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OCCURRENCE OF MULTIPLE ANTIBIOTIC RESISTANCES OF *ESCHERICHIA COLI* ISOLATED FROM DIARRHEAL CHILDREN LESS THAN FIVE YEARS IN BURKINA FASO.

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ABSTRACT

Diarrhea is a major public health and economic problem in Burkina Faso. The aim of this study was to evaluate antibiotic resistance of Diarrheagenic *Escherichia coli* (DEC) isolated from young children suffering from diarrhea in Ouagadougou, Burkina Faso. From August 2013 to May 2014, 119 diarrheal stool samples were collected from children. *E. coli* was isolate and identified by standard microbiological techniques. Antibiotic sensitivity and detection of extended spectrum β-lactamases (ESBLs) were performed according to European Committee of Antimicrobial Susceptibility Testing (EUCAST). This study showed a high prevalence (25.2%) of diarrhea during the month of September in Ouagadougou. Of the 21 DEC detected (69.7%), 13 were Enteropathogenic *Escherichia coli* (EPEC) (15.7%) and 8 Enterohemorrhagic *Escherichia coli* (EHEC) O157 (9.6%). DEC rates were resistant to erythromycin (100%), amoxicillin-clavulanic acid (95.2%), doxycyclin (95.2%), tetracyclin (85.7%), ampicillin (81%) and trimethoprim-sulfamethoxazole (76.2%). Most of the DEC (EPEC and EHEC O157) presented a Multidrug resistance (MDR) for β-lactam, cyclins, macrolides and sulfamides but all the DEC isolated in this study was not ESBLs producers. Those results indicated that it is imperative to develop strategies for prevention, epidemiological surveillance and control of MDR bacteria.

KEYWORDS: Antibiotics, Burkina Faso, Diarrheal Children, Diarrheagenic *Escherichia coli*, Multidrug resistance.

INTRODUCTION

Infectious diseases continue to be a major public health and economic problem worldwide, particularly in developing countries.[1] Among these diseases, diarrheal infections remain the second leading cause of death in children younger than 5 years globally, accounting for 1.3 million deaths annually.[2] In Burkina Faso, children diarrhea is a public health concern because it's the third cause of morbidity and mortality.[3] Moreover, according to a study conducted in Ouagadougou, 39% of hospitalizations due to diarrhea in children less than 5 years of age were caused by Rotavirus with 94.2% prevalence among those aged less than 2 years.[4]

The etiological agents include a wide range of viruses, bacteria and parasites. Among bacterial pathogens, Diarrhoeagenic *Escherichia coli* (DEC) are important agents of endemic and epidemic diarrhoea worldwide.[5,6] In developing countries, DEC are said to be commonly associated with endemic form of diarrhea. There is no standard classification of DEC.[7] Nowadays, there are six pathotypes which cause diarrhea: Enteropathogenic *E. coli* (typical or atypical EPEC), Enterotoxigenic *E. coli* (ETEC), Shiga toxin-producing *E. coli* (STEC), Enteroaggregative *E. coli* (EAEC), Enteroinvasive *E. coli* (EIEC) and Diffusely adherent *E. coli* (DAEC).[7] From those pathotypes, enterohemorrhagic *E. coli* (EHEC), a subtype or subgroup of STEC is associated to two major diseases in humans, the urine-hemolytic syndrome (UHS) and the hemorrhagic colitis (HC), being the serotype O157: H7 its main representative.[5,8] while EPEC is associated to childhood diarrhea, affecting especially children under 5 years in developing countries.[9, 10]

However, antimicrobial resistance in enteric bacteria as DEC complicates the situation in developing countries, where indiscriminate use of antimicrobial agents is common.[11] Determination of the serotype and the antimicrobial susceptibility pattern is usually adequate for defining outbreak strains of DEC in order to determine appropriate therapies for patients with suspected *E. coli* infections.[12]

Data exist on the prevalence of *E. coli* (EPEC and EHEC O157) in rural settings of Burkina Faso, using serotyping methods [13] but not in urban settings. This study was initiated to determine the prevalence and occurrence of multiple antibiotic resistances of DEC strains isolated from diarrheal children less than 5 years in Ouagadougou, Burkina Faso.

MATERIAL AND METHODS

Study design and site

This was a prospective study conducted from August 2013 to May 2014. Stool samples collection was done in "Centre Medical avec Antenne chirurgical (CMA)" Paul VI, a medical Centre of Ouagadougou, Burkina Faso (Fig. 1).

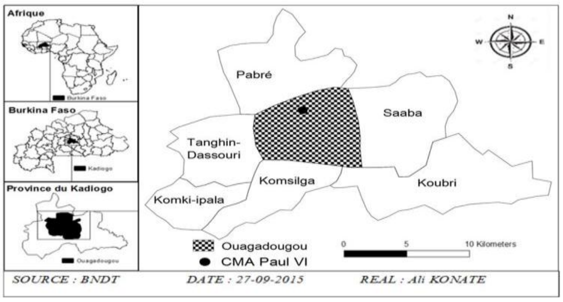

Figure 1: Study Area (CMA Paul VI of Ouagadougou)

Legend: CMA = Medical Centre with Surgical Antenna.

Samples and clinical data collection

Study population was composed of children less than 5 years of age with acute diarrhea and who were hospitalized or received as external consultation in the health center (CMA). All other contrast cases were excluded. Hundred and nineteen stool samples (119) were collected from children (less than 5 years of age) suffering from diarrhea. Samples and epidemiological investigations were carried out with the informed verbal consent obtained from the parents/guardians of every child before taking the stool samples. Stool samples were collected in sterile containers and transported to 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 Professeur Joseph KI-ZERBO") within 24 h in a cool box at 4°C for immediate analysis.

Isolation and identification of EPEC and EHEC O157

Escherichia coli were isolated according to the recommendations of the French Society for Microbiology.[14] Thus, the Levine's Eosin-Methylene-Blue agar (EMB) (Liofilchem-Italy) was used to isolate *Escherichia coli* (metallic green colonies). As relates to the isolation of EHEC O157, samples were plated onto Mac Conkey agar with Sorbitol (Liofilchem-Italy) plate (being colonies). After 24 hours incubation at 37°C, 2 or 3 suspected colonies of *E. coli* (metallic green colonies or being colonies) were subcultured onto Muller Hinton agar (Liofilchem-Italy) and then identified by classic biochemical tests (Catalase, Oxidase, Urease, Indole, Citrate, Lactose, Glucose, H_2S, Gas, Mannitol, Mobility). *E. coli* species biochemical confirmation was performed with API 20E (bioMérieux, France) and the interpretation was done using API 20E catalogue according to the manufacturer's instructions.

Serotyping of EPEC and EHEC O157

In this study, isolates biochemically identified as *E. coli* were serologically examined by slide agglutination test according to the instructions of the manufacturer (Bio-Rad, France). EPEC serotypes were determined using nonavalent and trivalent (I, II, III and IV) antisera (Bio-Rad, France). The strains tested positive with trivalent I, II, III and IV antisera were subjected to the tests with the monovalent antisera (Bio-Rad, France). EHEC, suspicious colonies (i.e. Sorbitol-negative) were confirmed as O157 using the Dry Spots test (DR0120M, Oxoid) specific for the identification of O157.[15]

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on Mueller-Hinton agar (Liofilchem-Italy) plates at 37°C for 24 h by the Kirby Bauer disc diffusion method and interpretation was done as per European Committee of Antimicrobial Susceptibility Testing (EUCAST) guidelines.[16] Twelve (12) different antibiotics were used: amoxicillin-clavulanic acid (20+10 µg), ceftriaxone (30 µg), ampicillin (10 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), gentamycin (15 µg), tetracycline (30 µg), doxycycline (30 µg), erythromycin (15 µg), colistin sulphate (10 µg) and trimethoprim-sulfamethoxazole (1.25/23.75 µg) (Liofilchem-Italy).

Detection of Extended-Spectrum β-lactamase (ESBL) producing *E. coli*

Resistant's strains to β-lactams were considered as a potential ESBL producer and were further confirmed by Double Disk Synergy Test (DDST).[16] This test is based on the detection of synergy between an amoxicillin-clavulanic-acid disc and two discs of third generation cephalosporin's (ceftriaxone and cefotaxime) separated by 2 to 3 cm from one another. The presence of ESBLs is denoted by an appearance of synergy between the discs, giving the appearance of "champagne cork" shape.[16]

Statistical analysis of the data

Statistical analysis was done using Sphinx version 5 software (Parc Altaïs, 74650 Chavanod, France). Chi-square test (Chi^2) was used for the comparison of two variables. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Patient characteristics

This study showed a high prevalence of diarrhea in September and October (Fig. 2). However, more samples of diarrheal stools were collected in September indicating high prevalence of diarrheal infections in this month (25.2%) (p = 0.3538). Breakdown of diarrhea cases by sex and age showed a slight predominance of male (63%) (p = 0.0481). The slice most affected age group was 0-11 months (61.4%) (p = 0.0356) (Table 1). In this age group affected by diarrheal disease, 47 were male (i.e. 39.5% of the samples) and 26 were female (i.e. 21.9% of the samples) (Table 1).

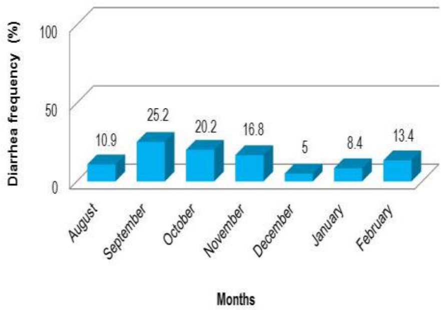

Figure 2: Monthly distribution of diarrhea

Table 1: Distribution of cases of diarrhea by age and sex

Age groups (months)	Sex		Total N (%)
	Male (n = 75) (%)	Female (n = 44) (%)	
0-11	47 (39.5)	26 (21.9)	73 (61.4)
12-23	23 (19.3)	14 (11.8)	37 (31.1)
24-36	3 (2.5)	3 (2.5)	6 (5)
37-48	2 (1.7)	1 (0.8)	3 (2.5)
Total N (%)	75 (63)	44 (37)	119 (100)

Legend: n = number of cases of diarrhea by sex, **N** = total number of cases of diarrhea.

The monthly distribution of diarrhea cases showed a high prevalence in rainy season, such as September (25.2%) to October (20.2%) (Fig. 2). The high prevalence of diarrhea cases in rainy season (June to August) was also observed in Burkina Faso during the survey of diarrheal diseases.[17] However, elevated incidence of bacterial diarrhea in Senegal was correlated with the occurrence of the cool dry season (December to February).[18] This high prevalence of diarrhea during the rainy season can be explained by the fact that, it is a time when important drainage filth observed charge of various germs rainwater. Also, infiltration is important, the rise of the water table during the rainy season can cause contamination of some groundwater sources and current consumption sinks.[19] Then, children's could contract diarrhea when they consume these contaminated waters. Diarrheal diseases repartition various according to climate and hygiene level.[18] However, there are important regional and temporal variations.[10]

Diarrhea cases distribution by age and sex showed that children less than 1 year accounted for 61.4% of patients in our study. Similar frequencies of diarrhea cases before 1 year age were also been reported with respective frequencies of 49% in Burkina Faso and 39% in Senegal.[17, 18] High incidence in this age group may also be a result of the increasing exposure to contamination by pathogens associated with premature introduction of water, solids and liquids foods in the children feeding.[20] Moreover, malnutrition and lack of hygiene could also explain the high prevalence at the low age patients.[21]

In this study, male children account 63% of cases against 37% female (p = 0.238). This slight predominance has been reported in Côte d'Ivoire with 63.1% of male against 36.9% of female.[22] High prevalence of diarrhea among male could be explained due the fact that male are much more active and thus turbulent, which increases their risk of contamination selected enteric pathogens. However, another study showed that the rate of diarrhea caused by DEC in female and male were respectively 30 (58.8%) and 21 (41.2%).[23] Therfore, it seems that male and female are both susceptible to diarrheal disease. Difference would depend on hygiene conditions of the study site.

Prevalence of DEC

Of 119 fecal specimens, 83 were positive for *Escherichia coli* detection (69.7%). Of these *Escherichia coli* strains, 21 were DEC (25.3%). Among DEC, 13 Enteropathogenic *Escherichia coli* (EPEC) (15.7% of the samples) and 8 Enterohaemorrhagic *Escherichia Coli* (EHEC) (9.6% of the samples) were isolated (Table 2). Eight serotypes of EPEC were identified as: O111, O26, O86, O125, O126, O124, O114 and O142. *Escherichia coli* O124 were isolated with a high prevalence (23.1%) (Fig. 3).

Table 2: EPEC and EHEC O157 prevalence according to age group

Age (months)	Samples number (N = 119)	*Escherichia coli* strains n (%)			
		E. coli	DEC	ECEP	ECEH O157
0-11	73	46 (38.7)	12 (14.5)	7 (8.4)	5 (6)
12-23	37	27 (22.7)	9 (10.8)	6 (7.2)	3 (3.6)
24-36	6	8 (6.7)	0 (0)	0 (0)	0 (0)
37-48	3	2 (1.7)	0 (0)	0 (0)	0 (0)
Total N (%)	119	83 (69.7)	21 (25.3)	13 (15.7)	8 (9.6)

Legend: DEC = Diarrheagenic *Escherichia coli*, **EPEC** = Enteropathogenic *Escherichia coli*, **EHEC** = Enterohaemorrhagic *Escherichia coli* **N** = Total number of recorded samples, **n** = Number of positive samples.

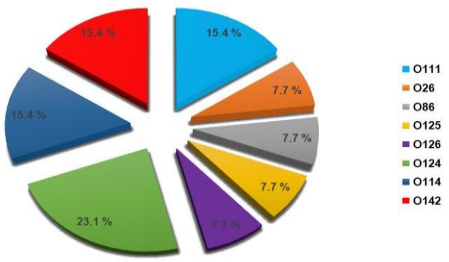

Figure 3: Serotypes of EPEC

Of the 119 children with diarrhea stool samples recorded, 21 were DEC (25.3%) with, 13 EPEC (15.7%) and 8 EHEC O157 (9.6%). The prevalence of DEC observed in our study is similar to those (21.4%) reported in Burkina Faso.[13] Other studies were previously reported EPEC detection in Burkina Faso (8%) and Senegal (10%).[17, 18] However, studies showed high prevalence of EPEC in Burkina Faso (19.3%) and China (22.2%).[13, 24] Also, another study in the equatorial zone showed a high prevalence of EPEC (57.7%). This prevalence is very high compared to our EPEC results typically predominate in equatorial regions.[25] This portrait is explained by the difference in regional climate. Several serotypes of EPEC were identified in this study including serotype O124 was most prevalent in urban Burkina Faso (Fig. 3). A similar recent study reported the same serotypes of EPEC in rural (Gourcy, Boromo) Burkina Faso (19.3%).[13] Unlike our results, O127 serotype was mainly isolated in rural areas.[13] Another study reported the serotypes O111, O126 and 0124 of EPEC (10%) in Senegal.[18] Regarding EHEC (*E. coli* O157), previous studies have reported their involvement in diarrheal diseases of the lower prevalence (2.1% and 2%) with in Burkina Faso [13, 25], in China (2.3%).[24] Domestic and wild animals are the sources of *E. coli* O157, but ruminants are regarded as the main natural reservoirs. Sporadic cases and outbreaks of human diseases caused by *E. coli* O157 has been linked to ground beef, raw milk, meat and dairy products, vegetables, unpasteurized fruit juices and water.[6] Infections can also be acquired by direct contact with animals and by person to person spread.[26] Unlike our results, according to a study in Senegal, EHEC O157 was not isolated in children diarrhea stool.[18] This could be explained by the environment, high level sanitation and hygiene of Senegal compared to Burkina Faso. Several previous studies have reported differentials health problems by age. He has been observed that children are particularly vulnerable to diarrhea before the age of one year. Two reasons typically cited for this finding. First, the very young ages (6 months to 12 months), the child's immune system develops gradually while a drop of maternal antibodies is performed.[27] Second, this period is that of the introduction of foods into the mouth, which coincides with an increased risk of diarrhea in children when hygienic precautions are not taken. The child may be exposed to contaminated food, and so have a greater probability of being malnourished, which could subsequently increase the risk of diarrhea.[28] Moreover, it is at this age that children begin to learn to walk, coming into contact with domestic animals. Then, they could be contaminated through the feces of animals which are often contaminated by pathogenic bacteria.

Distribution of EPEC and EHEC O157 by age and sex

This study showed that children less than one year were affected by the syndrome of EPEC (53.8%) against 62.5% of EHEC O157. Distribution of these germs by gender showed that male children were affected by EPEC diarrhea (69.2%) against 30.8% among females. Regarding the distribution of diarrhea cases with EHEC O157 according to sex, this study showed 62.5% males against 37.7% females.

Results showed that children less than 1 year were affected by EPEC diarrhea in our study was 53.8% (p = 0.56). According to the results of statistical analysis, distribution of EPEC according to age groups was not significant ($p > 0.05$). However, there is a slight predominance of those less than 1 year. Slight predominance frequency (51%) before 1 year age has also been reported in Burkina Faso.[17] Their distribution of diarrheal diseases by sex also showed that there is no significant difference ($p > 0.05$) infection among male (69.2%) and female (30.8%) ($p = 0.765$). Similar results were reported slight predominance of EPEC diarrheal diseases in male (63.1%) against 36.9% female.[22] However, contrarily to our study, EPEC diarrhea in male (41.9%) and female (58.1%) was reported in N'Djamena-Chad.[19] Several factors could explain EPEC diarrhea cases in this age: the immaturity of the immune system and early weaning. Indeed, contamination risks of children by adult could be linked to poor hygiene conditions related to the lack of environmental sanitation devices and the presence in the immediate environment of waste dumpsites habitations hosting pathogens.[29] High prevalence of these pathogens in boys could be due to the fact that, in general; boys are much more active and thus turbulent, which increases their risk of contamination selected enteric pathogens.

Antimicrobial susceptibility testing

All strains of DEC isolated were resistant to erythromycin (100%). DEC also have a high resistance to ampicillin, amoxicillin-clavulanic acid, doxycycline, tetracycline and trimethoprim-sulfamethoxazole (Table 3). No EHEC strain was resistant to colistin sulphate. No strain has responded positively to ESBLs production.

Table 3: *Escherichia coli* antibiotic resistance

Antibiotics	*Escherichia coli* antibiotic resistance n (%)		
	DEC	EPEC	EHEC
Ampicillin	17 (81)	12 (92.3)	5 (62.5)
Amoxicillin-clavulanic acid	20 (95.2)	13 (100)	7 (87.5)
Ceftriaxone	6 (28.6)	2 (15.4)	4 (50)
Chloramphenicol	6 (28.6)	2 (15.4)	4 (50)
Ciprofloxacin	8 (38.1)	4 (30.8)	4 (50)
Norfloxacin	7 (33.3)	3 (23.1)	4 (50)
Gentamicin	5 (23.8)	1 (7.7)	4 (50)
Erythromycin	21 (100)	13 (100)	8 (100)
Colistin sulfate	1 (4.8)	1 (7.7)	0 (0)
Doxycycline	20 (95.2)	12 (92.3)	8 (100)
Tetracycline	18 (85.7)	12 (92.3)	6 (75)
Trimethoprim-sulfamethoxazole	16 (76.2)	11 (84.6)	5 (62.5)

Legend: DEC = Diarrheagenic *Escherichia coli*, **EPEC** = Enteropathogenic *Escherichia coli*, **EHEC** = Enterohaemorrhagic *Escherichia coli*

Among 8 antibiotic class tested, 4 phenotype was observed: macrolides (100%), cyclins (90.5%), sulfamides (76.2%) and β-lactams (68.3 %) DEC were multidrug-resistant (Fig. 4).

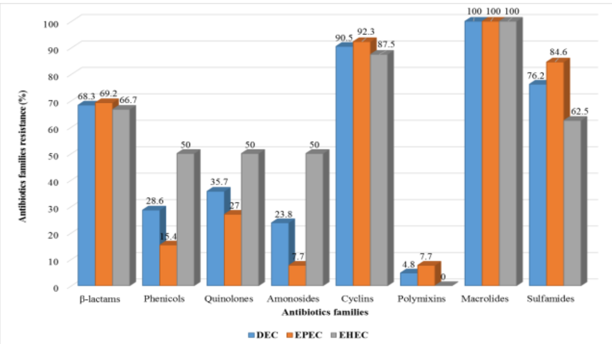

Figure 4: *Escherichia coli* antibiotics familly resistance

Legend: "**DEC** = Diarrheagenic *Escherichia coli*, **EPEC** = Enteropathogenic *Escherichia coli*, **EHEC** = Enterohaemorrhagic *Escherichia coli*, **β-lactams** (AMP, AUG, CRO), **Phenicol** (C), **Quinolones** (CIP, NOR), **Amonosides** (CN), **Cyclins** (TE, DXT), Polymixins (CS), **Macrolides** (E), **Sulfamides** (SXT), **AMP** = Ampicilline, **AUG** = Amoxicillin-clavulanic acid, **CRO** = Ceftriaxone, **C** = Chloramphenicol, **CIP** = Ciprofloxacin, **NOR** = Norfloxacin, **CN** = Gentamicin, **TET** = Tetracycline, **DXT** =Doxycycline, **CS** = Colistin sulfate, **E** = Erythromycin, **SXT** = Trimethoprim-sulfamethoxazole".

Antibiotic susceptibility of EPEC various serotypes allowed knowing what are the most resistant to common antibiotics used for the treatment of gastroenteritis in Burkina Faso. EPEC serotypes isolated were resistant to amoxicillin-clavulanic acid (100%) and erythromycin (100%) (Table 4). No strain of these serotypes was resistant to colistin sulphate and gentamicin except O126 serotype.

Table 4: EPEC serotypes antibiotic resistance

Antibiotics	ECEP serotypes resistance n (%)							
	O111	O26	O86	O125	O126	O124	O114	O142
Ampicillin	2 (100)	0 (0)	1 (100)	1 (100)	1 (100)	3 (100)	2 (100)	2 (100)
Amoxicillin-clavulanic acid	2 (100)	1 (100)	1 (100)	1 (100)	1 (100)	3 (100)	2 (100)	2 (100)
Ceftriaxone	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)
Chloramphenicol	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	1 (50)	0 (0)
Ciprofloxacin	1 (50)	0 (0)	1 (100)	1 (100)	0 (0)	1 (33.3)	0 (0)	0 (0)
Norfloxacin	1 (50)	0 (0)	0 (0)	1 (100)	0 (0)	1 (33.3)	0 (0)	0 (0)
Gentamicin	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)
Erythromycin	2 (100)	1 (100)	1 (100)	1 (100)	1 (100)	3 (100)	2 (100)	2 (100)

Colistin sulfate	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)
Doxycycline	2 (100)	1 (100)	1 (100)	1 (100)	1 (100)	2 (66.7)	2 (100)	2 (100)
Tetracycline	2 (100)	1 (100)	1 (100)	1 (100)	1 (100)	2 (66.7)	2 (100)	2 (100)
Trimethoprim-sulfamethoxazole	2 (100)	1 (100)	1 (100)	1 (100)	0 (0)	3 (100)	2 (100)	1 (50)

Legend: "**DEC** = Diarrheagenic *Escherichia coli*, **EPEC** = Enteropathogenic *Escherichia coli*, **EHEC** = Enterohaemorrhagic *Escherichia coli*, **n** = number of resistant strains.

After considering the antibiotic susceptibility of *E. coli* strains, we found that most EPEC isolated were resistant to multiple antibiotic families (β-lactam, cyclins, macrolides and sulfamides). As for EHEC, they were resistant to β-lactam, cyclins, macrolides and sulfamides (Fig. 4). According to multi-drug resistance definition, our isolates were multi-drug resistant. Indeed, a similar study conducted in Nigeria revealed EPEC resistance to ampicillin (100%), tetracycline (92%) and trimethoprim-sulfamethoxazole (70%).[30] Another study in Bolivia and Peru revealed *Escherichia coli* resistance to ampicillin (95%), tetracycline (93%) and trimethoprim-sulfamethoxazole (94%).[31] According to some studies, resistant bacteria and antibiotic residues have been detected in the environment (wastewater, surface water, groundwater, soil and sediment).[32] These antibiotic residues are released into environment after use in medicine (human, veterinary) and other fields. Resistance could be due to environmental conditions, such as transmission of resistant isolates between adults and children, or between animal and human. Latter implicates the use of antimicrobials in food animal, resulting in the selection of resistant bacteria that are transported by the food vehicle.

No strain of EPEC and EHEC has responded positively to ESBLs production. However, ESBLs producing *E. coli* strains (38.3%) were reported in Burkina Faso.[13] In Gram-negative bacilli, there are 3 types of mechanisms of resistance to β-lactam antibiotics: low affinity for penicillin-binding proteins (PLP), the impermeability and efflux phenomena, especially enzymatic inactivation the β-lactamases.

CONCLUSION

At the end of the study, children under 2 years of age were the most affected by diarrheal diseases at the CMA Paul VI of Ouagadougou in Burkina Faso. Of the eight EPEC serotypes identified, EPEC O124 was the most prevalent. DEC Isolated were resistant to several antibiotics of different families indicating their multiresistant character. Only colistin sulphate was still active on these DEC. These results challenge the urgent need to put in place strategies for antibiotics resistance monitoring and measures to prevent multi-resistant *E. coli* childhood diarrhea in hospitals and community settings.

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