Enteric infections due to *Escherichia coli*

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Readers are invited to use this article as a self-assessment exercise and to update their knowledge.

BACKGROUND

Bacterial diarrheas cause the death of 1.5 million children under 5 years old each year. *Escherichia coli* is one of the most common diarrheagenic bacteria in both developing and developed countries. *E. coli* serotype O157:H7, not previously recognized as diarrheagenic, was reported to be the causative agent of a multistate outbreak of hemorrhagic colitis (HC) in the USA in 1982. The illness was characterized by severe abdominal cramps, initially watery diarrhea followed by grossly bloody diarrhea and slight or no fever [1]. A number of outbreaks of *E. coli* O157:H7 have been reported in other developed countries (Canada, Japan and Europe) since 1982, sometimes associated with hemolytic uremic syndrome (HUS). The recent outbreak of *E. coli* O157:H7 in Scotland (November–December 1996) involved about 400 cases. Ten patients died as a result of the infection, and a number of patients were seriously ill for several weeks [2].

DISCUSSION POINTS

The increasing frequency of *E. coli* O157:H7 outbreaks in developed countries and the gravity of such food poisoning mean that early identification of these pathogenic strains in patients from the first clinical symptoms of infection is vital. Diarrheagenic *E. coli* comprises a heterogeneous group of pathogenic bacteria. They have been classified into six different pathotypes based on clinical aspects of the disease and identification of virulence factors produced by the isolates: enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), diffusely adherent *E. coli* (DAEC) and Shiga-like toxin-producing *E. coli* (SLTEC). The frequency of each pathotype depends on the geographic area and varies among epidemiologic studies.

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MULTIPLE-CHOICE QUESTIONS

In each of the numbered questions, at least one, and up to five, of the individual entries are correct. (The answers are at the end of this article.)

1. With regard to enteric *Escherichia coli*

(a) Enteropathogenic *E. coli* strains are the main cause of traveler's diarrhea. True/False

(b) All pathotypes produce toxins as virulence factors. True/False

(c) The LT toxin of enterotoxigenic *E. coli* is similar to cholera toxin. True/False

(d) Only enteropathogenic *E. coli* strains cause 'attaching and effacing lesions'. True/False

(e) Enteroinvasive *E. coli* infection typically presents as a dysentery-like illness. True/False

2. With regard to SLTEC infection

(a) Non-O157 strains are commonly implicated in cases in Europe. True/False

(b) Direct person-to-person transmission does not occur. True/False

(c) The level of infectious inoculum is high. True/False

(d) Cattle comprise the main reservoir of infection. True/False

(e) Hemolytic uremic syndrome occurs in 25% of cases. True/False

3. With regard to HUS

(a) It is characterized by thrombasthenia. True/False
(b) It is most frequently diagnosed in adults. True/False
(c) It usually develops 1 week after the onset of diarrhea. True/False
(d) It may result in chronic renal impairment. True/False
(e) It is only associated with SLTEC strains. True/False

4. With regard to the Shiga-like toxins (SLT)

(a) The type of SLT produced depends on the strain serotype. True/False
(b) Almost all SLTEC strains produce SLT-II. True/False
(c) All SLTs have a similar mechanism of action. True/False
(d) SLT-II is the most potent. True/False
(e) SLTs cause endothelial cell damage. True/False

5. With regard to the identification of SLTEC strains

(a) SLTEC strains are mostly detected in stools during the diarrheal phase of infection. True/False
(b) SLTEC strains are often detected in the stools of patients with HUS. True/False
(c) O157 strains can be reliably detected using sorbitol–MacConkey plates. True/False
(d) Serotyping of strains is only necessary during outbreaks. True/False
(e) Antibodies against the lipopolysaccharide are often detected in patients. True/False

6. With regard to the treatment of SLTEC infections

(a) Resistance to commonly used antibiotics is a growing problem with SLTEC. True/False
(b) ~10% of HUS patients require dialysis. True/False
(c) HUS patients often require transfusions of erythrocytes and/or platelets. True/False
(d) Antimotility agents increase the risk of progression to HUS. True/False
(e) Long-term antibiotic therapy may be needed to effect clearance of the organism. True/False

COMMENTS

Question 1

The mechanisms involved in altering intestinal electrolyte transport depend on the *E. coli* pathotype. They may involve toxins, adherence to epithelial cells, invasion of epithelial cells or a combination of several factors [3].

ETEC strains are a major cause of diarrhea in children in developing countries and are the most important agents responsible for traveler's diarrhea. They cause watery diarrhea and sometimes cholera-like syndromes with a risk of rapid dehydration, particularly in children. These *E. coli* strains produce two different types of toxins: LT (thermolabile toxin) and ST (thermostable toxin). Both toxins induce active fluid secretion. Detection of the toxins and toxin-encoding genes is used to identify an isolate as an ETEC strain. Production of these toxins requires prior colonization of the human intestinal epithelium which is mediated by colonization factor antigens or putative colonization factors; designation of these colonization factors varies depending on the investigators who have identified them. Adherence of ETEC strains to intestinal epithelium does not seem to affect enterocytes or cause acute inflammation or tissue damage in vivo, but some strains invade intestinal cells in tissue-culture cells. The real significance of the invasive properties observed in vitro remains to be determined in vivo. The LT toxin has structural (75% identity at the amino acid level) and functional similarities to the cholera toxin. The toxin binds to the specific cell receptor GM1. It is then transferred to the cytosol of the epithelial cell, where it irreversibly ADP-ribosylates a stimulatory GTP-binding protein. This activates the adenylate cyclase on the basolateral surface of the cell, causing an increase in the intracellular concentration of cyclic AMP. This may cause the selective opening of the chloride channels of the cell and the secretion of chloride and water into the intestinal lumen. The ST toxin binds to a specific receptor STaR, located on the apical surface of intestinal epithelial cells. It stimulates the guanylate cyclase activity of the STaR receptor. This causes an increase in intracellular cyclic GMP concentration which activates a cyclic AMP-dependent protein kinase. This causes chloride secretion and/or inhibits absorption of sodium and water.

EIEC strains are responsible for dysenteric syndromes in developing countries. They cause a disease clinically indistinguishable from shigellosis. Ability to invade intestinal epithelial cells is determined by the presence of a 140-MDa virulence plasmid similar to that of *Shigella* strains. Detection of the invasion genes (mapping to a 35-kb region of the plasmid) is used to identify EIEC strains. The full process of epithelial cell invasion may involve four stages: (1) entry of bacteria into cells; (2) multiplication of bacteria within epithelial cells; (3) intra- and intercellular spread of bacteria; and (4) killing of host cells. In addition, 75% of EIEC strains

produce the Sen toxin, a molecule also synthesized by *Shigella* strains.

A heterogeneous group of *E. coli* strains associated with acute or persistent watery diarrhea have been identified that bind to human tissue-culture HEp-2 or HeLa cells. Three subtypes of these enteroadherent *E. coli* are identified according to their adherence pattern to HEp-2 cells.

EAEC strains bind to epithelial cells in vivo and in vitro with an aggregative or 'stacked-brick' adherence pattern. This phenotype is associated with the presence of a 65-MDa virulence plasmid. This plasmid is also associated with cytotoxic effects such as exfoliation of mucosal epithelial cells in biopsy-derived intestinal mucosa from pediatric patients [4]. Detection of this plasmid allows identification of EAEC strains. A 120-kDa heat-labile enterotoxin secreted by EAEC strains has been identified that causes an increase in intracellular calcium concentration in HEp-2 cells and stimulates calcium-dependent protein phosphorylation. Some strains produce an ST-like toxin called EAST. Studies using human volunteers have demonstrated the pathogenicity of EAEC strains in human infections.

EPEC strains cause a localized adherence pattern (LA). This phenotype is associated with the production of Bfp (bundle-forming pilus) fimbrial adhesins. The determinants coding for Bfp map to the EAF (EPEC adherence factor) plasmid. EPEC strains were identified as the causative agents of infantile gastroenteritis in developed countries in the 1950s. They are still a major cause of diarrhea in infants in developing countries. EPEC strains induce diarrhea if given orally to adult volunteers even if no toxin is detected. The characteristic intestinal epithelial cell lesions seen in vivo and in vitro, are known as 'attaching and effacing lesions (A-E lesions)'. They are correlated with the presence of a chromosomal locus LEE (locus of enterocyte effacement) and with the intimin-encoding *eaeA* gene. The A-E phenotype results from an intimate adherence of bacteria associated with effacement of microvilli. Important rearrangements of the cytoskeleton are observed beneath the adherent bacteria, with the accumulation of cytoskeletal proteins, including actin, α-actinin, talin and ezrin. Diarrhea may partly be caused by the loss of microvilli and the decreased ability of infected cells to absorb fluids. EPEC strains were previously defined as *E. coli* strains of certain O:H serotypes associated with infantile diarrhea. Advances in molecular pathogenesis have demonstrated that pathogenicity is not restricted to the classical EPEC serogroups or serotypes. Some strains belonging to the 'classical EPEC' serotypes are non-pathogenic and some *E. coli* strains belonging to other serotypes but with LA or A-E phenotypes should be classed as EPEC. Classical EPEC strains are now identified by the presence of at least two out of three virulence factors (EAF plasmid, *bfpA* or *eaeA* genes) rather than by serotyping.

The only known virulence factor of DAEC strains is their diffuse pattern adherence to cultured epithelial cells. Two families of gene clusters encoding diffuse adherence have been identified in DAEC strains: *afa/daa* and the AIDA (adhesin involved in diffuse adhesion)-encoding genes. The *afa* (afimbrial adhesin) family includes closely related gene clusters that code for an adhesin and an invasin involved in bacterial–epithelial cell interactions. Recent epidemiologic studies have implicated DAEC strains carrying *afa* genes as a cause of acute and persistent diarrhea in 2–6-year-old children in developing countries [5,6]. Several epidemiologic studies have demonstrated that the AIDA-encoding genes are rare (0–1%) in DAEC strains [5,6].

SLTEC strains involved in outbreaks of bloody diarrhea in developed countries are characterized by the production of toxins. The presence of an LEE locus also causes A-E cellular lesions similar to those caused by EPEC strains. SLTEC strains can be classified as enterohemorrhagic *E. coli* (EHEC) when they cause diarrhea or HUS.

Question 2

E. coli O157:H7 was the first serotype implicated in outbreaks of bloody diarrhea and HUS. Over 100 other *E. coli* serotypes have now been associated with similar diseases. Among these, O113:H21, O111:NM, O103:H2 and O26:H11 have been isolated from stools of children with HUS. O157:H7 remains the most common serotype associated with HC in the USA. Recent studies indicate that SLTEC strains of the O157 serogroup are the main cause of HUS in childhood in Europe [7,8]. Antibodies to O26 and O55 antigens have also been detected in sera of patients with HUS [8,9]. It seems that O157:H7 causes major outbreaks more often and leads to higher rates of systemic disease than any other serotype [10].

Cattle are thought to be the major reservoir of SLTEC strains. The main source of contamination is bovine-derived food, especially ground beef insufficiently cooked in 'hamburgers'. However, raw milk, apple cider, mayonnaise and water have also been identified as sources of contamination. Direct person-to-person and cattle-to-person contacts can transmit the organisms, showing that SLTEC strains are highly infectious. Very few organisms (100 bacteria) are required to cause disease as is the case in shigellosis [10].

SLTEC strains cause a range of symptoms, some of which mimic non-infectious disorders such as

appendicitis, diverticulitis, ischemic colitis, intussusception and inflammatory bowel disease. The frequency with which SLTEC strains cause non-bloody diarrhea is unknown, as such illness can pass unnoticed. The incubation period for *E. coli* O157 infection is usually 3 or 4 days. SLTEC infection begins with watery diarrhea that becomes bloody within 1 or 2 days. There is then abdominal pain but, unlike classic dysentery, it is not accompanied by fecal leukocytes and patients have either no or low-grade fever. The bloody diarrhea lasts between 4 and 10 days. There is a risk of developing HUS after HC, according to estimates based on incidence data for *E. coli* O157:H7 infections. Some patients also develop thrombotic thrombocytopenic purpura (TTP), a thrombotic microangiopathic disorder resembling HUS, associated with major neurologic abnormalities. This syndrome is not usually preceded by a diarrheal episode.

Question 3

Few SLTEC outbreaks have been reported in Europe so the incidence rate of HUS in SLTEC infections is unknown. *E. coli* O157:H7 is identified as the causative agent of about 1000 cases of HUS each year in the USA. HUS affects people of all ages but is most often diagnosed in children under the age of 10. About 10% of *E. coli* O157:H7 infections in children younger than 10 years old progress to overt HUS [10]. Some authorities recommend that infected children should not return to group settings until at least two negative stool cultures have been obtained, as SLTEC strains are highly contagious [11]. These measures may prevent outbreaks in day-care centers or schools.

HUS typically develops 1 week after the onset of diarrhea. HUS is characterized by microangiopathic hemolytic anemia, thrombocytopenia and acute renal failure. It can be complicated by seizure, coma, hemorrhagic or non-hemorrhagic strokes, colonic perforation, pancreatitis, glucose intolerance, hepatobiliary disorders, hypertension, respiratory distress syndrome and cardiomyopathy. Sudden deaths account for about 15% of HUS-related fatalities. Some patients apparently recover from HUS but later develop chronic renal abnormalities [12].

Other bacteria such as *Citrobacter freundii* and *Enterobacter cloacae* have been implicated as causative agents for HUS in Germany and Australia [13,14]. These enterobacterial isolates were found to produce Shiga-like toxins. *Shigella dysenteriae* which produces the Shiga toxin, also causes HUS [15].

Question 4

EHEC strains (O157:H7 and non-O157:H7) produce the bacteriophage-encoded Shiga-like toxins (SLTs) that cause many of the pathologic effects of SLTEC infections.

SLTs are structurally and functionally related to the Shiga toxin. Two different antigenic types have been described. The SLT type produced is not dependent on the serotype of the *E. coli* strain. SLT-I is 99% identical to the Shiga toxin and SLT-II is 55% identical to SLT-I. The SLT-II toxin is produced by 97% of EHEC strains, either alone or with SLT-I. The mechanism of action of these two toxins is similar. SLTs are heterotetramers composed of five B subunits which bind to the cellular receptor and a single A subunit which is responsible for the enzymatic activity (*N*-glycosidase). The *N*-glycosidase cleaves an adenosine in the 3′ region of the 28S rRNA component of the eukaryotic ribosomal complex thus inhibiting protein synthesis [16]. The main targets of SLT are endothelial cell beds in the kidney, brain and gut. The detachment of endothelial cells may expose platelets to the subendothelium and initiate coagulation in the capillary beds. The way in which the SLTs gain access from the intestinal lumen to the target tissues via systemic dissemination is not understood, but SLTs have been shown to cross intact intestinal epithelial cell barriers in vitro. SLTs are enterotoxic, effecting fluid accumulation in ligated rabbit ileal loops. The colonic disease that progresses to HUS typical of humans is not observed in animals but some features of the disease can be reproduced in the mouse system. Streptomycin-treated mice orally infected with SLTEC die within 4–10 days (depending on the size of the inoculum). The toxicity of an *E. coli* isolate in mice is not related to the serotype but to toxin production. The virulence of SLTEC strains in mice depends on the SLT type produced by the isolate. SLT-II is much more toxic than SLT-I [17]. The amount of toxin produced by the isolate also affects the virulence of the strain.

Question 5

SLTEC infections are mostly detected during large outbreaks but they probably occur more frequently as sporadic cases. Identification of SLTEC strains is currently performed by microbiological, serologic, and molecular techniques but none is satisfactory.

The microbiological approach has several problems. The organism is usually sought only in stools that are obviously bloody, so milder SLTEC infections are underdiagnosed. The SLTEC strain is often not isolated from stool cultures because it is rapidly cleared from the gastrointestinal tract (two-thirds of patients with HUS no longer have pathogenic *E. coli* in their stools) and also because detectable excretion of the strain in the stools is often intermittent. The SLTEC strain often makes up less than 1% of the aerobic fecal flora when

excreted by patients. Stool cultures may thus test negative for the organism due to the low numbers of SLTEC bacteria in stools.

The *E. coli* O157:H7 serotype can be selectively screened in stool specimens using sorbitol–MacConkey (SMAC) medium. This selection is based on the fact that most fecal *E. coli* strains have a sorbitol-fermenting phenotype whereas *E. coli* O157:H7 strains do not ferment sorbitol. The inclusion of potassium tellurite, cefixime and rhamnose in SMAC medium increases selection for *E. coli* O157. SMAC medium is not suitable for detection of non-O157:H7 SLTEC strains or sorbitol-fermenting O157:H7 strains (some have been described). Some non-O157:H7 SLTEC strains have also been identified as non-sorbitol-fermenting strains.

Agglutination of a bacterial suspension by O antibody-coated latex particles is the usual method for detection of the O157 antigen. This test is not foolproof since non-O157:H7 SLTEC strains are not identified. O157 strains can be selectively isolated from stools using the immunomagnetic (super paramagnetic beads coated with anti-O157 antibody) separation culture enrichment method. These results must be confirmed biochemically because O157 antigens from other bacterial pathogens can cross-react with *E. coli* O157 antiserum.

Serologic identification is required when stool cultures from patients test negative during the diarrheal illness or when stool specimens cannot be obtained. Detection of antibodies against SLTs is not used, as the anti-SLT response in infected patients is poor. Antibodies directed against the O antigen have often been detected in the first few months after HUS. The magnitude of the response may be related to the severity of illness and is larger in patients who have developed HUS than in those who have not. Detection of antibodies against non-O157 O antigens is less well documented.

To detect SLTEC strains of both O157:H7 and non-O157:H7 serotypes, hybridization techniques and the polymerase chain reaction are also used. These techniques allow rapid and specific detection of virulence factor genes specific for SLTEC strains (the SLT-encoding genes) [18]. The intimin-encoding *eaeA* gene can also be sought, although not all SLTEC strains contain the gene [19].

Free fecal toxin can be identified in the stools of some patients. The production of SLTs and the cytotoxic activity in samples can be tested by determining the effect on the Vero cell line. However, this test is not easy to perform in clinical microbiological laboratories. Enzyme immunoassays allowing specific detection of SLTs in stool samples are being developed and should make identification of SLTEC strains of any serotype possible.

Question 6

Most *E. coli* O157:H7 isolates are susceptible to a broad spectrum of antibiotics such as ampicillin, trimethoprim–sulfamethoxazole, tetracycline, and quinolones. However, the use of antibiotic treatment to eradicate the strain from the intestine is controversial. Toxin production is one of the most important factors in SLTEC diseases, and antibiotic treatment which kills the bacteria may cause an increased release of toxins in the intestinal lumen. Thus, such treatment may worsen the illness. The risk of developing HUS is the same or greater in patients treated with antibiotics than in those not treated with these agents [20,21]. No significant effect of trimethoprim–sulfamethoxazole treatment on progression of symptoms, fecal pathogen excretion or the incidence of HUS was found in children with proven *E. coli* O157:H7 enteritis [22]. Thus, further studies are required to investigate the safety and efficacy of antibiotic therapy.

Administration of antimotility agents is not recommended for people with bloody diarrhea, because they may delay clearance of the pathogen from the gastrointestinal tract. Also, these agents may increase the risk of HUS following SLTEC infection [23].

Intravenous rehydration with isotonic saline solution is recommended for patients with diarrhea and vomiting. Seventy-five per cent of patients with HUS require transfusion of erythrocytes or platelets or both and 50% require dialysis. It is thus essential to monitor peripheral blood count, blood film and renal function of these patients.

CONCLUSION

E. coli is a bacterial pathogen of major importance in medical bacteriology. Unlike other pathogenic bacteria (such as *Vibrio cholerae* or *Yersinia enterocolitica*), *E. coli* is associated with different intestinal pathologies. Ideally, the diagnosis of enteric *E. coli* infection should be made with a combination of microbiological, biochemical, serological and genetic approaches. However, in practice, such a combination of diagnostic methods is rarely used. Each *E. coli* pathotype is defined by the production of specific virulence factors. Over the past few years, the polymerase chain reaction (PCR) has become a powerful technique for detection of these virulence factors. In the future, PCR assays which are specific, rapid and easy to perform may be recommended for routine clinical use to identify enteric *E. coli* of the ETEC, EIEC, EAEC, EPEC, and DAEC types. Immunoassays to detect SLTs in stools

should be developed and improved for detection of SLTEC strains, which are not always easy to isolate during infection. However, not all *E. coli* isolates from diarrheic stools can be classified into one of the six defined pathotypes. The development of DNA probes to identify these uncharacterized enteric *E. coli* strains is required for the determination of the role of these strains in the pathogenesis of diarrheal disease.

Answers to the multiple-choice questions

Q1: a. False; b. False; c. True; d. False; e. True
Q2: a. False; b. False; c. False; d. True; e. False
Q3: a. False; b. False; c. True; d. True; e. False
Q4: a. False; b. True; c. True; d. True; e. True
Q5: a. True; b. False; c. False; d. False; e. True
Q6: a. False; b. False; c. True; d. True; e. True

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