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EMERGING SHIGA CYTOTOXIN-PRODUCING *ESCHERICHIA COLI*
I. Enterohaemorrhagic *E.coli* O157: H7

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Abstract. One of several Shiga toxin-producing serotypes of *Escherichia coli* is O157 serotype. *E.coli* O157 was first identified as a human pathogen in 1982 and then has involved from a clinical novelty to a worldwide public health concern. This organism known to cause human illnesses develops from mild non-bloody diarrhoea to haemorrhagic colitis, haemolytic uremic syndrome and death. The bacterium evolved through acquisition of genes for Shiga toxin and many other virulence factors. The cattle and many other animal species represent the reservoir for human infection with *E.coli* O157. This serotype is transmitted by food, water and directly from one person to another. There are different laboratory methods for isolation and identification of *E.coli* O157. Surveillance, rapid reporting of cases and ready epidemiologic investigations are essential elements of timely public health response.
Key words: *E.coli* O157, virulence factor, clinical feature, epidemiology, laboratory diagnosis

Rezumat. Dintre multiplele serotipuri de *Escherichia coli* producătoare de toxină Shiga-like face parte şi *E.coli* O157. Acest serotip a fost identificat pentru prima dată în anul 1982 şi a devenit ulterior o problemă de sănătate publică. *E.coli* O157 este implicat în infecţii umane, ce pot evolua, de la starea de purtător asimptomatic până la colita hemoragică, sindromul hemolitic uremic şi deces. Acest serotip a achiziţionat gene ce codifică sinteza toxinelor Shiga, precum şi alţi factori de virulenţă. Rezervorul de bacterie este reprezentat în principal de bovine, dar, şi de alte specii animale. *E.coli* O157 se transmite prin alimente şi apă contaminate şi prin contact direct de la o persoană la alta. Au fost puse la punct mai multe metode de diagnostic pentru izolarea şi identificarea *E.coli* O157. Supraveghere, raportarea imediată a cazurilor şi investigaţiile epidemiologice sunt elemente esenţiale ale reacţiei sistemului de sănătate publică.
Cuvinte cheie: *E.coli* O157, factori de virulenţă, manifestări clinice, epidemiologie, diagnostic de laborator

INTRODUCTION

In the past decades, many Vero-cytotoxin-producing strains of *Escherichia coli* (VTEC) have been associated with the cases of haemorrhagic colitis (HC) and haemolytic uremic syndrome (HUS). The commonest serotype of these VTEC is *E.coli* O157: H7. During the past 20 years, *E.coli* O157: H7 has become a world - wide public health concern. One by one country, this once obscure pathogen is challenging clinicians, alarming food producers and transforming the public's perception about the safety of their food (1,2).

There are many questions about the evolution of *E.coli* O157: H7.A very

important one is why this pathogen suddenly emerged as a public health issue. Is this serotype a completely new pathogen, which appeared in the food supply or is it a pathogen present but unrecognized prior to 1982? (3).

Whittam S.T., 1998, pointed out that the crucial steps in the evolution and emergence of *E.coli* O157: H7 involve the acquisition of a suite of virulence determinants. There are included a pathogenicity island encoding factors for intestinal adherence, a large plasmid specifying haemolysin and other putative factors, and bacterio-phages carrying genes encoding powerful cytotoxins (1).

A doubtful element in the emergence of *E.coli* O157: H7 as a foodborne pathogen is its evolution of acid resistance, an attribute that promotes survival in acidic foods and give the capacity in an efficient transmission with low infective dose. The naturally selection of the resistant mutants give their the potential to emerge as a new foodborne and waterborne pathogen (1).

Short history

E.coli O157: H7 is so-named because it posses the 157 somatic antigen (O) and the 7 flagellar antigen (H). This bacterium was first recognized as a gastrointestinal pathogen during two outbreaks of haemorrhagic colitis in 1982 in Oregon and Michigan, USA. This *E.coli* strain has the capacity to synthesize so called Shiga toxin/ Vero cytotoxin (1,2,4).

Konwalchuk et al., 1977- cited by Whitman, described a cytotoxin active on Vero cells and produced by certain strains of *E.coli* and it was named/ termed Vero cytotoxin (VT). This cytotoxin is clearly distinguishable biologically and immunologically from the heat-labile and heat-stable enterotoxins of *E.coli* and has reported to be related to Shiga toxin, produced by strains of *Shigella dysenteriae* type 1 (1,3,5,7,8).

In 1983, Karmali and colleagues, in Canada, reported an association between infection with *E.coli* Shiga-toxin-producing (including O157: H7) and post-diarrhoeal haemolytic uraemic syndrome, a clinical condition defined by acute renal injury, thrombocytopenia and microangiopathic haemolytic anemia (2,9,18,20). In recognition of its distinct clinical manifestations, *E.coli* O157: H7 became the first of several strains referred to as enterohaemorrhagic *E.coli* or EHEC. Then the researches have indicated that *E.coli* O157: H7 is the cause of 85-90 % of all cases of HUS in North America and industrialized countries (2,3,9,10,11, 12,13).

By the years since the discovery of this pathogen, *E.coli* O157: H7 has become increasingly prominent, causing over 20,000 illnesses and 250 deaths each year in the United States alone. In 1993 in the western United States was a large multi-state outbreak resulted in more 700 illnesses and four deaths (3).

Outbreaks in the past few years have resulted in the illness of over 5000 Japanese schoolchildren, the death of 20 people in central Scotland and the many other outbreaks in all continents (1,2,3,10,11,12,13,21,22).

Nomenclature

E. coli O157: H7 produce toxins which are toxic to Vero (African green monkey kidney) tissue culture cells and are similar to Shiga toxin of *Shigella dysenteriae*. They have been known as Verotoxin 1 and 2 and as Shiga-like toxin I and II. The strains of *E. coli* that produce these toxins have been known as verotoxin- producing *E.coli* (VTEC) or as Shiga-like toxin-producing *E.coli* (STEC). "Stx-producing *E.coli* O157" is synonymous with *E.coli* O157: H7 (2,3,5,11,19,20). The term VTEC is still widely used in United Kingdom and many European scientific publications. The term STEC is used especially in American scientific papers. The term enterohaemorrhagic *E.coli* (EHEC) was originally coined to denote strains that cause HC and HUS.

Virulence factors of *E.coli* O157: H7

The *E.coli* O157: H7 strains have many virulence genes that are absent in nonpathogenic strains.

Among the most important virulence characteristics of this serogroup is its ability to produce one or more Shiga toxins. These toxins produce a cytotoxic effect on Vero cells in tissue culture, an effect that could be neutralized by polyclonal antisera to Shiga toxin. First of these, Shiga toxin 1 is indistinguishable from Shiga toxin produced by *Shigella dysenteriae* type 1. The second, Shiga toxin 2 is a more divergent molecule with only 56% aminoacid homology with Shiga toxin 1. Most *E.coli* O157: H7 strains produce Shiga toxin 2. The percentage that also produce Shiga toxin 1 ranges from less than 25% in Europe to greater than 80% in a series from North America and Japan (2,12,22,23).

The toxin is a 70.000 dalton protein composed of a single A subunit (32 kDal) and five B subunits (7.7 kDal). The A subunit has an N-glycosidase that inactivates the 28S ribosome, thus blocking protein synthesis. The B subunits provide tissue specificity by binding to globotriaosylceramide (Gb_3) receptors on the surface of eucariotic cells. Endotelial cells high in Gb_3 receptors are the primary target, accounting for the toxin's affinity for colon and renal glomeruli, associated with HC and HUS. The toxin can also indirectly damage cells by releasing cytokines, such as tumour necrosis factor (18).

VTEC strains isolated from HUS patients produce mostly Stx2 and/or Stx2e. Stx are considered the major virulence factors of VTEC involved in the pathogenesis of HUS. The toxins inhibit protein synthesis in target cells causing thus the cell death (21). There is an evidence that programmed cell death (apoptosis) may be the major pathway of Stx-mediated cytotoxicity towards susceptible microvascular endothelia (21). Stx can induce apoptosis in human epithelial cells derived from renal proximal tubuli *in vitro*. The tubular epithelial apoptosis has been demonstrated in renal biopsy samples from children with HUS (21).

The ability to produce Shiga toxins is encoded by a temperate bacteriophage inserted into the *E.coli* O157 chromosome (5,7,21,24). The Stx-converting phages are members of a diverse family of lambda-like phages wide spread in nature. The Stx genes

of this serotype have been transferred to nontoxigenic strains in laboratory conditions (1). Karmali, 1989–cited by (1) pointed out that bacteriophages have disseminated Stx genes in the *E.coli* population in nature. Related cytotoxins have also been discovered in *Citrobacter freundii*, indicating that Stx genes can spread among different bacterial species (25,26).

Toxin alone is not sufficient to make *E.coli* pathogenic. They must have and other virulence markers. There are the *eae* chromosomal gene (encoded for an outer membrane protein associated with attachment), a plasmid-encoded enterohaemolysin, which is ubiquitous among VTEC strains (1,5,7,18) and many other virulence determinants – table 1.

Table 1. Principal virulence determinants in *E.coli* O157 and VTEC (Adaptation after Sunders et al., 1999)

O – antigen – LPS
Virulence plasmid (pO157≈70-100kb)
Bundle – forming pili (btp) (type IV)
Ehx - enterohaemolysin
KatP – catalase/peroxidase
Regulators of virulence genes (Per)
Chromosomal pathogenicity island
Locus of enterocyte effacement (LEE) (43 kbp)
Signal transduction (EspA, EspB, EspD)
Intimin (EaeA- 94kDa)(colonic specificity?)
Translocated intimin receptor (Tir-78 kDa→ Hp 90)
Type III secretion system (Sep, Cfm)
Toxin-converting phages
Enterohaemolysin (Ehly 1, 2)
VT1/VT2 (Stx)

E.coli O157: H7, like the enteropathogenic *E.coli* (EPEC) that cause infantile diarrhoea, have the ability to disrupt the intestinal epithelium by intimately adhering to enterocytes (1,5,7,20). The close attachment of bacteria destroys the microvilli and intimate adherence of bacteria to the epithelial cell membrane creates a characteristic histopathology referred to as *attaching - effacing (A/E) lesions* (1,2,7,10,21). The bacteria appear to "sit" on a pedestal-like structure. 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) (1,7,21). LEE encodes the molecular machinery necessary for A/E lesion formation by *E.coli* O157: H7.

The LEE has three functional regions. The central region contains the *eae* gene encoding a 94-97 kDa outer

membrane protein called *intimin* is an EHEC adherence factor. The *tir* gene encoding a translocated intimin receptor (Tir protein) 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 extracellular secretion of the proteins encoded by the *esp* genes (1,7,21).

Virtually all VTEC strains possess a large plasmid (p O157) of approximately 75 to 100 kb in size which encodes determinants that may serve as additional virulence factors. The p O157 was once also thought to be involved in the production of an adhesin and in the adherence of VTEC to eucaryotic cells (1,7,21). The large plasmid - 90 kb - of VTEC O157: H7 carries the VTEC-*hly* gene encoding the VTEC haemolysin, which acts as a pore-forming cytolysin. 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 (21). Immunoblot analysis of patient sera indicates that this protease is immunoreactive and presumably expressed and secreted during VTEC infections (1,5,7,21,27).

The enterohaemolysin belongs to the RTX (repeat in toxin) family of haemolysin but differs in its target cell specificity and lytic activity from the prototypic *E.coli* α-haemolysin (Hly) associated with extraintestinal infections (1,2,26). The EHEC plasmid is strongly correlated in strains of serotypes O157: H7, O26: H11 and O111: H8 that have been associated with hemorrhagic colitis (1,7,19,22,28,29).

The mechanism by which *E.coli* O157 causes haemorrhagic colitis and HUS are not fully understood. The bacterium is believed to adhere closely to mucosal cells of the large bowel, disrupting the brush border. This process alone may be sufficient to produce non-bloody diarrhoea. Shiga toxins have both local and systemic effects on the intestine and are probably critical to the development of bloody diarrhoea. Histopathological changes associated with infection include haemorrhage and oedema in the *lamina propria* with areas of superficial focal necrosis (2).

Postdiarrhoeal HUS is primary a disease of the microvasculature, thought to develop when Shiga toxins produced in the intestine enter the blood and bind to Gb_3-rich endothelial cells of kidneys. Damage of the endothelial cells, mediated by Shiga toxins, may trigger platelet and fibrin deposition, leading to injury of passing erythrocytes (haemolysis) and occlusion of renal microvasculature (renal failure). Thrombocytopenia is believed to reflect trapping of plateletes in involved organs and removal by the liver and spleen. Inflammatory cytokines and circulating bacterial lipopolysaccharide may play an important part in augmenting this process (2).

Clinical features

The clinical manifestations of *E.coli* O157 and other VTEC serotypes infections range from symptom-free carriage to non-bloody diarrhoea, haemorrhagic colitis (a triade of severe abdominal pain, diarrhoea and frank red blood), HUS and death. The course of events in VTEC infection starts with the ingestion of the pathogen. Figure 1 achieved by Heuvelink, 2000, and assumed from Duffi (7) represents the evolution of infection with VTEC.

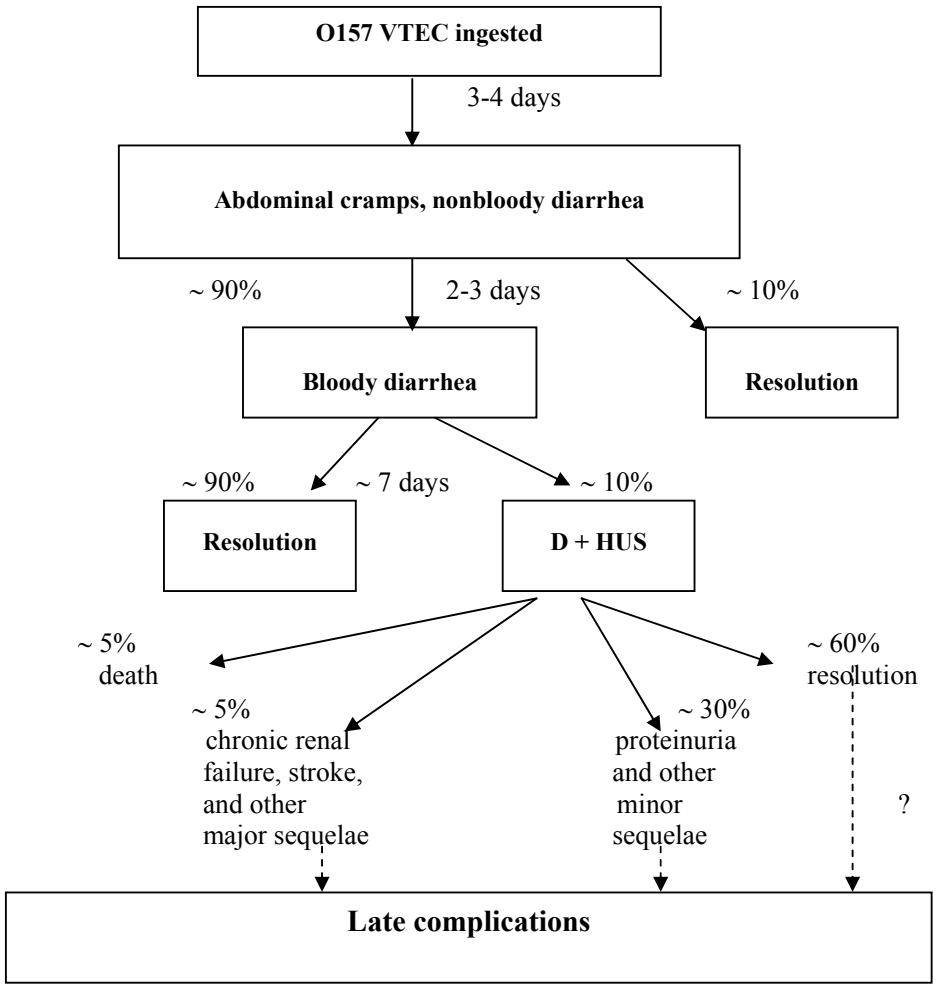

Fig. 1. Sequence of events involved in VTEC infection -Heuvelink, 2000- cited by Duffi (7)

The pathogen survives in acid environment of the stomach and progresses through into the large intestine. Colonization is achieved by the formation of a characteristic lesion on the cells of the large intestine (the attaching and effacing lesion). The VTEC cells multiply and release verocytotoxin into the blood stream. The target cells for the toxin are in kidneys, brain and large intestine. The infectious dose is lower than for other enteric pathogens and has been reported to be fewer than 50 organisms (1,2,6,7,22,27,28). The average interval between exposure and illness is about 3 days; incubation short periods as 1 day and as long as 8 days have been reported. Most patients with haemorrhagic colitis recover spontaneously within 7 days. Illness typically begins with abdominal cramps and non-bloody diarrhoea (1,5,26,27,28). Bowel movement may become bloody over the next 1-2 days, with the amount of bloody varying from a few small streaks to stools that are almost entirely blood. Vomiting occurs in 30-60% of cases, and fever, usually low grade, can be documented in only 30%. The absence of fever may lead clinicians to favour non-infectious diagnoses, such as intussuspicion ischaemic colitis, haemorrhage, or inflammatory bowel disease. Abdominal tenderness may be pronounced, prompting surgery for a presumed appendicitis (1,2,28).

The percentage of cases that progress to HUS, ranged from 3-7% in series of sporadic cases to about 20-30% or more in some outbreaks. Many studies indicated that this serotype of *E.coli* is present in 85-95 % of cases of HUS in North America (9,26). HUS is typically diagnosed 6 days after the onset of diarrhoea. Some patients have abnormal laboratory values, suggestive of an incomplete form of HUS. About 50% of patients with HUS need dialysis and 75% have erythrocyte transfusions (2,28). Among patients with HUS, about 3-17% die acutely and a similar percentage develop end-stage renal disease. Other long-term sequelae include cholelitiasis, colonic stricture, chronic pancreatitis, glucose intolerance. Acute neurological complications such as stroke, seizure and coma develop in 25% of patients (2,28,30).

It is unclear why *E.coli* O157: H7 is predominant serotype associated with HC and HUS when more than 100 STEC strains are known. In recent years, the incidence of HC and HUS associated with STEC strains other than O157: H7 has been increasing (30).

VTEC infection may also be complicated by thrombotic thrombocytopenic purpura (TTP), particularly in adults, a condition similar to HUS but with more prominent neurological findings and less renal involvement (2).

Epidemiology

The incidence of human infections with *E.coli* O157: H7 still appears to be increasing. Both sporadic cases and epidemic have been increasing throughout the world.

Human infections with *E.coli* O157 have been reported from over 30 countries on six continents. Annual incidence rates of 8 per 100,000 inhabitants or greater have been

reported in the region of Scotland, Canada and USA (1,2,9,12,21,27,31). High rates may also be present in regions of South America, especially Argentina, where HUS is endemic and has an incidence 5-10 times higher than in North America and where many patients with HUS have *E.coli* O157 infection (1,2,3,27). The incidence of VTEC O157 infection in the UK has a significant increased between 1981 when it was first described and 1997 when 1540 diagnosed cases were reported. In UK the reported incidence varies between 1.0 and 3.2 per 100,000 inhabitants. Most cases in England and Wales (over 80%) appear to be sporadic (22). Table 2 presents VTEC O157 infections in UK in period 1995-2000.

Table 2. VTEC O157 infections in England and Wales, 1995-2000

Year	No.cases	Rate per 100,000	% associated with recent aboard travel	% associated with general outbreaks	No. outbreaks
1995	792	1.64	5.2	14.3	11
1996	660	1.28	9.1	10.5	14
1997	1087	2.10	10.8	14.1	25
1998	890	1.71	10.1	8.1	17
1999	1084	2.08	7.4	20.5	18
2000	858	no data	no data	no data	19

From the morbidity associated with outbreaks of VTEC O157 infection in England and Wales 31% of cases required hospital admission and an overall mortality rate of 3.7% between 1992 and 1996 have been found. This proportion greatly exceeds that seen in outbreaks of infectious intestinal disease caused by other pathogens (1.6% admissions and 0.1% mortality) (28).

In Romania there are few cases with EHEC. In our studies, one strain verocytotoxigenic-EHEC O157:H7 was isolated from a 2 years child. Another 9 strains O157:H7 were isolated from children and adults with diarrhoeal disease. These strains were not tested for their capacity to produce verocytotoxins (32,33).

Outbreaks involving older patients may have substantial case fatality rates: 35% in a nursing home in Ontario in 1985, 6% in a community outbreak in Central Scotland in 1996 (28).

The peak in incidence of VTEC O157 infection is in the third quarter of the year.

The age specific incidence is highest in children under 5 years of age. About 10% of VTEC O157 infections are acquired abroad of industrialized countries (28).

Infection with *E.coli* O157 is more common in the warm summer months in both Northen and Southern hemispheres (1,2,3).

Health cattle are a major reservoir for human infection with *E.coli* O157. A bovine reservoir of *E.coli* O157 has

been suspected ever since the first human outbreak was linked with ground beef consumption in 1982. Since then, numerous studies in several countries have shown that this bacterium is present in cattle gastrointestinal tract of varying percentages. VTEC O157 was identified in different areas of the world: 0.4% of beef carcasses at slaughter and 0.83% of faeces from live cattle in England and Wales; 15.4% of faeces specimens from cattle for slaughter of one British abattoir and from 2.2% - 7.4% of sheep (3,6). In addition to cattle and sheep the organism has been isolated from deer, goats, horses, dogs, geese, wild birds and flies (1,2,3,28,34). Shedding by ruminants is particularly common, suggesting these animals provide a specific niche for the bacterium. Environmental studies have shown that the organism can persist in manure, water troughs and other places on farms VTEC O157 can survive for over 12 month in cattle faeces and for over 20 weeks in soil samples (35).

E.coli O157 is transmitted by food and water, directly from one person to another, and occasionally through occupational exposure. Most foodborne outbreaks have been traced to foods derived from cattle, especially ground beef and raw milk. Large outbreaks of VTEC O157 infection in the USA have been associated with eating undercooked beef burgers (3,6,28,31). Beef burgers have been linked to outbreaks and sporadic cases in the UK, also (2,28). Foods derived from other species may also transmit the bacteria, as demonstrated by the frequent contamination of lamb meat in some countries and by an outbreak linked to venison jerky (2). Meat probably becomes contaminated at the time of slaughter, grinding may compound the problem by introducing the pathogen into the interior of the meat, where it is more likely to survive cooking. Outbreaks involving commercial salami highlight the organism's tolerance to acid and its ability to survive fermentation and drying. Several outbreaks, including the large outbreak in central Scotland, have been traced to cooked meats that were probably cross- contaminated (1,2,3,28).

Other vehicles of infection implicated are unpasteurized milk, cream, cheese made from raw milk, milk following pasteurization failure, yoghurt (35-37). The pasteurized milk and diary produced also have been implicated in human infections, but only in relation to inadequate pasteurization or post-process contamination (31,38).

In the past few years, fruits and vegetables have accounted for a growing number of recognized outbreaks. Radish sprouts have been implicated in several outbreaks in Japan, including the massive Sakai city outbreak of 1996 (2). In the USA, fresh produces such as: lettuce, apple cider, unpasteurized apple juice, alfalfa sprouts, have been implicated. Vegetables may be contaminated from a variety of sources including cattle manure used as a fertilizer, run-off from cattle feed lots, irrigation/ washing water. Minimally processed vegetables and salads pose particular risks because once contaminated they

are not subject for future processes likely to reduce or eliminate such contamination. Particular care should be exercised during the production of fruit and vegetable juices, as low levels of contamination on the raw ingredient may well transfer into the final product, and may indeed be concentrated by the processing procedure (1,2,3,6,31,35-37).

While some produce-associated outbreaks may be due to cross-contamination from meat products, other are more likely to reflect direct contamination in the field with faeces of wild or domestic animals (2,3,31).

Waterborne outbreaks of *E.coli* O157 infection have occurred as a result of drinking and swimming in unchlorined water (2,3,28). In the United States the first reported drinking water outbreak of *E.coli* O157 infections occurred in 1989 in rural Missuri. Then, many others waterborne outbreaks were reported (3,39,40).

Person to person transmission occurs in day-care and chronic-care facilities. Outbreaks due to person to person, spread by the faecal oral route have occurred in households, child day care facilities, hospitals and nursing homes, particularly those caring for elderly people and people with learning disability (3,21,28).

Contact with infected animals has been identified as a source of infection, as occupational exposure among nurses and microbiologists (2,3). Laboratory acquired infection has been described by Coia (28).

Transmission through water and directly from one person to another suggests that *E.coli* O157 has a infectious dose, as low as similar of *Shigella sonnei*. This conclusion is supported by investigations of outbreaks in which implicated foods were cultured quantitatively. In one outbreak traced to salami, the average infectious dose was estimated at fewer than 50 bacteria (2).

The incubation period for diarrhoeal illness is one to eight days and is usually three to four days. 14 days incubations have been reported in some outbreaks (28).

E.coli O157 may shed in the human stool for several weeks after resolution of symptoms. In general, young children carry the organism longer than do older children or adults. Belongia (2), found a mean duration of faecal shedding among children younger than 5 years old of 17 days after resolution of symptoms and 38% of cases shed for over 20 days. In other study recorded shedding for an average of 29 days (range 11-57)-Shah, 1998-cited by (28).

Diagnosis

Isolation methods

Routine techniques for VTEC focus almost exclusively on the detection of *E.coli* O157: H7 in clinical specimens, animal faeces, food and environment. There are many problems with the developed techniques for *E.coli* O157: H7. A major challenge is the isolation of bacterium from different complex sample types. Often the sample will contain low numbers of VTEC, very high levels of background flora and natural inhibitors which interfere with isolation of *E.coli* O157: H7 (2,5,24,29).

Techniques to improve the speed of the testing protocols for serotype O157 has been developed based on immunological or DNA techniques (polymerase chain reaction-PCR). Typing of strains is a specialized skill, which is performed by national reference laboratories and is used as a research tool (24). The lack of standardization in cultivation's techniques, rapid detection methods and typing procedures remains a problem. We present an overview of the current methods being applied to *E.coli* O157: H7.

Sampling. Samples should be taken aseptically to ensure sample integrity. Because *E.coli* O157: H7 is categorized as hazard group 3 pathogens, there is necessary precaution to reduce the staff risks.

Samples should be placed in a sterile leak proof closed container and transported under chilled condition to the laboratory. Samples should be tested within 48 h although chilled storage up 72 h does not appear to reduce survival (24).

Enrichment. The pre-enrichment process may be needed for the samples with a small number of cells or cells of sub-lethal damage (heating, freezing, presence of inhibitory substances in the sample). These cells require a period of recovery in a medium, which permit repair of the damage and subsequently growth in an enrichment medium. Pre-enrichment media may be use: trypticase soya broth or modified trypticase soya broth. There are described many selective media as such as:

- Lauryl Tryptose Broth
- Modified Tryptone Soya Broth + Novobiocin
- Modified Tryptone Soya Broth + Acriflavin
- Modified *E.coli* Broth + Novobiocin
- Enterobacteriaceae Enrichment Broth + Novobiocin
- Modified Tryptone Soya Broth + Cefixime + Cefsulodine + Vancomycin
- Buffered Peptone Water + Vancomycin + Cefsulodine + Cefixime (24).

Isolation and identification

Most *E.coli* O157: H7 strains are non-sorbitol fermenters. This property is exploited to distinguish VTEC O157 from other *E.coli* sorbitol-positive.The selectivity of sorbitol containing agars may be increased by addition of low concentrations of cefixime, tellurite or rhamnose, although this may be unfavorable for its sensitivity (24). The *E.coli* O157: H7 including the sorbitol-fermenting strains are β-glucuronidase negative and this property is used in differential plating media. There are commercial developed media based on agar with D-glucoronide (24).

The Mac Conkey agar with sorbitol-SMAC is currently used for isolation of *E.coli* O157: H7. In SMAC media, inhibitory agents may be added to improve their selectivity. There are many commercialised solid media for isolation VTEC:

- Sorbitol McConkey Agar (SMAC)
- Modified McConkey Agar (MSMAC)
- Sorbitol McConkey Agar + Tellurite + Cefixime (CT-SMAC)

- Modified Eosin Methilene Blue Agar (MEMB)
- Haemorrhagic coli Agar (HC)
- Phenol Red Sorbitol Agar + MUG (PRS-MUG)
- Fluorocult™ *E.coli* O157: H7 agar

The plates are incubated for 24h at 37˚C.

Identification of presumptive VTEC isolates is traditionally archived by biochemical tests: carbohydrates utilization, β-glucuronidase production, API 20E strips or Biolog crystal ID kits. The isolates as *E.coli* will be tested for their toxigenic status (41-44).

Latex agglutination

A rapid latex agglutination assay was performed for the presumptive identification of *E.coli* O157. This test is based on latex based coated with an antibody specific to *E.coli* O157 or O157: H7. In the presence of the specific antigen the beads adhere to the cells causing visible clumping of latex particles (5,7,24).

Serotyping

The *E.coli* isolates may be grouped on the O- and H- antigens expressed on the cell surface. This is a classical microbiological method. Specific antisera have been raised to 173 O-antigen (O1-O173) and 56 H-antigens (H1-H56). Agglutination of these specific antisera in a slide or tube agglutination test confirms the presence of O- and H- antigens on the isolates (5,22,24,41,43). There are the isolates non-motile and supplementary motility tests are required.

Determination of verotoxigenic capacity

The carriage of VT gene may be determined using PCR assay and by the verocell monolayer assay. In the last case, killed suspensions of bacterial cultures are overlaid on a verocell monolayer. After incubation, the cell layer is examined microscopically. The damage to the monolayer indicates the presence of verotoxins (22,23,41-44).

Immunological procedures

In the past years, immunological procedures were developed.

Immunological techniques are based on the reaction between an antibody and antigen specific to VTEC. Some of these assays target surface antigens and thus detect VTEC cells of specific serogroups and others rely on the detection of the toxins produced by VTEC.

In general, immunological methods rely on the attachment of a label to the antibody to assist in the detection of the pathogen or its metabolite. This label may be a fluorescent tag or more usually an enzyme (ELISA). A fluorescent-labelled monoclonal antibody assay for *E.coli* O157: H7 has been developed in combination with direct microscope detection. This method has been applied to detection of the pathogen in faecal samples (5,8,21-23,27,41-44).

Enzyme Linked Immunosorbent Assay (ELISA).

There are many commercial ELISA kits formats but the sandwich assay is most commonly used. An antibody bound to a carrier surface (micro-titre plate or dip-stick) captures a specific antigen on *E.coli* O157. A second antibody with an enzyme label, binds to the captured antigen and an

appropriate substrate is added to give a colour reaction. The sensitivity of these assays is similar to traditional culture techniques, however they offer advantages in terms of rapidity, reduced labour costs and high volume throughput (21,22,23,27,41-44).

Serodiagnosis

Patients infected with VTEC produce immunological responses to the pathogen. Specifically, these antibodies target the lipopoly-saccharide (LPS) component of VTEC cell. Serology can play an important role in the diagnosis of infection with *E.coli* O157, but the results must be considered in the light of patients' symptoms and an understanding of known antibody cross-reactions between *E.coli* O157 and other bacteria. Shared epitopes on the LPS of *E.coli* O157 and *Brucella abortus*, *Yersinia enterocolitica* O9 and *E. hermanii* have been attributed (24,45,46). ELISA kits and immunoblotting assays have been commercially developed to detect the antibodies to VTEC LPS. Clinical diagnosis can be made from blood, faecal or saliva samples and they all contain the VTEC LPS antibodies (24).

Verotoxin Assay

ELISA based kits have been developed for the detection of verotoxin (VT) 1 and 2. The test is performed in a microtitre well assay and is commercially available (including Meridian Diagnostics and Oxoid) (24,29).

DNA based methods

DNA based methods are highly specific, based on the detection of a fragment of nucleic acid material unique to the target organism. Techniques include the polymerase-chain reaction (PCR) and less commonly, DNA hybridization (colony dot blot technique). Both techniques require enrichment to increase the levels of target DNA with the length of enrichment being dependent on sample type. The methods are then applied either directly to the enrichment medium or to colonies formed by subsequent culture on agar plates (24,45).

The PCR is a DNA based technique in which a particular sequence in the bacterial genome is amplified using a specific pair of primers. The primers are normally chosen to detect a characteristic virulence factor in VTEC- verotoxin gene *vt1* and *vt2* or *eae* (intimin) gene sequence. In the specific case of serotype O157, the rfb_{O157} gene for the O157 antigen may be targeted. PCR is a common used research and diagnostic tool and has been developed in commercially available formats (21,24,29).

Antibiotic sensitivity

The VTEC isolates from human clinical specimens are tested for sensitivity/resistance to panels of various antimicrobial agents. Their therapeutic value in management of VTEC infections is controversial. After some authors, the use of quinolones and thrimethoprim/ sulphamethoxazole has been implicated as a risk factor for HUS (49). Most studies have been showed for *E.coli* O157 strains the percentage of resistance to at least one antimicrobial agent has increased but still remains at a lower level compared with certain other enteric pathogens.

In combination with other methods the pattern of resistance to antimicrobial agents serve as useful epidemiological markers (47-49).

Phage typing

The scheme was developed in Canada and published by Ahmed et al., 1987- cited by Smith (46). Since then it has been used and expanded in a small number of centres in Canada and England. The scheme uses 16 phages and now identifies more than 80 phage types. The use of phage typing has been helpful in many epidemiological studies (24, 47).

Surveillance, rapid reporting of cases, and prompt epidemiologic investigations are essential elements of timely public health response. Surveillance that uses molecular subtyping methods has at least two advantages over traditional surveillance systems. Firstly, it is sensitive enough to identify outbreaks not detected by traditional surveillance or can detect them earlier. Secondly, it is specific enough to differentiate sporadic cases from outbreak-related cases and distinguish between single and multiple outbreaks (50).

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