Escherichia coli that Cause Diarrhea: Enterotoxigenic, Enteropathogenic, Enteroinvasive, Enterohemorrhagic, and Enteroadherent

Myron M. Levine

There are four major categories of diarrheagenic *Escherichia coli*: enterotoxigenic (a major cause of travelers' diarrhea and infant diarrhea in less-developed countries), enteroinvasive (a cause of dysentery), enteropathogenic (an important cause of infant diarrhea), and enterohemorrhagic (a cause of hemorrhagic colitis and hemolytic uremic syndrome). Besides manifesting distinct clinical patterns, these categories of *E. coli* differ in their epidemiology and pathogenesis and in their O:H serotypes. Common features (albeit distinct for each category) include plasmid-encoded virulence properties, characteristic interactions with intestinal mucosa, and elaboration of various types of enterotoxins or cytotoxins. A less-well-defined fifth category of diarrheagenic *E. coli* is that of enteroadherent *E. coli*, so far identifiable only by their pattern of adherence to Hep-2 cells in tissue culture.

As the predominant species among the facultative anaerobic normal flora of the intestine, *Escherichia coli* plays an important role in maintaining intestinal physiology [1]. Within this species, however, there are fully pathogenic strains that cause distinct syndromes of diarrheal disease. To the uninitiated, the plethora of terms and acronyms used to describe the diarrheagenic *E. coli* (table 1) represent a potential source of confusion; it is often difficult to discern which terms are redundant and which represent distinct entities. In this review, I shall attempt to clarify the situation and explain the four main categories of diarrheagenic *E. coli*; the categories are based on distinct virulence properties, different interactions with the intestinal mucosa, distinct clinical syndromes, differences in epidemiology, and distinct O:H serotypes (figure 1). The four major categories of diarrheagenic *E. coli* are (1) enterotoxigenic *E. coli* (ETEC), (2) enteroinvasive *E. coli* (EIEC), (3) enteropathogenic *E. coli* (EPEC), and (4) enterohemorrhagic *E. coli* (EHEC).

The EPEC category can be conveniently subdivided into two classes that, on a rational genetic basis, include strains referred to by some authors as attaching and effacing *E. coli* (AEEC) [2]. Class I EPEC exhibit localized adherence to Hep-2 cells, whereas class II EPEC exhibit either diffuse adherence or no adherence at all to Hep-2 cells. There is also a fifth category of diarrheagenic *E. coli*, enteroadherent *E. coli* (EAEC) [3, 4]. Although little is known about the pathogenesis, epidemiology, and serotypes of the strains in this category of *E. coli*, preliminary evidence suggests that they are indeed capable of causing diarrheal disease, and they clearly do not fit into the other four categories; therefore, they have been relegated to a fifth category, at least until further information becomes available.

Although the four main categories of diarrheagenic *E. coli* are quite distinct, they nevertheless have certain underlying commonalities with respect to pathogenesis. (1) Critical virulence properties are encoded in plasmids. (2) There is a characteristic interaction with intestinal mucosa. (3) Enterotoxins or cytotoxins are produced. (4) Within each category, the strains tend to fall within certain O:H serotypes.

In the 1940s, Kauffmann [5] proposed a scheme to differentiate *E. coli* on the basis of lipopolysaccharide O, flagellar H, and polysaccharide K antigens. We presently recognize 171 O and 56 H serogroups. Together, these constitute the O:H system, which has played an important role in studies of the epidemiology and pathogenesis of *E. coli* infection.

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Table 1. Plethora of descriptive terms for _E. coli_ associated with diarrhea.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Acronym</th>
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<tr>
<td>Enterotoxigenic <em>E. coli</em></td>
<td>ETEC</td>
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<tr>
<td>Enteroinvasive <em>E. coli</em></td>
<td>EIEC</td>
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<tr>
<td>Enteropathogenic <em>E. coli</em></td>
<td>EPEC</td>
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<tr>
<td>Classical serotype EPEC</td>
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<td>Traditional serotype EPEC</td>
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<td>Adherent enteropathogenic <em>E. coli</em></td>
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<td>Cytotoxin enteropathogenic <em>E. coli</em></td>
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<tr>
<td>Enterocyte-adherent enteropathogenic <em>E. coli</em></td>
<td>EAEPEC</td>
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<tr>
<td>Facultative enteropathogenic <em>E. coli</em></td>
<td>FEEC</td>
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<tr>
<td>Attaching and effacing <em>E. coli</em></td>
<td>AEEC</td>
</tr>
<tr>
<td>Enterohaemorrhagic <em>E. coli</em></td>
<td>EHEC</td>
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<tr>
<td>Verotoxin-producing <em>E. coli</em></td>
<td>VTEC</td>
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Recognition that _E. coli_ Cause Diarrhea

In the early 1940s, summer diarrhea was an important clinical problem in infants in Europe and North America. In England at this time, a microbiologist — John Bray — and a pediatrician — John Beavan — decided to study the problem [6, 7]. They were aware that the routine stool cultures in use at that time detected _Shigella_ and _Salmonella_, but failed to identify pathogens in most infants. These investigators hypothesized that some strains of _E. coli_ that appeared to be strains of normal flora by colonial morphology on agar might actually be pathogenic. To pursue this hypothesis, they used an immunizing strain of _E. coli_ isolated from an infant named Wickens who had summer diarrhea — with no other pathogens — to prepare an antiserum that might identify the homologous and related strains of _E. coli_. In an example of productive research despite meager resources, they immunized a pet rabbit named Snowy to obtain the typing serum [8]. This study was carried out before the Kauffmann O:H serotyping system for _E. coli_ was described.

Bray and Beavan examined cultures of stools from infants with summer diarrhea and from healthy control infants for strains of _E. coli_ that could be agglutinated by the rabbit antiserum. Agglutinating _E. coli_ were detected in cultures from 92% of 90 diarrheal infants but in only 6% of 180 controls [6, 7]. They referred to this strain as _Bacillus coli_ var. _neapolitanum_; it is now known to belong to serogroup O111. After these results were published, investigators in other countries pursued studies by using the same general approach [9–20]. As a consequence, by the mid-1950s, a series of _E. coli_ strains had been epidemiologically incriminated throughout the world as important causes of infant diarrhea [21–23]; the term _enteropathogenic E. coli_ (EPEC) was proposed by Neter [24] to refer to these serotypes. The major O serogroups considered to contain EPEC serotypes are shown in figure 1; except for O142, this list represents a compilation of reports from Ewing et al. [25] and Taylor [26], who headed the enteric reference laboratories at the Centers for Disease Control (Atlanta) and the Central Public Health Laboratory (Colindale, England), respectively, in the 1950s. O142 was identified as a major EPEC serotype in the late 1960s and early 1970s [27–30]. Only certain H types are incriminated within each O serogroup [25, 26]. I will henceforth refer to these O serogroups as “classical” EPEC O serogroups. Certain other O serogroups were recognized by both Ewing et al. [25] and Taylor [26] as having some incrimination as pathogens, but not to the same extent as the major classical O serogroups. These other O serogroups include O18, O44, O112, and O114, among others (figure 2).

Studies conducted with volunteers during the early 1950s clearly established the pathogenicity of strains from several of the most common classical EPEC O serogroups, including O55, O111 and O127 [31–35]. Nevertheless, although both epidemiological evidence and studies with volunteers established the classical serotype EPEC as causes of diarrhea, it was not possible to distinguish between these _E. coli_ and strains of normal flora by the tests and animal models available up to the mid 1960s, and the virulence properties by which they caused diarrhea remained unknown. Therefore, O serogrouping remained the only diagnostic tool for EPEC and was common until the early 1970s. Unfortunately, only

1. ENTEROTOXIGENIC

2. ENTEROTOXIGINAVSTE

3. ENTEROPATHOGENIC:

<table>
<thead>
<tr>
<th>CLASS</th>
<th>H type or ADRH</th>
<th>ADH</th>
<th>FAP</th>
<th>PROBE</th>
<th>SEROTYPES</th>
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<tr>
<td>I</td>
<td>Localized</td>
<td>+</td>
<td></td>
<td></td>
<td>Classical</td>
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<tr>
<td>II</td>
<td>Diffuse</td>
<td>-</td>
<td></td>
<td></td>
<td>Classical</td>
</tr>
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</table>

4. ENTEROHEMORRHAGIC

5. ENTEROADERENT

Figure 1. The distinct categories of diarrheagenic _E. coli_.
Diarrheogenic E. coli

Classical Enteropathogenic E. coli

Most Important (Class 1; usually EPEC)
(026)*, O55, O86, O111, O119, O129, O126, O127, O128abc, O142

Less Important (Class II; rarely EPEC)
O18, O44, O112, O114

Enterotoxigenic E. coli

O6, O8, O15, O20, O25, O27, O63, O78, O80, O95
O115, O128ac, O139, O148, O153, O159, O167

Enteroinvasive E. coli

O2hac, O29, O124, O136, O143, O144,
O152, O164, O167

Enterohemorrhagic E. coli

O157, O26, O111

Enteroadherent E. coli

O serogroups not yet defined

Figure 2. O serogroups associated with the major categories of diarrheagenic E. coli. * Serogroup O26 was originally categorized as an EPEC, but is now considered to be an EHEC. EAEC is a provisional, less-well-defined category of diarrheagenic E. coli.

ETEC

The second major class of E. coli to be associated with diarrhea are ETEC, which came to prominence in the late 1960s and early 1970s, largely on the basis of work carried out in Calcutta by Gorbach, Sack, and co-workers [36, 37]. ETEC are a major cause of infant diarrhea in less-developed countries (some reports of infants prospectively followed-up by frequent household surveillance suggest that as many as two to three clinical ETEC infections per child per year occur during the first two to three years of life) [38], one of the main bacterial causes of dehydrating infant diarrhea in developing areas [39], and an infection correlated with adverse nutritional consequences [40]. ETEC are also the agent most frequently responsible for travelers' diarrhea [41-43]. ETEC diarrhea within the United States and other developed countries is rare, although occasional outbreaks have been reported [44, 45].

ETEC infection is acquired by ingesting contaminated food or water. The bacteria colonize the proximal small intestine, the critical site of host-parasite interactions, where they elaborate heat-labile enterotoxin (LT) or heat-stable enterotoxin (ST). The reader is referred elsewhere for reviews of the properties of LT and ST [46]. The clinical features of ETEC infection are watery diarrhea, nausea, abdominal cramps, and low-grade fever.

When collections of ETEC from diverse geographic areas were serotyped, a limited number of O:H serotypes occurred repeatedly throughout the world and accounted for a majority of the ETEC strains [47-55]. Although many other serotypes can also be toxigenic, the recurrent O:H serotypes appear to be successful ETEC clones that have spread far and wide. Usually, these serotypes elaborate both LT and ST and possess fimbrial colonization factors [52-55]. The major O serogroups associated with ETEC are shown in figure 2.

Adherence of ETEC. ETEC possess attachment or colonization factors that allow them to overcome the peristaltic defense mechanism of the small intestine. Heretofore, all the characterized colonization factors have been fimbriae—hair-like, filamentous organelles (notably thinner than flagellae) on the surface of the E. coli. The fimbrial colonization factors of human ETEC have certain features in common [46]. (1) They consist of either rigid structures 6-7 nm in diameter or wiry, flexible structures 2-3 nm in diameter. (2) They are encoded by plasmids that almost always also encode ST and often LT as well. (3) They consist of protein subunits 14-22 kilodaltons (kDa) in size. (4) In the presence of mannose, many, but not all, fimbrial colonization factors mediate hemagglutination of certain erythrocytes (a characteristic differentiating them from type 1 fimbriae). (5) Fimbrial colonization factors are expressed in cultures grown at 37 C, but not at 18 C. (6) Particular colonization fimbriae are largely restricted to certain O:H serotypes.

There has been keen interest in the variety of fimbrial colonization factors present in human ETEC, because this information is directly relevant to the polyvalency of an anti-colonization factor vaccine against ETEC and to the breadth of protection that might be expected from such a vaccine [46].

The first fimbrial colonization factor in a human ETEC strain, described by Evans et al. [56, 57] in the mid 1970s and referred to as CFA/I, was found in ETEC of serotypes O15, O25, O63, O78, and O128 (table 2). Evans et al. reported that the presence of CFA/I could be suspected on the basis of a particular hemagglutination pattern and confirmed by using antiserum to CFA/I. Several other laboratories substantiated these findings.

Evans et al. [58] reported a second, antigenically
Table 2. Relation of O serogroup to colonization factor fimbriae in enterotoxigenic E. coli.

<table>
<thead>
<tr>
<th>Fimbrial antigen</th>
<th>O serogroup(s)</th>
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<tbody>
<tr>
<td>CFA/I</td>
<td>15, 25, 63, 78, 128, 153</td>
</tr>
<tr>
<td>CFA/II</td>
<td></td>
</tr>
<tr>
<td>CS1</td>
<td>6, 139</td>
</tr>
<tr>
<td>CS2</td>
<td>6</td>
</tr>
<tr>
<td>CS3</td>
<td>6, 8, 80, 85, 139</td>
</tr>
<tr>
<td>E8775</td>
<td></td>
</tr>
<tr>
<td>CS4</td>
<td>25</td>
</tr>
<tr>
<td>CS5</td>
<td>92, 115</td>
</tr>
<tr>
<td>CS6</td>
<td>25, 27, 92, 115, 148, 169</td>
</tr>
<tr>
<td>PCF0159</td>
<td>159</td>
</tr>
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</table>

distinct fimbrial colonization factor, referred to as CFA/II, that was associated with serogroups O6, O8, O80, and O85; CFA/II manifested a different hemagglutination pattern than did CFA/I. As other investigators followed the lead of Evans, it became clear that the situation with CFA/II was much more complicated than that with CFA/I. The initial breakthrough in clarifying this situation was made, essentially simultaneously, by Cravioto et al. [59] and Smyth [60]. Cravioto et al. showed that strains tentatively identified as CFA/II on the basis of hemagglutination patterns were in fact elaborate combinations of three distinct antigens, which they called components 1, 2, and 3. Smyth made identical observations and referred to the antigens as CS1, CS2, and CS3 (table 2). CS3 was present in virtually all the strains. However, depending on the serotype and biotype, they observed strains expressing CS1 and CS3, CS2 and CS3, or CS3 only. Strains simultaneously expressing both CS1 and CS2 were never found. Smyth [60] reported the subunit sizes of the antigens as 16.3 (CS1), 15.3 (CS2), and 14.7 kDa (CS1), and Smith et al. [61] reported that a single plasmid encodes the genes for CS1, CS2, and CS3 and that the determination of which fimbrial antigens are expressed is a function of the E. coli host bacteria and is related to serotype and biotype.

Both Mullaney et al. [62] and Smyth [63] described CS1 and CS2 as typical 6–7-nm diameter, rigid fimbriae that resembled CFA/I in morphology. However, they reported that CS3, the antigen common to virtually all CFA/II strains, was not fimbrial; instead, CS3 was described as amorphous and without detectable structure upon examination by electron microscopy.

Working with strains expressing both CS1 and CS3, as well as with strains expressing only CS3 in vitro, Levine et al. [64] and Knutton et al. [65] identified thin, wiry, flexible structures 2–3 nm in diameter that appeared in electron photomicrographs; these structures were shown to be CS3. The identification of these flexible fibrillae as CS3 was confirmed by purifying the antigen, by showing the specificity of its reactivity with antibody to CS3 by immunodiffusion and immunoblotting techniques, and by immunolabeling the structures with colloidal gold and visualizing them under the electron microscope [64].

These observations showed for the first time that, in addition to rigid 6–7-nm fimbriae such as CFA/I, human ETEC pathogens have flexible, 2–3-nm fibrillar-type fimbriae that resemble K-88 and F41 antigens of porcine strains [66, 67].

Clarifying the CFA/II family of antigens provided important basic information but did not expand the number of O serogroups that could be correlated with the presence of known colonization fimbriae. However, at approximately the same time, Thomas et al. [68] described a new putative colonization factor fimbria in prototype strain E8775. This new colonization factor was found in ETEC of serogroup O25, and for the first time in O115 and O167. More recently, these authors have shown that E8775, like CFA/II, consists of a family of three distinct antigens [69]. Two of these, CS4 and CS5, are rigid 6–7-nm fimbriae, whereas CS6, like CS3, is not. Furthermore, they have identified CS4, CS5, and CS6 in several of the ETEC O serogroups for which there previously had been no recognized colonization antigen, including the very common serogroups O27 and O148 (table 2).

The last of the important ETEC O serogroups not associated with a known colonization factor fimbria was O159 (table 2). Tacket et al. [70] have solved this problem. In contrast with other ETEC strains associated with colonization factor fimbriae, O159:H4 strains do not manifest mannose-resistant hemagglutination. Nevertheless, it was found that SDS-PAGE of disaggregated sheared preparations of an O159:H4 strain grown at 37 C revealed a distinct 19-kDa subunit band that was not visible in sheared preparations from cultures grown at 18 C [70]. This band apparently corresponded to a nonhemagglutinating fimbria. Tacket et al. [70] visualized this fimbria, purified it, showed that it is encoded on a 27-megadalton (MDa) plasmid that also encodes LT and ST, demonstrated that it is antigenically distinct from other colonization factor fimbriae, and showed
that it is present on O159:H4 strains from diverse parts of the world.

Several other putative colonization factors have been reported [71–73], some of which may share antigenic identity with the previously described factors. It is not known if the fimbria described by Honda et al. [72] is encoded on plasmids that also encode LT or ST (which would add credibility to its being a colonization factor), nor with what serotypes it is associated. The identity of the antigens described by Denke et al. [71] and whether they cross-react with CFA/I, CSI–CS6, or PCFO159 is not clear. A recent antigen described by Darfeuille et al. [73] is probably CS6, as evidenced by the serotype of the prototype strain, the size of the subunit, and the fact that it is not a rigid fimbrial structure.

Thus, colonization factor antigens have now been identified in all the main O serogroups associated with ETEC. An obvious related question is what proportion of human ETEC pathogens possess known colonization factor fimbriae. There has been debate on this point. Two recent studies from Asia suggest that the proportion is quite high [74, 75], particularly if fresh isolates are examined. Hereafter, no study has tested strains for all the fimbrial antigens described in this review. At least one such study will be initiated shortly in South America.

Considerable work is underway on the development of candidate oral vaccines to prevent ETEC diarrhea. Some of the suggested vaccines include purified CFA fimbriae, LT/ST toxoid, killed fimbriate E. coli, or attenuated strains of E. coli (A–B*, fimbriated, ST toxoid) or Salmonella typhi (modified Ty21a or 541Ty). Evans et al. [76], and Levine et al. [77, 78], previously worked with purified fimbriae; this approach has been largely abandoned. Kliopstein et al. [79] have recently reported the oral administration of a synthetic LT/ST toxoid to volunteers; it was well-tolerated and stimulated serum and intestinal antibodies to LT and ST. Killed, fimbriated E. coli are also being explored as an oral vaccine. Lastly, live oral vaccines are being developed. Clements and El-Morshidy [80] and Yamamoto et al. [81] have described the use of attenuated Salmonella typhi strain Ty21a as a carrier bacteria to express introduced genes encoding putative protective antigens of ETEC. Levine et al. [78, 79] have explored the use of nontoxigenic E. coli expressing fimbrial colonization factor antigens as live oral vaccines.

A group of volunteers were given a single oral dose of 10^9 organisms of strain El392/75–2A; this strain is serotype O6:H16 and bears CSI and CS3 fimbriae, but lacks the genes encoding LT and ST [78, 79]. Ten recipients of the vaccine, from whom paired prevaccination and postvaccination samples of intestinal fluids were obtained, showed significant rises in titer of antibody to fimbriae (prevaccination geometric mean titer, 5; postvaccination geometric mean titer, 416); the secretory IgA response was much greater than that observed in persons who received three evenly spaced enteral doses of purified fimbriae. The vaccinees were challenged one month later, along with a group of unimmunized control volunteers, with a toxigenic strain of a different O:H serotype bearing CSI and CS3 fimbriae. All controls but only three of 12 vaccinees developed diarrhea, a highly significant difference; furthermore, illness in the three vaccinees was milder. Bacteriologic studies showed that the mechanism of protection was prevention of colonization of the proximal small intestine. Duodenal cultures were positive in five of six controls (mean, 7,000 organisms/ml) versus only one of 12 vaccinees (mean, 10 organisms/ml; \( P < .004 \)).

**EIEC**

In 1971, DuPont et al. [82] described certain E. coli strains that caused an invasive, dysenteric form of diarrheal illness in volunteers. These strains, of serotypes distinct from ETEC and EPEC (figure 2), closely resembled Shigella in many ways. Like Shigella, their cardinal pathogenetic feature is the capacity to invade and proliferate within epithelial cells and cause eventual death of the cell [82]. The invasive capacity of both EIEC and Shigella is dependent on the presence of large (∼140 MDa) plasmids [83] coding for the production of several outer membrane proteins involved in invasiveness [84]; the proteins are antigenically closely related (if not identical) in EIEC and in Shigella. EIEC often resemble Shigella in being nonmotile and unable to ferment lactose. Furthermore, EIEC and Shigella O antigens show many cross-reactions.

EIEC have a predilection for colonic mucosa as the favored site of host-parasite interaction. Clinically, the illness is marked by fever, severe abdominal cramps, malaise, toxemia, and watery diarrhea followed by gross dysentery consisting of scanty stools of blood and mucus. A simple stain of the fecal mucus reveals sheets of PMNLs.

EIEC can be diagnosed by serotyping suspect E. coli strains [85], by an ELISA that detects the
outer membrane proteins associated with invasiveness [86], and by DNA probes that detect the genes for invasiveness [87].

**EPEC**

By the mid 1970s, the properties of LT, ST, and *Shigella*-like invasiveness in *E. coli* strains from certain patients with diarrhea (e.g., travelers) was well-established, as was the fact that these strains were only rarely of the classical EPEC O:H serotypes that had been epidemiologically incriminated in the 1950s as causes of infant diarrhea [88–94]. Conversely, several investigators examined stored EPEC strains from several outbreaks of infant diarrhea and recent isolates from patients with infant diarrhea for the presence of LT, ST, and epithelial cell invasiveness [90, 91, 93]; with rare exceptions, the strains lacked these properties. This precipitated a lively controversy. Some investigators concluded that EPEC were not pathogens because they did not manifest the known ETEC or EIEC properties of virulence, and they argued that in these strains the critical toxin or invasiveness plasmids were unstable and had been lost during subculture and storage [90–92, 95]. On the basis of epidemiological evidence, other investigators concluded that the classical EPEC strains were obviously pathogens, but caused diarrhea by mechanisms distinct from LT, ST, and *Shigella*-like invasiveness [89].

At the Center for Vaccine Development (University of Maryland School of Medicine, Baltimore), an attempt was made in 1977 to resolve this controversy by studying volunteers challenged with several EPEC strains lacking LT, ST, and invasiveness; these strains had been stored for up to seven years after isolation during outbreaks of infant diarrhea [97]. When these strains were fed to healthy young adult volunteers, they caused definite diarrhea (one individual developed severe, cholera-like illness and purged 5.6 liters of watery diarrhea). Therefore, EPEC were indeed pathogens, but they caused diarrhea by mechanisms unknown at that time. This study also stimulated renewed research on the pathogenesis of EPEC diarrhea and rapidly yielded results.

Polotsky et al. [98] noted that EPEC strains cause a distinctive ultrastructural histopathologic lesion in human intestines (visible by electron microscopy) that is not seen with ETEC strains. The distinctive lesion involves destruction of the microvilli by the EPEC bacteria, typically without further evidence of invasion. The bacteria are often closely adherent to the membrane of the enterocyte, with the membrane often cupping or partially enveloping the bacterium [99]. This same ultrastructural lesion was later described in biopsies from infants with classical serogroup O125 and O119 EPEC infections by Ulshen and Rollo [100] and Rothbaum [101]. This lesion has been studied in gnotobiotic piglets by Moon et al. [2] and Tzipori et al. [102].

In another hallmark study, Cravioto et al. [103] observed that approximately 80% of the EPEC strains in the Colindale Laboratory collection adhered to Hep-2 cells in tissue culture, a property rare in ETEC, EIEC, or normal flora strains. Shortly thereafter, Baldini et al. [104] reported that 30 of 31 classical serotype EPEC strains from patients with diarrhea possess a plasmid ~60 MDa in size that encodes the property of adhesiveness to Hep-2 cells described by Cravioto; the name EPEC adherence factor (EAF) was given to this property. Later, Scales et al. [105] and Nataro et al. [106] clearly differentiated localized adherence to Hep-2 and HeLa cells from diffuse adherence and showed that the former was plasmid-mediated.

Levine et al. [107] carried out a study to determine the role of the EAF plasmid in causing EPEC diarrhea in volunteers. Nine of 10 volunteers who ingested an O127:H6 strain with the EAF plasmid developed definite diarrhea; in contrast, only two of nine volunteers who ingested an EAF-negative variant of the strain developed diarrhea, and it was extremely mild (\(P < .006\)).

Preparations of the outer membranes of the O127 strain with and without its plasmid (compared by SDS-PAGE) revealed a prominent plasmid-encoded protein of 94-KDa [107]. By means of an immunoblotting technique, it was found that recipients of the EAF-positive strain made antibody to the 94-KDa protein in response to infection [107]. Furthermore, the one individual among the 10 challenged who failed to develop diarrhea was the only one who had antibody against this protein before challenge, a result suggesting that he was immune and that this protein may be a critical protective antigen. By using antiserum specific for the 94-KDa protein, this protein has been found in all the important EPEC serotypes, such as those in serogroups O55, O111, O119, O127, and O142. In contrast, this 94-KDa protein has not been found in ETEC, EIEC, EHEC, or in strains of *E. coli* that cause meningitis or pye-
lonephritis. An attempt to purify the 94-kDa protein is underway to prepare a potent and plentiful antibody that can be used in a simple diagnostic test, such as one based on agglutination.

In the meantime, an excellent test to identify EPEC already exists. Nataro et al. [108] have cloned a 1-kilobase segment of DNA from the EAF plasmid of strain E2348/69, and they have shown it to be a highly sensitive and specific DNA probe for detecting EPEC that carry the EAF plasmid. In a collaboration with Valeria Prado of Santiago, Chile, we found that the EAF probe hybridized with approximately 75% of E. coli strains identified as suspect EPEC by multi-step O serogrouping carried out by Dr. Prado’s laboratory (unpublished data). More recently, the question has arisen as to whether E. coli other than recognized EPEC serotypes also carry the EAF plasmid and whether they are diarrheagenic. This has been answered in a recent study carried out by Levine et al. (unpublished data) in collaboration with Dr. Valeria Prado in Santiago, Chile. In 11 (31%) of the 47 infants from whom EAF-positive E. coli were recovered, the E. coli were of non-EPEC serogroups. However, whereas EAF-positive E. coli of classical serogroups were recovered significantly more frequently from infected patients (35 [23%] of 154) than from controls (1 [2%] of 66; \( P = .0002 \)), EAF-positive strains of nonclassical serogroups were recovered with comparable frequency from infected patients (7 [5%] of 154) and controls (4 [6%] of 66). Thus, on the basis of preliminary epidemiological evidence from Chile, only EAF-positive E. coli of classical EPEC O serogroups appear to be capable of causing diarrhea. Presumably this is true because other virulence properties are required, and these accessory virulence properties appear to be restricted to classical serogroup strains.

EPEC serotypes that do not possess the EAF plasmid and do not give localized adherence to Hep-2 cells have also been incriminated by epidemiological studies and studies in volunteers as causes of diarrhea, even though their pathogenesis at the molecular level is not as precisely known as that for the EAF-positive EPEC. Nevertheless, at least for the present, these EAF-negative EPEC will be considered as a second class (class II) within the EPEC category. One such strain of classical serotype O114:H2 possesses two plasmids of ~60 MDa, exhibits no adherence to Hep-2 cells, and causes definite diarrhea. Moon et al. [2] found that this strain caused typical attaching and effacing lesions in enterocytes that were indistinguishable from those caused by EAF-positive EPEC. Thus, for both historical and pathological reasons, we have elected to refer to these strains as Class II EPEC [108], i.e., EAF-negative strains of classical EPEC serotypes (figure 2).

Marques et al. [109] and Cleary et al. [110] have reported that some EPEC strains elaborate moderate quantities of a cytotoxin very similar (or identical) to the toxin from Shigella dysenteriae type 1. It has been suggested that this toxin may play a role in the pathogenesis of EPEC disease. As I will show below, many of the cytotoxigenic strains referred to as EPEC serogroups and serotypes by Marques et al. and Cleary et al. should be more correctly categorized as enterohemorrhagic. In particular, this is true of O26:H11, which was heretofore considered a classical EPEC serotype.

Clinically, EPEC illness is characterized by fever, malaise, vomiting, and diarrhea with prominent amounts of mucus but without gross blood. EPEC illness tends to be clinically more severe than many other diarrheal infections in infants, some of whom develop prolonged diarrhea that persists for >14 days. Recent studies from several countries in South America have shown EPEC to be either the first or second most important bacterial cause of diarrhea in infants [111–113].

**EAEC**

The other diarrheagenic E. coli of interest are the EAF-negative, nonclassical serotype strains described by Mathewson [3, 4] and identified only by their property of adherence to Hep-2 cells. After their incrimination in an epidemiological study [3], challenge of volunteers showed that at least one strain caused mild but definite diarrhea without blood or fecal leukocytes [4]. Preliminary evidence suggests that these E. coli are identifiable by a particular pattern of adherence to Hep-2 cells that is clearly distinguishable from both localized adherence and diffuse adherence (J.P. Nataro et al., unpublished observations; M. M. L. et al., unpublished observations). EAEC do not elaborate LT, ST, or elevated levels of Shiga-like toxin, invade epithelial cells, or possess EAF plasmids. It is critical to determine whether the pathogenicity of EAEC and their adherence to Hep-2 cells is related to certain plasmids and whether they fall within a restricted set of O serogroups, as has been found with the other cate-
gories of diarrheagenic *E. coli*. It is also important to determine the relation, if any, between Mathewson's strains and the fimbria-mediated diffuse adherence described by Moseley et al. [114]. For the moment, at least, I suggest that the strains described by Mathewson et al. be considered a distinct, fifth category of diarrheagenic *E. coli*. Further data, however, must be generated before these strains can be fully accepted as diarrheagenic *E. coli*. One other epidemiological study carried out in Mexico [115] failed to identify diffuse Hep-2 cell–adherent *E. coli* any more commonly in children with diarrhea than in controls. It is very possible that this last, less-well-defined category is heterogeneous.

**EHEC**

In 1982, a multistate outbreak of hemorrhagic colitis drew attention to an unusual clinical syndrome of diarrheal disease and a new bacterial enteric pathogen [116]: the causative organism, *Escherichia coli* O157:H7, was a serotype not previously recognized as a cause of diarrheal disease in humans. The clinical syndrome was notable in that bloody but copious diarrhea, unaccompanied by fecal leukocytes, was seen in afebrile patients [116]; these features distinguish it from classic dysentery due to *Shigella* or EIEC, infections that are characterized by fever and scanty stools of blood and mucus containing many fecal leukocytes [82]. Since 1982, some knowledge has been gleaned on the epidemiology of O157:H7 infections as they occur in North America and Europe, and considerable progress has been made on elucidating its pathogenesis. There has also been a strong incrimination of O157:H7 as a cause of hemolytic uremic syndrome [117–124]. O157:H7 has emerged as an enteric pathogen of public health importance in Canada and the United States, with multiple reports of outbreaks of hemorrhagic colitis, hemolytic uremic syndrome, and diarrhea in nursing homes, day care centers, schools, and the community [117–129].

O157:H7 strains from persons with hemorrhagic colitis and hemolytic uremic syndrome elaborate phage-encoded potent cytotoxins active on HeLa and Vero cells [129–136]. One of these toxins, *Shiga-like toxin 1* or *Verotoxin 1*, is apparently identical to the potent cytotoxin/neurotoxin/enterotoxin produced by *S. dysenteriae* type I (Shiga toxin) [137, 138] and reacts with and is neutralized by antibody to Shiga toxin. Many strains also elaborate a second potent cytotoxin (Shiga-like toxin 2 or Verotoxin 2) that is not neutralized by antibody to Shiga toxin [130, 133, 135, 136]. In addition, O157:H7 strains possess a 60-MDa plasmid that plays a role in virulence. Recently, Karch et al. [139] have shown that this plasmid encodes the production of a newly recognized variety of fimbriae that appears to mediate attachment to Henle 407 gut-derived epithelial cells in tissue culture. They examined 14 O157:H7 strains isolated from patients with hemorrhagic colitis or hemolytic uremic syndrome for their plasmid content and for the presence of fimbriae. Thirteen of the strains possessed a 60-MDa plasmid and were fimbriated [139]. Three strains cured of the 60-MDa plasmid lacked fimbriae. Conversely, a nonfimbriated K-12 strain expressed fimbriae after being transformed with the 60-MDa plasmid from O157. A tissue culture assay with Henle 407 cells showed that O157:H7 bacteria containing the 60-MDa plasmid adhere to the cells in a characteristic way: small numbers of bacteria (usually two to four) attach in a central location, whereas plasmid-cured derivatives fail to adhere [139].

Several animal models have been developed to demonstrate the pathological features of O157:H7 infection [140–146]. In electron photomicrographs by Tzipori et al. [145], attached and effaced enterocytes are evident with destruction of the microvilli, a lesion resembling that due to classical serotype EPEC [2, 102]. Nevertheless, in gnotobiotic piglets, EHEC and EPEC infections can be clearly differentiated by anatomic site of involvement, severity of lesions, and degree of polymorphonuclear cell infiltration. EPEC involve the entire intestine of piglets, EHEC only the cecum and colon; EPEC lesions are generally less severe; some infiltration by leukocytes is seen with EPEC, but not with EHEC, infection.

The term EHEC refers to strains such as O157:H7, which manifest the above-mentioned clinical, epidemiological, and pathogenetic features.

Heretofore, it has been difficult to undertake studies of the epidemiology of EHEC infections, other than investigations of outbreaks, because of the lack of suitable methods for screening large numbers of stool cultures for O157:H7 strains, the lack of knowledge regarding what other serotypes may also be enterohemorrhagic, and an inability to identify these serotypes. We contend that one other serotype, in particular, O26:H11, should be classified as EHEC; this serotype is usually an abundant producer of verotoxin [147–151], possesses a 60-MDa
plasmid [149, 152-154], does not hybridize with the EAF gene probe from EPEC [108], and is sometimes associated with bloody diarrhea. Recognizing the importance of plasmids in encoding critical virulence properties in EPEC, EIEC, and ETEC and the usefulness of diagnostic DNA probes prepared from these plasmids, we undertook studies at the Center for Vaccine Development to compare the 60-MDa plasmids of O157:H7 and O26:H11 strains, on the hypothesis that the latter are also EHEC. These preliminary studies showed homology among the plasmids and led to the development of a sensitive and specific DNA probe to identify EHEC (M. M. L. et al., unpublished observations).

From the early days of the 1940s when *E. coli* were first convincingly associated with human diarrhea, much has been discovered about the several categories of diarrheagenic *E. coli*, including information on their clinical features, epidemiology, O:H serotypes, and most particularly, their pathogenesis. From a previous state of some confusion about their role as enteric pathogens, diarrheagenic *E. coli* are now recognized as being among the best understood bacterial enteropathogens.

References

7. Bray J. Beavan TED. Slide agglutination of *Bacterium coli var. neapolitanum* in summer diarrhoea. J Pathol 1948;60:395-401
17. Ørskov F. On the occurrence of *E. coli* belonging to O group 26 in cases of infantile diarrhoea and white scours. Acta Pathol Microbiol Immunol Scand 1951;29:373-8


61. Smith HR, Scotland SM, Rowe B. Plasmids that code for production of colonization factor antigen II and en-
66. de Graaf FK, Roorda I. Production, purification, and characterization of the fimbrial adhesive antigen F41 isolated from calf enteropathogenic Escherichia coli strain B41M. Infect Immun 1982;36:751–8
68. Thomas LV, Cravioto A, Scotland SM, Rowe B. New fimbrial antigenic type (E8775) that may represent a colonization factor in enterotoxigenic Escherichia coli in humans. Infect Immun 1982;35:1119–24
69. Tacket CO, Maneval DR, Levine MM. A new fimbrial putative colonization factor of enterotoxigenic Escherichia coli serotype O159:H4: purification, morphology and genetics. (Submitted for publication)
74. Evans DG, Graham DY, Evans DJ Jr, Opekun A. Administration of purified colonization factor antigens (CFA/1, CFA/II) of enterotoxigenic Escherichia coli to volunteers. Response to challenge with virulent enterotoxigenic Escherichia coli. Gastroenterology 1984;87:934–40
82. Toledo MRF, Trabulsi LR. Correlation between biochemical and serological characteristics of Escherichia coli and results of the Serény test. J Clin Microbiol 1983;17:419–21
89. Sack RB. Serotyping of E. coli. Lancet 1976;1:1132
90. Gurwith MJ, Wiseman DA, Chow P. Clinical and labora-
103. Cravioto A, Gross RJ, Scotland SM, Rowe B. An adhesive factor found in strains of Escherichia coli belonging to the traditional infantile enteropathogenic serotypes. Cur- rent Microbiology 1979;3:95–9


131. O'Brien AD, Newland JW, Miller SF, Smith HW, Fornal SB. Shiga-like toxin—converting phages from *Escherichia coli* strains that cause hemorrhagic colitis or infantile diarrhea. Science 1984;226:694-6


133. Scotland SM, Smith HR, Rowe B. Two distinct toxins active on Vero cells from *Escherichia coli* O157. Lancet 1985;2:885-6


138. Eiklid K, Olsnes S. Animal toxicity of *Shigella dysenteriae* cytoxin: evidence that the neurotoxic, enterotoxic, and cytotoxic activities are due to one toxin. J Immunol 1983;130:380-4


148. Scotland SM, Day NP, Rowe B. Production of a cytotoxin affecting Vero cells by strains of *Escherichia coli* belonging to traditional enteropathogenic serogroups. FEMS Lett 1980;7:15-7


