Gastroenteritis with *Eschericha coli* in pediatric hospital in N'Djamena-Chad

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ABSTRACT

Gastroenteritis with *Escherichia coli* diarrhea is severe and recurrent fatal diseases in children under 5 years especially in Africa. A prospective study of acute diarrhea was conducted at the General Hospital of National Reference of N'Djamena (HGRN). The aim of this study was to investigate the epidemiological, clinical and evolutionary characteristics of acute diarrhea with enteropathogenic *Escherichia coli*. This study involved 167 children aged 0-2 years having seen in outpatients or hospitalized for acute diarrhea in children. Stool samples were collected and analyzed according to standard procedures of clinical microbiology. Identification of pathotypes of *E. coli* was performed by Nonavalents serum agglutination, Trivalent I, Trivalent II, Trivalent III and mixture IV. Antimicrobial susceptibility was performed by disk diffusion method in solid medium according MH Kirby Bauer. Examination of 167 stool showed 26 cases (15.56%) bloody, 141 cases (84.83%), mucous, 122 cases (73.05%) and liquid 45 cases (26.94%) soft. The enteropathogenic *Escherichia coli* (EPEC) were detected in 58 cases (34.73%). On the other hand parasites (16.76%) were identified to direct microscopic observation that *Trichomonas intestinalis* 13 cases (7.78%), *Giardia intestinalis* 9 cases (5.38%), *Entamoeba histolytica* 6 cases (3.59%) and cases of intestinal candidiasis (15.56%). The sensibility of origins in antibiotics was varied, 89.65 % of the ECEP, were resistant in Sulpha drugs, 56% were it towards beta-lactamines. An average rate of 26% of resistance was observed by the families of fluoroquinolones and quinolones. This study allowed us not only to include the characteristics of diarrheas at the children of less than two years, but also, the necessity of an effective coverage as well as the implementation of a program of prevention against the diarrheas of the children in a hospital environment.

1. INTRODUCTION

Diarrhoeal diseases are very common in the world, especially in developing countries where they are endemic state [1]. Currently diarrheal diseases arrive in third place among the most deadly infectious diseases in the world with 2.5 million deaths any confused age. In terms of years of life lost adjusted for disability, diarrhea come in second with 73 million behind respiratory infections [2]. Some Shigatoxin producing *Escherichia coli* (STEC) are responsible for foodborne infections that result in diarrhea but also more serious syndromes in man as hemolytic uremic syndrome that can cause death [3;4]. Enterotoxigenic *Escherichia coli* (ETEC) are an important cause of diarrhea in children in developing countries and an estimated

380,000 deaths per year. They produce heat-labile and heat-stable toxins [5; 6] *Escherichia coli* enteroaggregants and enteroadherents (EAAGG, DAEC) are responsible for chronic diarrhea in children in underdeveloped countries and produce an enterotoxin and hemolysin [7]. The entero-invasive *Escherichia coli* (EIEC) cause bloody diarrhea [8; 9]. Currently, *Escherichia coli* are considered emerging pathogens, the leading cause of bacterial diarrhea in children and are a major problem of public health [10]. Instead of *Eschericha coli* diarrhea in developing countries, particularly in Chad is not well known because very few studies have been devoted. Chad has few reliable national statistics on the most frequently encountered enteropathogens in diarrheal diseases. However, data from the registers of stool cultures of some hospitals and typhoidal perforation recorded in the service of visceral surgery, these different hospitals attest diarrhoeal disease due to enteric pathogens (bacteria, viruses, parasites and fungal agents)

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posed a real public health problem in Chad. The objective of this work is to contribute to better understanding the epidemiological characteristics of diarrhoea due to *Escherichia coli* in pediatric hospital and the phenotype of the antibiotic susceptibility of the epidemiological characteristics of *Escherichia coli* diarrhea in pediatric hospitals and the phenotype of antibiotic sensitivity.

2. MATERIALS AND METHODS

2.1 Period of the study site

This is a prospective study carried out between 01 November 2009 to 30 October 2010, which took place in the Pediatric Unit and Bacteriology Laboratory of General National Hospital of Reference in N'Djamena (HGRN).

2.2 Population

The study involved 167 children aged 0-2 years, 97 girls (58.08%) and 70 males (41.91%) with acute diarrhea in hospitalized or come visit the Pediatric Department of HGRN. Cases of acute diarrhea were determined according to the WHO definition, namely the emission of at least three loose or liquid stools per day lasting for less than fourteen days.

2.3 Investigations

Investigations were made to parents or guardians of children after obtaining their informed consent. They were: the origin, sex, age, date of disease, ongoing treatment, number of stools per day, education level of the mother and the suspected origin of the contamination (the source drinking water, food and whole way of life of parents) signs and symptoms (diarrhea, abdominal pain, vomiting, fever, etc.) and conditions associated with diarrhea (acute respiratory infections, candidiasis, dehydration, anemia, etc.).

2.4 Sampling and analysis of stool

Feces were collected in sterile jars. Each sample was examined for macroscopic aspects (Purulent, mucus, presence of blood) and consistency (liquid, soft, molded). It has been researched parasites in association with diarrhea. The stools were also processed and analyzed using standard methods of clinical microbiology. The agar EMB (Eosin Methylene Blue) and MH (Muller-Hinton) (Bio-Rad ®) were used for the isolation of *Escherichia coli*. The Petri dishes containing the medium seeded were incubated at 37 °C in an oven for 24 hours. The reflective metallic green colonies were searched and re-isolated on MH for the subsequent identification of enteropathogenic *Escherichia coli*. The isolates were stored at -86 °C in brain heart broth with 15% glycerol for future molecular characterization.

2.5 Serotyping and antimicrobial susceptibility

On pure cultures of *E. coli*, it was proceeded to serotypagee as recommended by WHO. For this a colony of bacteria was deposited on a drop of saline water (0.85% Nacl) on a slide and crushed. Agglutination indicates the presence of a rough strain (rough). In the absence of agglutination, we proceed in the same way with Nonavalents serums, Ttrivalent I, Ttrivalent II, Trivalent III and mixture IV (Bio-Rad®). The antibiotic sensitivity tests were performed on solid Mueller-Hinton (Kirby-Bauer) by diffusion method using discs impregnated with known antibiotics as recommended by the Committee on Antimicrobial Technique doses of the French Society of Microbiology (CASFM). 58 strains of *E. coli* were tested with six antibiotics belonging to the family of beta-lactam antibiotics (amoxicillin (25μg): Amoxicillin + clavulanic acid ((20/10 μg), Ceftriaxone (30 μg), quinolones (nalidixic acid (30 μg), fluoroquinolones (ciprofloxacin (5 µg) and sulfonamides (trimethoprim-sulfamethoxazole (SXT) (1.25/23.75 μg).

2.6 Data processing

The data was entered and analyzed using Excel software. Statistical analysis was performed using the chi-square test (χ^2) for comparison of two variables. Differences were considered significant when $P \leq 0.05$.

3. RESULTS

3.1 Epidemiological characteristics

During the study period, out of 167 cases of children of 0-2 years of age with acute diarrhea reported in Pediatrics, 107 (64.07%) were hospitalized and 60 (35.92%) were followed on an outpatient basis. The evaluation of the temporal distribution of diarrhea is shown in Fig. 1. It appears a respective predominance of diarrhea cases in the months of January, March and June of 2010 with 18 (11%), 24 (14.4%) and 26 (16%) cases, respectively. The number of *Escherichia coli* isolated from the same month enteric was 7 (12.1%), 9 (15.5%) and 10 (17.2%) respectively. The average age of children was 12 months and ranged from 1 day to 24 months (Table 1). Cases of diarrhea were mostly observed in the age group 0 to 6 months (35.32%).

Table 1: Distribution of cases of diarrhea according to age and sex.

Age classes (Month)	Sex		Total (%)
	Male (%)	Female (%)	
0-6	25 (14.97)	34 (20.35)	59 (35.32)
7-12	19 (11.37)	23 (13.77)	42 (25.14)
13-18	11 (6.58)	13 (7.78)	24 (14.37)
19-24	15 (8.98)	27 (16.16)	42 (25.14)
Total (%)	70 (41.91)	97 (58.08)	167 (100)

3.2 Clinical Features

Fifty six (56) children (33.53%) were taken to the consultation within 1 to 3 days after event emission liquid stools while 71 (42.51%) were found within 4 to 7 days and 40 (29.95%) were seen in consultation after the first week. Total duration of diarrhea could be specified in 114 children: it was 5.8 days on average with the extremes of 1 to 14 days. Table 2 reported the results of the manifestations of diarrhea in the group of children observed. The number of stools per day ranged from 3 to 18; Altogether 86 children (51.49%) expressed 3-5 bowel movements per day, 65 children (38.92%) were 6-10 stools and 16 (9, 58%)

more than 10 stools per 24 days. The stools were bloody in 26 cases (15.56%), mucous in 141 cases (84.83%), liquid in 122 cases (73.05%) and soft in 45 cases (26.94%). Table 3 shows the distribution of signs and conditions associated with diarrhea. Fever was the most common symptom associated with 55.68% of cases, followed by vomiting in 72 cases (43.11%) and anemia in 15 cases (9%). Acute respiratory infections were the most common ailments associated with diarrhea (24.55%) followed by gastrointestinal candidiasis (15.56%) and malnutrition (10.17%).

Table 2: Distribution of cases of diarrhea by age according to the appearance and consistency of stools.

Age classes (Month)	Aspect		Consistence	
	Bloody	Mucus	Liquid	Soft
0-6	0	0	63	7
7-12	0	38	25	11
13-18	10	42	18	13
19-24	16	61	16	14
Total (%)	26 (15.56)	141 (84.43)	122 (73.05)	45 (26.94)

Table 3 : Signs and diseases associated with diarrhea.

Signs and symptoms	Case	Percentage (%)
Fever	93	55.68
Vomiting	72	43.11
Dehydration	10	5.98
hepato -aplemenogalle	11	6.58
Anemia	15	8.98
Affections	**Case**	**Percentage (%)**
Acute respiratory infections	41	24.55
Candidiasis	26	15.56
Malnutrition	19	11.37
Infection O.R.L	14	8.37
Dermatosis	12	7.18

3.3 Etiologic agents

E. coli was isolated in 34.73% of cases of diarrhea. 65.27% of cases were due to parasitic infections, acute respiratory, candidiasis and infection Ear Nose and Throat with proportions of 16.76%, 15.56%, 24.55 % and 8.37% respectively. Of 167 cultures performed, 58 enteropathogenic *Escherichia coli* were isolated (34.73%), 46 cases (42.99%) among 107 children hospitalized and 12 cases (20%) among children monitored externally. Of the 58 serotypes isolated from *Escherichia coli*,

there are 47 (81.03%) of trivalent 1 (O111 + O55 + O26) and 11 (18.96) enteropathogenic *Escherichia coli* (EPEC). Twenty-eight cases (16.76%) of diarrhea parasites were identified to direct microscopic observation of which 13 cases (7.78%) *Trichomonas intestinalis*, 9 cases (5.38%) of *Giardia intestinalis* and 6 cases (3.59%) of *Entamoeba histolytica*. Fungal agents in *Candida albicans* were isolated in 26 cases (15.56%). We highlighted six (3.6%) when combining *Entamoeba histolytica / Giardia intestinalis* and 4 (2.4%) when combining *Escherichia coli / Candida albicans*, an overall prevalence of association 6%.

3.4 Evolution

Thirteen cases (12.14%) deaths were recorded among 107 hospitalized children. The evolution towards healing was observed in 81 children (75.70%). Cases of escape were noted in 13 children (12.14%) escaped. The distribution function and evolution of nutritional status is reported in Table 4. The analysis shows that 61.53% of deaths (8 of 13) occurred in malnourished children. The observed difference is statistically significant. The risk of death was significantly greater in malnourished children than those with a normal nutritional status.

Table 4: Distribution of cases according to the evolution of the nutritional status.

Evolution	Nutritional status		Total
	Malnutrition	Normal	
Death	8	5	13
Healing	11	90	101
Total	19	95	114

3.5 Susceptibility testing

Table 5 shows the evaluation of the effectiveness of antibiotics with isolated bacterial agents. The study involved six antibiotics tested on 58 enteropathogenic *Escherichia coli* isolated. The proportions of resistance (R), intermediate (I) and sensitive (S) were obtained (Table 5). 56% of strains were resistant to beta-lactam antibiotics tested. The quinolones and fluoroquinolones were very active on 64% and 85% respectively of the strains.
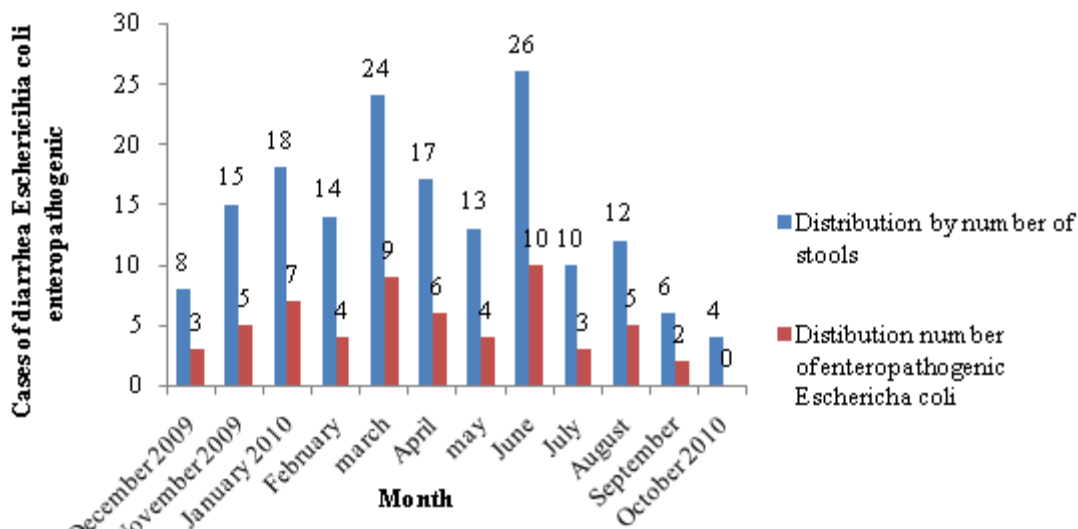

Figure 1: Monthly distribution of cases of diarrhea and enteropathogenic *Escherichia coli*.

Table 5 : Effectiveness of some antibiotics of the family of beta-lactam antibiotics, sulfonamides, quinolones and fluoroquinolones

bacterial agents	Nb tested	AM			AMX			CRO			SXT			Nal			CIP		
Eschericha coli		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
enteropathogenic	58	4	8	46	7	9	42	43	6	9	3	3	52	37	8	13	49	6	3

Nb = number

R = Resistance, I = Intermediate, S =Sensitive: Trimethoprim-sulfamethoxazole (SXT) : R < 10, $10 \leq I \leq 18$, $S \geq 19$; Nalidixic acid (Nal) : R< 15, $15 \leq I \leq 19$, $S \geq 20$; Ciprofloxacin (Cip) : R< 15, $15 \leq I \leq 20$, $S \geq 21$; Ampicillin (AM) :R<11, $11 \leq I \leq 16$, $S \geq 17$; Ceftriaxone (CRO) : R< 15, $15 \leq I \leq 20$, $S \geq 21$; Amoxicillin (AMX) : R< 14, $14 \leq I \leq 20$, $S \geq 21$(NCCLS, 1998, CASFM, 2010).

4. DISCUSSION

Diarrhoeal diseases are still a leading cause of hospitalization of children in developing countries. In this study children 0-6 months were the most affected by diarrhea 37.72% of cases. Maaroufi et *al*. [11] in Tunisia reported higher frequencies of 55.7%. The child before one year of age are particularly vulnerable to diarrhea for two main reasons. It was during this period that gradually develops the child's own immunity as it occurs a decline in maternal antibodies. The infant is more vulnerable to infections during this period [12]. This age corresponds to the period when food diversification of the child begins. When it is poorly managed, malnutrition and diarrhea may occur [13].The distribution of diarrhea by sex and age shows a predominance of diarrhea cases in the age group of 0-6 for females (20.35%) than males (14.97%) but difference is not statistically significant ($x^2 = 0.00781$, df = 1; $p \geq 0.90$).This could be explained by the fact that Chad, women's vital statistics are 51% against 49% for male according to INSEED (National Institute of Statistics and Economic and Demographic Studies) in 2010. Barro et *al*. [14] reported the infection rate among women (52.7%) which is slightly higher than men (47%) during an etiological study of gastroenteritis at the National Hospital Center Universitaire Yalgado Ouedraogo (CHU-YO) of Ouagadougou, Burkina Faso. The rate of 15.56% of bloody diarrhea in our study is slightly higher than the 10.2% reported by Diagne et *al*. [15] in Senegal. The mucous stools were observed in 84.83% of cases and loose stools in 73.05% of cases. The different aspects and consistency of stools were also reported by Dao et *al*. [16] at Bamako in Mali : aspects mucus in 14 cases (18.4%), the consistency of watery stools in 40 cases (52.6%), and polymorphic consistency in 8 cases (10.5%). Enteropathogenic *Escherichia coli* were much more isolated in the stools liquid consistency. Ingested bacteria adhere to enterocytes (intestinal cells) by a factor attachment and secrete enterotoxin (ST a thermostable toxin and heat-labile toxin LT) which are responsible for watery diarrhea [17]. The results of stool cultures were positive in 37.12% of cases. This rate is three times that observed Luki et *al*. [18] in Zaire, it was 12%. The high rate in our study could be explained by an unsatisfactory immunization status, parental neglect due to present infants in health centers. The frequency of enteropathogenic *Escherichia coli* isolated is relatively high (34.73%)) in our case study. Sanou et *al*. [19] reported a rate of 32% at the University Hospital Yalgado Ouedraogo of Ouagadougou in Burkina Faso. Bonkoungou et *al*. [20] showed that the major pathogroupes encountered is the ECEA (12%), followed by EPEC (8%), ETEC (4%), ECST (0.4%) and

EIEC (0.4%). The identification of parasites such as *Trichomonas intestinalis* and *Giardia intestinalis* in liquid stools in our study was also observed. By cons, in mucoid-bloody stools, were frequently reported the presence of *Entamoeba histolytica*. This would explain why, from the appearance and consistency of stools, one might suspect the presence of such parasites or bacterial infection such. The frequency of 16.76% of parasites found in the faeces in our study is relatively higher than that reported by Diouf et *al*. [21] in Senegal, which was 10.7%. Gendrel [22] in Gabon found a 15% prevalence of carriage in disadvantaged children. *Entamoeba histolytica* were found at a low rate of 3.59% of cases. The 6% of parasite/parasite or bacteria/candidiasis association could be explained by the level of adverse social parents to Chad or lack of food hygiene at the household level. Acute respiratory infections with 24.55% were disease most associated with diarrhea. It is followed by malnutrition in 11.37% of cases. Kangah et *al*. [23] in Côte d'Ivoire reported a rate increase of 22% association of malnutrition with diarrhea. These two conditions feed off each other, creating a vicious circle of malnutrition increases the risk of diarrhea and diarrhea worsens malnutrition [24; 25]. The average time taken to send the children in consultation noted in this study, 33.53% of children were consulted dice the first 3 days of diarrhea and two in five children after a week reflects the neglect of diarrhea and ignorance of its consequences for mothers. This could be explained by the conditions of precarious socio-economic life of the population in Chad. Access to health care due to the level of socio-economic life has also been reported by Poda et *al*. [26] in Burkina Faso. Most recorded deaths in our study occurred in malnourished children ($x^2 = 21.27$, df = 1; $p \geq 0.01$ significant difference). The association is diarrhea-malnutrition in our areas of major concern. These deaths could be explained by the fact that the department of pediatrics of HGRN does not have a Therapy Centre Oral Rehydration (CTRO) for the management of children with diarrhea and a Center for Recovery and Nutritional Education (CREN) dealing with malnourished children. Most isolates were resistant to beta-lactam antibiotics (Ampicillin, amoxicillin, ceftriaxone) at an average ratio of about 56%. Such resistance when observed, is linked to the production of beta-lactamase Amp C types and penicillinases by the strain in question [27; 28]. Our results can be explained by this phenomenon. This increase in resistance is due to the improper and inappropriate prescribing of antibiotics by health workers. This is what was observed during our investigation. By cons, our strains manifested good sensitivity to quinolones (64%) and fluoroquinolones (85%). Strains have developed a resistance rate

of 89.65% to trimethoprim-Sulfamoxazole. On the other hand, it appears, still, according to our surveys, that about 8% of mothers practiced self-medication by giving their child drugs such as amoxicillin, trimethoprim-sulfamethoxazole and metronidazole syrup before to the hospital. Moreover, Kam et *al.* [29] reported similar rates at the National Hospital Center of Yalgado (CHN-YO) of Ouagadougou.

5. CONCLUSION

The results of this study show that enteropathogenic *Escherichia coli,* representing 34.73% of all diarrheal diseases indicating the public health problem that cause enteric pathogens among children less than 2 years.

Several other parasites and enteric pathogens were identified in the stool. However, it has not been sought enterovirus in the stool. In this study, the antibiotics tested groups showed a very high resistance in beta-lactam and low in Quinolones and Fluoroquinolones. Actions favoured are needed to protect the health of infants, highly vulnerable to diarrheal diseases.

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