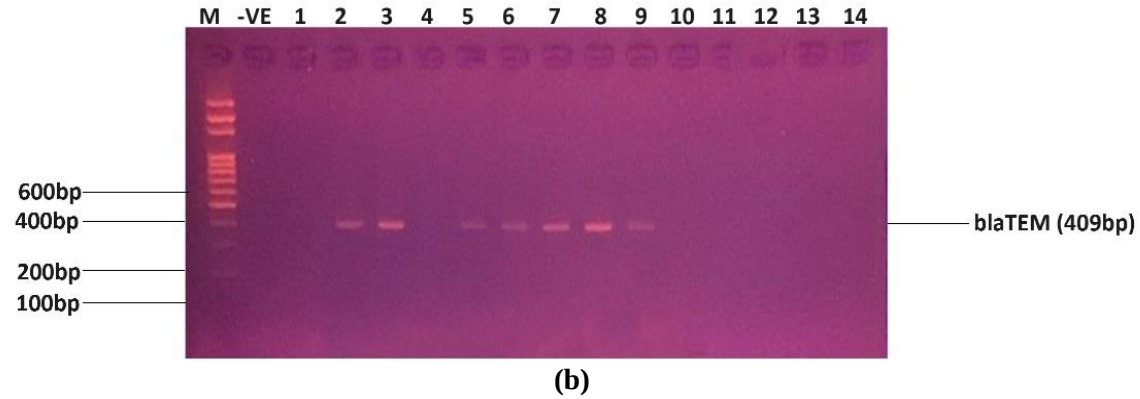
Note: R-Resistant, I- Intermediate and S- Sensitive.

Table 3: Multiple resistance profile with MAR index

S/N	Number of Antibiotics	Total number n= 75 (%)	MARI
1	Two	-	0.1
2	Three	1(1.3)	0.2
3	Four	3(4.0)	0.3
4	Five	4(5.3)	0.3
5	Six	10(13.3)	0.4
6	Seven	17(22.7)	0.5
7	Eight	18(24.0)	0.5
8	Nine	14(18.7)	0.6
9	Ten	4(18.7)	0.7
10	Eleven	2(2.7)	0.7
11	Twelve	1(1.3)	0.8



Lane M: 100bp DNA ladder, Lane –ve: Negative control, Lane 9: positive *blaTEM* gene (409bp), Lane 1-8 and 10-14: Negative *blaTEM* gene



(b)

Lane M: 100bp DNA ladder, Lane –ve: Negative control, Lane 2-3 and 5-9 are *blaTEM* positive, Lane 1, 4 and 10-14 are *blaTEM* negative

Figure 1: Gel electrophoresis of *blaTEM*

IV. DISCUSSION

This study focused on the genetic profiling of extended-spectrum beta-lactamase (ESBL) genes in uropathogenic E. coli isolates from Ekiti State, Nigeria, to understand their resistance patterns. The findings revealed that the highest resistance was against amoxicillin-clavulanate, with 72 isolates (59.0%) exhibiting resistance, while the lowest resistance was observed with imipenem, affecting 16 isolates (13.1%). These results highlight the significant antimicrobial resistance among uropathogenic E. coli in the region. The data indicates that while some antibiotics like carbapenems and aminoglycosides retain relatively good activity, there is significant resistance to many first-line and older antibiotics such as penicillins, tetracyclines, and cotrimoxazole. The observed resistance patterns underscore the importance of routine susceptibility testing to guide effective therapy, as well as the urgent need for antimicrobial stewardship programs to curb the spread of resistant strains. The high resistance rates to common antibiotics highlight the threat of multidrug-resistant organisms and the necessity for ongoing surveillance and prudent antibiotic use [19], [20].

Variations in resistance rates have been reported across different regions worldwide, primarily due to differing patterns of antibiotic use [21]. For comparison, E. coli isolates were most susceptible to imipenem (43.3%), meropenem (41.8%), levofloxacin (39.3%), and ceftazidime (33.6%). [22] Iran reported the highest resistance rates to ampicillin and the lowest to nitrofurantoin, whereas in Uganda, cefuroxime and ciprofloxacin exhibited the highest and lowest resistance rates, respectively [23]. Previous studies from Bangladesh, Pakistan, Nepal, Nigeria, and other countries also indicate varying levels of antibiotic resistance in E. coli strains [17], [19], [23], [24].

The data underscores the alarming presence of multidrug-resistant bacteria within the studied population, with nearly half of the isolates resistant to five or more antibiotics. The high prevalence of resistance to multiple antibiotics complicates treatment options and emphasizes the urgent need for antimicrobial stewardship programs [24]. Furthermore, the distribution suggests that resistance mechanisms are widespread and may be driven by factors such as overuse or misuse of antibiotics. Monitoring MARI values can aid in assessing the severity of antimicrobial resistance and in designing targeted interventions to curb the spread of resistant strains [24].

The gel electrophoresis results demonstrate that a subset of the tested isolates harbor the blaTEM gene, which is commonly associated with beta-lactam antibiotic resistance, particularly resistance to penicillins and early-generation cephalosporins. The identification of positive blaTEM genes aligns with phenotypic resistance patterns observed in antimicrobial susceptibility testing, emphasizing the genetic basis of

resistance in these isolates. The negative controls reinforce the reliability of the PCR assays, with no evidence of contamination or nonspecific amplification. Overall, these findings underscore the significance of blaTEM in mediating resistance within the studied bacterial population and highlight the necessity for molecular surveillance to inform appropriate antimicrobial stewardship strategies [25].

The prevalence of ESBL-producing E. coli isolates was 24%, suggesting an increasing trend in ESBL prevalence within the Ekiti State, aligning with reports from the South-western region of Nigeria [11]. A similar study in South Africa detected the blaTEM gene exclusively in 24% of isolates, with other genes such as blaSHV, blaCTX-M, and combinations thereof identified in varying proportions [26]. The high prevalence of ESBLs is comparable to findings from Ethiopia [27] and Egypt [28]. In Iran, [22] a reported that 61.5%, 45.0%, and 38.0% of E. coli isolates from UTI patients carried blaTEM, blaCTX-M, and blaSHV genes, respectively. China reported higher ESBL prevalence rates, ranging from approximately 39.7% to 53.0% over five years [29]. The variation in ESBL prevalence across studies may be attributed to differences in population sources and healthcare practices.

Compared to other regions, our observed prevalence is higher than North America (23.7%) [25], France (4.8%) [30], North Central Nigeria (18.6%) [31], and Korea (16.7%) [32]. The rising prevalence in our study may be linked to disparities in infection control strategies, socioeconomic factors, and treatment practices [31]. The presence of these genes, such as blaCTX-M, blaTEM, and blaSHV, confers the bacteria with the ability to break down antibiotics like amoxicillin-clavulanate, leading to high resistance rates [33].

V. CONCLUSION

Based on the findings, it is evident that ESBL-producing E. coli in Ekiti State exhibit high levels of resistance to commonly used antibiotics, particularly amoxicillin-clavulanate, ampiclox, nitrofurantoin, tetracycline, and cotrimoxazole. The significant multidrug resistance observed, coupled with the presence of blaTEM genes, underscores the genetic adaptability of these pathogens and the ongoing challenge they pose to effective treatment. The absence of other ESBL gene variants and carbapenemase genes suggests a limited but concerning prevalence of specific resistance mechanisms. To combat this rising threat, it is crucial to implement continuous surveillance, adopt rapid diagnostic methods, and update treatment protocols to promote rational antimicrobial use. Strengthening antimicrobial stewardship and infection control measures will be vital in managing ESBL-producing uropathogens and reducing their impact on public health in the region.

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