

## *Escherichia coli*

*Escherichia coli* is the predominant bacterium of the normal facultative anaerobic microflora of the human intestine. These commensal strains of *E. coli* usually remain harmlessly confined to the intestinal lumen and have shown to play an important role in maintaining intestinal physiology. However, given the right opportunities any *E. coli* strain can probably cause invasive disease, and *E. coli* has therefore aptly been called an opportunistic pathogen. On the other hand, several highly adapted *E. coli* clones have evolved the ability to cause a broad spectrum of human diseases. Some strains of *E. coli* are well equipped for extra-intestinal multiplication, causing urinary tract infections, neonatal meningitis, wound infections, peritonitis, and septicemia, however, inherently pathogenic strains of *E. coli* are generally considered as a cause of diarrhoeal diseases. Presently, six major groups of diarrhoeagenic *E. coli* strains have been defined based primarily on pathogenic mechanisms: enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli* (ETEC), enterohaemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC or EAaggEC), and diffusely adherent *E. coli* (DAEC) (Nataro and Kaper, 1998).

### Diarrhoeagenic *E. coli*

#### *Enteropathogenic E. coli*

The classical EPEC were the first *E. coli* strains that proved to cause human diarrhoea. In the 1940s and 1950s, investigators in North America and Europe demonstrated that *E. coli* of specific serotypes were responsible for several hospital and community outbreaks of neonatal diarrhoea with mortality rates often exceeding 50%. EPEC strains have long been defined as diarrhoeagenic *E. coli* of specific serogroups that historically have been incriminated in outbreaks of infantile diarrhoea, but whose pathogenic mechanisms have not been proven to be related to either heat-labile enterotoxins, heat-stable enterotoxins, or *Shigella*-like epithelial cell invasiveness. As the understanding of EPEC pathogenesis has improved, it has now become more appropriate to define EPEC strains based on their characteristic virulence determinants implicated in disease pathogenesis. The hallmark of infections due to EPEC is the attaching-and-effacing (AE) histopathology, which can be observed in intestinal biopsy specimens from patients or artificially infected animals and can be reproduced in cell culture. This striking phenotype is characterised by intimate adherence of the bacterium to the epithelial cell membrane and effacement of microvilli. EPEC strains are capable of invading a variety of epithelial cells *in vitro*. However, the clinical significance of cell entry in the pathogenesis of disease due to EPEC remains to be determined. Some EPEC strains can produce one or more toxins. While outbreaks of diarrhoea caused by EPEC are now uncommon in industrialised countries due to good standards of hygiene, EPEC strains are still an important cause of infantile diarrhoea in many developing countries.

#### *Enteroinvasive E. coli*

EIEC strains were first isolated in Italy during World War II. Since then, several foodborne and waterborne outbreaks of EIEC diarrhoea have been reported throughout the world. Person-to-person transmission does also occur. EIEC mimic *Shigella* strains in their ability to invade and multiply within intestinal epithelial cells. When the infection is severe a strong inflammatory response is being elicited which is manifested grossly as ulceration of the mucosa. The predominant site of bacterial invasion is the colon. A role for enterotoxins is unproven, but their presence may explain the characteristic watery diarrhoea attributed to EIEC. Presently, EIEC strains are rarely isolated in industrialised countries. However, in countries with poor sanitation and low socio-economic status, these strains are still a common cause of diarrhoea and dysentery.

#### *Enterotoxigenic E. coli*

ETEC strains were first isolated in 1968 during studies on the aetiology of non-cholera diarrhoea in India and Bangladesh. Later, ETEC strains were shown to be the most common cause of traveller's diarrhoea for individuals who travel from areas with good hygiene and temperate climate to areas with lower hygienic standards, particularly developing countries in the tropics. While ETEC strains are rare in industrialised countries, they are an important cause of endemic diarrhoea in developing countries especially among infants and young children. The mechanism of pathogenicity of ETEC is in many respects similar to that of *Vibrio cholerae* group O1. After ingestion, ETEC strains adhere to the proximal small intestine utilising fimbrial colonisation factors and elaborate heat-labile and/or heat-stable enterotoxins, which induce massive fluid and electrolyte secretion into the gut lumen resulting in watery diarrhoea. The heat-labile enterotoxin is structurally similar to cholera enterotoxin. ETEC strains do not invade the mucosa or cause histologic changes. ETEC strains causing disease in humans are considered to be human-specific,

but similar strains cause diarrhoea in calves and piglets.

#### *Enterohaemorrhagic E. coli*

EHEC strains comprise a subset of the so-called verocytotoxin (VT)- or Shiga toxin (Stx)-producing *E. coli* (VTEC or STEC), composed of those strains of VTEC which cause bloody diarrhoea in the preponderance of infected patients. STEC were first described during the late 1970s. However, the major breakthroughs occurred in 1983 with the publication of two epidemiological studies which linked STEC infection with two diseases of previously unknown aetiology, haemorrhagic colitis and the haemolytic-uraemic syndrome (HUS). Since then, there have been many reports from all over the world describing the association between STEC and human disease. At present, over 200 different serotypes of *E. coli* have been shown to produce Stx, but not all of these are pathogenic for humans. *E. coli* strains of serotype O157:H7, and the non-motile (NM) variant *E. coli* O157:NM, remain the most common in many parts of the world. *E. coli* O157 strains with other H antigens are not known to cause infections in humans. The most common non-O157:H7 STEC serotypes associated with human disease are O26:H11, O103:H2, O111:NM, and O113:H21. The mechanism of pathogenicity of EHEC strains is not yet fully elucidated, but is associated with their ability to produce one or more Stxs and the formation of AE lesions in the intestinal mucosa consistent with the AE lesions in human EPEC infection. EHEC strains are generally considered as not invasive. Most EHEC infections have been linked with the consumption of foods of bovine origin and cattle are regarded as the principal reservoir of these pathogens. EHEC strains seem to occur most frequently in developed countries in contrast to the other types of diarrhoeagenic *E. coli*.

#### *Enteroaggregative E. coli*

In 1987, Nataro et al. were the first who proposed that EAEC may be pathogenic after detecting these strains more frequently in faeces of children with diarrhoea than in those of controls. Following this initial study, numerous epidemiological studies in infants and children in developing countries have supported the association of EAEC with diarrhoea, most prominently with persistent diarrhoea. However, in some studies EAEC strains were isolated in similar numbers from cases and controls and some studies have not found an association with diarrhoea. It has been suggested that the ability to cause disease is a property of only certain EAEC strains. EAEC are currently defined as *E. coli* strains that do not secrete heat-labile or heat-stable enterotoxins and that adhere to HEp-2 cells in culture (of human laryngeal carcinoma origin) in a unique pattern of aggregative adherence. While the adherence pattern of EPEC strains is described as localised adherence, denoting the presence of clusters or microcolonies on the surface of the HEp-2 cells, EAEC strains adhere in an appearance likened to stacked bricks to the surface of the HEp-2 cells. The factors that are involved in causing disease are not yet understood. Putative virulence factors include an enterotoxin (EAST, for EAEC heat-stable enterotoxin) similar to the heat-stable enterotoxin of ETEC strains, haemolysins and cytotoxins, and various types of fimbriae and outer membrane proteins that may be involved in the adhesion process.

#### *Diffusely adherent E. coli*

The term DAEC was initially used to refer to any HEp-2-adherent *E. coli* strain that did not form EPEC-like microcolonies. With the discovery of EAEC strains, DAEC strains were recognised as an independent category of potentially diarrhoeagenic *E. coli* and were defined by the presence of the diffuse pattern in the HEp-2 adherence assay. In diffuse adherence, bacteria are seen dispersed over the surface of the HEp-2 cell, with little aggregation and little adherence to the glass coverslip free from the cells. Several studies have implicated DAEC strains as agents of diarrhoea, while other studies have not recovered DAEC strains more frequently from diarrhoeal patients than from asymptomatic controls. This may be explained by an age-dependent susceptibility. Recently, Jallat *et al.* (1993) reported that DAEC strains accounted for a large proportion of diarrheal cases among hospitalized patients in France who had no other identified enteropathogen. Therefore it has been suggested that DAEC strains may be important diarrheal pathogens in developed countries. Little is known about the pathogenetic features of DAEC-induced disease. Possible virulence factors include a surface fimbria, and a 100-kDa outer membrane protein. Furthermore, it has been shown that DAEC strains are able to induce finger-like projections extending from the surface of infected cell cultures.

#### **Importance of diarrhoeagenic *E. coli* as foodborne pathogens**

Infection with diarrhoeagenic *E. coli* is usually acquired by ingestion of the organisms via contaminated food or water. Different types of food including water have been associated with outbreaks of diarrhoea due to these *E. coli* (Table 1). The source of infection is usually not investigated in sporadic cases. In addition to the food

associated with outbreaks of *E. coli*-induced diarrhoea, several investigations of food and water as a potential vehicle have demonstrated the presence of pathogenic *E. coli* strains. Humans, symptomatic or symptom-free carriers, are presumed to be the principal reservoir of EPEC, EIEC, and ETEC strains that cause human illness. These bacteria are present in the intestinal tract of carriers and are excreted in their faeces. Infected foodhandlers with poor personal hygiene or water contaminated by human sewage are sources of food contamination. The principal reservoir of EHEC is the intestinal tract of cattle. Other animals used in the production of food have also been shown to carry the organisms, although less frequently. Animals carrying EHEC are usually asymptomatic. Raw foods of animal origin may be contaminated with the organisms via faecal contact during slaughter or milking procedures. Most EHEC infections are caused by ingestion of undercooked ground beef and cow's milk. In the past few years, however, fruits and vegetables have accounted for a growing number of recognised EHEC outbreaks. While contamination of fresh produce may be due to cross-contamination from meat products, contact with faeces from domestic or wild animals at some stage during cultivation or handling of fresh produce is another presumable route of contamination. Person-to-person contact has been shown to be an important cause of outbreaks in day-care and chronic-care facilities. Less is known about the reservoirs and routes of transmission of EAEC and DAEC. Although most reports have implicated EAEC in sporadic endemic diarrhoea, a growing number of reports have described EAEC outbreaks. However, the source of infection frequently remained unclear. In addition to the uncertainty about the contribution of DAEC to the human disease burden, the mode of acquisition of DAEC infection is also as yet undetermined.

**Table 1 Foods involved in outbreaks of infection with diarrhoeagenic *E. coli***

Type of <i>E. coli</i>	Known or presumed food vehicles of outbreaks
EPEC	Coffee substitute, cold port, meat pie, weaning foods or infant formula, water
EIEC	Brie and Camembert cheese, canned salmon, potato salad, water
ETEC	Brie cheese, curried turkey mayonnaise, tuna paste (school lunch), salads containing raw vegetables (airline flight, mountain lodge), prepared food (restaurant, cafeteria, cruise ship), water
EHEC	Undercooked ground beef, unpasteurised cow's milk, pasteurised cow's milk, goat's milk, unpasteurised cream, unpasteurised yoghurt, unpasteurised cheese, roast beef, salami, meat from poultry, sheep and deer, fresh potatoes, lettuce, radish and alfalfa sprouts, unpasteurised apple juice and cider
EAEC	Restaurant meal
DAEC	?

#### Isolation of diarrhoeagenic *E. coli* from foods

Because the species *E. coli* consists of both pathogenic and non-pathogenic strains, and due to the fact that the latter constitute a part of the normal intestinal flora, differentiation between pathogenic and non-pathogenic strains is very important. The detection and isolation of pathogenic *E. coli* from foods is quite difficult. Serological testing of *E. coli* isolates has limited value in identifying pathogenic strains. Traditional cultivation from food samples using selective enrichment broths at elevated temperatures (44 to 45.5°C) has been shown to favour non-pathogenic, "environmental" strains compared to pathogenic strains. In addition, some pathogenic strains do not have the typical characteristics of *E. coli*, such as fermentation of lactose and gas production. Adequate culture methods have been developed for EHEC, especially EHEC of serogroup O157. However, there are still no simple sensitive procedures available for the direct cultivation of the other diarrhoeagenic *E. coli* from foods. For the isolation of these organisms resuscitation in brain heart infusion broth for 3 h at 35°C, followed by enrichment in tryptone phosphate broth for 20 h at 44°C and plating on Levine's eosin-methylene blue agar and MacConkey agar is advised. Both typical (lactose-fermenting) and non-typical (non-lactose-fermenting) colonies have to be picked and characterised biochemically, serologically and on the basis of virulence properties (US Food and Drug Administration, 1995). A phenotypic approach to test for virulence properties requires the use of cell cultures and sometimes fluorescence microscopy, and a genotypic method requires the use of DNA hybridisation or the polymerase chain reaction (PCR). The above means that from one sample numerous isolates must be assayed.

#### Research needs / future directions

Since EHEC were first recognised as human pathogens, a lot of research has been initiated to learn more about the epidemiology of EHEC infections. However, the impact of the other diarrhoeagenic *E. coli* on causing

foodborne infections in Europe and their reservoirs and routes of transmission have not been looked at clearly so far. Future work should focus on the following.

- Determine the role of diarrhoeagenic *E. coli* other than EHEC in outbreaks of foodborne infections in Europe. *E. coli* strains isolated from foods involved in outbreaks of diarrhoeal illness should routinely be examined for (possible) virulence factors of the different groups of diarrhoeagenic *E. coli*.
- Determine the presence of diarrhoeagenic *E. coli* other than EHEC in food by examining *E. coli* strains isolated from food during routine analysis for (possible) virulence factors of the different groups of diarrhoeagenic *E. coli*.
- Determine the reservoirs and routes of transmission of EAEC and DAEC.
- (Collect information from Food Inspection Services throughout European on the frequency of isolation of *E. coli* during (1) routine analysis of foods and (2) examination of foods implicated in outbreaks of diarrhoea and on the typing results of these strains.)

### References

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