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Original Article

Molecular analysis of multidrug-resistant *E. coli* in pediatric UTIs: findings from a Nigerian Hospital

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Abstract

Introduction: This study aimed to isolate and characterize antibiotic-resistant *Escherichia coli* from urine samples of children at the Mother and Child Hospital in Ondo State, Nigeria, assessing antibiogram profiling and resistance genes.

Methodology: Three hundred urine samples (158 females, 142 males), aged 3-5 years, were collected, transported on ice, and analyzed bacteriologically. *E. coli* and Gram-negative bacteria were isolated using Eosin Methylene Blue agar and identified through colony morphology and biochemical tests. Antibiotic susceptibility was determined via Kirby Bauer's disc diffusion, and resistance genes were detected using Polymerase Chain Reaction (PCR).

Results: Of the 300 samples, 40 (13.3%) yielded *E. coli* with varying antibiotic resistance profiles. The highest resistance was against Amoxicillin-clavulanate (87.5%) followed by Ceftriaxone (80%). Susceptibility was observed to Nitrofurantoin, Erythromycin, and Chloramphenicol. Multiple resistance patterns against 3-4 antibiotic classes were recorded, with 12 distinct patterns observed. Eight isolates harbored blaCTX-M gene, while five carried the aac3-IV gene.

Conclusions: The study concluded a high occurrence of *E. coli* infection and multiple antibiotic resistance in the region. The presence of resistance genes suggests significant economic and health implications, emphasizing prudent antibiotic use under physician guidance to mitigate multiple antibiotic resistance.

Key words: *E. coli*; resistance; antibiotics; gene; UTI.

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Introduction

Urinary tract infection (UTI) is a bacterial infection that affects one or more parts of the urinary system, such as the urethra, bladder, ureters, and kidneys. The most common cause of UTI is the multiplication of bacteria at the urethra's entrance, which can then move up to the bladder or even the kidneys and spread to the bloodstream [1]. UTIs are a major public health concern in both hospital and community settings, particularly in developing countries, due to their high mortality rate and economic burden [2].

Enterobacteriaceae, specifically those possessing adhesins and hydrolytic enzymes like β-lactamases, are the primary cause of UTIs. β-lactamase production is the most common resistance mechanism used by these bacteria, and their increasing prevalence is a significant complication [2-5]. Children with UTIs can be challenging to diagnose and treat due to the nonspecific symptoms they exhibit, which can result in improper treatment.

The emergence and rapid spread of antibiotic-resistant *Enterobacteriaceae* is a significant public health threat. These bacteria can withstand lethal doses of antibiotics with various mechanisms of action and chemical structures, such as target alteration, decreased uptake, enzyme degradation, and overexpression of efflux proteins. Molecular genotyping and phenotyping techniques can be used to screen and confirm antimicrobial drug resistance expression in a population.

The prevalence of undiagnosed and improperly treated urinary tract infections (UTIs) in children is a major concern for the public and healthcare professionals due to the difficulty in diagnosing UTIs with nonspecific symptoms in pediatric patients. Although UTI complaints are rare in children,

symptoms usually only appear after five years of age, such as fever accompanied by rigors/chills, suprapubic/abdominal pain, and vomiting [6].

CTX-M β-lactamase enzymes are categorized into five groups based on amino acid similarity, with CTX-M-15 and CTX-M-14 being the most prevalent globally. These enzymes are often associated with genes conferring resistance to antibiotics like aminoglycosides and fluoroquinolones. *Escherichia coli*, a Gram-negative bacterium found in the human intestinal tract, is a significant cause of UTIs, bloodstream infections, septicemia, and meningitis. Multi-drug-resistant strains of *E. coli* are becoming increasingly prevalent worldwide, particularly in African countries with inadequate laboratory diagnostics and surveillance systems.

Given the significant impact of multidrug-resistant *E. coli* in pediatric UTIs, there is a critical need to investigate the genetic basis underlying antibiotic resistance in these bacterial strains. Understanding the prevalence of CTX-M β-lactamase enzymes and their association with multidrug resistance in *E. coli* isolates from pediatric UTI cases can provide valuable insights for improved diagnosis and treatment strategies.

Hence, this study aims to address the knowledge gap by characterizing the molecular profiles of multidrug-resistant *E. coli* isolates obtained from pediatric UTI cases in a specific healthcare setting. The findings from this study can contribute to a better understanding of antibiotic resistance patterns in pediatric UTIs and inform evidence-based strategies for effective antimicrobial therapy in the studied region. Ultimately, the research seeks to contribute to the control and management of multidrug-resistant *E. coli* infections in pediatric patients, safeguarding their health and promoting prudent antibiotic use in the hospital and community settings.

Methodology

Ethical approval and informed consent

The study obtained ethical clearance from the Mother and Child Hospital's ethical and research committee in Ondo, South-West, Nigeria, with the reference number MCHO/06/15/003. Informed consent was obtained from the parents or guardians of the children included in the study, and only those who provided consent were included in the research.

Inclusion and exclusion criteria

The study included asymptomatic children outpatients at the Mother and Child Hospital in Ondo, South-West, Nigeria, aged between 3 to 5 years.

Patients who declined to provide informed consent and those with medical conditions that could potentially interfere with the study objectives were excluded from the research.

Sample collection

A clean catch of urine samples was collected from 300 pediatric outpatients using sterile universal bottles. The samples were temporarily stored on ice packs at -4 ºC to preserve their integrity during transportation and were promptly transported to the laboratory for analysis.

Isolation of organisms

Using an aseptic technique, a loop full of each sample was streaked onto Eosin Methylene blue agar (manufactured by Lab M Ltd, UK), and the agar plates were then incubated at 37 °C for 24 hours. After the incubation period, distinct colonies were subcultured onto sterile nutrient agar plates, which were freshly prepared, and incubated again at 37 °C for 24 hours. The distinctive morphological properties of each pure culture on the agar plates were observed.

Identification of isolates

The isolates' morphological characteristics were appropriately recorded, and initial identification was conducted using Bergey's Manual of Determinative Bacteriology. To further identify the bacterial isolates, an Analytical Profile Index (API) 20E test kit (manufactured by bioMérieux, Inc.) was utilized.

Antibiotic susceptibility test

The Kirby-Bauer's disc diffusion method was utilized to test the susceptibility of the isolates to antibiotics. The antibiotic discs (manufactured by Oxoid Ltd, UK) that were used in the study include amoxicillin-clavulanate (Augmentin) (30 µg), Ceftriaxone (30 µg), Nitrofurantoin (300 µg), Gentamycin (10 µg), Cotrimoxazole (25 µg), Ofloxacin (10 µg), Amoxicillin (25 µg), Ciprofloxacin (10 µg), Ceftazidime (30 µg), Cefuroxime (30 µg), Cefixime (5 µg), Chloramphenicol (30 µg), Streptomycin (10 µg), Erythromycin (5 µg), and Pefloxacin (5 µg). These discs were placed aseptically onto Mueller-Hinton agar plates, which were previously seeded with a 24-hour-old culture (0.5 McFarland's standard- 107 cfu/mL) using sterile forceps. The plates were then incubated at 37 °C for 24 hours. The diameter of the zone of inhibition was measured using a transparent calibrated ruler to the nearest millimeter, and the results were interpreted according to the guidelines of the Clinical

Table 1. Primers for the detection of the resistance genes for *E. coli.*

Primer	Sequence 5'-3'	Gene	Product Size (bp)	Annealing Temp (°C)	Reference
CTX-M	F: CCATGTGCAGTACCAGTAA R: TTAGTGACCAGAATAAGCGG	*bla*CTX-M	585	58	[8]
aac3-IV	F: AGTTGACCCAGGGCTGTCGC R: GTGTGCTGCTGGTCCACAGC	*aac*3-IV	286	55	[9]

Laboratory Standards Institute [7]. Multiple antibiotic resistance was defined as resistance to more than two classes of antibiotics.

Molecular characterization of multiple antibiotic resistant E. coli isolates

Twelve *E. coli* isolates that were resistant to multiple antibiotics were selected based on their antibiotype and subjected to gene detection analysis. The resistance genes blaCTX (585bp) [8] and Tet A (954bp) [9] were detected using appropriate primers as described in Table 1. Bacterial DNA was extracted using the boiling method, whereby the samples were boiled at 100 °C for 7 minutes in a water bath and then chilled on ice for 2 minutes. The PCR reaction was carried out using the following thermocycling conditions: initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 58 °C for 30 seconds for blaCTX (585bp) gene and 55 °C (aac3-IV) for 1 minute, extension at 72 °C for 1 minute, and a final extension at the same temperature for 5 minutes. The PCR products were visualized using a short wave ultraviolet trans-illuminator and photographed using a gel bioimaging system.

Statistical analysis

Statistical analysis was carried out using R software (R 4.1.1). Descriptive statistics were employed to analyze the prevalence and resistance patterns of the isolated *E. coli* strains. One-way ANOVA was performed to determine if there is any significant difference in the occurrence of *E. coli* against the two age groups (ages 3-4 and 4-5). The null hypothesis for the one-way ANOVA was that there was no significant difference in growth rates between the two age groups. The alternative hypothesis was that there was a significant difference in growth rates between the two age groups, using a significance level of 0.05.

Results

Occurrence of E. coli in relation to age and sex of children patients

Out of the total 300 urine samples collected from the cohort of children (158 females and 142 males), 40 (13.3%) yielded *E. coli* isolates. The age and gender distribution of the participants is presented in Table 2. Among the 300 children, 147 (78 females and 69 males) were in the age range of 3-4 years, whereas 153 (80 females and 73 males) were between the ages of 4-5 years.

From the one-way ANOVA performed during this study, the calculated F-value was 2.08, and the critical F-value was 3.88. Since the calculated F-value was less than the critical F-value, we failed to reject the null hypothesis. This means that there is no significant difference in the occurrence of *E. coli* between the two age groups.

Antibiotic susceptibility profile of E. coli isolates cultured from urine samples of children attending mother and child hospital, Ondo

The *E. coli* isolates obtained from 40 of the children (13.3% of the total cohort) displayed varying degrees of antibiotic resistance, with the highest resistance rates observed for amoxicillin-clavulanate (35 isolates, 87.5%), ceftriaxone (32 isolates, 80%), and gentamycin (30 isolates, 75%). In contrast, none of the isolates were resistant to erythromycin, chloramphenicol, or nitrofurantoin. Intermediate resistance was observed in 16 isolates (40%) for amoxicillin and cotrimoxazole, while ceftazidime (32.5%), ciprofloxacin (30%), and streptomycin (25%) showed intermediate resistance in a smaller proportion of isolates. Among the 15 antibiotics tested, pefloxacin (8 isolates, 20%) and ceftazidime (4 isolates, 10%) showed the lowest levels of resistance. The percentage resistance of the *E. coli* isolates showed a significant difference ($p < 0.05$). None of the isolates displayed intermediate resistance to nitrofurantoin, gentamycin, ofloxacin, cefixime,

Table 2. Occurrence of *E. coli* in relation to age and sex of the children (n = 300).

Age	Number of children sampled	Number of samples with growth n (n %)	Number of females	Number of samples with growth n (n %)	Number of males	Number of samples with growth n (n%)
3-4 years	147	22(55)	78	13 (59.1)	69	9 (40.9)
4-5 years	153	18 (45)	80	11 (61.1)	73	7 (38.9)
Total	300	40 (13.3)	158	24 (15.2)	142	16 (11.3)

Table 3. Antibiotic susceptibility profile of *E. coli* isolates cultured from urine samples of children patients.

Antibiotic (µg)	No of Isolates	Number of Isolate Occurrence (n%)		
		Susceptibility	Intermediate	Resistant
Amoxicillin (25 µg)	40	13	16	11
Augmentin (30 µg)	40	2	3	35
Cefixime (5 µg)	40	29	0	11
Ceftazidime (30 µg)	40	23	13	4
Ceftriaxone (30 µg)	40	2	6	32
Cefuroxime (30 µg)	40	9	5	26
Chloramphenicol (30 µg)	40	40	0	0
Ciprofloxacin (10 µg)	40	19	12	9
Cotrimoxazole (25 µg)	40	14	16	10
Erythromycin (5 µg)	40	40	0	0
Gentamycin (10 µg)	40	10	0	30
Nitrofurantoin (300 µg)	40	40	0	0
Ofloxacin (10 µg)	40	12	0	28
Pefloxacin (5 µg)	40	30	2	8
Streptomycin (10 µg)	40	21	10	9

chloramphenicol, and erythromycin. The details of the resistance rates and intermediate resistance are provided in Table 3.

Multiple antibiotic resistance patterns of E. coli isolates from urine samples of children attending mother and child hospital, Ondo

The criterion for multiple antibiotic resistance was established as resistance to three or more different classes of antibiotics. The multiple antibiotic resistance profiles of the *E. coli* isolates are provided in Table 3. The antibiotic classes tested comprised Beta lactams (amoxicillin, amoxicillin-clavulanate, ceftriaxone, ceftazidime, cefuroxime, and cefixime), Fluoroquinolones (ciprofloxacin, ofloxacin, and pefloxacin), Aminoglycosides (gentamycin and streptomycin), Sulphonamides/Trimethoprim (cotrimoxazole), Nitrofurans (nitrofurantoin), Macrolides (erythromycin), and Chloramphenicol.

Seventy-five percent (30) of the 40 *E. coli* isolates recovered in this investigation exhibited multiple resistance to at least three antibiotic classes. A total of 12 different multiple antibiotic resistance patterns were identified (Table 4).

Molecular detection of resistance (blaCTX) genes in E. coli

Figure 1 shows the amplification products of blaCTX genes separated by agarose gel electrophoresis. Eight of the 12 representative isolates that was resistance to beta lactam antibiotics as depicted by Lanes 3, 4, 5, 7, 8, 10, 11, and 12 harbored blaCTX resistance gene of molecular weight of 585 bp.

Molecular Detection of aac6' Resistance Gene in E. coli Isolates

Agarose gel electrophoresis of the amplification product of *aac*3-IV (286 bp) gene in select multiple antibiotic resistant *E. coli* is presented in Figure 2. Five

Table 4. Multiple antibiotic resistance pattern of *E. coli* cultured from urine samples of children patients.

Isolate	No of antibiotic classes	Multiped antibiotic resistance patterns	Frequency (%)	Overall (%)
E. coli	4	AUG, COT, GEN, OFL	5 (12.5)	6 (15)
		CAZ, COT, OFL, STR	1 (2.5)	
	3	AMX, COT, OFL	1 (2.5)	24 (60)
		AUG, COT, GEN	1 (2.5)	
		AUG, GEN, OFL	11 (27.5)	
		AUG, GEN, PEF	3 (7.5)	
		CAZ, PEF, STR	2 (5)	
		CPX, GEN, OFL	2 (5)	
		CRO, PEF, STR	1 (2.5)	
		CRX, COT, OFL	1 (2.5)	
		CRX, GEN, OFL	1 (2.5)	
		CRX, OFL, STR	1 (2.5)	

COT: Cotrimoxazole 25 µg, CPX: Ciprofloxacin 10 µg, AMX: Amoxicillin 25 µg, OFL: Ofloxacin 5 µg, CRO: Ceftriaxone 30 µg, ERY: 5 µg, GEN: Gentamycin 10 µg, PFX: Pefloxacin 5 µg, AUG: Augmentin 30 µg, NIT: Nitrofurantoin 300 µg, CAZ: Ceftazidime 30 µg, CRX: Cefuroxime 30 µg, CXM: Cefixime 5 µg, CHL: Chloramphenicol 30 µg, ERY: Erythromycin 5 µg, STR: Streptomycin 10 µg, PEF: Pefloxacin 5 µg.

of the 12 representative isolates were resistant to gentamycin, as depicted by Lane 1, 2, 4, 11, and 12 harbored aac6 resistance gene weight of 286bp.

Discussion

E. coli is a well-known bacterial pathogen that is responsible for various infections worldwide. However, the emergence and spread of antibiotic resistance among *E. coli* isolates pose a significant threat to public health globally, leading to major health problems like prolonged hospitalization and treatment failure [10]. In this study, we investigated the prevalence and patterns of antimicrobial resistance among *E. coli* isolates obtained from urine samples of children under the age of five in Ondo, South Western Nigeria.

The study found a significant occurrence of antibiotic resistance among the *E. coli* isolates, indicating a wide spectrum of resistance to all routinely used antibiotics in the region, such as amoxicillin-clavulanate, amoxicillin, ciprofloxacin, gentamicin, cotrimoxazole, and ofloxacin. The number of *E. coli* isolates with multi-drug resistance was 30 (75%), and all the isolates displayed resistance to one or more antibiotic classes. Moreover, the resistance levels were higher than those reported by previous studies.

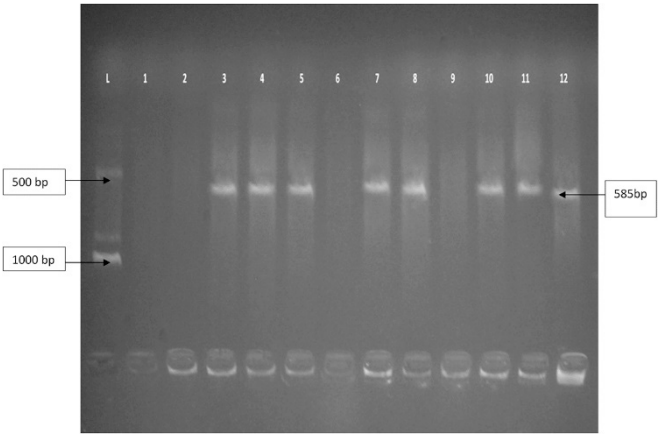
This study suggested that the widespread use of antimicrobial agents for infection treatment in the community could be a contributing factor to this serious antimicrobial resistance problem. This trend was observed in a study conducted at Rajah Muthiah Medical College and Hospital in Annamalainagar, Tamil Nadu, India, which discovered a high rate of drug resistance to commonly used antimicrobial agents in the treatment of *E. coli* infections [11].

A study that sought to discuss the rising concern of antibiotic resistance in pediatric UTIs by Esposito *et al.* (2022) emphasized the need for continuous monitoring of uropathogens' antibiotic resistance to guide effective therapy [12]. This aligns with the findings in the current study, where a significant proportion of *E. coli* isolates exhibited resistance to commonly used antibiotics, including Cefixime, Ceftazidime, and Erythromycin. The increased prevalence of extended-spectrum β-lactamase (ESBL)-producing strains in both studies reflects a global trend of concern.

Additionally, a single-center experience study by Samancı *et al.* (2020) also highlights the predominance of *E. coli* as the most common causative agent of UTIs in children [13]. Similar to the current study, the authors reported high resistance to antibiotics like ampicillin, ampicillin-sulbactam, amoxicillin-clavulanate, and TMP-SMX. The presence of multidrug resistance and low resistance to antibiotics like nitrofurantoin is consistent with the findings in the current study.

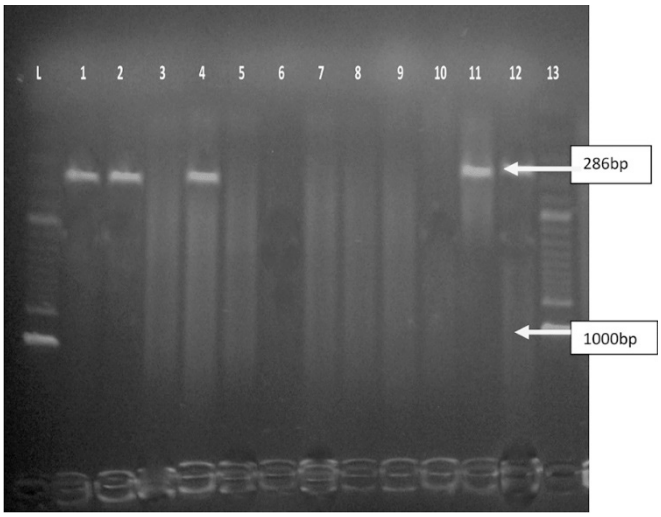
Another review by Mahony *et al.* (2020) discusses the global spread of MDROs and their implications on UTIs in children [14]. While most literature on drug resistance in UTIs has focused on ESBL-producing organisms, the review acknowledges the significance of multidrug resistance beyond beta-lactamase production. This aligns with the current study, where 60% of the *E. coli* isolates showed resistance to more than one antibiotic class, indicating multidrug resistance.

Figure 1. Agarose gel electrophoresis of the amplification product coding blaCTX gene in selected multiple antibiotic-resistant *E. coli* isolates.



L = 1000bp ladder, 3 = C23, 4 = C112, 5 = C91, 1 = C104, 2= C111, 7= C121, 8 = C14, 9 = C127

Figure 2. Agarose gel electrophoresis of the amplification product coding aac3-IVgene in selected multiple antibiotic-resistant *E. coli* isolates.



L = 100bp ladder, 1 = C23, 2 = C112, 9 = C91, 11 = C127, 12 = C14

Antimicrobial resistance in bacteria of medical importance imposes serious constraints on the treatment options for many infections, which concerns general practitioners and pediatricians in developing countries [15]. According to this study, amoxicillin-clavulanate, gentamycin, ceftriaxone, and ofloxacin should not be used to treat *E. coli* infections in the study location, due to their high-level resistance. The development of beta-lactamases enzymes may be the cause of amoxicillin-clavulanate resistance [16]. Moreover, the presence of extended-spectrum β-lactamase has been identified as the primary source of multiple resistance in *E. coli* [17]. The most common mechanism of cotrimoxazole resistance is the acquisition of plasmid-encoded, variant diaminopyrimidinefolatereductase enzymes [18], whereas the relatively high rates of gentamycin resistance in this study are most likely due to the antibiotic's indiscriminate use.

Interestingly, all *E. coli* isolates obtained from the urine samples of children under the age of five in the study showed a relatively high rate of sensitivity to nitrofurantoin, chloramphenicol, and erythromycin. The study found the existence of extended-spectrum β-lactamases and aminoglycoside 3-N-acetyltransferase, which contribute to multidrug resistance in *E. coli* isolates, which is in tandem to a study in Sudan that sought to identify the blaTEM, blaSHV and blaCTX-M genes among *Enterobacteriaceae* isolates from patients in Khartoum, Sudan [19]. The global incidence of *E. coli* infections has been recorded, and investigations have revealed the presence of several extended-spectrum β-lactamase (ESBLs) genes among Enterobacteriaceae members [19,20]. *Enterobacteriaceae* (mainly *E. coli*) generating CTX-M enzymes have been discovered as a source of urinary tract infections, primarily in the community [21].

The study's findings also suggested that antibiotic resistance could emerge due to patients' indiscriminate purchase of medications over the counter and physicians' incorrect prescription of antibiotics [22]. Therefore, the prescriber should strictly follow the five rights of medication administration: the appropriate patient, the right medicine, the right dose, the right route, and the right time.

Conclusions

In this study, the occurrence of *E. coli* infection is high, with a high level of multiple antibiotic resistance. This study's recovery of resistance genes (blaCTX and aac3-IV) in the multiple antibiotic resistant *E. coli* identified in this study is of great health and economic implications. Furthermore, *E. coli* susceptibility to nitrofurantoin, erythromycin, and chloramphenicol will assist clinicians in effectively managing *E. coli* infection in children in the research region.

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