MOLECULAR CHARACTERIZATION OF DIARRHEAGENIC *ESCHERICHIA COLI* IN CHILDREN LESS THAN 5 YEARS OF AGE WITH DIARRHEA IN OUAGADOUGOU, BURKINA FASO

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Diarrheagenic *Escherichia coli* (DEC) is important bacteria of children's endemic and epidemic diarrhea worldwide. The aim of this study was to determine the prevalence of DEC isolated from stool samples collected from children with acute diarrhea living in Ouagadougou, Burkina Faso. From August 2013 to October 2015, stool samples were collected from 315 children under 5 years of age suffering from diarrhea in the "Centre Médical avec Antenne Chirurgicale (CMA)" Paul VI and the CMA of Schiphra. *E. coli* were isolated and identified by standard microbiological methods, and the 16-plex PCR method was used to further characterize them. Four hundred and nineteen (419) *E. coli* strains were characterized, of which 31 (7.4%) DEC pathotypes were identified and classified in five *E. coli* pathotypes: 15 enteroaggregative *E. coli* (EAEC) (48.4%), 8 enteropathogenic *E. coli* (EPEC) (25.8%) with 4 typical EPEC and 4 atypical EPEC, 4 enteroinvasive *E. coli* (EIEC) (12.9%), 3 enterohemorrhagic *E. coli* (EHEC) 9.67%, and 1 enterotoxigenic *E. coli* (ETEC) 3.2%. The use of multiplex PCR as a routine in clinical laboratory for the detection of DEC would be a useful mean for a rapid management of an acute diarrhea in children.

Keywords: 16-plex PCR, virulence genes, Ouagadougou, diarrheagenic *Escherichia coli*

Introduction

Diarrheal diseases remain a global health issue, particularly in children in developing countries. The number of annual deaths due to these diseases is estimated around 2.5 million [1, 2]. Among the bacterial causes of diarrhea, diarrheagenic *Escherichia coli* (DEC) represents the most important etiologic agent of childhood diarrhea and constitutes a major public health concern in developing countries [3, 4]. DEC is one of the predominant facultative anaerobes species present in the human gut and usually known as harmless to the human host [5, 6]. However, a group of emerging pathogenic *E. coli* is responsible for several human diseases including diarrhea, urinary tract

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infections, and meningitis [6, 7]. *E. coli* strains associated with diarrhea were classified into six different groups based on the clinical, epidemiological, and molecular criteria [8, 9], namely, enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC) [10, 11]. Each of the classes of DEC is defined based on the virulence characteristics [12]. Most of the *E. coli* isolated from stool are nonpathogenic. The DEC pathotypes represent a main cause of the bacterial infection of pediatric diarrhea in developing regions [13, 14]. The prevalence and epidemiological features of these pathogens vary from different regions, even within the same country [15, 16]. Several studies on the incidence of diarrheal caused by different classes of DEC have been conducted mainly in Latin-America, Africa, South and South East Asia, and the Middle East [17]. However, little is known about the prevalence of DEC categories and their importance in children with diarrhea in Burkina Faso, particularly in Ouagadougou. Ouagadougou has 30 districts, and Bonkoungou et al. [16] conducted a study in one district (CMA 30). Therefore, to define the association of various categories of DEC with diarrhea, we undertook this study using the traditional culture technique and polymerase chain reaction (PCR) for the identification of DEC specific virulence genes in two districts of Ouagadougou, CMA Paul VI and CMA of Schiphra both with an even higher population density.

Materials and methods

Study area and target population

Ouagadougou (1,915,102 residents), the capital city of Burkina Faso, is composed of 30 districts. CMA Paul VI or CMA of Schiphra received most of residents with low and middle incomes seeking for health care services [18]. This study was conducted between August 2013 and October 2015 in CMA Paul VI and CMA of Schiphra located in peripheral areas and in the city center of Ouagadougou, respectively *(Fig. 1)*. The choice of these health centers is justified by the fact that they are located in different environments. The study population consisted of children less than 5 years of age with acute diarrhea and who were hospitalized or visited the health center as outpatient. Any child over the age of 5 years was excluded from the study.

Sample collection

Three hundred and fifteen (315) stool samples were collected in sterile containers and transported to the dedicated laboratory ("Laboratoire de Biologie Moléculaire, d'Épidémiologie et de Surveillance des Bactéries et virus Transmissibles par les Aliments (LaBESTA)/Centre de Recherche en Sciences Biologiques, Alimentaires et Nutritionnelles (CRSBAN)/Université Ouaga I, Pr Joseph
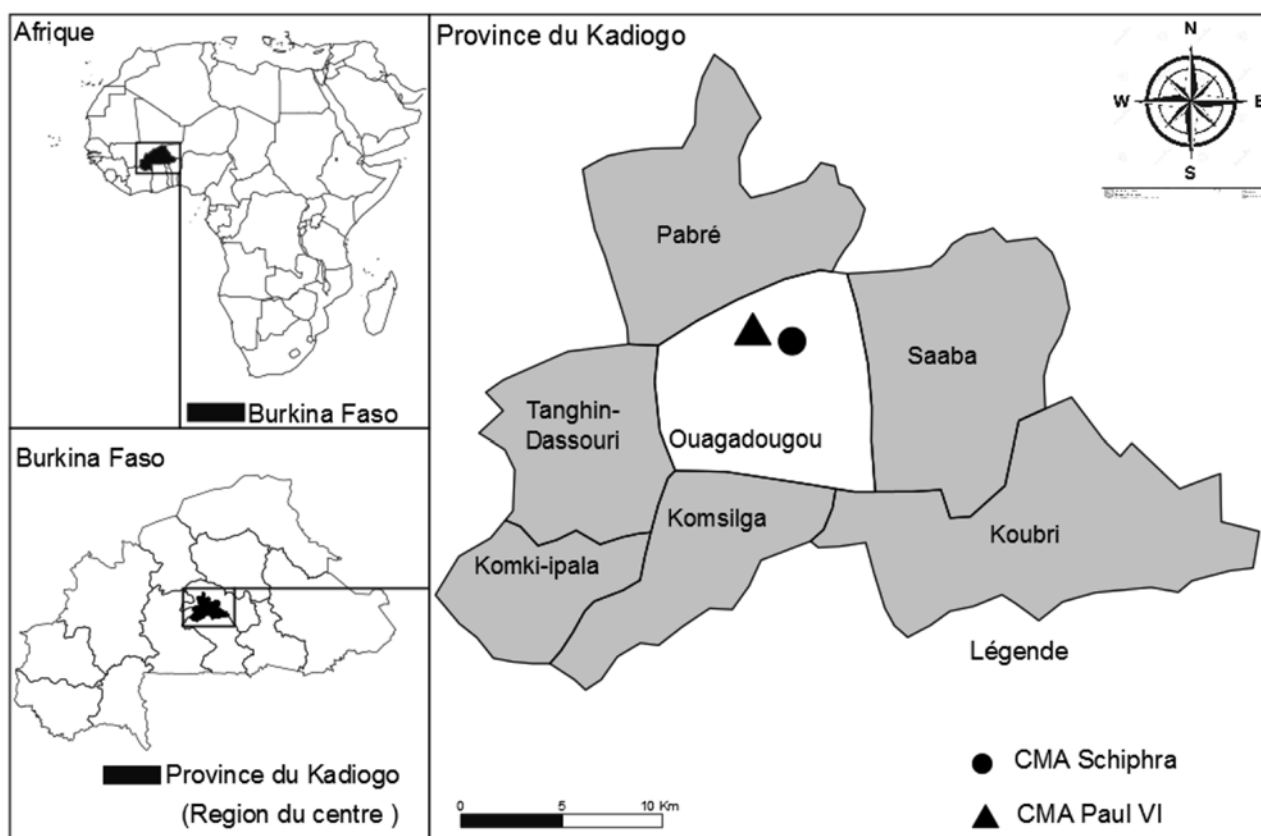

Fig. 1. Map of Ouagadougou with the sites where sampling was done (CMA Schiphra and CMA Paul VI)

Table 1. Oligonucleotides primers used for multiplex PCR reaction

Pathotype	Target gene	Primer sequence (5′ to 3′)	Product size (bp)	[C] (μM)	Reference
Typical EPEC	*bfpB*	MP3-bfpB-F: GACACCTCATTGCTGAAGTCG MP3-bfpB-R: CCAGAACACCTCCGTTATGC	910	0.1 0.1	Muller et al., 2007
	eaeA	eae-F: TCAATGCAGTTCCGTTATCAGTT eae-R: GTAAAGTCCGTTACCCCAACCTG	482	0.1 0.1	Müller et al., 2007
EHEC and EPEC	*escV*	MP3-escV-F: ATTCTGGCTCTCTTCTTCTTTATGGCTG MP3-escV-R: CGTCCCCTTTTACAAACTTCATCGC	544	0.4 0.4	Müller et al., 2007
	ent	ent-F: TGGGCTAAAAGAAGACACACTG ent-R: CAAGCATCCTGATTATCTCACC	629	0.4 0.4	Müller et al., 2007
	EHEC-*hly*	hlyEHEC-F: TTCTGGGAAACAGTGACGCACATA hlyEHEC-R: TCACCGATCTTCTCATCCCAATG	688	0.1 0.1	Antikainen et al., 2009
EHEC	*Stx1*	MP4-stx1A-F: CGATGTTACGGTTTGTTACTGTGACAGC MP4-stx1A-R: AATGCCACGCTTCCCAGAATTG	244	0.2 0.2	Müller et al., 2007
	Stx2	MP3-stx2AF: GTTTTGACCATCTTCGTCTGATTATTGAG MP3-stx2A-R: AGCGTAAGGCTTCTGCTGTGAC	324	0.4 0.4	Müller et al., 2007
	astA	MP-astA-F: TGCCATCAACACACAGTATATCCG MP2-astA-R: ACGGCTTTGTAGTCCTTCCAT	102	0.4 0.4	Müller et al., 2007
EAEC	*aggR*	MP2-aggR-F: ACGCAGAGAGTTGCCTGATAAAG MP2-aggR-R: AATACAGAATCGTCAGCATCAGC	400	0.2 0.2	Müller et al., 2007
	pic	MP2-pic-F: AGCCGTTTCCGCAGAAGCC MP2-pic-R: AAATGTCAGTGAACCGACGATTGG	1,111	0.2 0.2	Müller et al., 2007
EIEC	*invE*	MP2-invE-F: CGATAGATGGCGAGAAATTATATCCCG MP2-invE-R: CGATCAAGAATCCTAACAAGAAGAATCAC	766	0.2 0.2	Müller et al., 2007
	ipaH	ipaH-F: GAAAACCCTCCTGGTCCATCAGG ipaH-R: GCCGGTCAGCCACCCTCTGAGAGTAC	437	0.1 0.1	Vidal et al., 2005

Table 1. *(cont'd)*

Pathotype	Target gene	Primer sequence (5´ to 3´)	Product size (bp)	[C] (µM)	Reference
ETEC	*elt*	MP2-LT-F: GAACAGGAGGGTTCTGCGTTAGGTG	655	0.1	Müller et al., 2007
		MP2-LT-R: CTTTCAATGGCTTTTTTTTGGGAGTC		0.1	
	estIa	MP4-STIa-F: CCTCTTTTAGYCAGACARCTGAATCASTTG	157	0.4	Müller et al., 2007
		MP4-STIa-R: CAGGCAGGATTACAACAAAGTTCACAG		0.4	
	estIb	MP2-STI-F: TGTCTTTTTCACCTTTCGCTC	171	0.2	Müller et al., 2007
		MP2-STI-R: CGGTACAAGCAGGATTACAACAC		0.2	
E. coli	*uidA*	MP2-uidA-F: ATGCCAGTCCAGCGTTTTTGC	1,487	0.2	Vidal et al., 2005

Legend: EAEC = enteroaggregative *E. coli*, EPEC = enteropathogenic *E. coli*, EIEC = enteroinvasive *E. coli*, EHEC = enterohemorrhagic *E. coli*, ETEC = entero-toxigenic *E. coli*, µM = micromolaire, [C] = concentration, pb = "paire de base"

KI-ZERBO") within 24 h in a cool box at +4 °C for immediate analysis.

Isolation and identification of E. coli

Stool samples were plated on eosin methylene blue agar (Liofilchem, Italy), and the plates were incubated at +37 °C for 18–24 h. After incubation, the suspected colonies were selected and streaked onto Mueller Hinton agar plate (Liofilchem, Italy). Confirmation was carried out by a biochemical microbiology method based on negative urease (Bio-Rad, France), negative citrate (Liofilchem, Italy), positive indole (Bio-Rad, France), positive lactose (Liofilchem, Italy), and positive orthonitrophenyl-β-D-galactopyranoside (ONPG) (bioMerieux, France). *E. coli* strains isolated were confirmed by API 20E (bioMérieux, France).

Multiplex polymerase chain reaction (16 plex PCR)

The 16-plex PCR was used to detect simultaneously 16 genes from the five main pathogroups of *E. coli* (EHEC, EPEC, EAEC, EIEC, and ETEC) as described by Antikainen et al. [19]. The genes investigated and primers used are described in *Table 1*. DNA extraction was performed using heating method [20]. Briefly, a lapful of bacterial growth of Mueller Hinton agar (Liofilchem, Italy) plate was suspended in 1 ml of sterile water. The mixture was boiled for 10 min at 100 °C and centrifuged for 10 min at 12,000 rpm at +4 °C. The supernatant was collected and used in the PCR reactions. A volume of 2.5 µl of supernatant was added in 22.5 µl reaction mixture containing 5 U of Taq DNA polymerase (AccuPower, Korea), deoxyribonucleic triphosphate (10 mM), buffer GC (10×), MgCl$_2$ (25 mM), and PCR primers. Thermocycling conditions were as follows: 5 min at +94 °C, followed by 30 amplification cycles of +94 °C for 30 s, +63 °C for 60 s, and +72 °C for 60 s with a final extension of +72 °C for 7 min on a thermal cycler (AB, Applied Biosystems). Following PCR, the reaction products were separated by electrophoresis in (1.5% weight/volume) agarose gel, stained with a Redsafe solution (Prolabo, France) and visualized under ultraviolet (UV) light (Gel Logic 200).

Statistical analysis

The Fisher's exact test with two-tailed *p* of Open Epi version 7.1.2.0 was used to determine the statistical significance of the results. A *p* value of <0.05 was considered statistically significant.

Ethical considerations

Permission to conduct the study was obtained from the hospital authorities of Burkina Faso, and informed verbal

consent was obtained from the parents/guardians of every child before sample collection. The study protocol was approved by the National Ethical Committee(s) of Burkina Faso (N° 2009-39).

Results

Prevalence of diarrheagenic E. coli pathotypes

From 315 children with diarrhea, 192 stool samples were positive to one suspected *E. coli* detection (60.9%). Four hundred and nineteen (419) strains of *E. coli* were isolated, from which 31 DEC (7.4%) were characterized. The following *E. coli* pathotypes were identified with different proportion: EAEC 48.4% (15/31), EPEC 25.8% (8/31 with 4 typical EPEC and 4 atypical EPEC), EIEC 12.9% (4/31), EHEC 9.6% (3/31), and ETEC 3.2% (1/31). The highest prevalence was observed in EAEC and EPEC *(Fig. 2)*. The prevalence of DEC was higher in children from CMA of Schiphra (58.1%) than in those from CMA Paul VI (41.9%), but this difference is not statistically significant ($p = 0.08$; odds ratio [OR] = 0.48 (0.22–1.05)). In addition, 20/31 of DECs (64.5%) were characterized from children less than 1 year old. All EPEC and ETEC have also been identified in children less than 1 year of age.

Virulence genes of diarrheagenic E. coli pathotypes

The 16-plex PCR was used to detect a selected virulence genes carried by five pathotypes of *E. coli*. Virulence genes 35 (8.4%) of at least one DEC pathotypes was detected in 31 of the 419 *E. coli* strains, with 18 (58.1%) being positive for virulence genes of EAEC, 8 (25.8%) of EPEC with 4 (12.9%) of typical EPEC and atypical EPEC each, 4 (12.9%) of EIEC, 4 (12.9%) of EHEC, and 1 (3.2%) of ETEC *(Table 2)*.

Discussion

In this study, we investigated the occurrence of the major DEC pathogroups by 16-plex PCR in 315 stool samples from children with diarrhea in two health districts of Ouagadougou, Burkina Faso, namely, CMA Paul VI and CMA of Schiphra. Globally, the prevalence of DEC (7.4%) is quite comparable to the prevalence reported in Iran [21] but lower than results obtained previously in Burkina Faso [16], Mozambique [22], and Nigeria [12]. This lower prevalence may be due to the fact that nowadays, in developed and some developing countries, the improvement of the applied techniques in the recent years have greatly positively increased the level of detection of DEC infections. In fact, these improvements have consequently reduced the global death rate due to bacterial diarrhea diseases [17].

The present study concerned five pathotypes of *E. coli* (EAEC, EPEC [typical EPEC and atypical EPEC], EIEC, EHEC, and ETEC) responsible for children diarrhea in Burkina Faso. The majority of the previous studies carried out also implicated *E. coli* as the predominant bacterial agent in diarrheal diseases [13, 23–25]. Moreover, in Burkina Faso and many developing countries, DEC continues to be one of the major causes of morbidity and mortality among infants and young children [14, 26, 27]. This situation could be explained by the migration of persons within the same region and from one country to another, which increases the risk of transmission. Thus, this requires a better understanding of the epidemiology of the DEC infection in order to provide to the health policy makers and health care providers the information on this peculiar disease in each region or country.

EAEC was the most frequently detected pathotype in the present study. Nevertheless, some studies reported that EAEC might not be the primary cause of diarrhea [16, 28, 29]. Other authors have found EAEC to be associated with diarrhea, which is in concordance with our present results [30, 31]. EAEC has emerged as an important pathogen in several clinical scenarios including pediatric diarrhea
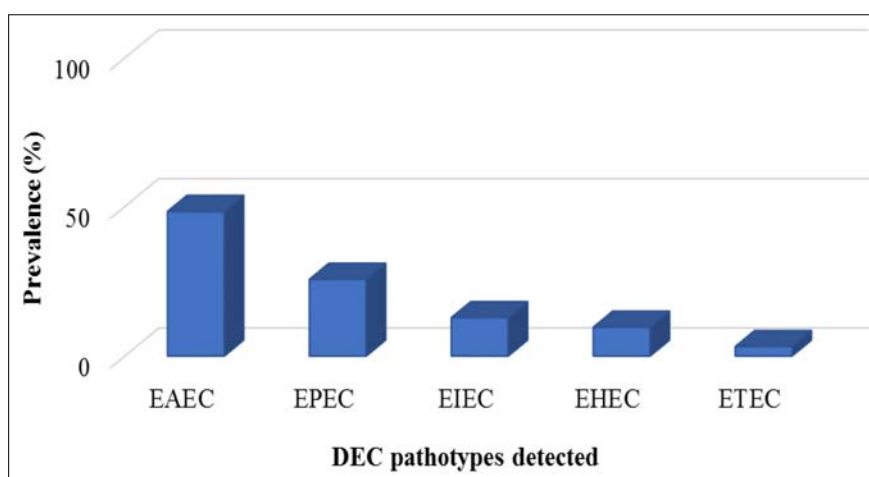

Fig. 2. Prevalence of diarrheagenic *E. coli* detected in stool samples. *Legend:* DEC = diarrheagenic *E. coli,* EAEC = enteroaggregative *E. coli,* EPEC = enteropathogenic *E. coli,* EIEC = enteroinvasive *E. coli,* EHEC = enterohemorrhagic *E. coli,* ETEC = Enterotoxigenic *E. coli*

Table 2. Prevalence of *E. coli* carrier of different virulence genes

Pathovars	uidA	pic	bfp	invE	EHEC-hly	elt	ent	escV	eae	ipaH	aggR	stx1	stx2	estla	estlb	astA
Control strains																
17.2 (EAEC)	+	+	–	–	–	–	–	–	–	–	+	–	–	–	–	+
EDL 933 (EPEC)	+	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–
H 907 (EIEC)	+	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–
E 2348-69 (EHEC)	+	–	–	–	+	–	+	+	+	–	–	+	+	–	–	–
Negative control	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
CMA Schiphra (n = 18)																
EHEC	–	–	–	–	3	–	–	–	–	–	–	–	–	–	–	1
Typical EPEC	–	–	4	–	–	–	–	–	–	–	–	–	–	–	–	–
Atypical EPEC	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–	–
EIEC	–	–	–	–	–	–	–	–	–	3	–	–	–	–	–	–
EAEC	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	7
ETEC	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
CMA Paul VI (n = 13)																
EHEC	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Typical EPE	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Atypical EPEC	–	–	–	–	–	–	–	3	–	–	–	–	–	–	–	–
EIEC	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–
EAEC	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	8
ETEC	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–

Virulence genes

Legend: CMA = Centre Médical avec Antenne churirgicale, – = absence, + = presence

which is endemic among children in developing countries [28, 29]. Thus, the high prevalence of the EAEC pathotype reported in our study could lead to public health concern.

The second pathotype with high prevalence was EPEC (25.8%). The EPEC prevalence observed in the present study was higher than those reported in a previous study which revealed that atypical EPEC was more prevalent than typical EPEC in Burkina Faso [16]. Typical EPEC and atypical EPEC differ in genetic characteristics, serotypes, and virulence properties. Indeed, typical EPEC possess a virulence plasmid *(bfpB),* while atypical EPEC do not possess this plasmid. In the past, typical EPEC were more frequently isolated than atypical EPEC in developing countries [32]. However, some recent reports indicate an increased isolation of atypical EPEC in developed countries as well as in developing countries [33–35]. In the present study, atypical EPEC constituted half of the total EPEC isolates, suggesting an epidemiological situation of possible transmission in the study area.

In this study, EIEC (12.9%) were detected, which is in agreement with the prevalence reported recently in Iran [36]. However, our result is higher than those reported by several other studies respectively in Tanzania, Nicaragua, and Burkina Faso [16, 20, 37]. This result may be related to the diversity of this pathotype reported during the last decade in Africa where different EIEC prevalence has been reported [22, 26, 29, 38, 39]. Our study suggests that EIEC may be an important and unrecognized cause of diarrhea in children.

EHEC (9.7%) was also characterized in our study. Similar prevalence was reported in South Africa as far the serotype O 157 is concerned [40]. However, the above-mentioned prevalence of EHEC is higher than the EHEC O157 prevalence (1.2%) reported in our previous study conducted in Burkina Faso [14]. One reason that could explain the differences might be that EHEC detection frequencies vary depending on countries. Moreover, in other African countries and in non-epidemic settings, EHEC appears to be more frequent in adults than children [26]. Furthermore, humans might acquire the pathogen from animal sources, given that EHEC can be transmitted primarily through the ingestion of fecal contaminated foods, particularly from the undercooked beef meat [41, 42]. Previous studies showed contamination of slaughtered animals with DEC pathotypes in Burkina Faso [6, 43]. However, in Iran, a large number of outbreaks of EHEC have also been associated with consumption of contaminated drinking water or contact with recreational water [44].

In the present study, ETEC was poorly detected (3.2%), while it is well-known as a causative pathogen of diarrhea in developing and developed countries [45]. Several countries in the world, including Bangladesh, Egypt, and Mexico, have reported ETEC as the most common cause of diarrhea among all *E. coli* intestinal pathotypes [11, 46–49].

Among the 8 isolated EPEC strains, 4 (50%) were typical EPEC (contained only *bfp* gene) and 4 (50%) were atypical EPEC (3 contained only *escV* gene and another 1 contained only *eae* gene). In contrast, a study reported in

Iranian, typical EPEC containing both *eae* and *bfp* gene, atypical EPEC containing only *eae* gene, and another containing only *bfp* gene [50]. The *estlb* gene was harbored by the only one (1) ETEC detected in our study. All 4 EIEC strains harbored the *ipaH* gene. However, detection of the EIEC-specific gene *ipaH* could also indicate the presence of *Shigella* sp. in the sample. For both EIEC and *Shigella,* the reservoir is considered to be the gut of infected humans [51].

Conclusion

The data generated in the present study with the use of 16-plex PCR assay, amplifying 16 virulence genes in a single reaction, is a practical and fast tool for DEC identification in routine clinical diagnostic and epidemiological studies. By using 16-plex PCR assay, DEC can be diagnosed in one PCR reaction that makes a conclusive diagnosis of diarrhea. Therefore, the use of 16-plex PCR as routine practice for detection of DEC in clinical laboratories would be an important diagnostic tool and thus resulting in better treatment of acute diarrhea in children although the cost would represent one major limitation for developing countries.

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Conflict of interest

The authors declare that they have no conflict interests.

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