The rise and rise of enteropathogenic *Escherichia coli*

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First described in 1885, *Escherichia coli* gradually achieved recognition as a cause of diarrhoea. Strains of *E. coli* which belonged to a limited number of O-serogroups and had been associated with outbreaks of diarrhoea in hospitalised children were designated 'enteropathogenic' *E. coli* (EPEC) to distinguish them from *E. coli* strains that cause other types of infection. The discovery that some strains of *E. coli* can produce toxins or invade epithelial cells in a similar fashion to established pathogens, such as *Vibrio cholerae* and *Shigella* species, shed new light on *E. coli* virulence. As EPEC do not exhibit either of these properties, however, their pathogenicity was brought into question. Further studies revealed that EPEC constitute a distinctive group of pathogenic bacteria that display characteristic adherence to cultured epithelial cells and produce distinctive histopathological changes, termed 'attaching-effacing lesions', in the intestinal epithelium. The ability to evoke these lesions, which are essential for virulence, is associated with a series of linked genes, known as the locus for enterocyte effacement, on the bacterial chromosome. In addition, the pathogenicity of some EPEC strains is associated with the presence of a plasmid-encoded, bundle-forming pilus. Naturally occurring strains of EPEC which lack this pilus are known as atypical EPEC and are an emerging cause of diarrhoea throughout the world.

Emergence of enteropathogenic *Escherichia coli*

Escherichia coli is named after Theodor Escherich (1857 - 1911), a German paediatrician, who identified this bacterium in the stools of healthy children and named it *Bacterium coli commune*. Escherich showed that *E. coli* could cause infection when inoculated parenterally into rabbits. On the other hand, the concept that *E. coli* could also cause disease in the gastrointestinal tract took much longer to gain acceptance.

Investigations during the early part of the 20th century in France, Denmark and the USA provided sound epidemiological evidence that *E. coli* was associated with diarrhoea in children.[1]

These observations were supported by reports from Adam and Goldschmidt in Germany who showed that certain strains of *E. coli* which were antigenically related to each other were linked to outbreaks of severe infantile gastroenteritis.[1] Despite this body of evidence, it was only after Bray and colleagues at the Hillingdon Hospital near London reported that a particular strain of *E. coli*, *Bacterium coli neapolitanum*, was significantly associated with diarrhoea in children, that the notion of diarrhoea induced by *E. coli* gained credence (Fig. 1).[2] Bray's seminal studies were followed by similar reports from Aberdeen and London which confirmed the epidemiological link between particular strains of *E. coli* and diarrhoea.[1]

A major breakthrough in this area stemmed from the work of Kaufmann and colleagues, who established a serotyping scheme for *E. coli* based on the bacterium's somatic (O) and flagellar (H) antigens (Fig. 2). This important work made it possible to compare the relatedness of *E. coli* isolates from different outbreaks and showed that diarrhoea-associated isolates of *E. coli* from Germany, England and Scotland that had been reported previously, belonged to a limited number of O-serogroups, in particular O111 and O55.[3] These early reports were followed by a series of studies, mainly in the USA and Japan, which showed that strains of *E. coli* that had been associated with outbreaks of severe diarrhoea in children caused diarrhoea when ingested by adult volunteers.[1] In 1955, Neter *et al*. coined the term 'enteropathogenic' *E. coli* (EPEC) to indicate those strains of *E. coli* that had been associated with outbreaks of childhood diarrhoea.[4] Some years later, Neter summarised the evidence favouring the pathogenicity of EPEC as follows: (*i*) during institutional outbreaks, a single O-serogroup has been found in the majority of affected infants,
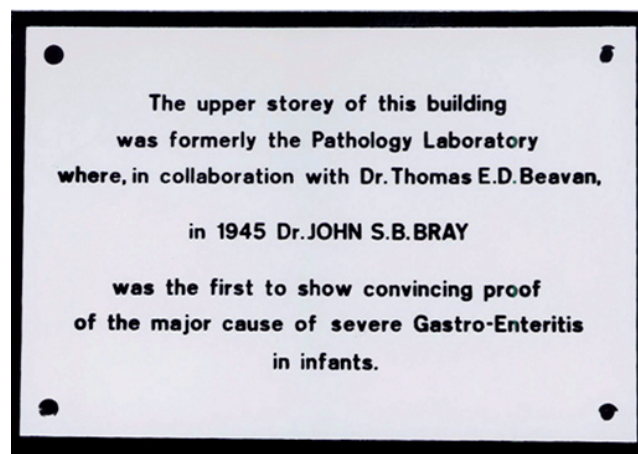
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Fig. 1. Plaque at the Hillingdon Hospital commemorating Bray's discovery of enteropathogenic E. coli.
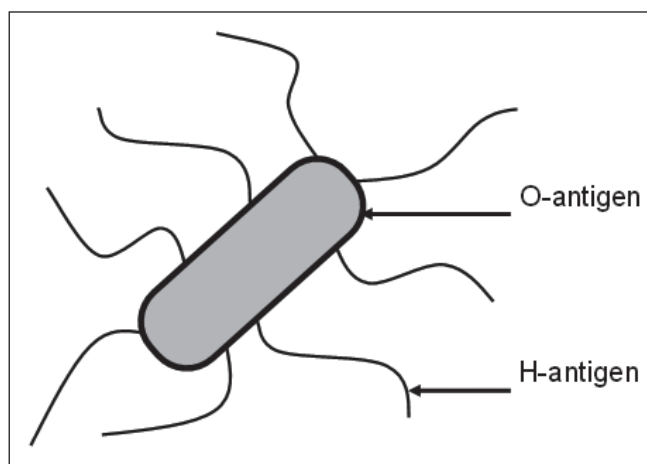
Fig. 2. Schematic diagram of an E. coli *cell showing the location of the somatic, lipopolysaccharide (O) antigen, and the flagellar, protein (H) antigen. Individual* E. coli *strains can be divided into serogroups according to their O-antigens and into serotypes according to the combination of their O- and H-antigens.*[1]

and the serogroup involved has usually been present as the predominant aerobic bacterium cultured from the stools of sick infants; (*ii*) EPEC strains have been recovered far less frequently from the stools of healthy infants, older children and adults than from those of infants with diarrhoea; (*iii*) volunteers develop diarrhoea when fed strains identified by serogroup alone; (*iv*) serogroup-specific antibodies may be detected in the serum of a proportion of affected patients and volunteers; (*v*) antibiotics that eliminate EPEC from the stool bring relief from illness in affected infants and prevent the development of illness in susceptible infants; and (*vi*) extensive bacteriological and limited virological investigations of outbreaks of *E. coli* diarrhoea have failed to show a strong association with any other microbial agent.[5]

Emergence of other varieties of diarrhoeagenic *E. coli*

In the years that followed Neter's review, interest in EPEC and infantile diarrhoea in general appeared to wane, probably because of a reduction in the number and impact of community and nursery outbreaks of life-threatening diarrhoea that had been a prominent feature of paediatric practice in industrialised countries during the 1930s, 1940s and early 1950s.[1] The role of *E. coli* as an enteropathogen became firmly established, however, by the discovery that some *E. coli* strains secreted a novel heat-stable enterotoxin and/or a heat-labile enterotoxin resembling cholera toxin, which accounted for their virulence. This variety of *E. coli* came to be known as enterotoxigenic *E. coli* (ETEC) and was shown to cause watery diarrhoea in volunteers.[6] Also, around this time, yet another pathogenic variety of *E. coli* was identified. This type of *E. coli* is referred to as enteroinvasive *E. coli* (EIEC) and causes dysentery that

is indistinguishable from that due to *Shigella* species, with which it shares a large number of virulence determinants. Also, during the early 1970s, Ruth Bishop, Ian Holmes and colleagues at Melbourne's Royal Children's Hospital and the University of Melbourne identified rotavirus, which was the first virus demonstrated to cause infantile gastroenteritis.[7]

At this time, I was a registrar in the School of Pathology studying microbiology under the tutelage of Hendrik Koornhof. Hendrik thought that we should look for these newly described varieties of *E. coli* and rotavirus, as well as traditional pathogens, in children with acute gastroenteritis in Soweto, where infantile diarrhoea was still a major health concern. In collaboration with Barry Schoub, we established the extremely laborious assays used at the time to identify rotaviruses, ETEC and EIEC.[8] We then applied these assays to an examination of faecal samples obtained from patients attending Baragwanath Hospital for management of acute gastroenteritis. To our surprise, we found that the most frequent pathogen identified in the stools of children with gastroenteritis was not ETEC or EIEC, but EPEC.[9] These findings were subsequently confirmed in a second study.[10]

At this time, no virulence factors of EPEC were known, and they had to be identified by determination of O-serogroup and their failure to produce known enterotoxins and to exhibit *Shigella*-like invasiveness. Because EPEC as a group lacks these virulence determinants, some authorities expressed doubts as to their pathogenicity.[11] These views were supported by studies which showed that even those EPEC strains which had been associated with outbreaks of diarrhoea and had caused diarrhoea in volunteers lacked known virulence determinants, causing some investigators to suggest that these strains were in fact ETEC or EIEC which had lost the capacity to produce enterotoxins or invade epithelial cells during storage.[1] Even an elegantly designed, volunteer study reported by Levine *et al*.[12] around this time, which confirmed the pathogenicity of outbreak-associated strains of EPEC that had been identified by O-serogroup alone and which lacked the virulence determinants of ETEC and EIEC, failed to convince many of the sceptics.[13]

Identification of EPEC-specific virulence determinants

The observation that EPEC must colonise the small intestine in order to cause diarrhoea[14] suggested that adhesive factors may contribute to the virulence of these bacteria. The suggestion was supported by studies which showed that EPEC adhere to tissue culture cells *in vitro* to a greater extent than other varieties of *E. coli*.[15] In addition, most EPEC strains in O-serogroups, which had been classified as containing EPEC because of their association with epidemics of diarrhoea, adhered to epithelial cells in a distinctive pattern termed

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localised adherence (Fig. 3A).[16] Investigations into the mechanisms of this type of adherence revealed that adhesion is mediated by a distinctive fimbrial adhesin, termed bundle-forming pili (Bfp; Fig. 3B),[17] which is encoded by plasmids known as the EPEC adhesive factor (EAF).[18] EPEC employ Bfp to bind to epithelial cells and to each other, thus accounting for the localised adherence pattern of EPEC strains which produce these pili. Studies in volunteers have demonstrated that the EAF plasmid and Bfp themselves are essential virulence determinants of EPEC, and that bacteria which lack either of these are markedly attenuated.[19,20]

Studies of the pathology of EPEC infection in animal models and naturally infected children have shown that EPEC adhere to the intestinal epithelium in a distinctive manner that is characterised by bacteria intimately attached to the plasma membrane of enterocytes where the microvilli are absent (or effaced).[21,22] In addition, at the sites where bacteria attach there is evidence of cytoskeletal rearrangements resulting in changes to the morphology of the cell surface, giving rise to formations termed cups and pedestals (Fig. 4). Collectively, these changes are referred to as attaching-effacing lesions.[21] Interestingly, EPEC strains which lack Bfp or the entire EAF plasmid retain the ability to induce the formation of these lesions indicating that the EAF is not required for attaching-effacing capacity.[23] A series of elegant experiments, from Jim Kaper's group, showed that the factors required for attaching-effacing capacity are encoded by genes co-located within a pathogenicity island, known as the locus for enterocyte effacement (LEE), which is completely absent from *E. coli* strains that are unable to produce attaching-effacing lesions, including ETEC and EIEC.[24]

The products of the approximately 40 genes which comprise the LEE act in concert to secrete proteins directly from the bacterial cytoplasm through a narrow protein channel, resembling a microsyringe, through the plasma membrane of the host cell into its cytoplasm (Fig. 5).[25] One of the proteins secreted in this way is called the translocated intimin receptor or Tir, because it acts as a receptor for a surface adhesive protein of EPEC, known as intimin. Once Tir has bound to intimin it acts as an anchor for host cytoskeletal proteins, thus completing the formation of the attaching-effacing lesions.[26] A series of volunteer studies and animal experiments using deletion mutants of human EPEC and analogous animal pathogens (such as rabbit-specific EPEC and *Citrobacter rodentium*, an attaching-effacing pathogen of mice) have shown that intimin, Tir and almost all other LEE-encoded proteins are required for virulence.[27-30]

Implications and applications of research findings

Recent advances in our understanding of EPEC biology and virulence have led to EPEC being re-defined. The revised definition is based primarily on the presence of the LEE
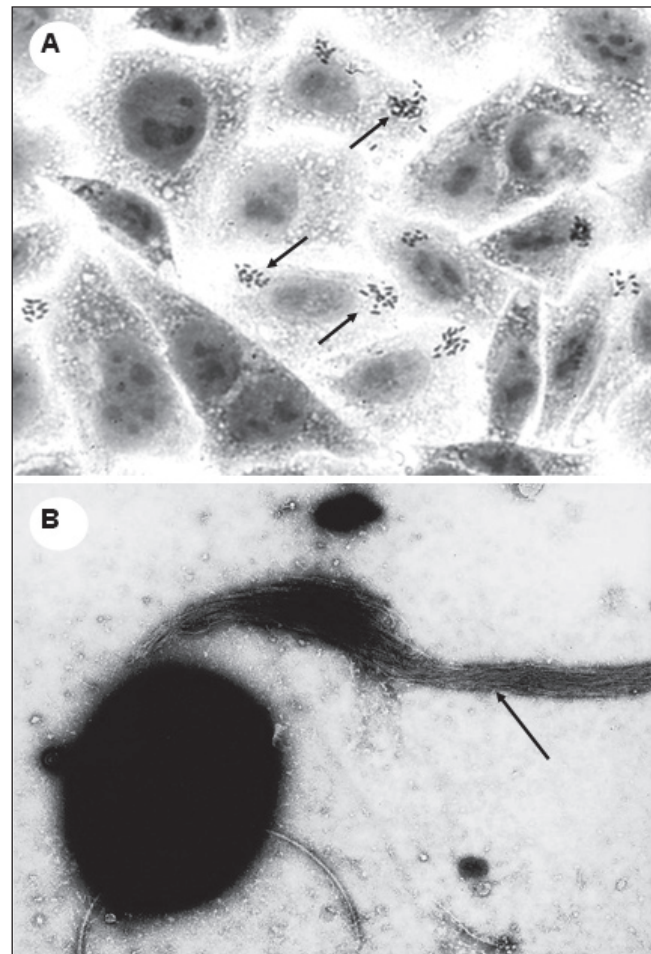

Fig. 3. A: Light micrograph showing E. coli *attached to HeLa cells in a localised pattern (arrows) (Giemsa stain). B: Electron micrograph showing bundle-forming pili aggregated together into rope-like bundles (arrow).*
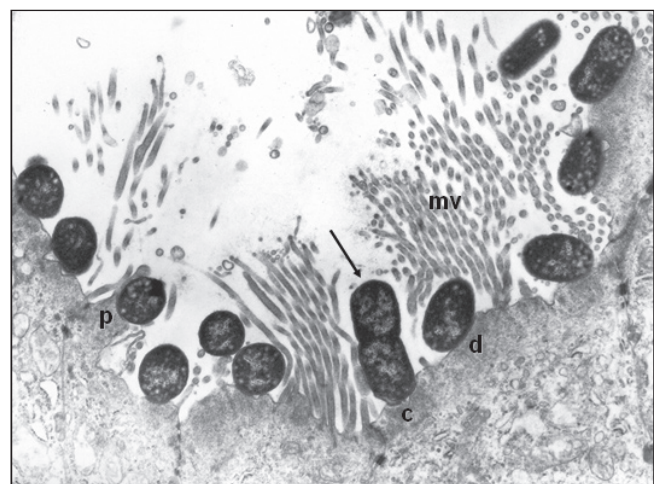

Fig. 4. Transmission electron micrograph of a section through the ileum of a rabbit 16 hours after inoculation with a strain of rabbit-specific enteropathogenic E. coli. *Note the attaching-effacing pathology comprising closely attached bacteria (arrow) accompanied by alterations to the enterocyte cytoskeleton, evidenced by pedestal formation (p), cupping (c), accumulation of electron-dense material (d), and the absence of microvilli (mv) at sites of bacterial attachment.*
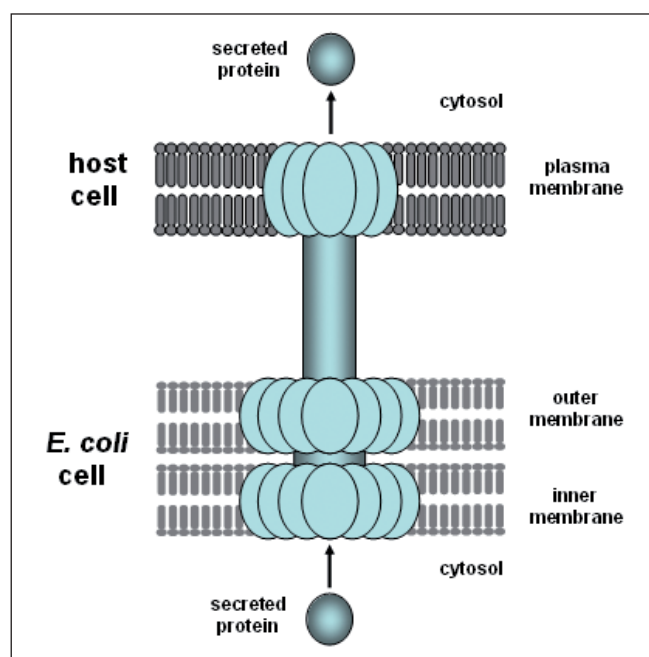
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Fig. 5. Schematic representation of the process employed by enteropathogenic E. coli *to transfer proteins from its cytoplasm directly into the cytoplasm of host cells. The secreted proteins and the structures used to translocate them from the bacteria to the host are encoded by genes within the chromosomal LEE pathogenicity island.*

pathogenicity island, which is a marker of attaching-effacing capacity. Only one other pathogenic variety of *E. coli* may carry LEE. This is enterohaemorrhagic *E. coli* or EHEC, which was first reported from the USA in 1982 and has subsequently been identified in a large number of countries worldwide.[31] Apart from diarrhoea, EHEC also cause haemorrhagic *coli*tis and the haemolytic uraemic syndrome.[31] In the laboratory, EHEC can be distinguished from EPEC by its ability to secrete one or more Shiga toxins (also known as Shiga-like toxins and Verotoxins).[32] Given the recent emergence of EHEC and its close genetic relatedness to EPEC it seems likely that EHEC evolved from EPEC in part by acquiring bacteriophages which carry the structural genes for the production of Shiga toxins.[33]

The re-definition of EPEC means that we no longer have to rely on O-serogrouping to identify these bacteria, although serotyping remains a useful epidemiological tool to track outbreaks of infection. Currently, identification of EPEC relies upon the demonstration of attaching-effacing capacity using tissue culture cells,[34] or more commonly by detection of the *eae* gene, which encodes intimin and is a marker of the presence of the LEE. Distinguishing EPEC from EHEC, which can also carry intimin, requires a demonstration of the absence of Shiga toxin production and/or the genes which encode these toxins. Alternatively, one can look for the presence of bundle forming pili or their genes. We and others now routinely use these assays, usually in the form of multiplex polymerase chain reactions, to identify EPEC-specific genes and those of other

varieties of diarrhoeagenic *E. coli* in patients with diarrhoea.[35,36] We have also used these assays to determine if *E. coli* strains which were isolated during the 1940s and 1950s that were labelled 'enteropathogenic' were truly EPEC, as defined currently, and showed that most were.[37]

One drawback of using Bfp as an identifying characteristic of EPEC is that there is a subtype of EPEC, known as atypical EPEC, which is naturally deficient in Bfp.[38] Although the role of atypical EPEC as a cause of diarrhoea is controversial, evidence is accumulating from widespread geographical regions that these bacteria are indeed pathogens which are particularly associated with the development of post-infectious persistent diarrhoea, i.e. diarrhoea lasting more than 14 days.[35,39]

Summary and conclusions

The suggestion that *E. coli*, a normal inhabitant of the intestinal tract of healthy humans, could also cause diarrhoea took several decades to gain credence. At the time, these varieties of diarrhoea-associated *E. coli* were designated 'enteropathogenic' to distinguish them from *E. coli* strains that were responsible for urinary tract infection, wound infection, meningitis, septicaemia, etc. Initially, EPEC were identified by serotyping. The emergence of ETEC and EIEC, which had readily identifiable virulence determinants at a time when EPEC did not, brought the pathogenicity of EPEC into question. Nevertheless, epidemiological studies including some in South Africa performed under the guidance of Hendrik Koornhof, suggested that EPEC identified by serotyping alone was significantly associated with diarrhoea.

Today, a considerable amount is known about the molecular pathogenesis of EPEC infections, although the mechanism by which EPEC cause diarrhoea is still unclear. EPEC are now recognised as a major cause of acute and persistent diarrhoea in children in less developed countries and a significant contributor to premature childhood mortality. EPEC are also the likely progenitor of EHEC, an important food-borne pathogen in many industrialised countries worldwide. The recent identification of a new subtype of EPEC, termed atypical EPEC, as a cause of persistent diarrhoea in children, has added another chapter to the EPEC story. Bioinformatic analysis of the genome of different *E. coli* strains points to their genetic plasticity and propensity to transfer genetic material between individual strains and other infectious agents, suggesting that new pathogenic varieties of *E. coli* will continue to arise in future.[40]

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