Enteropathogenic *Escherichia coli* infection: history and clinical aspects

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ABSTRACT

Diarrhoeagenic *Escherichia coli* remains an important cause of diarrhoeal disease worldwide. In terms of global public health, enteropathogenic *E. coli* (EPEC) and enterotoxigenic *E. coli* are the most important. However, enterohaemorrhagic *E. coli* has emerged as a cause of disease in developed countries in recent years, and a number of large outbreaks have been reported. Therefore, the importance of research into diarrhoeagenic *E. coli* remains an important issue. EPEC is the most widespread of the diarrhoeagenic *E. coli* and provides a good virulence model for other *E. coli* infections, as well as other pathogenic bacteria. Although the virulence mechanisms of *E. coli* are now better understood, there remains much to be learned before effective treatments can be developed. Type III secretion mechanisms, the locus of enterocyte effacement and various toxins are all involved in the pathogenesis of the various diarrhoeagenic *E. coli* and may provide targets for future therapies. This review aims to provide an update on the worldwide problem of diarrhoeagenic *E. coli* by focusing on EPEC, and describes the history of the organism, its incidence and the clinical aspects of infection.

KEY WORDS: Diarrhea. Escherichia coli.

Introduction

Diarrhoeal illness continues to be a major public health burden worldwide. Despite the fact that most children are now immunised against all the major childhood infectious diseases, the World Health Organisation (WHO) reports[1] that more than 10 million die each year before their fifth birthday. This amounts to almost half the total number of deaths before the age of 50. However, the figures were higher – the total mortality has been reduced successfully from around 21 million in 1995. Even so, more than 50 million deaths occurred in 1997, 40 million of which were in developing countries and approximately one-third were due to infectious and parasitic diseases. A large proportion of these deaths were due to diarrhoeal diseases, one of the most common being *Escherichia coli* infection.

Currently, there are seven recognised diarrhoeagenic *E. coli*: enteropathogenic *E. coli*, enterohaemorrhagic *E. coli*, enteroinvasive *E. coli*, enterotoxigenic *E. coli*, enteroaggregative *E. coli*, diffuse-adherent *E. coli* and cytolethal distending toxin-producing *E. coli*. In terms of global infection, enteropathogenic *E. coli* (EPEC) is the most important. This review provides a general background to the history of the EPEC bacterium and focuses on its worldwide incidence and the clinical aspects surrounding infection.

Enteropathogenic *Escherichia coli* – historical overview

'Infantile diarrhoea' was the term used to describe the symptoms of an infection that had been noted for a number of centuries. Over the past four centuries, infantile diarrhoea has had a number of synonyms including the terms 'griping in the guts', cholera infantum, 'summer diarrhoea' and 'gastro-enteritis'.[2] Up to and including the early part of the last century, infantile diarrhoea was a major problem worldwide, with high morbidity and mortality.

From 1920, diarrhoea-associated mortality in infants decreased in developed countries, resulting in a decline of interest in determining the aetiology of infantile diarrhoea;[3] however, during the 1930s, a number of severe nosocomial outbreaks of neonatal enteritis occurred in New York, USA, each of which was associated with high mortality.[4] In 23 outbreaks in 15 hospitals a total of 711 cases of infantile diarrhoea occurred in hospitalised infants, and the average morbidity and mortality rates were 15.5% and 7.3%, respectively (ranging from 4% to 49% and 0% to 21.5%, respectively). This led to a renewed interest in the aetiology of infantile diarrhoea.

Bray[5] reported the isolation of diarrhoeagenic *E. coli* from cases of infantile summer diarrhoea although the intestinal pathogenicity of *E. coli* had already been noted as early as 1889[6] and 1897.[7] Lesage[7] suggested that there were pathogenic and non-pathogenic strains of *E. coli* because convalescent serum from a patient with diarrhoea agglutinated bacteria from other patients with diarrhoea

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Table 1. *E. coli* serotypes commonly associated with infantile diarrhoea

018:H7	018:H14	020:H-	020:H26
026:H-	026:H11	028:H-	044:H18
044:H34	055:H-	055:H6	055:H7
086:H34	0111:H-	0111:H2	0111:H12
0111:H5	0111:H7	0112:H-	0114:H2
0119:H6	0124:H-	0124:H30	0125:H21
0126:H-	0126:H27	0127:H-	0127:H6
0127:H21	0128:H2	0128:H7	0128:H12
0142:H6	0158:H23		

during an epidemic. The same serum did not agglutinate bacteria from healthy individuals.[7]

Early epidemiological investigations used carbohydrate fermentation[5] or slide agglutination tests[8] to identify diarrhoeagenic *E. coli*. In 1947, Kauffman[9] published a serotyping scheme based on somatic (O), flagellar (H) and capsular (K) antigens, providing a reliable method of typing diarrhoeagenic *E. coli* that is still used today for the serotyping of *E. coli* and other members of the Enterobacteriaceae. In the UK in the first half of the last century, epidemiological studies to determine the source of diarrhoeagenic *E. coli* mostly were unsuccessful. In the mid 1950s, however, *E. coli* serogroups associated with infantile diarrhoea were isolated from cows' milk.[2] It is unlikely that they could be isolated from commercially-prepared milk today because of stringent bacteriological testing and pasteurisation, but some raw milks sold on farms could contain diarrhoeagenic *E. coli*.

The term 'enteropathogenic *E. coli*' (EPEC) was introduced in 1955 to describe strains of *E. coli* implicated epidemiologically with infantile diarrhoea.[10] The serotypes most commonly associated with EPEC are listed in Table 1. The work that provided much of this information[11] was important because it provided a list of diarrhoeagenic *E. coli* serotypes that has remained largely comprehensive, with only a few subsequent additions. Nevertheless, it should be noted that EPEC is not a homogeneous group of enteropathogens[11] because serotype does not always correlate with pathogenicity.[12,13]

Individual strains of some serotypes generally considered to be 'classical' EPEC serotypes produce a heat-labile enterotoxin, while others may be invasive. Strictly, such strains should not be classed as EPEC despite their serotypes. Moreover, as the early methods of identifying EPEC relied on serotyping alone, there was always some reluctance in accepting the organism as a pathogen. It could be argued that EPEC is non-pathogenic and that changes in the gut epithelium due, for example, to infection by an unknown bacterium or virus permit the multiplication and detection of EPEC serotypes. However, the pathogenic ability of EPEC was confirmed by Levine *et al*.[14] when they showed that strains of EPEC that did not produce enterotoxins were not invasive, were negative in the infant rabbit assay for gross fluid accumulation and caused diarrhoea when given to adult volunteers. No heat-labile or heat-stable enterotoxins were detected in *E. coli* isolated from volunteers with diarrhoea, and they did not show a rise in LT antitoxin titre.

Prior to the introduction of molecular microbiology, EPEC was defined generally as 'diarrhoeagenic *E. coli* belonging to serogroups epidemiologically incriminated as pathogens but whose pathogenic mechanisms have not been proven to be related either to LT enterotoxins or ST enterotoxins or to Shigella-like invasiveness. EPEC adhere in a seemingly pathognomic way to the intestinal epithelium'.[15] Although most of this holds true, molecular methods have provided more information on the virulence properties of EPEC. For example, they demonstrated that strains identified serologically as EPEC prior to 1960 and isolated from infants with diarrhoea are in fact EPEC.[16] This is an important finding because the study confirmed the identity of these isolates on the basis that they possessed virulence determinants now known to be associated with EPEC, whereas originally they were identified by criteria not associated with pathogenic determinants.

Worldwide incidence of EPEC infection

As little as three decades ago EPEC was a significant cause of infantile diarrhoea in developed countries, accounting for 16% of cases in one study.[17] However, the mortality rate for EPEC infection has fallen considerably since the beginning of the last century, apart from a significant rise during the First World War (Figure 1).[2] The mortality rate has continued to fall since then, from 70% to 25% in the two decades up to 1970.[18] EPEC infection is now uncommon in developed countries and is no longer regarded as a clinical problem, to the extent that in the UK it has been proposed that screening for the organism is not necessary.[19]

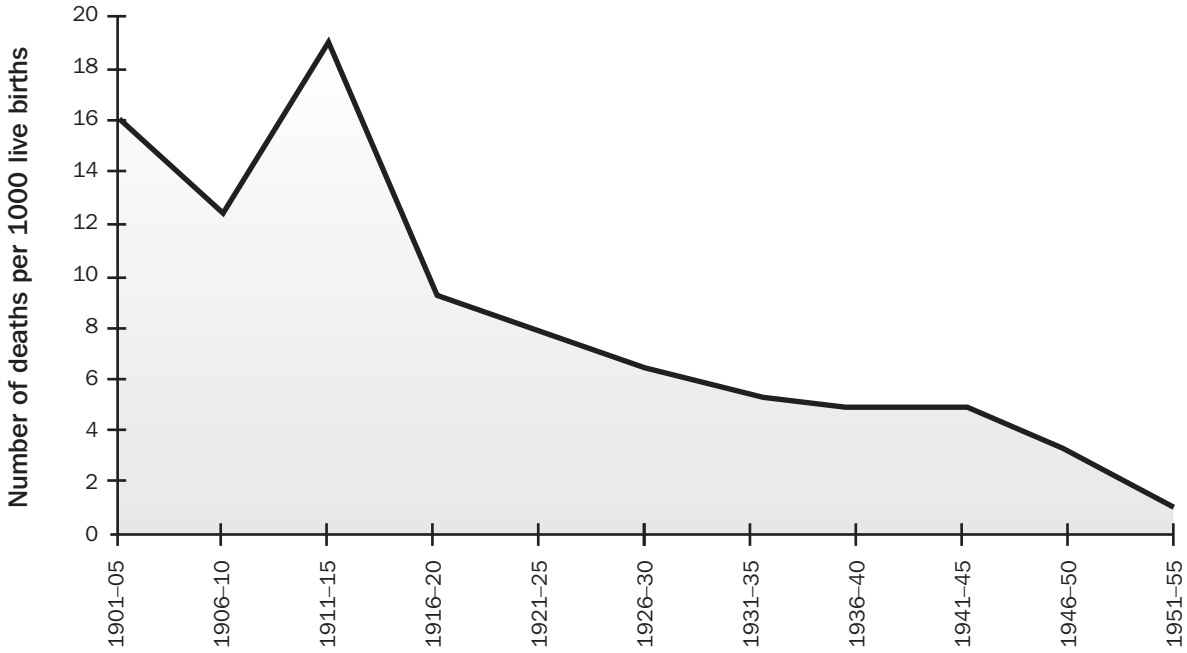
The last severe outbreak of infantile diarrhoea due to EPEC in the UK occurred in 1980 in a hospital paediatric unit, and was initiated by the introduction onto the ward of an infant suffering from diarrhoea. Thirty infants were affected and the outbreak resulted in two deaths. EPEC is still isolated from young children as sporadic cases.[20,21]

In a recent US study, it was found that 25 out of 445 children in a paediatric hospital were excreting EPEC,[22] but it was not known whether or not they were actually causing disease. In another study, in the UK,[21] the aetiology of gastroenteritis in children under five was investigated. *E. coli* was identified in 2% of reports, and 44% of these (totalling 1621 reports) were of classical EPEC serotypes. Many sporadic EPEC infections seen in developed countries are acquired during foreign travel, probably in developing countries.

Indeed, it is in the developing world that EPEC is a public health problem of massive proportions. A number of studies conducted between the 1960s and 1980s showed that the incidence of EPEC infection in developing countries ranged from 4% to 37%,[23] and it is unlikely that these figures have changed much since because populations have increased and sanitation has not improved significantly in such countries.

It is now estimated that there are 117 million diarrhoeal episodes attributable to EPEC each year in developing countries alone (excluding China). In some areas, EPEC remains the most prevalent enteropathogen isolated from children under the age of two.[24] However, there is little

Fig. 1. Summer diarrhoea as a cause of death in babies under one year of age in England from 1901 to 1953.



recent data on the incidence of EPEC infection worldwide and it is difficult to compare data from the few studies that have been performed over the last few decades.

A number of studies[25,26] have attempted to determine the incidence of EPEC in children with and without diarrhoea. In one study,[25] EPEC was isolated from 16% of children with diarrhoea and from 4% of those without. In another two studies, EPEC was isolated from children with diarrhoea in 9%[26] and 2.7%[27] of cases, respectively. Therefore, the actual incidence of EPEC varies according to the setting and may depend on a number of factors including the country, the time of year and the quality of the study.

E. coli gastrointestinal infections, apart from those due to EHEC, are not reportable to public health centres in either the UK or the USA, although outbreaks are reported. There are also no surveillance systems for *E. coli* infections, and many other infections, in developing countries where EPEC is most common. Thus, there is no accurate method for calculating the incidence of EPEC infection in either developed and developing countries.

Studies of the incidence of diarrhoeal pathogens in communities have been performed but these often omit EPEC. In such studies, up to 40% of infections are of unknown aetiology, a percentage to which EPEC may contribute. Those studies that include EPEC are still unrepresentative of the global situation because they are often performed in isolated communities.[25]

In line with most other gastrointestinal pathogens, there are many reservoirs of EPEC infection. These may include symptomatic or asymptomatic infants or children, and asymptomatic adult carriers or animals. However, the actual methods of transmission are poorly understood although, like other gastrointestinal pathogens, they probably rely on the faecal-oral route of infection. Thus, close contact between animals or people, sharing of food items and food itself can all act as sources of infection. For example, a recent study screened 402 *E. coli* isolates from various food items for the presence of EPEC. Surprisingly, a total of 19 EPEC isolates were found, 17 of which were from cooked foods.[28] Therefore, the simplest method of avoiding infection is good hygiene; however, as discussed above, the areas of the world in which EPEC remains a problem often do not have adequate access to clean water or facilities for hygienic disposal of faecal waste.

Clinical aspects and therapy

The infective dose of EPEC required to cause disease in infants has not been established. Volunteer studies in adults indicate that a high infective dose, between $5x10^8$ and 10^{10} organisms, may be required.[14,29] However, the infective dose in adults cannot be used to reflect the infective dose required in infants because of differences in physiology and immune status. Moreover, the incubation period for EPEC infection is not really known because infant studies have not been performed. In adult studies, one report claims that the incubation period is eight to 60 hours,[30] while another states that it varies from two to 12 days.[10] However, a recent volunteer study used 48-hour infection as the study end-point.[29] These figures should not be used to estimate the incubation period in infants because the adult intestine differs from the infant intestine.

Diarrhoeal disease due to EPEC is age-related, usually occurring in infants less than six months old. The reason for this age specificity is not understood, although factors such

as mucosal immunity and differences in gut structure between infants and adults (i.e. possession of receptors) could play a part. Lack of immunity appears to be the most probable reason for this, due to the association of infection with bottle-fed infants. The presence of IgA in breast milk may be a protective factor, although other factors in breast milk may also contribute. It would appear that contaminated feeding bottles do not account for all cases of EPEC infection, although they may play some part in transmission of the organism.

It is also interesting to note that EPEC infection is not alone in its age specificity. EHEC O157:H7 is only pathogenic in calves less than three weeks old,[31] although humans are affected at any age. A volunteer study in adults[32] showed that prior infection with EPEC can reduce disease after homologous rechallenge. This protection is thought to be due to serum IgG against the bacterial lipopolysaccharide; therefore, prior infection with serotype O55 may not protect against future infection with serotype O127.

EPEC infection results in an acute or persistent watery, non-bloody or mucoid diarrhoea,[30] often accompanied by fever and vomiting. The disease ranges from a fulminating diarrhoea to a subclinical infection,[10] presumably depending on host factors. After colonisation of the intestine with EPEC, bacteria can be isolated for some four to seven days before the onset of symptoms.[18] During the symptomatic stage, EPEC is present in pure culture in the faeces.[17,18] In most cases, if recovery occurs, then the organisms are also cleared; however, carriage may occur for some weeks in some cases.[18]

Most infants with diarrhoea caused by EPEC recover uneventfully if water and electrolyte disturbances are corrected promptly.[19,27] In addition, the introduction of a protein-hydrolysate, lactose-free formula in one study led to the prompt cessation of diarrhoea and nutritional recovery in two infants.[33] Antimicrobial therapy may also be of benefit to those in a life-threatening condition.[19]

The antimicrobial susceptibility patterns of EPEC are variable, as would be expected with the number of serotypes implicated in infantile diarrhoea. In one study of a nosocomial outbreak in Kenya,[34] 82% of EPEC strains belonged to two resistance patterns, although there was no consistent relationship between the plasmid profile and the antimicrobial resistance pattern. Most strains are susceptible to cefotaxime, colistin and amikacin, and are resistant to ampicillin,[35,36] but studies use different antibiotics and there is little overlap between them, making comparisons difficult.

In 1992, WHO and the United Nations' Children's Fund (UNICEF) introduced a strategy known as Integrated Management of Childhood Illness (IMCI), with the aim of reducing the morbidity and mortality associated with the major causes of childhood illness (diarrhoea, pneumonia, measles and malaria).[37] Initially, the project focused on improving care within primary health facilities. This is where millions of children arrive each day, most of whom are suffering from one of the major diseases. Patient management guidelines were drawn up by 1996 and they are now followed in more than 20 countries. Another 20 countries have shown interest in the IMCI. However, infections caused by EPEC (and also *Shigella* spp.) are likely to be less tractable to IMCI, and when there is a general fall in diarrhoea cases the proportion of EPEC infections will rise and EPEC will be seen as relatively more of a problem.

In summary

EPEC is an important gastrointestinal infection that is responsible for high morbidity and mortality worldwide, particularly in developing countries. Although epidemiological investigations and laboratory research has provided a large amount of information on this infection, there remains a lot to be learned about the virulence mechanisms of this pathogen before effective therapies or vaccines can be developed. □

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