Original Article

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Microbiology Section

Role of Extended Spectrum Beta Lactamases in Cephalosporin and Carbapenem Resistance in *Escherichia coli* from Inpatients and Outpatients in Nigeria

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ABSTRACT

Introduction: Antimicrobial resistance requires global coordinated action with a view to addressing its rising threat. Prevalence of antimicrobial resistance has been increasing worldwide in both developed and developing countries. Whilst cephalosporin and carbapenem resistance is a problem in *Escherichia coli* in Nigeria, there is a paucity of information regarding the mechanisms of resistance underpinning this in prevalence and types of extended spectrum in clinical *E. coli*.

Aim: To detect and characterise Extended Spectrum β Lactamases (ESBL) in clinical *E. coli* from inpatients and outpatients in Nigeria.

Materials and Methods: A total of 104 *E. coli* were obtained from 498 non-duplicate clinical specimens from Northern Nigeria between November 2017 and November 2018. Antibiotic susceptibility of third generation cephalosporin including other important antibiotics and phenotypic detection of ESBL of the isolates were determined. Genotypic detection of ESBL and randomly amplified polymorphic DNA to determine clonality were used to further characterise 44 *E. coli* isolates selected based on their phenotypes and clinical specimens. The data were analysed with the aid of statistical package for social sciences (IBM SPSS), version 21.0 and were reported in frequency tables and in percentages.

Results: Majority of the *E. coli* isolates showed no clonal relationships. More than half of *E. coli* were resistant to third generation cephalosporin class of antibiotics. There was no difference between MIC_{50} and MIC_{90} values for the majority isolates for most drugs where MICs ≥256 µg/mL were the norm except for carbapenems with low level resistance. In total, 44/104 (42.3%) *E. coli* were ESBL producers. bla_{CTX-M} was the dominant ESBLs seen in 75% (33/44) of isolates, of these $bla_{CTX-M-15}$ variant was most common and seen in 72.7% (24/33) of isolates followed by bla_{VEB}, 21/44 (47.7%) and bla_{PER} 6/44 (13.6%). No AmpC or carbapenemase genes were identified.

Conclusion: *E. coli* isolates from Northern Nigeria are highly multi-drug resistant with only carbapenems of common therapeutic drug classes retaining significant activity. Beta-lactam resistance is largely underpinned by carriage of CTX-M-15 and carbapenem resistance is likely to be a result of ESBL carriage with other mechanisms.

Keywords: Antibiotics, Bacteria, Infections, Resistance genes

INTRODUCTION

Antimicrobial resistance is a public health concern that requires a global coordinated action with a view to address its rising threat. Antimicrobial resistance has been increasing in prevalence worldwide in both developed and developing countries [1,2]. Most worrisome is limited resources in developing countries, which has inadvertently exacerbated the growing threat of antimicrobial resistance [2]. The annual death resulting from antibiotic resistant infections has been estimated globally at about 700,000 people and the deaths have been projected to hit 10 million by 2050 [3]. About half of those deaths are projected to occur in Africa because of non-effectiveness of currently available antibiotics and the non-availability and non-affordability of highly potent or alternative antibiotics [3].

Beta-lactamase production by Enterobacteriaceae is the most important single mechanism of resistance to beta-lactam antibiotics (penicillins, cephalosporins, monobactams and carbapenems), which are often used to treat nosocomial and community acquired infections. ESBL enzymes which are commonly produced by *E. coli* and *Klebsiella pneumoniae* hydrolyze third generation cephalosporins and monobactam thus rendering them ineffective against ESBL producing organisms, thus increasing costs, length of hospital stay, burden of disease and ultimately morbidity and mortality rates [4]. ESBL hydrolyze beta-lactams such as cefotaxime, ceftriaxone and ceftazidime, ampicillin, penicillins and aztreonam [5].

ESBL producing *E. coli* have been reported worldwide with varying prevalences although rates tend to be highest in lower income countries [6]. In Nigeria, there have been varying reports on the prevalence of beta-lactamase genes amongst Gram-negative pathogens from the South-West [7,8] and South-East [9] of the country. However, there is a dearth of information on the epidemiology of ESBL genes in *E. coli* from the North, though there are some pockets of phenotypic studies in some individual cities/regions like Maiduguri and Jos [10,11]. In order to bridge this gap between southern and northern Nigeria, this study was designed to detect and characterise ESBL resistance in clinical isolates of *E. coli* from northern Nigeria.

MATERIALS AND METHODS

This was a prospective convenient cross-sectional study. Four hundred and ninety-eight clinical specimens were obtained

using formulae, $N=Z^2Pq/d^2$ [12] based on the prevalence of 79.8% [13]. The specimens (included ear swab, wound, eye swab, urine, stool, high vaginal swab, cerebrospinal fluid and blood culture) were collected between November 2017 and November 2018 at the Aminu Kano Teaching Hospital, Kano, Federal Medical Centre, Katsina and Asokoro District Hospital, Abuja. Both outpatients and inpatients of these hospitals were recruited into the study following ethical approvals and informed consent from the patients. Ethical approvals were sought and obtained from the Research and Ethics Committee of the three hospitals with reference numbers; FHREC/2018/01/95/14-08-18 for Asokoro District Hospital, Abuja, NHREC/21/08/2008/AKTH/EC/2301 for Aminu Kano Teaching Hospital, Kano, while Federal Medical Centre, Katsina had no reference number but approval letter was obtained.

The patients recruited into the study had age range of <11 months to 71 years with both males and females. Clinical and demographic data, such as age, gender and patient status (Inpatient or Outpatient) were collected from all patients using a proforma.

Inclusion criteria: All patients that endorsed informed consent form and filled questionnaire (questions such as age, sex, recent use of antibiotics, whether it was self-medication or by prescription, history of travel and so on) were included in the study from the three hospitals.

Exclusion criteria: All patients that refused to sign or thumb print the informed consent forms and questionnaires were excluded from the study.

AKTH and FMCK are tertiary hospitals while ADHA is a secondary hospital.

Cultural Isolation and Identification

Clinical samples were cultured on Blood and MacConkey agars (Oxoid, U.K) overnight at 37°C and *E. coli* was identified using conventional microbiological procedures [14]. All *E. coli* isolates were further confirmed using Microbact 12A/12E (Oxoid, UK). This identification and other experiments were carried out at Molecular Biology laboratory of Department of Medical Laboratory Science, Ladoke Akintola University of Technology, Osogbo, Nigeria.

Antimicrobial Susceptibility Testing

The antibiotic susceptibility patterns of the 104 *E. coli* isolates to a panel of 9 antibiotics including representative of third generation cephalosporin, comprising ceftazidime (30 µg), cefotaxime (30 µg), meropenem (10 µg), imipenem (10 µg), ciprofloxacin (5 µg), gentamicin (10 µg), ertapenem (10 µg), amoxy-clavulanate (30 µg), Ampicillin (10 µg) were determined by the disc diffusion method (Oxoid, UK) using Mueller-Hinton agar (Oxoid, UK) according to CLSI guidelines [15]. Isolates were further selected for minimum inhibitory concentrations to ceftazidime, amoxyclav, ciprofloxacin, ampicillin, cefotaxime and gentamicin using agar dilution method. All runs included the control organism *E. coli* ATCC 25922.

Detection and Identification of Beta-lactamase Genes

All isolates were tested for production of ESBL/AmpC and carbapenemase based on susceptibility testing using the disc based 'ESBL/AmpC and Carbapenemase detection set' from Mast Diagnostics (Bootle, UK) following manufacturer's protocol and interpreted using the 'ESBL/AmpC and carbapenemase detection set calculator' tools also as per the manufacturer's guidelines. Polymerase Chain Reaction (PCR) was used to detect ß-lactam resistance genes from 44 isolates (selected to cover all phenotypes present) using primers shown in [Table/Fig-1] as previously described [7,16,17]. Control strains obtained from the Health Protection Agency (HPA) (now part of Public Health England, PHE) included

Primer	DNA sequence (5′ to 3′)	Annealing temp (°C)	Product size (bp)
NDM F NDM R	TTGATGCTGAGCGGGTG CTGTCCTTGATCAGGCAGC	56	578
KPC F KPC R	ATGTCACTGTATCGCCGTCT TAGACGGCCAACACAATAGG	56	785
OXA 48 F OXA 48 R	TTCGGCCACGGAGCAAATCAG GATGTGGGCATATCCATATTCATCGCA	56	240
VIM F VIM R	AGTGGTGAGTATCCGACAG ATGAAAGTGCGTGGAGAC	56	261
IMP F IMP R	CTACCGCAGCAGAGTCTTTG AACCAGTTTTGCCTTACCAT	58	587
GES F GES R	CGGTTTCTAGCATCGGGACACAT CCGCCATAGAGGACTTTAGCACAG	58	263
SME F SME R	AACGGCTTCATTTTTGTTTAG GCTTCCGCAATAGTTTTATCA	58	830
IMI F IMI R	GAGGGTATGACTAAATTCATGCGGTCGA GCAGGTGTAGATGTGTCACGTCATCG	58	116
PER-1F PER-1R	ATGAATGTCATTATAAAAGC AATTTGGGCTTAGGGCAGAA	51	590
VEB F VEB R	CGACTTCCATTTCCCGATGC GGACTCTGCACCAAATACGC	55	604
OXA-10 F OXA-10 R	GTCTTTCGAGTACGGCATTA ATTTTCTTAGCGGCAACTTAC	52	600
OXA F OXA R	ATATCTCTACTGTTGCATCTCC AAACCCTTCAAACCATCC	50	216
CTX-M-1 F CTX-M-1 R	GACGATGTCACTGGCTGAGC AGCCGCCGACGCTAATACA	60	499

[Table/Fig-1]: Primers used for amplification of β-lactamase genes.
NDM: New Delhi Metalobeta-lactamase; KPC: Klebsiella pneumoniae carbapenemase; OXA: Oxacillinase; VIM: Verona Integron-encoded Metalobetalactamase; GES: Guiana extended spectrum beta-lactamase; PER: Pseudomonas extended spectrum resistant; VEB: Vietnam extended spectrum beta-lactamase; CTX-M: Cefotaximase munich; SME: Serratia marcescens enzyme; IMI: Imipenem hydrolysing beta-lactamase; IMP: Imipenemase

for each gene. Amplimers resulting from these PCR reactions were sequenced to confirm the identity and specific variant of each gene identified and sequences were aligned to known reference sequences using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo).

Random Amplified Polymorphic DNA and Polymerase Chain Reaction Typing

The epidemiological relationships between strains of *E. coli* analysed by random amplified polymorphic DNA. The primers sequence and PCR running conditions used were according to Vogel I et al., [18], modified to use 1 µL of 100 µm of primers at a final concentration of 0.02 µm [19]. The experiment was repeated to ensure reproducibility. DNA fingerprints were compared by visual inspection to assign similar banding patterns to the same Random Amplified Polymorphic DNA (RAPD) type.

STATISTICAL ANALYSIS

Data from this study were reported in frequency tables and percentages. The data were analysed with the aid of statistical package for social sciences (IBM SPSS), version 21.0.

RESULTS

In total, 104 (60.5%) non-duplicate clinical *E. coli* strains were obtained from 172 Gram negative bacilli identified in the three hospitals (from 498 clinical specimens). Out of these 104 isolates, 61 (58.7%) were from females while 43 (41.3%) were from males. The age range of the participants in this study was between 11 months and 71 years, 70.2% was found between age range 11-40 years. Similarly, 51 (49.0%%) *E. coli* were found in Inpatients and 53 (51%) Outpatients. Disc susceptibility of 104 *E.coli* isolates showed that 93 were largely resistant to ampicillin (89.4%), 75 amoxicillin-clavulanic acid (72.1%), while ciprofloxacin

and gentamicin had resistance rate of 73 (70.2%) and 64 (61.5%) respectively. More than half of *E. coli* were resistant to third generation cephalosporin class of antibiotics used in this study, they comprised cefotaxime with resistant rate of 74 (71.2%) and ceftazidime 53 (51.0%). Carbapenem resistance was less common; ertapenem (7.7%), imipenem (4.8%) and meropenem (2.9%) [Table/Fig-2]. MIC of six antibiotics further confirmed resistance shown by disc susceptibility with the majority of isolates having an MIC 256 µg/mL with no difference between MIC_{50} and MIC_{90} for almost all the isolates [Table/Fig-3].

Antibiotic (µg/mL)	Sensitive n (%)	Intermediate n (%)	Resistant
Ceftazidime (30)	47 (45.2)	4 (3.8)	53 (51.0)
Meropenem (10)	101 (97.1)	0 (0.0)	3 (2.9)
Imipenem (10)	98 (94.2)	1 (1.0)	5 (4.8)
Cefotaxime (30)	30 (28.8)	0 (0.0)	74 (71.2)
Ciprofloxacin (5)	26 (25.0)	5 (4.8)	73 (70.2)
Gentamicin (10)	31 (29.8)	9 (8.7)	64 (61.5)
Ertapenem (10)	92 (88.5)	4 (3.8)	8 (7.7)
Amoxy-clavulanate (30)	22 (21.2)	7 (6.7)	75 (72.1)
Ampicillin (10)	7 (6.7)	4 (3.9)	93 (89.4)

[Table/Fig-2]: Summary of antimicrobial disc susceptibility testing of 104 *E. coli* isolates.
Numbers in parentheses are percentages

Isolate	Antimicrobial agents	MIC (0.06 -256) µg/mL)		Sensitive n (%)	Intermediate n (%)	Resistant n (%)
		MIC_{50}	MIC_{90}			
Escherichia coli	Cefotaxime	256	256	0 (0.0)	2 (1.9)	102 (98.1)
	Ceftazidime	128	256	10 (9.6)	15 (14.4)	79 (76.0)
	Amoxyclav	256	256	0 (0.0)	3 (2.9)	101 (97.1)
	Ampicillin	256	256	0 (0.0)	0 (0.0)	104 (100.0)
	Ciprofloxacin	256	256	2 (1.9)	0 (0.0)	102 (98.1)
	Gentamicin	128	256	17 (16.3)	4 (3.9)	83 (79.8)

[Table/Fig-3]: Minimum inhibitory concentrations of *E. coli* isolates.
Numbers in parentheses are percentages

Phenotypic Detection of Beta-lactamases

Phenotypic detection of beta-lactamases revealed 44 isolates (42.3%) to be ESBL-producers, AmpC enzymes were present in 15 isolates (14.4%) while 27 (26.0%) were predicted to be carbapenemase producers. Analyses of sources revealed that 28 out of 51 isolates (54.9%) from inpatients produced ESBLs while for outpatients only 16 of 53 (30.2%). Breakdown according to the hospital showed that *E. coli* from AKTH had more ESBL and carbapenemase resistance (59.3% and 40.7% respectively) while FMCK had more putative AmpC producers (19%) than others [Table/Fig-4].

Status	Number	ESBL present n (%)	ESBL absent n (%)	AmpC present n (%)	AmpC absent n (%)	Carba present n (%)	Carba absent n (%)
Inpatients	51	28 (54.9)	23 (45.1)	10 (19.6)	41 (80.4)	20 (39.2)	31 (60.8)
Outpatient	53	16 (30.2)	37 (69.8)	5 (9.4)	48 (90.6)	7 (13.2)	46 (86.8)
Total	104	44 (42.3)	60 (57.7)	15 (14.4)	89 (85.6)	27 (26.0)	77 (74.0)
Hospital							
FMCK	21	9 (42.9)	12 (57.1)	4 (19.0)	17 (81.0)	4 (19.0)	17 (80.9)
ADHA	56	19 (33.9)	37 (66.1)	6 (10.7)	50 (89.3)	12 (21.4)	44 (78.6)
AKTH	27	16 (59.3)	11 (40.7)	5 (18.5)	22 (81.5)	11 (40.7)	16 (59.3)
Total	104	44 (42.3)	60 (57.7)	15 (14.4)	89 (85.6)	27 (26.0)	77 (74.0)

[Table/Fig-4]: Phenotypic distribution of beta-lactamases in 104 *E. coli*.
Carba: Carbapenemase. Numbers in parentheses show percentages

Genotypic Detection of ESBL

PCR and sequencing identified different ESBL encoding genes in the 44 ESBL producers. bla_{CTX-M} were identified from 33/44 (75%) isolates, of which $bla_{CTX-M-15}$ has the dominant variant for 24/33 (72.7%) isolates, all isolates had multiple ESBL genes between 2 and 5. $bla_{CTX-M-2}$ had 18/33 (54.5%), $bla_{CTX-M-9}$ 11/33 (33.3%), $bla_{CTX-M-8}$ 10/33 (30.3%) and bla_{CTX-25} 6/33 (18.2%). Other ESBL genes identified were bla_{OXA} variants 17/44 (38.6%), of these bla_{OXA-10} was the dominant variant with 6/17 (35.3%), bla_{SHV} genes were seen in 11/44 (25.0%); bla_{VEB} from 21/44 (47.7%) and bla_{PER} in 6/44 (13.6%) [Table/Fig-5]. However, no AmpC or carbapenemase genes were detected. ESBL genes were detected in different proportions with varying diagnosis and sources in these hospitals [Table/Fig-6]. Percentage distribution of the ESBL genes between inpatients and outpatients showed bla_{CTX-M}/$bla_{CTX-M-15}$ to be more prevalent 93.8%/56.3% in outpatients compared to inpatients 89.3%/53.6%. Among the specimens, urine exhibited highest prevalence of ESBL genes 19/44 (43.2%) followed by stool and wound having 8/44 (18.2%) and 6/44 (13.6%) respectively, least prevalence was found in Semen, Ear and Eye swabs with prevalence of 2.3% each.

Typing of Isolates

RAPD typing was used to determine the degree of clonality among the ESBL producing *E. coli* isolates. The data revealed high diversity amongst all the species tested, with no identical RAPD patterns observed.

This suggests that the spread of resistance genes is underpinning the spread of resistance rather than expansion of a dominant clones.

DISCUSSION

It is a fact that ESBLs production is the most common mechanism of *E. coli* resistance to third-generation cephalosporins among Enterobacteriaceae and it mediates multidrug resistance, as shown in this study. Phenotypic production of ESBLs was seen in 42.3% of isolates tested which were not closely related. This was considerably higher than other reports from similar studies from different parts of the world including different cities/regions of Nigeria where prevalences of ESBL production between 18.6% and 42% had been reported in Nigeria [8,10,11]. While outside Nigeria; in Benin, Tanzania, Mexico, Nepal and India ESBL report was between 31.3 and 38.7% [20-24]. But in Turkey and USA, ESBL productions were extremely higher 84.0% and 72% respectively [25,26]. The differences in prevalence may be multifaceted ranging from varying sample size, ESBL detection method, population dynamics, literacy, economic, socio-cultural or lack or inadequate antimicrobial stewardship. The presence of ESBL-producers in an individual is a key indicator to increased antibiotic resistance because plasmid carries multiple resistance genes including ESBL.

The prevalence of ESBL production in both inpatients and outpatients were 54.9% and 30.2%, respectively. This was similar to the report obtained in Iran; inpatients and outpatients were 53.0% and 41.0% respectively [27], while different from that of Bosnia-Herzegovina with ESBL prevalence in inpatients of 12.5% [28]. High level ESBL producing isolates were originally confined to inpatients the trajectory of paradigm shift is very high in outpatients and community. This shows the extent of spread and level of resistance in the region. The isolates from outpatient in this case can be regarded as community isolates because the patients were not admitted they only visited these hospitals for treatment which in most cases were brief period spent in the facility. The very high prevalence of ESBL in these hospitals particularly the level in outpatients is a worrisome development as several other reports have indicated overlap between community and nosocomial ESBL.

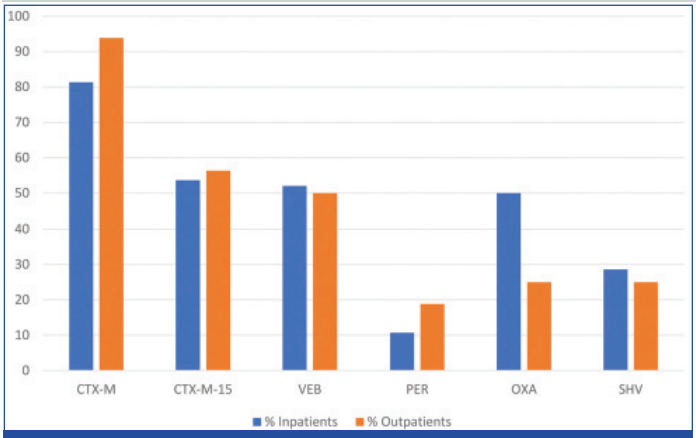
bla_{CTX-M} variant were the most common observed in this study followed by bla_{VEB}, bla_{OXA}, bla_{SHV} and bla_{PER}. This is in line with other

Serial #	ID no.	Diagnosis	Specimen	Source	MIC (µg/mL)						Phenotype			β-lactam gene
					CTX	CFZ	AMC	Amp	CIP	GEN	E	A	C	
1	AD 03	UTI	Urine	OP	256	128	256	256	256	256	+	-	+	CTX-M-15, CTX-M-2
2	AD 04	UTI	Urine	IP	256	256	256	256	256	256	+	-	-	PER, VEB, SHV, OXA
3	AD 05	GET	Stool	IP	256	128	128	256	128	256	+	+	-	CTX-M-15, CTX-M-9
4	AD 06	GET	Stool	OP	256	256	256	256	256	128	+	-	-	CTX-M-15, CTX-M-2, VEB
5	AD 07	Sepsis	Wound	IP	256	256	256	256	256	256	+	-	-	SHV, CTX-M-15, OXA
6	AD 08	UTI	Urine	IP	256	256	256	256	256	256	+	+	-	CTX-M-2, CTXM-8, CTX-M-15,
7	AD 09	GET	Stool	IP	256	256	256	256	256	256	+	-	-	CTX-M-15, OXA, CTX-M-2, VEB
8	AD 17	GTI	HVS	OP	256	256	256	256	128	256	+	+	+	CTXM-8, VEB, CTX-M-15
9	AD 21	Sepsis	Wound	IP	256	256	256	256	256	256	+	-	+	CTX-M-15, OXA, CTX-M-25
10	AD 22	UTI	Urine	IP	256	128	256	256	256	256	+	-	-	VEB, CTX-M-15
11	AD 26	Sepsis	Wound	OP	256	256	256	256	256	256	+	-	+	CTX-M, SHV, OXA-10, CTX-M-2
12	AD 30	GTI	HVS	IP	256	256	256	256	256	256	+	+	-	CTX-M-2 VEB,
13	AD 47	Infertility	Semen	OP	256	256	256	256	256	256	+	-	-	SHV, CTX-M-15
14	AD 52	UTI	Urine	IP	256	256	256	256	256	256	+	-	+	CTX-M-15, CTX-M-2,
15	AD 56	GTI	HVS	OP	256	256	256	256	256	256	+	-	-	CTX-M, VEB,
16	AD 60	UTI	Urine	OP	256	256	256	256	256	256	+	+	-	PER, VEB, CTX-M-15
17	AD 65	UTI	Urine	IP	256	256	256	256	256	128	+	-	+	CTX-M, OXA, VEB, OXA-10
18	AD 78	GTI	HVS	IP	256	256	256	256	256	256	+	-	+	SHV, VEB, CTX-M-2
19	AD 84	UTI	Urine	OP	256	256	256	256	128	256	+	+	-	VEB, SHV, CTX-M-8
20	AK 08	Septicaemia	Blood	IP	256	256	256	256	256	256	+	-	-	CTX-M-15, VEB, SHV, CTX-M-9
21	AK 11	UTI	Urine	OP	256	256	256	256	256	256	+	-	+	CTX-M-8, CTX-M-25, VEB, CTX-M-15
22	AK 16	GET	Stool	OP	256	256	256	256	256	256	+	-	+	CTX-M-15, CTX-M-25, PER
23	AK 17	UTI	Urine	IP	256	256	256	256	256	128	+	-	+	CTX-M-15, CTX-M-9, VEB, OXA
24	AK 20	Sepsis	Wound	IP	256	256	256	256	256	256	+	-	-	CTX-M-15, CTX-M-2, CTX-M-9,
25	AK 22	GTI	HVS	OP	256	256	256	256	256	256	+	-	+	SHV, CTX-M-8 CTX-M-2, OXA
26	AK 23	GTI	HVS	IP	256	256	256	256	256	256	+	-	-	CTX-M-15, CTX-M-8, OXA, OXA-10
27	AK 24	UTI	Urine	IP	256	256	256	256	128	256	+	-	+	CTX-M-9, CTX-M-2, SHV,
28	AK 27	UTI	Urine	OP	256	256	256	256	256	256	+	-	-	CTX-M-9, VEB, CTX-M-2
29	AK 28	GET	Stool	IP	256	256	256	256	256	256	+	-	-	CTX-M-2, VEB, OXA
30	AK 31	Septicaemia	Blood	IP	256	256	256	256	256	256	+	-	-	CTX-M-15 SHV, CTX-M-8, VEB
31	AK 32	Otitis media	Ear swab	IP	256	256	256	256	256	256	+	-	-	CTX-M-15, VEB, CTX-M-25
32	AK 41	Conjunctivitis	Eye swab	IP	256	256	256	256	256	256	+	-	+	CTX-M-2, CTX-M-9, OXA, PER
33	AK 49	UTI	Urine	IP	256	256	256	256	256	256	+	-	-	CTX-M-8, CTX-M-2, CTX-25
34	AK 53	UTI	Urine	OP	256	256	256	256	256	256	+	+	+	OXA, OXA-10, PER
35	AK 59	Sepsis	Wound	IP	256	256	256	256	256	256	+	-	-	OXA, CTX-M-2, CTX-M-9
36	FM 06	UTI	Urine	OP	256	256	256	256	256	256	+	+	-	CTX-M-15, CTX-M-25, OXA
37	FM 10	UTI	Urine	IP	256	256	256	256	256	128	+	-	-	CTX-M-2 SHV, VEB, CTX-M-9
38	FM 14	GET	Stool	IP	256	256	256	256	256	256	+	-	+	OXA, SHV, CTX-M-8
39	FM 15	Sepsis	Wound	OP	256	256	256	256	256	256	+	-	-	CTX-M-15, VEB, CTX-M-9
40	FM 19	GET	Stool	IP	256	256	256	256	256	256	+	-	-	OXA, OXA-10, PER
41	FM 21	UTI	Urine	IP	256	256	256	256	256	256	+	-	+	CTX-M-8, CTX-M-15, CTX-M-2
42	FM 25	UTI	Urine	IP	256	256	256	256	256	256	+	-	-	CTX-M-15, SHV, CTX-M-9, OXA
43	FM 31	GET	Stool	OP	256	256	256	256	256	256	+	-	+	CTX-M-8, CTX-M-2
44	FM 36	UTI	Urine	IP	256	256	256	256	256	256	+	+	-	VEB, OXA, OXA-10

[Table/Fig-5]: Carriage of beta-lactamases in *E. coli* isolates.
AD: Asokoro District Hospital Abuja; AK: Aminu Kano Teaching Hospital, Kano; FM: Federal medical centre, Katsina; CTX: Cefotaxime; CFZ: Ceftazidime; AMC: Amoxycillin-clavulanic acid; Amp: Ampicillin; CIP: Ciprofloxacin; GEN: Gentamicin; UTI: Urinary tract infection; GET: Gastroenteritis; GTI: Genital tract infection; HVS: High vaginal swab; OP: Outpatient; IP: Inpatient; E: Extended spectrum β-lactamase; A: AmpC; C: Carbapenemases; OXA: Oxacillinase; PER: Pseudomonas extended spectrum resistant; VEB: Vietnam extended spectrum beta-lactamase; CTX-M: Cefotaximase munich

studies showing SHV and TEM families were gradually reducing in prevalence and being supplanted by the CTX-M family [19,29]. The CTX-M family has become widespread in both hospital and community settings virtually in every continents of the world [8,26]. $bla_{CTX\text{-}M\text{-}15}$ was the dominant ESBL found among the $bla_{CTX\text{-}M}$ variants in accordance with previous reports all over the world [8,26,30]. $bla_{CTX\text{-}M\text{-}15}$ has frequently been seen to co-exist with other ESBL genes in the isolates. bla_{VEB} has been reported from most parts of the world since its first description in Vietnam [31] including south-western Nigeria [8,32]. To our knowledge, this was the first report of bla_{VEB} and bla_{PER} ESBLs from northern Nigeria. ESBLs production was found across varying clinical specimens and hospitals.

There was a high level of multidrug resistance to majority of the antibiotics including third-generation cephalosporins, ampicillin, beta-lactamase/inhibitor (amoxicillin-clavulanic acid), fluoroquinolones and aminoglycosides among the isolates. These findings have significant implications in the use of third-generation cephalosporins including fluroquinolones for the management



[Table/Fig-6]: Distribution of ESBL genes between inpatients and outpatients.

of patients with infections caused by *E. coli*. In this panel only carbapenems retained good efficacy as a therapeutic option. The low rate of resistance to carbapenems was mirrored by a lack of detection of carbapenemase genes, the most likely scenario may be that carbapenem resistance is being caused by $bla_{CTX\text{-}M\text{-}15}$ in conjunction with other mechanisms such as efflux and or porin loss. The absence of carbapenemase genes is very surprising compared to high resistance rates reported from different studies from Southern Nigeria [8,19]. Carbapenem resistance has been reported to be mediated by production of ESBL (largely CTX-M-15) accompanied by porin loss and or efflux activity [33]. The low resistance rate may not be unconnected with the high cost of carbapenem antibiotics which is directly related to the poverty and literacy level in the North of Nigeria in terms of affordability and awareness. Indiscriminate and empirical use of beta-lactam drugs in particular carbapenems should be avoided to guide against the development of carbapenem-resistant strains of *E. coli* leaving no viable therapeutic options in the future.

Limitation(s)

It was self-sponsored hence it was not possible to investigate further, for instance, confirm mechanisms of few isolates with carbapenem resistance with no carbapenemase genes.

CONCLUSION(S)

There is a significant challenge in the use of third-generation cephalosporins including other antibiotics except carbapenem in the management of patients with infections caused by *E. coli* in Nigeria, in the North of the country. This was threatened by widespread dissemination of CTX-M enzymes.

There is need for an effective antibiotic stewardship programme and regular antibiotic resistance surveillance studies enhanced by real time routine detection of ESBLs with a view to stemming the tide of antibiotic resistance and maintain low level resistance especially to carbapenems which remain critically important in this setting.

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