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Prevalence of Antibiotic Resistant Diarrheagenic *Escherichia coli* Isolated from Stool Samples of Diarrheic Children Under 5 Years in Sokoto, Nigeria

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Abstract

Diarrheal diseases continue to pose substantial public health challenges, especially in children under the age of 5. Diarrheagenic Escherichia coli (DEC) is the second most common cause of diarrhea in children after Rotavirus. This study aimed to assess the prevalence of antibiotics resistant DEC recovered from diarrheic children 0-5 years in Sokoto. Stool samples were obtained from 300 diarrheic kids attending two hospitals in Sokoto. Bacterial isolates that showed colonial morphology suggestive of E. coli were subjected to antibiotic susceptibility testing. PCR was carried out to confirm the presence of DEC and resistant genes among the multiple antibiotic-resistant isolates. Structured questionnaires were administered to determine the risk factors that predispose the children to diarrhea. The result revealed a 21% prevalence of E. coli isolates, out of which 75% displayed resistance to Ampicillin, 75% to Nalidixic acid, 30% to Gentamycin, 23% to Ofloxacin, 74% to Cefotaxime, 23% to Ceftriaxone, 18% to Nitrofurantoin, 10% to Imipenem and 73% to Cefuroxime. Out of the 30 E. coli isolates with a MAR index of ≥ 0.2, 12 were found to be multidrug-resistant (MDR). All four MDR E. coli selected were confirmed to be DEC using the UidA gene. Out of all the four MDR DEC confirmed, only one class 1 integron was detected. Raising concern about misuse of commonly used antibiotics. This study highlights the need for implementing antibiotic stewardship programs and infection control measures to combat the growing threat of antibiotic-resistant DEC within Sokoto.

Keywords: Diarrhea, Escherichia coli, Children, Antibiotic, Resistance

INTRODUCTION

Diarrhea is defined as the expulsion of three or more loose or watery feces occurring within 24 hours or a change in stool consistency compared to the patient's usual pattern (Mohammed *et al.*, 2013). This condition, recognized as a global public health challenge, significantly impacts the well-being of infants and children, particularly in low and middle-income countries, leading to substantial illness and death (Iguchi *et al.*, 2023). Diarrhea is often categorized into three main types: acute diarrhea, bloody diarrhea (also referred to as dysentery), and persistent diarrhea, known as chronic diarrhea. Intestinal pathogenic *E. coli*, known as diarrheagenic *E. coli*, are responsible for various types of diarrhea infections in children aged 0-5 years (Zelelie *et al.*, 2023). This strain of *E. coli* stands as a significant contributor to gastroenteritis, particularly impacting children in developing nations (Zelelie *et al.*, 2023). Following Rotavirus, diarrheagenic *Escherichia coli* stands as the second most prevalent cause of diarrhea among children under the age of five. There are six types of pathogenic *Escherichia coli* linked to intestinal infections: Enterohemorrhagic *E. coli* (EHEC), Shigatoxin-producing *E. coli* (STEC), Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), and Diffusely Adherent *E. coli* (DAEC). These classifications mainly vary in their genetic makeup concerning virulence (Zelelie *et al.*, 2023).

Bacterial infections continue to stand among the primary reasons for illness and death in children. The disease impact caused by these infections, coupled with the rise of antimicrobial resistance to commonly used treatments, further worsens this health burden (Zelelie *et al.*, 2023). Antimicrobial resistance (AMR) refers to the

consistently increasing trend where microorganisms demonstrate the ability to endure the impacts of a particular antimicrobial agent. Consequently, more than 2 million individuals contract infections that resist antimicrobials, leading to over 23,000 fatalities annually, making it a significant public health concern (CDC, 2008).

Diarrhea continues to be the second most common cause of death in children under the age of five, after pneumonia. (Soiza *et al.*, 2018). Nigeria experiences a loss of around 2,300 young lives every day, positioning the country as the second-largest contributor to the under-five mortality rate due to diarrhea (Boschi-Pinto *et al.*, 2008). Furthermore, there are over 2.5 billion cases of diarrhea annually among kids aged 0-5 years. This imposes significant economic burdens on both health systems and families (Boschi-Pinto *et al.*, 2008). Additionally, over 2 million people contract antimicrobial-resistant infections, resulting in more than 23,000 deaths annually, presenting a grave public health concern. Recurrent episodes of diarrhea can lead to malnutrition, hindered brain development, stunted growth, and immense stress within affected households. In Sokoto, there are overlooked factors contributing to this burden, including issues like contaminated food or water, poor hygiene, and sanitation. Additionally, aspects such as the duration of breastfeeding in relation to family social status or education level tend to be disregarded (Adegoke *et al.*, 2021). Furthermore, Sokoto's low completion rate of infant vaccinations in Nigeria leads to reduced immunity among infants, making them more susceptible to health risks (Madubu, 2021). Diarrheagenic *E. coli* stands as a significant cause of gastroenteritis in children within developing nations (Zelelie *et al.*, 2023). The use of antibiotics for children experiencing acute diarrhea due to this type of *E. coli* isn't entirely clear due to the absence of quick diagnostic tests in hospitals (O'Ryan *et al.*, 2005). Conducting surveys to gather information on antibiotic-resistant strains of diarrheagenic *E. coli* and their risks in children aged 0-5 could mitigate its impact. Recommendations from this research could prompt the government to establish rapid diagnostic facilities in various hospitals, aiding in diagnosing *E. coli*-induced diarrhea. Additionally, it would educate mothers on personal hygiene, the importance of exclusive breastfeeding for the first 6 months, and the significance of infant immunization. In many areas of study, parents often attribute childhood diarrhea to teething, leading to a lack of documented information on the microbial causes and associated risks in these regions. This gap highlights the necessity for further investigation into the prevalence of antibiotic-resistant diarrheagenic *Escherichia coli*, a primary cause of diarrhea in children aged 0-5, and its related risk factors. This thorough research aims to eradicate DEC infections in Sokoto. Determining the role of antibiotic treatment for children experiencing acute diarrhea from this *E. coli* strain remains unclear largely due to the absence of swift diagnostic tests available in hospitals (O'Ryan *et al.*, 2005). To alleviate its impact, providing information via surveys on antibiotic-resistant diarrheagenic *E. coli* and its risk factors in children aged 0-5 years is crucial. This research could prompt the establishment of rapid diagnostic facilities in various hospitals within the state to diagnose diarrhea caused by this strain more effectively. Furthermore, it aims to educate mothers on personal hygiene practices, the importance of exclusive breastfeeding for the first six months, and the significance of infant immunization. Misconceptions prevail in many areas, where parents attribute children's diarrhea to teething, compounded by a lack of documented data on the microbial causes and associated risk factors within the study area. Hence, increased investigation into the prevalence of antibiotic-resistant diarrheagenic Escherichia coli, a primary cause of diarrhea in children aged 0-5 years, is imperative to eliminate DEC infection in Sokoto. This study aimed to conduct a survey focusing on antibiotic-resistant diarrheagenic *Escherichia coli* (DEC) isolated from children between the ages of 0 to 5 who suffered from diarrhea in Sokoto, Sokoto State, Nigeria.

MATERIALS AND METHODS

Ethical approval and consent

A hospital-centered descriptive cross-sectional study took place in the study area. Before initiating the research, ethical approval was sought and obtained from the State Specialist Hospital and Usmanu Danfodiyo University Teaching Hospital Sokoto. Parents and guardians of the children gave their consent, both in writing and verbally, before any samples were collected.

Collection of stool samples

Stool samples were collected from individuals at the State Specialist Hospital and Usmanu

Danfodiyo University Teaching Hospital Sokoto. The two hospitals were selected for their capacity to provide the necessary information pertinent to this study.

Study Participants

Study participants' stool specimens were gathered, totaling 300 samples sourced from children aged 0 to 5 who had presented diarrhea symptoms at two selected hospitals. These cases encompass both outpatient visits and hospitalized patients in the pediatric ward, following examination by attending physicians. Over nine months (August 2022 to March 2023), the stool samples (5 mL each) were collected using sterile sample bottles.

These containers were appropriately labeled and then transported in transport media (Buffered Peptone Water) to the Microbiology laboratory at Usmanu Danfodiyo University Sokoto for further analysis (Dabo *et al.*, 2019).

Culture and Isolation

A small portion of every stool sample collected was carefully inoculated onto sterilized MacConkey agar within a labeled petri dish using a sterilized inoculation wire. These Petri dishes were then placed in an incubator at 37°C for 24 hours. Following this incubation period, each plate was examined. Any colony displaying a pink or reddish coloration was picked and inoculated on Eosin methylene blue agar (EMB) using a sterile inoculating wire and labeled accordingly. These petri dishes were then re-incubated at 37°C for an additional 24 hours.

Colonies showing a moist appearance with a distinctive green metallic sheen were singled out for further identification via gram staining and biochemical tests. The colonies suspected to be *E. coli,* identified by their moist appearance with a distinctive green metallic sheen, were selected. A smear of these colonies was prepared on a rinsed slide using a single drop of water. The slide was gently passed over the flame 2-3 times to fix the smear. Subsequently, the smear was stained with crystal violet for 1 minute, followed by a thorough water wash. Then, the smear was stained with iodine for 60 seconds before being exposed to alcohol. After rinsing off the alcohol, the slide was covered with safranin for 60 seconds, washed, gently dried, and examined under a microscope. Any isolates appearing pink and displaying a rod-like shape were categorized as gram-negative bacteria and were further subjected to subsequent biochemical tests (citrate, indole, and urease test).

Antibiotic Susceptibility Testing

The susceptibility/resistance of the *E. coli* isolates to various antimicrobial drugs was evaluated using the standard disc diffusion technique, as described by the Clinical and Laboratory Standard Institute (CLSI, 2020). The isolates underwent screening for resistance against a range of antibiotics, including Cefuroxime (30µg), ceftriaxone sulbactam (30µg), amoxicillin-clavulanate (30µg), Cefotaxime (25µg), nalidixic acid (30µg), imipenem/cilastatin (10/10µg), gentamicin (5µg), Ofloxacin (5µg), ampiclox (10µg), cefexime (5µg), levofloxacin (5µg), and Nitrofurantoin (30µg) based on the CLSI guidelines. The pure isolates, previously cultured on sterile MacConkey agar and EMB agar, were transferred onto sterile physiological-buffered saline. Adjusting the turbidity of these suspensions to match the 0.5 Mac Farland's standard, 100µl of the suspensions were evenly spread onto Mueller-Hinton agar plates. Paper disks saturated with various antimicrobial agents were then placed on the test isolates (*E. coli*) previously cultured on the Mueller-Hinton Agar plates. Subsequently, the plates were incubated at 37°C for 24 hours, and the diameter of the zones displaying inhibition was measured to the nearest millimeter. Multiple antibiotic resistance (MAR) index was calculated for each isolate by using the formula MAR= (number of resistant antibiotics)/(total antibiotics tested) (Afunwa *et al.*, 2020).

E. coli isolates with a (MAR) index of 0.2 or higher from the disk diffusion test were further tested using PCR to detect the presence of the diarrheagenic *E. coli* using primers specific to the UidA gene (Adzitey *et al.* 2022). Isolates confirmed to be DEC with the UidA gene were then further assessed through PCR for the presence of class 1 integron gene. These two PCR tests were used to confirm the isolates as multidrug-resistant diarrheagenic *E. coli*.

RESULTS

Three hundred (300) stool samples were gathered from the two selected medical facilities (200 samples from Specialist Hospital Sokoto and 100 from Usmanu Danfodiyo University Teaching Hospital). Out of the three hundred samples cultured on MacConkey agar, one hundred and twelve (112) samples showed

colonial morphology suggestive of *E. coli*. While one hundred and eighty-eight (188) samples showed features of isolates not *Escherichia coli*. The one hundred and twelve samples were then further subcultured on Eosin methylene blue (EMB) agar. Sixty-four (64) samples showed the green metallic sheen colonial feature suggestive of *E. coli* (Table 1).

The distribution of Gram-negative bacteria is shown in Table 2, with a visual summary in Figure 1. The most prevalent Gram-negative bacteria were *E. coli* isolates, which accounted for 21% (64 out of 300) of the total isolates, while *Serratia marcescens* isolates had the lowest frequency of occurrence with a total number of 2% (5 out of 300) of the total isolates (Table 2).

The occurrence of *E. coli* in the loose stool samples collected from the study participants is presented in Table 3. Out of the 300 stool samples, sixty-four 64 (21%) showed patterns and reactions suggestive of *E. coli* by microscopy and biochemical tests. (forty-four, 44 (14%) samples from Specialist hospitals and twenty, 20 (7%) from UDUTH). The remaining two hundred and thirty-six, 236 (79%) samples showed patterns and reactions suggestive of other bacteria (Table 3)

Antimicrobial susceptibility testing of *E. coli* isolates is presented in Table 4. A total number of 40 *E. coli* isolates were tested for antimicrobial susceptibility. Imipenem (88%) was found to be the most effective drug against diarrheagenic *E. coli,* whereas Ampiclox (75%) and Nalidixic acid (75%) showed the least activity against diarrheagenic *E. coli* (Table 4).

The determination of the Multiple Antibiotic Resistance (MAR) index for each isolate is presented in Table 5. Out of the 40 isolates tested on the antibiotic disc, four 4 (10%) were susceptible to all the 12 antibiotics on the disc having the MAR index of 0, while six 6 (15%) Isolates were resistant to only one to two antibiotics giving a MAR index of less than 0.2. All the remaining 30 had a MAR index of greater than or equal to 0.2, with four 4(10%) isolates having the highest MAR index of 0.92, followed by four 4 (10%) isolates of 0.83, one (2.5%) isolate with 0.67, four 4(10%) with 0.58, two 2 (5%) isolates with 0.5, three 3 (7.5%) isolates with 0.42, five 5 (12.5%) isolates with 0.33 and lastly seven 7 (17.5%) isolates with 0.25. (Table 5).

The pattern of antibiotic resistance of *E. coli* isolates recorded for each of the 36 isolates having resistance to at least one antibiotic is presented in Table 6. 26 different resistance patterns were observed, with only 6 being resistant to less than 3 antibiotics (MAR index of <0.2) (Table 6).

Occurrences of multi-drug resistance based on antibiotic classes are presented in Table 7. Among the 30 *E. coli* isolates with a MAR index (≥ 0.2), 12 showed multi-drug resistance, meaning they were resistant to at least 1 agent in 3 or more classes of antimicrobials. Out of these 12 isolates that were found to be multidrug-resistant, four isolates were picked for PCR based on their phenotypic representation of resistance to each of the classes of antibiotic used for the study (Table 7).

The result of PCR amplification for the confirmation of DEC by targeting the UidA gene is shown in Figure 2. The UidA gene was used as a positive control in PCR assays to validate the identification of *E. coli* and confirm the presence of diarrheagenic *E. coli* (DEC). Out of the four multidrug-resistant *E. coli* selected based on their phenotypic representation of four (4) classes of antibiotics. All the four isolates were confirmed to be DEC (Figure 2).

The gel electrophoresis image of one class one integron detected is shown in Figure 3. Out of the 4 multidrug-resistant DEC confirmed by PCR using the UidA gene, only one class 1 integron was detected (Figure 3).

Table 1: Bacteria obtained from the samples on MacConkey and EMB Agar

S/N	Sample site	Samples collected (n)	Positive samples on MacConkey n (%)	Negative samples on MacConkey n (%)	Positive samples on EMB n (%)	Negative samples on EMB n (%)
1	SHS	200	74 (24%)	126 (42%)	44 (14%)	158 (52%)
2	UDUTH	100	38 (13%)	62 (21%)	20 (7%)	78 (26%)
	Total	**300**	**112 (37%)**	**188 (63%)**	**64 (21%)**	**236 (78%)**

Key: Specialist Hospital Sokoto (SHS), Usmanu Danfodiyo University Teaching Hospital Sokoto (UDUTH), Eosin Methylene Blue (EMB).

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Table 2: Frequency of Occurrence of Gram-Negative Bacteria Isolated from Stool samples (n= 300)

S/N	Organisms	Frequency	Percentage
1	*Escherichia coli*	64	21%
2	*Salmonella typhi*	34	11%
3	*Acinetobacter baumanni*	18	6%
4	*Pseudomasauroginosa*	25	8%
5	*Klebsiella pneumonia*	25	8%
6	*Proteou mirabilis*	20	7%
7	*Aeromonas spp*	10	3%
8	*Morgenellamorganni*	20	7%
9	*Klebsiella oxytoca*	20	7%
10	*Enterobacter cloacae*	31	10%
11	*Enterobacter gergoviae*	8	3%
12	*Shigella spp*	20	7%
13	*Serratia marcesens*	5	2%
TOTAL	**ISOLATES**	**300**	**100%**

Table 3: Frequency of *E. coli* obtained from stool samples of the study subject in two sampling sites

S/N	Sample site	Number of samples collected (n)	Number of positive samples n (%)	Number of negative samples n (%)
1	SHS	200	44 (14%)	158 (67%)
2	UDUTH	100	20 (7%)	78 (33%)
	Total	**300**	**64 (21%)**	**236 (79%)**

Figure 1: Growth of *E. coli* after 24 hours incubation on EMB agar.

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Table 4: Antibiotic Suceptibility Test on *E. coli* isolates

S/N	Name of Antibiotics	Number of Isolates Tested	Antibiogram		
		DEC Isolates (n=40)	Susceptible (%)	Intermediate (%)	Resistant (%)
1	Ofloxacin		20 (50%)	11 (25%)	9 (23%)
2	Gentamycin		20 (50%)	8 (20%)	12 (30%)
3	Nalidixic acid		3 (8%)	7 (18%)	30 (75%)
4	Ampiclox		3 (8%)	7 (18%)	30 (75%)
5	Cefixime		16 (40%)	13 (33%)	11 (28%)
6	Levofloxacin		18 (45%)	10 (25%)	12 (30%)
7	Amoxicillin clavulanate		13 (33%)	14 (35%)	13 (33%)
8	Cefotaxime		5 (13%)	5 (13%)	30 (75%)
9	Ceftriaxone Sulbactam		27(68%)	4 (10%)	9 (23%)
10	Nitrofurantoin		16 (40%)	17 (43%)	7 (18%)
11	Imipenem		32(80%)	4 (10%)	4 (10%)
12	Cefuroxime		5(13%)	6 (15%)	29(73%)

Key: OFX- (Ofloxacin), GN-(Gentamycin), NA-(Nalidixic acid), ACX-(Ampiclox), ZEM- (Cefexime), LBC-(Levofloxacin), AUG-(Augmentin), CTX-(Cefotaxime), NF-(Nitrofurantoin), CRO-(Ceftriaxone sulbactam), IMP-(Imipenem), CXM- (Cefuroxime).

Table 5: MAR indices of *E. coli* isolates from the study subjects

S/N	MAR index	Number and Percentage (n %)
1	0	4 (10%)
2	<0.2	6 (15%)
3	0.25	7 (17.5%)
4	0.33	5 (12.5%)
5	0.42	3 (7.5%)
6	0.5	2 (5%)
7	0.58	4 (10%)
8	0.67	1 (2.5%)
9	0.83	4 (10%)
10	0.92	4 (10%)
Total	**40**	

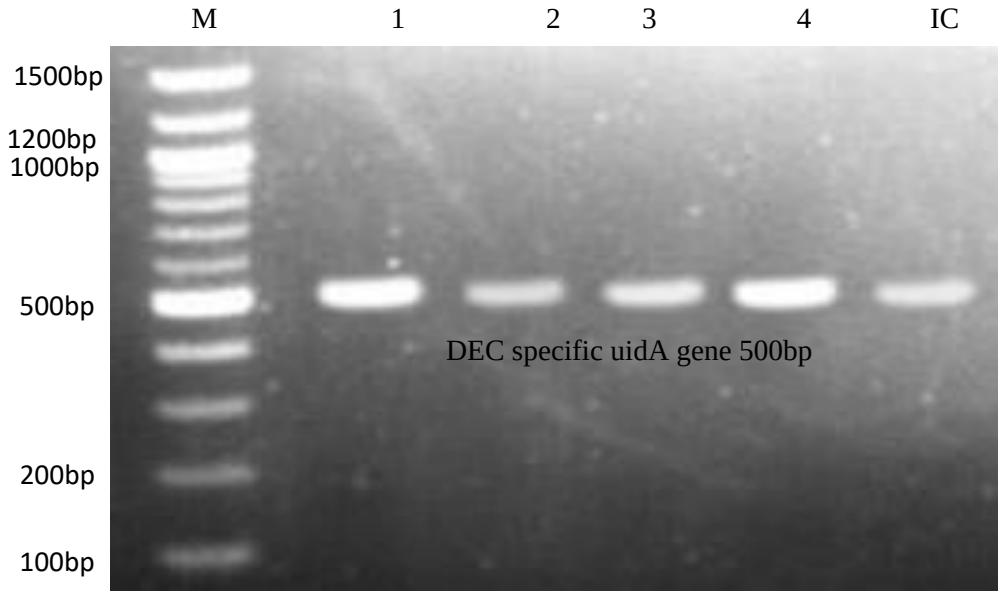

Figure 2: Representative Gel electrophoresis image of the confirmed DEC isolates recovered from diarrheagenic stool specimens.

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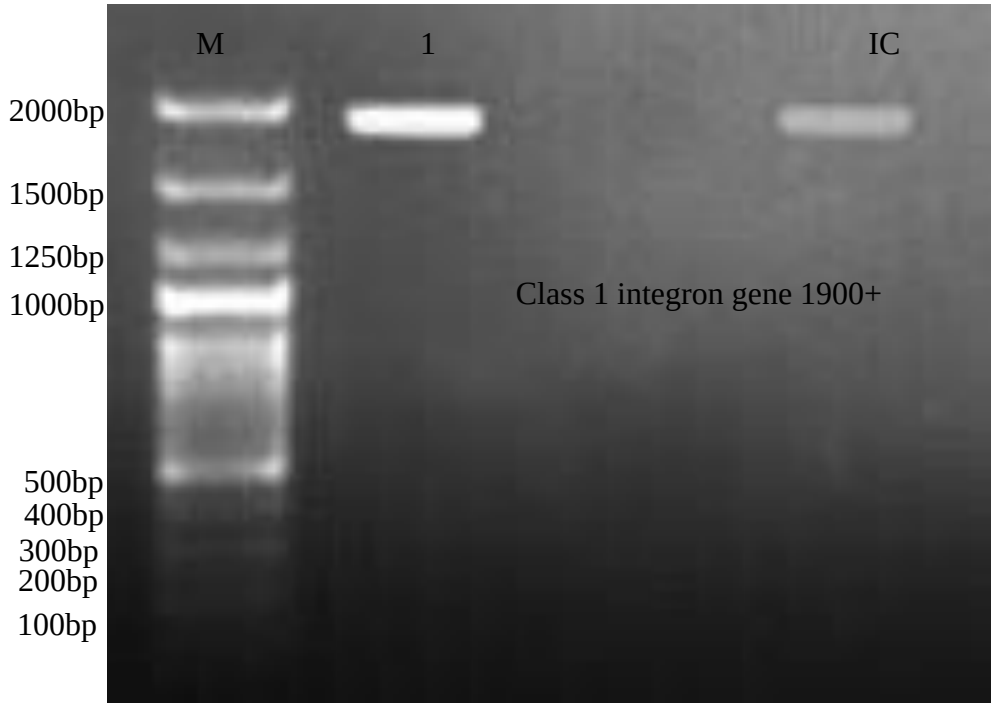

Figure 3: Representative Gel electrophoresis image showing one class 1 integron L1=, IC= internal control, M= 2000bpladder used as a marker.

Table 6: Pattern of antibiotic resistance exhibited by the *Escherichia coli isolates* on 12 antibiotics

S/N	Antibiotic Resistance	No. of isolates
1	ACX	1
2	CTX	1
3	CXM,AUG	1
4	CXM,CTX	2
5	CXM,NA	1
6	CXM,CRO,CTX	1
7	CXM,ACX,NA	3
8	CXM,CTX,NA	1
9	ACX,CTX,NA	2
10	CXM,ACX,CTX,NA	4
11	ACX,OFX,GN,NA	1
12	CXM,ACX,AUG,CTX,NA	2
13	CXM,ACX,CTX,IMP,NA	1
14	CXM,ACX,LBC,AUG,CTX,NA	1
15	CXM,ACX,CTX,IMP,GN,NA	1
16	CXM,CRO,ACX,ZEM,LBC,CTX,NA	1
17	CXM,CRO,ACX,ZEM,AUG,CTX,NA	1
18	CXM,ACX,ZEM,AUG,CTX,GN,NA	1
19	CXM,ACX,ZEM,CTX,OFX,GN,NA	1
20	NF,CXM,LBC,AUG,CTX,OFX,GN,NA	1
21	NF,CXM,CRO,ACX,ZEM,LBC,CTX,OFX,GN,NA	1
22	NF,CXM,ACX,ZEM,LBC,AUG,CTX,OFX,GN,NA	1
23	CXM,CRO,ACX,ZEM,LBC,AUG,CTX,OFX,GN,NA	1
24	CXM,ACX,ZEM,LBC,AUG,CTX,IMP,OFX,GN,NA	1
25	NF,CXM,CRO,ACX,ZEM,LBC,AUG,CTX,OFX,GN,NA	3
26	NF,CXM,CRO,ACX,ZEM,LBC,AUG,CTX,IMP,OFX,NA	1

Key: OFX- (Ofloxacin), GN-(Gentamycin), NA-(Nalidixic acid), ACX-(Ampiclox), ZEM- (Cefexime), LBC-(Levofloxacin), AUG-(Augmentin), CTX-(Cefotaxime), NF-(Nitrofurantoin), CRO-(Ceftriaxone sulbactam), IMP-(Imipenem), CXM- (Cefuroxime).

Table 7: Occurrence of Multi-drug Resistant *E. coli* isolates based on Antibiotic Classes

S/N	Isolates number	Antibiotic tested	Antibiotic class
1	13	ACX,AUG,CTX,CXM,CRO,ZEM,IMP	Beta-lactams
2	27	OFX,LBC,NA,CXM,CTX	Quinolones
3	11	NF, CXM,CTX,ACX,ZEM,LBC	Nitrofurantoin
4	3	ACX,OFX, GN,NA	Aminoglycosides

Key: OFX- (Ofloxacin), GN-(Gentamycin), NA-(Nalidixic acid), ACX-(Ampiclox), ZEM- (Cefexime), LBC-(Levofloxacin), AUG-(Augmentin), CTX-(Cefotaxime), NF-(Nitrofurantoin), CRO-(Ceftriaxone sulbactam), IMP-(Imipenem), CXM- (Cefuroxime).

DISCUSSION

The prevalence of *E. coli* from diarrheic children in the study area is 21%. This finding is similar to the finding of Dairo *et al.* (2017), where the rate of morbidity from Kaduna North Local Government Area was also reported as 21%. This finding agrees with the finding of Onanuga *et al.* (2014) from Gwagwalada, Federal Capital Territory Abuja, where the rate of diarrheagenic *E. coli* strains was 18.4%. This indicates a slightly higher burden of *E. coli* infection in the study area and an increased need to address the issue. The research found that *E. coli* strains were the most prevalent Gram-negative bacteria isolated from the stool of diarrheagenic children, while *Serratia marcescens* had the lowest frequency of occurrence (Table 2). In a similar study by Lengerh *et al.* (2013) at Gonder University Hospital Northwest Ethiopia, it was shown that *Campylobacter* species was the most prevalent Gram-negative bacteria at a frequency of 15.4%.

Antibiotic susceptibility tests of the bacterial isolates revealed that 75% of the diarrheagenic *E. coli* (DEC) isolates showed resistance to Ampicillin (75%) and Nalidixic acid (75%). This study disagrees with the finding of Gabresilasie *et al.* (2018) in Addis Ababa, Ethiopia, which stated that 86% of the diarrheagenic *E. coli* (DEC) were resistant to Ampicillin and Amoxicillin clavulanate. However, the study showed that DEC has the highest susceptibility to Imipinem, with 88%. Such differences in the resistance pattern of Ampicillin with that of Imipinem could be associated with the beta-lactamase inhibitors production as the predominant mechanisms of resistance in *E. coli* isolates. Generally, all members of the *Enterobacteriaceae* produce small amounts of the enzymes (Iredell *et al.*, 2016). However, the *E. coli* isolates are not extended-spectrum beta-lactamases (ESBL) producers because the isolates have a low level of resistance to Imipenem, which belongs to the group of third-generation Cephalosporin, Goyal *et al.* (2009). Resistance to Ampicillin (75%) could be overprescribing of the drug, persistent over-the-counter availability and misuse of the antibiotic.

The study also revealed the pattern of antibiotic susceptibility with 26 different resistance patterns which were observed with only 6 being resistant to less than 3 antibiotics. Taken on the whole, the MARI phenotypes showed that 75% of the *E. coli* isolates were resistant to three or more antibiotics, where 4(10%) isolates were found to be susceptible to all antibiotics, 6(15%) isolates show MAR index of <0.2, and 30(75%) *E. coli* isolates showed a MAR index of ≥ 0.2. This finding is similar to the finding of Titilawo *et al.* (2015) from Osun state, Nigeria, where the MAR index of *E. coli* isolates was 75%.

The suspected *E. coli* isolates were verified using PCR techniques that targeted the UidA gene. The UidA gene is a reliable target in PCR assays for detecting *E. coli* as it encodes an enzyme that breaks down a specific substrate (4-methylumbelliferyl-beta-D-glucuronide (MUG) among different strains Titilawo *et al.* (2015). One Class 1 integron was detected from the 4 *E. coli* isolates that PCR confirmed to have the UidA gene. This could be due to resistance factors other than horizontal gene transfer as several factors account for the development of antibiotic resistance in bacteria, for example production of enzymes that inactivate the antibiotic and the efflux mechanism that removes the antibiotic, etc.

CONCLUSION

The study findings identified a high prevalence of diarrhea morbidity due to *E. coli* among children aged 0-5 years in the study area. Most of the *E. coli* isolates showed a high level of resistance, with a significant number exhibiting multi-drug resistance (75%). This widespread prevalence of multi-drug resistant DEC poses a

serious and imminent threat to the health of young, vulnerable children and highlights the urgent need for antibiotic stewardship and infection control measures.

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