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ESCHERICHIA COLI ENTEROHEMORAGIC – AN EMERGED PATHOGEN OF HUMAN INFECTIONS
Part II. Non-O157 *Escherichia coli* enterohemorrhagic

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Abstract. The incidence of the non-O157 enterohemorrhagic *Escherichia coli* (EHEC) - non-O157:H7 Shiga toxin-producing *E. coli* (STEC) infections increased in the last years. Many serotypes of this toxigenic *E.coli* group were isolated from hemolytic uremic syndrome (HUS) and hemorrhagic colitis (HC) cases. The STEC strains possess many virulence factors codified by plasmids. Among the over 200 non-O157 STEC serotypes, more than 100 serotypes have been associated with HC or HUS in humans. The serogroups O26, O103, O111 and O145 are most commonly isolated from humans and are clearly recognized as human pathogens. STEC can be found in the fecal flora of a variety of animals. Domestic cattle and other ruminants constitute a natural reservoir for STEC. Usually STEC are transmitted to humans by food, water and from person to person. Foods, particularly foods of animal origin, the beef meat, represent a major vehicle of transmission of non-O157 STEC. The detection of the STEC is possible by many systems: Stx-specific PCR, Vero cell toxicity test, Enterohemolysin-agar, Stx-EIA on direct stool, Stx-EIA on stool culture grown overnight in TSB. Serological diagnosis is also possible. Antibodies to Stx or LPS have been proposed as markers of recent infection.

Key-words: non-O157:H7 Shiga toxin-producing *Escherichia coli* (STEC), infection, serotype, source, laboratory diagnosis, EHEC

Rezumat. În ultimii ani, în întreaga lume, a crescut semnificativ numărul îmbolnăvirilor cauzate de EHEC/STEC. Multe serotipuri aparţinând acestui grup de colibacili au fost izolate de la pacienţi cu sindrom hemolitic uremic (HUS) şi colită hemoragică (HC). Tulpinile STEC posedă diverşi factorii de virulenţă codificaţi de gene cu localizare plasmidică. Din cele peste 200 serotipuri STEC cunoscute, peste 100 sunt asociate cu HUS şi HC. Mai frecvent izolate din infecţii umane sunt serotipurile O26, O103, O111 şi O145, unanim recunoscute ca patogeni pentru om. Tulpini STEC sunt prezente în flora fecală la diverse specii de animale. Bovinele şi alte rumegătoare reprezintă rezervorul natural pentru STEC. Transmiterea la om se face pe calea alimentului, a apei şi prin contact direct interuman. Alimentele, în special cele de origine animală, şi mai ales carnea de vită, sunt recunoscute ca o cale importantă de contaminare cu non-O157 STEC. Diagnosticul de laborator al infecţiei cu STEC se poate face prin investigaţii bacteriologice şi serologice. Sunt puse la punct diverse sisteme de evidenţiere a bacteriei: testul de toxicitate pe culturi celulare Vero, amplificarea genică (PCR) pentru detectarea genelor Stx1 şi Stx2, teste imunenzimatice de decelare a toxinelor, evidenţierea enterohemolizinelor pe medii speciale. În diagnosticul serologic au fost propuşi ca markeri ai infecţiei anticorpi faţă de Stx şi LPS.

Cuvinte cheie: *Escherichia coli* producătoare de toxine Shiga non-O157:H7 (STEC), infecţie, serotip, rezervor de bacterie, diagnostic de laborator

INTRODUCTION

In recent years the incidence of *Escherichia coli* O157:H7 and non-O157:H7 Shiga toxin-producing *Escherichia coli* (STEC) infections increased. The emergence of new serotypes of the bacteria O111, O145, O26 and O103 has highlighted the need for an increase in epidemiological studies on these pathogens to monitor their incidence and spread (1-4). The morbidity and mortality associated with several recent large outbreaks of gastrointestinal disease caused by STEC has determined to consider these organisms as a public health problem (1-5).

The O157:H7 STEC is the dominating causative agent of numerous outbreaks and sporadic cases of food borne STEC infections in the USA, Canada, the UK and Japan. In continental Europe, non-O157 STEC seems play an important role as disease-causing agents, especially STEC O26, O103, O111 and O145. The STEC O118, a serogroup previously associated mainly with calf dysentery, has recently been recognized as another emerging STEC associated in human disease (1-4).

In this review some epidemiological and microbiological data concerning the non-O157 STEC serotypes, which may be implicated in human infections will be presented.

Clinical manifestations

The disease caused by STEC ranges from self-limiting diarrhea to hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (2,5,6,7).

Non-O157 associated intestinal disease includes watery diarrhea, usually with pain full cramps, and HC.

Extraintestinal manifestations include HUS, or incomplete forms of the syndrome that consist of only one or two of the three features associated with it, namely thrombocytopaenia, haemolytic anaemia and acute renal failure. About 75% of patients with HUS caused by non-O157 STEC require peritoneal or haemodialysis. Uncommon complications of STEC infections include urinary tract infection and, particularly in women after pregnancy and in elderly patients, thrombotic thrombocytopaenic purpura (TTP) (4,6-9). Either caused by O157 STEC or non-O157 STEC, the treatment for severe complications of infection (HC, HUS, TTP) is non-specific and generally insufficient. Most patients with HUS require extended ambulatory treatment, resulting in significant healthcare costs (2,4).

Pathogenesis

E.coli O157: H7 and non-O157 STEC, like the enteropathogenic *E.coli* (EPEC) that cause infantile diarrhea, have the ability to disrupt the intestinal epithelium by intimately adhering to enterocytes (2,10). Following attachment the shiga toxin, also known as Shiga-like toxin (SLT) or verotoxin (VT) is elaborated. The Stx family contains two major, immunologically non-cross-reactive groups called Stx1 ad Stx2, encoded by the stx_1 and stx_2 genes. The nomenclature of Shiga toxins proposed by Calderwood, 2000(2) is presented in table 1.

Table 1. Nomenclature of members of Shiga toxin family proposed by Calderwood – cited by Paton, 2000

Previous nomenclature	Proposed new nomenclature	
	Gene	Protein
Shiga toxin (Stx)	*Stx*	Stx
Shiga –like toxin I (SLT-I) or verotoxin 1(VT1)	stx_1	Stx1
SLT-II or VT2	stx_2	Stx2
SLT-IIc or VT2c	$stx_{2\ c}$	Stx2c
SLT-IIc or VT2e	$stx_{2\ e}$	Stx2e

Stx1 is virtually identical in amino acid sequence to the shiga toxin of *Shigella dysenteriae*. The details about the Stx and another virulence factors of STEC, especially for O157STEC serotype, were presented in another paper (11).

Diarrhea is thought to occur by toxin action on epithelial cells, and HUS as a result of absorbed toxin acting on renal endothelial cells.

Most STEC isolates from patients possess additional virulence characteristics such as the ability to cause attaching-and-effacing lesions (A/E) on intestinal epithelial cells (2,4,12,13).

The A/E lesions are characterized by the effacement of intestinal epithelial cell microvilli and intimate adherence of the bacteria to the epithelial cell membrane (2,12,13).

The genes encoding proteins involved in producing the A/E lesions are located on a 43-kb pathogenicity island termed LEE (locus of enterocyte effacement) (12,13). The LEE has three functional regions. The central region contains the *eae* gene, which encodes a 94-97 kDa outer membrane protein called intimin, which is an EHEC adherence factor. The *tir* gene encoding a translocated intimin receptor (Tir) is also located in the central region. Downstream of *eae* are located the *esp* genes which encode secreted proteins responsible for inducing the epithelial cell signal transduction leading to the development of A/E lesions. The region upstream of the *eae* and *tir* contains many genes (*esc* and *sep*) encoding a type III secretion apparatus involved in the extra-cellular secretion of the proteins encoded by the *esp* genes (2,13).

However, STEC strains, which are *eae*A negative, are known to cause disease including HUS and several other putative adhesin have been identified (2,4,12,13).

Other virulence factors of STEC are the production of enterohaemolysin (Ehly) and, possibly, heat-stable enterotoxin (EAST1). Genes encoding Ehly are located in the 60-Mda plasmid found in nearly all O157: H7 strains and also widely in non-O157 STEC strains. This enterohemolysin acts as a pore-forming cytolysin (2,6,7,12-16).

Approximately 90% of all STEC strains isolated in Germany from patients possessed genes encoding

Ehly (6,15,16). Eklund et al. in a study on 62 non-O157 STEC isolates (21 strains from patients with HUS or TTP) pointed out that the strains with *stx:eae:Ehly* present were significantly more frequently associated with HUS and bloody diarrhea than the strains without these virulence profiles (17). The enterohemolysin belongs to the RTX (repeat in toxin) family of hemolysin but differs in its target cell specificity and lytic activity from the prototypic E.coli α-hemolysin (Hly) associated with extraintestinal infections (15,16).

Beutin et al., classified a big number of the non-O157 isolates with regard to serotype, toxin pheno/genotype and enterohemolysin into two distinct groups of strains-Group 1, all *eae*A positive and, with one exception enterohemolysin producers. These were associated with more severe diarrhea disease, the development of HUS and a younger age range than group II strains which were *eae*A negative and which were associated with milder symptoms or asymptomatic carriage (14).

Virtually all EHEC strains possess a large plasmid of approximately 75 to 100 kb in size which encodes determinants that may serve as additional virulence factors (12). The *katP* gene is responsible for the bifunctional catalase-peroxidase (KatP), the *espP* gene encoding a serine protease (EspP), which cleaves human coagulation factor V, and the *etp* gene cluster that encodes a type II secretion pathway system (2,12).

EAST1, first described in EAEC, is also found in many STEC strains. The significance of EAST1 in the pathogenesis of STEC is unknown, but it might account for some of the non-bloody diarrhea frequently seen in persons infected with STEC. (18).

Epidemiology of STEC disease

Non-O157 STEC infections are found in 20-70% of patients with STEC – associated disease, depending on geographical location. The STEC infections were found more frequently in infants, children and elderly patients without gender differences (2,4-6).

Variations in the frequency of isolation of non-O157 STEC compared with O157 and other pathogens in diarrhea cases were related and the differences in the ecology and epidemiology of specific non-O157 STEC serotypes were pointed out (15,16). The incidence of non-O157 STEC is not well known. In a recent study of 3,289 diarrhea samples from clinical laboratories in the United States, non-O157 STEC were more frequent than O157 serotype. In a study concerning the prevalence of non-O157 STEC in the northen United States, where cattle and other animal reservoirs of STEC are abundant, 4.2% of the samples were positive for STEC (1,2). Studies from Europe have shown that the prevalence of STEC in diarrhea samples was 0.3% to 9.3% and serogroup O157 STEC prevalence of 0% to 2.7%. Five different non-O157 STEC were isolated: O111:NM, O26:H11, O145:NM, O103:H2 and O rough:H2. In Germany, STEC figures at the second rank among human infections caused by enterohemorrhagic *E.coli* (3,15,16,19).

Serotypes isolated from human infections

The most frequently implicated STEC causing HC and HUS are the serotype O157: H7.There are many other STEC serotypes implicated in diarrhea disease, and several non- O157: H7 serotypes have been implicated as the cause of foodborne outbreaks and HUS in the United States, Europa, Australia, Canada (2-6,14-16). Studies from Canada, Europe, Argentina and Australia suggest that non- O157: H7 STEC infections are present in the some frequency or more so than O157: H7 infections (5). Among the over 200 non-O157 STEC serotypes, more than 100 of *E.coli* serotypes have been associated with HC or HUS in humans and those in serogroups O26, O103, O111 and O145 are most commonly isolated from humans and are clearly recognized as human pathogens (1-4). The frequencies of non- O157 STEC isolated from HUS patients ranged between 7% to 90% (1-4,20-29).

In England and Wales, the informations on the incidence of infection caused by STEC belonging to serogroups other than O157 are very limited because very few laboratories screen for these organisms. It has been recommended that patients with bloody diarrhea or haemolytic uremic syndrome (HUS), whose stools are negative for E.coli O157, should be investigated for non-O157 STEC infection (20). During the period 1995-2000, 16 strains of non-O157 STEC were identified in stool samples, from patients by the Laboratory of Enteric Pathogens. By serotyping six strains were serogroup O26, and the rest were O8, O75, O76, O103, O116, O118, O128, O146 or "rough strain". Three strains (O8, O26, O103) were from patients who developed haemolytic uremic syndrome (20).

Among the non-O157 STEC strains, the nonmotile STEC O111 strains were most frequently found in HUS patients from Europe also. In Canada this serotype was associated with HUS as early as 1983 (4,21-23). Two outbreaks of HUS caused by STEC O111 in South Australia and Italy have recently been reported (4,21). In Italy from May 1988 to December 2000 were reported 342 HUS cases. Twenty-four cases were part of two outbreaks that occurred in northern Italy in 1992 and 1993, which were associated with *E.coli* O111 and O157. Other cases associated with O26 serotype infection occurred in spring 1997 in southern Italy (23). The magnitude of the public health problem rised by *E.coli* O111 and other non-O157:H7 STEC strains can be only roughly estimated since most laboratories do not screen stool samples for these pathogens (22). Table 2 summarizes the non-O157 STEC serotypes that have been isolated from humans.

Dr.K. Bettelheim from National *E.coli* Reference Laboratory, Melbourne, Australia realized an update list of STEC with literature references. As table data show; many serotypes of STEC have been implicated in HUS.

Several studies have emphasized the importance of non-STEC strains as a cause of human illness, including HUS/TTP, which may account for as many as 30% of all cases (24).

Table 2. Serotypes of non-O157 STEC isolated from humans* (WHO, 1999, completed)

O1:H-	O20:H7	O76:H19	O112:H21	**O145:H25**
O1:H1	**O21:H5**	O77:H4	O113:H2	**O145:H28**
O1:H2	**O21:H?**	O77:H18	O113:H4	O145:HNT
O1:H7	O22:H-	**O79:H7**	O113:H7	**O146:H8**
O1:H20	**O22:H1**	O80:H-	**O113:H21**	O146:H21
O1:HNT	**O22:H8**	O82:H-	O114:H4	O146:H28
O2:H1	O22:H16	O82:H5	O115:H10	O150:H10
O2:H5	O22:H40	O82:H8	O115:H18	O153:H2
O2:H6	O23:H7	O83:H1	O116:H19	O153:H11
O2:K1:H2	O23:H16	O84:H2	O117:H-	O153:H12
O2:H7	O25:H-	**O85:H-**	**O117:H4**	**O153:H25**
O2:H9	**O25:K2:H2**	**O85:H10**	O117:H7	**O154:H-**
O2:H27	**O26:H-**	**O85:H23**	O117:K1:H7	**O154:H4**
O2:H29	O26:H8	O90:H-	O117:H19	**O154:H19/20**
O2:H44	**O26:H11**	O91:H-	O118:H12	**O161:H-**
O4:H-	O26:H21	**O91:H10**	**O118:H16**	**O163:H19**
O4:H5	O26:H32	O91:H14	O118:H30	O165:H-
O4:H10	O27:H-	**O91:H21**	O119:H-	O165:H10
O4:H40	O30:H2	O91:HNT	**O119:H5**	**O165:H19**
O5:H-	O30:H21	O98:H-	O119:H6	**O165:H25**
O5:H16	O30:H23	O98:H8	**O121:H-**	O166:H12
O6:H-	O37:H41	O100:H25	O121:H8	O166:H15
O6:H1	O38:H21	O100:H32	O121:H19	O166:H28
O6:H2	O39:H4	**O101:H-**	O123:H49	**O168:H-**
O6:H4	O39:H8	O101:H9	O124:H-	O169:H-
O6:H28	O45:H-	**O103:H-**	O125:H-	**O171:H-**
O6:H29	O45:H2	**O103:H2**	O125:H8	O171:H2
O6:H31	O45:H7	O103:H4	O126:H-	O172:H-
O7:H4	**O48:H21**	O103:H25	O126:H2	**O173:H2**
O7:H8	**O49:H-**	O103:HNT	O126:H8	ONT:H-
O8:H-	**O49:H10**	O104:H-	O126:H21	**ONT:H2**
O8:H14	O50:H-	**O104:H2**	**O126:H27**	ONT:H18
O8:H21	**O50:H7**	O104:H7	O128:H-	ONT:H21
O9ab:H-	O52:H23	O104:H21	**O128ab:H2**	**ONT:H25**
O11:H49	**O55:H-**	**O105ac:H18**	O128:H8	ONT:H47
O14:H-	**O55:H6**	O105:H20	O128:H12	O-rough:H-
O15:H-	**O55:H7**	O109:H2	**O128:H25**	**O-rough:H5**
O15:H2	**O55:H10**	O110:H-	O132:H-	**O-rough:H11**
O15:H27	**O55:H?**	O110:H19	O133:H53	**O-rough:H16**
O16:H-	O60:H-	**O111:H-**	O134:H25	O-rough:H20
O16:H6	O60:H16	**O111ac:H-**	**O137:H41**	O-rough:H21
O17:H18	**O69:H-**	**O111:H2**	O141:H-	**OX3:H2**
O18:H-	O70:H11	**O111:H8**	O143:H-	**OX3:H2**
O18:H7	O73:H34	O111:H30	O144:H-	* The bold faced are strains isolated from patients with HUS.
O18:H12	**O75:H-**	O111:H34	**O145:H-**	
O18:H?	O75:H5	O112ab:H2	O145:H16	

Sources of STEC

STEC can be found in the fecal flora of a variety of animals including cattle, sheep, goats, pigs, cats and dogs, horses and even seagulls.

Domestic cattle and other ruminants constitute a natural reservoir for STEC and healthy animals can excrete these organisms in their feces. The main route of STEC into the food chain is through contamination of meat by intestinal contents and feces in the abattoir (1,2,4-6,19).

Many studies have shown that cattle constitutes a worldwide reservoir for *E.coli* O157 and other STEC, and is a primary source of infection for humans. It was suggested that changes in lifestyle, consumer habits and food production contributed to the increase of infections with E.coli O157 and other STEC in industrialized countries (1,2,4,32-34).

Non-O157 STEC can also cause diseases in animals, such as diarrhea (or dysentery) in calves and oedema disease in pigs. Information is limited for other animal species. Non-O157 STEC associated with disease in animals belonging to a limited number of serotypes, some of which have also been associated with human infections. The STEC causing disease in cattle are frequently serotypes O5:H-, O26:H11, O103:H2, O111:H- and O145:H-(4,34). STEC strains have also been detected in cats and dogs with diarrhea (2). Natural and experimental infection of calves with a O111 STEC strain results in colitis with attachment and effacement of the colonic mucosa (2). Piglet edema disease, on the other hand, is a serious, frequently fatal STEC-related illness. It is characterized by neurological symptoms including ataxia, convulsions, and paralysis; edema is typically present in the eyelids, brain, stomach, intestine, and mesentery of the colon. This disease is associated with particular STEC serotypes (most commonly O138:K81, O139:K82, and O141:K85) (2,4,35).

The most STEC serotypes were found to be associated with only one animal species, indicating that they might be specific for their hosts (32,34,39).

The investigations carried out in geographically different places in Europe and North America revealed that 10 to 80% of cattle were infected with STEC (32,34). Serological analysis of O:H types of STEC isolated from different sources revealed that STEC are present in a high number of different *E.coli* serotypes. More than 100 different O:H serotypes of STEC have been isolated from cattle. Certain serotypes of STEC seem to be adapted for colonizing the intestine of their animal hosts well and become residents over long time periods.

The STEC serotypes isolated from different animal species are presented in table 3.

Usually STEC are transmitted to humans by food, water and from person to person. Foods are recognized as a major vehicle of transmission of O157 STEC and are likely to play the same role for non-O157 STEC, but there is limited information on the occurrence of non-O157 STEC in the food supply. Most cases are caused by ingestion of contaminated foods, particularly foods of animal origin, the beef meat, being a major vehicle of infection (1,2,4,5, 39,40).

Most foodborne outbreaks associated with E.coli O157:H7 have been traced to foods derived from cattle, especially ground beef and milk (1,2).

Although, most STEC cases are linked to undercooked hamburger and contact with food animals (1,2,5).

Table 3. Serotypes of non-O157 STEC isolated from animals (WHO, 1999, completed)

O1:H20	O17,77:H18	O69:H11	O103:H-	O128:H35	O163:H-
O2:H-	O18:H11	O69:H28	O103:H2	O130:H38	O163:H2
O2:H1	O20:H19	O71:H12	O104:H21	O131:H2	O163:H19
O2:H5	O21:K5:H4	O73:HNT	O105:H18	O132:H-	O168:H8
O2:H7	O22:H8	O74:HNT	O110:H2	O136:H-	O169:HNT
O2:H25	O22:H16	O74:H29	O110:H16	O136:H12	O170:H8
O2:H27	O22:H21	O75:H8	O111:H-	O136:H16	O171:H2
O2:H29	O22:H40	O76:H21	O111:H2	O136:H20	O172:H-
O2:H39	O26:H-	O76:H25	O111:H8	O139:H8	ONT:H-
O2:H49	O26:H11	O76:HNT	O111:H11	O139:H19	ONT:H2
O3:H-	O26:H21	O77:H4	O112:H2	O141:H4	ONT:H7
O4:H-	O27:H-	O80:H-	O113:H-	O145:H-	ONT:H8
O4:H4	O35:H21	O82:H2	O113:H4	O145:H8	ONT:H17
O4:H25	O38:H16	O82:H8	O113:H21	O145:H16	ONT:H18
O5:H-	O39:H7	O82:H40	O115:H8	O145:H28	ONT:H19
O5:H10	O39:H21	O84:H-	O115:H18	O146:H8	ONT:H21
O5:H11	O39:H40	O84:H28	O116:H-	O146:H21	ONT:H25
O6:H3	O39:H48	O85:H-	O116:H21	O147:H11	ONT:H31
O6:H10	O40:H8	O87:H16	O117:H4	O147:H29	ONT:H34
O6:H34	O43:H2	O87:H21	O118:H-	O152:H-	ONT:H42
O7:H4	O45:H-	O88:H25	O118:H16	O153:H-	O-rough:H7
O8:H2	O45:H2	O90:H24	O119:H8	O153:H9	O-rough:H8
O8:H9	O46:H2	O91:H-	O119:H25	O153:H12	O-rough:H14
O8:H19	O46:H38	O91:H10	O120:H-	O153:H19	O-rough:H19
O8:H25	O49:H-	O91:H14	O120:H2	O153:H21	O-rough:H21
O8:H35	O53:H2	O91:H21	O120:H18	O153:H25	O-rough:H34
O9:H-	O54:H21	O91:H49	O120:H42	O153:H31	O-rough:H38
O10:H21	O55:H17	O92:H-	O121:H7	O156:H-	O-rough:H42
O11:H8	O65:H-	O98:H25	O123:H10	O156:H7	O-rough:H47
O15:H-	O65:H48	O100:H-	O126:H20	O156:H21	OX3:H8
O15:H4	O68:H-	O101:H-	O126:H21	O156:H25	
O15:H27	O68:H14	O101:H14	O128:H2	O156:H46	

Outbreaks and sporadic cases of human infections with STEC are frequently associated with consumption of fecally contaminated foodstuff and water (1-4, 11,35-40). STEC serotypes isolated from foods of animal origin are summarized in table 4.

The environment is now recognized as increasingly important in the epidemiology of O157 and non-O157 STEC, in relation to persistence of the pathogen outside its main animal reservoir, the movement through animate and inanimate vectors, (re)contamination of food and (re)infection of animal hosts, including humans (40). STEC surviving in feces and in/on contaminated soil of farms and related environment may be washed by surface and ground water where they can persist for long periods (several weeks and/or months). Other water born outbreaks of STEC have been

Table 4. Serotypes of non-O157 STEC isolated from foods of animal origin (WHO, 1999)

Serotype	Food	Serotype	Food	Serotype	Food
O2:H32	sausage	O57:H-	beef	O117:H4	beef
O4:H21	retail beef	O60:H9	pork sausage	O117:H8	retail beef
O5:H-	beef	O62:H-	minced beef	O125ab:H-	minced beef
O6:H-	lamb	O62:H8	minced beef	O128:H2	pork sausage
O6:H10	lamb,milk,cheese	O65:H-	milk filters,	O128ab:H2	beef
O6:H34	milk,beef		lamb	O128:H30	minced meat
O7:H-	minced beef,lamb	O71:H21	minced meat	O128:H35	beef
O7:H16	minced beef	O73:H-	minced beef	O138:H-	sausage
O8:H-	minced beef,lamb	O73:H16	minced beef	O139:H19	beef
O8:H9	beef	O73:H31	minced beef	O146:H8	pork sausage
O8:H16	beef	O74:H-	minced beef	O146:H28	beef
O8:H19	beef	O74:H37	minced beef	O148:H8	minced meat
O8:H25	pork sausage	O74:H39	minced beef	O149:H45	retail beef
O8:H30	beef	O75:H5	lamb	O151:H12	beef
O8,60:H-	lamb	O79:H-	beef	O153:H25	milk filters, beef
O8,60:H51	lamb	O82:H8	beef	O156:H25	milk
O9:H-	pork	O84:H21	lamb	O166:H-	beef
O14:H-	minced beef	O87:H16	beef	O171:H2	beef
O15:H27	beef	O91:H-	pork sausage,	O171:H25	beef
O17:H18	minced beef		sausage, beef	ONT:H-	soft cheese,
O21:H21	milk	O91:H21	milk, beef		retail beef,
O22:H-	minced beef	O96:H-	lamb		lamb, milk,
O22:H4	minced beef	O100:H-	pork sausage,		sausage, beef
O22:H5	minced beef		beef	ONT:H2	milk, beef
O22:H8	unpasteurized milk,	O100:H16	retail chicken	ONT:H5	minced beef
	milk, sausage, beef	O103:H2	milk, beef	ONT:H7	milk, beef
O22:H16	beef	O103:H21	beef	ONT:H8	pork sausage,
O22:H54	beef	O104:H-	minced beef		milk filters, beef
O23:H-	minced beef	O104:H12	minced beef	ONT:H9	sausage
O23:H15	minced beef	O107:H7	minced beef	ONT:H10	pork
O26:H11	milk filters	O111:H-	meat	ONT:H16	beef
O26:H32	cheese	O112ac:H-	lamb	ONT:H18	sausage
O28:H4	beef	O112:H2	beef	ONT:H19	milk filters, beef
O30:H-	minced beef	O112:H21	meat	ONT:H21	sausage,beef
O30:H8	milk	O113:H-	minced beef,	ONT:H23	minced beef
O38:H30	minced beef		beef	ONT:H28	beef
O43:H2	milk filters	O113:H4	milk,sausage,	ONT:H47	minced meat,
O44:H-	milk filters		beef		beef
O46:H-	minced beef	O113:H19	beef	O-rough:H23	minced beef
O46:H8	minced beef	O113:H21	beef	O-rough:H48	beef
O49:H-	pork	O114:H4	milk	O-n:H8	meat
O54:H21	retail chicken	O115:H-	pork sausage	O-n:H8	meat
O55:H9	beef	O115:H10	pork sausage		
O56:H56	beef	O116:H-	cheese		

associated with accidental consumption of infective doses of the pathogen present in a wide range of recreational and unchlorinated waters (1,2,40).

In order to prevent outbreaks that could arise through contamination of widely distributed commercial food products, EHEC infections need to be monitored continuously in order to detect outbreaks early and to identify risk factors. Prevention is particularly important because antibiotic treatment of EHEC infections is not recommended on the grounds, which may cause the release of toxins predisposing to HUS (1,2,4,5,11,40).

Only seven European countries have a surveillance systems. EHEC infections are statutorily notable in three countries: Austria, Finland and Sweden. Five countries: Belgium, Finland, Italy, Netherlands and the United Kingdom have sentinel systems (40). 67 outbreaks caused by STEC have been reported. Fifty-six were in beginning 1992 in United Kingdom and 11 in others european countries. *E.coli* O157 was identified in 42 of the 47 outbreaks and in six of them was identified other serotypes: O111, O103, O86 (1-4,11,40,41).

Diagnosis

In many countries the STEC diagnostics are based on the detection of sorbitol-negative STEC O157: H7 strains only (1-10)**.** If *E.coli* O157: H7 may be easy differentiated from other *E.coli* by its incapacity to ferment sorbitol, the non- O157: H7 STEC was the phenotypically similar to commensal non-pathogenic *E.coli* and is not detected with sorbitol MacConkey agar (5,6,11,22). There are many difficulties associated with diagnosis of STEC infections (1,2). In the early stages of infections STEC is in a very large proportion in feces; in many cases the STEC represents more than 90% of the aerobic flora (2). In patients with HUS, the typical signs may become apparently only a week or more following the onset of gastrointestinal symptoms, and the number of the STEC is very small or the bacteria may have been eliminated from the gut. The rapid diagnosis is important to prevent unnecessary invasive and expensive surgical and investigative procedures or administration of antibiotic therapy, which may be contraindicated for STEC infection (2,5,6,12).

Diagnostic procedures are based on detection of the presence of *Stx* or Stx in fecal extracts or fecal cultures, and/or isolation of the STEC (2,4).

A method, which is suitable for identification and isolation of all STEC strains, should be based on the detection of Shiga-toxins or their genes. However, the detection of Stx-production or of Stx-related genes in clinical and other samples without attempting the subsequent isolation of an STEC strain is an incomplete investigation and should only be considered as a presumptive result (42-44).

Testing for STX

Tissue culture cytotoxicity assay. The large sensitivity of Vero cells to STEC was observed by Konowalchul et al-cited by Paton, and this cytotoxicity test for this cell line is considered the "gold standard" for confirmation the

Stx-producing isolates. The Vero cell cytotoxicity assay permits to demonstrate the presence of Stx in extract of feces or fecal culture filtrates. The assay consists of treatment of Vero monolayers with sterile extract of filtrates and examining cells for cytopathic effect after 48 to 72 h of incubation (2,4,6,12,24,43). The detection of Stx by tissue culture cytotoxicity is a good diagnostic method but it is labor-intensive and time-consuming (2).

ELISA for detection of Stx. During the past years many enzyme-linked immuno-sorbent assays (ELISA) have been developed for the direct detection of Stx1 and Stx2 in fecal cultures (2). The sensitivity of the various ELISA is affected by the avidity of the antibodies used and the type and amount of Stx produced by strains (2,6,12,24,44).

VTEC-RPLA - reverse passive latex agglutination. Another commercially available toxin-detection kit is the VTEC-RPLA (Oxoid), the principle of which is reverse passive latex agglutination. This kit can only be performed on bacterial colonies from solid media and not directly on fecal specimens (24). This method is not sufficiently sensitive for screening fecal cultures for the presence of Stx-producing bacteria (2,4,6,44).

Detection of STX genes

Hybridisation with DNA and oligonucleotide probes. The availability of cloned *stx1* and *stx2* genes enable the development of DNA probes for the detection of STEC (2,4,6,42-44).

PCR. The possibility to sequence data for the various *Stx* genes permitted the design a variety of oligonucleotide primer sets for amplification of *Stx* genes by PCR (2,4,6).

PCR has also used for the detection of genes encoding other virulence factors, such as *eaeA* and EHEC-*hlyA* in STEC strains (2,4,6,43,44).

Multiplex PCR assays, which detect genes of other virulence markers such as *eae*A, have been developed and, more recently, multiplex PCRs, which include primers for the detection of O-antigen coding genes have been described (22,23). Many authors suggest that stx PCR is as sensitive and specific as CT-SMAC culture and EIA combined, and therefore may be used as an alternate method to diagnose diarrhea infections caused by STEC. The results of some STEC strains tested by multiplex PCR amplification are included in the table 5 (5).

Table 5. Results from multiplex PCR amplification - Fey et al. 2000

Isolate	Serotype	*stx1*[a]	*stx2*	*eae*	*ehx*A
A3	O26:H11	+	-	+	+
B1	O145:NM	+	-	+	+
B2	O103:H2	+	-	+	+
D1	O111:NM	+	+	+	+
D2	O111:NM	+	+	+	+
E1	O rough:H2	+	+	+	+
E2	O26:H11	+	-	+	+

a = the presence of gene as assessed by PCR; -= absence of gene as assessed by PCR, NM= non motile.

Multiplex PCR was performed on isolated Shiga toxin-positive colonies to detect specific genes encoding Shiga toxins 1 and 2 (*stx*1 and stx_2), intimin (*eae*A) and enterohemolysin A (*ehx*A). All isolates, regardess of serotype, encoded *eae* and *ehxA*; two of seven non-O157 isolates encoded stx_2 (both O111:NM) (5).

Beutin et al., 1997, made a comparative study on 477 human clinical stool samples that were investigated for STEC. The majority of the samples were from patients with gastro-intestinal symptoms and 24 stool samples were from HUS-patients. Different STEC detection systems were used: Vero cell toxicity test, Stx-specific PCR, Shiga-toxin enzyme immunoassay (Stx-EIA), enterohemolysin-agar and characterization of fecal coliform isolates and VTEC-RPLA test (reverse passive latex agglutination test for detection of verocytotoxins 1 and VT2). Out of the 477 stool samples, 145(30.4%) were positive for STEC. None of the four different test systems was able to identify all the 145 stool specimens, which were positive for an STEC -table 6.

Table 6. Comparative evaluation of different STEC- detection systems (Beutin et al, 1997)

Test system	No. of samples	Relative sensitivity[a] %	Relative specificity [b]%	Relative agreement[c] %
Stx-specific PCR	477	82.1	93.7	90.1
Vero cell toxicity test	477	95.9	84.0	87.6
Enterohemolysin-agar	477	85.5	89.2	88.1
Stx-EIA on direct stool	131	61.5	84.8	74.6
Stx-EIA on stool culture grown overnight in TSB	136	78.9	91.1	86.0

a) relative sensitivity: number of samples reacting positive diveded by the total number of "gold-standard" positive samples. b) relative specificity: number reacting negative divided by the total number of "gold-standard" negative samples. c) relative agreement: sum of the true positive and negative test samples divided by the total number of samples examined.

The Vero cell toxicity test showed the highest sensitivity by detecting cytotoxic activity in 139 (95.9%) of the 145 samples, which were positive for STEC. The Stx-EIA ("Premier EHEC") was found less sensitive than the Vero cells. Only 32 (61.5%) of 52 tested STEC positive samples were detected by examination of direct stool specimens with this assay. Enrichment of bacteria by growing stool overnight in TSB improved the detection rate. By this means, 45 (78.9%) of 57 STEC positive samples were identified as Stx-positive with "premier EHEC" assay (43).

By Stx-PCR, 119 (82.1%) of 145 STEC positive stool samples were identified. A minimal amount of 100 bacteria per PCR reaction was found necessary for visible detection of an Stx-specific PCR product on agarose-gels (44).

A close association between the enterohemolytic phenotype and production of Stx was found with STEC strains belonging to the high virulent types (O157, O145, O111, O103, O26). These STEC types carry the *eae*-gene and are frequently associated with hemorrhagic colitis and HUS in humans. (43,44). The *eae*A gene was present in all STEC strains belonging to some common serogroups (O26, O103, O111, O118 and O145) and in O-rough strains. The enterohemolytic phenotype was expressed in many strains and was highly associated with *eae*A positive STEC. The *eaeA*-gene was closely associated with severe illness and young age.

A combination of different test systems is recommended for identification and isolation of STEC strains. Further work is necessary to increase the sensitivity and to improve the specificity of both, commercially available and laboratory based STEC detection systems.

Isolation of Shiga toxin-producing E. coli

The isolation of the STEC strain represents the definitive diagnostic procedure. The isolation of the strains permits characterization of STEC by O:H serotyping, phage typing, restriction fragment polymorphism, pulsed-field gel electrophoresis and amplification based DNA-typing (2).

In Europe is recommended the culture on Sorbitol - MacConkey - Agar (SMAC) (52-55).

Culture methods. The non-O157 STEC can't be differentiate by biochemical characteristics from commensal fecal *E.coli* strains. The significant proportion of non-O157 STEC strains produce EHEC-hly. The strains producing EHEC-hly are not hemolytic on standard blood agar. They produce small, turbid hemolytic zones on washed sheep agar supplemented with Ca^{2+} after 18 to 24 h of incubation at 37°C.The production of EHEC-hly has a high positive predictive value (2). Schmidt H et Karch investigated the enterohemolytic genotypic and phenotypic profiles of 36 O111:H^- STEC strains isolated from patients with HUS and diarrhea.20 strains were positive for EHEC-hly and the fact that a significant proportion of disease-causing STEC strains are EHEC-hly negative diminishes the usefulness of washed sheep agar in screening for primary isolation of STEC (22).

Immunomagnetic separation for isolation of STEC. Immunomagnetic separation –IMS- techniques have been developed to assist in the isolation of STEC.The procedure involves coating magnetic beads with anti-LPS antibody and mixing them with broth cultures or suspensions of feces or suspect food homogenates. There are the studies in which magnetic beads were coated with anti-O157 LPS and O111 LPS (2).

Serological diagnosis of STEC infection

Serological tests may be of value if STEC infection is presumed clinically but fecal examination is negative. These techniques have also proved of value in epidemiological investigations

and have been largely based on detection of anti-O157 antibodies, although they assays to detect a response to the O antigens of other serotypes have been developed.

Antibodies to Stx or LPS have been proposed as markers of recent infection (2). Many authors demonstrated the utility of anti-LPS passive hemagglutination assays specific for serogroups O26, O111, O128 and O157 for establishing a diagnosis of STEC infection in HUS patients whom STEC had not been isolated. By Western immunoblotting, the presence of antibodies to O111, O137 and O145 LPS in sera from HUS patients was demonstrated (2,4,45,46). Serum antibodies were detected to the LPS of *E. coli* strains belonging to serogroups O2, O5, O26, O55, O103, O111, O115, O145, O153 and O165. Antibodies to the secreted proteins were detected in 16 of 70 sera from patients with HUS, HC or diarrhea, but without bacteriological evidence of infection with STEC and which did not contain antibodies to STEC serogroups O5, O115, O145, O153 and O157 (45,46).

Although non-O157 STEC are an important cause of HUS and diarrhea, infections are markedly under-recognized because clinicians do not request testing of stools for STEC and few laboratories screen stools for non-O157 STEC.

Currently approaches to the surveillance of STEC related disease and the laboratory methods used in the diagnosis are different from a country to another. In order to prepare for future international events, "Enter-net", a European Union established a common action under the BIOMED 2 program for extending the activities on STEC (40).

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