South Asian Journal of Research in Microbiology

4(3): 1-10, 2019; Article no.SAJRM.51119

Phenotypic Detection of Extended Spectrum Beta-lactamase Resistance of *Escherichia coli* from Patients Attending Selected Healthcare Facilities in Nasarawa State, Nigeria

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Authors' contributions

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

Article Information

DOI: 10.9734/SAJRM/2019/v4i330108
Editor(s):
(1) Dr. Lidija Kozacinski, Professor, Department of Hygiene, Technology and Food Safety, Faculty of Veterinary Medicine, University of Zagreb, Croatia.
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(4) Rajesh Kumar Shah, DHSK College, India.
Complete Peer review History: http://www.sdiarticle3.com/review-history/51119

Original Research Article

Received 01 July 2019
Accepted 02 September 2019
Published 10 September 2019

ABSTRACT

Aims: This study investigated the phenotypic detection of extended spectrum beta-lactamase resistance of diarrheagenic *E. coli* isolated from diarrheic patients attending some major health facilities in Nasarawa State, Nigeria.
Place and Duration of Study: Department of Microbiology, Nasarawa State University, P.M.B 1022, Keffi, Nasarawa State, Nigeria; between December, 2017 to March, 2019.

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Methodology: A total of 207 confirmed *E. coli* isolates from loose stool samples of patients with suspected cases of diarrhea (69 from Federal Medical Centre Keffi [MCK] 69 from General Hospital Akwanga [GHA] and 69 from Dalhatu Araf Specialist Hospital Lafia [DASHL]) were included in this study.
Results: *E. coli* was isolated and identified using standard microbiological methods. The antibiotic susceptibility testing for the isolates was carried out and interpreted in accordance with Clinical and Laboratory Standards Institute protocol. Phenotypic detection of ESBL production in isolates resistant to ciprofloxacin, cefotaxime and ceftazidime) was carried out using double disc synergy test. The occurrence of *E. coli* was 100% in all the hospitals. Age groups 0-5 and 6-10 years have the highest occurrence than age group 35 – >45 years. Isolates from DASHL were more resistant to amoxicillin/clavulanic acid (86.9%), Streptomycin (75.0%) and sulphamethoxazole/trimethoprim (68.1%), isolates from FMCK were more resistant to amoxicillin/clavulanic acid (84.1%), sulphamethoxazole/trimethoprim (69.6%), isolates from GHA were more resistant to amoxicillin/clavulanic acid (85.5%) and sulphamethoxazole/trimethoprim (73.0%). Multiple antibiotic resistance (MAR) was observed with the order of occurrence: FMCK (98.6%) > DASHL (92.8%) > GHA (89.9%). The most common MAR index of 0.2 in DASHL was 0.4 (20.3%); FMCK was 0.4 (15.9%) and GHA was 0.3 (17.4%). The order of occurrence of classes of antibiotic resistance in *E. coli* isolates in DASHL was MDR (84.0%) > XDR(7.2%) > PDR and NMDR (4.3%); in FMCK was MDR (91.3%) > XDR(4.3%) > NMDR (2.9%) and PDR(1.4%); and in GHA was MDR (88.8%) > NMDR(5.8%) > XDR and PDR(2.9%). Detection rate of ESBL was 53.6% (30/207), distributed in relation to the location as DASHL (60.0%), FMCK (50.0%) and GHA (52.6%).
Conclusion: Most of the isolates from the study locations were antibiotic resistance. Further studies on molecular detection of ESBL, diversity and characterization of the *E. coli* into pathotypes are ongoing.

Keywords: Escherichia coli; extended spectrum beta-lactamase; antibiotic.

1. INTRODUCTION

Escherichia coli (E. coli) is the predominant facultative anaerobe and commensal microbiota in the mammalian gastrointestinal gut; and some strains can cause severe diarrhea illnesses in humans [1,2]. Various classes of antibiotics have been used to treat diarrhea caused by Diarrheagenic *E. coli* (DEC) and their continued usefulness is limited by the acquisition of resistance mechanisms in the bacteria [3]. The use of antibiotics has been reported to be one of the factors contributing to the emergence of bacterial resistance [4,5].

Antibiotic resistance is a global public health issue that is impacted by both human and nonhuman antimicrobial usage. The continuing emergence, development, and spread of pathogenic organisms that are resistant to antibiotics are a cause of increasing concern to health care practice [5].

Beta-lactam antibiotics have wide application in the treatment of infectious diseases; and constitute more than 50% of prescribed antibiotics [6]. Resistance mechanisms in bacteria against β-lactam antibiotics include: β-lactamase production and alteration of the penicillin-binding protein (PBP) target site [7]. The production of β-lactamases, which hydrolyzes the β-lactam ring, is among the most frequently encountered mechanisms in *E. coli* [7]. The phenotypic characteristics of ESBL facilitate the identification of ESBLs-producing organisms using routine laboratory tests such as double disk diffusion test or E-test. However this study investigated the extended spectrum beta-lactamase resistance of *E. coli* isolated from patients attending selected healthcare facilities in Nasarawa State, Nigeria.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 207 (69 from Federal Medical Centre Keffi, 69 from General Hospital Akwanga and 69 from Dalhatu Araf Specialist Hospital Lafia) loose stool samples of patients with suspected cases of diarrhea were randomly collected over a period of three (3) months using sterile container and transported using ice pack to Microbiology Laboratory, Nasarawa State University, Keffi for analysis. The consents of the suspected

diarrheic patients were obtained before sample collection.

2.2 Isolation and Identification of *Escherichia coli*

Escherichia coli were isolated from loose stool samples of patients with suspected cases of diarrhea: With the aid of a wire loop, the stool sample was streaked on MacConkey agar (Oxoid Ltd., Basingstoke, UK) plate and incubated at 37°C for 24 h. Pinkish colonies that grew on MacConkey agar were further inoculated on Eosin Methylene Blue agar (Oxoid Ltd., Basingstoke, UK) and incubated at 37°C for 24 h. Greenish metallic sheen colonies that grew on the Eosin Methylene Blue agar plate were selected as presumptive *E. coli* based on method already described [8]. Presumptive *E. coli* were identified by microscopical (Gram stain) and minimum biochemical tests for *E. coli* identification namely "IMViC" (Indole, Methyl red, Voges-Proskauer, Citrate). Indole positive, Methyl red positive, Voges-Proskauer negative and citrate negative isolates were further confirmed as *E. coli* using a commercial kit B004HI^{TW} (HiMedia Ltd, India) in accordance with the manufacturer's instructions. The bacterium was stored in the refrigerator at 4°C on nutrient agar slants and reactivated by sub-culturing on MacConkey agar and used in the further experiments.

2.3 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of the confirmed *E. coli* isolates was carried out as earlier described [9]. Briefly, (3) pure colonies of isolated *E. coli* from loose stool samples of patients with suspected cases of diarrhea was inoculated in to 5 ml sterile 0.85% (w/v) NaCl (BDH Chemicals Ltd., England) and the turbidity of the bacteria suspension was adjusted to the turbidity equivalent to 0.5 McFarland's standard. The McFarland's standard was prepared as follows; 0.5 ml of 1.172% (w/v) $BaCl_2.2H_2O$ (BDH Chemicals Ltd., England) was added into 99.5 ml of 1% (w/v) H_2SO_4 (BDH Chemicals Ltd., England).

A sterile swab stick was soaked in the standardized bacteria suspension and streaked on Mueller- Hinton agar (Oxoid Ltd., Basingstoke, UK) plates and the antibiotic discs (Oxoid Ltd., Basingstoke, UK) were aseptically placed at the center of the plates and allowed to stand for 1 h for pre-diffusion. The plates were placed in an incubator (Model 12-140E, Quincy Lab Inc.) set at 37ºC for 24 h. The diameter zone of inhibition in millimeter was measured and the result of the susceptibility was interpreted in accordance with the susceptibility break point earlier described [10].

2.4 Extended Spectrum β-Lactamase (ESBL) Production Test

The confirmatory test for Extended Spectrum β-Lactamase (ESBLs) Production against *E. coli* isolates jointly resistance to cefotaxime, ceftazidime and ciprofloxacin was carried using two-disc method earlier described [9]. Briefly, 10^8 CFU *E. coli* suspensions jointly resistance to cefotaxime, ceftazidime and ciprofloxacin were streaked on sterilized Mueller Hinton agar plates and Amoxicillin-clavulanic acid (30µg) disc was placed in the centre of the plate and cefotaxime (30µg), cefpodoxime (10µg), ceftaxidime (30µg) and ceftriaxone (30µg) disks were placed 15mm (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 disks in comparism 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 test strain.

3. RESULTS AND DISCUSSION

3.1 Isolation and Identification of *Escherichia coli*

The cultural, morphological and biochemical finger print of *E. coli* isolated from stool of suspected diarrheic patients in Dalhatu Araf Specialist Hospital, Lafia (DASHL), Federal Medical Centre, Keffi (FMCK) and General Hospital, Keffi, Nigeria is as shown in Table 1. Pinkish colony on MCA which grew with greenish metallic sheen on EMB agar was Gram negative rod and had biochemical reactions namely: indole-positive, methyl red-positive, Voges-Proskauer-negative, citrate-negative, ONPG-positive, among others indicated *E. coli*.

3.2 Occurrence of *Escherichia coli*

The occurrence of *Escherichia coli* from stool of patients with suspected cases of diarrhea in the selected health facilities in Nasarawa State, Nigeria is as shown in Fig. 1. All (100%) stool samples collected (207) harbored *E. coli* in all the hospitals. The occurrence in relation to age and gender is distributed as shown in Tables 2 and 3 respectively.

Table 1. Cultural, morphological and biochemical characteristics of *Escherichia coli* from stool of patients with suspected cases of diarrhea in Nasarawa State

Cultural characteristics	Morphological characteristics		Biochemical characteristics												Inference
	Gram reaction	Morphology	IND	MR	VP	CT	TDA	ONPG	LYS	ORN	UR	NT	H_2S	MAL	
Pinkish colonies on MCA and Greenish metallic sheen on EMB agar	-	Rod	+	+	-	-	-	+	+	+	-	+	-	-	*E. coli*

+ = Positive, - = negative, IND = Indole; MR = Methyl red; Vp = Voges-Proskauer, CT = Citrate, LYS = Lysine, ORN = Ornithine; ONPG = Ortho-Nitrophenyl-β-galactosidase, UR = Urease, NT = Nitrate, H2S = Hydrogen Sulphide, Mal = Malonate, TDA = Phenylalanine deaminas

Table 2. Occurrence of *Escherichia coli* in the stool of patients in relation to age

Age (Years)	No. of samples			No. (%) *Escherichia coli*		
	DASHL	FMCK	GHA	DASHL	FMCK	GHA
0-5	28	23	29	28(100.0)	23(100.0)	29(100.0)
6-10	17	18	16	17(100.0)	18(100.0)	16(100.0)
11-15	5	6	5	5(100.0)	6(100.0)	5(100.0)
16-20	8	6	1	8(100.0)	6(100.0)	1(100.0)
21-25	4.0	0.0	2.0	4.0(100)	0.0(0.0)	2.0(100)
26-30	6.0	3.0	5.0	6.0(100)	3.0(100)	5.0(100)
31-35	0.0	0.0	6.0	0.0(0.0)	0.0(0.0)	6.0(100)
36-40	0.0	1.0	0.0	0.0(0.0)	1.0(100)	0.0(0.0)
41-45	0.0	5.0	0.0	0.0(0.0)	5.0(100)	0.0(0.0)
>45	1.0	7.0	5.0	1.0(100)	7.0(100)	5.0(100)
Total	**69**	**69**	**69**	**69(100)**	**69(100)**	**69(100)**

DASHL= Dalhatu Araf Specialist Hospital, Lafia; FMCK= Federal Medical Centre Keffi; GHA= General Hospital, Akwanga; No.= Number, %= Percentage

Table 3. Occurrence of *Escherichia coli* in the stool of patients in relation to gender

Gender	No. of sample			No. (%) *E. coli*		
	DASHL	FMCK	GHA	DASHL	FMCK	GHA
Male	27	33	29	27(100.0)	33(100.0)	29(100.0)
Female	42	36	40	42(100.0)	36(100.0)	40(100.0)
Total	**69**	**69**	**69**	**69(100.0)**	**69(100.0)**	**69(100.0)**

DASHL= Dalhatu Araf Specialist Hospital Lafia; FMCK= Federal Medical Centre, Keffi; GHA= General Hospital, Akwanga; No. = Number; % = Percentage

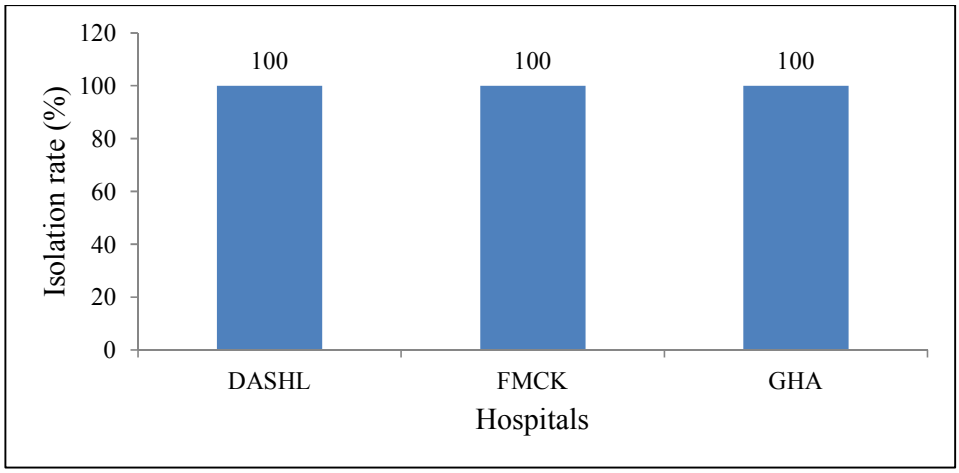

Fig. 1. Occurrence of *Escherichia coli* from stool of patients with suspected cases of diarrhea in Nasarawa state in relation to hospital (DASHL= Dalhatu Araf Specialist Hospital Lafia, FMCK = Federal Medical Centre Keffi, GHA= General Hospital Akwanga)

3.3 Antimicrobial Resistance Profile of *Escherichia coli*

The antimicrobial resistance profile of the *E. coli* isolated from the patients is as shown in Table 4. Isolates from DASH were more resistant to Amoxicillin/Clavulanic acid (86.9%), Streptomycin (75.0%) and Sulphamethozazole/ Trimethoprim (68.1%); but less resistant to Imipenem (11.6%), Cefotaxime (13.0%) and Ceftazidime (20.3%). Similarly, isolate from FMCK were more resistant to Amoxicillin/ Clavulanic acid (84.1%), Sulphamethozazole/ Trimethoprim (69.6%), but less resistant to Imipenem (72.0%), Gentamicin (24.6%) and Ceftazidine (26.1%). For GHA, the isolates were more resistant to Amoxicillin/Clavulanic acid (85.5%) and Sulphamethoxazole/Trimethoprim (73.0%), but less resistant to cefotaxime (15.9%), Ceftazidine (18.8%) and Gentamicin (21.7%).

3.3.1 Antimicrobial resistance phenotypes

The antimicrobial resistance phenotypes in the isolates from the patients are as shown in Table 5. The commonest phenotype in DASHL was AMC-S-SXT-CTX-CAZ-FOX-CIP-AMP (7.2%); FMCK was S-SXT-CTX-CAZ-AMP-AMC-S-SXT-CTX-CAZ-IPM-CIP-AMP (5.8%); and GHA were S-SXT-CTX-CN-AMP-S-SXT-CTX-CAZ-FOX-AMP and AMC-S-SXT-CTX-CAZ-IPM-AMP (5.8%).

Table 4. Antimicrobial resistance profile of *Escherichia coli* from stool of patients with suspected cases of diarrhea in Nasarawa State

Antibiotics	Disc content (µg)	No. (%) resistance		
		DASHL (n=69)	FMCK (n=69)	GHA (n=69)
Amoxicillin/Clavulanic acid (AMC)	10/20	60(86.9)	58(84.1)	59(85.5)
Ampicillin (AMP)	10	52(75.4)	47(68.1)	44(63.8)
Cefoxitin (FOX)	30	39(56.5)	37(53.6)	30(43.5)
Cefotaxime (CTX)	30	9(13.0)	19(27.5)	11(15.9)
Ceftazidime (CAZ)	30	14(20.3)	18(26.1)	13(18.8)
Gentamicin (CN)	10	22(31.9)	17(24.6)	15(21.7)
Ciprofloxacin (CIP)	5	23(33.3)	28(40.5)	20(28.9)
Imipenem (IPM)	30	8(11.6)	5(7.2)	19(27.5)
Streptomycin (S)	30	52(75.4)	46(66.7)	30(43.5)
Sulphamethoxazole/Trimethoprim (SXT)	25	47(68.1)	48(69.6)	51(73.9)

DASHL= Dalhatu Araf Specialist Hospital Lafia, FMCK= Federal Medical Centre Keffi, GHA= General Hospital Akwanga, No. = Number, % = Percentage

Table 5. Antimicrobial resistance phenotypes of *Escherichia coli* from the stool of the patients

Antibiotic resistance phenotypes	Frequency (%)		
	DASHL(n=69)	FMCK(n=69)	GHA(n=69)
SXT,FOX,CN,AMP	1(1.4)	2(2.9)	1(1.4)
SXT,FOX,AMP	3(4.3)	1(1.4)	1(1.4)
SXT,CTX,FOX,AMP	2(2.9)	0(0.0)	1(1.4)
S,SXT,FOX,AMP	1(1.4)	2(2.9)	1(1.4)
S,SXT,CTX,FOX,IPM,AMP	2(2.9)	1(1.4)	1(1.4)
S,SXT,CTX,FOX,CN,IPM,AMP	1(1.4)	2(2.9)	0(0.0)
S,SXT,CTX,FOX,CIP,AMP	2(2.9)	1(1.4)	3(4.3)
S,SXT,CTX,CN,CIP,AMP	1(1.4)	3(4.3)	1(1.4)
S,SXT,CTX,CN,AMP	4(5.8)	2(2.9)	4(5.8)
S,SXT,CTX,CAZ,FOX,IPM,CIP,AMP	1(1.4)	1(1.4)	1(1.4)
S,SXT,CTX,CAZ,FOX,IMP	2(2.9)	1(1.4)	1(1.4)
S,SXT,CTX,CAZ,FOX,CN,IPM,CIP,AMP	2(2.9)	3(4.3)	1(1.4)
S,SXT,CTX,CAZ,FOX,AMP	1(1.4)	1(1.4)	4(5.8)
S,SXT,CTX,CAZ,FOX	1(1.4)	1(1.4)	2(2.9)
S,SXT,CTX,CAZ,CN,CIP,AMP	1(1.4)	3(4.3)	2(2.9)
S,SXT,CTX,CAZ,CN,AMP	2(2.9)	1(1.4)	2(2.9)
S,SXT,CTX,CAZ,CIP,AMP	0(0.0)	1(1.4)	1(1.4)
S,SXT,CTX,CAZ,AMP	1(1.4)	4(5.8)	2(2.9)
S,SXT,CTX,AMP	2(2.9)	1(1.4)	3(4.3)
S,SXT,CIP,AMP	1(1.4)	2(2.9)	3(4.3)
S,SXT,CAZ,FOX,CIP,AMP	3(4.3)	2(2.9)	2(2.9)
S,FOX,AMP	1(1.4)	1(1.4)	3(4.3)
S,CTX,CAZ,FOX,CN,IPM,AMP	1(1.4)	2(2.9)	1(1.4)
S,CAZ,FOX,AMP	2(2.9)	1(1.4)	1(1.4)
AMC,SXT,CTX,CAZ,CN,IPM,AMP	0(0.0)	3(4.3)	1(1.4)
AMC,SXT,CTX,CAZ,CIP,AMP	1(1.4)	1(1.4)	2(2.9)
AMC,S,SXT,CTX,FOX,CN,CIP,AMP	3(4.3)	2(2.9)	1(1.4)
AMC,S,SXT,CTX,FOX,AMP	1(1.4)	1(1.4)	2(2.9)
AMC,S,SXT,CTX,CN,CIP,AMP	2(2.9)	1(1.4)	3(4.3)
AMC,S,SXT,CTX,CAZ,IPM,CIP,AMP	1(1.4)	4(5.8)	1(1.4)
AMC,S,SXT,CTX,CAZ,IPM,AMP	3(4.3)	1(1.4)	4(5.8)
AMC,S,SXT,CTX,CAZ,FOX,IPM,AMP	1(1.4)	1(1.4)	2(2.9)
AMC,S,SXT,CTX,CAZ,FOX,CN,IPM,CIP,AMP	2(2.9)	2(2.9)	0(0.0)
AMC,S,SXT,CTX,CAZ,FOX,CN,IPM,AMP	2(2.9)	2(2.9)	2(2.9)
AMC,S,SXT,CTX,CAZ,FOX,CIP,AMP	5(7.2)	1(1.4)	3(4.3)
AMC,S,SXT,CTX,CAZ,FOX,AMP	1(1.4)	1(1.4)	1(1.4)
AMC,S,SXT,CTX,CAZ,CN,CIP,AMP	2(2.9)	1(1.4)	0(0.0)
AMC,S,SXT,CIP,AMP	1(1.4)	1(1.4)	1(1.4)
AMC,S,SXT,AMP	1(1.4)	4(5.8)	1(1.4)
AMC,S,CTX,FOX,IPM,AMP	1(1.4)	1(1.4)	2(2.9)
AMC,S,CTX,CAZ,FOX,CN,IPM,CIP,AMP	3(4.3)	1(1.4)	0(0.0)
AMC,S,CTX,CAZ,FOX,CN,CIP,AMP	1(1.4)	2(2.9)	1(1.4)

AMP = Ampicillin; AMC = Amoxicillin/Clavulanic acid; S = Streptomycin; CN = Gentamicin; SXT = Cotrimoxazole; CAZ = Ceftazidime; CTX = Cefotaxime; FOX = Cefoxitin; CIP = Ciprofloxacin; IPM = Imipenem, DASHL= Dalhatu Araf Specialist Hospital Lafia, FMCK= Federal Medical Centre Keffi, GHA= General Hospital Akwanga, No. = Number, %= Percentage

3.3.2 Multiple Antibiotic Resistance (MAR) index

Multiple antibiotic Resistance is defined here as resistance to two or more of the antibiotics tested. The occurrence of MAR isolates is as shown in Table 6. The order of occurrence is: FMCK (98.6%) > DASHL (92.8%) > GHA (89.9%). The difference in the multiple antibiotic resistances of the isolates in relation to their location was statistically insignificant ($p>0.05$).

The MAR indices of the isolates from DASHL, FMCK, and GHA are as given in Table 7. All the isolates in DASHL, FMCK, and GHA were MAR isolates with MAR index of 0.2 and the most common MAR index in DASHL was 0.4 (20.3%), FMCK was 0.4(15.9%) while GHA, the common MAR index was 0.3 (17.4%) as shown in Table 7.

Table 6. Occurrence of multiple antibiotic resistant *Escherichia coli* from the stool of the patients

Hospital	No. (%) MAR isolates (n= 69)
DASHL	64(92.8)
FMCK	68(98.6)
GHA	62(89.9)

DASHL= Dalhatu Araf Specialist Hospital, Lafia; FMCK= Federal Medical Centre, Keffi; GHA= General Hospital, Akwanga

3.3.3 Classes of antimicrobial resistance

The *E. coli* isolates from DASHL, FMCK and GHA were classified into different categories of antibiotic resistance namely; Multi-drug resistance (MDR), Extensive-drug resistance (XDR) and Pandrug resistance (PDR) as shown in Table 8. The order of occurrence of categories of antibiotic resistance in *E. coli* isolates in DASHL were, MDR (84.0%) > XDR(7.2%) > PDR and NMDR (4.3%), FMCK were; MDR (91.3%) > XDR(4.3%) > NMDR (2.9%) and PDR(1.4%) while in GHA, the order of occurrence of the classes of antimicrobial resistance was MDR (88.8%) > NMDR(5.8%) > XDR and PDR(2.9%) as shown in Table 8.

The difference in the multiple antibiotic resistant of the isolates in relation to location was statistically insignificant (p>0.05).

3.4 Phenotypic Detection of Extended Spectrum Beta-lactamase

The phenotypic detection of ESBL production in *E. coli* isolates jointly resistant to third generation cephalosporins (cefotaxime and/or ceftazidime) and ciprofloxacin is as shown in Table 9. Out of 56 isolates jointly resistant to cefotaxime and/or ceftazidime and ciprofloxacin from DASHL, FMCK and GHA, 53.6% (30/56) were ESBL producers, distributed in relation to the hospitals as follows: DASHL (60.0%), FMCK (50.0%) and GHA (52.6%).

The number of infections due to ESBL *E. coli* is increasing, especially in African countries [9]. This study evaluated the extended spectrum beta-lactamase resistance of *Escherichia coli* from patients attending selected healthcare facilities in Nasarawa State, Nigeria. The isolation of *E. coli* in all stool samples (100%) as shown in Fig. 1 is in agreement with studies reported [11,12,13]; and confirms the fact that *E. coli* is a common bacteria isolated in stool of human [14].

The occurrence of *E. coli* from the stool of patients with suspected cases of diarrhea in the study was an indication that the *E. coli* is among the pathogens that may be responsible for diarrheic infection and this is in agreement with the study earlier reported [14,15,16].

Table 7. Multiple Antibiotic Resistance (MAR) index of *Escherichia coli* from the stool of the patients

No. of antibiotic resistance to (a)	No. of antibiotic tested (b)	MAR index (a/b)	No. (%) MAR isolates		
			DASHL (n= 64)	FMCK (n= 68)	GHA (n= 62)
10	10	1.0	4(6.3)	6(8.8)	2(3.2)
9	10	0.9	8(12.5)	8(11.8)	8(12.9)
8	10	0.8	3(4.7)	8(11.8)	6(9.7)
7	10	0.7	5(7.8)	9(13.2)	9(14.5)
6	10	0.6	10(15.6)	5(7.4)	9(14.5)
5	10	0.5	7(10.9)	10(14.7)	6(9.7)
4	10	0.4	14(21.8)	11(16.2)	7(11.3)
3	10	0.3	2(3.1)	7(10.3)	12(19.4)
2	10	0.2	11(17.2)	4(5.9)	3(4.8)

DASHL= Dalhatu Araf Specialist Hospital, Lafia; FMCK= Federal Medical Centre, Keffi; GHA = General Hospital Akwanga; No. = Number; % = Percentage

Age group 0-5 and 6-10 years have the highest number of samples collected while age group 35 – >45 have the least number collected as shown in Table 2. However, it was observed that between age groups the presence of the bacterial isolates with age group 0-5 and 6-10 years having the highest occurrence of bacterial isolates and the least is age group 35 – >45. This follows the same trend with a study done in Abuja by [11,17], which shows that diarrhea is statistically associated with age and majority of the cases occurring in children between 7 months and 2 years of age. The reason for high incidence of bacteria isolates in age group 0-5 and 6-10 years could be due to the fact that children within this age group on their own cannot differentiate between what to eat and what not to eat; they have not learnt the rudiment of adherence to aseptic or hygienic practice; they can barely express themselves. Most diarrhea occur during the first 2 years of life due to combined effects of declining levels of maternally acquired antibodies, the lack of active immunity in the infant, the introduction of food that may be contaminated with faecal bacteria and direct contact with human or animals faeces when the infant start to grow [11,17]. Most enteric pathogens stimulate at least partial immunity against repeated infection or illness, which helps to explain the declining incidence of disease in older children and adults.

The isolates from all the study locations were resistance to Amoxicillin/Clavulanic acid, Streptomycin and Sulphamethozazole/ Trimethoprim but less resistance to Imipenem Gentamicin and Ceftazidine and is in tandem with similar study [7,18,19] observed high percentage of drug resistance against ceftazidime (100%), cefotaxime (100%), cefepime (100%), ofloxacin (97.56%), amoxicillin/clavulanic acid (97.56%) and norfloxacin (85.36%) as shown in Table 4.

The occurrence of MAR isolates observed in this study was expected and is in a tandem with similar study reported [7,20]. The resistance of isolates to these antibiotics may be due to antibiotic misuses, ineffective empiric antibiotic therapy, poor dosing regimen of antimicrobial agent, and prolong therapy of infection caused by this organism may also likely being the reason for the resistance of antibiotics mentioned [20]. The occurrence of MDR resistance isolates in the all the study locations was not different from the study earlier reported [7,21], that MDR *E. coli* responsible for diarrheic infection difficult to treat with antibiotics. The percentage occurrence of MDR isolates observed in this study was 92.8% in DASHL, 98.6% in FMCK and 89.9% in GHA higher than 64.9% reported [21] as shown in Table 8. The occurrence of XDR and PDR resistant isolates observed in this study was also similar with the study earlier described [20,21]. The occurrence of ESBL producers in *E. coli* isolates jointly resistant to ceftazidine and cefotaxime observed in this study was higher than 22.2% reported [3,20,21], 26.3% reported [7], 48.7% reported, 16.5% reported by Ahmed et al. [22].

Table 8. Classes of antimicrobial resistance in *Escherichia coli* from the stool of the patients

Classes of antimicrobial resistance	No. (%) *E. coli*		
	DASHL (n=69)	FMCK (n=69)	GHA (n=69)
NMDR	3(4.3)	2(2.9)	4(5.8)
MDR	58(84.0)	63(91.3)	61(88.8)
XDR	5(7.2)	3(4.3)	2(2.9)
PDR	3(4.3)	1(1.4)	2(2.9)

NMDR= Non-multi-drug resistance; MDR= Multi-drug resistance (non-susceptible to ≥1 agent in ≥3 antimicrobial categories); XDR = Extensive drug resistance (non-susceptible to ≥1 agent in all but ≤2 antimicrobial categories); PDR=Pan drug resistance (non-susceptible to all antimicrobial listed) DASHL= Dalhatu Araf Specialist Hospital Lafia; FMCK= Federal Medical Centre, Keffi; GHA= General Hospital, Akwanga. No. = Number, %= Percentage

Table 9. Phenotypic detection of extended spectrum beta-lactamase production in the *Escherichia coli* from the stool of the patients

Isolates	No. (%) cefotaxime/ceftazidime resistant isolates	No. (%) ESBL producers
DASHL	15	9(60.0)
FMCK	22	11(50.0)
GHA	19	10(52.6)
Total	**56**	**30(53.6)**

DASHL= Dalhatu Araf Specialist Hospital, Lafia; FMCK= Federal Medical Centre, Keffi; GHA= General Hospital, Akwanga; No.= Number, %= Percentage

4. CONCLUSION

Most of the isolates from the study locations were multidrug resistance and ESBL resistant. The resistance of the isolates to antibiotics may be due to antibiotic misuses, ineffective empiric antibiotic therapy, poor dosing regimen of antimicrobial agent, and prolong therapy of infection caused by the *E. coli.*

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.

ACKNOWLEDGEMENTS

Authors are grateful to the management and staff of Nasarawa State Ministry of Health and Hospital Management Board, Lafia, Nigeria for approving the use of their health care facilities for the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle3.com/review-history/51119