

# Molecular Characterization of Diarrhoeagenic *Escherichia coli* Pathotypes Isolated from Under Five Years Children in Katsina State

<sup>1</sup>Anas Umar, <sup>2</sup>Abdulkadir Bashir, <sup>3</sup>Abdulahadi Kumurya Sale, <sup>2</sup>Mukhtar Gambo Lawal,

<sup>1</sup>Adamu Nafisa Abduljalil and <sup>1</sup>Timothy Auta

<sup>1</sup>Department of Microbiology, Federal University Dutsin-Ma, 821101, Dutsin-Ma, Katsina, Nigeria

<sup>2</sup>Department of Microbiology, Umaru Musa Yar'adua University, 820102, Batagarawa, Katsina, Nigeria

<sup>3</sup>Department of Medical Laboratory Science, Faculty of Health Allied Sciences, Bayero University Kano, Kano, Nigeria

## ABSTRACT

**Background and Objective:** Diarrhoea, majorly caused by diarrhoeagenic *Escherichia coli* (DEC) is the second leading cause of death in children globally, particularly in developing countries like Nigeria. The study molecularly characterized diarrhoeagenic *Escherichia coli* pathotypes isolated from children under five years in Katsina State. **Materials and Methods:** A total of 165 stool samples were collected from 135 (diarrheic) and 30 (non-diarrheic) children attending five selected hospitals in Katsina State, within a period of nine months, from October, 2021 to June, 2022. The samples were screened for diarrhoeagenic *E. coli* pathotypes using uniplex and multiplex conventional PCRs. Specific primers were used for the detection of seven virulence genes (*eae*, *stx1*, *stx2*, *est*, *elt*, *aggR* and *ipaH* genes), respectively, which are peculiar to the DEC strains. **Results:** Of 165 stool samples examined, 40 (57.97%) tested positive for DEC. Enteroaggregative *E. coli* (EAEC) was found to be the most frequently encountered pathotype (36.23%) followed by enteropathogenic *E. coli* (EPEC, 8.69%) then enteroinvasive *E. coli* (EIEC, 7.25%) and enterotoxigenic *E. coli* (ETEC) which was the pathotype that least prevailed among the study population (5.79%). **Conclusion:** The findings of this study revealed that enteroaggregative *E. coli* (EAEC) and enteropathogenic *E. coli* (EPEC) are the most circulating DEC pathotypes among children under the age of five years in Katsina State.

## KEYWORDS

*Escherichia coli*, diarrhoea, malnutrition, pathotypes, PCR, virulence factors

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## INTRODUCTION

With about 525,000 young children dying and 1.7 billion cases of diarrhoea being recorded worldwide each year, diarrhoeal illness is a serious health issue and is considered a significant source of morbidity and mortality, especially in infants and children under the age of five<sup>1</sup>.

In the entire world, diarrhoea is a severe health burden. Poor water supply, sanitation and hygiene conditions are intimately related to it and are typical in developing nations<sup>2</sup>. Diarrhoea is the second



leading cause of death among children under the age of 5 years after respiratory diseases worldwide, particularly in developing countries. Diarrhoea is defined as the passage of three or more loose or watery stools in a 24 hrs period or more frequent passage by an individual than he/she usually does<sup>3,4</sup>.

Different pathogens, including viruses, bacteria and parasites are responsible for diarrhoea. Such diarrhoeal agents can be acquired from faecally contaminated food, water, fingers, etc., through the faecal-oral route<sup>5</sup>.

*Escherichia coli* is one of the most famous bacteria among those that cause diarrhoea<sup>2</sup>. *Escherichia coli* is a member of the *Enterobacteriaceae* family and is a rod-shaped, oxidase-negative, facultative anaerobic bacterium. The bacterium is discovered as a typical resident of the intestines of mammals, including humans. After German paediatrician and bacteriologist, Theodor Escherich, who discovered *E. coli* in 1885, the genus *Escherichia* was named<sup>6</sup>. The organism typically colonizes the infants' gastrointestinal tract within hours of life after birth and, thereafter, *E. coli* and the host derive mutual benefit<sup>7</sup>. *Escherichia coli* bacterium is normally isolated from faeces. Various well-adapted *E. coli* pathotypes have, however, evolved specific virulence factors which increase their pathogenicity<sup>2</sup>. *Escherichia coli* pathotypes implicated in diarrhoea are called diarrhoeagenic *E. coli* (DEC). The (DEC) accounts for about 30-40% of acute diarrhoea episodes in children <5 years in developing countries and is an important cause of both sporadic cases and diarrheal outbreaks worldwide.

About six pathotypes of DEC are identified on the basis of their specific virulence factors and are implicated in causing diarrhoea. These include enterotoxigenic *E. coli* (ETEC), enter-invasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC) (including shiga toxin-producing *E. coli* (STEC), diffusely adherent *E. coli* (DAEC) and enteroaggregative *E. coli* (EAEC)<sup>8</sup>. However, some strains, such as shiga toxin-producing *E. coli* (STEC), can result in serious foodborne illness. It is mainly spread to people by eating contaminated foods including raw or undercooked ground beef, contaminated raw milk and contaminated raw vegetables. Foods should be thoroughly cooked until they reach a temperature of at least 70°C throughout or higher to get STEC destroyed. In terms of public health, *E. coli* O157: H7 is the most significant STEC serotype, nevertheless, other serotypes are commonly involved in isolated cases and outbreaks<sup>8</sup>. These strains are identified using molecular techniques with primers unique to each pathotype. These strains differ primarily in their preferred host colonization sites, virulence mechanisms and the ensuing clinical symptoms and consequences<sup>9</sup>.

Nigeria was ranked second among the top 15 countries that recorded high child death rates due to diarrhoea and pneumonia as of 2015<sup>9</sup>.

The occurrence of diarrhoeagenic *E. coli* (DEC) in children is rarely investigated in Nigeria and Katsina State in particular and even the very few studies that isolated *E. coli* in diarrheic children did not characterize the *E. coli* to strains level and also did not investigate the genetic background, particularly the presence or absence of virulence factors (VFs), is not routinely studied and consequently, the proportion of diarrhoea related to DEC in Katsina State remains unclear.

In order to establish a baseline understanding of the prevalent DEC pathotype(s) in the State and their clinical significance, this study set out to examine the prevalence and incidence of diarrhoeagenic *E. coli* pathotypes as a cause of infectious diarrhoea in children under the age of five in Katsina State, as well as their antibiotic susceptibility profile.

## MATERIALS AND METHODS

**Study area:** The study was conducted in five health facilities across the three senatorial districts in Katsina State. Katsina State is located at 11.30' North Latitude, 13.15' North Latitude of the equator and 6°52'

East Longitude and 9°20' East Longitude of the prime Meridian of Greenwich. It borders the Republic of Niger to the North, Kaduna to the South, Zamfara to the West and Kano and Jigawa to the East. Katsina State covers an area of 23,938 km<sup>2</sup>, equivalent to about 2.7% of Nigeria's total land area.

The average annual temperature is 30°C. In general, the climate varies greatly depending on the month and season. From December to February, from March to May there is a cooler dry season (harmattan). A hot dry season, from June to September and a warm and less pronounced rainy season from October to November, characterized by less precipitation and a gradual drop in temperature. State Strata consist of basic complexes and sedimentary layers.

According to the 2006 census, Katsina State had a population of 5,792,578, an annual growth rate of 2.2%. Katsina is primarily a Hausa Fulani Province and most people speak only Hausa. The majority of people are farmers and traders. A significant number of immigrants from Southern Nigeria, particularly the Yoruba and Igbo, have been found and mainly live there and dwell mostly in cities<sup>10</sup>. Agriculture is the State's primary economic activity, with little livestock or food processing. The state's agricultural system is based on rain-fed subsistence production of annual crops, with the nomadic Fulani people raising cows and falcons to produce fermented milk locally. About 95% of the population is engaged in subsistence agriculture. A variety of crops are grown including maize, millet, cowpea, rice, sorghum, sugar cane and peanuts. Livestock farming is also widely practised in the area. These include cattle, goats, sheep, horses, donkeys, camels, poultry and pigeons. Cities and node villages are known for trade, business activities and handicrafts<sup>10</sup>.

**Study design:** The study was a cross-sectional, hospital-based conducted over 9 months, between October, 2021 and June, 2022.

**Ethical approval:** Katsina State Ministry of Health gave permission to collect stool samples from the study subjects attending the selected medical facilities. Written informed consent was gotten from the parents/guardians of the study participants. For uneducated participants' parents/guardians, verbal informed consent and willingness to provide socio-demographic information were obtained prior to the commencement of sample collection. A structured questionnaire was administered to patients/guardians of the participants in the study for the collection of socio-demographic information and clinical characteristics including sex, age, feeding patterns, patients' categorical classification (in patients or outpatients), drugs used in diarrhoea treatment, onset of diarrhoea, nature of stool, etc. that in one way or the other contributed to the success of this study.

**Study population:** Subjects in this study were recruited from Mallam Mande General Hospital Dutsin-Ma (MMGHD), Turai Yar'adua Maternity and Children Hospital Katsina (TYMCHK), General Hospital Mani (GHMN), General Hospital Malumfashi (GHML), Karfi Primary Health Centre (KPHC) representing the three senatorial districts of Katsina State, Nigeria. Children under the age of five years with diarrhoea (characterized by the occurrence of three or more loose or watery stools in the last 24 hrs period) were enrolled in the study as a diarrheic group and children without diarrhoea in the last 14 days were enrolled as a control group.

**Inclusion criteria:** All diarrheic subjects below the age of five years after obtaining verbal assent from the parents/guardians of the children that had not commenced antibiotic treatment with or without vomiting were recruited into the diarrheic group. Subjects without diarrhoea were considered as a control group.

**Exclusion criteria:** Children without diarrhoea, above five years of age, children already on antibiotics and children whom the parents/guardians refused assent were excluded from the diarrheic group. In the control group, children who had an episode of diarrhoea in less than 14 days were excluded as well as children whose parents refused consent.

**Sample collection and transportation:** A total of 165 stool samples were collected from 135 (diarrheic) and 30 (non-diarrheic) under five years' children attending five different selected hospitals in Katsina State, into sterile, transparent, wide-mouthed sample bottles and transported on the ice-packed container to the laboratory of the Department of Microbiology Umaru Musa Yar'adua University Katsina for analyses by adopting laboratory procedures used by Adeniyi and Yahaya<sup>10</sup>.

**Isolation and identification of *E. coli*:** A sterile wire loop was used to aseptically streak the stool samples collected from the study participants on prepared Eosin Methylene Blue (EMB) agar plates after which the plates were incubated at 37°C for 24 hrs in an incubator for the primary isolation of the bacterium<sup>2,10</sup>. Afterwards, the plates were observed for colony formation after 24 hrs of incubation. In order to obtain distinct colonies, colonies from the primary plates were sub-cultured EMB agar plates. Distinct green metallic sheen colonies were aseptically picked, streaked and stored on a nutrient agar slant for further biochemical characterization of the isolates<sup>10</sup>.

**Molecular characterization of *E. coli* pathotypes:** The molecular analysis was conducted at the Molecular Biology Laboratory of the Nigerian Institute for Trypanosomiasis Research (NITR), Kaduna, Kaduna State.

**Oligonucleotide primers:** The oligonucleotide primers used for the molecular analysis are shown in Table 1.

**DNA extraction:** The DNA was extracted from the bacterial culture as previously described by Ali *et al.*<sup>11</sup> using the AccuPower® genomic DNA extraction kit (Bioneer Corporation) following the manufacturer's instruction. Briefly, the bacterial cells were transferred into a clean labelled 1.5 mL Eppendorf tube. About 200 µL of tissue lysis buffer, 20 µL of proteinase K and 10 µL of RNase were added to the tube. The tube was vortexed and incubated at 60°C for 1 hr. After the incubation, 200 µL of genomic DNA extraction buffer was added and immediately mixed by vortexing, 400 µL of absolute ethanol was then added and mixed. The lysate was transferred to a binding column attached to a collecting tube and centrifuged at 8000 rpm for 1 min. The lysate in the collection tube was discarded and the tube was reattached to the binding column. Two stages of washing were followed by using wash buffers 1 and 2. After washing, the empty binding column was centrifuged at 13000 rpm for one min to remove excess ethanol. As 50 µL of elution buffer was added to the binding column to elute the DNA.

The extracted DNA was quantified using a Nanodrop spectrophotometer (Thermo Fisher Scientific, UK) to obtain the quantity and purity of the DNA.

Table 1: PCR primer sequences used for detection of different virulence genes in DEC

Target gene	Primer pair (5'-3')	PCR conditions for 35 cycles	Amplicon size (bp)
<i>eae</i>	ATGAAAAAGCTAATGTTGGCA TTAATAACATCCAGCACAGGCA	30 sec 94°C, 45 sec 53°C,	454
<i>stx1</i>	CAACTGGATGATCTCAG CCCCCTCAACTGCTAATA	30 sec 94°C, 45 sec 56°C,	350
<i>stx2</i>	ATCAGTCGTCACACTCACTGGT CTGCTGCACAGTGACAAA	30 sec 94°C, 45 sec 56°C,	110
<i>est</i>	ATGAAAAAGCTAATGTTGGCA TTAATAACATCCAGCACAGGCA	40 sec 95°C, 45 sec 59°C,	402
<i>elt</i>	CATAATGAGTACTTCGATAGAGGAAC GAAACCTGCTAATCTGTAACCATCC	40 sec 95°C, 45 sec 59°C,	239
<i>aggR</i>	CTAATTGTACAATCGATGTA ATGAAGTAATTCTTGAAT	45 sec 94°C, 50 sec 42°C,	308
<i>ipaH</i>	GTTCTTGACCGCTTCCGATACCGTC GCCGGTCAGCCACCCTGAGAGTAC	40 sec 94°C, 45 sec 52°C,	619

For all PCR conditions, a final extension step of 7 min at 72°C was performed<sup>2,11</sup>

**Polymerase chain reaction (PCR) for detection of *E. coli* virulence genes:** The primers used in this study for the detection of virulence genes were described previously by Saka *et al.*<sup>2</sup> and Dashti *et al.*<sup>12</sup> with minor modifications as follows: Denaturation for five min at 94 and 95 °C, 35 cycles of different annealing temperatures (Ta), 72 °C for one min and a final extension step of seven min at 72 °C. The samples were subjected to polymerase chain reactions for the detection of seven virulence genes (*eae*, *stx1*, *stx2*, *est*, *elt*, *aggR* and *ipaH* genes), respectively.

The following *E. coli* reference strains were used as positive controls for the conventional PCR: STEC O157: H7 strain CNM 2686/03 (positive to *stx1/stx2*), EPEC strain CNM764 (positive to *eae*), ETEC strains 117-86 HYMS007 (positive to *elt* and/or *est*), ATCC 43892 for EIEC strain (positive to *ipaH*) and EAEC O104: H4 strain 55989 (positive to *aggR*). Distilled water was used as the negative control in all the PCR reactions.

**Agarose gel preparation and samples load:** Agarose gel (1%) was prepared by dissolving 1 g of agarose powder in 100 mL of 1X TBE buffer (Tris-Borate-EDTA). The solution was then boiled in a microwave oven to completely dissolve so as to obtain a clear liquid. After boiling, the temperature of the solution was allowed to cool down to about 50 °C after which 4-5 µL of ethidium bromide was added to the mixture and shaken well to homogenize. The gel was then carefully dispensed into the gel casting tray with a tightly fitted 20-well comb and the gel was allowed to solidify. After the solidification of the gel, the combs were carefully removed to avoid cracks in the gel. The prepared gel was carefully transferred into the gel tank/chamber containing the 1X TBE buffer used in its preparation and samples were loaded into the wells. The electrophoresis machine was then connected to an electric source. The gel was run at 100V for 1 hr. After 1 hr of separation, the gel was observed under UV light for the presence or absence of bands and the bands present were photographed using a UV-Pro Trans illuminator (Thermo Fisher Scientific, United Kingdom) camera.

**Statistical analysis:** One-way Analysis of Variance (one-way ANOVA) and percentage prevalence were performed using SPSS statistical software version 20.0 (IBM, Colorado, United States of America) to determine statistical significance for the distribution of Socio-demographic and clinical characteristics of the study participants, estimation of associations between virulence gene and tested antibiotics. A p-value of less than or equal to 0.05 was considered statistically significant.

## RESULTS

**Distribution of DEC pathotypes among the study population based on gender:** The distribution of diarrhoeagenic *Escherichia coli* pathotypes according to the gender of both diarrheic and non-diarrheic under-five children in Katsina State in this study is presented in Table 2. As can be seen, EAEC was the most frequently encountered pathotype among the study participants and occurred in both the diarrheic and non-diarrheic subjects (31.88%) and (4.35%), respectively and was higher in the case group than in the control group. There was a significant difference among the diarrheic and non-diarrheic subjects (p-value = 0.037). Also, EAEC was higher in female children in both case and control subjects (17.39 and 2.89%) than in male children (14.49 and 1.45%). There was however no significant difference between male and female subjects (p-value = 0.154).

**Distribution of DEC pathotypes among under five years diarrheic and non-diarrheic children in Katsina State according to age:** The distribution of diarrhoeagenic *Escherichia coli* pathotypes according to the age of both diarrheic and non-diarrheic under-five children in Katsina State in the study is shown in Table 3. As can be seen, EAEC occurred most among the study population, both the diarrheic and non-diarrheic subjects (31.88%) and (4.35%), respectively and was higher in the case group than in

Table 2: Distribution of DEC Pathotypes among under five years children in Katsina State according to gender

Parameters	Number examined	Pathotypes positive					p-value
		EPEC (%)	EAEC (%)	ETEC (%)	EIEC (%)	Total DEC n (%)	
<b>Diarrheic subjects</b>							
Gender							0.154
Male	28	0 (0)	10 (14.49)	1 (1.45)	1 (1.45)	12 (17.39)	
Female	41	5 (7.25)	12 (17.39)	2 (2.90)	2 (2.90)	21 (30.43)	
Total <sub>d</sub>	69	5 (7.25)	22 (31.88)	3 (4.35)	3 (4.35)	33 (47.83)	
<b>Control subjects</b>							
Gender							
Male	7	1 (1.45)	1 (1.45)	0 (0)	0 (0)	2 (2.89)	
Female	10	0 (0)	2 (2.89)	1 (1.45)	2 (2.89)	5 (7.25)	
Total <sub>c</sub>	17	1 (1.45)	3 (4.35)	1 (1.45)	2 (2.89)	7 (10.14)	
Grand Total	86	6 (8.69)	25 (36.23)	4 (5.79)	5 (7.25)	40 (57.97)	

EPEC: Enteropathogenic *E. coli*, EIEC: Enteroinvasive *E. coli*, EAEC: Enteroaggregative *E. coli*, ETEC: Enterotoxigenic *E. coli*, DEC: Diarrhoeagenic *Escherichia coli*, <: Less than, n: Number, d: Diarrheic and c: Control

Table 3: Distribution of DEC pathotypes among under five years diarrheic and non-diarrheic children in Katsina State according to age

Parameters	Number examined	Pathotypes positive					p-value
		EPEC (%)	EAEC (%)	ETEC (%)	EIEC (%)	Total DEC n (%)	
<b>Diarrheic age group (months)</b>							
0-12	28	3 (4.35)	11 (15.94)	1 (1.45)	1 (1.45)	16 (23.19)	0.045*
13-24	25	2 (6.45)	9 (13.04)	2 (6.45)	2 (6.45)	15 (21.74)	
>24-59	16	0 (0)	2 (6.45)	0 (0)	0 (0)	2 (6.45)	
Total <sub>d</sub>	69	5 (7.25)	22 (31.88)	3 (4.35)	3 (4.35)	33 (47.83)	
<b>Control age group (months)</b>							
0-12	6	0 (0)	1 (1.45)	1 (1.45)	2 (6.45)	4 (5.79)	
13-24	6	1 (1.45)	2 (6.45)	0 (0)	0 (0)	3 (4.35)	
>24-59	5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Total <sub>c</sub>	17	1 (1.45)	3 (4.35)	1 (1.45)	2 (2.65)	7 (10.14)	
Grand total	86	6 (8.69)	25 (36.23)	4 (5.79)	5 (7.25)	40 (57.97)	

EPEC: Enteropathogenic *E. coli*, EIEC: Enteroinvasive *E. coli*, EAEC: Enteroaggregative *E. coli*, ETEC: Enterotoxigenic *E. coli*, DEC: Diarrhoeagenic *Escherichia coli*, <: Less than, n: Number, d: Diarrheic and c: Control

the control group. The EAEC was most prevalent among diarrheic children in the 0-12-months age group (17.39%), while it was most prevalent (6.45%) among non-diarrheic subjects in the 13-24 month's age group. A significant difference existed among the age groups (p-value = 0.045).

## DISCUSSION

The findings of this study revealed a high frequency of diarrhoeagenic *Escherichia coli* pathotypes among children younger than five years in Katsina State. The study was the first to characterize *E. coli* into strain levels in Katsina State. It shows that enteroaggregative *E. coli* is the most prevalent diarrhoeagenic pathotype recorded in the State (31.88%) and thus, the pathotype that circulates most among children under 5 years of age.

Diarrhoea is one of the leading causes of morbidity and remains the second leading cause of death in children younger than 5 years globally, particularly in developing countries, accounting for 1.3 million deaths annually<sup>13,14</sup>. Diarrhoeagenic *Escherichia coli* (DEC) is one of the major causes of diarrhoea in Nigeria<sup>15</sup>.

In this study, the prevalence of DEC pathotypes was significantly higher among children with diarrhoea (47.83%) than those without diarrhoea (10.14%). Similar results have also been reported from other parts of Nigeria, for example, from Kano State, FCT Abuja, Ile Ife, Osun State and of course, from other countries

across the world, including Switzerland, Iraq, Burkina Faso<sup>2,15-17</sup>. The ETEC, EAEC and EPEC strains of *E. coli* are the most commonly encountered of all the strains<sup>15</sup>.

The prevalence of *E. coli* pathotypes was higher in the diarrheic group (47.83%) than in the non-diarrheic group (10.14%). The distribution of the pathotypes among the various children age groups in this study showed that children in the age group 0-12 months had the highest prevalence rate (23.19%) followed by participants in the 13-24 months age class in the diarrheic category (21.74%) and was higher among non-diarrheic children in 0-12 months age group (5.79%). The DEC pathotypes were also most prevalent in female children (30.43%) than in male children (17.39%), (7.25%) and (2.89%) for both diarrheic and non-diarrheic groups, respectively. This is however dissimilar with 44.80 and 44.40% overall DEC prevalence in the 0-12 month age group for both case and control subjects, respectively, reported by Saka *et al.*<sup>2</sup> in a study conducted in Kano State, Nigeria.

The results of the current study indicated that EAEC (31.88%) is the most frequently detected diarrhoeagenic *E. coli* pathotype among under five diarrheic children in Katsina State and 4.35% among non-diarrheic group followed by EPEC (7.25%) and (1.45%), respectively. This correlates with the findings of Saka *et al.*<sup>2</sup>, who reported 36.75% frequency of EAEC among diarrheic and 33.3% among non-diarrheic children younger than 5 years in Kano State and that of Onanuga *et al.*<sup>15</sup> in a study conducted at Gwagwalada, FCT, Abuja, who reported a prevalence of EAEC pathotype (34.40%). The result of the study is however contrary to the findings of Mandeel *et al.*<sup>14</sup> in their study conducted on diarrhoeagenic *E. coli* among young children in Iraq who reported the lowest EAEC (5.30%) pathotype prevalence and higher than 11.90% prevalence of EAEC recorded by Pabst *et al.*<sup>16</sup> in a study on the prevalence of diarrhoeagenic *E. coli* among under-five children in Switzerland. The high frequency of DEC pathotypes (EAEC and EPEC) among the study population could be attributed to the fact that a high proportion, about half of the population, of the children had mixed feeding patterns (50.00%) instead of exclusive breastfeeding which, could have protected the children in the first six months of their life by antibodies in their mothers' milk from getting infected by the diarrhoeagenic *E. coli* and other agents implicated in diarrhoea.

Further research should be conducted to establish the main circulating diarrhoeagenic *Escherichia coli* pathotypes among young children in Katsina State by exploring other sample sources other than stool samples, for example, by screening rectal swabs of children younger than five years of age. A more methodological work, for example, one involving the use of next-generation sequencing, is needed on how to robustly characterize diarrhoeagenic *E. coli* isolated from children under five years in the State.

## CONCLUSION

The findings of this study revealed that enteroaggregative *E. coli* (EAEC) and enteropathogenic *E. coli* (EPEC) are the most circulating DEC pathotypes among under five years children in Katsina State. Enteroaggregative *E. coli* was found to resist treatment with three or more antibiotics. Further research should be conducted to establish the main circulating diarrhoeagenic *Escherichia coli* pathotypes among young children in Katsina State by exploring other sample sources other than faecal specimens, for example, by screening rectal swabs of children younger than five years of age. Parents should maintain good personal and environmental hygiene, for example, hand washing, use of clean cooking utensils and sanitation which is key to the prevention of diarrhoea.

## SIGNIFICANCE STATEMENT

At the time of embarking on this research, there were few available published literature on bacterial diarrhoea among young children in Katsina State, especially one caused by diarrhoeagenic *E. coli*. The study therefore, was carried out to investigate the occurrence and frequency of diarrhoeagenic *E. coli* pathotypes as a cause of infectious diarrhoea in children below 5 years of age in Katsina State, as well as their antibiotic susceptibility profile. The findings of the study revealed that enteroaggregative *E. coli* is

the most frequently encountered *E. coli* pathotype among children under five years of age in Katsina State and was found to resist treatment with three or more antibiotics.

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