JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Yearly incidence of acute childhood gastroenteritis in Nigeria: Implicated pathogens predominantly harbor *bla$_{CTXM}$* and *bla$_{TEM}$* genes

Ebuka Elijah David[1,2], Ikechuku Okorie Igwenyi[2], Ifeanyichukwu Romanus Iroha[3], Eghosa Lucky Emumwen[4], Christian Emmanuel Offor[2], Okechukwu Jerry Orji[3], Franklyn Chinwe Igwe[2], David Chukwu Obasi[5], Jeff Onochie Nkama[6], Boniface Oke[3], Moses Eji Ogbanshi[1]

[1] *Department of Biochemistry, Alex Ekwueme Federal University, Ndufu-Alike, Ikwo, Nigeria*
[2] *Department of Biochemistry, Ebonyi State University, Ebonyi State, Nigeria*
[3] *Department of Microbiology, Ebonyi State University, Ebonyi State, Nigeria*
[4] *Department of Pediatrics, St Patrick's Hospital, Mile 4 Abakaliki, Ebonyi State, Nigeria*
[5] *Department of Biochemistry, Evangel University, Akaeze, Ebonyi State, Nigeria*
[6] *Akanu Ibiam Federal Polytechnic, Unwana Afikpo*

Abstract

Background and Objectives: Routine use of antibiotics for infectious diarrhea in children is associated with the risk of increasing antibiotic resistance in developing countries. This work aimed to study the predominant extended spectrum beta-lactamase (ESBL) genes among bacteria pathogens implicated in acute childhood gastroenteritis in a tertiary hospital in Nigeria.

Materials and Methods: The stool samples of children diagnosed with acute gastroenteritis were collected. Isolation and identification of bacterial pathogens from the stool samples using standard microbiological and molecular sequencing methods. Pure cultures of the probable bacteria pathogens were subjected to antibiotics susceptibility profiling using the Kirby-Bauer Disk Diffusion Method and also screened for ESBL and AmpC using the Modified Double Disc Synergy Test. Primers for 5 different ESBL genes associated with beta-lactam antibiotic resistance were amplified and sequenced.

Results: Out of the 62 isolates, the highest number of organisms identified within the isolates were *Bacillus sp* at 38.7% (24) followed by *Alcaligenes sp* at 37% (23). Resistance to cefepime and ceftazidime were recorded at 50.8% (30) each. Ceftriaxone and cefotaxime were resisted in 47.4% (28) of the isolates. Out of 34 isolates resistant to all the cephalosporins used, 41.2% (14) were ESBL-producing, of which *bla$_{CTXM-1}$* and *bla$_{CTXM-2}$* were detected in 85.7%, while *bla$_{TEM}$* was seen in 64.3%.

Conclusions: *bla$_{CTXM}$ and bla$_{TEM}$* may be the predominant ESBL genes haboured in the bacteria pathogens implicated in the yearly incidence of acute childhood gastroenteritis in Nigeria. This may be due to the widespread use of antibiotics in treating this disease.

Key words: *Bacillus sp*; emesis; gastroenteritis; enterotoxigenic; extended-spectrum beta-lactamases; gastroenteritis.

J Infect Dev Ctries 2025; 19(1):40-48. doi:10.3855/jidc.18716

(Received 10 June 2023 – Accepted 22 December 2023)

Introduction

Gastroenteritis is an inflammation of the lining of the intestine caused by a virus, bacteria, or parasites. It causes watery diarrhea, pain or abdominal cramps, nausea, vomiting, and sometimes fever [1]. Worldwide, about 3-5 billion cases of acute gastroenteritis occur yearly with nearly 2 million deaths in children below 5 years. This makes diarrhea disease the fifth leading cause of death in children and the eighth leading cause of death among all ages [2]. Nigeria is one of the two countries accounting for 42% of global deaths attributable to gastroenteritis in children under five years [3]. Due to the high mortality rate of gastroenteritis in children, treatment decisions are mostly made on clinical outcomes while the choice of antimicrobial drugs is empirically made based on narrow drugs that cover most of the possible pathogens [4]. The necessity for this empirical treatment method is because; the cost-effective means of identifying these pathogens involve conventional stool culture and antimicrobial susceptibility testing of isolated organisms which usually takes not less than 72 hours. The use of ciprofloxacin (fluoroquinolone) was recommended by WHO as the first-line drugs in the treatment of bloody diarrhea [5]. Empirically, ceplalosporins, especially cefixime and ceftriaxone are considered the best treatment for acute childhood gastroenteritis [6]. However, reports have confirmed that ciprofloxacins are equally safe to be used in pediatrics with some restrictions, like in severe cases [7].

Unless in severe and in bloody diarrhea cases, antibiotics are not prescribed in the treatment of acute childhood diarrhea disease [8]. Routine use of antibiotics for infectious diarrhea in children is associated with the risk of increasing antibiotic resistance in developing countries [4,9,10]. Without consideration to the WHO guidelines, ciprofloxacin (fluoroquinolone) and cephalosporins (cefriaxone and cefepime) are routinely used by physicians in the treatment of this disease in children in Nigeria [11-13]. Unlike in developed nations, intravenous rehydrations are commonly used [14-16]. This indiscriminate use of antibiotics has resulted in the emergence of both multidrug and cephalosporins (extended spectrum beta-lactamase, ESBL) resistant enteric pathogens [17]. Center for Disease Control has categorized ESBL-producing *Enterobacteriaceae* as an "urgent threat" [18]. Multidrug-resistant and ESBL producing pathogens isolated from diarrhea stools have been reported in developing countries [19-26]. ESBL-producing *enteropathogens*, $bla_{CTX\text{-}M}$ type, amongst hospitalized preschool children with gastroenteritis have been characterized as most prevalence in Bilhar, India, Poland, and Ethiopia [26-28]. bla_{TEM} and bla_{OXA} were found to be the most common amongst isolated pathogens from the stools of diarrheal children in Ghana and Burkina Faso [29,30]. In Nigeria, there is a scarcity of data with reference to the prevalence of ESBL genotypes in *enteropathogens* implicated in acute childhood diarrhea. While the prevalence of ESBL-producing pathogens from diarrheal stools of children have been reported in Nigeria, the results were only based on phenotype. This work aimed to study the predominant ESBL genes among bacteria pathogens implicated in acute childhood gastroenteritis in a tertiary hospital in Abakaliki, Ebonyi State, Nigeria.

Materials and Methods

Sample collection

The study recruited stool samples of 62 children between the ages of 0-5 years who were diagnosed with gastroenteritis from January to March 2020. These months covered the window period for the yearly outbreak of gastroenteritis in Abakaliki, Ebonyi State, Nigeria. The stools were collected from the pediatrics unit of the hospital. Ethical clearance (Ref.No: RE/M4H/48/19) was obtained from Ethical and Research Committee of the hospital, after which informed consent was obtained from the parents/guardians/attendants of the children. Stool samples were immediately transported in ice pack to the Microbiology Laboratory, Alex Ekwueme Federal University, Ndufu-Alike, for isolation of bacteria.

Antibiogram tests and Double-disk synergy test (DDST)

Antibiotics susceptibility testing was performed by the Disk Diffusion Method according to the Clinical and Laboratory Standards Institute guidelines (CLSI) on Muller-Hinton (MH) agar. After 24 hours of incubation at 37 ºC, the microorganisms were tested for their susceptibility to ciprofloxacin (5 µg), levofloxacin (5 µg), cefepime (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), amoxicillin-clav (20/10 µg), ampicillin (10 µg), imipinem (10 µg), fosfomycin (200 µg). The CLSI Performance Standards for these antibiotics were used for interpretation (Supplementary Table 1).

Isolates with zone of inhibition diameter less than 27 mm for cefotaxime and 25 mm for ceftriaxone were selected for DDST. A modified DDST (Modified Double Disc Synergy Test) with the use of cefepime (4^{th} generation cephalosporin) improved the detection of ESBL-producing isolates which co-produce AmpC as described previously. Briefly, the lawn culture of the isolates was made on a Mueller-Hinton agar plate while placing an AMC (20/10 µg) disc at the center of the plate. The discs of 3GC-cefotaxime, ceftriaxone, ceftazidime, and 4GC-cefepime were placed 15 mm and 20 mm apart respectively, center to center to that of the AMC. Any increase in the zone towards the disc of AMC was considered positive for ESBL production.

Genomic DNA extraction and amplification of 16S rRNA region

The genomic DNA was extracted using the Wizard Genomic Purification kit (Promega, USA) as described in the manufacturer's instructions. The DNA solutions were stored at 4 °C until electrophoresis. The bacterial pathogens were identified by amplification of the 16S rRNA V_2-V_4 hypervariable regions which are approximately 1500 bp using specific primers (Supplementary Table 2) as previously described. A total volume of 25 µL reaction mixture were as follows: 8.5 µL deionized diethylpyrocarbonate (DEPC) treated and 0.22 µm membrane-filtered water (ThermoFisher, USA), 12. 5µL 2X KAPA HiFi Hot Start Ready Mix (KAPA Biosystems, South Africa), 1 µL each of forward and reverse primers, and 2 µL (5 ng/µL) template DNA. The amplification conditions were programmed in a GeneAmp PCR System 9700, Applied Biosystem, USA as follows: initial denaturation at 95 ºC for 3 minutes, followed by 25 cycles of denaturation

at 95 ºC for 30 seconds, annealing temperature of primers at 55 ºC for 30 seconds and extension at 72 ºC for 30 seconds. The final extension was conducted at 72 ºC for 5 minutes. PCR reactions were kept at 4 ºC until electrophoresis and sequencing reactions.

Amplification of ESBL genes

ESBL genes (including *blaSHV*, *blaTEM*, *blaCTX-M-1*, *blaCTX-M-2*, and *blaCTX-M-9*) were amplified using specific primers (Supplementary Table 2). The PCR parameters were as follows in a total volume of 25 µL: initial denaturation at 94 °C for 5 minutes followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 60, 50, 52, 55, and 57 respectively for 30 seconds, and extension at 72 °C for 40 seconds, with a final extension step at 72 °C for 5 minutes.

Purification and eluting of amplified products

A MinElute PCR Purification Kit with Cat number 28004 (Qiagen, Brazil) was used to purify and elute the amplified product according to manufacturer's instructions. Briefly, 125 µL of Buffer PB was added to 25 µL PCR reaction. The PCR reaction samples were applied to a MinElute column and centrifuged for 1 minute at 13,000 rpm (17,900 × g). The flow-through was discarded and the MinElute column was placed back into the same tube. To wash, 750 µL Buffer PE was added to the MinElute column and centrifuged as above. The flow-through was discarded and the MinElute column placed back in the same tube. Additional centrifugation was performed as above and the MinElute column placed in a clean 1.5 mL microcentrifuge tube. To elute the amplicon, 10 µL Buffer EB was added directly to the center of the membrane and allowed to stand for 1 minute before centrifuging as above. The average eluted volume was 9 µL.

Table 1. Taxonomic identification of 62 stool isolates based on 16S rRNA V3-V4 sequence.

Taxonomic identification	Number of isolates
Bacillus sp	24
Alcaligenes sp	23
Lysinibacillus sp	2
Enterococcus faecalis	2
Providencia sp	2
Bacterium	1
Proteus sp	2
Psychrobacter	1
Brevundimonas sp	1
Burkholderia sp	1
Lost sample	3

Agarose gel electrophoresis

Bacterial DNA samples and PCR amplicons were analyzed by electrophoresis in 2% agarose gels in TBE buffer at 100V using GIBCO-BRL Electrophoresis Power System Model 250 (Life Technologies, UK) for 30 minutes. The gels were stained with 5 µL ethidium bromide (10 mg/mL). A mixture of 4 µL Loading dye (PCRBIO 6x Sample Loading Buffer A, PCR Biosytems, USA) and 2 µL DNA samples were loaded in the gel. The DNA ladder (PCRBIO Ladder III, PCR Biosytems, USA) was used as reference. A MiniBis gel documentation system (DNR Bio-Imaging System, Isreal) was used to photograph the gel.

Sanger Sequencing

Forward and reverse sequence reactions were prepared in a total volume of 15 µL following the protocol described in the BigDye Terminator v3.1 Cycle Sequencing Kit User Guide. The PCR products were diluted to 20ng/µL and used to prepare the 0.5X diluted sequencing reaction: containing 8.8 µL ultra-pure water, 3 µL 5X BigDye Terminator Sequencing Buffer, 1 µL 2.5X BigDye Terminator Ready Reaction Mix (Life Technologies, USA), 1.2 µL primer and 1 µL template DNA. The sequencing reactions were run in a thermal cycler, GeneAmp PCR System 9700, Applied Biosystem, USA as follows: incubation at 96 ºC for 2 minutes, followed by 35 cycles of denaturation at 96 ºC for 45 seconds, annealing temperature of primers at 55 ºC for 30 second and extension at 60 ºC for 4 minutes and held at 12 ºC. Purification was done with 5 µL 125 mM EDTA solution and 60 µL 70% ethanol. Genetic Analyser 3130 xl sequencer from Applied Biosystems was used for Sanger sequencing.

Results

Molecular identification of isolates

The number of organisms identified within the isolates were *Bacillus sp* at 38.7% [24] followed by *Alcaligenes sp* at 37% [23]. *Proteus spp*, *Providencia spp* and *Enterococcus feacalis* were 3.2% [2] each. Three isolates (6.5%) were unidentified while another three were lost (Table 1). The BLAST analysis results

Table 2. Antibiotics resistance profile of isolates (n = 59).

Antibiotics (µg)	Resistance (%), N
CIP	1.7 [1]
LEV	1.7 [1]
CPM	50.8 [30]
CRO	47.4 [28]
CTX	47.4 [28}
CTZ	50.8 [30]
IPM	0 [0]

CIP: Ciprofloxacin; LEV: Levofloxacin; COM: Cefepime; CRO: Ceftriaxone; CTX: Cefotaxime; CTZ: Ceftazidime; IPM: Imipenem.

Table 3. Drug resistance per organism (n = 59).

Organisms	CIP, % [N]	LEV, % [N]	CPM, % [N]	CRO, % [N]	CTX, % [N]	CTZ, % [N]
Bacillus sp [24]	0 [0]	0 [0]	54 [13]	54 [13]	54 [13]	54 [13]
Alcaligenes sp [23	0 [0]	0 [0]	57 [13]	57 [13]	57 [13]	57 [13]
Lysinibacillus sp [2]	50 [1]	50 [1]	50 [1]	50 [1]	50 [1]	50 [1]
Enterococcus faecalis [2]	50 [1]	50 [1]	50 [1]	50 [1]	50 [1]	50 [1]
Providencia sp [2]	50 [1]	50 [1]	50 [1]	50 [1]	50 [1]	50 [1]
Bacterium [1]	100 [1]	100 [1]	100 [1]	100 [1]	100 [1]	100 [1]
Proteus sp [2]	50 [1]	50 [1]	50 [1]	50 [1]	50 [1]	50 [1]
Psychrobacter [1]	100 [1]	100 [1]	100 [1]	100 [1]	100 [1]	100 [1]
Brevundimonas sp [1]	100 [1]	100 [1]	100 [1]	100 [1]	100 [1]	100 [1]
Burkholderia sp [1]	100 [1]	100 [1]	100 [1]	100 [1]	100 [1]	100 [1]

CIP: Ciprofloxacin; LEV: Levofloxacin; COM: Cefepime; CRO: Ceftriaxone; CTX: Cefotaxime; CTZ: Ceftazidime; IPM: Imipenem.

with obtained accession numbers can be seen in Supplementary Table 3.

Antibiotics resistance profile of isolates

Having lost 3 isolates, 59 were subjected to antibiotics susceptibility testing. In Table 2, only 1.7% [1] of the 59 isolates was found to be resistant to ciprofloxacin and levofloxacin, second and third generation fluoroquinolones. Resistance to cefepime, a fourth-generation cephalosporin and ceftazidime, a third-generation were recorded at 50.8% [30] each. Ceftriazone and cefotaxime, third-generation cephalosporins were resisted in 47.4% [28] of the isolates. No isolate was found to be resistant to imipenem. As seen in Table 3, about 54% [13] of the 24 *Bacillus sp* isolates were found to be resistant to all the β-lactam drugs used while 57% [13] of the 23 *Alkaligenes sp* were resistant.

Extended beta-lactamase production

Out of the 34 isolates resistant to the cephalosporins, the double disc synergy test revealed that 47.1% [16] were non-ESBL producing while 41.2% [14] were ESBL-producing. Four isolates representing 11.8% were seen to produce both ESBL and cephalosporin hydrolyzing enzyme, AmpC (Table 4). Out of the 14 ESBL producing isolates, 64.3% [9] was *Bacillus sp* while 35.7% [5] was *Alkaligene sp*. Out of the four isolates that showed the ability to co-produce ESBL/AmpC, 50% [2] was *Bacillus sp* while the other 50% [2] was *Alkaligene sp*.

Detection of extended spectrum beta-lactamase genes

In Table 5, the percentage detection of ESBL genes in the 14 ESBL-producing isolates was shown. bla_{CTXM-1} $bla_{TEM,}$ and bla_{CTXM-2} genes were detected in 85.7% [12], 85.7% [12], and 64.3% [9] respectively. bla_{CTXM-9} and bla_{SHV} were the least detected in 35.7% [5] each.

Discussions

Empirical treatment of childhood gastroenteritis and gender distribution

The high mortality rate of gastroenteritis in children has led to treatment decisions mostly made on clinical outcomes. Hence, empirical treatment with antimicrobial drugs is based on narrow drugs that cover most of the possible pathogens [4]. In this health facility, while a higher number of children was treated without antibiotics, the guidelines established by WHO on the use of antibiotics were not fully followed. Ciprofloxacin (fluoroquinolone) was only recommended by WHO as the first-line drug in the treatment of bloody diarrhea [5]. While the hospital rightly used ceftriaxone which is considered the best treatment for acute childhood gastroenteritis [6], they equally used cefepime, a fourth-generation

Table 4. ESBL-production profile of the isolates.

TOTAL (n = 34)	NON-ESBL	ESBL	ESBL/AmpC
	47.1% [16]	**41.2% [14]**	**11.8% [4]**
Bacillus sp	25% [4]	64.3% [9]	50% [2]
Alkaligene sp	50% [8]	35.7% [5]	50% [2]
Other organisms	25% [4]	0% [0]	0% [0]

Table 5. Detection of *ESBL* genes in the isolates.

Genes	*Bacillus sp*	*Alkaligenes sp*	Total
CTXM-1	6 [42.9%]	6 [42.9%]	12 [85.7%]
TEM	7 [50%]	5 [35.7%]	12 [85.7%]
CTXM-9	5 [35.7%]	0 [0.0%]	05 [35.7%]
SHV	5 [35.7%]	0 [0.0%]	05 [35.7%]
CTXM-2	6 [42.9%]	3 [21.4%]	09 [64.3%]

cephalosporin which was not recommended (Supplementary Figure 1). Routine use of antibiotics for infectious diarrhea in children is associated with the risk of increasing antibiotics resistance in developing countries [4,9]. In a multi-stage random sampling study involving different health facilities in Rivers State, Nigeria, antibiotics were prescribed in 78.6% of the children with acute gastroenteritis. Unlike our result, metronidazole was prescribed in 50.9% of the cases while ceftriaxone was used in 0.2% [41]. In another tertiary institution in Abakaliki Nigeria and Accra Ghana, 88.9% and 95% of the children were treated with antibiotics respectively [11,42]. In an observational study of 210 children with acute gastroenteritis in Enugu, Nigeria, the use of antibiotics was found to start from the caregivers even before hospital admission, where 46.7% of the children were given un-prescribed antibiotics [13].

Isolates and antimicrobial resistance

The number of organisms identified within the isolates in this study was *B. cereus* at 38.7% [24] followed by *Alcaligenes feacalis* at 37% [23]. *Proteus spp*, *Providencia spp*, and *Enterococcus feacalis* were seen in 3.2% [2] each. Though a neglected human soil pathogen, *B. cereus* is responsible for two types of gastrointestinal diseases; the emetic type characterized by nausea and vomiting, and the diarrheal form, which manifests as frequent watery stools and abdominal cramps [43]. The incidences of toxigenic *B. cereus* in fecal samples from infants and children with diarrhea cases have been reported in developing countries [44]. Yearly incidences of acute childhood gastroenteritis that occur in Nigeria coincide with the "dry season" (November to March), characterized by Harmattan haze, which carries a large amount of dust particles. This dust particle may act as a vehicle of the transmission of *B. cereus* from the soil into food particles. Hence, *B. cereus* may be responsible for a greater number of yearly incidence of acute childhood gastroenteritis that goes unreported in Nigeria. *Alcaligenes feacalis,* though usually considered a harmless human intestinal saprophyte, mostly cause opportunistic infections in humans ranging from cystitis, diabetic foot infections, pneumonia, acute pyelonephritis, bacteremia [45]. Hence*, A. feacalis* has been established to be pathogenic. Infections with *A. feacalis* in infants and children have been sufficiently rare to warrant reporting its occurrence. However, there have been reports of meningitis, bacteremia, and bloody diarrhea in children and infants [46]. While *A. feacalis* has not been reported around childhood gastroenteritis in Nigeria, it has been consistently described as being an extensively drug-resistant bacteria species [47]. Similar to our result*, Proteus spp* has been implicated in acute childhood gastroenteritis in Nigeria. In a descriptive and cross-sectional study involving 135 and 222 children with gastroenteritis in Ilorin and Ile-Ife, Nigeria, respectively, *Proteus spp* was implicated in 3.7% and 2.2% of the cases, respectively [48-49]. In Abeokuta, Nigeria, and Ethiopia, a higher incidence (9% and 9.7%, respectively) of the cases involving *Proteus spp* in acute childhood gastroenteritis was reported [50-51]. While many children die yearly as a result of acute gastroenteritis, *Proteus spp* has not been reported and implicated as one of the etiology of this disease, majorly due to a lack of proper molecular identification techniques in developing countries. Similar to *Proteus spp*, *Providencia spp* also has a low prevalence in acute gastroenteritis both in this study and elsewhere. In Japan and Bangladesh, the prevalence of *Providencia spp* among diarrheal children was found to be 1.4% and 2.1% respectively [52-53]. In Nigeria, there is scarcity of reports implicating *Providencia spp* in childhood gastroenteritis. One of the few available reports was from Ondo, Nigeria, where 2.4% prevalence rate was found [54]. *Enterococci* are opportunistic pathogens that may cause various infections like urinary tract infections, sepsis, bacteremia, and endocarditis outside of their typical commensal gut habitats [55]. Although *Enterococcus feacalis* is mostly a hospital-acquired infection, its contamination in drinking water and food has been reported in South East Nigeria and may contribute to the etiology of gastroenteritis [56].

The indiscriminate use of antibiotics has resulted in the emergence of both multidrug and cephalosporins (extended spectrum beta-lactamase, ESBL) resistant enteric pathogens [17]. Center for Disease Control has categorized ESBL-producing *Enterobacteriaceae* as an "urgent threat" [18]. Multidrug-resistant and ESBL producing pathogens isolated from diarrhea stools have been reported in developing countries [19-25] In this study, out of the 36 isolates resistant to the cephalosporins, double disc synergy test revealed that 47.2% [17] were non-ESBL producing while 41.7% [15] were ESBL-producing. Four isolates representing 11.1% were seen to produce both ESBL and cephalosporin hydrolyzing enzyme, AmpC. Out of the 15 ESBL producing isolates in this study, 60% [9] was *Bacillus sp* while 40% [6] was *Alkaligene sp*. Four of the isolates showed the ability to co-produce AmpC, out of which 50% [2] was *Bacillus sp* and 50% [2] was *Alkaligene sp*. Extended-spectrum β-lactamase

(ESBL)-producing pathogens majorly cause resistance to expanded-spectrum β-lactam antibiotics. Their worldwide spread and isolation from both community-acquired and hospital-associated infections makes them a source of global public health concern [57]. ESBLs are class A serine β-lactamases with the ability to hydrolyse expanded spectrum β-lactam antibiotics, and inhibition by β-lactamase inhibitors, like clavulanate. While they confer resistance to most β-lactam antibiotics, including cephalosporins and monobactams, they are susceptible to carbapenems and cephamycins [58].

Extended beta lactamase resistance genes

In this study, amplification of *ESBL* genes from a total of 14 *Bacillus sp* and *Alkaligene sp.* ESBL-producing isolates show that *bla*$_{CTXM-1}$, *bla*$_{CTXM-2}$ and *bla*$_{TEM}$ genes were mostly detected while *bla*$_{SHV}$ was the least detected. As the *bla*$_{CTX-M}$-type β-lactamases became the most predominant ESBL worldwide, the frequency of *bla*$_{TEM}$-type enzymes was reduced. In a survey of European isolates, *bla*$_{TEM}$-type ESBLs were detected in less than 1% of ESBL-producing *E. coli* and *Klebsiella pneumoniae* [59]. In clinical isolates of 44 *E. coli* from out-patients in a teaching hospital in Nigeria, *bla*$_{CTXM-1}$ was also found to be the most prevalent, followed by *bla*$_{TEM}$, while *bla*$_{SHV}$ was not detected [60]. Similarly, in India, clinical isolates of *E. coli* and *Klebsiella spp* were shown to habour *bla*$_{CTX-M}$ (82.5%), *bla*$_{TEM}$ (67.5%), and *bla*$_{SHV}$ (57.5%) [61]. Unlike our result, in Kuala Lumpur, Malaysia, clinical isolates of *Alkaligene sp* were reported to predominantly harbor *bla*$_{TEM}$ ESBL [62]. In a survey of raw and pasteurized milk samples contaminated with *B. cereus*, *bla*$_{TEM}$ type of ESBL was mostly found in *B. cereus* from raw milk while *bla*$_{CTX-M}$ type was mostly found in pasteurized milk [63]. In a recent cross-sectional study of 122 diarrheal patients in Accra Ghana where *E. coli* was the predominant pathogen (67.2%), analysis of *ESBL* genes revealed that *bla*$_{TEM}$ was the most common, followed by *bla*$_{CTX-M}$ and bla$_{SHV}$ [29]. Manda *et al*. determined and characterized ESBL-producing *E. coli* pathotypes from 633 randomly selected diarrheal stools of hospitalized preschool children living in low socioeconomic level communities in Bihar India. The most common β-lactamase was *bla*$_{CTX-M}$ followed by *bla*$_{SHV}$ and *bla*$_{TEM}$ [26]. Another study involving the stools of children with acute diarrhea in Poland with *E. coli* as the predominant pathogen also detected *bla*$_{CTX-M}$ as the major β-lactamase [27]. In Nigeria, there is scarcity of report where β-lactamase genes were detected in pathogens implicated in acute childhood gastroenteritis. Most research was limited to phenotypic detection of ESBL-producing isolates. However, in Kano State Nigeria, the prevalence of phenotypic and genotypic ESBLs in 296 *E. coli* isolates from stools of diarrhea children younger than 5 years were determined. Similar to our result, *bla*$_{CTX-M}$ and bla$_{TEM}$ were found in 73.3% of the isolates while bla$_{SHV}$ was the least predominant in 6.66% of the isolates [34]. Again, in a systematic review and meta-analysis to identify different *ESBL* genes reported in published literature from Nigeria, *bla*$_{CTX-M}$ was reported to be the predominant gene [35].

Conclusions

Yearly incidences of acute childhood gastroenteritis that occur in Nigeria coincide with the "dry season" (November to March) characterized by Harmattan haze. Within this period, there is a sudden increase in acute childhood gastroenteritis in children below 5 years which normally leads to death. The hamattan haze carries dust particles which may act as vehicles of transmission of pathogenic organisms from the soil into food particles. For the first time in Nigeria, our study used a molecular biology approach to establish that this yearly incidence of gastroenteritis may be mediated by *Bacillus sp* and *Alcaligene sp.* These organisms were found to show extended spectrum beta lactamase activities against the second, third, and fourth generations of cephalosporins routinely used in the treatment of gastroenteritis. This may be due to their empirical use in the treatment of childhood acute gastroenteritis.

Funding

This research was funded by International Center for Genetic engineering and Molecular Biology (ICGEB) under Arturo Falaschi ICGEB Fellowship with funding number S_NGA20-01-1. The funders had no role in study design, data collection, and analysis; in the decision to publish; or in the preparation of the manuscript.

Authors' contributions

All authors contributed to the study concept and design. Conceptualization, E.E.D, I.O.I and I.R.I.; methodology, E.E.D, E.L.E and C. E. O; formal analysis, E.E.D, O. J. O, M. E. O and F. C. I.; investigation: E.E.D, J. O. N, B. O, and D. C. O.; writing—original draft preparation, E.E.D.; writing—review and editing, I. O. I and I. R. I.; supervision, I.O.I and I.R.I.; funding acquisition, E.E.D. All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Ethics Committee of St Patrick's Hospital (RE/M4H/48/19).

Data availability

The gene sequences have been deposited in DDBJ/ENA/GenBank databases and publicly available under the following accession numbers: OP113777-OP113790, OP131838-OP131851, OP132416-OP132419, OP164754, OP216524-OP216525, OP219795-OP219796, OP650091-OP650109.

Corresponding author

Dr Ebuka Elijah David
Department of Biochemistry, Alex Ekwueme Federal University Ndufu-Alike,
PMB 1010 Abakaliki, Ebonyi State, Nigeria
Tel: +2348033188823
Email: david.ebuka@funai.edu.ng

Conflict of interests

No conflict of interests is declared.

References

1. Hartman S, Brown E, Loomis E, Russell HA (2019) Gastroenteritis in children. Am Fam Physician 99: 159–165.
2. Troeger C, Blacker BF, Khalil IA, Rao PC, Cao S, Zimsen SRM, Albertson SB (2018) Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect Dis 18: 1211–1228. doi: 10.1016/S1473-3099(18)30362-1.
3. Arowolo KO, Ayolabi CI, Lapinski B, Santos JS, Raboni SM (2019) Epidemiology of enteric viruses in children with gastroenteritis in Ogun State, Nigeria. J Med Virol 91: 1022–1029. doi; 10.1002/jmv.25399.
4. Diniz-Santos DR, Silva LR, Silva N (2006) Antibiotics for the empirical treatment of acute infectious diarrhea in children. Brazilian J Infect Dis 10: 217–227. doi: 10.1590/S1413-86702006000300011.
5. World Health Organization (2005) Guidelines for the control of shigellosis, including epidemics due to Shigella dysenteriae type 1. Available: https://www.who.int/publications/i/item/9241592330. Accessed: 20 Feb 2024.
6. Lieberman JM (2003) Appropriate antibiotic use and why it is important: the challenges of bacterial resistance. Pediatr Infect Dis J 22: 1143–1151. doi: 10.1097/01.inf.0000101851.57263.63.
7. Bruzzese E, Giannattasio A, Guarino A (2018) Antibiotic treatment of acute gastroenteritis in children. F1000 Res 7: 193. doi: 10.12688/f1000research.12328.1
8. Qureshi S, Resham S, Hashmi M, Naveed AB, Haq Z, Ali SA (2021) A retrospective review on antibiotic use in acute watery diarrhea in children in a tertiary care hospital of Karachi, Pakistan. PLoS one 16 :e0253712. doi: 10.1371/journal.pone.0253712.
9. Britto CD, Wong VK, Dougan G, Pollard AJ (2018) A systematic review of antimicrobial resistance in *Salmonella enterica* serovar Typhi, the etiological agent of typhoid. PLoS Negl Trop Dis 12: e0006779. doi: 10.1371/journal.pntd.0006779.
10. Ayukekbong JA, Ntemgwa M, Atabe AN (2017) The threat of antimicrobial resistance in developing countries: causes and control strategies. Antimicrob Resist Infect Control 6: 1–8. doi: 10.1186/s13756-017-0208-x.
11. Efunshile AM, Ezeanosike O, Nwangwu CC, König B, Jokelainen P, Robertson LJ (2019) Apparent overuse of antibiotics in the management of watery diarrhoea in children in Abakaliki, Nigeria. BMC Infect Dis 19: 1–7. doi: 10.1186/s12879-019-3899-1.
12. David EE, Igwenyi IO, Iroha IR, David CN, Mbah PC, Okpala OF, Ukeh NU, Ogbaji O, Ugwurauma CE, Chukwu GC (2021) Trends in empirical treatment of hospitalized children with acute gastroenteritis in Nigeria. Recent Adv Anti-Infective Drug Discov 16: 237–244. doi: 10.2174/2772434416666211022155438.
13. Ekwochi U, Chinawa JM, Obi I, Obu HA, Agwu S (2013) Use and/or misuse of antibiotics in management of diarrhea among children in Enugu, Southeast Nigeria. J Trop Pediatr 59: 314–316. doi: 10.1093/tropej/fmt016.
14. Burstein B, Rogers S, Klassen TP, Freedman SB (2022) Trends in management of children with acute gastroenteritis in US Emergency Departments. JAMA Netw Open 5: e2211201--e2211201. doi: 10.1001/jamanetworkopen.2022.11201.
15. Stanyevic B, Sepich M, Biondi S, Baroncelli GI, Peroni D, Di Cicco M (2022) The evolving epidemiology of acute gastroenteritis in hospitalized children in Italy. Eur J Pediatr 181: 349–58. doi: 10.1007/s00431-021-04210-z.
16. Weghorst AAH, Bonvanie IJ, Holtman GA, de Boer MR, Berger MY (2022) Course of uncomplicated acute gastroenteritis in children presenting to out-of-hours primary care. BMC Prim care 23: 1–8. doi: 10.1186/s12875-022-01739-2.
17. Wallace MJ, Fishbein SRS, Dantas G (2020) Antimicrobial resistance in enteric bacteria: current state and next-generation solutions. Gut Microbes 12: 1799654. doi: 10.1080/19490976.2020.1799654.
18. Center for Disease Control (2019) Antibiotic resistance threats in the United States, 2019. Available: https://www.cdc.gov/antimicrobial-resistance/data-research/threats/index.html. Accessed: 13 Mar 2024.
19. David EE, Yameen MA, Igwenyi IO, Okafor AC, Obeten UN, Obasi DO, Ezeilo UR, David CN (2020) The frequency of virulent genes and antimicrobial resistance patterns of diarrheagenic *Escherichia coli* isolated from stools of children presenting with diarrhea in a tertiary hospital in Abakaliki, Nigeria. Int J One Heal 6: 147–152. doi: 10.14202/IJOH.2020.147-152.
20. Kayode A, Okunrounmu P, Olagbende A, Adedokun O, Hassan A-W, Atilola G (2020) High prevalence of multiple drug resistant enteric bacteria: Evidence from a teaching hospital in Southwest Nigeria. J Infect Public Health 13: 651–656. doi: 10.1016/j.jiph.2019.08.014.
21. Subbiah M, Caudell MA, Mair C, Davis MA, Matthews L, Quinlan RJ, Quinlan MB (2020) Antimicrobial resistant enteric bacteria are widely distributed amongst people, animals and the environment in Tanzania. Nat Commun 11: 1–12. doi: 10.1038/s41467-019-13995-5.
22. Beyene AM, Gezachew M, Mengesha D, Yousef A, Gelaw B (2022) Prevalence and drug resistance patterns of Gram-negative enteric bacterial pathogens from diarrheic patients in Ethiopia: A systematic review and meta-analysis. PLoS One 17: e0265271. doi: 10.1371/journal.pone.0265271.
23. Kibwana UO, Majigo M, Kamori D, Manyahi J (2020) High fecal carriage of extended beta lactamase producing Enterobacteriaceae among adult patients admitted in referral

hospitals in Dar es Salaam, Tanzania. BMC Infect Dis 20: 1–7. doi: 10.1186/s12879-020-05272-4.
24. Kantele A, Lääveri T (2022) Extended-spectrum beta-lactamase-producing strains among diarrhoeagenic *Escherichia coli*—Prospective traveller study with literature review. J Travel Med 29: taab042. doi: 10.1093/jtm/taab042.
25. Aminshahidi M, Arastehfar A, Pouladfar G, Arman E, Fani F (2017) Diarrheagenic *Escherichia coli* and Shigella with high rate of extended-spectrum Beta-lactamase production: two predominant etiological agents of acute diarrhea in Shiraz, Iran. Microb Drug Resist 23: 1037–1044. doi: 10.1089/mdr.2017.0204.
26. Mandal A, Sengupta A, Kumar A, Singh UK, Jaiswal AK, Das P, Sushmita D (2017) Molecular epidemiology of extended-spectrum β-lactamase-producing *Escherichia coli* pathotypes in diarrheal children from low socioeconomic status communities in Bihar, India: emergence of the CTX-M Type. Infect Dis Res Treat 10: 1178633617739018. doi: 10.1177/1178633617739018.
27. Franiczek R, Sobieszczańska B, Turniak M, Kasprzykowska U, Krzyżanowska B, Jermakow K, Mokracka-Latajka G (2012) ESBL-producing *Escherichia coli* isolated from children with acute diarrhea--antimicrobial susceptibility, adherence patterns and phylogenetic background. Adv Clin Exp Med. 21: 187–192.
28. Worku M, Getie M, Moges F, Mehari AG (2022) Extended-spectrum beta-lactamase-and carbapenemase-producing *Enterobacteriaceae* family of bacteria from diarrheal stool samples in Northwest Ethiopia. Interdiscip Perspect Infect Dis 8: 7905350. doi: 10.1155/2022/7905350.
29. Dela H, Egyir B, Majekodunmi AO, Behene E, Yeboah C, Ackah D, Bongo RNA, Bonfo B (2022) Diarrhoeagenic *E. coli* occurrence and antimicrobial resistance of extended spectrum beta-lactamases isolated from diarrhoea patients attending health facilities in Accra, Ghana. PLoS One 17: e0268991. doi: 10.1371/journal.pone.0268991.
30. Dembele R, Kaboré WAD, Soulama I, Traoré O, Ouédraogo N, Konaté A (2021) Molecular characterization of β-lactamase producing genes and integrons in diarrheagenic *Escherichia coli* from diarrheal children less than five years of age in Ouagadougou, Burkina Faso. Benign Anorectal Disorders - An Update. IntechOpen. Available: http://dx.doi.org/10.5772/intechopen.103169. Accessed: 3 Jan 2024.
31. Khairy RMM, Fathy ZA, Mahrous DM, Mohamed ES, Abdelrahim SS (2020) Prevalence, phylogeny, and antimicrobial resistance of *Escherichia coli* pathotypes isolated from children less than 5 years old with community acquired-diarrhea in Upper Egypt. BMC Infect Dis 20: 1–9. doi: 10.1186/s12879-020-05664-6.
32. Ogefere HO, Ibadin EE, Omoregie R, Ilerhunwa I (2016) Prevalence of extended spectrum β-lactamase among diarrheagenic strains of *Escherichia coli* among children in Yenagoa, Nigeria. Sokoto J Med Lab Sci 1: 7–12.
33. Zakou A, Nkene I, Abimiku R, Yahaya I, Bassey B, Ngwai Y (2020) Antimicrobial resistance profile and detection of extended spectrum and Amp C β-Lactamase resistance genes in *Escherichia coli* isolated from diarrheic children in Lafia, Nasarawa State, Nigeria. South Asian J Res Microbiol 7: 15–27. doi: 10.9734/sajrm/2020/v7i330172.
34. Saka HK, Garcia-Soto S, Dabo NT, Lopez-Chavarrias V, Muhammad B, Ugarte-Ruiz M (2020) Molecular detection of extended spectrum β-lactamase genes in *Escherichia coli* clinical isolates from diarrhoeic children in Kano, Nigeria. PLoS One 15: e0243130. doi: 10.1371/journal.pone.0243130.
35. Awosile BB, Agbaje M, Adebowale O, Kehinde O, Omoshaba E (2022) Beta-lactamase resistance genes in *Enterobacteriaceae* from Nigeria. Afr J Lab Med 11: 1371. doi: 10.4102/ajlm.v11i1.1371.
36. Monica C (2006) District laboratory practice in tropical countries, 2nd Edition. London. Cambridge University Press. doi: 10.1017/CBO9780511543470.
37. Humphries R, Bobenchik AM, Hindler JA, Schuetz AN (2021) Overview of changes to the clinical and laboratory standards institute performance standards for antimicrobial susceptibility testing, M100. J Clin Microbiol 59: e0021321. doi: 10.1128/JCM.00213-21.
38. Kaur J, Chopra S, Mahajan G (2013) Modified double disc synergy test to detect ESBL production in urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae*. J Clin Diagn Res 7: 229-233. doi: 10.7860/JCDR/2013/4619.2734.
39. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M (2013) Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic Acids Res 41: e1. doi: 10.1093/nar/gks808.
40. Cui L, Zhao J, Lu J (2015) Molecular characteristics of extended spectrum ß-lactamase and carbapenemase genes carried by carbapenem-resistant Enterobacter cloacae in a Chinese university hospital. Turkish J Med Sci 45: 1321–1328. doi: 10.3906/sag-1407-62.
41. Udoh EE, Meremikwu MM (2017) Antibiotic prescriptions in the case management of acute watery diarrhea in under-fives. Int J Contemp Pediatr 4: 691–695. doi: 10.18203/2349-3291.ijcp20171685.
42. Abdul-Mumin A, Ervin S, Halvorson EE (2019) Clinical characteristics associated with increased resource utilization of hospitalized children under 5 years with acute gastroenteritis at a tertiary hospital in the northern region of Ghana: a retrospective study. Pan Afr Med J 33: 186. doi: 10.11604/pamj.2019.33.186.13133.
43. Dietrich R, Jessberger N, Ehling-Schulz M, Märtlbauer E, Granum PE (2021) The food poisoning toxins of *Bacillus cereus*. Toxins (Basel) 13: 98. doi: 10.3390/toxins13020098.
44. Organji SR, Abulreesh HH, Elbanna K, Osman GEH, Khider M (2015) Occurrence and characterization of toxigenic Bacillus cereus in food and infant feces. Asian Pac J Trop Biomed 5: 515–520. doi:10.1016/j.apjtb.2015.04.004.
45. Huang C (2020) Extensively drug-resistant *Alcaligenes faecalis* infection. BMC Infect Dis 20: 1–11. doi: 10.1186/s12879-020-05557-8.
46. Sherman JD, Ingall D, Wiener J, Pryles CV (1960) *Alcaligenes faecalis* infection in the newborn. Am J Dis Child 100: 212–216. doi: 10.1001/archpedi.1960.04020040214009.
47. Alharbi M, Alshammari A, Alasmari AF, Alharbi S, ul Qamar M, Abbasi SW (2022) Whole proteome-based therapeutic targets annotation and designing of multi-epitope-based vaccines against the Gram-Negative XDR-*Alcaligenes faecalis* bacterium. Vaccines 10: 462. doi: 10.3390/vaccines10030462.
48. Afolabi OF, Saka AO, Ojuawo A (2019) Acute diarrhoea in hospitalized under-five children in Ilorin, Nigeria: Relationship between isolated enteropathogens and clinical outcome. Niger J Paediatr 46: 182–188.
49. Akinwumi FO, Igbeneghu OA, Oyelami OA, Lamikanra A (2021) A study of bacterial pathogens associated with

diarrhoea in children under 2 years in Ile-Ife, Nigeria. African J Microbiol Res 15: 82–88. doi: 10.5897/AJMR2020.9462.
50. Akingbade OA, Akinjinmi AA, Olasunkanmi OI, Okerentugba PO, Onajobi BI, Okonko IO (2013) Bacterial organisms isolated from children with diarrhoea in Abeokuta, Nigeria. Stem Cell J 4: 5–9.
51. Zelelie TZ, Gebreyes DS, Tilahun AT, Craddock HA, Gishen NZ (2019) Enteropathogens in under-five children with diarrhea in health facilities of Debre Berhan Town, North Shoa, Ethiopia. Ethiop J Health Sci 29: 203-214. doi: 10.4314/ejhs.v29i2.7.
52. Shima A, Hinenoya A, Asakura M, Nagita A, Yamasaki S (2012) Prevalence of *Providencia* strains among children with diarrhea in Japan. Jpn J Infect Dis 65: 545–547. doi: 10.7883/yoken.65.545.
53. Albert MJ (2001) Association of *Providencia alcalifaciens* with diarrhea in children. J Clin Microbiol 34: 1433-1435. doi: 10.1128/JCM.36.5.1433-1435.1998.
54. Adegunloye DV (2006) Carrier rate of enteric bacteria associated with diarrhoea in children and pupils in Akure, Ondo State, Nigeria. African J Biotechnol 5: 162–164.
55. Krawczyk B, Wityk P, Gałecka M, Michalik M (2021) The many faces of *Enterococcus* spp.—Commensal, probiotic and opportunistic pathogen. Microorganisms 9: 1900. doi: 10.3390/microorganisms9091900.
56. Ehiri JE, Azubuike MC, Ubbaonu CN, Anyanwu EC, Ibe KM, Ogbonna MO (2001) Critical control points of complementary food preparation and handling in eastern Nigeria. Bull World Health Organ 79: 423–433.
57. Castanheira M, Simner PJ, Bradford PA (2021) Extended-spectrum β-lactamases: An update on their characteristics, epidemiology and detection. JAC-antimicrobial Resist 3: dlab092. doi: 10.1093/jacamr/dlab092.
58. Bush K, Jacoby GA (2010) Updated functional classification of β-lactamases. Antimicrob Agents Chemother 54: 969–76. doi: 10.1128/AAC.01009-09.
59. Kazmierczak KM, de Jonge BLM, Stone GG, Sahm DF (2013) Longitudinal analysis of ESBL and carbapenemase carriage among Enterobacterales and *Pseudomonas aeruginosa* isolates collected in Europe as part of the International Network for Optimal Resistance Monitoring (INFORM) global surveillance programme, 2013-17. J Antimicrob Chemother 75: 1165–1173. doi: 10.1093/jac/dkz571.
60. Iroha IR, Esimone CO, Neumann S, Marlinghaus L, Korte M, Szabados F (2012) First description of *Escherichia coli* producing CTX-M-15-extended spectrum beta lactamase (ESBL) in out-patients from south eastern Nigeria. Ann Clin Microbiol Antimicrob 11:1–5. doi: 10.1186/1476-0711-11-19.
61. Sharma M, Pathak S, Srivastava P (2013) Prevalence and antibiogram of extended spectrum β-lactamase (ESBL) producing Gram negative bacilli and further molecular characterization of ESBL producing *Escherichia coli* and *Klebsiella spp.* J Clin diagnostic Res 7: 2173-2177. doi: 10.7860/JCDR/2013/6460.3462.
62. Puah SM, Puthucheary SD, Chua KH (2019) First report of extended-spectrum β-lactamases TEM-116 and OXA-10 in clinical isolates of Alcaligenes species from Kuala Lumpur, Malaysia. Jpn J Infect Dis 72: 266–269. doi: 10.7883/yoken.JJID.2018.031.
63. Ghazaei C (2019) Phenotypic and molecular detection of beta-lactamase enzyme produced by *Bacillus cereus* isolated from pasteurized and raw milk. J Med Bacteriol 8: 1–7.

Annex – Supplementary Items

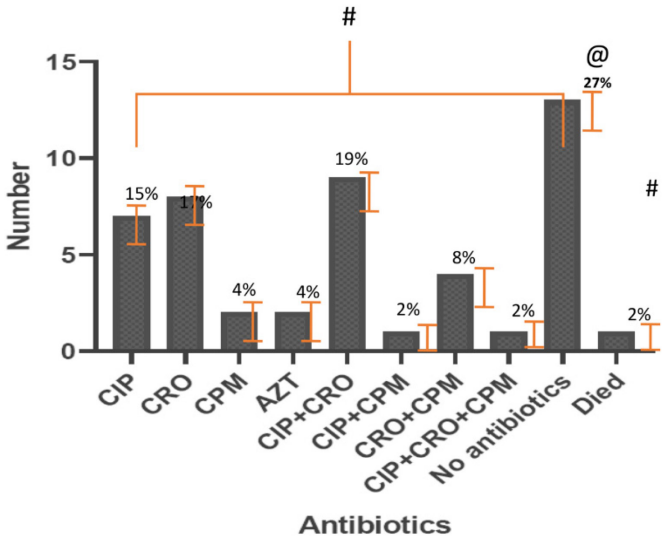
Supplementary Table 1. CLSI Performance Standards for the antibiotics.

Class of drug	Antibiotics	Disk content	Zone diameter breakpoints (mm)		
			S	I	R
Penicillin	Ampicillin	10µg	≥ 17	14-16	≤ 13
β-lactam combination	Amoxicillin-clavulanate	20/10µg	≥ 18	14-17	≤ 13
Cephems	Cefepime	30µg	≥ 25	19-24	≤ 18
	Cefotaxime	30µg	≥ 26	23-25	≤ 22
	Ceftriaxone	30µg	≥ 23	20-22	≤ 19
	Ceftazidime	30µg	≥ 21	18-20	≤ 17
Carbapenems	Imipenem	10µg	≥ 23	20-22	≤ 19
Fluoroquinolones	Ciprofloxacin	5µg	≥ 21	16-20	≤ 15
	Levofloxacin	5µg	≥ 17	14-16	≤ 13
Fosfomycin	Fosfomycin	200µg	≥ 16	13-15	≤ 12

Supplementary Table 2. Primers used in PCR amplifications of 16S rRNA and ESBL genes.

Target gene	Sequence (5′ to 3′)	Size (bp)
CTX-M -1	GGTTAAAAAATCACTGCGTC TTGGTGACGATTTTAGCCGC	863
CTX-M -2	ATGATGACTCAGAGCATTCG TGGGTTACGATTTTCGCCGC	865
CTX-M -9	ATGGTGACAAAGAGAGTGCA CCCTTCGGCGATGATTCTC	869
SHV	CGCCGGGTTATTCTTATTTGTCGC CGCCGGGTTATTCTTATTTGTCGC	795
TEM	ATAAAATTCTTGAAGACGAAA GACAGTTACCAATGCTTAATCA	1079
16S	27F (5′AGAGTTTGATCMTGGCTCAG-3′) 1525R (5'- AAGGAGGTGWTCCARCCGCA-3')	1500

Supplementary Figure 1. Antibiotics used in empirical treatment of childhood gastroenteritis (n = 48).



Supplementary Table 3. BLAST results for 16S rRNAV3-V4 sequence of 62 stool isolates.

SN	Sample ID	Accession number 16S rRNA V3-V4	Reference gene (best blast hit)	Strain	% ID	% Coverage	Accession number of reference gene	Query length
1	1	OP131838	*Alcaligenes faecalis*	5 NA	94.35	87	OM912041.1	469
2	2	OP650091	*Alcaligenes faecalis*	2	99.67	100	MN636316.1	299
3	3	OP131839	*Alcaligenes faecalis*	rajabi2	91	96.64	MW165751.1	477
4	4	OP113777	*Bacillus cereus*	12b	96.31	90	LC501403.1	413
5	5	OP113778	*Lysinibacillussp*	L10	100	94	MH628183.1	407
6	6	OP164754	*Alcaligenes faecalis*	BFE13	89.44	92	MT415328.1	453
7	7	OP113779	*Bacillus cereus*	12b	97.08	90	LC501403.1	408
8	8	OP650092	*Burkholderiasp*	tkp12	99.67	100	AJ971388.1	305
9	9	OP132416	*Providencia sp*	N17-1-1	94.52	88	MN696506.1	496
10	10	OQ472527	*Bacillus sp*	MRHKN 15	100	100	OQ354206.1	560
11	11	OQ472528	*Bacillus sp*	ASP6	100	100	OQ352634.1	426
12	12	OP216524	*Psychrobacter*	RSAP27	98.65	90	MH348992.1	496
13	13	OP219795	*Lysinibacillussp*	ZJ-2016-1	91.04	96	MF037826.1	438
14	14	OP216525	*Lysinibacillussp*	H73	87.31	97	KU057067.1	457
15	15	OP131840	*Alcaligenes sp*	SMC Bioinfo 1	95.96	89	MG430281.1	500
16	16	OP131841	*Alcaligenes sp*	p21(2011)	94.8	89	HQ652591.1	494
17	17	OP650093	*Enterococcus faecalis*	HASOB12b	100	99.2	MH291406.1	441
18	18	OP113780	*Bacillus sp*	FJAT-29853	99.14	86	MF948327.1	401
19	19	OP131842	*Alcaligenes faecalis*	AFMUI	96.22	91	MN250293.1	493
20	20	OP113781	*Bacillus sp*	CEN6E	97.14	94	JN628291.1	398
21	21	OP650094	*Alcaligenes sp*	20-B17	90.91	72	KT893463.1	466
22	22	OP113782	*Bacillus cereus*	B1GW	95.44	93	MN204613.1	443
23	23	OP131843	*Alcaligenes sp*	L3102	88.37	90	OM920553.1	493
24	24	Lost	Lost					
25	25	OP132417	*Providencia sp*	K1	96.89	91	KP836255.1	490
26	26	OP113783	*Bacillus wiedmannii*	HYN6-4	98.44	94	MN527227.1	402
27	27	OP650095	*Bacillus thuringiensis*	SK03P	82.81	81	MH210880.1	221
28	28	OP650096	*Proteus sp*	G32	93.95	75	CP053371.1	457
29	29	OP131844	*Alcaligenes sp*	NAY2	87.43	91	MZ424620.1	399
30	30	OP650097	*Alcaligenes faecalis*	WS-5	89.73	90	LN870277.1	488
31	31	OP113784	*Bacillus sp*	D5(2014)	92.1	95	KM609881.1	426
32	33	OP113785	*Bacillus sp*	WS001	87.77	95	KP313873.1	431
33	34	OP650098	*Bacillus sp*	AW43	76.97	89	JX076856.1	494
34	35	OP650099	*Alcaligenes faecalis*	LZU-52	89.89	72	KT262984.1	515
35	36	OP113786	*Bacillus thuringiensis*	NMCC-195	97.53	98	MN448379.1	409
36	37	OP650100	*Bacillus sp*	SPM2	100	98.33	MK779777.1	419
37	38	OP132418	*Enterococcus faecalis*	Y181	96.46	91	HM776199.1	495
38	39	Lost	Lost					
39	40	OP132419	*Proteus sp*	KY072916.1	92	92	MH346230.1	488
40	42	OP650101	*Bacillus sp*	PS25(6)	100	96.75	MF449178.1	461
41	45	OP113787	*Bacillus cereus*	RSN07	100	95	MH045981.1	426
42	46	OP650102	*Bacillus sp*	CF-S21	84.59	71	KJ781392.1	474
43	47	Lost	Lost					
44	48	OP113788	*Bacillus anthracis*	a	99.76	95	MF754137.1	442
45	49	OP113789	*Bacillus thuringiensis*	NMCC-195	97.04	94	MN448379.1	427
46	50	OP650103	*Alcaligenes faecalis*	L3102	83.3	91	OM920553.1	503
47	51	OP650104	*Bacillus sp*	B-Tb	79.91	88	JN975097.1	501
48	52	OP650105	*Alcaligenes faecalis*	NJ-17	92.4	91	MW138074.1	498
49	53	OP650109	*Alcaligenes faecalis*	L3102	100	98.25	OM920553.1	458
50	54	OP131845	*Alcaligenes faecalis*	NJ17	95.58	91	MW138074.1	492
51	55	OP219796	*Brevundimonassp*	BIO-TAS2-2	89.46	86	NR_116722.1	486
52	57	OP131846	*Alcaligenes sp*	SMC Bioinfo 1	87.58	90	MG430281.1	505
53	58	OP131847	*Alcaligenes sp*	G20	94.69	91	KX344028.1	494
54	59	OP650106	*Bacillus sp*	SOYG 3	98	99.67	MH485365.1	524
55	60	OP650107	*Bacillus thuringiensis*	SS2	98	99.42	MK389411.1	350
56	61	OP650108	*Uncultured bacterium*	FL3Bc12_21659	100	98.66	JQ368687.2	447
57	63	OP650109	*Alcaligenes sp*	L3102	93.89	91	OM920553.1	501
58	65	OP131848	*Alcaligenes sp*	30ft3	92.51	91	MG602714.1	496
59	66	OP131849	*Alcaligenes faecalis*	NJ17	90.49	90	MW138074.1	497
60	73	OP131850	*Alcaligenes sp*	SMC Bioinfo 1	90.31	91	MG430281.1	494
61	74	OP131851	*Alcaligenes faecalis*	14	86.52	90	KC605328.1	507
62	98	OP113790	*Bacillus anthracis*	APBSCS17	86.35	97	MG733392.1	396