

Co-existence Pattern of *ESBL* Resistance Genes in Multidrug Resistant *E. coli* and *Klebsiella* species Isolated from a Nosocomial Environment in South-Eastern Nigeria

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Abstract

This study investigated the co-existence pattern of *ESBL* resistance genes in *Escherichia coli* and *Klebsiella* species isolated from patients visiting a university tertiary hospital in Ebonyi State, Nigeria. Clinical samples of urine and feces (n=246) were bacteriologically analyzed for the presence of *E. coli* isolates and *Klebsiella* species using standard microbiological techniques. Phenotypic detection of *ESBL*-producing test isolates was done using the double disk synergy test technique, while antimicrobial studies were carried out using the Kirby-Bauer disk diffusion method. 80 (50%) and 30 (18.75%) isolates of *E. coli* and *Klebsiella* species were recovered from in-patients respectively. 28 (17.5%) and 22 (13.76%) isolates of *E. coli* and *Klebsiella* species were obtained from the outpatients, respectively. Phenotypic confirmation of *ESBL* revealed that 26 (31.71%) and 15 (18.29%) isolates of *E. coli* and *Klebsiella* species were obtained from inpatients, whereas 7 (8.54%) and 4 (4.88%) isolates of *E. coli* and *Klebsiella* species were obtained from outpatients, respectively. All *E. coli* and *Klebsiella* species isolates displayed varied resistance to the antibiotics used, but were completely susceptible to imipenem. *E. coli* strain NRC124 16S, *E. coli* strain SD134209, *K. aerogenes* strain CB461, *E. coli* strain 80, *E. coli* strain Mui T6, *E. coli* strain 71, *E. coli* strain AKRC4, *E. coli* isolate AV14, and *E. coli* strain H-*ESBL* were identified using DNA sequencing techniques. There was co-existence of *OXA/SHV* gene in 4 isolates, *ACC/CTXM* gene in 1 isolate, *TEM/SHV* gene in seven isolates, *CTX-M/SHV* gene in 6 isolates, and *CTXM/OXA/SHV/TEM* gene in 6 isolates.

Keywords: *ESBL*, bacteria, antimicrobial resistance, genes, nosocomial environment

Резюме

Това проучване изследва модела на съвместно съществуване на *ESBL* резистентни гени в видове *Escherichia coli* и *Klebsiella*, изолирани от пациенти, посещаващи университетска третична болница в щата Ебони, Нигерия. Клинични проби от урина и фекалии (n=246) бяха бактериологично анализирани за наличие на изолати на *E. coli* и видове *Klebsiella*, използвайки стандартни микробиологични техники. Фенотипното откриване на *ESBL*-продуциращи тестови изолати беше извършено с помощта на техниката на двоен дисков синергичен тест, докато антимикробните изследвания бяха проведени с помощта на дисково-дифузионния метод на Кърби-Бауер. 80 (50%) и 30 (18.75%) изолати на видове *E. coli* и *Klebsiella* бяха изолирани съответно от хоспитализирани пациенти. Двадесет и осем (17.5%) и 22 (13.76%) изолати на видове *E. coli* и *Klebsiella* бяха получени съответно от амбулаторни пациенти. Фенотипното потвърждение на *ESBL* показва, че 26 (31.71%) и 15 (18.29%) изолати от видове *E. coli* и *Klebsiella* са получени от хоспитализирани пациенти, докато 7 (8.54%) и 4 (4.88%) изолати от видове *E. coli* и *Klebsiella* са получени от амбулаторни пациенти, съответно. Всички изолати от тези два вида показват различна резистентност към използваните антибиотици, но са напълно чувствителни към имипенем. Щамове *E. coli* NRC124 16S, *E. coli* SD134209, *K. aerogenes* CB461,

E. coli 80, *E. coli* Mui T6, *E. coli* 71, *E. coli* AKRC4, изолат на *E. coli* AV14 и *E. coli* H-ESBL са идентифицирани с помощта на техники за ДНК секвениране. Наблюдавано е едновременно съществуване на *OXA/SHV* гена в 4 изолата, *ACC/CTXM* гена в 1 изолат, *TEM/SHV* гена в седем изолата, *CTX-M/SHV* гена в 6 изолата и *CTXM/OXA/SHV/TEM* гена в 6 изолата.

Introduction

Extended-spectrum beta-lactamases (*ESBLs*) are cephalosporin-hydrolyzing enzymes produced by Gram-negative bacteria, which confer antibiotic resistance on certain bacteria, especially in the *Enterobacteriaceae* family (Yan *et al.*, 2000). They are penicillin-degrading enzymes capable of hydrolyzing penicillins, narrow-spectrum cephalosporins, many extended-spectrum cephalosporins, and the oxymino cephalosporins such as ceftriaxone, cefotaxime, and ceftazidime (Paterson *et al.*, 2010). The existence and emergence of new beta-lactamases are important mechanisms by which bacteria develop resistance to beta-lactam antibiotics. Bacteria species, including *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *K. oxytoca*, under certain conditions can become resistant to antibiotics of different classes, and are generally called multi-drug-resistant Gram-negative bacteria (MDRGNB) (Okafor *et al.*, 2022; Adie *et al.*, 2024).

ESBL-producing Gram-negative bacteria such as *E. coli* and *Klebsiella* species pronounce serious health challenges because these organisms have become multidrug resistant in nature. The detection of *ESBL*-positive Gram-negative bacteria, especially those harboring the widely distributed *bla*_{CTX-M-15} gene, is important because it helps to provide effective guidance to therapy when treating infections caused by these organisms. Many MDRGNB produce beta-lactamases such as *ESBLs* that can inactivate or destroy expanded/broad-spectrum antibiotics such as the 3rd generation cephalosporins and monobactams (e.g., Aztreonam). *ESBLs* are detected mostly in *Enterobacteriaceae*, especially *K. pneumoniae*, *E. coli*, and *K. oxytoca*. They often harbor resistance determinants for other classes of antibiotics like the aminoglycosides, sulfonamides, and fluoroquinolones, which are readily transmissible from one strain of organism to another and across different species of Gram-negative bacteria (Munday *et al.*, 2004a). Apart from the *TEM*- and *SHV*- type *ESBLs*, other classes of *ESBLs*, such as including cefotaxime (*CTX*)-M type *ESBLs*, penicillinase (*PER*)- and oxacillinase or *OXA*- type *ESBLs* have also been described (Munday *et al.*, 2004b). Although there is little or no information on the relationship between the consumption of the 3rd-generation cephalosporins and the occurrence

of *bla*_{CTX-M} type beta-lactamases, the high frequency of occurrence observed in the community and worldwide dissemination may indicate that oxymino-cephalosporins are available and abused outside the hospital settings, especially in over-the-counter (OTC) treatment and self-medication purposes.

This study aimed to investigate the co-existence pattern of *ESBL* resistance genes in *E. coli* and *Klebsiella* species from patients visiting Alex Ekwueme Federal University Teaching Hospital (AE-FETHA) in Abakaliki, Ebonyi State, Nigeria. *bla*_{CTX-M-15} *ESBL* types amongst bacteria have been previously reported by Iroha *et al.* (2012) in South-eastern Nigeria. However, the frequency of many other *ESBL* types in Abakaliki, Nigeria, is still under-reported. This informed the decision to undertake this study.

Materials and Methods

Ethical consideration

Ethical clearance was obtained from Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Ebonyi State, Nigeria, before the commencement of this research work.

Study area

This research work was carried out in the Laboratory of Applied Microbiology Department of Ebonyi State University, Abakaliki, Ebonyi State. Ebonyi State is located in the south-eastern part of Nigeria. The state lies approximately within longitude 7°30' and 7°E and, latitude 5°40' and 6°45' N with a population of about 3 529 683, and a land mass of about 6 488 square kilometers. The State is bounded to the north by Benue State, to the south by Abia State, to the east by Enugu State, and to the west by Cross River State, respectively. Abakaliki, the State capital, has a tropical climate with an average relative humidity of 75% and may reach 80% during the rainy season. The vegetation characteristics are predominantly rainforest with an atmospheric temperature of 30°C. Two seasons are distinguishable in Ebonyi State: a dry season (November to March) and a wet season (April and October).

Sample size determination

Sample size was determined based on previously reported prevalence rates of *ESBL*-producing Gram-negative bacteria in some parts of Ni-

geria (Iroha *et al.*, 2012; Ejikeugwu *et al.*, 2017). The prevalence of *ESBL*-producing bacteria was pegged at 20%. Sample size was determined at 95% confidence interval (CI) at a 0.05 precision using Cochran's formula. Therefore, 246 clinical isolates of *E. coli* and *Klebsiella* species were used for this study.

Sample collection, characterization, and identification of isolates

A total of 246 clinical isolates of bacteria from urine and fecal samples were collected from inpatients and outpatients, female and male patients between the ages of 1 and 90 years, attending the Microbiology laboratory unit of Alex Ekwueme Federal University Teaching Hospital, Abakaliki, for clinical investigation. The study was carried out between September, 2018 and October, 2024. The bacterial isolates were identified and characterized using standard microbiological techniques, which include culturing, Gram staining, biochemical and sugar fermentation test such as the indole test, methyl red test, Voges-Proskauer test, citrate utilization test, motility test, and urease test. The colony from nutrient agar media was re-inoculated on MacConkey agar and CLED agar and incubated at 37°C for 18-24 hours. The colonial morphology of the isolates was observed and recorded. The colonies were purified by sub-culturing to obtain pure cultures. The pure cultures of the isolates were inoculated on nutrient agar slants and incubated at 37°C for 24 hours. This was stored in a refrigerator at 4°C as a stock culture for future use (Cheesbrough, 2006).

Antimicrobial susceptibility testing

The antimicrobial resistance and susceptibility patterns of the *ESBL*-positive *E. coli* and *Klebsiella* species clinical isolates were determined using the Kirby-Bauer disk diffusion method from an overnight culture of the test bacteria that was adjusted to 0.5 McFarland turbidity standards. The antibiotic disks that was used includes amoxicillin/clavulanic acid (20/10 µg), ceftriaxone (30 µg), cefepime (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), ciprofloxacin, (5 µg), gentamicin (10 µg), ertapenem (10 µg), imipenem (10 µg), meropenem (10 µg), ofloxacin (5 µg), amikacin (10 µg), levofloxacin (10 µg), aztreonam (30 µg), nitrofurantoin (10 µg) and sulphamethoxazole/trimethoprim (25 µg) (Oxoid, UK). The plates were incubated at 37°C for 18-24 hours, and the inhibition zone diameters (IZDs) produced by the antibiotic disks were measured and recorded to the nearest millimeter.

The recorded inhibition zone diameters were evaluated and interpreted using the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2024).

Determination of multiple antibiotic resistance indexes

Multiple antibiotic resistance index (MARI) was determined for all *ESBL*-positive *E. coli* and *Klebsiella* species isolates in order to determine their multiple antibiotic resistance nature. MARI will be determined by the method of Okafor *et al.* (2022) using the formula: $MARI = a/b$; where: a = total number of antibiotics to which the resistant isolate was resistant; b = total number of antibiotics for which the resistant isolate was evaluated for

Screening of bacterial isolates for ESBL production using Double Disk Synergy Test (DDST)

ESBL production was phenotypically confirmed in all the *E. coli* and *Klebsiella* species clinical isolates through the DDST method (Ejikeugwu *et al.*, 2017). DDST was performed as a standard disk diffusion assay on Mueller-Hinton (MH) agar (Oxoid, UK) plates. Antibiotic disks of amoxycillin-clavulanic acid (20/10 µg) were placed at the center of the MH agar plate, and antibiotic disks containing cefotaxime (30 µg) and ceftazidime (30 µg) were each placed at a distance of 15 mm from the central disc (amoxicillin/clavulanic acid). The plates were incubated at 37°C for 18-24 hours. *ESBL* production was inferred phenotypically when the zones of inhibition of the cephalosporins (Cefotaxime 30 µg and ceftazidime 30 µg) were expanded by the amoxicillin/clavulanic acid disk (20/10 µg). However, a ≥ 5 mm increase in the inhibition zone diameter for either of the cephalosporins tested in combination with amoxicillin/clavulanic acid versus its zone when tested alone confirms the phenotypic production of *ESBL*.

Statistical analysis

Statistical analysis for this study was conducted using SPSS version 22. Comparative analysis of categorical variables such as age groups, gender, and prevalence of multidrug-resistant (*MDR*) strains was performed using the Chi-square test with Bonferroni post hoc correction for multiple comparisons and Fisher's Exact tests

Molecular studies

Molecular studies in this research were carried out at the Bioinformatics Laboratory of the International Institute of Tropical Agriculture (IITA),

Ibadan, Nigeria. After the extraction of total DNA, the quality and purity of the extracted DNA were assessed. This was followed by polymerase chain reaction (PCR) analysis using 16S rRNA primer, assessment of gel integrity, purification of amplified product, DNA sequencing and analysis, phylogenetic tree construction, and detection of antibiotic resistance genes, namely bla_{CTX-M}, ESBL, bla_{TEM}, bla_{SHV}, bla_{aac(6')} I_b-cr, and bla_{OXA-1} using primers.

Extraction of total DNA

Genomic DNA from fifteen presumptively identified isolates was extracted. A 1000 µL of freshly prepared broth culture was centrifuged at 10,000 rpm for 5 minutes to get pellets. The supernatant was decanted, and 1 ml of DNA Extraction Buffer (DEB) containing proteinase K (0.05 mg/ml) was added and vortexed. About 50 µl of 20 % Sodium Dodecyl Sulphate (SDS) was added and incubated in a water bath at 65°C for 30 minutes. The tubes were allowed to cool to room temperature, after which 100 µl of 7.5 M Potassium Acetate was added and mixed briefly. The mixture was centrifuged at 13000 rpm for 10 minutes. The supernatant was transferred into new, fresh autoclaved tubes. To the supernatant was added 2/3 volumes of cold Isopropanol/Isopropyl alcohol. The tubes were gently inverted 5 times and incubated at -20°C for 1 hour. The tubes were centrifuged at 13000 rpm for 10 minutes, and the supernatant was discarded. 500 µl of 70% ethanol was added and centrifuged for 5 minutes at 13000 rpm. The supernatant was discarded carefully, with the DNA pellet intact. Traces of ethanol were removed, and the DNA pellets were dried at 37°C for 15 minutes. The DNA pellets were re-suspended in 50 µl of Tris-EDTA (TE) buffer. After this aliquot of DNA was extracted was stored at -20°C for further laboratory analysis. Thus, an ultra-pure DNA was ready for use (Edet *et al.*, 2018).

Assessing the quality and purity of the extracted DNA

The quality of the extracted DNA was assessed and confirmed using 1% agarose gel for electrophoresis. This was carried out at 100 Volts, 400 amps for 2 hours. All the isolated DNA samples were found intact and devoid of any form of shearing or degradation effects.

Polymerase Chain Reaction (PCR) analysis using 16s rRNA primer

The PCR preparation cocktail consisted of 10 µl of 5 x GoTaq colourless reaction, 3 µL of 2.5 mM MgCl₂, 1 µL of 10 mM of dNTPs mix, 1 µL of 10 pmol each of the 16S rRNA gene forward primer

(16S rRNA: GTGCCAGCAGCCGCGCTAA) and reverse primer (16S rRNA: AGACCCGGAACG-TATTAC) and 0.3 units of Taq DNA polymerase (Promega, USA) made up to 42 µL with sterile distilled water and 8 µL DNA template. The PCR master mixture used in the DNA amplification assay is presented in Table 2. PCR was carried out in a GeneAmp 9700 PCR System Thermal cycler (Applied Biosystem Inc., USA) with a PCR profile consisting of an initial denaturation at 94°C for 5 minutes; followed by a 30 cycles consisting of 94°C for 30 seconds, 30 seconds annealing of primer at 56°C and 72°C for 1 minute 30 seconds and a final termination at 72 °C for 10 minutes was maintained at 4 °C as its soaking temperature as described by Edet *et al.* (2018) with little modification on the reagents mix, initial temperature and cycles.

DNA sequencing and analysis

DNA sequencing and analysis were carried out in accordance with the procedures described by Olowe *et al.* (2017) with little modification by removing chloroform in the extraction process. The amplified fragments were sequenced using a Genetic Analyzer 3130 xl sequencer from Applied Biosystems using manufacturers' manual, while the sequencing kit used was that of the BigDye terminator version 3.1 cycle sequencing kit. Bio-Edit software and MEGA 6 were used for all genetic analysis. The samples were loaded on the machine, and data in the form of adenine (A), cytosine (C), thymine (T), and guanine (G) were released. The amplified 16S rRNA gene sequence was compared with the sequences in the National Centre for Biotechnology Information (NCBI) sequence database using the Basic Local Alignment Search Tool (BLAST) search program.

Construction of phylogenetic trees

The optimal tree was determined using the distance matrix methods. A phylogenetic/evolutionary tree was inferred using the unweighted pair group method with arithmetic mean (UPGMA). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown above the branches. All positions with less than 95 % site coverage were eliminated. That is, fewer than 5 % alignment gaps, missing data, and ambiguous bases were allowed at any position. Evolutionary analyses were conducted in MEGA 5 (Tamura *et al.*, 2011).

Characterization of antibiotic resistance genes

Isolates were tested for the presence of resistance genes by PCR analysis according to published

procedures described by Prabhat *et al.* (2013). The primers *bla*_{CTX-M}-ESBL, *bla*_{TEM}, *bla*_{SHV}, *bla* *aac*(6') *Ib-cr*, and *bla**OXA-1*, corresponding annealing temperature used in the PCR analysis are listed in Table 1. The PCR consisted of the following steps: 1 amplification cycle at 95°C for 15 minutes; 30 cycles at 94°C for 30 seconds, 60°C for 90 seconds, and 72°C for 60 seconds; and a final extension step at 72°C for 7 minutes (Tamura *et al.*, 2011).

Oligonucleotide primer sets used for detection of the ESBL gene

The oligonucleotide primers used for the PCR amplification/detection of CTX-M-15 ESBL genes and other co-resistant genes, namely *OXA-1*, *TEM-1*, *SHV-1*, and *aac*(6') *Ib-cr*, are shown in Table 1. The detection of the ESBL gene was carried out in accordance with the procedures described by Olowe *et al.* (2017). The samples were loaded on the machine, and data in the form of adenine (A), cytosine (C), thymine (T), and guanine (G) were released. The amplified 16S rRNA gene sequences were compared with the sequences in the National Centre for Biotechnology Information (NCBI) sequence database using the Basic Local Alignment Search Tool (BLAST) search program.

Results

Age and gender-related bacterial prevalence of inpatients and outpatients attending Alex Ekweme Federal Teaching Hospital, Abakaliki, Ebonyi State, Nigeria

The result for age and gender related bacterial prevalence of in- and outpatients attending Federal teaching hospital, Abakaliki, Ebonyi State, Nigeria are shown in Table 2. The total number of isolates was 160, after re-characterizing, 108 (67.5 %) and 52 (32.5 %) were identified as *E. coli* and *Klebsiella* spp. respectively.

Frequency of distribution of bacterial isolates from inpatients and outpatients

Table 3 shows the frequency of distribution of bacterial isolates from inpatients and outpatients. The total occurrences of the test isolates for *E. coli* were higher than those of *Klebsiella* spp. from both inpatients and outpatients in urine and fecal samples.

Antibiotic susceptibility patterns of the ESBL-producing *E. coli* and *Klebsiella* species from inpatients and outpatients

The results obtained from the antibiotic susceptibility testing for the ESBL-positive clinical isolates of *E. coli* and *Klebsiella* species by the disc diffusion method are presented in Tables 4 - 7. Results are presented according to the different isolates collected with respect to the different organisms isolated. The test isolates from urine and feces from both inpatients and outpatients visiting AEFE-THA show varied resistance patterns to the various commercial antibiotic discs used in the study.

Total and average multiple antibiotic resistance (MAR) index analysis of ESBL-producing *E. coli* and *Klebsiella* species in and outpatient

Table 8 reveals the results for the total and average multiple antibiotic resistance (MAR) index for bacterial isolates. The results revealed that the four isolate categories had a total MARI of 10.45, 5.07, 6.21, and 1.86 for *E. coli* inpatients, *Klebsiella* species inpatients, *E. coli* outpatients, and *Klebsiella* species outpatients, respectively. It also showed that the isolates had an average MARI of 0.80, 0.39, 0.48, and 0.14 for isolates from in-patients and outpatients, respectively.

BLAST results of sequenced organisms

A total of nine strains of *E. coli* strain NRC124 16S, *E. coli* strain SD134209, *K. aerogenes* strain

Table 1. Primer and PCR condition for amplification of CTX-M-15 ESBL gene

PCR target	Primer name	Primer sequence	Amplicon size (bp)
blaCTX-M-15	CTX-M-15F	CCGTCACGCTGTTGTTAGG	481
	CTX-M-15R	GACGATTTTAGCCGCCGAC	
OXA-1	OXA-1-F	GCGTGGTTAAGGATGAACAC	369
	OXA-1-R	CATCAAGTTCAACCCAACCG	
TEM-1	TEM-1-F	CATTTCCTGTCGCCCTTATTC	800
	TEM-1-R	CGTTCATCCATAGTTGCCTGAC	
SHV-1	SHV-1-F	AGCCGCTTGAGCAAATTAAAC	713
	SHV-1-R	ATCCCGCAGATAAATCACCAC	
aac(6') Ib-cr	aac(6') IbFw 1	TTGCGATGCTCTATGAGTGGCTA	482
	aac(6') IbFw 2	CTCGAATGCCTGGCGTGTTT	

Key: F = Forward primer, R = Reverse primer

Table 2. Age and Gender-related bacterial prevalence of in patients and outpatients

Groups	Sex	Age interval (Years)	Number of isolates	Number positive with <i>E. coli</i> (%) $\pm$ SD	Number positive with <i>Klebsiella</i> spp. (%) $\pm$ SD
1a	Male	1–10	5	3 (8.11 $\pm$ 2.91)a	2 (5.41 $\pm$ 2.27)a
		11–20	10	6 (16.22 $\pm$ 5.12)b	4 (10.81 $\pm$ 3.86)b
		21–30	22	15 (40.54 $\pm$ 6.32)c	7 (18.92 $\pm$ 4.87)C
1b	Female	1–10	3	2 (4.17 $\pm$ 1.85)a	1 (2.08 $\pm$ 1.01)a
		11–20	15	10 (20.83 $\pm$ 5.79)b	5 (10.42 $\pm$ 3.94)b
		21–30	30	20 (41.67 $\pm$ 6.45)C	10 (20.83 $\pm$ 5.11)C
2a	Male	31–40	16	12 (42.86 $\pm$ 6.30)C	6 (21.43 $\pm$ 5.18)C
		41–50	8	5 (17.86 $\pm$ 4.21)b	3 (10.71 $\pm$ 3.26)b
		51–60	4	3 (10.71 $\pm$ 3.79)b	1 (3.57 $\pm$ 1.79)a
2b	Female	31–40	23	15 (44.12 $\pm$ 6.49)C	8 (23.53 $\pm$ 5.67)C
		41–50	9	6 (17.65 $\pm$ 4.35)b	3 (8.82 $\pm$ 2.94)b
		51–60	2	1 (2.95 $\pm$ 1.60)a	1 (2.95 $\pm$ 1.60)a
3a	Male	61–70	4	2 (25.00 $\pm$ 5.42)b	2 (25.00 $\pm$ 5.42)b
		71–80	3	2 (25.00 $\pm$ 5.42)b	1 (12.50 $\pm$ 3.54)a
		81–90	1	1 (12.50 $\pm$ 3.54)a	0 (0.00 $\pm$ 0.00)a
3b	Female	61–70	5	1 (20.00 $\pm$ 4.00)b	1 (20.00 $\pm$ 4.00)b
		71–80	3	1 (20.00 $\pm$ 4.00)b	1 (20.00 $\pm$ 4.00)b
		81–90	1	1 (20.00 $\pm$ 4.00)b	0 (0.00 $\pm$ 0.00)a

Key: Superscripts (a,b,c) denote statistically significant differences between age groups ($p < 0.05$).

Table 3. Frequency distribution of bacteria isolates from inpatients and outpatients

Sample Source	<i>E. coli</i>		<i>Klebsiella</i> spp.	
	Inpatients	Outpatients	Inpatients	Outpatients
Urine	50 (46.3% $\pm$ 4.8)	20 (18.5% $\pm$ 3.7)	20 (38.5% $\pm$ 6.8)	15 (28.8% $\pm$ 6.4)
Feces	30 (27.8% $\pm$ 4.3)	8 (7.4% $\pm$ 2.5)	10 (19.2% $\pm$ 5.3)	7 (13.5% $\pm$ 4.8)

Key: Pearson χ^2 (1) = 0.097, $p = 0.756$; Fisher's Exact $p = 0.859$. Values are counts with percentages and standard deviations ($\pm$ SD) calculated assuming a binomial distribution.

Table 4. Antibiotic susceptibility pattern of ESBL-producing *E. coli* and *Klebsiella* spp. from urine isolates of inpatients

Antibiotics (μ g)	Number tested	<i>E. coli</i>		Number tested	<i>Klebsiella</i> spp.	
		Number sensitive (%)	Number resistant (%)		Number sensitive (%)	Number resistant (%)
CAZ (30)	26	10 (38.46)	16 (61.54)	15	3 (20.00)	12 (80.00)
IMP (10)	26	42 (100.00)	0 (0.00)	15	5 (33.33)	10 (66.67)
CRO (30)	26	5 (19.23)	21 (80.76)	15	8 (53.33)	7 (46.67)
CTX (30)	26	4 (15.38)	22 (84.61)	15	9 (60.00)	6 (40.00)
FOX (30)	26	21 (80.76)	5 (19.23)	15	5 (33.33)	10 (66.67)
CEP (30)	26	14 (53.84)	12 (46.15)	15	8 (53.33)	7 (46.67)
ATM (30)	26	9 (34.61)	17 (65.38)	15	9 (60.00)	6 (40.00)
SXT (5)	26	18 (69.23)	8 (30.76)	15	2 (14.29)	12 (85.71)
DO (30)	26	19 (73.08)	7 (26.92)	15	5 (33.33)	10 (66.67)
NOR (10)	26	23 (88.46)	3 (11.54)	15	11 (73.33)	4 (26.67)
NA (30)	26	6 (23.08)	20 (76.92)	15	10 (66.67)	5 (33.33)
CN (10)	26	23 (88.46)	3 (11.54)	15	14 (93.33)	1 (6.67)
CIP (5)	26	24 (92.31)	2 (7.69)	15	15 (100.00)	0 (0.00)

Key: ATM = aztreonam, CAZ = ceftazidime, CRO = ceftriaxone, CTX = cefotaxime, FOX = cefoxitin, IMP = imipenem, CEP = Cefepime, SXT = sulfamethoxazole\ trimethoprim, DO = Doxycycline, NOR= Norfloxacin, NA = Nalidixic acid, CN = gentamicin and CIP = ciprofloxacin

CB46l, *E. coli* strain 80, *E. coli* strain Mui T6, *E. coli* strain 71, *E. coli* strain AKRC4, *E. coli* isolate AV14, and *E. coli* strain H-ESBL were identified using DNA sequencing techniques (Table 9).

Coexistence of resistance genes in ESBL-producing *E. coli* and *Klebsiella* species.

Table 10 showed the co-existence pattern of resistance genes in ESBL-producing isolates of *E.*

Table 5. Antibiotic susceptibility pattern of ESBL *E. coli* and *Klebsiella* spp. from feces isolates of inpatients

Antibiotics (µg)	Number Tested	<i>E. coli</i>		Number Tested	<i>Klebsiella</i> spp.	
		Number Sensitive (%)	Number Resistant (%)		Number Sensitive (%)	Number Resistant (%)
CAZ (30)	14	5 (37.71)	9 (64.29)	8	3 (37.50)	5 (62.50)
IMP (10)	14	13 (92.86)	1 (7.14)	8	8 (100.00)	0 (0.00)
CRO (30)	14	3 (21.43)	11 (78.57)	8	2 (25.00)	6 (75.00)
CTX (30)	14	4 (28.57)	10 (71.43)	8	0 (0.00)	8 (100.00)
FOX (30)	14	12 (85.71)	2 (14.29)	8	7 (87.50)	1 (12.50)
CEP (30)	14	3 (21.43)	11 (78.57)	8	2 (25.00)	6 (75.00)
ATM (30)	14	2 (14.29)	12 (85.71)	8	1 (12.50)	7 (87.50)
SXT (5)	14	8 (57.14)	6 (42.86)	8	5 (62.50)	3 (37.50)
DO (30)	14	7 (50.00)	7 (50.00)	8	4 (50.00)	4 (50.00)
NOR (10)	14	9 (64.29)	5 (35.71)	8	6 (75.00)	2 (25.00)
NA (30)	14	12 (87.50)	2 (12.50)	8	7 (87.50)	1 (12.50)
CN (10)	14	10 (71.42)	4 (28.57)	8	6 (75.00)	2 (25.00)
CIP (5)	14	13 (92.86)	1 (7.12)	8	7 (87.50)	1 (12.50)

Key: ATM = aztreonam, CAZ = ceftazidime, CRO = ceftriaxone, CTX = cefotaxime, FOX = ceftioxitin, IMP = imipenem, CEP = Cefepime, SXT = sulfamethoxazole\ trimethoprim, DO = Doxycycline, NOR= Norfloxacin, NA = Nalidixic acid, CN = gentamicin and CIP = ciprofloxacin

Table 6. Antibiotic susceptibility pattern of ESBL *E. coli* and *Klebsiella* spp. from urine isolates of outpatients

Antibiotics (µg)	Number tested	<i>E. coli</i>		Number tested	<i>Klebsiella</i> spp.	
		Number sensitive (%)	Number resistant (%)		Number sensi- tive (%)	Number resistant (%)
CAZ (30)	7	3 (42.86)	4 (57.14)	4	1 (37.50)	3(62.50)
IMP (10)	7	7 (100.00)	0 (0.00)	4	4 (100.00)	0 (0.00)
CRO (30)	7	2 (28.57)	5 (71.43)	4	1 (25.00)	3 (75.00)
CTX (30)	7	0 (0.00)	7 (100.00)	4	0 (0.00)	4 (100.00)
FOX (30)	7	6 (85.71)	1 (14.29)	4	3 (75.00)	1 (25.00)
CEP (30)	7	2 (28.57)	5 (71.43)	4	1 (25.00)	3 (75.00)
ATM (30)	7	1 (14.50)	6 (87.71)	4	1 (25.00)	3 (75.00)
SXT (5)	7	5 (71.43)	2 (28.57)	4	3 (75.00)	1 (25.00)
DO (30)	7	3 (42.86)	4 (57.14)	4	2 (50.00)	2(50.00)
NOR (10)	7	5 (71.43)	2 (28.57)	4	3 (75.00)	1(25.00)
NA (30)	7	6(87.50)	1 (12.50)	4	3 (75.00)	1 (25.00)
CN (10)	7	5 (71.43)	2 (28.57)	4	3 (75.00)	1 (25.00)
CIP (5)	7	6(85.71)	1 (14.29)	4	3 (75.00)	1 (25.00)

Key: ATM = aztreonam, CAZ = ceftazidime, CRO = ceftriaxone, CTX = cefotaxime, FOX = ceftioxitin, IMP = imipenem, CEP = Cefepime, SXT = sulfamethoxazole\ trimethoprim, DO = Doxycycline, NOR= Norfloxacin, NA = Nalidixic acid, CN = gentamicin and CIP = ciprofloxacin

Table 7. Antibiotic susceptibility pattern of *ESBL E. coli* and *Klebsiella* spp. from feces isolates of outpatients

Antibiotics (µg)	Number tested	<i>E. coli</i>		Number tested	<i>Klebsiella</i> spp.	
		Number sensitive (%)	Number resistant (%)		Number sensi- tive (%)	Number resistant (%)
CAZ (30)	5	1 (20.00)	4 (80.00)	3	1 (33.33)	2 (66.67)
IMP (10)	5	5 (100.00)	0 (0.00)	3	3 (100.00)	0 (0.00)
CRO (30)	5	2 (40.00)	3 (60.00)	3	1 (33.33)	2 (66.67)
CTX (30)	5	2 (40.00)	3 (60.00)	3	0 (0.00)	3 (100.00)
FOX (30)	5	3 (60.00)	2 (40.00)	3	1 (33.33)	2 (66.67)
CEP (30)	5	2 (40.00)	3 (60.00)	3	0 (0.00)	3 (100.00)
ATM (30)	5	1 (20.00)	4 (80.00)	3	1 (33.33)	2 (66.67)
SXT (5)	5	2 (40.00)	3 (60.00)	3	0 (0.00)	3 (100.00)
DO (30)	5	3 (60.00)	2 (40.00)	3	1 (33.33)	2 (66.67)
NOR (10)	5	1 (20.00)	4 (80.00)	3	3 (100.00)	0 (0.00)
NA (30)	5	3 (60.00)	2 (40.00)	3	2 (66.67)	1 (33.33)
CN (10)	5	4 (80.00)	1 (20.00)	3	3 (100.00)	0 (0.00)
CIP (5)	5	4 (80.00)	1 (20.00)	3	3 (100.00)	0 (0.00)

Key: ATM = aztreonam, CAZ = ceftazidime, CRO = ceftriaxone, CTX = cefotaxime, FOX = ceftiofur, IMP = imipenem, CEP = Cefepime, SXT = sulfamethoxazole\ trimethoprim, DO = Doxycycline, NOR = Norfloxacin, NA = Nalidixic acid, CN = gentamicin, and CIP = ciprofloxacin.

Table 8. Mean average MARI values by bacterial type and patient category

Group	Mean Average MARI ± SD
ECIP & ECOP	0.65 ± 0.21a
KSIP & KSOP	0.25 ± 0.21a
ECIP & KSIP	0.60 ± 0.28b
ECOP & KSOP	0.30 ± 0.28b

Keys: ^{a,b} = Comparison superscripts, ECIP = *E. coli* from in-patients, ECOP = *E. coli* from outpatients, KSIP = *Klebsiella* from in-patient, KSOP = *Klebsiella* from outpatient, $p > 0.05$ for independent samples t-test.

coli and *Klebsiella* species. It revealed that there was a co-existence of the *OXA/SHV* gene in 4 isolates (1K, 6K, 10, and 6). There was a co-existence of the *ACC/CTXM* gene in 1 isolate (9). Seven (7) isolates (1K, 4K, 6K, 10K, 4, 6, and 22) had a co-existence of *TEM/SHV* gene. There was a co-ex-

istence of *CTX-M/SHV* gene in 6 isolates (1K, 4K, 6K, 10K, 4, and 6). There was a co-existence of *CTXM/OXA/SHV/TEM* genes in 6 isolates (1K, 6K, 10K, and 6).

Phylogenetic tree of sequenced organisms

Sequences of organisms obtained from DNA sequencing reactions of the 16S rRNA marker resolved them into three clusters (groups), which are further divided into subgroups as shown in Figure 2. Related isolates are clustered together.

Discussion

In this study, the co-existence pattern of *ESBL* resistance genes in multidrug-resistant *E. coli* and *Klebsiella* species isolated from inpatients and outpatients in a Federal University Teaching Hospital in Nigeria was investigated. The aim of providing an update on the antimicrobial resistance pattern of

Table 9. BLAST results of sequenced organisms

S/N	Isolate Codes	% Pairwise Identity	NCBI Accession	Identified Organism
1	13	89.70	KP313046	<i>E. coli</i> strain NRC124 16S
2	1K	93.30	CP029692	<i>E. coli</i> strain SD134209
3	6	96.10	MK014300	<i>K. aerogenes</i> strain CB461
4	16	98.50	MK621272	<i>E. coli</i> strain 80
5	14	95.90	KJ660338	<i>E. coli</i> strain Mui T6
6	9	95.60	MK621266	<i>E. coli</i> strain 71
7	4	88.40	MK332563	<i>E. coli</i> strain AKRC4
8	19	94.10	MH040908	<i>E. coli</i> isolate AV14
9	23	93.70	KT153190	<i>E. coli</i> strain H-ESBL

Table 10. Co-existing pattern of resistance genes in isolates

S/N	Co-existence Pattern	No of isolates	Isolate Codes
1.	OXA / SHV	4	1K, 6K, 10K, 6
2.	AAC / CTXM	1	9
3.	TEM / SHV	7	1K, 4K, 6K, 10K, 4, 6, 22
4.	CTX-M/SHV	6	1K, 4K, 6K, 10K, 4, 6
5.	CTXM/OXA/SHV/TEM	4	1K, 6K, 10K, 6

Key: 1K = KUI, 4K = KFI, 6K = KUO, 10K = KFO, 2, 4, 6 = EUI, 9, 12 = EFI, 13, 14, 16 = EUO, 19, 22, 23 = EFO, KUI = *Klebsiella* spp. from urine of in-patient, KFI = *Klebsiella* spp. from feces of in-patient, KUO = *Klebsiella* spp. from urine of out-patient, KFO = *Klebsiella* spp. from feces of out-patient, EUI = *E. coli* from urine of in-patient, EFI = *E. coli* from feces of in-patient, EUO = *E. coli* from urine of out-patient, EFO = *E. coli* from feces of out-patient.

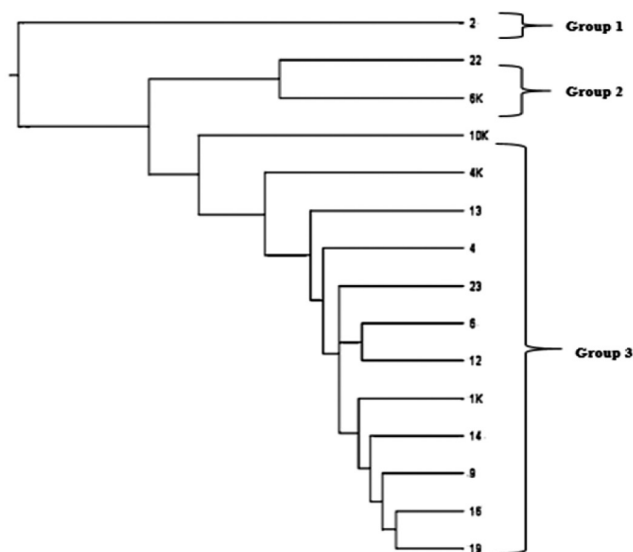


Fig. 1. Phylogenetic relationship between the isolates
Key: 1K = KUI, 4K = KFI, 6K = KUO, 10K = KFO, 2, 4, 6 = EUI, 9, 12 = EFI, 13, 14, 16 = EUO, 19, 22, 23 = EFO, KUI = *Klebsiella* spp. from urine of in-patient, KFI = *Klebsiella* spp. from feces of in-patient, KUO = *Klebsiella* spp. from urine of out-patient, KFO = *Klebsiella* spp. from feces of out-patient, EUI = *E. coli* from urine of in-patient, EFI = *E. coli* from feces of in-patient, EUO = *E. coli* from urine of out-patient, EFO = *E. coli* from feces of out-patient.

these multidrug-resistant bacteria and contributing towards clinicians' /physicians' knowledge on the optimal options for antibiotic recommendation for infected individuals by these organisms justified the study. Antimicrobial resistance (AMR) due to *ESBL* is seriously contributing to the challenges in controlling the spread of infectious diseases in the public healthcare systems. AMR is a global health threat that warrants urgent steps to mitigate its growing, ugly trend. We advocate for strong surveillance of *ESBL*-producing bacteria in order to lessen the complications arising from the infection and spread of diseases caused by these multidrug resistant pathotypes in the healthcare setting.

Age and gender-related bacterial prevalence among inpatients and outpatients

This study investigated age and gender relat-

ed bacterial prevalence of in- and out-patients attending Federal teaching hospital, Abakaliki, Ebonyi State, Nigeria (Table 3). It was observed that female patients across all age groups had the highest occurrence of both *E. coli* and *Klebsiella* spp. The highest occurrence of *E. coli* and *Klebsiella* spp among the female patients was observed between the ages of 31-40 years, followed by those between ages 21-30 years for both in- and out-patients attending the federal teaching hospital. The higher number of urine cultures from female patients than from male patients may be the reason for the differences. It was also observed that microbial growth was higher in samples from female patients than in samples from male patients. Urethral openings in females are short and can result in complicated physiology, especially during pregnancy, and can be considered as a reason for the differences (Iroha *et al.*, 2012). The constant use of contraceptives by females can also increase the risk factor for urinary tract infections (UTIs). Statistical analysis revealed significant differences in the prevalence of *E. coli* and *Klebsiella* spp. across the age groups ($p < 0.05$). The highest mean prevalence of both bacteria was observed among patients aged 21–40 years, with significantly lower prevalence in older age categories, particularly among those aged 51 years and above. There were no statistically significant differences observed between male and female patients within the same age category ($p > 0.05$). These findings confirm that age is a statistically significant factor influencing the prevalence of *E. coli* and *Klebsiella* spp., whereas gender does not significantly impact prevalence in the study population. Although men are usually less prone to UTI compared to women, owing to the longer course of the urethra and bacteriostatic properties of prostate secretions, a barrier that most microorganisms cannot overcome (Mahesh *et al.*, 2010). There are an estimated 150 million urinary tract infections per annum world-

wide. In a community, women are more prone to developing UTIs. About 20 % of women experience a single episode of UTI during their lifetime, and 3% of women have more than one episode of UTI per annum (Dalela, 2012).

In a related study by Darioush *et al.* (2015), bacterial species of the genera *Klebsiella*, *Proteus*, *Pseudomonas*, *Enterobacter*, *Citrobacter*, *Acinetobacter*, and *Moraxella* were detected more often in females than in males with UTIs. An Iranian study by Farajnia *et al.* (2009) reported that *E. coli* occurred significantly more frequently in women than in men ($P < 0.05$), whereas the prevalence of UTIs due to *K. pneumoniae* and *P. aeruginosa* was higher in men than in women, respectively. Differences in the reported isolation rates of uropathogens from different regions may be due to differences in age, gender, and the number of patients and types of uropathogens examined. Another factor may be the type and number of media used in the isolation of the uropathogens.

Bacterial isolates of E. coli and Klebsiella species collected from inpatients and outpatients

The result of the distribution of the bacterial isolates from in-patients and out-patients revealed that urine isolates from in-patients harbored a higher bacterial prevalence than urine from out-patients. Although it was also observed that *E. coli* and *Klebsiella* spp. in urine samples had a higher occurrence than *E. coli* and *Klebsiella* spp. in fecal samples, statistical analyses using Chi-square and Fisher's Exact tests showed no significant difference in isolate distribution by specimen source (Table 3). This finding correlates with several reports from previous studies carried out on the test isolates in other places within and outside Nigeria. Similar findings on the occurrence of *E. coli* and *S. aureus* have been reported among pregnant women in some parts of Ebonyi State by Iroha *et al.* (2012). These bacteria isolate among other enterobacteriaceae has also been identified mostly from women having UTI in countries such as Brazil (Guerra *et al.*, 2012), India (Mahesh *et al.*, 2010; Nalini *et al.*, 2012), Iran (Saeide *et al.*, 2013), Pakistan (Ayub *et al.*, 2016), and Turkey (Altun *et al.*, 2014). This indicates that their spread and implications in UTI are on the increase in the past decade in Nigeria, as Onwue-zobe & Orok (2015) have also reported *E. coli*, *K. pneumoniae*, *K. oxytoca*, *Citrobacter* spp., *Proteus mirabilis*, *Enterobacter* species, and *Acinetobacter baumannii* as a cause of nosocomial infection in a teaching hospital in Uyo, Nigeria. This strengthens the hypothesis that nosocomial bacterial infections

constitute a substantial cause of morbidity and mortality in developing countries where there are inadequate healthcare facilities and poor management systems. The presence of these organisms is suggestive of impending health hazards (Okafor *et al.*, 2022; Adie *et al.*, 2024). *E. coli* and *Klebsiella* can be life-threatening in immunocompromised individuals, especially children and the elderly (Onuoha *et al.*, 2023). They are known to be one of the major organisms causing infections within the hospital environment (Ovia *et al.*, 2023).

E. coli and *Klebsiella* are the most common uropathogenic bacteria that have been isolated in different countries and are responsible for confirmed UTI cases in nosocomial settings. Other bacteria associated with the disease are *P. aeruginosa* and *A. baumannii*. *Enterobacter aerogenes*, *P. mirabilis*, *S. aureus*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, *Acinetobacter calcoaceticus*, *Pantoea agglumaranace* and *Serratia*. These organisms have all been reported as predominant bacteria in urine culture (Al-Sweih *et al.*, 2005; Bercion *et al.*, 2009; Kashef *et al.*, 2010; Nalini *et al.*, 2012; Altun *et al.*, 2014; Riffat *et al.*, 2014; Abujnah *et al.*, 2015). Among all these bacteria, *E. coli* has been identified as the most common causative agent of urinary tract infections in 90-80% of patients across all age groups (Bakhtiari *et al.*, 2020). Infections caused by this organism, especially in complicated and uncomplicated UTIs, are common bacterial infections that pose a serious public health threat that calls for an urgent, efficacious approach in controlling over 150 million cases per annum, which signifies their rapid spread and pathogenesis (Zhou *et al.*, 2023).

Antibiotic resistance analysis of ESBL-producing E. coli and Klebsiella species isolated from Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Ebonyi State, inpatient and outpatient

The result of antibiotic susceptibility testing showed that *ESBL* producing of *E. coli* and *Klebsiella* spp. isolates displayed varied resistance and susceptibility to the antibiotics used (Table 4–7). *ESBL*-producing *E. coli* had the highest susceptibility to imipenem and ciprofloxacin, followed by amoxicillin/clavulanic acid and ceftazidime, but were resistant to most of the drugs used as first-line drugs. Low susceptibility was observed with third-generation cephalosporin (cefotaxime, ceftazidime, and ceftriaxone), cephamycin (cefoxitin), monobactam (aztreonam), piperacillin, and cefepime used in the study. *Klebsiella* species were completely susceptible to imipenem and ciprofloxacin, while showing varied susceptibility to nali-

dixic acid and gentamicin. The organism showed complete resistance to cefotaxime, with varied resistance to aztreonam, ceftriaxone, cefepime, and ceftazidime. In general, the test isolates displayed significant resistance to the various antibiotics used in the study. In related studies, *ESBL*-producing *E. coli* from clinical isolates has been reported to display high resistance to antibiotics belonging to different classes. Some of the antibiotics include amikacin, gentamicin, trimethoprim/sulfamethoxazole, levofloxacin, tetracycline, carbenicillin, ampicillin, azithromycin, piperacillin, cefuroxime, ceftriaxone, ofloxacin and ciprofloxacin (Aruna and Mobashshera, 2012; Maina *et al.*, 2013; Parviz *et al.*, 2014; Fosson *et al.*, 2018). The high level of resistance to different antibiotics observed in this study also complies with previous studies by other researchers across the globe (Maina *et al.*, 2013; Parviz *et al.*, 2014; Fosson *et al.*, 2018; Nagarjuna *et al.*, 2018; Ngong *et al.*, 2021).

Multiple antibiotic resistance index (MARI) of *ESBL*-producing *E. coli* and *Klebsiella* species isolated in this study from in- and out-patients showed that *E. coli* from in-patients had the highest MARI value, while the lowest MARI value was recorded by *Klebsiella* species from out-patients. There was no significant difference among the groups examined in this study ($p > 0.05$) (Table 8). The high resistance to antibiotics observed in this study may be due to the selective pressure exerted by the use of antibiotics in the management of bacterial infections. Genetic diversion and high consumption of antibiotics are other key factors that contribute to the emergence and wide distribution of bacterial resistance to these commonly used antibiotics (Bakhtiari *et al.*, 2020). Some of the ways adaptive evolution of uropathogenic *E. coli* has been observed are in their ability to invade the urothelium and survive intracellularly through changes in colonization, attachment, invasion, and intracellular replication.

Rapid choice of good and inexpensive antibiotics is one of the extremely important factors to consider in the treatment of UTIs caused by strains of *E. coli* and *Klebsiella*, but the major challenge about it is the consistent acquisition of resistance to many known antibiotics by these bacteria. Over the years, antimicrobial chemotherapy has recorded a historic success in vivo in the treatment of UTIs caused by these causative agents. This progress is due to the in-vitro susceptibility. The findings in this study, as seen with the isolates' sensitivity to therapeutic agents such as imipenem, ciprofloxacin,

tazobactam, cefoperazone/sulbactam, amoxicillin/clavulanic acid, and ceftazidime, corroborate this claim. But the rising trend in the recurrence and prevalence of these causative agents and antimicrobial resistance among these uropathogenic organisms is a threat to the efficacy of these known antibiotics used for the treatment of UTIs. Also, the gradual emergence of multidrug-resistant uropathogenic *E. coli* across the globe calls for urgent attention to further investigate the disease-causing mechanism of the organism to seek more efficacious therapeutic and preventative approaches. It also calls for more research efforts towards an in-depth understanding of the clinical status and pathogenesis of UTIs. To achieve this, an in-depth awareness of the merits and demerits of antimicrobials as a known and accepted treatment option could invigorate a positive surge in the discovery of other alternative treatment options. These options may include vaccines, phage, and nature-based treatment. In combating antibiotic resistance, these new approaches that target multiple disease-causing mechanisms in uropathogenic *E. coli* should be the research interest for the management of UTIs in the future (Zhou *et al.*, 2023).

Co-existing pattern of resistance genes in isolates

This study revealed a high prevalence of *ESBL*-producing organisms, with 82 (51.26 %) isolates positive for *ESBL* enzymes. The spread of *ESBL*-producing organisms is usually high in communities with poor hygiene and poor sanitary conditions, fecal contamination of soil and water. Most patients infected with *ESBL* producers may have had their gastrointestinal tracts colonized for a long period by these organisms (Paterson *et al.*, 2010). The persistent occurrence of *ESBL*-producing *E. coli* and *Klebsiella* species is one of the most common causes of morbidity and mortality associated with hospital-acquired infections. Many studies have attempted to identify different risk factors that could be helpful in predicting true infections caused by *ESBL*-producing organisms so that effective strategies may be developed to limit outbreaks of diseases due to these infections. Additionally, identifying risk factors associated with high rates of mortality also helps in ascertaining the prognosis of patients with infections caused by *ESBL*-producing organisms (Gudiol *et al.*, 2013; Fadil *et al.*, 2017).

PCR result for *ESBL* genes in nine bacterial isolates identified in this study revealed that the selected *ESBL*-producing isolates of *E. coli* and *Klebsiella* species harbored resistance genes such as *AAC*, *bla*_{OXA}, *bla*_{CTX-M}, *bla*_{SHV}, and *bla*_{TEM}. These

resistance genes co-existed indiscriminately in *ESBL*-producing isolates of *E. coli* and *Klebsiella* species (Table 9, 10). Eight out of the nine test isolates harbored at least two of the *ESBL* resistance genes. Phylogenetic analysis of the organisms obtained from the study resolved them into three clusters (groups), which are further divided into subgroups as shown in Figure 1. The result suggests that *bla*_{CTX-M} genes have evolved from chromosomal *bla* genes of *Kluyvera* sp. (Sid Ahmed *et al.*, 2016). The frequency of each *ESBL*-producing organism isolated varied between study groups, as shown in Figure 1. The Group 1 cluster represents one *Klebsiella* spp. isolate from the feces of an outpatient. This group displayed a 69.23% resistance to the antibiotics used in the study. The Group 2 cluster represents one *E. coli* isolate from the urine of an out-patient and one *Klebsiella* spp. isolated from the urine of outpatients. Each of these isolates in this cluster displayed a resistance pattern of 92.31% to the antibiotics used. The Group 3 clusters represent one *Klebsiella* spp. isolate from the urine of an in-patient with a 92.31% resistance to the antibiotics used, three *Klebsiella* spp. isolates from the feces of out-patient with a 69.23% resistance to the antibiotics used in the study, one *Klebsiella* spp. isolate from feces of in-patient with 92.31% resistance, three *E. coli* isolates from urine of out-patient with a 92.31% resistance to the antibiotics used in the study, two *E. coli* isolates from feces of in-patient with 100% resistance to the antibiotics used in the study and two *E. coli* isolates from feces of out-patient with a resistance pattern of 92.31% to the antibiotics used in the study. The co-existence pattern of the resistance genes among these clusters is shown in Table 10.

In most parts of the world, CTX-M-type replaced TEM- and SHV-type and became the predominant *ESBL* types found among Enterobacteriaceae, particularly in community-acquired infections caused by *E. coli* (Cantón *et al.*, 2012). In a similar study by Al-Mayahie & Al Kuriashy (2016), it was reported that CTX-M-type was the most frequent *ESBL* (accounting for 70.3 %), with CTX-M-1 being the only subtype discovered in the isolates. The prevalence of OXA- and SHV-type was 32.9 % and 10.9 %, respectively. None of the isolates produced TEM β -lactamase. All OXA-type *ESBLs* were produced concomitantly with CTX-M-1.

The increasing prevalence of antibiotic-resistant microorganisms, especially those that produce *ESBLs*, is of global concern. Since β -lactam antibiotics came into clinical use after their discovery

some decades ago, enzymes that destroy them and cause resistance to the β -lactam drugs have also evolved alongside them (Jacoby and Munoz-Prize, 2005). The introduction of these broad-spectrum antibiotics into clinical practice was largely heralded as a breakthrough in the fight against bacterial resistance to drugs owing to the fact that these antibiotics are more stable than the earlier beta-lactamases, and are an important tool for the treatment of severe bacterial infections (Cindy, 2011). Beta-lactamases such as *ESBLs* with variable success destroy and confer resistance to the latest β -lactam antibiotics, including the cephalosporins, monobactams, and carbapenems (Jacoby and Munoz-Prize, 2005).

We observed that inpatients had a greater prevalence of *ESBL*-producing isolates than outpatients. Also, it was revealed that *ESBL*-producing organisms in urine isolates had a higher frequency than in fecal isolates. The spread of *ESBL* in the study setting is mainly reported due to inappropriate infection control practices, contaminated intravenous catheters, feeding tubes, and various surfaces (door handles, sucker machine, incubators, mattresses, wash basins, floor, sink, emergency trolley, ventilator, ambo bag, laryngeal scopes) and colonized hands of staff. Another major factor is the substandard practices that are very common and lower standard hygienic conditions in the hospital settings. This observation therefore confirms the assertion that *ESBL* producers are indeed a problem in the communities and the hospitals (Dalela, 2012; Dinesh *et al.*, 2014).

Nosocomial bacterial infections constitute a substantial cause of morbidity and mortality in developing countries. *E. coli* and *Klebsiella* species are known to be the major organisms causing nosocomial infections within the hospital and have also been implicated in community-acquired *ESBL*-resistant strains.

Conclusion and Recommendations

This study has shown that clinical isolates obtained from inpatients and outpatients harbored *E. coli* and *Klebsiella* species. The results obtained from the antibiotic profile of isolates showed high resistance to cefotaxime, doxycycline, ceftriaxone, ceftazidime, aztreonam, and colistin sulphate. However, antibiotics such as imipenem, ciprofloxacin, and gentamicin were generally effective against isolates. Data on multiple antibiotic resistance profiles of bacterial isolates suggests that there has been an indiscriminate use of the antibiotics tested. The result of PCR analysis revealed that the *ESBL*-producing isolates of *E. coli* and *Klebsiella*

species harbored resistance genes. Antibiotic resistance is a growing public health problem in many bacterial pathogens. It is of serious concern for both hospital-acquired and community-acquired infections. Antibiotic-resistant bacteria may also increase the length of hospitalization of a patient, the severity of illness, and the overall cost of treatment. The microbiology laboratory should be up and doing in producing and disseminating meaningful local surveillance data with respect to the predominant pathogens and their antibiotic resistance profiles. Incorrect or delayed laboratory diagnostic data can lead to lengthened empiric antibiotic therapy or blind treatment, which is capable of allowing resistant strains to thrive. Correct identification of *ESBL*-positive enterobacteriaceae in due time is mandatory not only for optimal patient management but also for immediate institution of appropriate infection control measures to prevent the spread of these organisms (Mofolorunsho *et al.*, 2021). The emergence and spread of *ESBL*-producing bacteria, which initially looked benign, has become one of the major problems that now overwhelms the healthcare sector across the globe, hampering the efficacy of the available antibiotics for the treatment of bacterial-related infections. It is therefore imperative to detect *ESBL*-producing Enterobacteriaceae such as *E. coli* and *Klebsiella* species from clinical specimens to forestall the plethora of damage they cause to our therapeutic armamentarium. Prompt and accurate detection of these organisms will help to keep antibiotic-resistant bacteria at bay, and infection control strategies that will help to avert future occurrences can be developed; thus, the need for undertaking this study.

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