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Molecular Prevalence and Epidemiological Characteristics of Diarrheagenic *E. coli* in Children under 5 Years Old in the City of Koula-Moutou, East-Central Gabon

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Abstract

Background and Purpose: Diarrhoeagenic *E. coli* (DEC) is one of the germs responsible for childhood diarrhea in developing countries. This study aims at determining the prevalence of the five main pathotypes of DEC isolated from faeces of children under five years old with diarrhea or not, living in the city of Koula-Moutou. **Methodology:** Isolates of *E. coli* were phenotypically screened on chromID™ agar and molecularly by multiplex PCR to detect the presence of enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohemorragic *E. coli* (EHEC) and enteroinvasive *E. coli* (EIEC). The evaluation of their sensitivity to 12 β-lactam antibiotic molecules was carried out by Kirby Bauer method. This method has also made it possible to characterize phenotypically the different β-lactamases produced. **Results and Conclusion:** Overall, at least one DEC pathovar was detected in the 63 *E. coli* strains with phenotypic and molecular frequencies of 63.5% and 68.5% respectively. Thus, ETEC (28.3%) and EHEC (28.3%) were the most frequent DEC in diarrheal isolates. ETEC/EHEC hybrid was recorded in both groups with rates of 7.5% in diarrheal cases and 10.0% for controls. The results showed produced carbapenemase type β-lactamases (31.7%), followed by ESBL (24.4%) and few produced high level penicillinases

(4.9%). The DEC, in particular ETEC and EHEC are most likely the epidemiological agents responsible for childhood diarrhea in this study.

Keywords

Diarrhea, Children, Diarrheagenic *E. coli*, β-Lactamases, Multiplex PCR

1. Introduction

Diarrhea remains the leading source of morbidity and mortality in children under 5 years of age worldwide with approximately 760,000 annual deaths [1] [2]. According to the World Health Organization, diarrheal infections are the second leading cause of death in children under 5 years of age after the Human Immunodeficiency Virus [3]. However, diarrheagenic *Escherichia coli* (DEC) is the main enteric pathogen involved in diarrheal disease [4] [5]. DEC strains are mainly responsible for causing gastroenteritis in children and account for 30% to 40% of childhood diarrhea episodes reported in developing countries [6]. In addition, recent work conducted in Africa, Spain, India, China, Mexico and Japan have revealed the evolution of the atypical and hybrid variants of the DEC strains, but also that these variants had a higher severity of infection than those of typical DEC [7] [8]. Moreover, DEC are involved in many infections due to their various virulence factors and pathogenicity mechanisms. Thus, they pose a serious public health problem [7] particularly among children and adults of developing countries.

In addition, the frequency of DEC is often high in Africa and varies with the geographical area. Indeed, in Nigeria, prevalences of 18.4% and 35% were respectively recorded in Abuja, [9] and Ile-Ife [10]. Whereas, DEC recorded prevalences were 37.5% in India [11], 16.4% in Southeast China, 21.4% in Eastern Iran and 23.3% in Northeast Mexico [12] [13] [14]. In the United States, approximately 20,781 cases of food-borne illness are caused each year by DEC, with a mortality rate of 0.8% [15].

These very large groups of *E. coli* strains, which have different pathogenicity mechanisms in diarrheal episodes, are classified into six (6) major pathotypes. According to numerous studies, we distinguish enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC) including shiga toxin producing *E. coli*, enteroinvasive *E. coli*, enterotoxinogenic *E. coli* (ETEC), Enteroaggregative *E. coli* (EAEC) and diffuse adherence *E. coli* (DAEC) [16] [17].

In Africa and particularly in Gabon, these pathogens are not routinely investigated. Moreover, epidemiological data on DEC is still rare and whenever *E. coli* strains are isolated and identified, the pathotypes are not identified. Therefore, this study was undertaken to determine phenotypically and genotypically the prevalence of diarrheagenic *E. coli* isolated from childhood diarrheal feces at the Paul Moukambi Regional Hospital Center in Koula-Moutou, East-central Gabon.

2. Methodology

2.1. Study Design and Population and Clinical Strains

Stool samples from 132 children aged 0 to 5 years old with acute diarrheal or not were collected in the city of Koula-Moutou over a two-year period (May 2016-Fevrier 2018). Samples were screened for *Enterobacteria* and parasites. Among the various presumptive pathogens cryopreserved in the Cellular and Molecular Biology Laboratory (LABMC), 63 strains of *E. coli* formed the biological material, of which 53 strains were isolated from children with diarrhea and 10 from healthy children (control). The details of the various characteristics and criteria of the patients recruited were previously described [18].

2.2. Characterization of *E. coli* Isolates

Phenotypic research of presumptive strains of *E. coli* O157:H7

Phenotypic research for enterohemorrhagic *E. coli* (EHEC) serotype O157: H7 in diarrheal and normal stool sample was carried out on the agar medium chromIDTM O157:H7. The young colonies of the *E. coli* strains were subcultured on the same medium and incubated at 37˚C for 18 - 24 h. *E. coli* O157:H7 colonies gave the characteristic Green color.

Molecular identification and classification of diarrheagenic *E. coli* (DEC)

All strains of *E. coli* were analyzed as described in previous studies with some modifications [19] [20] and the summary description is detailed below.

DNA Extraction

Genomic DNA was extracted bacterial colonies as described below. Colonies were resuspended in 200 µl of 1X DNA/RNA reagent (Biolabs New England). The bacterial suspensions were lyzed in 200 µl of 2X lysis buffer (1% SDS, 20 mM Nacl, 20 mM Tris pH 8, 20 mM EDTA) and 40 µl of 100 µg/ml proteinase K solution. Then, the preparations were incubated in a heat block at 56˚C for 1h. The resulting lysates obtained were centrifuged at 12,000 rpm for 10 min. Then, released DNA was precipitated by adding 350 µl of isopropanol and 50 µl of sodium acetate at 3M and centrifuging at 14,000 rpm for 15 min. The DNA containing pellets were resuspended in 70 µL of ultra-pure water and used later for PCR amplification.

Multiplex Polymerase Chain Reaction

E. coli pathogens (EAEC, EIEC, EHEC, EPEC, ETEC and STEC) were identified using primers listed in the Table 1.

PCR was performed in a final volume of 20 µl containing 1X master mix PCR multiplex (Qiagen® Multiplex PCR Kit), 1X Q-Solution (Qiagen® Multiplex PCR Kit) and 2 µl of genomic DNA diluted 1000 fold, and 0.3 µM of each primer set. The amplification was carried out in a thermocycler (Bio-RAD, T100TM, USA) under the program described by Vilchez *et al.* [20]. The *E. coli* strains carrying both *eaeA* and *stx* genes were considered as STEC O157: H7 (Table 1). On the other hand, those carrying only the *eaeA* gene were considered as atypical EPEC (aEPEC). While those carrying the *bfpA* gene or both (*eaeA* and *bfpA*) were

Table 1. Primers used for the detection of the various DEC.

Type of DEC	Genes	Primer	Primer sequence (5' - 3')	Size (bp)	References
ETEC	*eltB*	LT-F	TCTCTATGTGCATACGGAGC	322	[20]
		LT-R	CCATACTGATTGCCGCAAT		
	estA	ST-F	GTCAAACCAGTA(G/A)GGTCTTCAAAA	147	[20]
		ST-R	CCCGGTACA(G/A)GGAGGATTACAACA		
EHEC	*vit*1	VT1-F	GAAGAGTCCGTGGGATTAC	130	[20]
		VT1-R	AGCGATGCAGCTATTAATAA		
	*vit*2	VT2-F	ACCGTTTTTCAGATTTT(G/A)CACATA	298	[20]
		VT2-R	TACACAGGAGCAGTTTCAGACAGT		
EPEC	*eaeA*	eae-F	CACACGAATAAACTGACTAAAATG	376	[20]
		eae-R	AAAAACGCTGACCCGCACCTAAAT		
	bfpA	bfpA-F	TTCTTGGTGCTTGCGTGTCTTTT	367	[20]
		bfpA-R	TTTTGTTTGTTGTATCTTTGTAA		
EAEC	pCVD432	EA-F	CTGGCGAAAGACTGTATCAT	630	[20]
		EA-R	AAATGTATAGAAATCCGCTGTT		
EIEC	*ial*	SHIG-F	CTGGTAGGTATGGTGAGG	320	[20]
		SHIG-R	CCAGGCCAACAATTATTTCC		
Shigella ipaH	*ipaH*	ipaH-F	GTTCCTTGACCGCCTTTCCGATACCGTC	619	[21]
		ipaH-R	GCCGGTCAGCCACCCTCTGAGAGTAC		
	eaeA	eae-F	CACACGAATAAACTGACTAAAATG	376	
		eae-R	AAAAACGCTGACCCGCACCTAAAT		
STEC O157	*stx1*	X1-F	CAGTTAATGTGGTGGCGAAG	894	[22]
		X1-R	CTGCTAATAGTTCTGCGCATC		
	stx2	X2-F	CTTCGGTATCCTATTCCCGG	478	
		X2-R	GGATGCATCTCTGGTCATTG		

identified as typical EPEC (tEPEC). The amplicons were separated on a 1.5% (m/v) agarose gel for 2 h at 300 V. The 100 bp ladder was used as a molecular weight marker (100 bp; Qiagen®, GelPilot®). The DNA bands were visualized and photographed under UV light following the gel staining with ethidium bromide.

Diversity of β-lactamases produced by characterized diarrheagenic *E. coli*

The Antibiogram profile was determined by the agar dilution method according to the reference technique of diffusion in agar medium (Mueller-Hinton) against 12 specific antibiotics of the β-lactam family [23] to determine their sensitivity profiles and categorize phenotypically the different β-lactamases likely to be produced by the DEC. The detection of extended-spectrum β-lactamase isolates (ESBL) was not only done by screening on MacConkey agar supplemented with concentrated Cefotaxime at 2 mg/ml; but also, by the double disc synergy test

(phenotypic confirmation test for ESBL) according to the standards of the Antibiogram Committee of the French Society of Microbiology v.2.0. Mai 2019.

2.3. Statistical Analyzes

Statistical analyzes were performed using R software version 3.2.2. The significance threshold set was 5%. Fisher's exact test compared the prevalence of DEC isolates between the two population categories.

3. Results

3.1. Phenotypic Detection of *E. coli* O157:H7

The characterization of the 63 *E. coli* strains isolated from children with diarrhea and controls was carried out phenotypically by looking for serovar O157: H7 and genotypically by looking for genes of interest. The results of the phenotypic detection of the *E. coli* O157: H7 strains are presented in Figure 1.

In total, 63.5% (40/63) of the *E. coli* isolates were phenotypically positive for the serotype O157:H7, of which 61.9% were detected in diarrheic children and 1.6% in healthy ones. In diarrhea cases, out of 53 *E. coli* strains screened, 39 strains (74%) were presumptive of the O157:H7 phenotype compared to 1 strain (10%) in control cases (see Figure 1).

3.2. Genotypic Detection of DEC

Of the 63 strains of *E. coli*, the detection rate of genes of interest for DEC characterization was 68.3% (43/63). The various pathovars thus detected after PCR screening are presented in Table 2.

The results of Table 2 revealed that 67.9% (36/53) of the isolates from diarrheal cases were positive DEC against 10% (1/10) of the isolates for the control

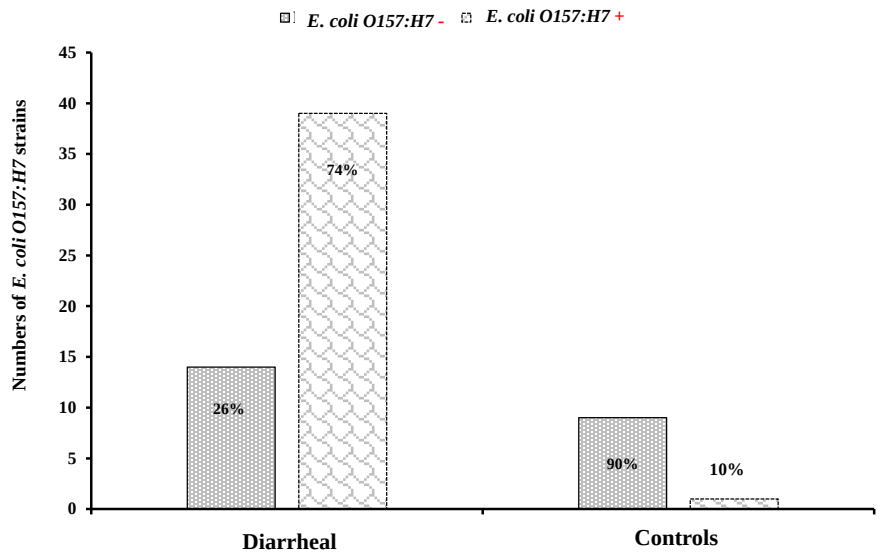

Figure 1. Proportion of *E. coli* O157:H7 strain in children with and without diarrhea (control group).

Table 2. Frequency of detection of DEC in diarrheal cases and controls in the city of Koula-Moutou.

Type of DEC	N (%) isolated *E. coli* in cases and controls		OR	Fisher's exact test p-value
	E. coli isolated from diarrheic children (n= 53)	*E. coli* isolated from healthy children (n = 10)		
Mono-detection	**36 (67.9)**	**1 (10.0)**	**16.76**	**0.001**
EAEC	2 (3.8)	0 (0.0)	NA	NA
EHEC	15 (28.3)	1 (10.0)	3.18	0.4
EPEC	4 (7.5)	0 (0.0)	NA	NA
ETEC	15 (28.3)	0 (0.0)	NA	NA
EIEC	0 (0.0)	0 (0.0)	NA	NA
STEC	0 (0.0)	0 (0.0)	NA	NA
Shigella ipaH	0 (0.0)	0 (0.0)	NA	NA
Co-detection	**5 (9.4)**	**1 (10.0)**	**1.36**	**1**
ETEC/EHEC	4 (7.5)	1 (10.0)	0.54	0.5
EAEC/ETEC	1 (1.9)	0 (0.0)	NA	NA

OR: odds ratio; **NA:** not applicable.

group for mono-detection. In addition, Fisher's exact test showed a significant difference between the two groups (p = 0.001). Among the different DEC detected in diarrheic children, ETEC and EHEC pathotypes were the most common with a detection rate of 28.3% each. The least representative pathotype were the EPEC and the EAEC with frequencies of 7.5% and 3.8%, respectively. While for the control group, the only type of DEC identified was the EHEC pathotype with a frequency of 10%. On the other hand, no significant difference was observed between the cases and the control group for the type of DEC (p = 0.4). Furthermore, no strain is STEC or producer of the virulence factor of *Shigella* ipaH (Table 2).

The co-detection of DEC pathogroups was also recorded in the two groups with an overall prevalence of 9.4% for diarrheal cases and 10% for the control group without any significant difference (p = 1.0) (see Table 2) ETEC/EHEC hybrids were detected in 7.5% of cases of diarrhoea and 10.0% for controls with no significant difference (p = 0.5). The detection of EAEC/ETEC hybrids was found only in diarrheic cases with a rate of 1.9%.

3.3. Different Genes of Intra-Pathotype Determinants and Classification of DEC

The classification of DEC into standard pathovars was carried out by specific research into their virulence determinants, the profile of which is presented in Table 3.

Table 3 analysis revealed a heterogeneity in the virulence factors of the four

Table 3. Frequency of detection of selected virulence factors within DEC.

Pathotypes	N = 43	*virulence genes*	Number of strains (n)	Percentage (%)
EAEC	**2**			
		EA	2	100.0
EHEC	**16**			
		vit1	2	12.5
		vit2	11	68.8
		vit1-vit2	3	18.8
EPEC	**4**			
tEPEC	4	*bfpA*	3	75.0
		bfpA + eae	1	25.0
aEPEC	0	eae	0	0.0
ETEC	**15**			
		ST	12	80.0
		LT	3	20.0
ETEC/EHEC	**4**			
		vit1-ST	3	75.0
		vit2-ST	1	25.0
EAEC/ETEC	**1**			
		EA-LT	1	100.0

tEPEC: EPEC typical; **aEPEC:** EPEC atypical.

(4) pathotypes within the different *E. coli* strains. The EHEC pathogen was mainly carrier of the *vit2* verotoxin gene (68.8%), followed by the combination of *vit1* and *vit2* verotoxin genes (18.8%) and finally those carrying the *vit1* gene (12.5%). Eighty percent (80.0%) of ETEC were carriers of the thermostable *ST* toxin genes versus 20.0% for the thermolabile LT toxin genes. Finally, for the EPEC pathotype, only typical EPEC were recorded, 75.0% of which possesses the *Bfp* determinant gene while 25% the *Bfp* and *Eae* determinant genes (see Table 3). Regarding *E. coli* hybrid pathovars, the association of EA-*LT* virulence genes (100%) was the most common for EAEC/ETEC, followed by the association of the vit1-ST determinant genes (75.0%) for ETEC/EHEC.

3.4. Classification of Diarrheagenic *E. coli* According to *β*-Lactamases

Among the 41 characterized diarrheagenic *E. coli* strains, a classification of the *β*-lactamases they produce was performed, the results are presented in Table 4.

The results of Table 4 revealed a diversity of *β*-lactamases produced by DEC. Thus, it appeared that the EAEC of this study produced the association of ESBL + Case and Carbases enzymes with frequencies of 50% each. The EHEC produce

Table 4. Diarrheagenic *E. coli* and their *β*-lactamase enzyme diversity.

Type of DEC	Number of strains	PHN n (%)	PRI n (%)	ESBL n (%)	Case n (%)	ESBL + Case n (%)	Carbase n (%)
EAEC	**2**	-	-	-	-	1 (50.0%)	1 (50.0%)
EHEC	**15**	4 (26.7)	-	3 (18.8)	1 (6.3)	4 (25.0)	3 (18.8)
EPEC	**4**	-	-	-	-	-	4 (100.0)
ETEC	**15**	-	2 (13.3)	6 (40.0)	2 (13.3)	1(6.7)	4 (26.7)
ETEC/EHEC	**3**	1 (25.0)	-	1 (25.0)	1 (25.0)	-	1 (25.0)
EAEC/ETEC	**1**	-	-	-	-	1 (100.0)	-
Total	**41**	**5 (12.2)**	**2 (4.9)**	**10 (24.4)**	**4 (9.8)**	**7 (17.1)**	**13 (31.7)**

PHN: high-level penicillinases; **PRI:** inhibitor resistant penicillinase; **Case:** cephalosporinase; **ESBL:** extended spectrum β-lactamase; **Carbase:** carbapenemase; **EAEC:** enteroaggregative *E. coli*; **EHEC:** enterohemorragic *E. coli*; **EPEC:** enteropathogenic *E. coli*; **ETEC:** enterotoxinogic *E. coli*.

mostly PHN (26.7%) and the association ESBL + Case (26.7%). These strains also produced ESBL + Carbase, and Case with frequencies of 18.8% and 6.3%, respectively. All EPEC were producers of Carbases and the ETEC in this study were mostly producers of ESBL (40.0%). The other isolates in this group produced 26.7% of Carbases, 13.3% of each PRI and Case, and 6.7% of ESBL + Case (see Table 4). ETEC and EHEC hybrids produced the enzymes of the PNH, ESBL, Case and Carbase types with the same frequency (25.0%). Whereas, the only EAEC/ETEC hybrid only produced the association ESBL + Case (100.0%).

4. Discussion

The objective of this study was to determine the epidemiological profile of *E. coli* pathovars isolated from stool samples of children aged 0 - 5 years with acute diarrhea or not living in the city of Koula-Moutou. Diarrheagenic *E. coli* (DEC) in childhood diarrhea in developing and developed countries has a heavy burden on the morbidity and mortality rates of this class age.

The high frequency of the different DEC pathovars found in this study, both phenotypically (63.5%) and molecularly (68.3%) is similar to that reported by d'Iijima *et al.*, in Kenya [24], Darbandi *et al.* in Iran [25] and higher than that reported in other developing countries [14] [26]. The impact of these results clearly highlights the public health threat posed by diarrheal diseases due to DEC for children in these developing countries [14]. Particularly, the serogroup O157, which is part of serogroup O111, is the most frequently isolated and often associated with one or more of these pathotypes [27] [28]. This health threat is especially higher in rural and even urban areas due to poor hygiene conditions. Indeed, the many rivers in the city of Koula-Moutou, are generally used for domestic needs by local populations. However, their pollution by human and animal wastes or other forms of pollution such as poor maintenance of public water pumps are all risk factors for transmission of these pathogens [29] [30]. Currently, an increase in these DEC is described in particular by the phenomenon of

cross-transmission relating to environmental factors such as the contamination of irrigation water [16] [31].

In this study, the results obtained underline the importance of ETEC (28.3%) and EHEC (28.3%) as the dominant agents of DEC responsible for childhood diarrhea in the city of Koula-Moutou as reported in the literature [31]. These results are different from those reported by other authors in the cities of Libreville and Lambaréné in Gabon [33] [34]. This observed difference could be explained by the spatio-temporal variability of the epidemiological character concerning the infectious etiologies of childhood diarrhea [1] [35]. The prevalence of ETEC recorded is similar to that reported in Nicaragua among children in the same age group which was 27.9% [20]. This could be explained by the fact that most *E. coli* isolates were from children over 6 months of age who loses the immunity [antibodies] transmitted through breast milk overtime [9]. Indeed, it has been stressed in the literature that breast milk could give infants protection against infectious diseases [36]. In addition, the practice of exclusive breastfeeding during the first 6 months or up to the 11th month of birth is an effective preventive means against child mortality related to diarrheal diseases [37]. This prevalence was higher than the one reported among children in Malawi (10.6%) [38], in a rural area of Egypt (18.9%) [39], in Kenya (7.2%) [32], in Northern India (6%) [40] and Perou (4%) [41].

Although previous studies have described a low prevalence or absence of EHEC in diarrheal infections in developing countries [20] [42] [43], their prevalence was significant in this study (28.3%) and they all produced verocytoxins. These results corroborate the rapid expansion of the involvement of this group of DEC in diarrheal diseases in children [44] [45]. This expansion could probably be favored by the presence of numerous rivers used by populations prone to human and/or animal faecal contamination.

The cases of detection of EAEC in our study was very low compared to current data [43] [45] [46]. Indeed, a prevalence of 3.8% was recorded in this study which is lower than the prevalences of 34.4% reported by Onanuga *et al.*, in Nigeria [9], 14.7% in South India [45], 14% in Tehran [22], 9.5% in Burkina Faso [26] and 7.86% in Lambaréné, Gabon [33]. However, it is comparable to that found in India (3.8%) [47] and Iran (4.0%) [25]. Their isolation in this study could corroborate the fact that these pathogens are considered as emerging pathogens currently in cases of diarrheal diseases especially in developing countries [9] [26]. Also, they could be involved in acute and chronic diarrhea in all age groups [7]. Their frequent detection could be linked to their ubiquitous character of any environment that could be favored by poor hygiene conditions in developing countries [26] [47]. Finally, they are associated with diarrhea known as traveler's diarrhea in developed and developing countries [15] [46].

The frequency of EPEC cases (7.5%) in this study is similar to that recorded in other studies in developing countries [47] [48]. However, the latter is higher than that recorded in Lambaréné (Gabon) where no EPEC isolates were found

[33], However, our EPC frequency was lower than the frequencies of 57.7% and 50.0% reported respectively by Koko *et al.*, in Libreville, the capital city of Gabon, and, Zhou *et al.*, in China [34] [46], reflecting the epidemiological variability of pathogens. In addition, these EPEC isolates could also be one of the very important pathogens in children with diarrhea in developing countries even though other pathogens are currently involved [49] [50].

Ability to acquire virulence genes by horizontal transfer in DEC to other pathogens leads to the development of hybrids or mixed virulence profiles [8]. Thus, the hybrid ETEC/EHEC is one of the peculiarities of this work corroborating many studies [11] [51]. This result could be explained by the fact that these two types of DEC are the most common germs by their mode of transmission. Indeed, these are transmitted through the ingestion of contaminated water and food, in addition to the geo-epidemiological variability of DEC [26] [52] [53]. Although poorly identified, EAEC/ETEC hybrid strains were also recorded in Burkina Faso and Kenya with similar prevalence [26] [43] and in India in larger proportions [48]. However, the increase in the hybridization phenomenon between these two strains has been suggested in several studies [51] [54], precisely because EAEC can integrate other virulence genes in order to generate epidemic isolates [55], this emphasizes the fact that this phenomenon is expanding and therefore it would be wise to implement monitoring methods.

The absence of identification of EIEC isolates (0.0%) in this study is perfectly in line with the literature that reports a weak prevalence of this class of DEC in young children [9] [40]. Indeed, they are known to cause diarrheal symptoms similar to those of *Shigella* in adults and children sometimes associated with malnutrition [7] [56]. The registered results are similar to those found in India (1.0%), Iran (0.7%), Nigeria (0.6%), Kenya (0.6%), Libya (0%), and Gabon (0%) [9] [24] [32] [41] [42] [44].

4.1. Different Genes of Intra-Pathovar Determinants and Classification of DEC

The characterization of virulence factors not only makes it possible to classify the different pathotypes of the *E. coli* strains, but also these factors are of particular interest as potential targets in therapeutic and/or vaccine trials [57]. For the ETEC pathotype, the prevalence of the enterotoxin *ST* gene was highest and there were no isolates combining both enterotoxin types in this study. This profile is comparable to that obtained by Saka *et al.*, in Nigeria [58], from Cabal *et al.*, in Spain [59], and other studies [25] [57]. Although, in the latter ones isolates combining the two types of enterotoxins were found

The low prevalence of verocytotoxic O157 EHEC isolates in developing countries makes epidemiological characterization of circulating strains difficult [43]. However, the verocytotoxin *vit2* gene and the combination of both types were the most common genetic markers in the EHEC pathogen in this study. This profile is similar to previous studies [20] [57]. In addition, the virulence markers of the

EHEC island-O like shiga toxins (stx/vt) marked by a high diversity of variants of this family [7] [32]. Virulence factor stx2/vt2 is most frequently reported in the literature [44] [60] corroborating the results of this study on the most recurring virulence determinant.

EPEC is one of the most frequently isolated DEC pathotypes in developing countries, particularly in children under 2 years of age [46] [47] [61]. Current advances in their characterization demonstrate the emergence of atypical strains (aEPEC) in addition to the so-called typical strains [50] [62]. The EPEC strains detected in this study were all typical EPEC and were significantly higher in children with diarrhea than in controls. Their high prevalence compared to atypical would be the fact that they are still prevalent in the poor regions of sub-Saharan Africa [63] [64] while atypical are predominant in developed countries [65]. Our results are in agreement with those reported by Saka and collaborators in Nigeria [58]. But, different from those found by Zhou *et al.*, in China, Singh *et al.*, in India and Darbandi *et al.*, in Iran who reported aEPEC prevalences of 77.8%; 20.0%, and 39,0%, respectively, [25] [46] [64].

4.2. Comparison between Phenotypic and Molecular Detection

The *E. coli* O157: H7 serotype is the main agent implicated in hemorrhagic diarrhea, the molecular characteristic is the presence of the genes coding for a shiga-toxin (stx) by these so-called STEC strains and the capacity for attachment and effacement induced by intimin, an adhesion receptor encoded by the *eaeA* gene [66] [67]. Although 63.5% of strains had phenotypic characteristics, no PCR detection of associated genetic markers coding for the *stx1* and *stx2* variants associated with the *eae* gene was recorded in this study. Our results are different from several studies that found high levels of *stx* genes [44] [68]. However, 28.3% of the strains were carriers of the *vit1* and *vit2* genes coding for verocytotoxins whose O157 serotype is also one of the *E. coli* strains producing verocytotoxins known to be harmful to humans [69]. The strong presence of this serotype of DEC suggests that the origin of diarrhea is food. Indeed, an incidence of 801 cases of foodborne *E. coli* O157 infection in the province of Alberta in Canada was estimated by the public health database [60] and the Centers for Disease Control and Prevention estimated that in the United States, *E. coli* O157: H7 causes more than 63,000 cases per year, resulting in more than 2100 hospitalizations and 20 deaths [67]. The rural nature of the study city, consumption of local and imported meats, stream water would contribute to the high rate of *E. coli* O157 recorded.

4.3. Classification of *β*-Lactamases by Diarrheagenic *E. coli*

The expansion of antibiotic resistance, particularly in enteric isolates in developing countries, is of great concern, and an improvement in our knowledge of the different susceptibility profiles in the latter is essential [70] [71]. Literature reports many cases of multiresistant DEC strains [14] [61] [72] [73]. The phe-

notypic characterization of the possible resistance mechanisms responsible for the multidrug resistance observed in the DEC of this study reveals significant levels of isolates producing carbapenemase type β-lactamases (31.7%), extended spectrum β-lactamases (ESBL) (24.4%) and the ESBL-cephalosporinases association (17.1%). Indeed, previous studies on the analysis of multidrug resistance phenotypes of DEC certainly favor the production of various ESBLs with variable prevalences; however, other β-lactamases are also involved. In Burkina-Faso, a study revealed 38.7% of DEC-ESBL, 6.4% DEC-ESBL + Case and 9.7% DEC-ESBL + carbapenemases [5]. In India, Mandal *et al.* reported a DEC-ESBL prevalence of 37.6% of [74], while two different studies in Iran reported prevalences of 43.8% [73] and 69.2% [75], comparable to that found in our study. However, the ETEC pathotype was the most common producer of ESBL, a profile similar to other studies [57] [74]. The frequency of carbapenemases recorded in this study, particularly for EPEC, ETEC and EHEC pathotypes, is likely related to the current pattern of *E. coli* enzyme release in *Enterobacteriaceae*. In addition, this diversity of β-lactamases could be the consequence of horizontal transfer of resistance and virulence genes involving mobile genetic elements such as resistance plasmids and class 1 integuments [76] [77] which contribute to the increase of new pathogens and their multiresistance.

4.4. Limitations of the Study

The data from this study do not allow for clear profiling of the different pathovars of circulating *E. coli*. In addition, the low detection of DEC in control children, probably correlated with the sample size, does not allow a strict distinction between strains with high clinical importance and those with minor clinical importance.

5. Conclusion

The results of this study demonstrate the prevalence of different of diarrheagenic *E. coli* pathotypes, particularly EHEC and ETEC, are highly correlated with childhood diarrhea in the city of Koula-Moutou. However, the detection of EHEC and ETEC strains in apparently healthy children would indicate that they could then be sources of transmission of these pathogens to other children. This study also shows that virulence genes associated with different *E. coli* pathotype can coexist in the same strains. Ultimately, this study demonstrates the high prevalence of DEC in rural areas, the development of new hybrid DEC strains and those producing ESBL and carbapenemase β-lactamases. This could be a new risk factor for morbidity and mortality among young children in developing countries, and could challenge traditional diagnoses of *E. coli* infections, thereby increasing the risk of therapeutic deadlock.

Author Contributions

All authors contributed to the study conception and design. Material prepara-

tion, data collection and analysis were performed by Rolande Mabika Mabika, Jean Fabrice Yala, Franck Mounioko and Sandrine Lydie Oyegue Liabagui. The first draft of the manuscript was written by Rolande Mabika Mabika, Jean Fabrice Yala, Hilaire Kenguele Moundounga, Alain SOUZA and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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