Available online at www.ujpronline.com
Universal Journal of Pharmaceutical Research
An International Peer Reviewed Journal
ISSN: 2831-5235 (Print); 2456-8058 (Electronic)

RESEARCH ARTICLE

PREVALENCE OF MULTIDRUG RESISTANT *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE* SPECIES ISOLATED FROM URINE SAMPLES OF PATIENTS AT ALEX EKWUEME FEDERAL UNIVERSITY TEACHING HOSPITAL ABAKALIKI, EBONYI STATE, NIGERIA

Onu Henrietta Chinaemerem[1], Idoko Alexander[2], Iroha Ifeanyichukwu Romanus[3]

[1]*Alex Ekwueme Federal University, Ndufu-Alike, P.M.B. 1010. Ebonyi State, Nigeria.*
[2]*Department of Biochemistry, Faculty of Natural Sciences, Caritas University, Amorji – Nike, P.M.B. 01784, Enugu, Nigeria.*
[3]*Department of Applied Microbiology, Faculty of Science, Ebonyi State University, Abakaliki, P.M.B. 053, Ebonyi State, Nigeria.*

Article Info:

Article History:
Received: 3 December 2022
Reviewed: 9 January 2023
Accepted: 24 February 2023
Published: 15 March 2023

Cite this article:
Onu HC, Idoko A, Iroha IR. Prevalence of multidrug resistant *Escherichia coli* and *Klebsiella pneumoniae* species isolated from urine samples of patients at Alex Ekwueme Federal University Teaching Hospital Abakaliki, Ebonyi state, Nigeria. Universal Journal of Pharmaceutical Research 2023; 8(1):15-22.
https://doi.org/10.22270/ujpr.v8i1.892

***Address for Correspondence:**
Idoko Alexander, Department of Biochemistry, Faculty of Natural Sciences, Caritas University, Amorji – Nike, P.M.B. 01784, Enugu, Nigeria; Tel: +2348032354823.
E-mail: *idokoalexander1@gmail.com*

Abstract

Aim and Objective: In hospitals and community, morbidity and mortality are attributed to urinary tract infections (UTIs). This study investigated the prevalence of multi-drug resistant isolates of *Escherichia coli* and *Klebsiella* species from urine samples of patients visiting Alex Ekwueme Federal University Teaching Hospital Abakaliki (AE-FUTHA), Ebonyi State.

Method: With the use of standard microbiological and biochemical techniques for analysis, a total of 300 mid-stream samples of urine were collected in sterile bottles. Phenotype screening for extended spectrum beta lactamase (ESBL) production was achieved by double disc synergy test (DDST). Disc diffusion method was used to check for ESBL producing bacteria susceptible to antibiotics. With specific primers, the presence of temoniera (TEM) and sulfhydryl variable (SHV) beta lactamases genes was determined using polymerase chain reaction (PCR).

Results: 88 isolates of *E coli* and *Klebsiella* species were isolated from the urine samples; 29 were *E coli* and 59 were *K. pneumonia* species. 47 ESBL positive isolates were identified with *E. coli*, 19 (40%) and *Klebsiella*, 28 (60%). *Escherichia coli* isolated from GOPD patients, gynecology, and men's surgery ward were 100% resistant to ofloxacin, ceftazidime, amoxicillin, nitrofurantoin, chloramphenicol, and aztreonam, while Klebsiella species isolated from gynecology department, maternity ward, and psychiatric ward were 100% resistant to ofloxacin, Nitrofurantoin and chloramphenicol. Multiple antibiotics resistance index (MARI) of *E. coli* and *Klebsiella* species isolated recorded an average of 0.64 for *E. coli* and 0.41 for *Klebsiella* species.The molecular analysis revealed that 44.4% of SHV beta lactamase and 65.6% TEM-type β-lactamase genes were present in ESBL producing *E. coli* while 54.5% of SHV beta lactamase gene and 45.5% TEM-type β-lactamase genes were present in ESBL producing *Klebsiella* species.

Conlusion: In conclusion, TEM and SHV-type β-lactamase genes are the primary cause of β-lactam antibiotic resistance in *E. Coli* and *Klebsiella* species resulting in increased infections caused by organisms harboring the ESBL gene. Nitrofurantoin, ceftazidime, cefalexin, and cefotaxime may be the antibiotic of choice in the treatment of UTIs.

Keywords: Antibiotics, β-lactamase, *Escherichia coli*, *Klebsiella* species, multiple antibiotics resistance, sulf-hydryl variable, temoniera.

INTRODUCTION

Attention is gradually shifted to antimicrobial resistance as a global public health concern and emergency[1]. Exhaustive use of antimicrobials for food animals may pose challenge in the treatment of infections because resistance among bacterial pathogens from animals and humans could be selected[2]. Resistant bacteria from animals to humans could be transferred through environment, direct interaction with animals or food chain, and resistant infections may result[3]. In Africa, such as in Nigeria,

resistance to gentamicin was seen in isolates of *E. coli* in connection with urinary tract infections[4]. The arrival of the new generation of quinolones antimicrobial agents, fluoroquinolones provided a choice option for the treatment of various life threatening infections resulting from multi-drug resistant bacteria[5]. Meanwhile, the widespread use of these drugs in clinical settings gave rise to bacteria resistance[4]. Treatment of UTIs in hospitals and communities was often handled with fluoroquinolones and their application has resulted in increased resistance in *E. coli* responsible for UTIs[6]. In *E. coli* strains, DNA replication is inhibited by quinolones. These agents mark the *E. coli* DNA enzymes such as topoisomerase IV (parC) and topoisomerase II (gyrase). Meanwhile, *E. coli* resistance to quinolones occurs due to alteration in single amino acid in topoisomerase IV or gyrase as a result of *gyrA*, *gyrB*, *parC*, and *parE* mutated genes[1]. Human gastrointestinal tracts (HGITs) are frequently colonized by *E. coli* strains, and are responsible for several infections including diarrhea, UTIs, meningitis and septicemia[5]. However, *E. coli's* harmless strains persist as commensals in HGITs but become virulent and thereby cause infection and diseases when they become genetically modified[1]. In both hospital and community, these *E. coli* strains are increasingly reported to have acquired antibiotic resistance genes mostly for UTIs, yet with scarce therapeutic intervention[5].

The *E. coli* strains also encodes resistance to oxyiminocephalosporins in the expression of Extended Spectrum Beta Lactamases (ESBLs), thus treatment to its infections may be difficult[7]. In another case, *E. coli* strains was recently reported to pose resistance to carbapenems, which are known to be effective in treating infections from ESBL-producing *E. coli*, by generating a hydrolyzing enzyme, called carbapenem-hydrolases[4]. In the antibiotic munitions, aminoglycosides essentially useful for treating infections and UTIs caused by *E. coli*, that are serious threat to life but the devastating widespread antibiotic resistance has made some them ineffective in treating infections caused by *E. coli*[7]. The combined rates of biofilm formation in Uropathogenic *E. coli* (UPEC) isolates were achieved as 84.6% (95% CI: 72.7-91.9)[8]. Clinicians well recognized *Klebsiella* as a cause of community-acquired bacterial pneumonia, seen in chronic alcoholics and revealing peculiar radiographic abnormalities as a result of relentless pyogenic infection with a high fatality rate if not treated[9]. Most of the infections of *Klebsiella* are reported to be hospitalization associated. Majorly, the pathogenic *Klebsiella* spp. attacks the hospitalized individual whose immunity has been compromised due to existing chronic pulmonary obstruction or diabetes mellitus[9]. The immunity of patients in intensive care unit (ICU) tend to be compromised most time and are at risk of infections due to nosocomial as a result of existing untreated disease or may be due to the exposure to invasive procedures. Similarly, such patients who are also administered broad spectrum antibiotics, could be vulnerable to pseudomembranous colitis and candidiasis infections since their protective microbiota has been damaged[1]. Penicillin, broad and narrow spectrum cephalosporins and monobactams (Aztreonam), are reported to be hydrolyzed effectively by plasmid-mediated β-lactamase, which are also known as extended spectrum Beta lactamase (ESBLs), while they cannot hydrolyze cephamycin or carbapenems (imipenem, meropenem)[1]. ESBL generating strains frequently found in *K. pneumoniae* and *Escherichia coli* are hindered by inhibitors of β-Lactamase, which include tazobactam, clavulanic acid and sulbactam[1]. A resistant strain to inhibitors of β-Lactamase is the plasmid-mediated AmpC β-lactamase, found in the chromosome, inhibits clavulanic acid and hydrolyzes cephamycin[1]. Carbapenemase is reported to to exhibit multipurpose hydrolytic abilities on carbapenems, cephalosporins, monobactams and penicillins[9]. The therapeutic intervention of *E coli* and *Klebsiella* species acquire antibiotic resistance genes for UTIs and other bacterial infections seem to be limited. Therefore this study was aimed at investigating the prevalence of multidrug resistant isolates of *Escherichia coli* and *Klebsiella* species from urine samples of patients visiting Alex Ekwueme Federal University Teaching Hospital Abakaliki, Ebonyi State.

MATERIALS AND METHODS

Media for Bacteriological Screening

Nutrient broth, Nutrient agar, Mueller-Hinton agar and MacConkey agar (Oxoid, UK), were the media used and their preparation was based on instruction of the manufacturer.

Equipment

The following are the equipment used; microscope (Olympus optical Co. Ltd. UK), autoclave (Searchtech, England), thermocycler, refrigerator (Gallenkamp Germany), weighing balance (Ohaus, USA) and incubator (Newlife England).

Reagents and Chemicals

Chemicals and reagents used include; crystal violet, normal saline, lugols iodine, oxidase reagent, methyl-red reagent, immersion oil, Kovacs reagent, hydrogen peroxide, acetone alcohol and decolourizer.

Antibiotics disk

Antibiotics used are all of Oxoid Ltd, Basingstoke, United Kingdom and they include: ampicillin (10 µg), amoxicillin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefoxitin (30 µg), nitrofurantoin (10 µg), chloramphenicol (10 µg), azetreonam (5 µg), cefepime (25 µg), ertrapenem (10 µg) and ofloxacin (30 µg).

Determination of sample size

In this study, Cochran's sample size formula was employed for the determination of sample size. Using 95% confidence interval (CI) at a 0.05 precision, the sample size was calculated and the Cochran formula;

$$n=Z^2pq/e^2$$

Where: n=sample size; Z=standard normal deviation at 95% confidence interval (which was 1.96); p=proportion of target population (prevalence estimated at 50%; this implies 75/100=0.75); q=alternate proportion (1-p), which was calculated as: 1–0.5=0.5; e=desired level of precision (degree of precision/significance).

This was taken as 0.05**;** Thus, using the sample size determination formula:

$n=Z^2pq/e^2$**;** $(1.96)^2$ x 0.75(1-0.75)=291.8 0.05^2

Thus, in this study, the sample size of 300 was approximately and randomly collected.

Ethical Clearance

The ethical committee of Alex Ekwueme Federal University Teaching Hospital Abakaliki, Ebonyi State approved the design of this study.

Sample collection

The microbiology laboratory unit of the hospital was the sample collection centre and 300 mid-stream urine samples were collected. In order to carry out bacterio-logical analysis, these samples were transported to Microbiology laboratory unit of Applied microbiology department of Ebonyi State University Abakaliki.

Phenotypic Determination of ESBL Production by Double disk synergy test (DDST)

The phenotypic confirmation of ESBL producing *E. coli* from the isolates was done by the method of double disc synergy test (DDST) as described in a previous study[6]. Briefly, adjustment to 0.5 McFarland turbidity standards of the overnight suspensions of the 2nd and 3rd generation Cephalosporin resistant bacteria was achieved and on a solidified MH agar (Oxoid, UK), they were aseptically swabbed. At the center of Mueller-Hinton agar plates, antibiotic disks containing amoxicillin acid (10 µg) was placed and adjacent to the central disk (amoxicillin acid) at a distance of 15 mm, was the antibiotic disk containing ceftazidime (30 µg) and cefotaxime (30 µg) positioned. For 18- 24 hours, the incubation of the plates at 37ºC was done. And increase ($a \geq 5$ mm) in the inhibition zone diameter for either of the cephalosporins tested in combination with the central disk versus its zone when tested alone confirms ESBL production phenotypically by the DDST method[6].

Antibiotic susceptibility testing

Employing the Kirby-Bauer disc diffusion Method described by the Clinical and Laboratory Standards Institute[11], ESBL producing isolates of *E. coli* and *Klebsiella* species's antibiotic susceptibility test of was carried out. On the entire Mueller-Hinton agar plate was the bacteria inoculum equivalent to 0.5 McFarland standard of the isolate smeared and pre-diffusion was allowed for 5 minutes. At 37ºC and for 24 hours, the antibiotics aseptically saturated on the agar plates and incubated include; Amoxicillin (10 µg), Ceftazidime (30 µg), Ampicillin (30 µg), Ertapenem (10 µg), Nitrofurantoin (10 µg), Chloramphenicol (10 µg), Cefepime (25 µg), Aztreonem (5 µg), Cefotaxime (10 µg), Ofloxacin (30 µg) and Cefalexin (30 µg), (Oxoid Ltd, Basingstoke, United Kingdom). And the level of susceptibility to each antibiotic was measured and interpreted using the diameter of zone of inhibition[11].

Determination of Multiple Antibiotic Resistance (MAR) index

The formula MAR=a/b, was used to measure the multiple antibiotic resistance (MAR) index. Where **a** is the number of antibiotics to which test isolate displayed resistance and **b** is the total number of antibiotics it was subjected to[12].

Molecular studies

Preparation of bacterial stock prior to PCR analysis

Screening for the presence of SHV and TEM beta lactamases was achieved by selecting 6 ESBL producing *E. coli* which were representative of each sample source and 7 ESBL producing *K. Pneumoniae* which represented each sample source. Using standard microbiological culturing technique[10], preparation of stock cultures for ESBL producing *E. coli* and *K. pneumoniae* isolates for gene amplification was done. *E. coli* and *K. pneumoniae* cultures were reassigned from stock cultures in the refrigerator and inoculated on double strength nutrient broth (Oxoid, UK). In order to resuscitate the isolates, inoculation of the culture tubes at 37ºC were done overnight. Then on nutrient agar plates, a looping through the turbid suspension, inoculation was done and purification achieved after overnight incubation, and the plates were subsequently incubated for 18-24 hours at 37ºC. A stock culture for DNA template and bacteria cells for molecular studies was prepared from 8 colonies of the overnight cultures which were inoculated in 5 ml peptone water.

DNA Extraction using *Quick*-DNA™ Miniprep Plus Kit (Catalog Nos. D4068 & D4069)

With modification, the *Quick*-DNA™ Miniprep Plus Kit, genomic DNA was extracted. Before us, isotonic buffer was used to re-suspend the cultured cells. Into a micro-centrifuge tube, 200 µl of the sample was pipette. This was then followed by addition of 200 µl of biofluid lysis buffer and 20 µl of Proteinase K, meticulously mixed for 10-15 seconds using a vortex mixer. Incubation was done for 10 minutes at 55°C in to this mixture was 420 µl of genomic binding buffer (GBB) added and meticulously mixed for 10-15 seconds using a vortex mixer. In a Zymo-Spin™IIC-XLR column in, the mixture was transferred to a collection tube and centrifuged at 12,000 x g for 1 minute. The collection tubes were discarded with the flow through after centrifuging. Into a new collection tube of 400 µl of DNA pre-wash buffer was added to the spin column and for 1 minute, centrifuged at 12,000 x g, and then the tubes were emptied. Into the spin column, 700 µl of g-DNA wash buffer was added and centrifuged at 12,000xg for 1 minute and the collection tubes were emptied. To the spin column again, 200 µl of g-DNA wash buffer was added and centrifuged again for 1 minute at 12,000 x g. The collection tubes were discarded with the flow through after centrifuging. Into a clean micro-centrifuge tube, the spin column was transferred and then direct addition of 65 µl of DNA elution buffer on the matrix and at room temperature, this was incubated for 5 minutes. In order to elude the DNA, for 1 minute, the mixture was centrifuged at maximum speed, and at -20°C the eluted DNA was stored until further amplified by PCR.

PCR screening for ESBL genes

By the aid of PCR technique with specific primers as described[13], different ESBL genes that encode ESBL production in the resistant ESBL producing *E. coli* and *K. pneumonia* isolates was were detected.

Agarose Gel Electrophoresis of PCR products

Of the agarose powder, 2 g was weighed and mixed with 100 ml of 1xTAE in a microwaveable flask. In a

microwaveable flask, 2 g of agarose powder was weighed and mixed with 100 ml of 1xTAE for the preparation of electrophoresis. After 3 minutes microwaving, the agarose gel mixture in a flask dissolved completely and was cooled to 50ºC. Under the ultraviolet (UV) light, a 10 µl EZ vision DNA stain was added which bound to the PCR product to allow its visualization. Into a gel tray with the well comb in place, was the agarose transferred and at room temperature and for 30 minutes, it was allowed to solidify. By adding gel, the well comb was removed with care. To each of the PCR fragment, the loading buffer was added and inserted in the electrophoresis unit. The gel box covering the gel surface was sealed using 1xTAE buffer. Into the first lane of the gel was the molecular weight ladder packed and into the additional wells of the gel was the PCR product meticulously packed. For about 1hour 30 minutes, the gel was run at 80-150 V. Under UV trans illumination, the DNA fragment was visualized after electrophoresis was completed[14].

Statistical Analysis

In the course of this study, the raw data collected were presented in tables and bar charts as mean ± standard deviation while simple descriptive statistics such as minimum, maximum, and one-way analysis of variance (ANOVA) was used to interpret relevant data with the help of Microsoft Excel 2013 software and IBM Statistical Package for Social Sciences (SPSS) version 22. And statistically significant was set at $p<0.05$.

Table 1: Amplification conditions for the PCR technique and Oligonucleotide primers selection.

Target Gene	Primer sequence	PCR conditions	Amplicon size	Reference
^{bla}*TEM*	F-5'-ATTCTTGAAGACGAAAGGGC-3'	Initial denaturation at 94°C x 5mins, followed by 34 cycles of denat. at 94°C x 30 secs	1350	Saenz *et al.*,
	R-5'-ACGCTCAGTGGAACGAAAAC-3'	42°C x 30 secs (annealing) 72°C x 5 mins secs (elongation)		
^{bla}*SHV*	F-5'-CACTCAAGGATGTATTGTG-3'	Initial denaturation at 94°C x 5mins, followed by 34 cycles of denaturation at 94°C x 30 secs	700	Jouini *et al.*,
	R-5'-TTAGCGTTGCCAGTGCTCG-3'	47°C x 30 secs (annealing) 72°C x 5 mins secs(elongation)		

RESULTS

Amplification conditions for the PCR technique and Oligonucleotide primers Selection

Table 1 reveals the specific primers that were used to determine the presence of ESBL resistance genes.

Morphology, microscopy and biochemical characteristics of bacterial isolates from urine samples of patients in different wards of AE-FUTHA

Out of the 300 urine samples collected from patients in Alex Ekwueme Federal University Teaching Hospital (AE-FUTHA), 88 isolates of bacteria were identified. Table 2 shows the microscopic, morphological and biochemical characteristics of the bacteria isolated. It showed the biochemical test, the colour, shape and Gram staining reaction.

Distribution of Bacteria from Urine samples of Patients in Different Wards in AE-FUTHA

Table 3 shows the occurrence of isolated bacteria from different wards in AE-FUTHA is shown. The highest *E. coli* frequency of 7 (24%) is from female medical ward, while Labour ward, Male medical, Orthopedic and Male surgical wards all with a frequency of 1 (3%) had the least isolated. Isolation of *K. pneumonia* was mostly from Female medical ward with a frequency of 30 (59%), while Psychiatric ward had the least isolates with a frequency of 1 (2%).

Table 2: Morphological, microscopic and biochemical characteristics of bacterial isolates from urine samples of patients in different wards of AE-FUTHA.

Morphological Characteristics		Microscopic Characteristics		Biochemical Tests						Suspected Organisms
Shape	Colour	Gram RXN	Motility test	IN	CAT	CIT	UR	VP	MR	
Rod	Pink	-	+	+	+	-	-	-	-	*E. coli*
Rod	Pink	-	-	-	+	+	+	+	-	*K. pneumoniae*
Rod	Green	-	+	-	+	+	-	NA	NA	*P. aeruginosa*

Key: IN=Indole test, CAT=Catalase test, CIT=Citrate test, UR=Urease Test, VP=VogesProskauer test, MR=Methyl Red test, SH=Starch Hydrolysis, Colour=Colour on MacConkey Agar, Gram RXN=Gram staining reaction, -=Negative, +=positive, NA=Not applicable

Table 3 shows result of detected ESBL. This result showed that from 300 urine samples screened for presence of ESBL, 47 ESBL positive bacteria was gotten in which 19 (40%) were *E. coli*, and *K. pneumonia* had the highest number of ESBL positives with a frequency of 28 (60%), out of the 88 isolated bacteria.

Antibiotic Susceptibility Pattern of ESBL Producing *E. coli* and *K. pneumonia* Isolated from Urine Samples of Patients in Different Wards in AE-FUTHA

Table 4 and Table 5 shows the antibiotic susceptibility pattern of *E. coli* and *K. pneumonia* isolated from urine samples of patient in different wards in AE-FUTHA.

Table 3: Frequency of Isolation of *E. coli* and *Klebsiella* species and distribution of extended spectrum Beta-lactamase producing *E. coli* and *Klebsiella* species from urine samples of patients in different wards in AE-FUTHA.

S.N.	Ward	No. (%) of *E. coli* Isolated	ESBL positive *E. coli*	No. (%) of *Klebsiella sp.* Isolated	ESBL positive *Klebsiella*
1	GOPD	4 (14)	2 (10)	2 (3)	2 (7)
2	A&E	4 (14)	3(15)	10 (18)	2 (7)
3	Female Medical	7 (24)	7 (40)	30 (49)	17(63)
4	Gynaecology	3 (11)	3 (15)	3 (5)	3 (10)
5	Male Medical	1 (3)	-	-	-
6	Male Surgical	1 (3)	-	-	-
7	Orthopaedic	1 (3)	-	-	-
8	Female Surgical	2 (7)	2 (10)	10 (18)	3 (10)
9	Labour	2 (7)	2 (10)	3 (5)	1 (3)
10	Psychiatric	4 (14)	-	1 (2)	-
	Total	**29 (33)**	**19 (66)**	**59 (67)**	**28 (47)**

Key: GOPD=General Outpatient Department, A & E=Accident & Emergency. -=Nil

Table 4: Antibiotic Susceptibility Pattern of ESBL producing *E. coli* isolated from urine samples of patients in different wards in AE-FUTHA.

	GOPD (n=4)	A&E (n=4)	FM (n=7)	GYN (n=3)	MM (n=1)	MS (n=1)	OTH (n=1)	FS (n=2)	LAB (n=2)	PSY (n=4)
Antibiotic (µg)	**R (%)**	**R (%)**	**R (%)**	**R (%)**	**R (%)**	**R(%)**	**R (%)**	**R(%)**	**R(%)**	**R(%)**
OFX (30)	100	50	71	100	100	100	0	100	100	100
CAZ (30)	100	25	14	100	100	100	0	100	100	0
AM (30)	100	50	57	100	100	100	0	50	100	0
F (10)	50	25	57	100	100	100	0	100	100	100
C (10)	50	50	71	100	0	100	100	50	100	100
FEP (25)	25	25	100	100	100	100	100	50	100	100
AMC (10)	100	50	28	66.3	100	100	0	50	100	100
CN (30)	100	50	42	66.3	0	0	100	50	0	0
ATM (5)	100	50	42	100	100	100	100	50	50	0
CTX (10)	100	50	85	33.3	100	100	100	50	50	0
ETP (10)	0	0	0	0	0	0	0	0	0	0

Key: OFX=Ofloxacin, CAZ=Ceftazidime, AM=Ampicilli, F=Nitrofurantoin, C=Chloramphenicol, FEP=Cefepime, AMC=Amoxycillin, CN=Cefalexin, ATM=Aztreonem, CTX=Cefotaxime**,** ETP- Ertapenem, n=Number tested, R (%)=Percentage resistance.

There was 100% resistant to the different antibiotics used in this study which includes; ofloxacin, ceftazidime, amoxicillin, nitrofurantoin, chloramphenicol and aztreonam (Table 4), by *E. coli* isolated from patients in GOPD, Gynaecology and Men Surgical ward. While in Table 5, there was 100% resistant to ofloxacin, nitrofurantoin and chloramphenicol by *K. pneumonia* isolated from Gynecology ward, Labour ward and Psychiatric ward.

Multiple Antibiotic Resistance Index (MARI) of ESBL producing *E. coli* and *K. pneumonia* Isolated from Urine Samples of Patients in AE-FUTHA

The results of multiple antibiotics resistance index (MARI) of *E. coli* and *K. pneumonia* isolated from different hospital wards in AE-FUTHA is shown in Table 6. For *E. coli*, the average MARI was read at 0.64 *E. coli* and *Klebsiella* specie was read at 0.41.

Table 5: Antibiotic susceptibility pattern of ESBL producing *Klebsiella* spp. isolated from urine samples of patients in different wards in AE-FUTHA.

	GOPD (n=2)	A&E (n=10)	FM (n=30)	GYN (n=3)	FS (n=10)	LAB (n=3)	PSY (n=1)
Antibiotic (µg)	**R (%)**	**R (%)**	**R (%)**	**R (%)**	**R (%)**	**R (%)**	**R (%)**
OFX (30)	100	80	90	100	40	100	100
CAZ (30)	100	100	100	100	40	33.3	100
AM (30)	100	100	100	100	60	33.3	100
F (10)	50	60	87	100	40	100	100
C (10)	50	60	90	100	100	100	100
FEP (25)	100	60	90	100	20	66.3	0
AMC (10)	100	100	100	66.3	100	100	0
CN (30)	50	40	77	0	20	0	0
ATM (5)	100	80	100	100	80	33.3	0
CTX (10)	50	80	100	100	40	33.3	0
ETP (10)	0	0	0	0	0	0	0

OFX=Ofloxacin, CAZ=Ceftazidime, AM=Ampicilli, F=Nitrofurantoin, C=Chloramphenicol, FEP=Cefepime, AMC=Amoxycillin, CN=Cefalexin, ATM=Aztreonem, CTX=Cefotaxime**,** ETP- Ertapenem, n=Number tested, R (%)=Percentage resistance.

Table 6: Multiple Antibiotic Resistance Index (MARI) of ESBL-producing *E. coli* isolated from different hospital wards.

S. N.	Ward	MARI of ESBL *E. coli*	MARI of ESBL *Klebsiella sp.*
1	GOPD	0.8	0.7
2	A&E	0.4	0.7
3	Female Medical	0.5	0.8
4	Gynaecology	0.8	0.9
5	Male Medical	0.8	-
6	Male Surgical	0.9	-
7	Orthopaedic	0.5	-
8	Female Surgical	0.6	-
9	Labour	0.7	0.5
10	Psychiatric	0.5	0.5
	Average MARI	0.64	0.41

Molecular Analysis

Figure 1 displayed the gene image of SHV amplifications of SHV beta lactamases. Isolates 1-6 represent *E. coli* from GOPD, A&E, Female Medical, Gynecology, Female Surgical and Labor wards. Isolates 1, 2, 3 and 6 (44.4%) were positive for SHV among ESBL producing *E. coli* while isolate 4 is negative. Isolates 7-13 from GOPD, A&E, Female Medical, Gynecology, Female Surgical, Labor and Psychiatric wards are ESBL producing *K. pneumoniae* and all were positive for SHV except isolate 8. Figure 2 shows the gene amplifications of TEM beta lactamases. Isolates 1-6 represent *E. coli* from GOPD, A&E, Female Medical, Gynecology, Female Surgical and Labor wards. Isolates 1,2,3,5 and 6 (65.6%) were positive for TEM among ESBL producing *E. coli* while isolate 4 was negative. Isolates 7-13 are ESBL producing *K. pneumoniae* and all were positive for TEM except isolates12 and 13 from GOPD, A&E, Female Medical, Gynecology, Female Surgical, Labor and Psychiatric wards.

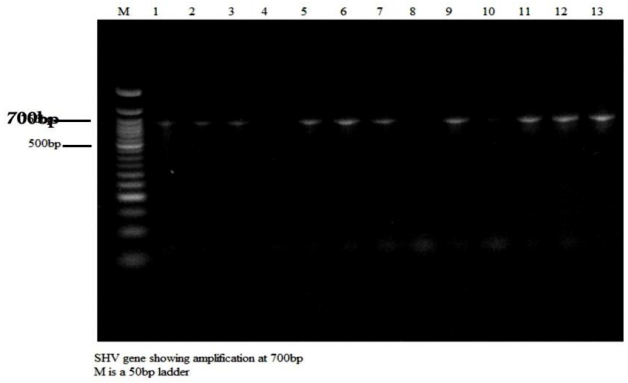

Figure 1: SHV gene showing amplification at 700bp (M is a 50bp ladder).

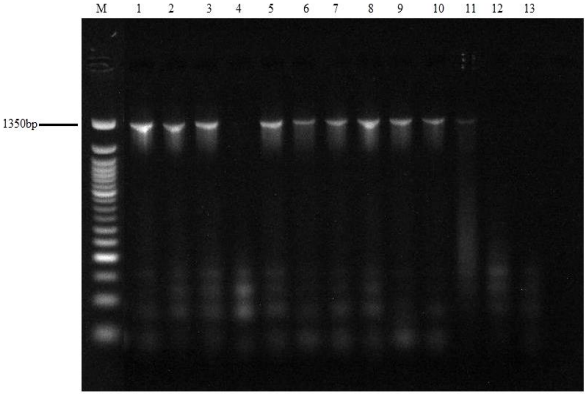

Figure 2: TEM gene showing amplification at 1350bp (M is a 50bp ladder).

DISCUSSION

ESBLs producing MDR pathogen *E. coli* and *Klebsiella* species were respectively 40% and 60% as revealed in this study. Several reports showed varied prevalence rates of ESBLs producing Entrobacteriaceae from countries and regions, capable of causing UTIs. The popularly reported ESBL-positive species are *K. pneumoniae* and *E. coli*, while plasmid-mediated ESBL genes are hosted by Enterobacteriaceae[14,16]. ESBL were produced by 40.9 and 40.3% *E. coli* and *Klebsiella* species respectively as reported by by[16] from Egypt and Blair[15] in Saudi Arabia, whose findings were lower than this finding.

It was reported that *E. coli* was 10% prevalent among 100 patients studied, of the age group of ≤17 years, females (11.5%) had higher prevalence than males (8.3%)[17]. In this study, the isolates of *E. coli* were found to be highly susceptible to nitrofurantoin (70%) and cefotaxime/clavulanic acid (100%) but were resistance to nalidixic acid (90%), cefuroxime (100%), ciprofloxacin (90%) and ceftazidime (100%). It was reported that ESBL production was 71.5% and 82.3% of the Gram-negative bacilli (*E. coli* and *Klebsiella* species), which was higher than results of this study[19]. This study showed that form the 88 isolates of *E. coli* and *Klebsiella* species, 47(53%) were phenotypically confirmed as ESBL producers. ESBL producing Enterobacteriaceae prevalence highly differs among country and among the hospitals within the country, as below 1% to more than 70% ESBLs producers is reported across the globe[20].

Table 7: Percentage occurrence of amplified genes through PCR.

S. N.	Molecular Gene	*E. coli* (% occurrence)	*Klebsiella* (% occurrence)
1	SHV	4 (44.4)	6 (54.5)
2	TEM	5 (65.6)	5 (45.5)
	Total	9 (100)	11 (100)

In 400 urine samples (68.6%) out of which 134 *E. coli* isolates were identified, Uropathogens were reported to be present and 80 (59.7%) of the uropathogenic *E. coli* (UPEC) were recognized as ESBL-producers and while they were all susceptible to meropenem, multi-drug resistance (62%) was observed in more than half of the ESBL-producers[20]. The prevalence of ESBL production as observed in *E. coli* was higher than *K. pneumonia* in this study. Similar report was given by Anago[18], but contradicted in a previous study[12], where 19 (66%) *E. coli* isolates were ESBL producers from a total of 29. This also agrees with the findings of a previous study[18,19]. This study also showed that 28 (47%) of *Klebsiella* spp. isolates were ESBL producers out of 59 samples. This is in agreement with the report of Rossolini[19], but disagrees[12,18]. From the analyzed samples, it was reported that 283 (29.5%) *E coli* and *K. pneumonia* were the most frequently isolated organisms, with positive cultures[21].

All isolates were susceptible to carbapenems in this study and for treatment of ESBL producing bacteria, carbapenems are the most common alternative drugs employed. This was in agreement with another study greater than 89% of the ESBL producers were susceptible to imipenem and meropenem[18]. Carbapenem resistance in another organism could result in the use of other drugs as alternative in the broad spectrum as first line for treatment of ESBL-positive bacteria which treatment cost might be very high[12,19]. It was also reported that in ESBLs producing *E. coli* and *Klebsiella* species, presenting therapeutic problem, resistance to non- beta lactams classes of antibiotics occured[18,22]. For consistent epidemiological analysis of anti-microbial resistance drug, molecular characterization of β-lactamase gene is imperative[18]. Several studies showed that the predominant of ESBL gene was dissimilar[18]. It was reported that with PCR, 35 isolates of *E. coli* were confirmed positive from the 58 ESBL screened positive *E. coli*[23]. The ESBL gene reported predominant was blaTEM while among MBL genes, blaSPM was the most prevalent. CTX-M, SHV and TEM genes were said to be the most prevalent type of ESBL genes. Meanwhile, the most common types of β-lactamase genes reported in the past were SHV and TEM genes types but lately, CTX-M type has reportedly said to have a wide range of global spread than SHV and TEM[19].

Several studies reported the predominance of CTX-M in many countries such as 74% in Iran, 70% in Morocco and North Africa, and 93.7% in India[18,19]. This study is in agreement with other studies since the predominant ESBL gene in uropathogenic *E. coli* was TEM-type β-lactamase gene (65.6%)[12,19]. Similarly, in Portugal (40.9%), Italy (45.4%) and Turkey (72.7%), the predominance of TEM-type β-lactamase gene was reported[12,24]. In *E. coli* and *K. pneumonia* from UTIs TEM was predominance was reported in india followed by that of SHV[18], which is consistent with this study. In 14.5% (29/200) participants, UTIs were confirmed and *E. coli* was 30.6% (11/36) predomimant[25].

CONCLUSIONS

In conclusion, the two ESBL-producing *E. coli* and *Klebsiella* species are a rising problem and spread is occurring in the study area. The dominant variant in our study was the TEM gene, but the SHV gene was also on the increase. The TEM and SHV beta-lactamase genes are the main cause of beta-lactam resistance in *E. coli* and *Klebsiella*, which may lead to an increase in urinary tract infections and thus pose a major challenge to the management of ESBL infection. Ertapenem, nitrofurantoin, ceftazidime, cefalexin, and cefotaxime constitute a reasonable treatment option for UTI based on the status of the findings. As the prevalence of MDR- and ESBL-producing *E. coli* and *Klebsiella* isolates reduces treatment options and increases hospital cost, it is imperative to update the prevalent pattern of resistance in any setting that will aid in appropriate antimicrobial therapy.

ACKNOWLEDGEMENTS

Appreciation goes to I.R. Iroha and A. C Nwuzo for their academic guardians and support, in making this works a reality.

AUTHOR'S CONTRIBUTION

Onu HC: designed the study, literature searches, writing original draft, and statistical analysis. **Idoko A:** writing original draft, literature searches. **Iroha IR:** editing, methodology. Final manuscript was read and approved by all authors.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

Authors declare that no conflict of interest exist.

REFERENCES

1. Laxminarayan RDA, Wattal C, Zaidi AK, Wertheim HF Sumpradit N. Antibiotic resistance, the need for global solutions. The Lancet Infectious diseases 2013; 13(12): 57–98. *http://dx.doi.org/10.1016/S1473-3099(13)70318-9*
2. Ibrahim Y, Sani Y, Saleh Q, Saleh A, Hakeem G. Phenotypic detection of extended-spectrum beta-lactamase and carbapenemase co-producing clinical isolates from two tertiary hospitals in Kano, northwest Nigeria. Ethiopian J Health Sci 2017; 27: 3–10. *http://dx.doi.org/10.4314/ejhs.v27i1.2*
3. De Kraker MDP, Grundmann H. BURDEN study group. Mortality and hospital stay associated with resistant Staphylococcus aureus and *Escherichia coli* bacteria: estimating the burden of antibiotic resistance in Europe. PLoS medicine 2011; 2: 11-17. *http://dx.doi.org/10.1371/journal.pmed.1001104*
4. Muhammad MH, Swedan S. Molecular and phenotypic characterization of carbapenem resistance and extended spectrum beta-lactamases among urinary *Escherichia coli* isolates. Int J Sci Tech 2015; 5(9): 21-29.

5. Röderova M, Halova D, Papousek I, *et al*. Characteristics of quinolone resistance in *Escherichia coli* isolates from humans, animals, and the environment in the Czech Republic. Front Microbiol 2017; 7: 21-47. *http://dx.doi.org/10.3389/fmicb.2016.02147*
6. Iroha IR, Esimone CO, Neumann S, Marlinghaus L, Korte M, Szabados F. First description of *Escherichia coli* producing CTX-M-15-extended spectrum beta lactamase (ESBL) in out-patients from south eastern Nigeria. Annals Clin Micro Antimicro 2012; 11: 19-23. *http://dx.doi.org/10.1186/1476-0711-11-19*
7. McLaughlin M, Advincula MR, Malczynski M, Qi C, Bolon M, Scheetz MH. Correlations of antibiotic use and carbapenem resistance in enterobacteriaceae. Antimicro Agents Chemother 2013; 57(10): 5131-3. *http://dx.doi.org/10.1128/AAC.00607-13*
8. Zhao B, Chao N, Ran G, *et al*. Recapitulation of SARS-CoV-2 infection and cholangiocyte damage with human liver ductal organoids. Protein Cell 2020; 11:771–775. *http://dx.doi.org/10.1007/s13238-020-00718-6*
9. Rothe C, Schlaich C, Thompson S. Healthcare associated infections in sub-Saharan Africa. J Hospital Infect 2013; 85 (4): 257-267. *http://dx.doi.org/10.1016/j.jhin.2013.09.008*
10. Cheesbrough M. Tropical health technology. Medical laboratory manual for tropical countries 2004; 7: 35-45.
11. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution of antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard 2015; 10: 7-10.
12. Dia ML, Ngom B, Diagne R, Ka R, Lo S, Cisse MF. Molecular detection of CTX-M-15- type β-lactamases in *Escherichia coli* strains from Senegal. New microbes and New Infection 2016; 9: 45-46.
13. Savelkoul PH, Aarts HJ, de Haas J, Dijkshoorn L Duim B. Amplified-fragment length polymorphism analysis: The state of an art. J Clin Microbiol1999; 37: 3083–3091. *http://dx.doi.org/10.1128/jcm.37.10.3083-3091.1999*
14. Mukonzo JK, Namuwenge PM, Okure G, Mwesige B, Namusisi OK, Mukanga D. Over-the-counter sub-optimal dispensing of antibiotics in Uganda. J Multidisc Healthcare 2013; 6:303–10. *http://dx.doi.org/10.2147/JMDH.S49075*
15. Blair JMA, Mark A, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJV. Molecular mechanisms of antibiotic resistance. Nature Rev Microbiol 2015; 13 (1): 42-51. *https://doi.org/10.1038/nrmicro3380*
16. Brink AJ. Epidemiology of carbapenem-resistant Gram-negative infections globally. Current Opin Infect Dis 2019; 32: 609–616. *https://doi.org/10.1097/QCO.0000000000000608*
17. Innes GK, Randad PR, Korinek A, Davis M.F, Price LB, So AD, Heaney CD. External societal costs of antimicrobial resistance in humans attributable to antimicrobial use in livestock. Annual Rev Public Health 2020; 41 (1): 141–157. *https://doi.org/10.1146/annurev-publhealth-040218-043954*
18. Anago E, Ayi-Fanou L, Akpovi CD, Hounkpe WB, Tchibozo MA, Bankole HS. Antibiotic resistance and genotype of beta-lactamase producing *Escherichia coli* in nosocomial infections in Cotonou, Benin. Annals Clin Microbiol Antimicro 2015; 14: 5-17. *https://doi.org/10.1186/s12941-014-0061-1*
19. Rossolini GM. Extensively drug-resistant carbapenemase-producing Enterobacteriaceae: An emerging challenge for clinicians and healthcare systems. J Internal Med 2015; 277: 528–531. *https://doi.org/10.1111/joim.12350*
20. Hassuna NA, Ahmed SK, Eman MF, Adel MH, Medhat A. Molecular characterization of extended-spectrum β lactamase- producing *E. coli* recovered from community-acquired urinary tract infections in Upper Egypt. Sci Reports 2020: 10(2772). *http://dx.doi.org/10.1016/j.afju.2015.02.005*
21. Asuquo SE, Kadija MT, Kathryn EM, *et al*. Youth engagement in HIV prevention intervention research in sub-Saharan Africa: A scoping review. J Int AIDS Soc 2021: 24(2):e25666. *http://dx.doi.org/10.1002/jia2.25666*
22. Okolie SO, Iroha IR, Ikpe VPO, Peter UO, Victor EJ, Alexander I. Characterization of multi-drug resistance livestock acquired methicillin resistant *Staphylococcus aureus* (LA-MRSA) isolates from different swine farms in Enugu Metropolis, Nigeria. Universal J Pharm Res 2022; 7(5):32-41. *http://dx.doi.org/10.22270/ujpr.v7i5.843*
23. Ugwu MC, Shariff M, Nnajide CM, *et al*. Phenotypic and molecular characterization of β-Lactamases among enterobacterial uropathogens in Southeastern Nigeria. Canadian J Infect Dis Medical Microbiol 2020;1-9. *http://dx.doi.org/10.1155/2020/5843904*
24. Bush K. Alarming β-lactamase-mediated resistance in multidrug-resistant Enterobacteriaceae. Curr Opin Microbiol 2012; 13(5):58–64. *http://dx.doi.org/10.1016/j.mib.2010.09.006*
25. Testimony JO, Oluwafolajimi AA, Iyanuoluwa JF, *et al*. Infectious disease outbreak preparedness and response in Nigeria: History, limitations and recommendations for global health policy and practice. AIMS Public Health 2020; 22; 7(4):736-757. *http://dx.doi.org/10.3934/publichealth.2020057*