Journal of Bacteriology and Mycology

Open Access

Austin Publishing Group

Research Article

Extended-Spectrum B-Lactamase (ESBL)-Producing *Escherichia coli* Isolated from Children under 5 years with and without Diarrhoea in Two Hospitals in Dschang, Cameroon

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Received: May 10, 2023
Accepted: June 02, 2023
Published: June 09, 2023

Abstract

Introduction: Extended-Spectrum B-Lactamase (ESBL) mediating resistance in Enterobacterales is a global public health issue, especially in Low-and Middle-Income Countries (LMICs) such as Cameroon. ESBL-producing Enterobacterales reduce therapeutic options and lead to the use of last resort drugs such as carbapenems even in vulnerable populations like children under five years. This study aims at determining the phenotypic and genotypic characteristics of ESBL-producing *Escherichia coli* (ESBL-*Ec*) isolated from children under five years with and without diarrhoea in two health care facilities in Dschang.

Materials and Methods: A cross-sectional study was conducted from 3 February to 19 May 2022 in two hospitals in the city of Dschang, Cameroon. Stool collected were cultured on Eosine Methylen Blue (EMB) medium. Enterosystem 18R kit was used for bacterial identification. Evaluation of the resistance patterns and detection of ESBL production were performed with, the Kirby Bauer disk diffusion method and CHROMagar® ESBL medium, respectively. The genomic DNA of ESBL-Ec was extracted using the boiling method and subjected to conventional and multiplex PCRs for detection of bla_{SHV}, bla_{CTX}-M and bla_{TEM} genes. Data were entered into Excel™ 2016. Epi info and R software were used for statistical analyses with a p-value <0.05 considered statistically significant.

Results: Out of the 125 children enrolled, 67.2% (84/125) were colonized by E. coli. Among these, 57.14% (48/84) were colonized by ESBL-*Ec*. The prevalence of ESBL-Ec per hospitals, was higher in H1 than that of H2 although without statistical significance (60.42% *vs* 39.58%, p=0.24). ESBL-Ec isolates showed high levels of resistance to amoxicillin-clavulanic acid (96.22%), cefotaxime (75.47%), ceftriaxone (73.58%), ofloxacin (67.92%), levofloxacin (56.6%) and ciprofloxacin (54.71%). The majority of ESBL-Ec isolates (52.83%; 28/53) were co-producers of bla_{CTX-M} and bla_{TEM}.

Conclusion: Infection prevention and control measures coupled with antimicrobial stewardship strategies need to be strengthened to reduce emergence and dissemination of ESBL-Ec among this vulnerable population.

Keywords: ESBLs; CTX-M; *Escherichia. coli*; Diarrhoea; Children;

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Citation: Mafo LD, Founou RC, Noubom M, Dimani BD, Zemtsa JR, et al. Extended-Spectrum B-Lactamase (ESBL)-Producing *Escherichia coli* Isolated from Children under 5 years with and without Diarrhoea in Two Hospitals in Dschang, Cameroon. J Bacteriol Mycol. 2023; 10(2): 1206.

Introduction

Diarrhoeal diseases are one of ten leading causes of death among children under five years, particularly in Low and Middle-Income Countries (LMICs) where inadequate Water, Sanitation and Hygiene (WASH) prevails [1-3]. Foodborne diseases caused 600 million illnesses and 420,000 deaths globally in 2010 [4]. Enterotoxigenic and enteropathogenic *Escherichia coli* were the leading bacteria involved in the largest number of cases and deaths, respectively [5,6].

The emergence and escalation of Extended-Spectrum ß-Lactamase producing *Enterobacterales* (ESBL-E) is a major public health concern worldwide. ESBL-E including ESBL-producing *E. coli* (ESBL-Ec) exhibit resistance to third generation cephalosporins and also usually to several other antibiotic families such as aminoglycosides and fluoroquinolones leading to the use of last resort drugs [2,7,8]. ESBL-Ec has been incriminated in both nosocomial and community-acquired infections [5]. Numerous studies focusing on faecal carriage revealed a high prevalence of ESBL-Ec in Nepal (74%) [8], Egypt (71%) [9] and Morocco (58%) [10] in community settings; while, lower rates were observed in hospitals in Gabon (12%) and Ethiopia (17%) [11,12]. A study performed in Cameroon reported a 54% prevalence of ESBL-E faecal carriage in participants including outpatients, inpatients and hospital workers [13].

However, there is limited comprehensive data on ESBL-Ec colonizing or causing diarrhoeal diseases in children under five in Cameroon. This study aimed at determining the prevalence, risk factors, phenotypic and genotypic characteristics of ESBL-Ec isolated from faecal samples of children with or without diarrhoea in two hospitals of Dschang, Cameroon.

Material and Methods

Study population

Children under five years old with and without diarrhoea, attending the pediatric departments of two major hospitals, encoded for ethical consideration as H1 and H2, in the Western region of Cameroon were considered; during a four-month period (the 03rd February 2022 to 19th May 2022). H1 is a district hospital which receives an average of 300 children per year with about 20 cases of gastroenteritis per month. It is equipped with a biomedical analysis laboratory divided into three buildings and the hospital has 200 beds and provides care for more than 1,000 patients per year. The H2 which is a confessional hospital, receives an average of 30 children per month with about 5 children suffering from gastroenteritis. The hospital has 100 beds and provides care for more than 700 patients per year.

Recruitment and sample collection

The parent or legal guardian of any child that met the inclusion provided written informed consent and answered a questionnaire about the socio-demographics and clinical information of participants. Stool samples were collected in a sterile container after informed consent. Stool samples were cultured onto EMB agar (produced by Rapid Lab) and, incubated for 18-24h at 37ºC in presence of oxygen. The growing colonies on EMB agar were analysed using biochemical tests through Enterosystem 18R as per the manufacturer instructions.

Antimicrobial susceptibility testing and Screening Confirmation for ESBL

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method [14]. A panel of nine antibiotics were tested including amoxicillin/clavulanic acid (30µg), ceftriaxone (30µg), cefotaxime (30µg), chloramphenicol (30µg), ciprofloxacin (30mcg), levofloxacin (5µg), ofloxacin (5µg), tobramycin (10µg) and gentamicin (10µg). The different diameters of the inhibition zones were measured and interpreted as susceptible (S), intermediate (I) or resistant (R) according to the criteria defined by CA-SFM 2021 [14]. In addition, two screening methods were used for ESBL detection namely the champagne cork or funnel shaped synergies using double discs (clavulanic acid and ceftazidime) and chromogenic media CHROMagar™ ESBL.

E. coli ATCC 35218, *K. pneumoniae* ATCC 700603 and *P. aeruginosa* ATCC 27853 were used as quality control strains for the antimicrobial susceptibility testing and ESBL screening.

Genomic DNA Extraction

The genomic DNA of ESBL-Ec was extracted using a modified boiling method as described previously [15]. Briefly, one pure ESBL-Ec colony was suspended into 400µL of Tris-EDTA (10mMTris, 0.1mMEDTA) and then vortexed for five seconds. The suspension was then incubated for 25 min at 95ºC in a dry bath digital (MIULab DKT200-1, Lasec International Ltd., Johannesburg, South Africa). After incubation, the suspension was centrifuged for 5 min at 9500rpm. The supernatant containing DNA (300µl) was subsequently transferred to a new eppendorf tube and stored at -40ºC for future analysis.

Conventional Singleplex-Polymerase Chain Reaction (PCR)

Amplification of bla_{SHV} gene was performed by conventional PCR using a thermal cycler BIO-RAD T100 (Bio-Rad Laboratories, Marnes-la-Coquette, France). The reaction took place in a 10µL reaction mixture consisting of 5µL of Dream Taq Green Polymerase Master Mix 2x (ThermoFisher Scientific™, Vilnius, Lithuana), 2.8µL of nuclease-free water, 0.1µL of each forward and reverse primer [10µM] and 2µL of DNA. The amplification steps were as follows: initial denaturation (95ºC for 3 min), 30 cycles of denaturation at 95ºC for 4s, annealing at 46.9°C for 45s, elongation at 72°C for 60 s, and final elongation at 72°C for 5 min as previously described [16,17].

Conventional Multiplex-Polymerase Chain Reaction (PCR)

The detection of bla_{CTX-M} and bla_{TEM} genes among ESBL-Ec isolates was performed by multiplex PCR method using a thermal cycler BIO-RAD T100 (Laboratoires Bio-Rad, Marnes-la-Coquette, France). The reaction was done in a 10µL reaction mixture consisting of 5 µL of Dream Taq Green Polymerase Master Mix 2x (ThermoFisher Scientific™, Vilnius, Lithuana); 2.6µL of nuclease-free water, 0.1 µL of each forward (CTX-Mu-F and TEM-F) and reverse (CTX-Mu-R and TEM-R) primers [50µM] and 2µL of DNA. Amplification steps were as follows: initial denaturation (95°C for 3 min), 30 cycles of denaturation at 95ºC for 4 s annealing at 46.9ºC for 45s, elongation at 72ºC for 60 s and final elongation at 72ºC for 5 min. The annealing temperatures and primer sequences of primers are shown in Table 1.

Agarose electrophoresis gel and visualization

PCR products were subjected to electrophoresis analysis performed on an agarose gel of 1.5% (w/v) that was run at 90V for 45min along with a 100bp molecular ladder (New England Biolabs, MA, USA). After electrophoresis, the gel was stained in an ethidium bromide solution (0.5µg/mL) for 15 min and briefly unstained with water. PCR products were then visualised under UV light using a gel documentation system G-BOX Chemi-XL (Syngene, Cambridge, UK).

E. coli ATCC 35218 (bla_{TEM}), K. *pneumoniae* ATCC 700603 (bla_{SHV}) and P. aeruginosa ATCC 27853 ($blaC_{TXM}$ + bla_{TEM}) were used as internal positive controls.

Ethical considerations

This research was approved by the Regional Ethics Committee for Research in Human Health, West, Cameroon (Nº 2022/11/105/CE/CRERSH-OU/VP). Written informed consent to participate in this study was provided by the legal guardian/nearest relative of the participants.

Data management and analysis

Data analysis was performed using R software (version 4.1.0) and RStudio (version 2021.09.0). Proportions were compared using the Fischer exact test, chi square test and two-sample T-test as appropriate. A participant was considered positive to ESBL-Ec when at least one ESBL colony was detected. A participant was considered multidrug resistant when an *E. coli* isolate showed resistance to at least three antibiotics of three or more family of antibiotics with or without the presence of an ESBL phenotype. A p-value<0.05 was considered statistically significant.

Results

Population characteristics

A total of 132 children were enrolled in the study and among these, 125 provided samples. Of the 125 children who provided sample, 85 were from H1 and 40 from H2. Seventy children (38 girls and 32 boys) reported having diarrhoea and 55 children (24 girls and 31 boys) had at least one other clinical sign than diarrhoea. The majority of children were aged between 1-3 years (42.4%, 53/125) and lived in an urban area (56.80%, 71/125; Table 2).

Pathogen recovery

Among the 125 children sampled, 67.2% (84/125) were colonized by E. coli. The prevalence of *E. coli* per hospitals, was higher in H1 (57.14%, 48/84) than in H2 (42.86%, 36/84) with high statistical significance (p=0.00005). Children without diarrhoea were more colonized by *E. coli* than those with diarrhoea with statistical significance (53.57% vs 46.43%, p=0.001). The overall prevalence of *E. coli* among children having diarrhoea associated with other clinical signs was 52% (65/125) while the prevalence of children having other clinical signs than diarrhoea was 44% (55/125) (p=0.006). Hand washing (p=0.25) was associated with *E. coli* colonisation although without statistical significance. The socio-demographic characteristics associated with *E. coli* colonisation are reported in Table 2.

Prevalence of ESBL-*E. coli*

Among the 84 children positive to *E. coli*, 57.14% (48/84) were colonized by ESBL-*Ec*. The prevalence of ESBL-Ec per hospitals, was higher in H1 than that of H2 although without statistical significance (60.42% *vs* 39.58%, p=0.24). Female were more positive than male with statistical significance (56.26 *vs* 43.75, p=0.02). The prevalence of ESBL-Ec among children with diarrhoea was lower than that of those without diarrhoea (41.67% *vs* 58.33%, p=0.16). A 70.83% prevalence of ESBL-Ec was observed among children having received an antibiotic with statistical significance (p=0.01). The socio-demographic characteristics associated with ESBL-Ec colonisation are reported in Table 3.

Antibiotic resistance profile of ESBL-*E. coli*

Among the 84 children positive to *E. coli*, 6 were colonized by two isolates leading to a total of 90 *E. coli* isolates. Table 4 presents the resistance profile of ESBL-Ec and non ESBL- *Ec* to a panel of nine antibiotics. The majority of isolates were resistant to amoxicillin-clavulanic acid (96.22%), followed by cefotaxime (75.47%), ceftriaxone (73.58%), ofloxacin (67.92%), levofloxacin (56.6%) and ciprofloxacin (54.71%). Tobramycin (35.84%), gentamicin (32%) and chloramphenicol (30.18%) displayed the lowest resistance although it was still above 30%.

Figure 1 presents the resistance profile of ESBL-*E. coli* per hospital to a panel of nine antibiotics. It appears that the majority of isolates from H1 were resistant to amoxicillin+clavulanic acid (60.38%), cefotaxime (49.06%), ceftriaxone (47.17%) and ofloxacin (45.28%). Likewise, in H2, the antibiotics that were mainly resistant were amoxicillin+clavulanic acid (35.87%), cefotaxime (27%) and ceftriaxone (27%) although with lower rates.

Multi-drug resistance and resistance patterns of ESBL-*E. coli* isolated from stool samples

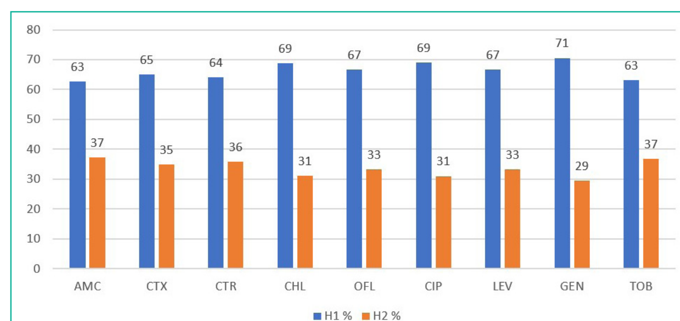

Figure 1: Distribution of antibiotic resistance of ESBL-*E. coli* per Hospital. AMC: Amoxicillin/Clavulanic Acid, CTX: Cefotaxime, CTR: Ceftriaxone, CHL: Chloramphenicol, OFL: Ofloxacin, CIP: Ciprofloxacin, LEV: Levofloxacin, GEN: Gentamicin, TOB: Tobramycin.

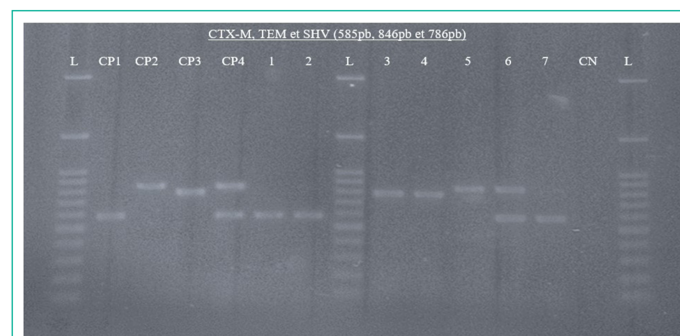

Figure 2: Agarose gel electrophoresis of amplified *bla* genes (bla_{CTX-M}, bla_{SHV}, and bla_{TEM}) from seven (07) ESBL-*E. coli* isolated from stool among children five years. L: 100bp molecular weight marker, CP1: Positive control bla_{CTX-M}, CP2: Positive control bla_{TEM} (*E. coli* ATCC 35218), CP3: Positive control bla_{SHV} (*K. pneumoniae* ATCC 700603), CP4: Positive control bla_{CTX-M} and bla_{TEM} (*P. aeruginosa* ATCC 27853), CN: Negative control (sterile water).

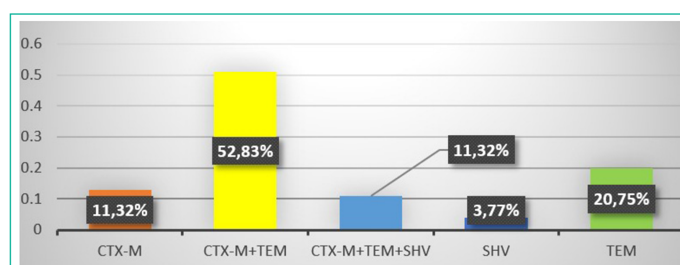

Figure 3: Distribution of *ESB*L genes in ESBL-*E. coli* isolates

Table 1: Characteristics of PCR primers.

Targeted gene	Primer Name	Sequence (5’-3’)	Amplicon Size (bp)	Annealing	References
TEM	TEM-F	CATTTCCGTGTCGCCCTTATTC	846 pb	46.9ºC	(16) (p. 712)
	TEM-R	CCAATGCTTAATCAGTGAGGC			
CTX-M	CTX-MU F	CGATGTGCAGTACCAGTAA	585 pb	46.9ºC	(17) (p. 1319)
	CTX-MU R	TTAGTGACCAGAATCAGCGG			
SHV	SHV-F	AGCCGCTTGAGCAAATTAAAC	786 pb	46.9ºC	(16) (p. 29)
	SHV-R	GTTGCCAGTGCTCGATCAGC			

Table 2: Sociodemographic characteristics and clinical symptoms of study participants associated with *E. coli* status.

Variables		Total n(%)	Positive *E.coli* n(%)	Negative *E. coli* n(%)	*p-value*
Overall		**125(100)**	**84(67.2)**	**41(32.8)**	
Gender	Male	63(50.4)	45(57.53)	18(43.9)	0.15
	Female	62(49.6)	39(46.43)	23(56.1)	
Age	< 1 year	50(40)	28(33.33)	22(53.66)	**0.09**
	1–3 years	53(42.4)	40(47.62)	13(31.71)	
	3-5 years	22(17.6)	16(19.05)	6(14.63)	
Residence	Rural area	54(43.20)	36(42.86)	18(43.9)	0.45
	Urban area	71(56.80)	48(57.14)	23(56.1)	
Child's education level	Kindergarten	18(13.6)	13(15.48)	5(12.2)	
	Pre-nursery	6(4.8)	3(3.57)	3(7.32)	0.77
	Nursery	18(14.4)	13(15.48)	5(9.76)	
	Primary	7(5.6)	5(5.95)	2(4.88)	
	Not applicable*	77(61.6)	50(59.52)	27(65.85)	
Monthly income of parents (XAF)	<38500	69(55.2)	42(50)	27(65.85)	0.38
	38500-100.000	31(24.8)	23(27.38)	8(19.51)	
	100.000- 250.000	19(15.2)	14(16.67)	5(12.2)	
	>250.000	6(4.8)	5(5.95)	1(2.44)	
Marital status of parents	Single	30(24)	19(22.62)	11(26.83)	
	Married	95(76)	65(77.38)	30(73.17)	0.3
Hospital	H1	85(68)	48(57.14)	37(90.24)	
	H2	40(32)	36(42.86)	4(9.76)	**5.00E-05**
Food consumption habits					
Yogourt	Yes	83(66.4)	56(66.67)	27(65.85)	
	No	42(33.6)	28(33.33)	14(34.15)	0.46
Eggs	Yes	93(74.4)	65(77.38)	28(68.29)	
	No	32(25.6)	19(22.62)	13(31.71)	0.14
Porridge	Yes	89(71.2)	57(67.86)	32(78.05)	
	No	36(28.8)	27(32.14)	9(21.95)	0.12
Natural Fruit Juice	Yes	100(80)	66(78.57)	34(82.93)	
	No	25(20)	18(21.43)	7(17.07)	0.29
Artificial juice	Yes	59(47.2)	41(48.81)	18(43.9)	
	No	66(52.8)	43(51.19)	23(56.1)	0.3
Breast milk	Yes	53(42.4)	34(40.48)	19(46.34)	
	No	72(57.6)	50(59.52)	22(54.66)	0.26
Artificial milk	Yes	79(63.2)	55(65.48)	24(58.54)	
	No	46(36.8)	29(34.52)	17(41.46)	0.22
Hygienic measures					
Disposable nappies	Yes	86(68.8)	56(66.67)	30(73.17)	0.23
	No	39(31.2)	28(33.33)	11(26.83)	
Hand washing of parents	Yes	93(74.4)	64(76.19)	29(70.73)	0.25
	No	32(25.6)	20(23.81)	12(29.27)	
Type of consumed water	Mineral	70(56)	45(53.57)	25(60.98)	0.25
	Tap	15(12)	11(13.1)	4(9.76)	
	Drilling	28(22.4)	21(25)	7(17.07)	
	Source	12(9.6)	7(8.33)	5(12.2)	
Cut finger nail (parents)	Yes	93(74.4)	63(75)	30(73.17)	0.41
	No	32(25.6)	21(25)	11(26.83)	
Clinical status					
Diarrhoea	Yes	70(56)	39(46.43)	31(75.61)	
	No	55(44)	45(53.57)	10(24.39)	**0.001**
Symptoms	Diarrhoea with others	65(52)	36(42.86)	29(70.73)	
	Only diarrhoea	5(4)	3(3.57)	2(4.88)	
	Other clinical signs without diarrhoea				**0.006**
		55(44)	45(53.57)	10(24.39)	
Antibiotic	Yes	76(60.8)	51(60.71)	25(60.98)	
	No	49(39.2)	33(39.29)	16(39.02)	0.49

*Not applicable represents out-of-school children

Table 3: Sociodemographic characteristics and clinical symptoms of study participants associated with ESBL-*E. coli* status.

Variables		Total n(%)	ESBL-*Ec* positive n(%)	EBSL-*Ec* negative n(%)	*p-value*
Overall		**84(100)**	**48(57.14)**	**36(42.85)**	**0**
Gender	Male	45(53.57)	21(43.75)	24(66.67)	**0.02**
	Female	39(46.43)	27(56.25)	12(33.33)	
Age	< 1 year	28(33.33)	14(29.17)	14(38.89)	0.63
	1-3 years	40(47.62)	24(50)	16(44.44)	
	3 -5 years	16(19.05)	10(20.83)	6(16.67)	
Residence	Rural area	36(42.85)	22(45.83)	14(38.89)	0.26
	Urban area	48(57.14)	26(54.17)	22(61.11)	
Child's education level	Kindergarten	13(15.48)	7(15.4)	6(16.67)	0.98
	Pre-nursery	3(3.57)	3(3.57)	1(2.78)	
	Nursery	13(15.48)	13(15.48)	5(13.89)	
	Primary	5(5.95)	5(5.95)	2(5.56)	
	Not applicable*	50(59.52)	50(59.52)	22(61.11)	
Monthly income of parents (XAF)	< 38500	42(50)	19(39.58)	23(63.89)	0.1
	38500-100.000	23(27.38)	17(35.42)	6(16.67)	
	100.000- 250.000	14(16.67)	8(16.67)	6(16.67)	
	>250.000	5(5.95)	4(8.33)	1(2.78)	
Marital status of parents	Single	19(22.62)	9(18.75)	10(27.78)	
	Married	65(77.38)	39(81.25)	26(72.22)	0.17
Hospital	H1	48(57.14)	29(60.42)	19(52.78)	
	H2	36(42.86)	19(39.58)	17(47.22)	0.24
Food consumption habits					
Yogourt	Yes	56(66.67)	32(66.67)	24(66.67)	
	No	28(33.33)	16(33.33)	12(33.33)	0.49
Porridge	Yes	57(67.86)	34(70.83)	23(63.89)	
	No	27(32.14)	14(29.17)	13(36.11)	0.25
Eggs	Yes	65(77.38)	38(79.17)	27(75)	
	No	19(22.62)	10(20.83)	9(25)	0.32
Natural Fruit Juice	Yes	66(78.57)	39(81.25)	27(75)	
	No	18(21.53)	9(9.75)	9(25)	0.25
Artificial juice	Yes	41(48.81)	22(45.83)	19(52.78)	
	No	43(51.19)	26(54.17)	17(47.22)	0.26
Breast milk	Yes	34(40.48)	21(56.25)	13(36.11)	
	No	50(59.52)	27(43.75)	23(63.89)	0.24
Artificial milk	Yes	55(65.48)	29(60.42)	26(72.22)	
	No	29(34.52)	19(39.58)	10(27.78)	0.13
Hygienic measures					
Disposable nappies	Yes	56(66.67)	35(72.92)	21(58.33)	0.08
	No	28(33.33)	13(27.08)	15(41.67)	
Hand washing	Yes	64(76.19)	35(72.92)	29(80.56)	0.21
	No	20(23.81)	13(27.08)	7(19.44)	
Type of consumed water	Mineral	39(46.43)	21(43.75)	18(50)	0.72
	Tap	11(13.10)	7(14.58)	4(11.11)	
	Drilling	27(32.14)	15(31.25)	12(33.33)	
	Source	7(8.33)	5(10.42)	2(5.56)	
Clinical status					
Diarrhoea	Yes	39(46.43)	20(41.67)	19(52.78)	
	No	45(53.57)	28(58.33)	17(47.22)	0.16
Symptoms	Diarrhoea with others	36(42.86)	18(37.5)	18(50)	
	Only diarrhoea	3(3.57)	2(4.17)	1(2.78)	0.51
	Other clinical signs without diarrhoea	45(53.57)	28(58.33)	17(47.22)	
Antibiotic	Yes	51(60.71)	34(70.83)	17(47.22)	
	No	33(39.29)	14(29.17)	19(52.78)	**0.01**

Table 4: Distribution of antibiotic resistance among all ESBL-*E. coli* and non-ESBL-*Ec* isolated from stools.

Antimicrobial Agents	Overall N=90, n(%)	Resistance, n(%)	
		ESBL-*Ec (n=53)*	Non-ESBL-*Ec(n=37)*
Amoxicillin/Clavulanic Acid	87(96.66)	51(96.22)	36(97.29)
Cefotaxime	47(52.22)	40(75.47)	7(18.91)
Ceftriaxone	47(52.22)	39(73.58)	8(21.62)
Chloramphenicol	30(34.48)	16(30.18)	14(37.83)
Ofloxacin	53(58.88)	36(67.92)	17(45.94)
Ciprofloxacin	36(40)	29(54.71)	7(18.91)
Levofloxacin	40(44.44)	30(56.6)	10(33.33)
Gentamicin	27(30)	17(32.07)	10(33.33)
Tobramycin	34(37.77)	19(35.84)	15(40.54)

Table 5: Resistance patterns of ESBL-*E. coli* resistant to three or more antibiotics.

Resistance patterns	Number of Antibiotics	Number of family of Antibiotics	Number of Isolates(%)
AMC-CHL-OFL	3	3	1(4.16)
AMC-OFL-CIP-GEN-TOB	5		1(4.16)
AMC-CTX-CTR-CHL-OFL-LEV	6		2(8.33)
AMC-CTX-CTR-OFL-GEN-TOB			1(4.16)
AMC-CTX-CTR-OFL-CIP-LEV-GEN	7		1(4.16)
AMC-CTX-CTR-CHL-CIP-OFL-LEV			2(8.33)
AMC-CTX-CTR-OFL-CIP-LEV-TOB			3(12.5)
AMC-CTX-CTR-OFL-CIP-LEV-GEN-TOB	8		4(16.66)
AMC-CHL-OFL-GEN	4	4	1(4.16)
AMC-CTX-CHL-CIP-OFL-LEV-TOB	7		2(8.33)
AMC-CTX-CTR-CHL-OFL-GEN-TOB			1(4.16)
AMC-CTX-CTR-CHL-OFL-LEV-GEN->TOB	8		1(4.16)
AMC-CTX-CTR-CHL-OFL-CIP-LEV-TOB			2(8.33)
AMC-CTX-CTR-CHL-OFL-CIP-LEV-GEN			1(4.16)
AMC-CTX-CTR-CHL-OFL-CIP-LEV-GEN-TOB	9		2(8.33)
TOTAL			**25(100)**

AMC: Amoxicillin-Clavulanic acid, **CTX**: Cefotaxime, **CTR**: Ceftriaxone, **TOB**: Tobramycin, **GEN**: Gentamicin, **CHL**: Chloramphenicol, **LEV**: Levofloxacin, **OFL**: Ofloxacin; **CIP**: Ciprofloxacin.

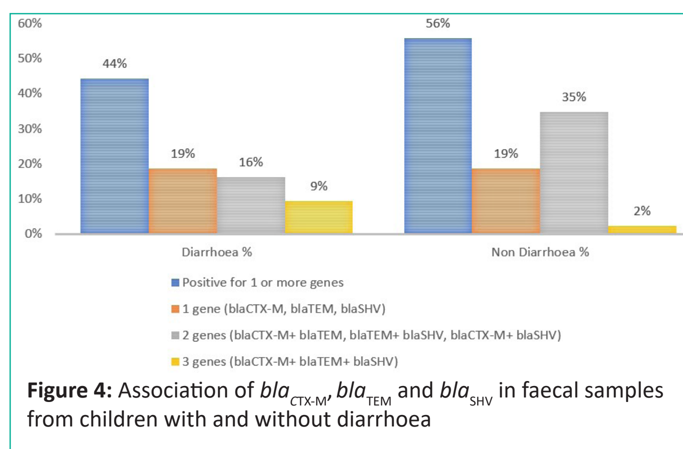

Figure 4: Association of bla_{CTX-M}, bla_{TEM} and bla_{SHV} in faecal samples from children with and without diarrhoea

A total of 24 different resistance patterns were observed among the ESBL-Ec isolates (Table 5). In the 53 ESBL-Ec isolates, 47.17% (n=25/53) isolates were concomitantly Multi-Drug Resistant (MDR, resistance to at least one antibiotic of three or more families of antibiotics) and ESBL producers.

Genotypic resistance profiles

Figure 2 represents the detection of the different ß-lactamase genes (bla_{CTX-M}, bla_{SHV}, and bla_{TEM}) after amplification and visualisation. Altogether, 52.83% (28/53) of ESBL-Ec isolates were co-producers of bla_{CTX-M} and bla_{TEM}, while 11.32% (6/53) harboured all three resistance genes (Figure 3).

ESBL-*E. coli* isolates carrying two resistance genes were more common in non-diarrhoeal stool samples (35%). The percent number of resistance genes detected in *E.coli* isolates from children with and without diarrhoea are reported in Figure 4.

Discussion

The aim of this study was to determine the prevalence, risk factors, phenotypic and genotypic characteristics of ESBL-Ec isolated from children under five years with and without diarrhoea in two hospitals in the Western region of Cameroon.

Our study revealed a high prevalence of *E. coli* (72%) among children under five. This prevalence is consistent that study of Tola et al (2021), who reported an 84.4% prevalence of *E. coli* faecal carriage among children under five years in Addis Ababa, Ethiopia (12). Of the 90 *E. coli* isolates detected in our study, 57.14% were ESBL producers. This is higher than that reported by Saka et al (2020) in a study conducted in the Kano area of Nigeria were 17.83 % of ESBL-Ec were detected in stools of female patients under five years of age [18]. Our result is similar to a study conducted in Iran by Haghighatpanah et al (2016), where a prevalence 51.9% of ESBL-Ec was reported in clinical samples in the north of the country [19]. These discrepancies may be explained by the regional geographic differences in the availability and consumption of antibiotics, in the implementation of programmes conducted for surveillance, control measures and appropriate use of antibiotics.

Therapeutic options for the infections caused by the ESBL producers are becoming increasingly limited and; if available, expensive for patients living in LMICs. It was found that over 70% of all ESBL-Ec were resistant to third generation cephalosporins (cefotaxime and ceftriaxone). These results agree with previous studies in Burkina Faso (60%) [2] and in Nepal (100%) among patients attending Bir hospital [8]. Furthermore, it was observed that resistance to fluoroquinolones in ESBL-Ec was high especially for ciprofloxacin with 54.71%. This is higher than that was previously reported in Yaounde (2020) from in- and out-patients at three referral hospitals, where 36.6% of *Enterobacterales* were resistant to ciprofloxacin [20]. In contrast, out finding agrees a with previous study conducted in Gabon (52,8%) where risks factors for ESBL-Ec carriage were age under five years of age, hospitalization for above five days and a hospital stay during the past year [21]. Numerous reports have already shown that in LMICs, community and hospitalized patients receive antibiotic treatment without antibiotic susceptibility testing, which contribute to the selective pressure on the microbiome [22]. It is thus indispensable to implement antimicrobial stewardship and surveillance programs to curb the dissemination of antimicrobial resistance in Cameroon.

bla_{CTX-M} in combination with bla_{TEM} was found in 52.83% of ESBL-*E. coli,* which is higher than that was detected in Iran among diarrheal children aged 0-60 months (42.1%) in 2014 [5]. These results disagree with previous studies conducted in many countries including Egypt (89.04%) [23], United States (70%) [24], Nigeria (47%) [25] and Burkina Faso (7.14%) [2], where the bla_{CTX-M} was the most represented ß-lactamase genes. Isolates with multiplex bla gene combinations and in particular those carrying bla_{CTX-M}+bla_{TEM} and bla_{CTX-M}+bla_{TEM}+bla_{SHV} were resistant to a greater number of antibiotics from β-lactams and non ß-lactam antibiotics (>55% resistance). These results could be explained by the fact that ß-lactam genes can be carried by multi-drug resistant plasmids as described by Tawfick et al (2022) [6]. The

high level of MDR isolates observed could also be due to the excessive or inappropriate use of drugs in Dschang, where antibiotics are easily accessible over the counter without a prescription, the absence of legislation and limited infection prevention control measures favours the emergence of resistance.

Risk factors such as antibiotic use one prior sampling, age and gender, were common factors associated with ESBL-Ec infections in this study. Continuous and consistent adoption of hygienic practices and mass education through all available public and social media as well as regular hand washing are also important in the control of resistant infectious diseases especially in children under five [26]. Strengthening epidemiological surveillance is also essential for better management of infections including diarrheal diseases among children.

Notwithstanding, the present study has some limitations. First, the limited sample size precludes any robust conclusion on the real burden of ESBL-Ec in children under five in the Western region of Cameroon. Second, the role of *E. coli* as causative agent of gastroenteritis among children could not be ascertained since its pathogenicity was not investigated. Third, children could not be followed up to assess the outcome of the disease. Finally, the selected healthcare structures were not of similar type (confessional *vs* public), hence the comparative results may not reflect the reality. Despite these limitations, the study adds significant data on the burden and molecular characteristics of ESBL-Ec circulating in a vulnerable population and a neglected region of the Cameroon.

Conclusion

The high prevalence of ESBL-Ec observed in children under five years in this study revealed the need to implement real time surveillance of this important foodborne pathogen across vulnerable populations using the One health approach. Our study further pointed out some potential sources of transmission of these resistant bacteria among children under five years and highlighted the necessity for stringent infection prevention control measures to curb the dissemination of ESBL-*Ec*. Finally, it shows that it is imperative to implement antimicrobial stewardship guidelines in community as well as in hospital settings to contain antimicrobial resistance and mitigate its public health impact on children morbidity and mortality.

Author Statements

Acknowledgments

We would also like to thank all the parents who gave their consent for their children to participate in this study, as well as the staff of the various health facilities who helped with the recruitment of the study subjects.

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