Original Research Article

Antimicrobialresistance and phenotypic detection of extended spectrum beta-lactamase in *Escherichia coli* from children with cases of diarrhoea in Nasarawa-south, Nasarawa State, Nigeria

ABSTRACT

Aims: This study investigates the antimicrobial resistance and phenotypic detection of extended spectrum beta-lactamase in *Escherichia coli* from children with cases of diarrhoea in Nasarawa-south, Nasarawa State, Nigeria

Place and Duration of Study: Nasarawa South, Nigeria, in 2023.

Methodology:A total of 251 non-duplicate *E. coli* isolates were collected from Dalhatu Araf Specialist Hospital Lafia (DASHL), General Hospitals Awe (GHA), General Hospital Doma (GHD), General Hospital Obi (GHO) and General Hospital Keana (GHK); and confirmed using standard microbiological methods. Antimicrobial susceptibility testing and phenotypic detection of ESBL production in the isolates were carried out using disc diffusion methods. **Results:**The isolates were highly resistant (74.0-100.0%) to ampicillin, amoxicillin/clavulanicacid and sulfamethoxazole/trimethoprim, ceftriaxone and streptomycin in all the selected hospitals, but less resistant (0-7.8%) to Cefotaxime and Ceftazidime. All the antibiotic resistant isolates were multiple antibiotic resistant (MAR) with MAR indices above 0.2; and more than 90.0% in all hospitals were multidrug resistant (MDR) isolates. The occurrence of ESBL producing isolates was highest in DASHL (7.8%), GHK and GHD (2.0%) but none of the isolates from GHA were ESBL producers.**Conclusion:**Extended spectrum third generation cephalosporins cefotaxime and ceftazidime were very effective against the isolates, even though most of the isolates were multidrug resistance. The molecular diversity of the ESBL producing isolates is being investigated.

Keywords:*Escherichia coli, Antimicrobial, diarrhoea, Extended-spectrum Beta-lactamase*

1. INTRODUCTION

Acute diarrheal diseases are an important health problem among children and are among the commonest deaths among infants and children in developing countries [1]. About 70% of the cases of acute diarrheal illness occur in the first 5 years of life [2]. Bacteria and viruses are responsible for over 20.0% of fatal cases of gastroenteritis in children. Among the bacterial pathogens, diarrheagenic *E. coli* is the most common cause of acute diarrhea [3, 4, 2]. Diarrheagenic *E. coli* (DEC) is a significant cause of gastroenteritis (diarrhea) and a major public health threat [2], causing over 500,000 deaths per year worldwide, especially in children [1].

Antimicrobials such as β-lactams and fluoroquinolones are commonly used as therapeutic options for infections caused by Gram-negative Enterobacteriaceae in both human and veterinary medicine [5]. Antimicrobial resistance in enteric bacteria is a serious global problem associated with prolonged hospitalization, increased morbidity and mortality, and high treatment costs [6, 7]. Few studies in Africa have reported over 50% of diarrheagenic *E. coli* resistance to groups of antimicrobials such as penicillins, third-generation cephalosporins, fluoroquinolone, aminoglycoside, carbapenem, and other

classes (8, 9), and several studies have reported less than 40% resistance of suspected diarrheagenic *E. coli* to third-generation cephalosporins, fluoroquinolone, aminoglycoside, and carbapenem [4, 10, 11].
The emergence of ESBL strains of diarrheagenic *E. coli* is a serious challenge to clinicians because beta-lactam antibiotics are widely used as therapeutic options for the treatment of *E. coli* infections [1]. The resistance to beta-lactam antibiotics such as third-generation cephalosporins is due to the production of ESBL that inactivates the beta-lactam ring of the drugs [12]. Over the years, the burden of ESBL-producing DEC has increased, ranging from 22.2-74.0% in Nigeria [1, 4, 2, 13]. Few studies from 2019-2020 in the Tertiary Health care facilities and secondary facilities in the Northern senatorial district have reported the prevalence of ESBL-producing *E. coli* from the stool of suspected diarrheic patients of all age groups [4, 10]. The focus of the present study is on the antimicrobial resistance profile and phenotypic detection of ESBL-producing *E. coli* from the stool of suspected cases of diarrhea among children of ≤ 5 years in Nasarawa South Senatorial district, Nasarawa State, Nigeria.

2. MATERIAL AND METHODS

2.1 Collection of *Escherichia coli*

A total of 251 (50 from each of the facilities) non-duplicate presumptive E. coli isolates stool of children with suspected diarrhea were collected using Nutrient agar (NA: Oxoid Ltd, UK) slants and transported using an ice pack to Microbiology Laboratory, Nasarawa State University, Keffi for analysis.
Confirmation of *Escherichia coli*

2.2 Gram Staining

The Gram staining of the presumptive organism was carried out as earlier described by Cheesbrough (2006). Briefly, a smear of three (3) pure colonies of the organism was made on a drop of normal saline on a clean grease free slide and allowed to air-dry. The slide was passed twice through the flame to heat fix, then flooded with crystal violet solution for 30 sec and rinsed under slow running tap water. The washed slide was decolorized briefly with acetone, then immediately rinsed under slow running tap water and counter-stained with safranin solution for 60 sec. The slide was rinsed under slow running tap water, then allowed to air dry and then examined under x100 oil immersion objective.

2.2.1 Commercial Biochemical Kit (KB003 H125TM) Identification of *Escherichia coli*

The presumptive *Escherichia coli* isolates that was Gram negative, rod shape was confirmed using KB003 H125TM Kit following the manufacturer's instruction as follows. Following purification, 2 pure colonies of suspected isolates from NA plate were transfer to 5 ml of sterile normal saline in a tube to prepare a suspension and the turbidity of the suspension was adjusted to the turbidity equivalent to the turbidity of 0.5 McFarland standard.
The kit was aseptically opened by sealing off the sealing foil and 50 µl of the adjusted suspension was inoculated into each well of the kit and it was sealed back using the sealing foil and incubated at 37ºC for 24 h. After incubation, 3 drops of reagent R036 and 1 drop of reagent R015 was added to well No 5; 2 drops of reagent R009 was added to well No. 6; 3 drops of reagent R029 and 1 drop of reagent R030 was added to well No. 9; 1 drops of reagent 1007 was added to well No. 10 and finally 1 drops of reagent R008 was added to well No. 11. The results were read and interpreted as per the standard given in the identification index.

2.2.2 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing of the bacterial isolates was carried out as earlier described by Clinical and Laboratory Standards Institute (CLSI, 2021). 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 bacteria suspension will be adjusted to the turbidity equivalent to 0.5 McFarland standard. The McFarland's standard was prepared as follows: 0.5 ml of 1.172% (w/v) BaCl2.2H2O was added into 99.5 ml of 1% (w/v) H2SO4.
A sterile swab stick was soaked in standardized bacteria suspension and streaked on Mueller-Hinton agar (MHA: Oxoid Ltd, UK) plates and the antibiotic discs namely; Amoxicillin (AMX) 10 µg, Amoxicillin-Clavulanic acid (AMC) 30 µg, Cefotaxime (CTX) 30 µg, Imipenem (IMP) 30 µg,

Ceftazidime (CAZ) 30 µg, Ceftriaxone (CRO) 30 µg, Ciprofloxacin (CIP) 5 µg, Sulfamethoxazole/Trimethoprim (SXT) 25 µg, Gentamicin (CN) 10 µg and Streptomycin (S) 10 µg all from Oxoid Ltd, UK were aseptically placed at the center of the plates and allowed to stand for 1 h for pre-diffusion. The plates were incubated at 37ºC for 24 h. The diameter zone of inhibition in millimeters was measured and the result was interpreted in accordance with the susceptibility breakpoint earlier described by Clinical and Laboratory Standards Institute (CLSI, 2021).

2.2.3 Determination of Multiple Antibiotic Resistance (MAR) Index

The MAR index of the isolates was determined using the formula [14]: MAR Index= Number of antibiotics isolate is resistant to/ Number of antibiotics tested

2.2.4 Classification of Antibiotic Resistance

Antibiotic resistance in the isolates was classified into multidrug resistance (MDR: non-susceptible to ≥1 agent in ≥3 antimicrobial categories); extensive drug resistance (XDR: non-susceptible to ≥1 agent in all but ≤2 antimicrobial categories); pan drug resistance (PDR: non-susceptible to all antimicrobial listed) [15].

2.2.5 Phenotypic Detection of Extended Spectrum β-Lactamase Production

Phenotypic detection of Extended-Spectrum Beta-Lactamase (ESBL) production was conducted on isolates resistant to third-generation cephalosporins using the double disk synergy test (DDST) in accordance with CLSI guidelines. Ceftazidime (30 µg) and cefpodoxime (30 µg) antibiotic disks were positioned on Mueller-Hinton agar (MHA: Oxoid Ltd, UK) plates, spaced 16 mm apart center to center from a central amoxicillin-clavulanic acid disk (20/10 µg), and then incubated for 18–24 hours at 37°C. A ≥5 mm increase in the zone of inhibition diameter for any cephalosporin tested in combination with amoxicillin-clavulanic acid compared to its zone when tested alone was indicative of ESBL production, as confirmed phenotypically [16].

3. RESULTS AND DISCUSSION

3.1 Antibiotic Resistance Profile

The antibiotic resistance profile of the *E. coli* isolated from stool of suspected diarrhea patients in Nasarawa South Senatorial District as is shown in Table 1. The isolates from all the selected hospitals were resistance to all the antimicrobials. Most notably, the isolates were highly resistance to Ampicillin, Amoxicillin/Clavulanic acid and Sulfamethoxazole/Trimethoprim, Ceftriaxone and Streptomycin with percentage resistance ranges from 74.0-100.0% in all the selected hospitals but less resistance to Cefotaxime and Ceftazidime with percentage resistance ranges from 0-7.8%. Similarly, the isolates from DASHL and GHK were also resistance to imipenem with percentage resistance accounts for more than 45.0%.

3.1.1 Antibiotic Resistance Phenotypes

The antimicrobial resistance phenotypes of the isolates from suspected cases of diarrhea are as shown in Table 2. The isolates were distributed into different resistant phenotypes of which the most common phenotype which dominant other phenotypes (Table 2) in the secondary health care facilities such as GHA, GHD, GHK and GHO was AMP-AUG-CRO-SXT-CN-S and accounted for highest percentage than other phenotypes with percentage prevalence ranges from 28.0-40.0%. Similarly, AMP-AUG-CRO-SXT-CN-IMP-CIP-S phenotype also dominated other phenotypes in the Tertiary healthcare Centre (DASH) percentage prevalence of 24.0%.

3.1.2 Multiple Antibiotic Resistance Index

The *E. coli* isolates from stool suspected diarrheic patients were distributed into different MAR index with all having MAR of > 0.2 and the most common MAR index in DASH, GHD, GHO, GHA and GHK were 0.6(19.6%), 0.6(50.0%), 0.7(60.0%), 0.6(40.0%) and 0.5(52.0%) respectively as Table 3.

3.1.3 Categories of Antibiotic Resistance

The categories of antibiotic resistance in the antibiotic resistant *E. coli* isolates from suspected diarrheic patients are as shown in Figure 1. Multidrug resistance (MDR) is the most common (98.4%), while the occurrence of XDR was low (1.2%). The occurrence MDR in relation to hospital was highest in DASH and GHD (100.0%) but low in GHK (96.0%) as shown.

3.1.4 Occurrence of Extended Spectrum β-Lactamase (ESBL) producers

The occurrence of ESBL-producing isolates in selected hospitals at Nasarawa South Senatorial District, Nigeria is summarized and presented in Figure 2. Most notably, the overall occurrence of ESBL producers was 8(3.2%) with DASHL (7.8%) having the highest proportion followed by GHK and GHD (2.0%), and none of the isolates from GHA were ESBL producers. Our study investigates the antimicrobial resistance profile and molecular detection of extended-spectrum beta-lactamase (ESBL) resistance genes, as well as the molecular diversity of ESBL-producing isolates from selected hospitals in Nasarawa North Senatorial District, Nigeria. The results revealed a 100% occurrence of E. coli isolates from stool samples of suspected diarrhea cases in the selected hospitals, consistent with the 100% prevalence reported by Abimiku et al. [4] in a similar study. This high occurrence suggests that *E. coli* may be a significant causative agent of diarrhea in the studied population.

A striking result of our study is the high resistance of the isolates to ampicillin, amoxicillin-clavulanic acid, ceftriaxone, and sulfamethoxazole-trimethoprim. This suggests that these antimicrobials are ineffective against the isolates. The high resistance rates could be attributed to inappropriate or overuse of these antimicrobials as therapeutic options for infections caused by Enterobacteriaceae. Specifically, the resistance rates to sulfamethoxazole-trimethoprim, amoxicillin-clavulanic acid, and streptomycin were higher than the 84.1%, 73.0%, and 75.0% reported by Abimiku et al. [4]. Additionally, our finding that 97.5% of isolates were resistant to amoxicillin-clavulanic acid aligns with the report by Dufour (2013).

Table 1: Antimicrobial Resistance of *Escherichia coli* isolated from stool of patients attending selected Hospitals in Nasarawa South Senatorial District, Nasarawa state, Nigeria

Antimicrobials	Disc Content (µg)	GHA n=50	GHD (n=50)	GHK (n=50)	DASHL (n=51)	GHO (n=50)	Total (n=251)
Ampicillin (AMP)	10	49(98.0)	50(100.0)	40(80)	51(100.0)	50(100.0)	240(95.6)
Amoxicillin/Clavulanic acid (AUG)	30	49(98.0)	50(100.0)	48(96.0)	46(90.0)	50(100.0)	243(96.8)
Cefotaxime (CTX)	30	0(0.0)	2(4.0)	1(2.0)	4(7.8)	1(2.0)	8(3.1)
Ceftazidime (CAZ)	30	0(0.0)	2(4.0)	1(2.0)	4(7.8)	1(2.0)	8(3.1)
Ceftriaxone (CRO)	30	48(96.0)	50(100.0)	48(96.0)	38(74.5)	50(100.0)	234(93.2)
Sulfamethoxazole/ Trimethoprim (SXT)	25	47(94.0)	49(98.0)	47(94.0)	51(100.0)	47(94.0)	241(96.0)
Gentamycin (CN)	10	39(78.0)	40(80.0)	14(28.0)	23(45.1)	45(90.0)	161(64.1)
Ciprofloxacin (CIP)	5	9(18.0)	26(52.0)	12(24.0)	48(94.1)	39(78.0)	134(53.3)
Imipenem (IMP)	30	0(0.0)	9(18.0)	48(96.0)	25(49.0)	2(4.0)	84(33.4)
Streptomycin (S)	30	41(82.0)	37(74.0)	39(78.0)	40(78.4)	48(96.0)	205(81.6)

GHA: General Hospital Awe, GHD: General Hospital Doma: GHK: General Hospital Keana, GHO: General Hospital Obi, DASHL: Dalhatu Araf Specialist Hospital Lafia

Table 2: Multiple Antibiotic Resistance (MAR) index of *Escherichia coli* isolated from stool of patients attending selected hospitals in Nasarawa South Senatorial District, Nasarawa State, Nigeria

No. of antibiotic resistance (a)	No. of antibiotics tested (b)	MAR index (a/b)	Frequency (%)				
			DASHL	GHA	GHO	GHD	GHK
10	10	1.0	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
9	10	0.9	0(0.0)	3(6.0)	1(2.0)	2(3.9)	1(2.0)
8	10	0.8	0(0.0)	0(0.0)	0(0.0)	8(15.7)	2(4.0)
7	10	0.7	1(2.0)	16(32.0)	1(2.0)	6(11.8)	30(60.0)
6	10	0.6	20(40)	25(50.0)	9(18.0)	10(19.6)	15(30.0)
5	10	0.5	19(38.0)	3(6.0)	26(52.0)	9(17.6)	1(2.0)
4	10	0.4	10(20.0)	2(4.0)	2(4.0)	5(9.8)	0(0.0)
3	10	0.3	0(0.0)	0(0.0)	3(6.0)	1(2.0)	1(2.0)
2	10	0.2	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
1	10	0.1	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

GHA: General Hospital Awe, GHD: General Hospital Doma: GHK: General Hospital Keana, GHO: General Hospital Obi, DASHL: Dalhatu Araf Specialist Hospital Lafia

Table 3: Antibiotic Resistance Phenotypes of *Escherichia coli* isolated from stool of patients in selected hospitals in Nasarawa South Senatorial District, Nasarawa State, Nigeria

Antimicrobial Resistance Phenotypes	DASH (n=51)	Awe (n=50)	Doma (n=50)	Keana (n=50)	Obi (n=50)
AMCAug CRO	0(0.0)	0(0.0)	0(0.0)	1(2.0)	1(2.0)
Amp SXT CIP	2(3.9)	0(0.0)	4(8.0)	0(0.0)	3(6.0)
AMCCRO SXT	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
AMCAMPCN SXT	0(0.0)	1(2.0)	0(0.0)	0(0.0)	0(0.0)
AMPCRO SXTCN	0(0.0)	1(2.0)	0(0.0)	0(0.0)	0(0.0)
AMPAMC CRO S	0(0.0)	0	4(8.0)	3(6.0)	0(0.0)
AMC CRO SXT S	0(0.0)	2(4.0)	0(0.0)	0(0.0)	0(0.0)
AMC Amp CRO SXT	2(3.9)	6(12.0)	8(16.0)	0(0.0)	5(10.0)
AMPCROSXT CIP	4(7.8)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Aug AmpSXT Imp	0(0.0)	0(0.0)	0(0.0)	6(12.0)	0(0.0)
CROSXTCNS	0(0.0)	0(0.0)	0(0.0)	4(8.0)	0(0.0)
AMPSXTCIPImp	2(3.9)	0(0.0)	0(0.0)	3(6.0)	0(0.0)
AMPAMC CRO CN S	0(0.0)	2(4.0)	0(0.0)	0(0.0)	0(0.0)
AMP AMC CROSXT S	0(0.0)	7(14.0)	0(0.0)	0(0.0)	5(10.0)
AMPAAMC SXTCN CIP	6(11.8)	2(4.0)	0(0.0)	0(0.0)	0(0.0)
AMPAMCCRO SXTCN	0(0.0)	3(6.0)	12(24.)	0(0.0)	3(6.0)
AMPAMC CROCIPS	0(0.0)	1(2.0)	0(0.0)	6(12.0)	0(0.0)
AMPAMC CROSXT CIP	4(7.8)	0(0.0)	5(10.0)	0(0.0)	0(0.0)
AMPImp CRO S	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
AMPAugCRO SXTCNS	0(0.0)	20(40.)	14(28.)	15(30.0)	19(38.0)
AMPAugCAZCRO SXT S	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
AMPAug CRO SXT CN CIP S	0(0.0)	5(10.0)	0(0.0)	5(10.0)	2(4.0)
AMPAugCROSXT CN Imp	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
CRO SXTCNCIPImpS	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(4.0)
AMPAMC CRO SXT Imp S	0(0.0)	0(0.0)	0(0.0)	2(4.0)	0(0.0)
AMPAMCCRO SXT CIP CN	8(15.7)	0(0.0)	0(0.0)	0(0.0)	8(16.0)
AMPAMCSXTCN CIP IMP	2(3.9)	0(0.0)	3(6.0)	0(0.0)	0(0.0)
AMPAMC SXT CIP IMP S	4(7.8)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
AMPAMCCTX CAZCRO SXT CIP	4(7.8)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Amp AMC CTX CAZ CRO SXT CIP CN	4(7.8)	0(0.0)	0(0.0)	0(0.0)	2(4.0)
AMPAMC CRO SXT CN IMP CIP S	12(24.)	0(0.0)	0(0.0)	5(10.0)	0(0.0)
AMPAMC CTX CAZ CRO SXT CIP IMP S	1(2.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)

Amp: Ampicillin, Aug: Ampicillin/clavulanic acid, CAZ: Ceftazidime, CTX: Cefotaxime, CIP: Ciprofloxacin, CN: Gentamycin, SXT: Sulphamethaxazole/trimethaprime, CRO: Cetriazone, S: Streptomycin, Imp: Imipenem, DASH: Dalhatu Arab Specialist Hospital

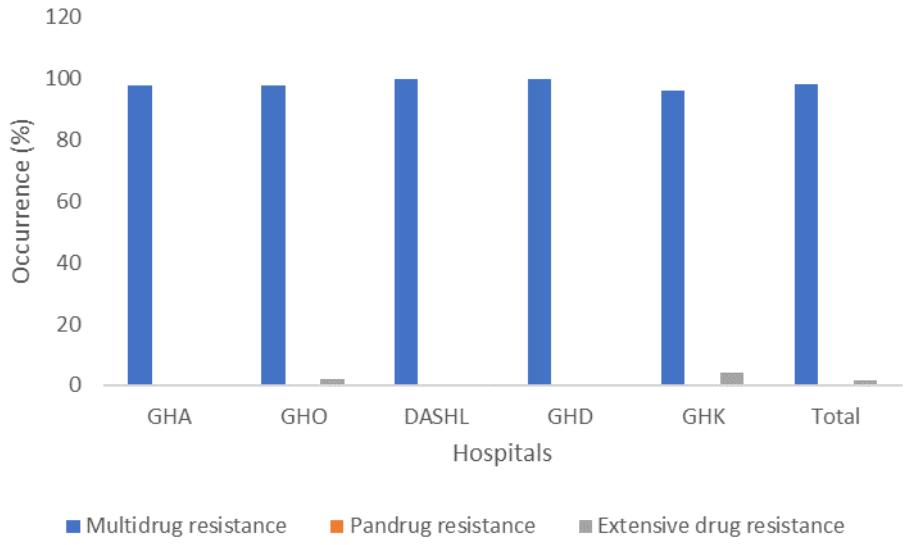

Figure 1: Categories of Antibiotic Resistance in antimicrobial resistant *Escherichia coli* isolated from patients attending selected Hospitals in Nasarawa South Senatorial District, Nasarawa State, Nigeria
(GHA=General Hospital Awe; GHO=General Hospital Obi).

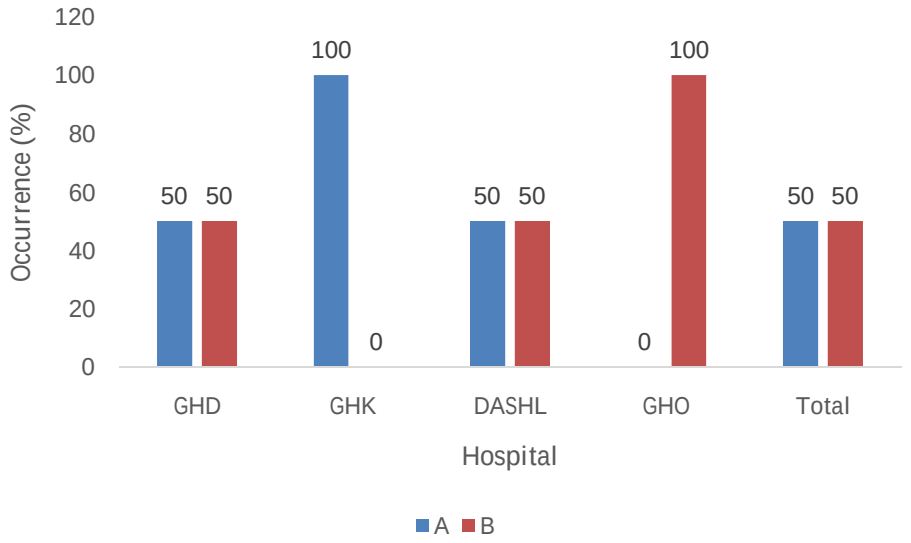

Figure 2: Occurrence of strains of Extended spectrum β-lactamase producing *Escherichia coli* isolated from stool of diarrheic patients in Nasarawa South Senatorial District, Nasarawa State, Nigeria (GHD= General Hospital Doma; GHK= General Hospital Keana).

Our study also revealed that over 90.0% of the isolates were multidrug-resistant (MDR), and this finding is consistent with the results of Dalas *et al*. [11], who reported over 90.0% of MDR *E. coli* isolates from suspected diarrhea cases. The high prevalence of MDR isolates in the study area has significant public health implications, as infections caused by MDR organisms are challenging to treat and may lead to prolonged hospital stays and increased disease burden.

Conversely, the low resistance rates to ciprofloxacin, imipenem, ceftazidime, and imipenem suggest these antimicrobials have not been misused as therapeutic options for *E. coli* infections in the study centres. This low resistance also indicates that these antimicrobials may be effective for treating intestinal infections caused by *E. coli* in the study area.

The percentage occurrence of ESBL-producing isolates in our study was lower than the 13.2%, 22.2%, and 16.5% reported by Dalas et al. [11], Fordy *et al*. [12], and Ahmed et al. [2013]. Similarly, the percentage prevalence of ESBL-producing isolates (50.0%) from our present study is less than the 66.6% in our previous study conducted in 2020 [10]. This, however, suggests a decline in the prevalence of ESBL-producing isolates from the study centre.

The occurrence of ESBL production in the cefotaxime and ceftriaxone-resistant isolates suggests that the ESBL enzymes may be responsible for inactivating these antibiotics.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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