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Identification and Characterization of Extended Spectrum β-Lactamase *E. coli* from Clinical Samples at Selected Hospitals in Maiduguri, Nigeria

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Abstract

The prevalence of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*) and their antibiotic resistance patterns were investigated in clinical samples from selected hospitals in Maiduguri, Nigeria. A total of 400 samples, including urine and stool, were collected and analyzed for the presence of ESBL-producing *E. coli*. Microbiological analysis and antimicrobial susceptibility testing were conducted following standard procedures. The results revealed a significant prevalence of *E. coli*, with higher rates in stool samples (80.0%) compared to urine samples (59.6%). Multi-drug resistance (MDR) was prevalent among *E. coli* isolates, with varying resistance levels observed across different antibiotics and hospitals. ESBL-producing *E. coli* strains were detected in both urine and stool samples, indicating the growing challenge of antibiotic resistance in clinical settings. The findings underscore the urgent need for comprehensive strategies to combat antibiotic resistance and enhance infection control measures in healthcare settings. Enhanced surveillance, strict adherence to hygiene protocols, and judicious use of antibiotics are essential to mitigate the spread of antibiotic-resistant pathogens and their impact on public health. These results provide valuable insights into the dynamics of antibiotic resistance and inform evidence-based interventions to address this global health threat.

Keywords: ESBL, *E. coli*, MDR, Prevalence and Antibiotics

Introduction

Escherichia coli, first identified by a German-Australian paediatric pathologist in 1835, belong to the order Enterobacteriales. This gram-negative, rod-shaped bacterium commonly inhabits the intestinal tracts of warm-blooded organisms, contributing to gastrointestinal diseases and neonatal meningitis, affecting both animals and humans (Emanuela*et al.*, 2019). While often associated with foodborne illnesses and urinary tract infections, *E. coli* is a highly

versatile bacterium with significant implications for human health. Many strains residing in the human gut are non-pathogenic commensals, assisting in essential functions such as synthesizing vitamin K2 and aiding digestion while competing with pathogenic bacteria for resources. However, certain pathogenic *E. coli* strains possess virulence factors that enable them to cause infections, including urinary tract infections (UTIs) (Manel*et al.,* 2021). *E. coli* is also known as a motile bacterium that contributes to hospital-acquired infections (Claire*et al.*, 2017), posing a threat not only at the individual level but also within communities due to its association with multidrug-resistant infections. Aminoglycosides, a class of antibiotics derived from soil bacteria, have been instrumental in combating various infections for decades. Examples include gentamicin, kanamycin, and streptomycin, commonly used in the treatment of tuberculosis (Becker and Matthew, 2013).

Worldwide, microbial pathogens cause approximately 300 million deaths annually, surpassing mortality rates from cardiovascular diseases (Joseph *et al.,* 2021). This increase in resistant infections is intricately linked to the overutilization of antimicrobials across human, animal, and plant sectors. Antimicrobial drugs play a crucial role in inhibiting bacterial growth to manage infections in both humans and animals. However, certain bacteria exhibit innate resistance due to genetic alterations (AMR, 2022). Notably, antibiotic resistance, especially among bacterial pathogens like *E. coli*, poses a formidable threat to global health. The emergence of multi-drug resistant (MDR) strains of *E. coli*, demonstrating resistance to a broad spectrum of antibiotics, significantly complicates effective infection treatment. According to the World Health Organization (WHO), antimicrobial resistance (AMR) contributed to approximately 1.27 million deaths worldwide in 2019 (WHO, 2019), underscoring its status as a pervasive menace to public health.

The rise of multidrug resistance (MDR) among human pathogens presents a significant global health challenge. It renders previously potent antibiotics ineffective, complicating the treatment of infections and heightening both morbidity and mortality rates (Wakil *et al.,* 2021). MDR E. coli pose a substantial public health risk due to their resistance to multiple classes of antibiotics, resulting in treatment failures and escalated healthcare expenses. Identifying the underlying resistance genes is vital for effective treatment and control measures. Molecular detection techniques provide rapid, precise, and sensitive tools for this purpose. MDR infections often entail more severe and prolonged courses, necessitating costlier and more intricate treatment approaches. Consequently, this difficulty in treatment, such as in cancer chemotherapy, jeopardizes numerous medications (Lito *et al.*, 2018)

The production of β-lactamase in Gram-negative bacteria presents a significant challenge to the efficacy of β-lactam antibiotics. Treatment with broad-spectrum antibiotics fosters the development of broad-spectrum enzymes known as β-lactamases, which can degrade penicillins, cephalosporins, and monobactams. These enzymes, primarily derived from TEM and SHV-type enzymes, include extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, responsible for global infection outbreaks. Mutations in the β-lactamase gene sequence lead to the production of various enzymes, classified into four groups: A, B, C, and D. Over the past two decades, Gram-negative bacteria have increasingly developed resistance to broad-spectrum beta-lactam antibiotics. ESBLs, exceeding 400 types, are mostly found in the Enterobacteriaceae family, with *E. coli* being a prominent producer (Mashwal *et al.,* 2017).

The global reporting of ESBLs is on the rise, with prevalence varying based on geographic location, directly influenced by antibiotic usage patterns (Nwafuluaku *et al.,* 2021). However, laboratory detection of ESBLs can be intricate and is not commonly conducted in many facilities. Recognizing these challenges, this study aimed to assess the prevalence of ESBL-producing *E. coli* in clinical samples from selected hospitals in Maiduguri, Nigeria, and to phenotype the ESBL carriage of these isolates.

Materials and Methods

Study Area

The research was carried out across three hospitals situated in Maiduguri Metropolis: Borno State Specialist Hospital (BSSH), UmaruShehu Ultra-Modern Hospital (USUH), and MammanShuwa Memorial Hospital (MSMH). Maiduguri serves as the capital city of Borno state, located in the North-East geopolitical zone of Nigeria. Borno State shares borders with Yobe to the west, Gombe to the southwest, and Adamawa to the south. To the east, it shares a border with Cameroon, while its northern and northeastern borders extend to Niger and Chad, respectively. Maiduguri experiences an annual average temperature ranging from 19.1°C to 34.7°C, with an average annual precipitation of 562 mm (NPC, 2006).

Collection of Sample

A total of 400 clinical samples were collected under sterile conditions using sterile universal containers. Among these, 200 urine specimens were obtained from patients diagnosed with urinary tract infections across the three hospitals, while the remaining 200 stool samples were acquired from patients diagnosed with diarrhea within the same hospital cohort. Subsequently, all collected samples underwent analysis to detect the presence of ESBL-producing *E. coli*.

Microbiological analysis

The urine and stool samples were aseptically collected using sterile universal containers (200ml) and labelled appropriately with participant's study number. All the clinical samples collected werestreak separately onto CLED Agar (urine) and MacConkey agar (stool) plates under aseptic techniques and incubated at 37 °C for 24 hours. The resulting colonies were further sub-cultured for confirmatory onto Eosin Methylene Blue agar and incubated at 37 °C for 24 hours. The colonies were identified using gram staining and biochemical techniques.

Antimicrobial susceptibility testing

The isolates underwent antimicrobial susceptibility testing using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Hi-Media), interpreted following CLSI guidelines (Hout*et al.,* 2019). A specific panel of antibiotics, adhering to CLSI guidelines, was chosen based on their demonstrated high sensitivity. Following incubation at 37°C for 24 hours, antimicrobial activity was assessed by observing inhibition zones, with their diameters measured in millimetres using a calibrated scale (Asati, 2013). Antibiotics assessed included amikacin, gentamicin, ciprofloxacin, norfloxacin, nalidixic acid, nitrofurantoin, co-trimoxazole, cefoperazone, cefoperazone/sulbactam, tazobactam/piperacillin, imipenem, ertapenem, meropenem, colistin, cefotaxime, and cefotaxime/clavulanic acid (Becton Dickinson, USA) (CLSI, 2021).

Phenotypic detection of extended spectrum β-lactamase (ESBLs)

ESBL production screening in all Escherichia coli isolates was conducted using a modified double disc synergy test (MDDST). The phenotypic detection involved the use of three 3rd generation cephalosporins: ceftazidime (CAZ 30µg), cefotaxime (CTX 30µg), and ceftriaxone (CRO 30µg). Individual discs of these antibiotics were placed on Mueller Hinton agar plates inoculated with the isolates, then incubated at 37°C for 24 hours to detect ESBL enzymes. ESBL production was confirmed if the zone diameter around each disc measured 5 mm or more (CLSI, 2021).

Confirmatory test for detection of extended spectrum β-lactamase

The confirmatory test for detecting extended spectrum β-lactamase (ESBL) involved the placement of an Augmentin (AMC 30µg) disc at the center of a Mueller Hinton agar plate containing streaked colonies of the positive isolate. Three additional discs of ceftazidime (CAZ 30µg), cefotaxime (CTX 30µg), and

ceftriaxone (CRO 30µg) were positioned around the Augmentin disc, with a 15mm distance from the center. Following overnight incubation at 37°C for 24 hours, the zones of inhibition around the Augmentin disc were measured to confirm ESBL production (CLSI, 2021).

Results

The results provided herein stem from a comprehensive investigation into *E. coli* isolated from clinical specimens, predominantly urine and stool samples, obtained from patients receiving care at diverse medical facilities in Maiduguri. The analysis unveils a noteworthy prevalence of *E. coli* within the studied samples. Out of a total of 400 samples examined, a substantial 245 were found to harbor *E. coli*. The breakdown of prevalence rates illustrates a slightly higher occurrence in urine samples, with 125 out of 214 samples yielding positive results, equating to a prevalence rate of 59.6%. Conversely, stool samples exhibited a notably higher prevalence rate of 80.0%, with 120 out of 150 samples testing positive for *E. coli* (Table 1).

Table 1: Prevalence of *E. coli* isolated from clinical samples among patients attending hospitals in Maiduguri.

Sample	Source	Number (%) of collected samples	Number (%) positive for *E.coli*
Urine	B.S.S.H	101 (47.2)	54 (53.5)
	U.S.U.H	54 (25.2)	32 (59.3)
	M.S.M.H	59 (27.6)	39 (66.1)
	Total	**214 (100)**	**125 (59.6)**
Stool	B.S.S.H	60 (40.0)	50 (41.7)
	U.S.U.H	40 (26.7)	30 (25.0)
	M.S.M.H	50 (33.3)	40 (33.3)
	Total	**150 (100)**	**120 (80.0)**

Table 2 presents the prevalence of multi-drug resistant (MDR) E. coli isolated from urine samples collected from patients attending different hospitals in Maiduguri. The table delineates the various combinations of antibiotics to which the *E. coli* isolates have exhibited resistance across three hospitals: Borno State Specialist Hospital (B.S.S.H), UmaruShehu Ultramodern Hospital (U.S.U.H), and MammanShuwa Memorial Hospital (M.S.M.H). Furthermore, a detailed investigation into the multi-drug resistance (MDR) patterns of *E. coli* isolates obtained from urine samples obtained from three distinct hospitals elucidates varying levels of resistance across different combinations of antibiotics employed. Upon combining the findings from the 141 isolates, it is discerned that MDR is prevalent, with the highest level observed at 26 (18.4%) and the lowest at 5 (3.5%) (Table 2).

Similarly, an equivalent analysis focusing on stool samples from the same trio of hospitals highlights the MDR patterns among the 120 isolates examined. The cumulative MDR rates portray a comparable scenario, with the highest resistance level recorded at 16 (13.3%) and the lowest at 1 (1.7%) (Table 3).

Moreover, a comprehensive table is presented, encapsulating the total count and percentage of extended spectrum beta-lactamase (ESBL)-producing and non-ESBL-producing *E. coli* isolates derived from both urine and stool specimens gathered across the three hospitals. Among the 243 positive isolates identified, 46 were categorized as ESBL-producing, with varying proportions noted across urine and stool samples (Table 4).

Table 2: Prevalence of Multi-drug resistant *E. coli* isolated from Urine among patients attending different hospital in Maiduguri

S/No.	Number of Combination of Antibiotics	B.S.S.H Isolâtes (%) (n=52)	Number of combination of Antibiotics	U.S.U.H Isolâtes (%) (n=45)	Number of combination of Antibiotics	M.S.M.H Isolâtes (%) (n=44)	MDR Isolates Combined (%) (n=141)
1.	OFX	9 (17.3)	PN	9 (20.0)	CN	8 (18.2)	26 (18.4)
2.	CN, S	8 (15.4)	CPX, OFX	7 (15.6)	NA	5 (11.4)	20 (14.2)
3.	CPX, CN	7 (13.5)	CPX, S	6 (13.3)	S,CN	8 (18.2)	21 (14.9)
4.	AU, CPX, OFX	6 (12.0)	CPX, OFX	3 (6.7)	S,SXT	5 (11.4)	14 (10.0)
5.	AU, CEP, PEF, PN	3 (5.8)	CPX, S	4 (8.9)	CPX, OFX	2 (4.5)	9 (6.4)
6.	CPX, SXT, PN, PEF	3 (5.8)	CPX, SXT, PN, PEF	3 (6.7)	AU, CPX, SXT	3 (6.8)	9 (6.4)
7.	AU,S,PN,CEP	3 (5.8)	AU,CPX,SXT,S,CN	4 (8.9)	AU, CPX,SXT,S	5 (11.4)	12 (8.5)
8.	AU,CPX,PN,PEF	4 (7.7)	CPX, SXT, S, OFX, PEF	5 (11.1)	PN, SXT,CPX, AU, PEF	4 (9.1)	13 (9.2)
9.	CPX, SXT, S, OFX, PEF	4 (7.7)	AU,CPX,SXT,PN,PEF	0 (0.0)	S,PN,OFX,PEF,CN,CPX, SXT	3 (6.8)	7 (5.0)
10.	CPX, SXT, S, PN, CEP,OFX	3 (5.8)	AU,CPX,SXT,S,OFX,PEF	2 (4.4)	AU,SXT,PN,OFX,PEF,S, CPX,CN	0 (0.0)	5 (3.5)
11.	AU,CPX,SXT,S,PN, OFX	2 (3.8)	AU,CPX, SXT, S, PN,OFX,PEF	2 (4.4)	S,PN,OFX,CN,CEP,PEF, CPX,SXT	1 (2.3)	5 (3.5)

Key: **B.S.S.H**= Borno State Specialist Hospital, Maiduguri, **U.S.U.H**= Umaru Shehu Ultramodern Hospital, Maiduguri. **M.S.M.H**= Mamman Shuwa Memorial Hospital, Maiduguri, OFX= Tarivid, PEF= Reflacine, CPX= Ciproflox, AU= Augumentin, CN= Gentamycin, S= Streptomycin, CEP= Ceporex, NA= Nalidixic acid, SXT= Septrin, PN= Amplicin

Table 3: Prevalence of Multi-drug resistant *E. coli* isolated from stool among patients attending different hospital in Maiduguri

S/ No	Number of Combination of Antibiotics	B.S.S.H Isolâtes (%) (n=50)	Number of combination of Antibiotics	U.S.U.H Isolâtes (%) (n=30)	Number of combination of Antibiotics	M.S.M.H Isolâtes (%) (n=40)	MDR Isolates Combined (%) (n=120)
1.	PN, CEP	7 (14.0)	PN, NA, AU, SXT	3 (10.0)	CEP, NA, AU	6 (15.0)	16 (13.3)
2.	PN, SXT	5 (10.0)	PN, AU, CPX, SXT,	5 (16.7)	PN, AU, SXT	5 (12.5)	15 (12.5)
3.	PN, NA, SXT	6 (12.0)	S, NA, AU, CPX	3 (10.0)	PN, NA, AU, SXT	3 (7.5)	12 (10.0)
4.	S, SXT, CPX	7 (14.0)	PN, NA, AU, CPX, SXT	4 (13.3)	PN, NA, CEP, SXT	4 (10.0)	15 (12.5)
5.	PN, NA, AU, SXT	3 (6.0)	OFX, NA, AU, CPX, SXT	5 (16.7)	PN,CEP, NA, AU,	5 (12.5)	13 (10.8)
6.	OFX, NA, AU, CPX, SXT	5 (10.0)	PN, NA, AU, CPX, SXT	2 (6.7)	PN, NA, PEF, AU, SXT	3 (7.5)	10 (8.3)
7.	S, CPX, OFX, AU, SXT	4 (8.0)	PN, NA, CN, AU, CPX, SXT	3 (10.0)	PN, NA, PEF, AU, OFX	4 (10.0)	11 (9.2)
8.	S, PN, NA, AU, CPX, SXT	3 (6.0)	S, PN, NA, AU, CPX, SXT,	3 (10.0)	S, PN, CEP, NA, PEF, AU	3 (7.5)	9 (7.5)
9.	PN, NA, PEF, CN, AU, CPX,	2 (4.0)	S, PN, CEP, OFX, NA, PEF	0 (0.0)	S,PN,CEP,NA, PEF,OFX	3 (7.5)	5 (4.2)
10.	S, NA, CPX, OFX, AU, SXT	4 (8.0)	PEF, CN, NA, PEF, OFX, AU, SXT	2 (6.7)	PN,CEP,NA,PEF,AU,SXT	4 (10.0)	10 (8.3)
11.	S, PN, CEP, OFX, NA, PEF, AU	1 (2.0)	S, CPX, AU, CN, PEF, NA, OFX, SXT	0 (0.0)	-	-	1 (1.7)
12.	S, PN, NA, PEF, AU, CPX, SXT	3 (6.0)			-	-	3 (2.5)

Key: OFX= Tarivid, PEF= Pefloxacin, CPX= Ciprofloxacin, AU= Augmentin, CN= Gentamycin, S= Streptomycin, CEP= Ceporex, NA= Nalidixic acid, SXT= Septrin, PN= Ampicillin

Table 4: Prevalence of ESBL- producing and non-ESBL *E. coli* isolated from clinical samples of patients attending hospitals in Maiduguri.

Source of isolates	B.S.S.H	U.S.U.H	M.S.M.H	ESBL producing *E. coli* (%)	Non-ESBL producing *E. coli* (%)
Urine	54	32	39	28 (31.1)	62 (68.9)
Stool	50	30	40	18 (34.0)	35 (66.0)
Total	**104**	**62**	**79**	**46 (32.2)**	**97 (67.8)**

Discussion

Treating infectious diseases is crucial for human health, and the growing resistance of bacteria has led to increased costs for patients. The emergence of Extended-Spectrum Beta-Lactamases (ESBLs) poses a significant threat to the effectiveness of newer cephalosporins. Over the past two decades, there has been a notable rise in ESBL production among Enterobacteriaceae bacteria (Gholipour *et al.,* 2014; Khorshidi *et al.,* 2012). Specifically, *E. coli* are prominent causes of hospital-acquired infections. Hence, the bacterial strains were chosen for our investigation. The prevalence of infections caused by ESBL-producing *E. coli* has been widely documented worldwide, largely due to the widespread usage of extended-spectrum cephalosporins (Pathak *et al.,* 2012).The results of the study shows that urine samples collected from patients across different hospitals exhibited a varying prevalence of *E. coli*. The highest prevalence was observed in samples from Mamman Shuwa Memorial Hospital (M.S.M.H), followed by Umaru Shehu Ultramodern Hospital (U.S.U.H) and Borno State Specialist Hospital (B.S.S.H). This variation could be attributed to differences in patient demographics, environmental factors, or healthcare practices among the hospitals (Muhammad *et al.,* 2013).Similarly, stool samples also showed variations in *E. coli* prevalence across the three hospitals, with the highest prevalence observed in samples from B.S.S.H, followed by M.S.M.H and U.S.U.H. Again, factors such as patient population, hygiene practices, and hospital protocols may contribute to these differences (Muhammad *et al.,* 2013).The high prevalence of *E. coli* in clinical samples, particularly urine and stool, underscores the significance of *E. coli* infections in healthcare settings. These findings emphasize the importance of infection control measures, such as hand hygiene, sanitation, and proper waste management, to prevent the spread of *E. coli*-related illnesses among patients and healthcare workers (Wakil *et al.,* 2021). Understanding the prevalence rates in different hospital settings can guide decisions regarding diagnostic testing, empirical antibiotic therapy, and infection control strategies.

The ESBL-producing *E. coli* strains were detected in both urine and stool samples collected from patients across different hospitals. The prevalence of ESBL-producing *E. coli* varied among the hospitals, with the highest percentage observed in urine samples from B.S.S.H and stool samples from M.S.M.H. Similarly, non-ESBL-producing *E. coli* strains were also detected in urine and stool samples, with varying prevalence rates across the hospitals. Notably, the percentage of non-ESBL-producing *E. coli* was higher than ESBL-producing strains in both sample types and across all hospitals. The high prevalence of ESBL-producing *E. coli* in clinical samples is concerning as it indicates resistance to commonly used antibiotics, such as beta-lactams. This resistance can compromise the effectiveness of antibiotic treatment and lead to treatment failures, prolonged illnesses, and increased healthcare costs (Wakil *et al.,* 2021).The detection of ESBL-producing *E. coli* in both urine and stool samples highlights the importance of infection control measures in healthcare settings. Enhanced surveillance, strict adherence to hygiene protocols, and judicious use of antibiotics are crucial for preventing the spread of antibiotic-resistant pathogens among patients and healthcare workers (Gholipour *et al.,* 2014).

The prevalence rates of 32.2% ESBL-producing observed in this study can be compared with those reported in previous studies conducted in similar healthcare settings or patient populations. Such comparisons can help assess the consistency of ESBL prevalence over time and identify any emerging trends or changes in resistance patterns.In Iraq, certain researchers (Al-mayahie, 2013) have documented the prevalence of multi-drug resistant Enterobacteriaceae and gram-negative bacteria that produce extended spectrum beta-lactamases (ESBLs). Previous findings by Adam and Turgut (2019) also noted that *E. coli* exhibited the highest resistance among all tested drugs. Notably, quinolone and gentamicin, traditionally effective against many bacterial infections in the past decade, were found to be largely ineffective against these *E. coli* isolates in the current study. The escalation of resistance has been documented in recent research from other developing nations where antibiotic usage policies are not rigorously enforced in their communities (Muhammad and Swedan, 2015).In Tehran, ESBL-positive rates of 34.8% among Gram-negative bacterial strains were reported by Ramazanzadeh et al. (2009), while Mobasherizadeh et al. (2012) indicated that out of 2035 consecutive clinical isolates identified as E. coli in Al-Zahra Hospital, 44.1% and 21.2% were ESBL producers among hospitalized and non-hospitalized patients, respectively. Discrepancies or variations between the findings of this study and previous research may reflect regional differences in antibiotic usage, infection control practices, or the prevalence of antibiotic-resistant bacteria. Temporal trends in ESBL prevalence can also be examined to assess the impact of interventions aimed at reducing antibiotic resistance. Therefore, these findings underscore the urgent need for comprehensive strategies to combat antibiotic resistance and enhance infection control measures in healthcare settings. Comparisons with previous studies can provide valuable insights into the dynamics of antibiotic resistance and inform evidence-based interventions to mitigate its impact on patient outcomes and public health.

Conclusion

In conclusion, the findings of this study shed light on the prevalence and antibiotic resistance patterns of *E. coli* isolated from clinical specimens, primarily urine and stool samples, collected from patients attending various hospitals in Maiduguri. The investigation revealed a significant prevalence of *E. coli* across the sampled population, with 245 out of 400 samples testing positive for the bacteria. Notably, urine samples exhibited a prevalence rate of 59.6%, while stool samples showed a higher rate of 80.0%. These results underscore the substantial burden of E. coli infections in healthcare settings. Moreover, the analysis of multi-drug resistance (MDR) patterns among *E. coli* isolates obtained from urine and stool samples elucidated varying levels of resistance across different combinations of antibiotics. The prevalence of MDR was notable, with varying resistance levels observed among different hospitals. Similarly, the detection of extended spectrum beta-lactamase (ESBL)-producing *E. coli* strains in both urine and stool samples highlights the growing challenge of antibiotic resistance in clinical settings. Therefore, the findings emphasize the urgent need for comprehensive strategies to combat antibiotic resistance and enhance infection control measures in healthcare settings. Enhanced surveillance, strict adherence to hygiene protocols, and judicious use of antibiotics are essential to prevent the spread of antibiotic-resistant pathogens and mitigate their impact on patient outcomes and public health. Therefore, these results provide valuable insights into the dynamics of antibiotic resistance and inform evidence-based interventions to address this global health threat.

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