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New insights into the epidemiology of enteropathogenic *Escherichia coli* infection

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Abstract

Summary—Enteropathogenic *Escherichia coli* (EPEC) are among the most important pathogens infecting children worldwide and are one of the main causes of persistent diarrhea. EPEC were originally serogroup-defined *E. coli* associated with infantile diarrhea. As various mechanisms of pathogenesis have been discovered, EPEC classification has come to be based on the presence of specific genes. The *eae* (intimin) and *hfpA* (bundle-forming pilus) genes have both been used for identification of EPEC and for subdivision of this group of bacteria into typical and atypical strains. For many years typical EPEC have been considered to be the leading cause of infantile diarrhea in developing countries and were considered rare in industrialized countries. However, current data suggests that atypical EPEC are more prevalent than typical EPEC in both developing and developed countries. Moreover, the duration of diarrhea in patients infected with atypical EPEC is significantly longer than that caused by other pathogens. When comparing the isolation rates of EPEC among children with diarrhea and healthy controls without diarrhea, in general, there is a higher isolation rate in diarrhea, although not significantly higher in all studies. These inconsistencies probably are related to the study patient populations, reflecting a possible age-related susceptibility to infection.

Keywords

Escherichia coli; EPEC; Diarrhea; Children; Infantile diarrhea; Epidemiology

1. Introduction

Diarrhea is one of the leading causes of death in children, accounting for approximately 2 million deaths each year worldwide (Bryce et al., 2005). As improvements in treating children with acute diarrheal disease (particularly oral rehydration therapy) have led to decreased morbidity and mortality related to fluid and electrolyte loss, persistent diarrhea has emerged as a common cause of death. Persistent diarrhea is an infection-induced illness that can result from multiple consecutive infections, an unresolved infection, secondary malabsorption or a post-gastroenteritis syndrome. Although many viruses, bacteria and parasites can produce persistent diarrhea, *Giardia*, *Cryptosporidium*, enteroaggregative *Escherichia coli* (EAEC) and enteropathogenic *E. coli* (EPEC) are the most important of these agents (Ochoa et al., 2004). The purpose of this review is to summarize current information regarding the epidemiology of

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EPEC infection in children and its role in persistent diarrhea, and to highlight the emerging role of atypical EPEC.

2. EPEC pathogenesis

EPEC induce a distinctive histopathology known as the attaching and effacing (A/E) lesion, which is characterized by the intimate attachment of bacteria to the epithelial surface and effacement of host enterocyte microvilli. There are three stages in EPEC pathogenesis: (i) initial adherence to the host cell, (ii) production and translocation of bacterial proteins through a needle complex via a type III secretory system, and (iii) intimate bacterial attachment with pedestal formation ([Chen and Frankel, 2005], [Garmendia et al., 2005], [Nataro and Kaper, 1998] and [Vallance and Finlay, 2000]).

The major virulence proteins shared by all EPEC are encoded on a 35-kb chromosomal pathogenicity island termed the locus of enterocyte effacement (LEE). Among these proteins is intimin, which is necessary for the attachment of the bacteria to the epithelial host cells ([Chen and Frankel, 2005] and [Vallance and Finlay, 2000]). Among the non-LEE encoded proteins is bundle-forming pilus (BFP), a type IV pilus, encoded on a plasmid, called EPEC adherence factor (EAF) ([Chen and Frankel, 2005] and [Vallance and Finlay, 2000]). The BFP is responsible for the localized adherence (LA) phenotype of EPEC, characterized by the ability of bacteria to form microcolonies on the surface of the intestinal epithelial cells. There are a number of other EPEC virulence proteins (invasion and effector proteins and toxins) that might be important for the clinical manifestations of intestinal infection in children. However, the association and relevance of these proteins to the development of prolonged diarrhea and other clinical manifestations is not well established.

3. EPEC classification

EPEC were originally serogroup-defined *E. coli* associated with infantile diarrhea. Current EPEC classification has come to be based on the presence of specific virulence genes. Using new molecular techniques, typical virulence traits have also been found in strains not belonging to classic serogroups/serotypes (Vidotto et al., 2000). The *eae* (intimin) and *bfpA* (bundle-forming pilus) genes have both been used for identification of EPEC and for subdivision of this group of bacteria into typical and atypical strains (Nataro and Kaper, 1998). By definition all EPEC lack genes to produce shigatoxin (*stx*). *Escherichia coli* strains that are *eae+* *bfpA* + *stx*⁻ are classified as typical EPEC; most of these strains belong to classic O:H serotypes. On the other hand, *E. coli* strains that are *eae+* *bfpA*⁻ *stx*⁻ are classified as atypical EPEC. The frequency of typical and atypical EPEC and their association with acute or persistent diarrhea, and asymptomatic carriage is not well defined (Scaletsky et al., 2002b; [Trabulsi et al., 2002], [Vidotto et al., 2000] and [Vieira et al., 2001]).

4. Epidemiology of EPEC infection

EPEC are among the most important pathogens infecting children under 2 years of age in the developing world. EPEC prevalence varies related to differences in study population, age group, diagnostic criteria, methods used for diagnosis, etc. Based on molecular methods (intimin gene identification), currently EPEC is responsible, on average, for 5–10% of pediatric diarrheal episodes in the developing world. When diagnosis was made based on the HEp-2 adherence pattern or serotyping, the estimated prevalence rates of EPEC were higher, on average by 10–20%, with large variability among studies.

Typical EPEC have been considered for many years as the leading cause of infantile diarrhea in developing countries and were considered rare in industrialized countries where atypical EPEC seemed to be a more important cause of diarrhea ([Afset et al., 2003] and [Trabulsi et

al., 2002]). However, recent data suggests that atypical EPEC are more prevalent than typical EPEC in both developing and developed countries. Table 1 summarizes recent studies of the prevalence of EPEC in developing countries, based on molecular diagnosis. Of interest, atypical EPEC strains were isolated more frequently than typical strains in most of these studies. Overall atypical EPEC were responsible for 78% (131/169) of all EPEC cases in children younger than 5 years of age with diarrhea (based on the studies that reported data only for children younger than 5 year of age in Table 1). These findings reinforce the need for further investigation of the virulence properties of atypical EPEC strains.

When comparing the isolation rates of EPEC among children with diarrhea and healthy controls without diarrhea, in general, there is higher isolation rate in diarrhea, although not significantly higher in all studies (Table 2). These trends without statistical significance may relate to the study patient population, perhaps reflecting an age-related susceptibility to infection. The pathogenicity index, calculated as the percentage of case patients shedding EPEC divided by the percentage of control patients with positive test results for EPEC (Nataro et al., 2006), is remarkably variable in these studies, with no correlation with the children's age. However, these studies have different patient populations, recruitment methods, inclusion and exclusion criteria, and different definitions for their control subjects. Therefore, in order to understand the significance of EPEC isolation in control samples and to determine the age-specific variations in EPEC infection, large epidemiological studies are needed. Observations suggest that the predilection of EPEC infection to occur among young persons in developing countries may not be because an inherently increased susceptibility of young patients; rather, it may be because of acquired immunity in areas where the pathogen is highly endemic.

Each year EPEC are responsible for thousands of deaths worldwide, mostly in infants and young children. Comparing the clinical features of diarrhea caused by EPEC versus other pathogens, children with EPEC are more likely to fail to respond to oral rehydration therapy, have cow's milk intolerance, require hospitalization and develop persistent diarrhea (Fagundes-Neto and Scaletsky, 2000). Although some studies have shown that patients infected with atypical EPEC have a mild, non-dehydrating and non-inflammatory diarrhea (Nguyen et al., 2006), typically the duration of diarrhea in patients infected with atypical EPEC is significantly longer than that caused by other pathogens ([Afset et al., 2004] and [Nguyen et al., 2006]). Among children with persistent diarrhea (defined as diarrhea lasting more than 14 days), atypical EPEC was the most common pathogen in recent studies from Australia (43% [12/28]; Nguyen et al., 2006) and Norway (22% [20/89]; Afset et al., 2004). Likewise, persistent diarrhea was the most common clinical presentation in atypical EPEC cases, accounting for more than half of the episodes in children in Brazil, Australian and Norway ([Afset et al., 2004], [Nguyen et al., 2006] and [Scaletsky et al., 1999]). These findings indicate that atypical EPEC may have an innate propensity to persist longer in the intestine than other diarrheagenic *E. coli* which cause diarrhea that is more transient in nature. EPEC adhere tightly to epithelial cells and disrupts normal cellular process (Chen and Frankel, 2005), and some evidence suggest that atypical EPEC may decrease apoptosis of intestinal epithelial cells (Heczko et al., 2001) possibly because of the lack of BFP (Melo et al., 2005). These features may favor prolonged intestinal colonization by atypical EPEC compared with other intestinal pathogens. These data suggest that interventions targeted towards atypical EPEC may be beneficial in managing children with prolonged diarrhea.

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Table 1
Enteropathogenic *Escherichia coli* (EPEC) prevalence in children with diarrhea in developing countries

Country	Sample collection period	Children's age (years)	No. of samples	EPEC ^a (%)	EPEC type		Reference
					Typical ^b (%)	Atypical ^c (%)	
Tanzania	1996–1997	<5	451	24 (5.3)	9 (37.5)	15 (62.5)	Vargas et al., 2004
Thailand	1996–2000	<12	2629	85 (3.2)	24 (28.2)	61 (71.8)	Ratchrachenchai et al., 2004
Mozambique	1998–1999	<7	548	13 (2.4)	4 (30.8)	9 (69.2)	Rappelli et al., 2005
Brazil	1998–1999	<2	237	34 (14.3)	21 (61.8)	13 (38.2)	Saletsky et al., 2002a
Mongolia	2001–2002	<16	238	9 (3.9)	5 (55.6)	4 (44.4)	Sarantuya et al., 2004
Brazil	2001–2002	<5	175	13 (7.4)	1 (7.7)	12 (92.3)	Franzolin et al., 2005
Vietnam	2001–2002	<5	587	39 (6.6)	0 (0)	39 (100.0)	Nguyen et al., 2005
Mexico	2000–2004	<5	430	16 (3.7)	3 (18.7)	13 (81.3)	Estrada-Garcia et al., 2005
Tunisia	2001–2004	<15	115	6 (5.2)	2 (33.3)	4 (66.7)	Al-Gallas et al., 2007
Brazil	2002–2003	<5	446	25 (5.6)	2 (8.0)	23 (92.0)	Araujo et al., 2007
Chile	2004–2005	<9	509	54 (10.6)	14 (25.9)	40 (74.1)	Vidal et al., 2005
Peru	2006–2007	<1	461	18 (3.9)	2 (11.1)	16 (88.9)	Barletta et al., 2007
Iran	Nd ^d	<10	247	45 (18.2)	29 (64.4)	16 (35.6)	Alikhani et al., 2006

^a Detected by PCR.

^b Typical EPEC: *eae*⁺/*hlyE*⁺.

^c Atypical EPEC: *eae*⁺/*hlyE*[−].

^d ND: no data.

Table 2
Frequency of enteropathogenic *Escherichia coli* (EPEC) isolation in children with diarrhea and healthy controls without diarrhea

Country	Children's age (years)	Rate of EPEC isolation ^a n/N (%)		p ^b	Pathogen city index ^c	Reference
		Patients with diarrhea	Control subjects			
Brazil	<1	23/100 (23.0)	2/100 (2.0)	<0.001	11.5	Scaletsky et al., 2002b
Peru	<1	18/461 (3.9)	9/166 (5.4)	NS ^d	0.7	Barletta et al., 2007
Brazil	<2	34/237 (14.3)	20/231 (8.6)	0.05	1.7	Scaletsky et al., 2002a
United States	<5	45/684 (6.5)	19/486 (3.9)	0.05	1.7	Cohen et al., 2005
Vietnam	<5	39/587 (6.6)	11/249 (4.4)	NS	1.5	Nauven et al., 2005
Brazil	<5	25/446 (5.6)	2/139 (1.4)	0.05	4.0	Aratijo et al., 2007
Norway	<5	38/251 (15.1)	21/210 (10.0)	NS	1.5	Afset et al., 2004
United States	<5	20/317 (6.3)	6/56 (10.7)	NS	0.6	Nataro et al., 2006
Mozambique	<7	13/548 (2.4)	3/380 (0.8)	NS	3.0	Rappelli et al., 2005
Iran	<10	45/247 (18.2)	16/110 8 (1.4)	<0.001	13.0	Alikhani et al., 2006

^a Detected by PCR.
^b For the difference between EPEC isolation in cases and controls.
^c The pathogenicity index was calculated as the percentage of case patients shedding EPEC (*eae*+), divided by the respective percentage of control patients with positive test results for EPEC (*eae*+).
^d NS; not significant.