Escherichia coli and persistent diarrhea
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Persistent diarrhea due to *Escherichia coli*

Review of the literature

Various microbiological and parasitological causes of persistent diarrhea, especially in children, have been identified. In infants with a stunted growth, rotavirus and norovirus may be the cause of diarrhea lasting longer than 14 days. Also, gastrointestinal infection due to enteropathogenic *Escherichia coli* may be caused by long-lasting diarrhea in children. Enterotoxigenic *Escherichia coli* causes persistent diarrhea in patients with impaired host defense, including patients with AIDS. *Salmonella* and *Campylobacter* species, *Cryptosporidium* and *Microsporidia* may cause persistent diarrhea.

*Enterotoxigenic* *Escherichia coli* are an important cause of acute gastro-intestinal illness in children and adults in developed and developing countries (1). The role of *enteropathogenic* *E. coli* in the etiology of persistent diarrhea is less well established. Six categories of *enteropathogenic* *E. coli* are recognized: *enterotoxigenic* *E. coli* (ETEC), *enteropathogenic* *E. coli* (EPEC), *enterohemorrhagic* *E. coli* (EHEC), *enterohemorrhagic* *E. coli* (EAEC), *enteroinvasive* *E. coli* (EIEC), and *enteroinvasive* *E. coli* (O157:H7). In this chapter, the evidence data are reviewed which address the association of the presence of *enterotoxigenic* *E. coli* in different patient groups with the occurrence of persistent diarrhea.
**Introduction**

Most infectious diarrheal episodes resolve within one week. However, in a proportion of patients, infectious diarrhea may continue for up to 2 weeks or more. In those cases, diarrhea is persistent, a term encompassing a diarrheal episode which begins acutely and continues for longer than 2 weeks. The term persistent diarrhea does not include patients with chronic diarrhea due to non-infectious causes, such as gluten sensitive enteropathy, granulomatous disease or tumour producing gastro-intestinal hormones (1).

Various bacteriological and parasitological causes of persistent diarrhea, sometimes in addition to host defense impairment, have been identified. In hosts with a normal host defense, infection with *Yersinia enterocolitica*, *Shigella* species or *Aeromonas* species may be the cause of diarrhea lasting longer than 14 days. Also gastrointestinal infection due to microorganisms usually causing systemic disease, such as Mycobacteria species or various fungi, may be manifested by long lasting diarrhea. In addition, protozoa such as *Giardia lamblia*, *Cryptosporidium* and cyclospora may be the cause of persistent diarrhea. In patients with impaired host defense, including patients with AIDS, *Salmonella*, *Campylobacter* species, *Clostridium difficile* and protozoa, such as microsporidia, may cause persistent diarrhea.

Diarrheagenic *Escherichia coli* are an important cause of acute gastro-intestinal illness in children and adults in developed and developing countries (2). The role of diarrheagenic *E. coli* in the etiology of persistent diarrhea, is less well established. Six categories of diarrheagenic *E. coli* are recognized: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAggEC), enteroinvasive *E. coli* (EIEC), and diffuse adherent *E. coli* (DAEC) (see also chapter 1). In this chapter the available data are reviewed which address the association of the presence of diarrheagenic *E. coli* in different patient groups with the occurrence of persistent diarrhea.
Persistent diarrhea in children

In children in developing countries, persistent diarrhea is an important cause of morbidity and mortality (3-5). The mortality is particularly high among severely malnourished children with persistent diarrhea below 3 years of age (3,4). The bacteriological etiology of persistent diarrhea in children in developing countries has been studied by several investigators in different geographic areas.

EAggEC has been associated with persistent diarrhea in a number of investigations. These studies are summarized in the table. Bhan et al. studied the epidemiology of persistent diarrhea among children aged under 6 in rural northern India prospectively (6,7). EAggEC, as detected by the Hep-2 adherence test, was found in a significantly higher number of children with persistent diarrhea during the first week of illness when compared with children with acute diarrhea and controls. In two studies among children who presented with persistent diarrhea in a tertiary referral centre in New Delhi (8,9), EAggEC was isolated as a single pathogen in a significant number of patients with persistent diarrhea when compared with children with acute diarrhea and controls. In both studies, stool samples were taken on admission and the presence of EAggEC was determined using the HEp-2 adherence test.

In a study on the etiology and epidemiology of persistent diarrhea in northeastern Brazil (10), EaggEC, hybridizing with the fragment DNA probe, was the only microorganism isolated in a significant number from stool samples of patients with persistent diarrhea when compared with children with acute diarrhea and controls. EAggEC which did not hybridize with the probe but which showed the characteristic stacked brick-like aggregates on HEp-2 cells, was also associated with persistent diarrhea. However, this association was not analyzed after correction for simultaneous infection with other pathogens. In addition, probe-negative EAggEC was isolated with high frequency from quantitative cultures of small bowel aspirates from patients with both persistent and acute diarrhea.

In a prospective study in an urban slum in Brazil (11), Wanke detected EAggEC, using the HEp- adherence test, as a sole pathogen in 20% of children with persistent diarrhea, 8% of children with acute diarrhea and 5% of controls. Of the children with persistent diarrhea, stool samples were cultured from 25 within the first 14 days of illness and from 15 after 14 days of illness. EAggEC was present in 18% of stools cultured within 14 days of illness and in 27% of stools collected more than 14 days into illness, suggesting persistence of pathogens into the diarrheal illness.
EAggEC, as detected in the HEp-2 adherence test, was also associated with persistent diarrhea in a study in Mexico (12). However, no mention is made of other pathogens which may have been isolated during the episodes of diarrhea in this study.

In a 2 year prospective study among children in rural Bangladesh, EAggEC was associated with persistent diarrhea when compared with children with acute diarrhea (4). However, EAggEC was isolated in similar frequency from non-diarrheal stool samples and diarrheal stool samples. Furthermore, no mention is made of co-infections with other pathogens. Also in this study, the HEp-2 adherence test was used to detect EAggEC.

The association between the presence of EAggEC in stool samples and persistent diarrhea could not be confirmed by other investigators. In a group of 705 children in Bangladesh, who were studied prospectively for a period of one year, Baqui et al. found similar isolation rates of EAggEC among children with acute or persistent diarrhea and age matched healthy controls (13). In less than 15% of children with follow-up stool samples, identical pathogens were isolated in the first 3 days and in the persistent phase of a diarrheal episode (13). In a longitudinal study in urban Peru among 677 children under three years of age, no association was found between any enteropathogen and persistent diarrhea (14). Also in a study among children under five in a Cambodian refugee camp, EAggEC was found in similar frequency among 408 children with acute diarrhea and 79 children with persistent diarrhea (15). In all three studies mentioned, EAggEC was detected using the HEp-2 cell adherence test. Finally, in a recent study among a cohort of Israeli Bedouin infants, none of the enteric pathogens examined, including EAggEC as detected by fragment DNA probe, were associated with persistent diarrhea (16). In this study, family and environmental factors, in particular the age of the mother and the level of education, appeared to be associated with the occurrence of persistent diarrhea.

In the only controlled study performed among children in Europe, EAggEC was isolated in 16 (2%) of 298 children with diarrhea and in none of 580 children without diarrhea (17). Four children with EAggEC infection had persistent diarrhea. The numbers in this study are too small to reach statistical significance. EAggEC was detected using PCR and the HEp-2 adherence test.

The differences found in the above mentioned studies can partly be ascribed to methodological differences. In studies in which both the fragment DNA probe and HEp-2 adherence test were applied, the sensitivity of the probe varied (10,17,18). In addition, volunteer studies have shown that EAggEC strains vary in their ability to cause diarrhea (19).
Proportion of enteroaggregative *E. coli* among children with persistent and acute diarrhea and controls in the various studies

<table>
<thead>
<tr>
<th>Country</th>
<th>Author</th>
<th>Reference</th>
<th>Test</th>
<th>Acute N/total</th>
<th>Persistent N/total</th>
<th>Controls</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>India</td>
<td>Bhan</td>
<td>(6)</td>
<td>HEP-2 test</td>
<td>22/179</td>
<td>15/43</td>
<td>nd</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>India</td>
<td>Bhan</td>
<td>(7)</td>
<td>HEP-2 test</td>
<td>23/179</td>
<td>18/61</td>
<td>20/201</td>
<td>&lt;.006*; &lt;.0052**</td>
</tr>
<tr>
<td>India</td>
<td>Bhatnagar</td>
<td>(9)</td>
<td>HEP-2 test</td>
<td>nd</td>
<td>18/92</td>
<td>6/92</td>
<td>.016</td>
</tr>
<tr>
<td>Brazil</td>
<td>Fang</td>
<td>(10)</td>
<td>HEP-2 test</td>
<td>24/52</td>
<td>38/56</td>
<td>13/42</td>
<td>&lt;.01**</td>
</tr>
<tr>
<td>Brazil</td>
<td>Wanke</td>
<td>(11)</td>
<td>DNA-probe</td>
<td>4/52</td>
<td>8/40</td>
<td>2/38</td>
<td>&lt;.03***</td>
</tr>
<tr>
<td>Mexico</td>
<td>Cravioto</td>
<td>(12)</td>
<td>HEP-2 test</td>
<td>49/579</td>
<td>29/57</td>
<td>5/100</td>
<td>&lt;.001**</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>Henry</td>
<td>(4)</td>
<td>HEP-2 test</td>
<td>5/28</td>
<td>17/62</td>
<td>nd</td>
<td>&lt;.05</td>
</tr>
<tr>
<td>Bangladesh</td>
<td>Baqui</td>
<td>(13)</td>
<td>HEP-2 test</td>
<td>39/175</td>
<td>33/177</td>
<td>34/159</td>
<td>ns</td>
</tr>
<tr>
<td>Peru</td>
<td>Lanata</td>
<td>(14)</td>
<td>HEP-2 test</td>
<td>5/130</td>
<td>4/130</td>
<td>nd</td>
<td>ns</td>
</tr>
<tr>
<td>Cambodia</td>
<td>Arthur</td>
<td>(15)</td>
<td>HEP-2 test</td>
<td>16/408</td>
<td>3/79</td>
<td>nd</td>
<td>ns</td>
</tr>
<tr>
<td>Israel</td>
<td>Fraser</td>
<td>(16)</td>
<td>DNA probe</td>
<td>25/111</td>
<td>2/21</td>
<td>215/827</td>
<td>ns</td>
</tr>
<tr>
<td>Germany</td>
<td>Huppertz</td>
<td>(17)</td>
<td>PCR/HEP-2 test</td>
<td>12/280$</td>
<td>4/280$</td>
<td>0/580</td>
<td>nd</td>
</tr>
</tbody>
</table>

- * persistent vs acute diarrhea
- ** persistent vs controls
- *** persistent vs acute and controls
- \$ no distinction made between acute and persistent diarrhea in total number of cases
- nd: not determined; ns: not significant
It is also known that the characteristic adherence to Hep-2 cells does not predict the pathogenicity of EAggEC (see also chapter 1). Therefore, it may be that differences between isolation rates of pathogenic EAggEC strains in different geographic areas, are due to the variable occurrence of pathogenic EAggEC clones. However, no data are available to substantiate this hypothesis.

In none of the above mentioned studies, an association between the presence of enteropathogenic E. coli (EPEC) and persistent diarrhea was found (4,6,7,9-16). However, isolated cases of persistent diarrhea in infants, associated with E. coli of EPEC serotypes, have been reported. These cases occurred in the United States (20), Great Britain (21) and Brazil (22). EPEC infection was associated with travel to a developing country in one study (21). Small bowel biopsies revealed adherent bacteria in all reported cases. In one report, bacteria were observed intracellularly (22) and E. coli O18ab H14 was isolated from stool samples and jejunal aspirates. This strain hybridized with the eae probe but not with the EAF probe, daaC probe or Inv probe. EPEC strains may thus be associated with persistent diarrhea in isolated cases.

Enterotoxigenic E. coli (ETEC) was isolated from children with persistent diarrhea at similar rates as from children with acute diarrhea in studies done in Brazil (11), Peru (14) and Thailand (15). ETEC was associated with acute diarrhea only in two studies (4,7), or no association with diarrhea at all was found (10,13,16).

Diffusely adherent E. coli (DAEC) was associated with persistent diarrhea in one study in Bangladesh (13). DAEC was detected in 16.4% of 177 specimens from children with persistent diarrhea compared with 10.3% of 175 specimens from children with acute diarrhea (p < 0.05) and 8.2% of 159 specimens from healthy controls (p < 0.05). However, mixed infections with other pathogens were common and the number of single DAEC infections was not reported (13). In 10 other studies, no association of DAEC with persistent diarrhea was observed (4,6-10,12,14-16).

In a number of the aforementioned studies, repeat stool samples were investigated during an episode of persistent diarrhea(13-16). In only 1-15% of cases, repeat samples yielded the same pathogen during such an episode, even without antibiotic treatment (13-16). However, the overall isolation rates of all pathogens were similar when culture results of samples taken during the first days of illness were compared with results from samples taken 2-4 weeks into the illness (13,14,16). These studies suggest that persistent infection with a single pathogen is uncommon in children with persistent diarrhea. Reinfection with one or more of
the common pathogens is more likely and episodes of persistent diarrhea probably occur by sequential infection due to continuous or repeated exposure. It has therefore been suggested to shift attention from the search for a specific enteric agent that causes persistent diarrhea to determination of potential risk factors which are associated with increased and continuous exposure to enteric pathogens, in order to design appropriate interventions (16).

There are currently no studies available which address the association of diarrheagenic *E. coli* with persistent diarrhea in adults, neither in developing nor in developed countries.

**Persistent diarrhea in travelers**

Millions of people travel from industrialized to developing countries each year. Traveler’s diarrhea is the most common disorder encountered by travelers during their journey. Traveler’s diarrhea generally lasts less than one week (23). However, in a minority of patients traveler’s diarrhea develops into persistent diarrhea. By definition persistent diarrhea lasts at least 14 days, but diarrhea may persist for several months to one year. It is estimated that approximately 3% of international travelers to high-risk areas develop persistent diarrhea, of which approximately 50% lasts more than 30 days (24). Persistent diarrhea is also a major cause of morbidity in expatriates and other long-term foreign residents in developing countries (25,26).

The etiology of persistent diarrhea in travelers remains unknown in at least 50% of cases (24,27). In contrast with the large number of studies on acute traveler’s diarrhea, there are only a few studies which address the etiology of persistent diarrhea in travelers and foreign residents. In addition, these studies are sometimes limited to a specific pathogen, such as *Aeromonas* species (28). Although enterotoxigenic *E. coli* (ETEC) is considered the most important cause of acute traveler’s diarrhea (23), ETEC has rarely been implicated as a cause of persistent diarrhea in travelers (25). A recent Spanish study in returned travelers identified ETEC in 15.2% of 165 patients with diarrhea and in 1.8% of 165 controls (p < 0.0001). However, the duration of ETEC associated diarrhea was not specified (29).

Since EPEC, similar to ETEC, is a common cause of childhood diarrhea in developing countries, several studies have investigated the role of EPEC in acute traveler’s diarrhea (29-32), using either serotyping or DNA probes for detection of EPEC. In none of these studies, EPEC was significantly associated with diarrhea. EPEC has only rarely been implicated as a cause of persistent diarrhea in travelers: Hill (21) et al. isolated EPEC from 6 of 54 children
with persistent diarrhea in the UK, using serotyping. The 6 children were less than 12 months of age and 3 of them had been traveling from the Indian subcontinent to the UK recently. Their diarrhea had started during or shortly after their stay abroad. Persistent diarrhea after travel due to EPEC may be limited to infants, similar to the occurrence of EPEC infections observed in developing countries, which is also limited to infants.

The possible association of enteroaggregative *E. coli* (EAggEC) with persistent diarrhea in children in developing countries (7,10,33) has led to the suggestion that EAggEC may be a cause of persistent diarrhea in travelers as well. Cohen et al. studied the presence of diarrheagenic *E. coli* in short term travelers shortly before and shortly after their journey to Central or South America, the Caribbean or Mexico, using fragment DNA probes (31). Travelers took either placebo, ciprofloxacin or trimethoprim/sulfamethoxazole for prevention of traveler’s diarrhea. In contrast with the other categories of diarrheagenic *E. coli*, colonization rates with EAggEC had risen sharply after travel, but no association with diarrhea was observed. Follow-up data were not available and it is therefore not known whether diarrhea may have become persistent in some travelers who were infected with EaggEC (31).

In a case-control study in 165 returned travelers in Spain (29), controls were recruited on voluntary basis among relatives or among travel companions from the case-patients. EAggEC, as detected by PCR, was isolated from 23 of 165 cases (13.9%) and 4 of 165 controls (2.4%, p = .0003). In 16 patients, EAggEC was the only pathogen identified and in 5 of these patients diarrhea lasted for more than 14 days. The clinical presentation and duration of diarrhea did not differ from ETEC diarrhea. ETEC and *Shigella* were the only other pathogens significantly associated with diarrhea in this study.

Enterohemorrhagic *E. coli* (EHEC) and enteroinvasive *E. coli* (EIEC) are rarely isolated from travelers with diarrhea (29-32,34). It is unlikely that an association of infection with EHEC or EIEC and persistent diarrhea in travelers will be found since large numbers of travelers will need to be studied to find a significant difference in isolation rates of EHEC or EIEC between patients with persistent diarrhea and patients with acute diarrhea or controls.

An association of diffusely adhering *E. coli* (DAEC) and persistent diarrhea in travelers has never been observed. Mathewson reported an association between enteroadherent *E. coli* and traveler’s diarrhea in travelers in Mexico (35). However, at that time, no distinction was made between the aggregative and diffuse adherence pattern in the HEp-2 adherence test. Therefore, these data can not be compared with later studies. Furthermore, no follow-up was available regarding the development of persistent diarrhea in these travelers.
Persistent diarrhea in human immunodeficiency virus-infected individuals

Persistent diarrhea and weight loss are common in human immunodeficiency virus (HIV)-infected patients. Although various enteric pathogens are implicated in the majority of cases, the diarrhea remains unexplained in 30-50% of patients (36).

Several investigators reported a possible association of persistent diarrhea in HIV-infected patients and diarrheagenic *E. coli*. These studies were performed in both developing and developed countries. Thea et al. determined the presence of diarrheagenic *E. coli* in HIV-seropositive and HIV-seronegative children with diarrhea in Zaire (37). Although a high prevalence of EAggEC was found, as determined by DNA hybridization, no association between EAggEC and diarrhea was observed. Mathewson et al. studied the prevalence of HEp-2 cell adherent *E. coli* in HIV-infected and HIV-uninfected adults with diarrhea in Zambia (38). In 55 of 80 (69%) HIV-infected patients with diarrhea, HEp-2 adherent *E. coli* were found, compared with 3 of 12 (25%) of HIV-uninfected patients with diarrhea (p < 0.007). Of the HIV-infected patients, 30 (79%) of 38 patients had persistent diarrhea and were HEp-2 adherent *E. coli* positive, compared with 1 (16%) of 6 HIV-uninfected patients. However, the various adherence patterns (localized, aggregative and diffuse) were equally distributed among the adherent isolates and no correlation of a specific adherence pattern with acute or chronic diarrhea was observed. None of the adherent isolates hybridized with the EAF probe, which was the only DNA probe used. Neither in the Zairian, nor in the Zambian study, a control group of HIV-infected individuals without diarrhea and with comparable CD4 cell counts was included.

Kotler et al. studied persistent diarrhea in HIV-infected patients in the United States. They observed adherent bacteria in colonic biopsy specimens from an HIV-infected patient with severe persistent diarrhea. (39). In a subsequent study, adherent bacteria were observed in 11 of 51 patients with diarrhea and in none of the 15 patients without diarrhea (not significant) (40). In 4 patients with diarrhea, other pathogens were isolated which could have caused the diarrhea. *E. coli* were present in cultures from 10 of 12 rectal biopsies from cases, and in 2 of 6 biopsies from controls. Strains isolated from 5 cases and 1 control displayed aggregative adherence to HEp-2 cells. Strains from 1 patient and 1 control hybridized with the EAggEC DNA probe.
Polotsky et al. (41) studied the HEp-2 cell adherence of *E. coli* isolates obtained from stool samples of 8 HIV-infected patients with persistent diarrhea and compared these isolates with stored control isolates with known adherence pattern and serotypes. Three patients harboured *E. coli* which respectively showed a localized, diffuse or mixed adherence pattern, while isolates from 5 patients demonstrated an aggregative adherence phenotype. None of these isolates hybridized with the recognized DNA probes used for detection of the specific adherence genotype. Although these data are interesting and warrant further studies, no conclusions can be drawn about an association between *E. coli* and persistent diarrhea in HIV-infected patients since only a small number of patients was studied and no control group was included.

In a recent study from Wanke et al. (42), stool samples from HIV-infected patients with and without diarrhea were studied for the presence of EAggEC using the HEp-2 adherence test. EAggEC was present in stools of 30 (44%) of 68 patients with and 18 (30%) of 60 patients without diarrhea (p = .05). EAggEC was isolated in similar numbers from patients with acute and persistent diarrhea. CD4 cell count was significantly lower in patients with diarrhea than in patients without diarrhea but no significant difference in CD4 count was observed between patients with diarrhea associated with EAggEC and patients with EAggEC-negative diarrhea. Remarkably, none of the EAggEC isolates was positive in a PCR for detection of fimbriae encoding AAF/I genes, which has comparable sensitivity as the DNA probe. Eleven of 59 isolates tested were positive for toxin encoding EAST/1 genes as determined by PCR. None of the isolates showed cytotoxicity for Vero cells. However, in only 21 of 68 patients with diarrhea, stool samples were evaluated for other gastrointestinal pathogens. Although in 17 patients no other pathogens were found, no conclusion can thus be drawn about the etiological significance of EAggEC in diarrhea in this group of patients.

Finally, one report has been published, describing recurrent diarrhea and bacteremia due to EIEC in one patient with advanced HIV-infection (43).

From the studies described above, it can be concluded that it is quite well possible that certain strains of diarrheagenic *E. coli* are associated with persistent diarrhea in HIV-infected patients. However, these strains apparently do not belong to any of the categories of diarrheagenic *E. coli* described so far since they are not of serotypes which are associated with these categories and they do not hybridize with probes for detection of specific virulence factors (40-42). However, the association of *E. coli* which show aggregative adherence to HEp-2 cells, with persistent diarrhea in HIV-infected patients deserves further exploration.
Inflammatory bowel disease

Although the etiology of inflammatory bowel disease (IBD) is not known, evidence from a variety of animal models suggests that the presence of bacteria in the intestine is required to sustain the inflammatory reaction (44,45). A number of studies have addressed the potential role of \textit{E. coli} in the etiology of IBD. A variety of materials and methods have been applied, including measurement of serum antibodies to \textit{E. coli} (46), determination of the presence of \textit{E. coli} antigen in intestinal resection specimens, and adherence properties of \textit{E. coli} strains isolated from stool samples or rectal biopsies from patients with IBD. The latter studies will be summarized here.

Dickinson et al. reported that in fecal samples from patients with ulcerative colitis (UC), the incidence of \textit{E. coli} which adhered to or invaded HeLa cells was increased, when compared with controls (47). In 35% of 23 patients with active UC and in 27% of 15 patients with quiescent UC, adherent or invasive strains of \textit{E. coli} were isolated. Such \textit{E. coli} strains were isolated in only 5% of 20 patients with various other forms of colitis, including infectious, and 5% of 20 normal controls (p < 0.05).

Pinder et al. studied the incidence of toxigenic, adherent, invasive, or hemolytic \textit{E. coli} strains, in stool samples from 28 consecutive patients with a first manifestation of clinical colitis and from 40 controls (48). Adhesive or invasive properties were found in 8 colitic patients (29%) compared with 1 control (3%, p < 0.01). However, no mention has been made of the composition of the control group nor of the methods used for determining adherence and invasiveness in this study, which is only reported in abstract form.

Burke and Axon extensively studied the adherence of \textit{E. coli} strains isolated from stool samples of patients with UC and Crohn's disease (CD), using a buccal epithelial cell adherence assay (49-52). Isolates from 18 patients with UC showed a high index of adherence (number of cells with >50 adherent bacteria minus number of cells with >50 adherent bacteria without \textit{E. coli} added/100) when compared with isolates from 15 controls. Those results were independent of the source of the buccal epithelial cells, i.e. either from patients or from controls (50). Subsequent comparison of adherence to buccal epithelial cells and HeLa cells between isolates from 25 patients with UC and 13 controls, yielded similar results. The median index of adherence of \textit{E. coli} from patients with UC to buccal epithelial cells was higher than the median index of adherence to HeLa cells (51). A more extensive study included 50 patients
with a relapse of UC, 9 with UC in remission, 13 with CD, 11 patients with infectious diarrhea due to *Salmonella* or *Campylobacter jejuni*, and 22 healthy controls (49). This study again showed that the median index of adherence of *E. coli* to buccal epithelial cells in patients with relapse of UC (43%, range 5-81%) was significantly higher than that in controls (5%, range 0-16%, $p < 0.001$) or patients with infectious diarrhea (14%, range 0-68%, $p < 0.005$) (49). No significant difference was observed between patients with CD (53%, range 30-71%) and UC in remission (30%, range 2-75%). Adherence to buccal epithelial cells appeared to correlate with high cell surface hydrophobicity of bacteria, as measured by the salt aggregation test (SAT), which in its turn is associated with the presence of adhesins (52). The authors suggest a possible role of adherent *E. coli* in the pathology of IBD, either as the primary etiologic agent or secondary because of the exposure of host cell receptors that are unmasked due to the inflammatory process.

Using the same buccal epithelial cell adherence assay, Giafferi et al. also found significantly higher indices of adhesion of *E. coli* isolated from stool samples of 40 patients with CD and 34 patients with UC compared with 18 controls (53). There was no influence on the adherence indices of disease activity, bowel site of disease, sulphasalazine treatment or previous intestinal resection.

Hartley et al. studied the adherence to buccal epithelial cells, HEp-2 cells and SAT of *E. coli* isolated from rectal biopsies of 25 patients with a first episode of UC, 20 patients with a relapse of UC, 44 patients with quiescent UC and 15 controls (54). They found no significant difference in the distribution of adherent strains to HEp-2 cells between the respective colitis patient groups and there was no association with disease activity. The majority of the strains showed a diffuse adherence pattern. In addition, the hydrophobicity of the strain did not differ between groups and did not correlate with adherence activity. Patients with active colitis were colonised less frequently and with lower numbers of *E. coli* than were control patients. The buccal epithelial cell assay yielded highly irreproducible results. In addition, the buccal epithelial cells which bound *E. coli* also tended to have resident salivary flora attached, which was not readily removed by washing, and which made the assessment of *E. coli* adherence even less reliable (54).

In a recent publication, *E. coli* strains isolated from resected chronic ileal lesions of 20 patients with CD, neoterminal ileum of 19 patients with and 11 patients without recurrence of CD, and from the ileum of 13 controls, were studied (55). None of the strains harbored any of the virulence factor-encoding genes of *E. coli* involved in acute enteric diseases, as determined
by DNA hybridization or PCR. However, adhesion to differentiated Caco-2 cells of *E. coli* isolated from patients and controls differed. Adhesion was found for 11 of 13 (84.6%) strains isolated from chronic ileal lesions and 15 of 19 (78.9%) strains isolated from patients with recurrent CD compared with 4 of 7 (57.1%) strains isolated from patients with CD without recurrence and 3 of 9 strains (33.3%) isolated from control specimens (*p* < 0.02 for chronic lesions and recurrences vs controls). A number of adherent strains were shown to possess genes encoding hemolysin production, Pap or Sfa adhesins which are characteristic of strains involved in urinary tract infections, or genes which hybridized with the *daaC* probe, associated with diffuse adherence to HEp-2 cells. The authors suggested that the adherence of the isolates not possessing known adhesins, to Caco-2 cells, is mediated by the presence of as yet unidentified adhesins. The adhesive ability and hemolysin production of *E. coli* would allow the bacteria to colonize intestinal epithelium, damage intestinal cells, and participate in the inflammatory disease (55).

In another study, *E. coli* strains isolated from rectal biopsies of 20 patients with UC, 2 patients with CD, 5 patients with colitis of unknown cause and 24 healthy subjects were compared with respect to expression of cell surface hydrophobicity, carriage of intestinal virulence factors as determined with DNA probes, adhesion to tissue culture cells, and expression of binding of extracellular matrix proteins and plasma proteins (56). Most of the strains isolated from patients with colitis expressed low surface hydrophobicity compared with isolates from controls. Strains from 8 patients with UC or CD, 1 patient with proctitis and 1 control hybridized with synthetic oligonucleotides for detection of shiga toxins. Strains from one of these patients hybridized with the *eae* probe of EPEC and EHEC. *E. coli* obtained from patients with IBD adhered to HT-29 cells in higher numbers than strains from healthy controls. In addition, significantly more strains from patients with IBD than from healthy subjects expressed binding to fibronectin, collagens, laminin, vitronectin, plasminogen, thrombospondin and fibrinogen. These proteins are likely to be present in the extracellular matrix of the colonic epithelium during inflammation. It is suggested that binding of *E. coli* to these proteins enhances adhesion to colonic mucosa and contributes to tissue damage. A subset of adherent *E. coli* may express virulence factors which may aggravate a relapse of IBD.

The isolation of verocytotoxin producing *E. coli* from patients with UC has been reported by several authors (56-58). However, several other investigators have not been able to isolate such strains of *E. coli* from patients with IBD or from controls (53,55,59,60). In a prospective study, the microbiologic findings during 61 first attacks of IBD showed that
classical EHEC was not isolated from stool samples or rectal biopsies (60). Other E. coli which hybridized with DNA probes for detection of virulence factor-encoding genes from EPEC,EAggEC or EIEC were also not isolated. However, it can not be excluded that in isolated cases, infection with EHEC is mistaken for UC or that UC or CD are initiated by infection with EHEC.

The differences in isolation rates of potentially pathogenic E. coli between the various studies can largely be explained by differences in methodology. In particular, in the majority of the studies, strains isolated from either stool specimens or rectal biopsies were studied. Furthermore, adherence of E. coli was determined phenotypically in most studies. Test results could vary because of differences in the type of epithelial cells to which the bacteria were allowed to adhere, the duration of the incubation period with the epithelial cells, the number of isolates tested from each patient, the bacterial growth media used and observational differences. Only results obtained with objective methods, such as hybridization with DNA probes, are comparable.

In conclusion, E. coli strains which adhere to intestinal epithelial cells or to epithelial matrix proteins may contribute to an inflammatory response. Whether a specific pathogenic E. coli strain, harboring as yet unknown virulence factors, causes IBD remains to be determined. To date, the possible contribution of E. coli in the pathogenesis of IBD remains obscure.

Conclusions

While there is little doubt about the increasing importance of diarrheagenic E. coli as a cause of acute diarrhea, the role of E. coli in the pathogenesis of persistent diarrhea has not been established. Very few studies have been published providing evidence that the presence of diarrheagenic E. coli is related to chronic disease. In addition, results of the various studies on diarrheagenic E. coli are difficult to compare since the methods used for detection of diarrheagenic E. coli are diverse. In this context it is important to realize that for the detection of adherent E.coli, such as EAggEC and DAEC, the HEp-2 adherence test is considered the gold standard.

Studies investigating the etiology of persistent diarrhea are often hampered by the fact that only single stool samples from patients with diarrhea are collected and cultured. An association between a certain pathogen and persistent diarrhea requires the presence of the
pathogen in sequential samples, obtained during the acute as well as during the persistent phase of the diarrheal episode. Few of the reported studies fulfill this requirement.

Further studies, which incorporate the aforementioned issues, may shed more light on the association of diarrheagenic *E. coli* with persistent diarrhea.
Reference List


