

Enteropathogenic *Escherichia coli* Associated with Diarrhea in Children in Cairo, Egypt

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Received 11 October 2011; Accepted 15 November 2011

Academic Editors: E. L. Jarroll and A. Ouwehand

In this study we isolate and identify the Enteropathogenic *Escherichia coli* (EPEC) causing diarrhea in children less than five years in Cairo, Egypt, during different seasons. Children younger than five years with diarrhea, attending the Pediatric Gastroenterology Intensive Care Unit of the Cairo University Pediatric Hospital in one year period were our group of study. Our control group was age and sex matched concurrent healthy children. The identified *E. coli* isolates were subjected to antimicrobial disc diffusion susceptibility test and further identified for EPEC serotype by slide agglutination test, using antiserum *E. coli* somatic trivalent I (O111, O55, O26) according to the instructions of the manufacturer. Out of 134 patients 5.2% of them revealed EPEC in the fecal sample, while the 20 children control group showed no EPEC isolates in their samples. Our EPEC frequency showed variations from the compared results of other studies. Higher rate of EPEC (18.7%) was found in patients between 2 to 3 years, while EPEC rate was (7.5%) in patients less than 6 months old, with $P < 0.05$. EPEC was identified from fecal specimens as a unique pathogen or associated with other pathogens in acute and chronic diarrhea in children. EPEC were detected in all seasons except in winter, and was predominant in summer season. Four (57%) EPEC isolates were resistant to ampicillin, ticarcillin, and cotrimoxazole, and (14.3%) to the third generation cephalosporins.

KEYWORDS: enteropathogenic *E. coli*, children less than 5 years, slide agglutination test

1. Introduction

The diarrheagenic *E. coli* pathotypes that cause diarrhea include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), Shiga toxin-producing *E. coli* (STEC), diffusely adherent *E. coli*, and enteroaggregative *E. coli*. These pathotypes are defined by the presence or absence of one or more definable *E. coli* virulence factors [1]. Enteropathogenic *E. coli* (EPEC) strains are diarrheagenic *E. coli*, which usually are classified by a combination of oligosaccharides (O), flagella (H), and capsular (K) antigens. They are associated with outbreaks of infantile diarrhea among children in developing countries. In contrast to the limited importance of EPEC in industrialized countries, EPEC is a major cause of diarrhea in developing countries. Studies in Brazil, Mexico, and South Africa have shown that 30–40% of infant diarrhea can be accredited to EPEC. These strains are also an important cause of disease in nosocomial outbreaks, outpatient clinics, patients referred to hospitals, and urban and rural areas [2].

It had been demonstrated that the incidence of community-acquired EPEC infection was highest in the six-month period following childbirth and that the infection was more severe in younger children. In developing countries, the situation was much different; EPEC organisms were a major cause of endemic infantile diarrhea, exacerbated by seasonal outbreaks. There are certain serotypes, which are much more likely to be encountered in diarrhea than others.

Serotypes O55:H12/45, O86:H48, O127:H21, O142:H48, O126:H48, and O126:H19 were significantly associated with diarrhea in children [3]. Serotypes O55:H6 and O111:H2 [4], O111:H2 and O111:H—were reported as most frequent isolates in different geographical areas [5]. Fortunately, EPEC diarrhea is usually self-limited and rehydration is the most effective treatment. The use of antibiotics in general is of minor importance and has been criticised on the grounds of drug toxicity and the risk of increased wide-spread antimicrobial resistance [6, 7].

Although diarrheagenic *E. coli* pathotypes are of public health relevance, they are not routinely sought as enteric pathogens in clinical laboratories worldwide; thus, their incidence in children less than 2 years of age and their importance in community-acquired diarrhea are generally unknown, particularly in areas of endemicity [1].

In the present study, we isolated and identified the enteropathogens *E. coli* causing diarrhea among children less than five years old in Cairo, Egypt, during the different seasons, and study their patterns of resistance to different antimicrobial agents used for the treatment of patients with diarrhea.

2. Materials and Methods

2.1. Study Population

This cross-sectional study included infant and young children younger than five years with diarrhea, attending the Pediatric Gastroenterology Intensive Care Unit of the Cairo University Pediatric Hospital from January 2009 to December 2009. Information was also obtained from each patient regarding age, sex, onset of diarrhea, antibiotic intake, other relevant clinical information, and laboratory results. Diarrhea was defined as three or more liquid or semiliquid stools defecation per day. Our exclusion criteria were age more than 5 years, no diarrhea, incomplete data, and dry or suspected contaminated sample. Twenty age and sex matched concurrent healthy children who had had no diarrheal complaints during the previous month and were not on antibiotics for 1 week were included in this study as a control group.

Data collection was performed using references of other studies, for comparison between our results and other results in Egypt and other countries.

2.1.1. Microbiological Study

Stool specimens were collected from the patients in clear, transparent, wide-mouthed bottles and transported to the microbiology department of the laboratory. The specimens were examined for consistency, color,

TABLE 1: Age and sex of 134 children with diarrhea and positive EPEC in different age groups.

Age/month	Number of samples	Females no. (%)	Males no. (%)	Total positive no. (%) $P = 0.018$
<6	53	23 (42)	30 (38)	4 (7.5)
7–12	40	18 (33)	22 (28)	0 (0.0)
13–24	25	9 (16)	16 (20)	0 (0.0)
25–60*	16	5 (9)	11 (14)	3 (18.7)
Total	134	55 (41)	79 (59)	7 (5.2)

*2 EPEC isolated from 2-year-old boy and 1 from 3-year-old. $P < 0.05$.

and atypical components such as mucous, blood, and parasites, examined by light microscope for the presence of red blood cells, pus cells, parasitic ova, and protozoa, inoculated onto Blood, MacConkey, Salmonella Shigella (SS) agar media, and Selenite F broth, and incubated at 37°C for 24 h. The isolates were subjected to the following tests: Gram staining, citrate utilization, oxidase test, and subcultured on lysine iron agar (LIA), motility indole ornithine (MIO), and triple sugar iron agar (TSI) (Oxoid LTD, Basingstoke, Hampshire, England). All tests were done using the methods described by [8].

2.2. Serotyping

Three to five colonies of each sample isolates were identified by biochemical assay, and *E. coli* were selected for subculture on blood agar medium for serogrouping. Determination of the EPEC serogroups was performed by slide agglutination test using antiserum *E. coli* somatic trivalent I (O111, O55, O26) according to the instructions of the manufacturer (BIO-RAD, Marnes-la-Coquette, France).

2.2.1. Antimicrobial Susceptibility Testing

Antimicrobial drug susceptibility testing was done using standard methods (disc diffusion method) using Mueller-Hinton agar according to the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2009) [9] using the following antibiotic discs: amikacin (30 µg), amoxicillin-clavulanic acid (30 µg), ampicillin-sulbactam (20 µg), ampicillin (10 µg), aztreonam (30 µg), ceftriaxone (30 µg), cefepime (30 µg), cefoperazone (30 µg), cefoxitin (30 µg), ceftazidime (30 µg), cephalexin (30 µg), cotrimoxazole (25 µg), gentamicin (10 µg), imipenem (10 µg), and ticarcillin (75 µg) (Mast group, Merseyside, U.K.).

2.3. Statistical Analysis

Statistical analysis was done with Excel add-in Megastat, version 10.1 software (2007) using Chi-squared test. P values < 0.05 were considered significant.

3. Results and Discussion

A total of 156 consecutive nonrepeat samples from children under 5 years old with diarrhea were included in our study. Only appropriate samples from 134 patients with complete data were examined for the presence of EPEC. Our control group revealed no EPEC or other enteric pathogens ($P < 0.05$). Males in this study constituted 59% of the studied group with diarrhea, and females were 41%. All cases positive for EPEC were males. The total percentages of *E. coli* isolates were 51.5% (69/134); of which, 46% (62) were *E. coli* not EPEC, and 5.2% (7) were EPEC (Table 1).

Two cases with prolonged diarrhea had EPEC, and the other 5 cases with acute diarrhea had *E. histolytica* in association with EPEC (of which one case only was feverish and revealed *E. histolytica*,

TABLE 2: Clinical and laboratory data of cases with EPEC.

Sample no.	Month	Age	Sex	Stool analysis			Culture result	Antibiotic resistance
				Consistency	pus	Protozoa		
1*	April	2 y	♂	Loosed	0-1	<i>E. histolytica</i>	EPEC + <i>Pseudomonas</i>	No resistance
2	June	3 y	♂	Loosed	0-1	Negative	EPEC	No resistance
3	June	2 m	♂	Loosed	0-1	Negative	EPEC	A, TS, TC
4	July	5 m	♂	Loosed	>100	<i>E. histolytica</i>	EPEC + <i>Klebsiella</i> <i>spp.</i>	A, TS, TC, ATM, CRO, CPM, CPZ
5	July	2 y	♂	Watery	0-1	<i>E. histolytica</i>	EPEC + <i>Proteus</i>	No resistance
6	Aug.	36 d	♂	Watery	>100	<i>E. histolytica</i>	EPEC + <i>Salmonella</i>	A, TC, TS
7	Sep.	3 m	♂	Loosed	>50	<i>E. histolytica</i>	EPEC	A, TS, TC

*Only this patient was not on antibiotic at time of sampling; other patients were on β -lactam antibiotics.

All cases have acute diarrhea except no. 2 and 3 which have prolonged diarrhea.

TC: ticarcillin; TS: cotrimoxazole; A: ampicillin; CPZ: cefoperazone; CPM: cefepime; CRO: ceftriaxone.

TABLE 3: Comparison between different previous studies and our study.

Place	Year	No. of cases	No. of EPEC	% of EPEC	Reference	
Iran	2009	111	50	44.9	[2]	
Bangladesh	1983	104	24	23.1	[11]	
Jordan	2000	265	34	12.8	[12]	
Nigeria	2009	100	15	15	[3]	
	Cairo	1982	266	21	8	[13]
	Ismailia	1990	65	9	13.8	[14]
Egypt	Assiut	1992	150	47	31.3	[15]
	Tanta	1994	100	22	22	[16]
	Cairo	2009	134	7	5.2	Our study

EPEC, and *Salmonella spp.*) (Table 2). Only one patient with EPEC was not on antibiotic therapy before sample release, while the all other (6) patients were on antibiotic therapy (β -lactam).

Four (57%) isolates of EPEC were resistant to ampicillin, ticarcillin, and cotrimoxazole, 1 (14.3%) to the third-generation cephalosporins, and no resistance (0%) were detected to imipenem, amikacin, and gentamicin.

Estrada-Garcia et al. [1] demonstrated a significant association of an enteropathogenic *E. coli* (EPEC) with community-acquired acute diarrhea lasting 7 to 12 days among Mexican children, suggesting that EPEC is more associated with protracted diarrhea than the other diarrheagenic *E. coli* pathotypes. In our study, a total of 134 stool specimens were analyzed in this study; *E. coli* species were isolated at a relatively high rate (51.5%) with 5.2% of samples positive for enteropathogenic *E. coli*. This is lower than the prevalence in other studies at Nigeria and Iran (15% and 44.9%, resp.) [3, 10]. Also, it was lower than the prevalence documented in previous studies in Egypt (Table 3).

One probable reason for this difference between our study and other studies in Nigeria and Iran may be due to the number of children being exclusively breast fed in those studies as in Egypt most of the mothers exclusively breast fed their children till the age of 1 to 2 years. Breast milk (colostrum) from mothers living in endemic areas has been reported to contain high levels of immunoglobulin A (IgA) antibodies against the EPEC virulence factors [10]. The reason for the difference between our study and previous studies in Egypt

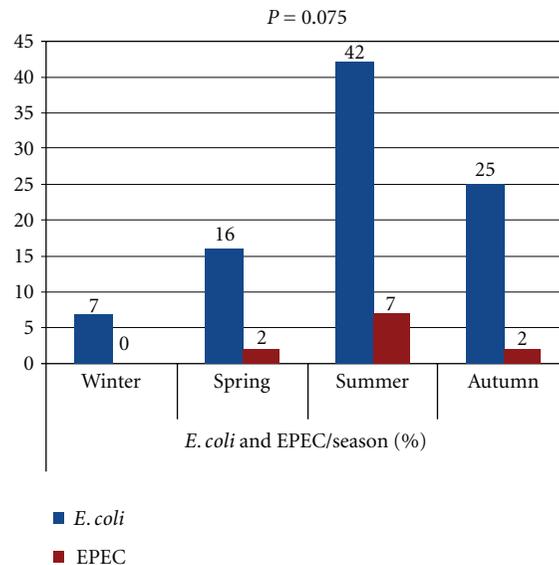


FIGURE 1: Percent of *E. coli* and EPEC in children with diarrhea/season.

could be due to increased awareness to clean food preparations and proper hand hygiene, as a result of intensive education programs carried out by the ministry of health and the media after H5N1 (Avian flu) and H1N1 (Swine flu) outbreaks in 2006 and 2008, respectively. However, further studies could be indicated to rule the effect of the educational programs on reduction of the infections caused by diarrheagenic pathogens. In this study, the highest rate of EPEC was found in children between 2 and 3 years (18.7%), followed by children below 6 months old (7.5%). Our statistical analysis suggests that EPEC was significantly associated with diarrhea in children in this two age groups ($P < 0.05$). This trend agrees with several studies which had shown that the peak of incidence of enteritis was always in the few months after the beginning of the weaning period and most of EPEC infections occur in the first 3 years of life [17]. However, in other studies, they demonstrated that the incidence of community-acquired EPEC infection is highest in the six-month period following childbirth and that the infection is more severe in younger children [18, 19].

In this study, non-EPEC *E. coli* species showed highest peak in summer followed by autumn. EPEC isolates were detected in all seasons except winter, but they appeared as predominant in the summer season (warm and dry season) (Figure 1).

This trend agrees with the findings in other studies [17, 20], which reported that EPEC infections show a marked seasonality and are associated with warm season peaks and [21], reported that peak rates of EPEC infection occurred mainly in the dry summer months. However, the difference was not found to be statistically significant.

Five patients with acute diarrhea revealed EPEC in association with other pathogens in their fecal samples, while the other two patients with prolonged diarrhea revealed EPEC as a unique pathogenic isolate, which demonstrates the importance of EPEC as single pathogen of infection or associated in coinfection.

Six (85.7%) patients in the current study were on β -lactam antibiotic therapy before stool sampling; of which, 57% of them revealed EPEC resistant to ampicillin, ticarcillin, and cotrimoxazole and 14.3% to third-generation cephalosporins. The misuse of β -lactam antibiotic largely is because they are inexpensive and can be obtained easily without a doctor's prescription. Resistance is probably due to indiscriminate antibiotic usage (drug abuse) which could result in plasmid-mediated antibiotic resistance found to be common in *E. coli* [8].

High rate of resistance of EPEC was also recorded for ampicillin, and cotrimoxazole in a study in Nigeria [3]. This study determined the significance and the association of the strains of *E. coli* as a predominant isolates with diarrhea in children younger than 5 years in Cairo, Egypt. EPEC was identification

from fecal specimens to be considered as a unique pathogen or associated with other pathogens in acute diarrhea in children, to prevent the prolonged sequences of diarrhea. The trivalent *E. coli* antiserum is a rapid and easy test for laboratory screening for detection of EPEC. Also multi-drug-resistant enteropathogenic *E. coli* can be associated with infantile diarrhea.

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This article should be cited as follows:

Iman K. Behiry, Emad A. Abada, Entsar A. Ahmed, and Rania S. Labeeb, “Enteropathogenic *Escherichia coli* Associated with Diarrhea in Children in Cairo, Egypt,” *TheScientificWorldJOURNAL*, vol. 11, pp. 2613–2619, 2011.



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