Escherichia coli 0157: An emerging new challenge in the developing world-A review

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Abstract

Escherichia coli O157:H7 strains are the most prevalent group of Shiga toxin (Stx) producing *E. coli* (STEC) worldwide. Transmission frequently occurs through ingestion of raw or undercooked beef, but other contaminated foods and water have also been implicated, and person-to-person transmission also occurs. *Escherichia coli* O157:H7 was first associated with severe gastroenteritis in 1982 when it caused two major outbreaks of HUS from the same restaurant chain in Oregon and Michigan in the United States of America. The frequency of sporadic and outbreak cases of *E. coli* O157 infection appears to be on the increase worldwide especially the developing countries. This review describes in detail epidemiology, clinical manifestations and current methods to diagnose this pathogen.

Keywords: Escherichia coli, Gastroenteritis, STEC, Infection, O157:H7, Shiga toxins

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Shiga toxin producing *Escherichia coli* (STEC) is an emerging pathogen which has been reported from over 30 countries across several continents and drawn immense international drive towards it. It is a major cause of widespread epidemics as well as sporadic cases of gastrointestinal illness. STEC infections cause a spectrum of human illness ranging from symptom-free carriage to severe bloody diarrhea and even to life-threatening sequelae such as HUS. Infection, especially in infants and young children, with mainly serotype O157:H7 has recently become a public-health problem of serious concern.[1]

Historical Background

In the year 1977 verotoxigenic *E. coli* strains were discovered by Konowalchuk and O'Brien on Vero and HeLa cells respectively. It was termed Shiga-like toxin as it affected the reactivity of the serum of *Shigella dysenteriae* type1. In 1975 from a patient with bloody diarrhoea the *E. coli* O157:H7 was first isolated in USA. In 1982 *E. coli* O157: H7 associated with two outbreaks of haemorrhagic gastroenteritis.[2]

Epidemiology

WHO 2010 Report demonstrated the incidence of EHEC infections varies according to age groups more commonly affecting the children less than 15 years of age (0.7 cases per 100 000 in the US). *E. coli* O157 was associated with approximately 73,000 illnesses each year in the US. The food-borne cases (52%) and water-borne cases (9%) caused 350 outbreaks of *E. coli* O157 during the 1982 and 2002.[3] The outbreak studies of *E. coli* O157:H7 infection highlights that majority cases are from Western countries like United States, Canada and United Kingdom, but cases have also been documented from other parts of the world. The isolation of STEC from clinical sources has also been reported from India world as well. Among the SEAR counties only Japan has higher incidence of *E. coli* O157.[4]

Introduction

The STEC/VTEC belongs to a broad family with variety of serotypes. Currently more than 200 serotypes of STEC are known and about 160 identified in humans with HC or HUS. Among various serotypes, O157: H7 dominates the other ones like O26: H11, O111: H-, O145: H-, O45: H2 and O4: H-.

Histopathology

The haemorrhage and oedema in the lamina propria are most common histo-pathological changes associated with *E. coli* O157:H7 infection.[5] There is oedematous and sub-mucosal hemorrhagic changes in the ascending and transverse colon recognized as "thumb printing" pattern on radiological studies.[6]

Shiga toxins of *Escherichia coli*

Shiga toxins was identified as a potent cytotoxin as the major virulence factor which is characteristic of EHEC.

STEC strains have been found to produce a family of related cytotoxins known as Shiga toxins (Stx). They have been classified into two major classes, Stx1 and Stx2 coded by *stx1* and *stx2* genes respectively. It has holotoxin of 70 kDa with 32 kDa A-subunit and a 7.7 kDa multimeric B-subunit helps in toxin to bind to target cell receptor.[7] Stx damages the glomerular endothelial cells by destruction of absorptive villi and narrows of capillary lumen.[8] In HUS patient acute renal failure occurs due to decreased glomerular filtration rate. The occlusion in vessels also causes RBC fragmentation.[9]

Other potential virulence factors:

The significance of EAST1 in the pathogenesis of disease due to EHEC is unknown but it could possibly account for some of the non-bloody diarrhoea frequently seen in persons infected with these strains.[10] The two other genetically distinct phage encoded haemolysins, called *Ehly1* and *Ehly2*, have been reported to be produced by many Stx-producing *E. coli* strains but there are no data to suggest *in vivo* expression or any role in pathogenesis for these haemolysins.[11] It *E. coli* O157:H7 grows better in the presence of heme and haemoglobin, and the lysis of erythrocytes by one or more of the haemolysins reported for this pathogen could release these sources of iron, thereby aiding infection.[12] Intestinal adherence factors plays a role in intestinal colonization *in vivo* in an animal model is the 94 to 97kDa OMP intimin, encoded by the *eae* gene. O157 LPS enhances the cytotoxicity of Stx on human vascular endothelial cells in vitro, but its effects *in vivo* are not clear. The O157:H7 has plasmid pO157 of size 93.6 to 104 kb is highly conserved. A/E phenotype and the pO157 plasmid expressing EHEC haemolysin are also probably pathogenic.[10]

Pathogenesis of STEC

The Shiga toxin causes vascular injury in the intestinal epithelium.[13] Stxs causes changes at the level of cytokine expression patterns as of epithelial cells. The bacteria adhere to the microvilli, transduces signal into and aggregates the molecular proteins of the cell and cause effacement of microvilli.[10] The attaching and effacing lesion is caused by surface bacterial proteins by a pathogenicity island called the locus for enterocyte effacement (LEE).[14] 97 kb plasmid encodes STEC hemolysin, which acts as a pore-forming cytolysin on eukaryotic cells; the bifunctional catalase

peroxidase *KatP*; a secreted serine protease (*espP*) and the *etpD* gene cluster which probably encodes a type II secretion pathway.[15]

General Characteristics

EHEC is a rather robust pathogen. It can survive extreme temperatures from 7 to 50°C, with an optimum temperature of 37°C. It has been found to grow in acidic foods at a pH-4.4 and in foods with a minimum water activity of 0.95. It can be destroyed by thorough cooking of foods until an internal temperature of 70°C (155°F) or higher has been reached.[16]

Reservoir and modes of transmission

The cattle, pigs, buffaloes, horses, deer, wild boar, antelope, reindeer, birds, *etc* are the reservoirs.[10] The organism may also be transmitted from person to person through the faecal-oral route.[13] An increasing number of outbreaks are associated with the consumption of fruits and vegetables.

Clinical manifestations

The incubation period of EHEC diarrhoea varies from 1-8 days and usually non bloody diarrhoea with abdominal cramps and fever. In 50% cases vomiting occur and after 1 or 2 days bloody diarrhoea follows for about a week. In severe cases, faecal specimens are described as "*all blood and no stool*".[2] In majority cases bloody diarrhoea resolves with no sequelae. In about 10% of patients younger than 10 years the disease progress to HUS. The thrombocytopenic purpura (TTP) is also seen in few cases.

HUS is defined by a triad of hemolytic anemia, thrombocytopenia, and renal failure and earlier in the course of disease oligouria, anuria, oedema, pallor and, seizures occur.[17]The threat of HUS is more in patients who have bloody diarrhoea, fever, elevated WBC counts, very young or old or were treated with anti-motility agents. According to WHO (2010) report the percentage of EHEC infections which progress to HUS varies between sporadic cases and outbreaks cases, 20% or more associated with outbreaks and mortality between 3%-5%. The neurological complications include seizure; stroke and coma in around 25% of HUS patients.[18]There are few case reports of isolation of EHEC from other sites such as blood, urine and glans.[17]

Diagnosis and Detection of STEC

The diagnosis of STEC is by culture, cytotoxic assays, serological assays and molecular techniques.

Culture isolation

It must be considered the definitive diagnostic procedure. It permits additional characterization of STEC like O: H serotyping, antimicrobial drug resistance, phage typing, restriction fragment length polymorphism and amplification-based DNA typing.

Culture media

The CDC has recommended that clinical microbiology laboratories routinely culture stool specimens from persons with bloody diarrhoea or HUS for *E. coli* O157:H7 with SMAC agar (CDC 1993).[19] The agar medium most commonly used for the isolation of *E. coli* O157:H7 is Sorbitol MacConkey agar (SMAC) and is available from multiple commercial sources and which incorporates 1% sorbitol instead of lactose in the MacConkey medium.[20] The serotype 0157:H7 strain of E. coli does not ferment sorbitol and therefore testing for sorbitol fermentation is as a simple method to screen this bacterium.[21] [22] The Sorbitol non-fermenting (SNF) colonies which are colourless on SMAC agar medium are usually indicate E. coli O157:H7. It has been suggested that multiple (3-10) colonies of SNF should be tested for E. coli O157. Various researchers have found an overall sensitivity of around 50-60% of SMAC plates for the detection of SNF E. coli O157.[23] [24]

The isolation rate of O157 was increased by supplementing SMAC with cefixime and rhamnose to allow its fermentation by sorbitol negative non-O157 E. coli strains.[25] The presumptive identification of E. coli O157:H7 can be reported for confirmed E. coli strains that are sorbitol negative on SMAC agar and agglutinate in O157 and H7 antiserum.[17] The previous studies have shown that the anti- O157 sera cross-reacts with *Citrobacter freundii* and other bacterial species.[26] [27] SMAC agar is is useful for O157:H7 but not other Stx-producing E. coli strains as there is no association of Stx production and sorbitol fermentation at gene level.[10] The yield E. coli O157:H7 on SMAC agar is improved by enrichment in selective broth (GN Broth Hajna and trypticase soy broth) for few hours to

overnight instead of direct plating of stool specimens. There are certain strains of E. coli O157 which are not picked up on SMAC because of their ability to ferment sorbitol which makes them indistinguishable from normal microbiota.[28] [29] The BBL CHROMagar O157 (CHROM) had a higher sensitivity of 96.30% and negative predictive value of 100% and a better diagnostic efficiency than SMAC was found to be cost effective.[30] The detection by hyperspectral imaging to detect STEC serogroups on Rainbow Agar has also been evaluated.[31]

Immunomagnetic separation (IMS)

IMS techniques was developed to assist in the isolation of STEC especially O157 to avoid the problem of false negative results from low abundance specimens.[32]

Idenification of isolates by biochemical characteristics

O157:H7 strains do not ferment D-sorbitol in the sugar fermentation test in comparison to about 75-95% other *E. coli* strains.[33]The characteristic of *E. coli* O157:H7 which separates it from most other serotypes of *E. coli* is the inability to produce β-glucuronidase, which hydrolyzes 4-methyl- umbelliferyl-D-glucuronide (MUG) and related substrates. It is believed about 96% of *E. coli* isolates produce the enzyme β glucuronidase which cleaves the MUG substrate and provides a fluorogenic end product called methylumbelliferone which is identified by a UV light source. In the test chromogen instead of a fluorogen substrate that is used and also known as glucuronidase test.[34] *Escherichia hermanii* resembles *E. coli* O157 both biochemically as well as serologically. In cellobiose fermentation the *E. coli* isolates are negative but *E. hermanii* is positive.[35]

Cytotoxic assays

The evaluation of the cytotoxic effects of shiga toxins to vero cells has been considered as the gold standard for confirmation of strains possessing shiga toxins.[14] In the assay vero monolayers in a 96-well microtitre trays having extracts/filtrates of test materials and examined for cytopathic effects after incubation period of 24-48h. The performance of cell culture cytotoxic assays s improved by use of Stx1 and Stx2 specific neutralizing antisera.[36] The vero cells (Gb3 and Gb4 receptors) and HeLa cells (lacks Gb4 moiety) have been used.

Serological assays: There are numerous immunoassays developed to detect shiga toxins in culture; faecal samples. The sandwich based ELISA using immobilized monoclonal Abs or Gb3 receptors have been developed. The reverse passive latex agglutination assay is also available.[14]Various other methods such as colony blot and passive agglutination assays are also in commercial use for detection. The most important ability that these immunological methods offer is the easy and flexible use besides being rapid. In majority of ELISA methods the sandwich technique is incorporated with immobilized monoclonal or polyclonal antibodies to the toxins used.

Rapid tests: The three commercial latex reagent kits which CDC has evaluated to detect the O157 antigen (Oxoid, Remel and Richmond Hill,) and one latex reagent kit for detecting the H7 antigen (Remel).The performance of tests showed 100% correlation of all the commercial kits with the reference antisera used by CDC.[37]

ELISA: The antisera directed against the O157 lipopolysaccharide and the H7 antigen have been used by various diagnostic kits like ELISA, latex formats, colloidal gold labeled formats. The direct testing by ELISA kits in stool requires time less than 1 hour to detect antigen. The commercial kits are accurate, easy and simple to use in laboratory settings.[38 39] In a study involving 605 stool samples *E. coli* O157 antigen detection ELISA; LMD Laboratories, Inc. was found more sensitive to direct plating done on SMAC agar. In comparison to colony sweeps and immune-fluorescence (IF) microscopy the ELISA had a sensitivity of about 90% and a specificity of 99%.[40] There are several commercially available ELISA kits to detect O157 antigen in stools directly (Premier *E. coli* O157; Meridian Diagnostics, Inc.). The positive results from the ELISA are only presumptive and must be confirmed by culture, toxin assays, or molecular PCR tests.[38]The antibodies to the O157 antigen have also been used in several assays to detect O157:H7 isolates such as clinical and food samples. The studies have reported cross reactions of *E. coli* O157 LPS with antibodies to several other pathogens, such as *E. hermanii, Citro-bacter freundii,*

Yersinia entercolitica O9, Salmonella O: 30, and *Brucella abortus*.[26 27 41 42 43]

The ELISA which can detect toxins Stx1 and Stx2 with the use of antibodies against toxins to capture the ligand antigen has also been developed. The Gb3 receptor capture technique available is the VeroTest (MicroCarb, Gaithersburg, Md.). The method has the ability to detect small quantities of about10 pg of Stx which is comparable to cell culture assays.

Western immunoblot assay

A Western immunoblot assay to detect Stx1 antibodies is more sensitive and specific in comparison to the ELISA or cytotoxic assays. There are several kits available which also have epidemiological importance.[44]

Molecular tests

The DNA based tests are colony hybridization and polymerase chain reaction (PCR) assays. There are probes for colony hybridization assay and PCR. The PCR-based assays have used lysates or DNA extracts from colony cultures, broth cultures and direct faeces or foods extracts. A simple multiplex-PCR assay to detect *stx1*, *stx2* and their variants has been evaluated. The limitations with the use of direct PCR in faecal samples are that it can contain inhibitory substances giving false results.[45] There are multiplex PCR to detect *rfbO157* gene and virulence genes such as *stx*1, *stx*2 and *eaeA* of STEC in clinical samples.[46] In the direct PCR technique to detect STEC from environmental sample is directly is also available.[47]

The real-time PCR has become very useful for quantification which was not possible with the conventional methods.[48] The introduction of the reverse transcriptase PCR has added greater dimensions and a useful tool for diagnosis.[49] The approach using combined PCR with ELISA is also a sensitive and specific tool for detection of STEC in various products. The digoxigenic labelled and biotin-labelled primers specific for the *VT* genes are added in the PCR amplification cycle.[50]The labelled PCR products, bound to streptavidin coated wells of a microtitre tray through the biotin, are then detected by an ELISA technique.[51]

An easy magnet nanoparticle embedded silica nanotube for the nucleic acid based detection method, the detection sensitivities of E. coli O157:H7 (102 cfu/1 g of seed sprout and 102 cfu/5 mL of water)

were 80% and 100%, respectively, whereas that was 0% using the commercial method.[52] In a novel, high throughput typing platform using microarray multiwell plate substrates and laser-induced fluorescence of the nucleic acid intercalating dye/stain SYBR Gold for detection of EHEC has been evaluated.[53] A luminex microbead-based suspension array has been developed to identify important STEC serogroups including O157.[54]

Treatment

The various study data showed have shown no benefit of antibiotics.[55] The fosfomycin trial data has also been questioned.[56] The treatment of HUS includes supportive measures like management of anemia, controlling bleeding, fluid and electrolyte imbalances, and other complications requiring renal replacement therapy required in 30–60% of cases.[57]The Stx-receptor (Gb3) and Stx-neutralizing monoclonal antibodies have been tried with success.[58] Human vaccines are unlikely to be utilized. Cattle vaccines may prove the most significant approach to this disease.[59]

Conclusion

The numerous recent outbreaks associated with *E. coli* O157:H7 reminds us of the threat that these microorganisms pose to human health which is indeed quite grave. There is a diligent need to search of better diagnostic tools for detection of STEC from clinical as well as environmental samples. There are various hurdles to the successful treatment strategy plans of HUS due to the complexity associated with the pathogenesis of *E. coli* O157:H7. But still there is an urgent need to keep a strict vigil on the antibiotic susceptibility pattern of the isolates obtained to initiate prompt control measures and accurate treatment.

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