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Antimicrobial Resistance Profile and Detection of Extended Spectrum and Amp C β-Lactamase Resistance Genes in *Escherichia coli* Isolated from Diarrheic Children in Lafia, Nasarawa State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AZ and YBN designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RHA and IHN managed the analyses of the study. Authors IY and BEB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study evaluated the presence of extended spectrum β-lactamase (ESBL) and AmpC β-lactamase resistance genes in *E. coli* from stool of diarrheic children in some hospitals in Lafia metropolis, Nigeria.
Methodology: A total of 70 stool samples of children were obtained from Dalhatu Araf Specialist Hospital, Lafia, M & D Hospital, Olivet Medical Centre and Sandaji Medical Centre, Lafia. *Escherichia coli* were isolated and identified using standard microbiological methods. Antimicrobial

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susceptibility of the isolates was tested using Clinical and Laboratory Standards Institute (CLSI) method. The phenotypic detection of ESBL and AmpC β-lactamase production in some antibiotic resistant isolates were carried out using disc method. The molecular detections of ESBL and AmpC resistance genes were carried out using Polymerase Chain reaction (PCR) method.

Results: Of the 70 samples, the occurrence of *E. coli* was 100%. The isolates were highly resistant to ampicillin (97.14%), ciprofloxacin (90.00%), sulfamethoxazole/trimethoprim (84.29%), streptomycin (78.57%), amoxicillin/clavulanic acid (70.00%); moderate to gentamicin (38.57%), ceftazidime (37.14%) and cefotaxime (30.00%); and less resistant to cefoxitin (15.71%) and imipenem (8.57%). Twenty-one (30.00%) isolates were jointly resistant to both cefotaxime and ceftazidine. Of this number, 66.67% (14/21) were phenotypically confirmed ESBL producers; and the occurrences of ESBL resistance genes were: 7.14% (*SHV*), 42.86% (*CTX-M*) and 50.00% (*TEM*). Out of 11isolates resistant to cefoxitin, 4(36.36%) were phenotypically confirmed as AmpC β-lactamase producers; and the occurrence of AmpC genes were: 50.00% (*CIT*), 25.00% (*FOX*) and 25.00% (*MOX*).

Conclusion: The isolates were least resistant to imipenem and cefoxitin and highly resistant to ampicillin, ciprofloxacin and sulfamethoxazole/trimethoprim. *TEM and CTX-M* ESBL genes were more frequent than *SHV*. *CIT* AmpC gene was more frequent than *FOX* and *MOX*.

Keywords: Escherichia coli; extended spectrum; AmpC; β-lactamase; stool; diarrhea; children.

1. INTRODUCTION

Diarrhea has been reported to be a leading cause of morbidity and mortality in children [1] and *Escherichia coli* (*E. coli*) is a major bacteria cause, particularly in developing countries [1,2].

Treatment of bacterial diarrhea has employed antibiotics [3,4]. However, the use of antibiotics has been reported to be one of the factors contributing to the emergence of bacterial resistance, a global health challenge [5,4,6]. Beta-lactam antibiotics, which have a beta-lactam ring nucleus as part of their molecular structure, and typically include penicillin derivatives (penams), cephalosporins (cephems), monobactams, and carbapenems [7], are the most widely used class of drugs for the treatment of bacterial infections [8,9,10,11,12].

Resistance mechanisms in bacteria against β-lactam antibiotics include: β-lactamase production and alteration of the penicillin-binding protein (PBP) target site [13,14]. The production of β-lactamases, which hydrolyzes the β-lactam ring, is among the most frequently encountered mechanisms in *E. coli* [15].

Extended-spectrum β-lactamases (ESBLs) are a group of β-lactamases that are usually plasmid-encoded, and have the capacity to hydrolyze many antibiotics including penicillins, cephalosporins, and aztreonam and are inhibited by clavulanic acid [16]. Extended-spectrum β-lactamases are frequently encoded by genes located on different transferable genetic elements, a variety of epidemiological situations have been documented, ranging from sporadic cases to large outbreaks [17]. Moreover, ESBL-producing strains are often resistant to antibiotics of other classes (sulfonamides, aminoglycosides, quinolones) which complicates the treatment strategies in many hospitalized patients [5,18].

Amp-C beta-lactamases are bacterial enzymes that hydrolyze third-generation extended spectrum cephalosporins and cephamycins engendering resistance to these categories of antibiotics [19]. Thus, the onslaught of AmpC resistance represents a major challenge for clinicians as it renders third-generation cephalosporins increasingly ineffective [20].

Several studies worldwide have reported on ESBL resistance in *E. coli* from stool of diarrheic children [5,14,21-29]. There are few reports in Nigeria on ESBL production in *E. coli* isolated from stool of diarrheic patients [30-34]. However, no report is known to us on ESBL and AmpC β-lactamase resistance in *E. coli* from diarrheic patients of any age group in the study area. This study thus investigated the antibiotic resistance profile and molecular basis ESBL and AmpC β-lactamase resistance in *E. coli* from diarrheic children accessing selected hospitals in Lafia, Nigeria.

2. MATERIALS AND METHODS

2.1 Antibiotic Discs

The antibiotic discs (potency) used in this study were from Oxoid Ltd (U.K.) and include:

Amoxicillin (AMX: 10 µg), Amoxicillin-Clavulanic acid (AMC: 30 µg), Cefotaxime (CTX: 30 µg), Ceftazidime (CAZ: 30 µg), Imipenem (IPM: 30 µg), Ciprofloxacin (CIP: 5 µg), Co-trimoxazole (SXT: 25 µg), Gentamicin (CN: 10 µg), Streptomycin (S: 10 µg) and Cefoxitin (FOX: 30µg).

2.2 Equipment

The equipment used for this study include: Autoclave (Certoclav, Model SM280E, Surgi friend Medicals, England), Gel electrophoresis machine (Max Fill Scie-plas Model HU10 serial no 5237), Laminar air flow cabinet (PCR-8 re-circulating laminar flow pre station, Labcaire product 220/240v), Microscope (Model CME 1349522X, Leica, USA), Spectrophotometer (Eppendorf Biophotometer 8.5mm, Lichtstrahihohe), UV illuminator (VilberbLourmat TFX-35-M serial no NoV02 8104), Centrifuge (Model 5417R: Lab-line Instrument Inc USA), Microwave oven (HINARI Life Style 800watts model MX310TCSL), Oven (Hotbox Size One, Galengkamp, U.K.), Incubator (Model 12-140E, Quincy Lab Inc), Refrigerator/Freezer (Model PRN 1313 HCA, BEKO, Germany), Thermocycler (Model TC-312, Techne, England), Electronic weighing balance (Model QT 600: Lab-line Instrument Inc USA),Vortex machine (Touch plate Super Mixer, CAT No 1291, Lab-line Instrument Inc USA), and Gel Doc system (Biorad, U.K.).

2.3 Chemicals and Reagents

The reagents and chemicals used were: acridine orange, carbolfuschin, crystal violet, ethanol, xylene, creatinine, pottasium hydroxide and Kovac's reagents, obtained from BDH chemical Ltd, England; ethydium bromide, iodine solution, EDTA and Glycerol, obtained from Sigma chemical Ltd, England; and agarose gel from Schwarz/ Mann Biotech., Germany.

2.4 Primers

The primers used in this study are as given in the Table 1.

2.5 Study Location

This study was carried in some selected hospitals namely: Dalhatu Araf Specialist Hospital, Olivet Medical Center, Sandaji Medical Center, M&D Hospital, within Lafia Metropolis, Nigeria.

2.6 Sample Collection

A total of seventy (70) stool samples of diarrheic patients,5 years and below, attending the selected hospitals were collected randomly using sterile container and transported using ice pack to Microbiology Laboratory of Dalhatu Araf Specialist Hospital, Lafia for analysis.

2.7 Isolation of *Escherichia coli*

Escherichia coli was isolated from the stool of the diarrheic children as earlier described [2]: A loopful of stool was streaked on MacConkey agar (Oxoid Ltd, U.K) and incubated at 37ºC for 24h. A pinkish colony from MacConkey agar plates were further streaked on Eosine methylene blue agar (Oxoid Ltd, U.K) and incubated at 37ºC for 24 h. Greenish metallic sheen colonies that grew on the Eosine methylene blue agar were presumptively selected as *E. coli*.

Table 1. Primers and expected amplicon size for each gene

S/N	Target genes	Sequence	Amplicon size	References
1	bla_{TEM}	5´-TCGGGGAAATGTGCGCG-3´ 5´-TGCTTAATCAGTGAGGCACC-3´	972	[15]
2	bla_{SHV}	5´-GGGTTATTCTTATTTGTCGC-3´ 5´-TTAGCGTTGCCAGTGCTC-3	615	[15]
3	bla_{CTX-M}	5´-ACGCTGTTGTTAGGAAGTG-3´ 5´-TTGAGGCTGGGTGAAGT-3´	857	[15]
4	*MOX-M (F)* *MOX-M (R)*	GCTGCTCAAGGAGCACAGGAT CACATTGACATAGGTGTGGTGC	520	[35]
5	*CIT-M (F)* *CIT-M (R)*	TGGCCAGAACTGACAGGCAAA TTTCTCCTGAACGTGGCTGGC	462	[35]
6	*FOX-M (F)* *FOX-M (R)*	AACATGGGGTATCAGGGAGATG CAAAGCGCGTAACCGGATTGG	190	[35]

F = Forward; R = Reverse

2.7.1 Identification of *Escherichia coli*

The presumptive *E. coli* was Gram-stained, and biochemically tested using Indole, Methyl red, Voges-Proskauer and Citrate tests ("IMViC") to confirm its identity as *E. coli* as earlier described [36].

2.8 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing of the bacterial isolates was carried out as earlier described [37]. Briefly, three (3) pure colonies of the isolates were inoculated in to 5 ml sterile 0.85% (w/v) NaCl (normal saline) and the turbidity of the bacterial suspension was adjusted to that equivalent to 0.5 McFarland's standard.

Mueller-Hinton agar (Oxoid Ltd, U.K) plates were inoculated with the standardized culture; antibiotic discs were aseptically placed at the center of the plates and allowed to stand for 1 h for pre-diffusion. The plates were incubated at 37°C for 24 h. The diameter zone of inhibition in millimeter was measured and the result was interpreted in accordance with the susceptibility break point earlier described [37].

2.9 Determination of Multiple Antibiotic Resistance (MAR) Index of the Isolates

The MAR index of the isolates was determined using the formula [6]:

$$\text{MAR Index} = \frac{\text{No. antibiotics isolate is resistant to}}{\text{No. of antibiotics tested}}$$

2.10 Extended Spectrum β-Lactamase Production Test

The phenotypic confirmatory test for ESBL production by isolates resistant to cefotaxime and ceftazidime was carried out using Double-Disc Synergy Test (DDST) method earlier described [38]. Briefly, 10^5cfu/ml bacterial suspension was streaked on sterile Mueller-Hinton agar plates and amoxicillin-clavulanic acid (30 µg) disc was placed at the centre of the plate. Cefotaxime (30 µg) and ceftazidime (30 µg) discs were then placed 15 mm (edge-to-edge) from the centre disc. Enhancement of zone of inhibition in the area between the amoxicillin-clavulanic acid disc and any one of the β-lactam discs compared with the zone of inhibition on the far side of the drug disc was interpreted as indicative of the presence of an ESBL in the tested strain.

2.11 Confirmatory Test for AmpC β-Lactamase Production

The confirmatory Test for AmpC β-Lactamase against *E. coli* isolates whose diameter zone of inhibition of cefoxitin were <18mm was carried out as follow; the swab stick was soaked in a standardized *E. coli* isolates resistance to cefoxitin suspension (10^5cfu/ml) and streaked on MHA plates and 30µg cefoxitin disks were placed at the centre of the plates and 30 µg cefotaxime disk was placed 20 mm away from cefoxitime disks and allowed to stand for 1 h for pre-diffusion at room temperature before they were incubated at 37°C for 24 h. A clover leaf zone of inhibition against the isolates after 24 h incubation was confirmed AmpC β-lactamase producers.

2.12 Molecular Detection of Extended Spectrum β-Lactamase and AmpC Genes

The confirmed ESBL isolates were subjected to a singleplex polymerase chain reaction to detect three (3) ESBL genes: bla_{SHV}, bla_{TEM}, $bla_{CTX\text{-}M}$ and the confirmed *AmpC* isolates were also subjected to multiplex PCR to detect three (3) genes: *MOX*, *CIT* and *FOX* genes.

2.12.1 DNA extraction

DNA extraction was performed by boiling method as described [2]. Following purification on MacConkey agar, bacterial DNA was isolated from a 24-h culture in Luria-Bertani broth (LB broth) prepared according to the manufacturers' protocol.

The bacterial cells were harvested by centrifugation at 3200 rpm in a micro centrifuge for 2 min at room temperature and the supernatant was discarded. The pelleted cells were re-suspended in 1ml of sterile normal saline and the micro-centrifuge tubes were placed in the vortex for five seconds. Centrifugation was carried out at 3200 rpm for 1 min and the supernatant was discarded. 0.5 ml of sterile normal saline was added to the pellets and the tubes were vortexed for 5 sec after which they were heated in the block heater at 90°C for 10 min. immediately after heating, rapid cooling was done by transferring the tubes into the freezer for

10 minutes. Cell debris was removed after centrifugation was done at 3200 rpm for 1 min and 300 µl of the supernatant was transferred into a sterile 2 ml Eppendof tube as DNA and stored at -10°C until use.

Estimation of the concentration, purity and yield of the DNA sample was accessed using absorbance method (measurement of absorbance) with the spectrophotometer (Nanodrop 1000). For DNA concentration, absorbance readings were performed at 260nm (A_{260}) and the readings were observed to be within the instrument's linear range (0.1 – 1.0). DNA purity was estimated by calculating the A_{260}/A_{280} ratio and this was done by the spectrophotometer's computer software (where A_{260}/A_{280} ratio ranges from 1.7 – 1.9).

2.12.2 DNA amplification of extended spectrum β-lactamase genes

Simplex Polymerase Chain Reaction (PCR) was performed in order to amplify the ESBL genes present in the isolates. The presence of bla_{CTX-M}, bla_{SHV} and bla_{TEM} genes were tested for using previously published primer sets and conditions. The primer sequences and expected amplicon size for each gene are listed in Table 1.

The reactions were carried out in 20 µl reaction volume which was made up of 10 µl of Mastermix (Qiagen), 0.32 µl of primers (0.16 µl each of forward and reverse primers), 3 µl of DNA and 6.68 µl of nuclease free water. The primer concentration stood at 0.2 M. The reaction tubes were placed in the holes of the thermal cycler and the door of the machine was closed.

Conditions during the reactions were set as: 3 minutes of initial denaturation at 95°C, followed by 35 amplification cycles of denaturation at 95°C for 30 sec, annealing at 56°C for 40 sec, initial extension at 72°C for 50 sec, final extension at 72°C for 3 min and a hold at 4°C infinitely.

2.12.3 DNA amplification of AmpC genes

Multiplex Polymerase chain reaction (PCR) was performed in order to amplify the AmpC genes present in the isolates. The presence of *MOX*, *CIT* and *FOX* genes were tested for using previously published primer sets and conditions. The primer sequences and expected amplicon size for each gene are listed in Table 1.

The reactions were carried out in 25 µl reaction volume which was made up of 5 µl of Mastermix (Qiagen), 2.4 µl of primers (0.4 µl each of forward and reverse primers), 0.5 µl of $MgCl_2$, 1.5 µl of DNA and 15.6 µl of nuclease free water. The primer concentration stood at 0.4 M. The reaction tubes were placed in the holes of the thermal cycler and the door of the machine was closed.

Conditions during the reactions were set as: 3 min for initial denaturation at 94°C, followed by 35 amplification cycles of denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec, initial extension at 72°C for 30 sec, final extension at 72°C for 7 min and a held at 4°C infinitely.

2.12.4 Agarose gel electrophoresis

Seven microliters of the amplified DNA were transferred into the wells of a 1.5% Agarose gel by stabbing the wells using a micropipette and this was done carefully to ensure that each well had only one sample. Each gel had one well which contained a DNA ladder (100 bp, Thermo Scientific) in order to estimate the size of the DNA amplicons. Electrophoresis was run at 125 volts for 20 min, after which the gels were viewed using ultra-violet trans-illuminator.

3. RESULTS AND DISCUSSION

3.1 Occurrence of *Escherichia coli*

The cultural, morphological and biochemical characteristics for identification of *Escherichia coli* is as shown in Table 2. The combined occurrence of *E. coli* in the study centers was100%.

3.2 Antibiotic Resistance Profile

The occurrence of resistance was 100%. The antibiotic resistance profile of the isolates is as given in Table 3. The isolates were more resistant to ampicillin (97.1%), ciprofloxacin (90.0%) and sulphamethoxazole/trimethoprim (84.3%) but less resistant to cefotaxime (30.0%), cefoxitin (15.7%) and imipenem (8.6%).

3.2.1 Antibiotics resistance phenotypes

The various antibiotic resistance phenotypes of the isolates are as given in Table 4. The most

common resistance phenotype was SXT-AMC-AMP-S-CIP with an occurrence of 10(14.3%).

3.2.2 Multiple Antibiotic Resistance (mar) index

The MAR index of *E. coli* isolates from stool of diarrheic children of underage attending selected hospitals in Lafia metropolis is as given in Table 5. All the *E. coli* isolates were MAR isolates MAR index of ≥0.2 and the most common MAR index were 0.4 and 0.5 and their percentage occurrence were 17.1%and 34.3% respectively as shown in Table 5.

3.2.3 Extended spectrum β-lactamase production

The phenotypic and genotypic confirmatory tests of ESBL production in the isolates jointly resistant to both cefotaxime and ceftazidine is as shown in Table 7. Out of 21 *E. coli* isolates jointly resistance to both ceftazidime and cefotaxime, 14 (66.7%) of the *E. coli* isolates were phenotypically confirmed ESBL producers as given in Table 7. The order of occurrence of ESBL resistance genes was: bla_{SHV} (76.5%)>bla_{TEM} (50.0%)>bla_{CTX-M} (42.9%) as given in Table 7. The DNA bands for *SHV, CTX-M* and *TEM* genes are as given in Plate 1, 2 and 3.

3.2.4 AmpC β-Lactamase production

The result of phenotypic and genotypic confirmatory test of AmpC β-lactamase Production in *E. coli* isolates resistant to cefoxitin from stool of diarrheic children attending some selected hospitals in Lafia metropolis, Nigeria is as shown in Table 7. Whereas the DNA bands for AmpC genes is as shown in Plates 1, 2 and 3 respectively. Out of 11 *E. coli* isolates resistant to cefoxitin, 4(36.4%) were phenotypically confirmed AmpC β-lactamase producers as given in Table 7. The OF percentage occurrence of the AmpC genes were; *CIT* (50%)>*MOX* (25%) and *FOX* (25%) respectively as shown in Table 7.

Table 2. Cultural, morphological and biochemical characteristics of *Escherichia coli* isolated from stool of diarrheic children attending selected hospitals in Lafia, Nigeria

Clinical characteristics	Morphological characteristics		Biochemical characteristics								Inference
	Gram stain	Morphology	Ind	Mr	Vp	Ct	Lac	Glu	Gal	Suc	
Pinkish colonies on MCA and greenish metallic sheen colonies on EMB agar	-	Rod	+	+	-	-	+	+	+	+	*E. coli*

MCA= Mac Conkey Agar; EMB= Eosin Methylene blue; - =Negative; + =positive; Ind =Indole; Mr = Methyl Red; Vp =Voges-Proskauer; Ct=Citrate; Lac=Lactose; Glu= Glucose; Gal =Galactose; Suc=Sucrose

Table 3. Antibiotic Resistance Profile of *Escherichia coli* from stool of diarrheic children attending selected hospitals in Lafia, Nigeria

Antibiotics	Disc Content (µg)	No. (%) Resistance(n=70)
Ampicillin (AMP)	30	68 (97.1)
Amoxycillin/clavunate (AMC)	30	49 (70.0)
Ciprofloxacin (CIP)	5	63 (90.0)
Ceftazidime (CAZ)	30	26 (37.1)
Cefotaxime (CTX)	30	21 (30.0)
Cefoxitin (FOX)	30	11 (15.7)
Gentamicin (CN)	10	27 (38.6)
Imipenem (IPM)	30	6 (8.6)
Streptomycin(S)	30	55 (78.6)
Sulphamethoxazole/trimethoprim (SXT)	25	59 (84.3)

Table 4. Antibiotic Resistance Phenotypes of *Escherichia coli* from diarrheic stool of children attending selected hospitals in Lafia, Nigeria

Antibiotic Resistance Phenotypes	No. (%) *E. coli* isolates (n=70)
AMP,CIP	1(1.43)
SXT,AMP	1(1.43)
SXT,AMP,S	1(1.43)
AMC,AMP,S	1(1.43)
SXT,AMP,CIP	2(2.86)
SXT,CN,AMP,CIP	3(4.29)
SXT,AMP,S,CIP	5(7.1)
SXT,CN,AMP,CIP	1(1.43)
SXT,AMC,AMP,S	1(1.43)
SXT,AMC,AMP,CIP	2(2.86)
SXT,CN,AMP,S,CIP	5(7.14)
FOX,AMC,AMP,S,CIP	1(1.43)
SXT,FOX,AMC,AMP,CIP	1(1.43)
SXT,CAZ,AMP,S,CIP	1(1.43)
SXT,CAZ,AMC,AMP,CIP	1(1.43)
CN,AMC,AMP,S,CIP	1(1.43)
CAZ,CTX,AMC,AMP,CIP	1(1.43)
SXT,AMC,AMP,S,CIP	10(14.29)
SXT,CAZ,AMC,AMP,S,CIP	1(1.43)
SXT,AMC,AMP,S,CIP,IPM	1(1.43)
SXT,CAZ,CN,AMP,S,CIP	1(1.43)
SXT,CN,AMC,AMP,CIP	2(2.86)
SXT,CAZ,CTX,AMC,S,CIP	1(1.43)
SXT,CN,AMC,AMP,S,CIP	3(4.29)
SXT,FOX,AMC,AMP,S,CIP	1(1.43)
CAZ,AMC,AMP,S,CIP	1(1.43)
CAZ,CTX,AMC,AMP,S,CIP	1(1.43)
SXT,CAZ,CN,AMC,S,CIP	1(1.43)
FOX,CNAMC,AMP,S,CIP,IPM	2(2.46)
SXT,CAZ,CTX,AMC,AMP,S,CIP	4(5.71)
SXT,FOX,CAZ,CTX,AMC,AMP,S	2(2.86)
SXT,CAZ,CN,AMC,AMP,S,CIP	1(1.43)
SXT,CTX,CN,AMC,AMP,S,CIP,IPM	1(1.43)
SXT,CAZ,CTX,AMC,AMP,S,CIP,IPM	1(1.43)
SXT,CAZ,CTX,CN,AMC,AMP,S,CIP	3(4.29)
SXT,FOX,CAZ,CTX,AMC,AMP,S,CIP	1(1.43)
SXT,FOX,CAZ,CTX,CN,AMC,AMP,S	1(1.43)
SXT,FOX,CAZ,CTX,AMC,AQMP,S,CIP,IPM	1(1.43)
SXT,FOX,CAZ,CTX,CN,AMC,AMP,S,CIP	1(1.43)

Table 5. Multiple Antibiotics Resistance (MAR) Indices of *Escherichia coli* isolated from stool of diarrheic children attending selected hospitals in Lafia, Nigeria

No of Antibiotics Resistance (a)	No. of Antibiotics tested (b)	MAR index (a/b)	Frequency (%)
9	10	0.9	2 (2.86)
8	10	0.8	7 (10.00)
7	10	0.7	9 (12.86)
6	10	0.6	10 (14.29)
5	10	0.5	24 (34.29)
4	10	0.4	12 (17.14)
3	10	0.3	4 (5.71)
2	10	0.2	2 (2.86)

Table 6. Extended Spectrum β-lactamase production and genes in *Escherichia coli* jointly resistant to cefotaxime and ceftazidime from stool of diarrheic children attending selected hospitals in Lafia, Nigeria

Isolates	No. (%) ESBL producers	No. (%) ESBL genes (n= 14)		
		CTX-M	SHV	TEM
E. coli (n=21)	14(66.67)	6(42.86)	1(7.14)	7(50.00)

Table 7. Phenotypic and Genotypic confirmation of AmpC β-lactamase production in cefoxitin resistance *Escherichia coli* from stool of diarrheic children attending selected hospitals in Lafia, Nigeria

Isolates	No. (%) AmpC β-lactamase producers	No. (%) AmpC genes (n = 4) *MOXCITFOX*		
E. coli (11)	4 (36.36)	1(25.00)	2(50.00)	1(25.00)

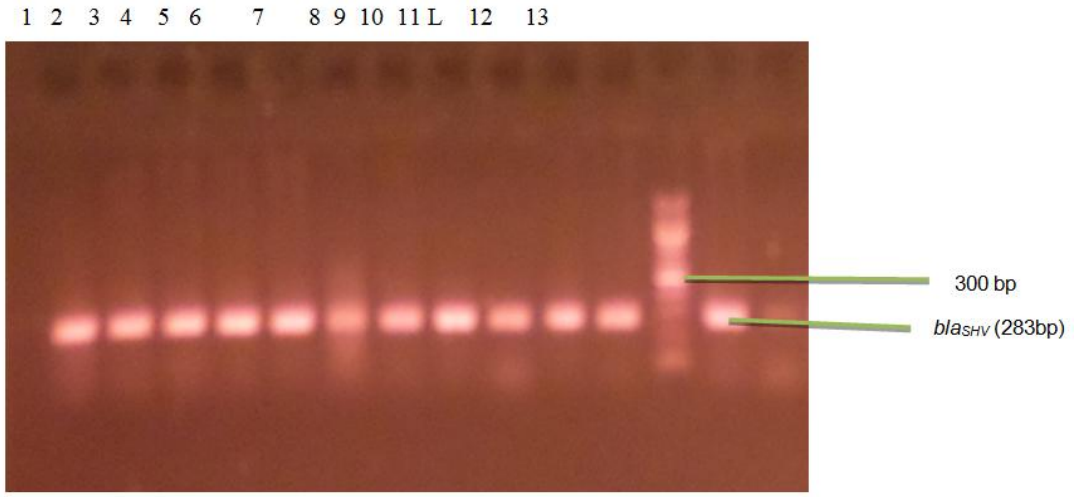

Plate 1. Agarose gel electrophoresis showing the blaSHV bands, lane 1-13 represent blaSHV gene bands while L represents the 1000bp molecular ladder

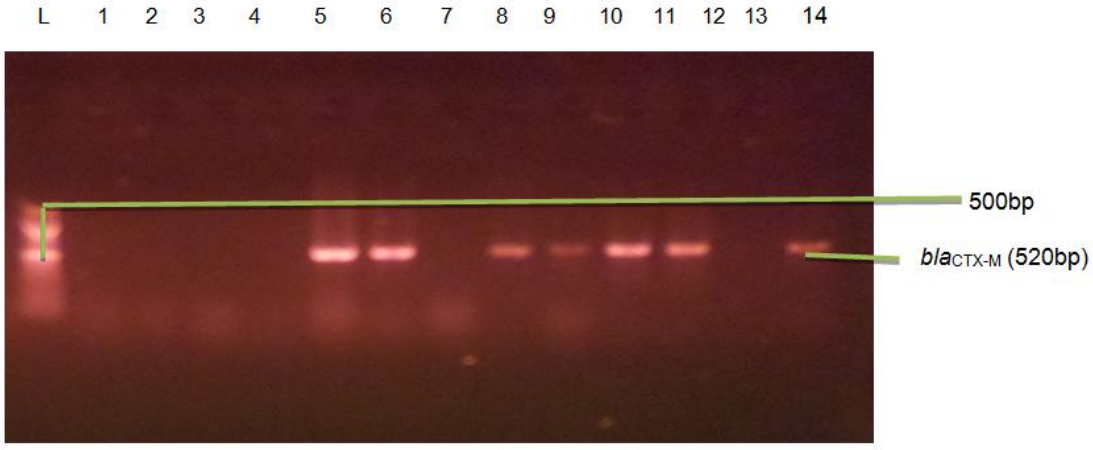

Plate 2. Agarose gel electrophoresis showing the blaCTX-M bands, lanes 5, 6, 8, 9, 10, 11, 12 and 14 represent blaCTX-M gene bands while L represents the 1000bp molecular ladder

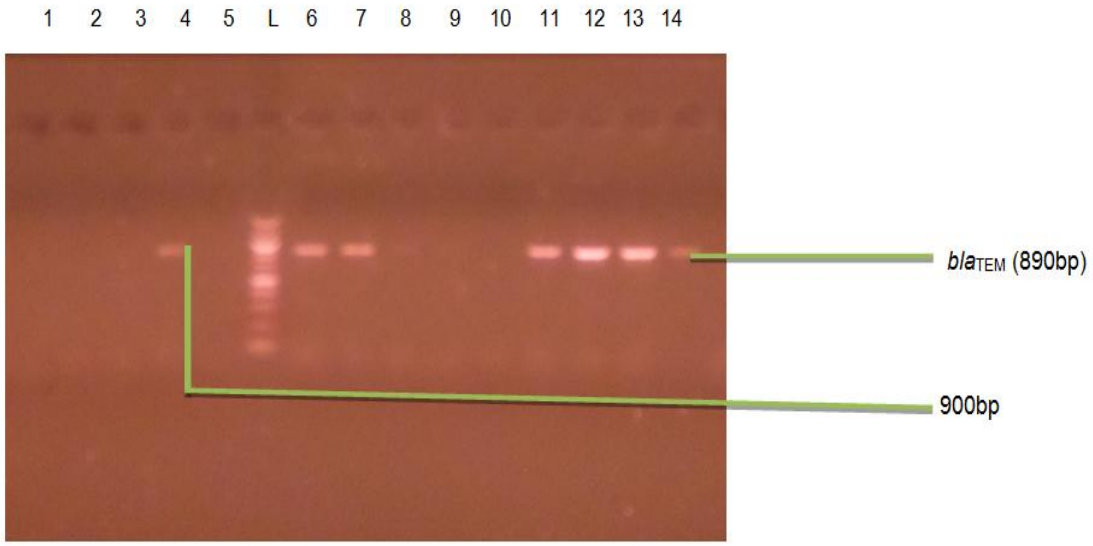

Plate 3. Agarose gel electrophoresis showing the blaTEM bands, lanes 4, 6, 7, 11, 12, 13 and 14 represent blaTEMgene bands while L represents the 1000bp molecular ladder

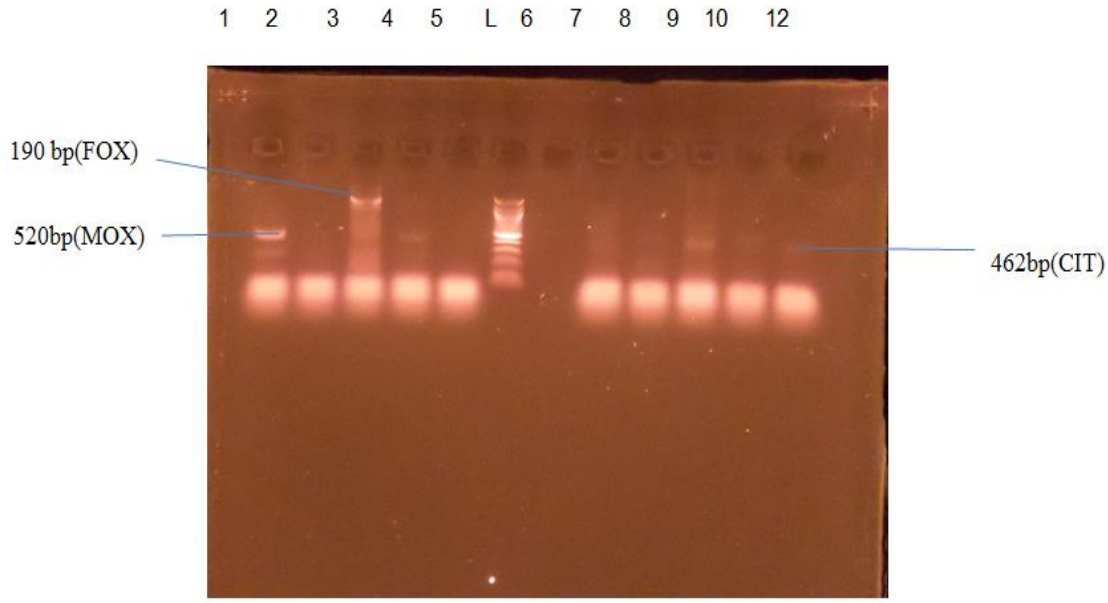

Plate 4. Agarose gel electrophoresis showing the MOX, FOX and CIT bands, while L represents the 1000bp molecular ladder

The study investigated the antibiotic susceptibility and presence of ESBL and AmpC genes in *E. coli* from stool of diarrheic children attending selected hospitals in Lafia, Nigeria. From this study we observed that the detection rate of *E. coli* in stool of children with diarrhea was 100% and this however is in agreement with available data elsewhere [2] that *E. coli* is one of the major causes of diarrhea in diseases in children. From this study we also observed that the *E. coli* isolates from stool of children with cases of diarrhea were more susceptible to imipenem, cefoxitin, cefotaxime, ceftazidine and gentamicin. The high susceptibility of *E. coli* isolates to

antibiotics mention was expected and this may be due to the fact that imipenem, cefoxitin, cefotaxime, ceftazidine, are very costly and not commonly prescribed and they are not likely to be abused and this however is in agreement with the early study reported [6] that antibiotics that are very costly are not likely to be abused. In another relation high susceptibility of *E. coli* isolates to gentamicin observed in this study may be due to the fact that drug is in injectable form and because of the pains and the discomfort of the injection they are not likely to be abused [39]. This finding is also in agreement with the study earlier reported [5]. The high susceptibility of *E. coli* isolates to ceftazidine and cefotaxime observed in this study is not in agreement with the study earlier reported [4,5] reported high level of resistance of *E. coli* to cefotaxime (92.6%), ceftazidine (97.2%). Also, 89.7% and 79.5% *E. coli* isolates resistance to cefotaxime and ceftazidine was reported [4]. The high susceptibility of *E. coli* isolates observed in this study to cefotaxime and ceftazidine is in agreement with the study earlier described [40]. The low susceptibility of *E coli* isolates from stool of children with cases of diarrhea to ampicillin, amoxicillin/clavulanic acid, ciprofloxacin, streptomycin, and sulphamethoxazole/trimethoprim and this however may be due to misused, abused and used of substances and antibiotics. The low susceptibility of *E. coli* isolates to antibiotics mention is in agreement with study earlier described [5]. The occurrence of ESBL and AmpC β-lactamase production in *E. coli* isolates from stool of children with diarrhea cases observed in this study was not surprising and this finding is also in agreement with other reports [4], [40]. The occurrence of ESBL producers in *E. coli* isolates jointly resistant to ceftazidine and cefotaxime observed in this study is higher than 26.3% reported [5] and 48.7% [4]. The occurrence of *SHV* genes was higher *TEM and CTX-M* and this finding seems to disagree with the study reported [10,14] reported that *CTX-M* gene is more prevalent in Enterobacteriaceae than *SHV* and *TEM* genes. The occurrence of *CTX-M, SHV* and *TEM* genes observed in this study is higher than that reported [38,39]. Although from our study we observed that not all the *E. coli* isolates jointly resistance to both cefotaxime and ceftazidime were ESBL producers and this finding is also in agreement with the study earlier reported [40].

The detection of AmpC β-lactamase production in *E. coli* isolates resistance to cefoxitin observed in this study was expected and this however is in agreement with the study earlier reported [4]. The percentage occurrence of AmpC β-lactamase producers in *E. coli* isolates in this study was higher than 7.2% reported [4,40].

4. CONCLUSION

The *E. coli* isolates were more susceptible to gentamicin, imipenem, ceftazidine, cefotaxime, and cefoxitin. Most of the isolates were confirmed ESBL and AmpC β-lactamase producers; ESBL genes (*SHV, CTX–M* and *TEM*) were detected; and *CIT* AmpC gene was more frequently detected than *MOX* and *FOX*. This suggest the need for further studies on molecular typing of ESBL and AmpC genes in *E. coli* isolates in the study area using a larger sample size.

CONSENT

All authors declare that written informed consent was obtained from the patients.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The ethical approval for this study was obtained from the ethical committees of the selected hospitals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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