Diagnostic Methods for Shiga Toxin Producing E. Coli

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ABSTRACT

Shiga toxin producing E. coli (STEC), a newly emerged pathogen that has been the focus of immense international research, is often associated with large epidemic outbreaks as well as the Hemolytic Uremic Syndrome (HUS), the leading cause of acute renal failure in children. STEC is a global problem, and more than 60 serotypes have been associated with human disease. A variety of foods have been identified as vehicles of STEC associated illness. The pathogenesis of STEC is multifactorial and involves several levels of interaction between the bacterium and the host. After ingestion, STEC colonize enterocytes of the large bowel with a characteristic attaching and effacing pathology, which is mediated by components of a type III secretion apparatus encoded by the LEE pathogenicity island. Two different types of shiga toxins produced by this organism enters the renal glomeruli and other organs through blood causing the pathophysiological changes that result in HUS. Many laboratories do not take steps to identify this organism as it is a laborious process and it is not cost effective. Specific therapeutic strategies are presently not available. The recent sequencing of the genome of epidemic strain of E. coli (O157 strain) offers a unique resource that will help to identify additional virulence genes. These research efforts in turn, should lead to development of new potent and cost effective therapies or vaccines. This brief review of the literature discusses the Diagnostic Methods for shiga toxin producing E. coli, published in the past few years.

INTRODUCTION

Diagnostic Methods

Infections caused by STEC are so severe that it is important and essential to detect the infections at the onset and adopt necessary preventive measures. For many years, laboratory detection of food–borne pathogens has relied on direct isolation, which is still the preferred method, as it represents the gold standard as compared to newly developed rapid detection methods. However, several methods available for rapid detection are discussed that shortens the detection time and initiate therapeutic measures.

Isolation of E.coli O157 strains from stool samples

The isolation of E.coli O157: H7 or other stx – producing E.coli strains from stool specimens depends upon culturing early in the course of disease. When stool samples obtained within 2 days of the onset of diarrhoea were cultured for E.coli O157:H7, the recovery rate was 100%. When stools were cultured 3 – 6 days or more than 6 days after diarrhoea began, the recovery rate decreases to 91.70 and 33.3% respectively (Tarr et al., 2005).

Cultural and Biochemical characteristics

The agar medium most commonly used for the isolation of E.coli O157:H7 is Sorbitol Mac Conkey (SMAC) agar (Boyce et al., 1995; Al- Gallas et al., 2006). Sorbitol non fermenting colonies, indicative of E.coli O157:H7, are colourless on this medium. Multiple sorbitol non fermenting colonies (at least 3 and up to 10) should be selected for testing as potential E.coli O157. The recovery rate of E.coli O157:H7 on SMAC agar can be improved by prior enrichment in selective broth for 4hrs to overnight rather than direct plating of stool specimens. Trypticase soy broth (Adwan and Adwan, 2004; Wani et al., 2004) or Trypticase soy broth supplemented with cefixime (50ng/ml) and vancomycin (40mg/ml) have been used with success. Due to their ability to ferment sorbitol during 34 hrs of incubation, SF STEC O157 and most of the non O157 STEC strains cannot be distinguished from normal intestinal E.coli flora on SMAC agar and are thus missed if only this procedure is used to investigate stools for STEC strains (Martina and Helge, 2000). Various agar media based on one or more properties are commercially available (CT – SMAC agar, MUG EC 0157 agar). There is little published information on side-by-side comparison of these various media. A non selective but differential media detects the enterohemolysin expressed by about 90% of stx – producing E. coli isolates from human (Smith and Scotland, 1993; Beutin et al., 1989; Schmidt et al., 1995).

Whatever plating and enrichment method is
used, suspected EHEC colonies should be confirmed as E.coli by conventional tests and by serotyping. Presumptive identification of E.coli O157:H7 can be reported for confirmed E.coli strains that are sorbitol negative and agglutinates with E.coli O157 antiserum. Strains can be forwarded to a reference laboratory for toxin testing and H typing. There are no common biochemical characteristics that are associated with the great majority of EHEC serotypes. However there are some biochemical characteristics of E.coli O157:H7 that have been exploited in the isolation and identification of this serotype. E. coli O157:H7 strains do not ferment d-sorbitol rapidly in contrast to about 75- 94% of other E.coli strains. Also E.coli O157:H7 strains do not ferment rhamnose on agar plates, whereas 60% of non-sorbitol fermenting E.coli belonging to other serotypes ferment rhamnose.

Another characteristic of E.coli O157:H7 that distinguishes it from most other serotypes of E.coli is the inability to produce β-glucuronide (MUG) and related substrates (Thompson et al., 1990). E.coli O157:H7 also does not grow well at 44-45°C, which is the temperature commonly used to grow E.coli from food and water samples.

**Immunosassays**

Commercially available ELISA kits to detect E.coli O157 antigen directly in stool samples offer testing times of less than 1Hr. These kits are accurate and easy to use in clinical laboratory settings. Positive results from this ELISA should be considered presumptive and should be confirmed by culture, stx tests or PCR (Dyilla and Thompson et al., 1995; Park et al., 1996; Wani et al., 2004).

Latex agglutination assay, Reversed Phase Latex Agglutination Assays (RPLA kit) for O157 and H7 antigens have greatly facilitated the screening of STEC O157:H7 isolates (Saeed et al., 2009). Numerous immunological assays using various formats have been developed for the detection of Stx1 and Stx2 – producing E.coli (Martin and Helge, 2000; Adwan and Adwan, 2004; Wani et al., 2004).

Western immunoblot assays for stx antibodies also have been described by Reymond et al., 1999. This is a sensitive method than conventional ELISA. The first test to be approved by the food and Drug administration is the premier EHEC test (Meridian Diagnosis, Inc). This test uses monoclonal antibodies directed against stx1 and stx2 to capture antigens and polyclonal anti-stx antibodies and horse radish peroxidase for detection. It is capable of detecting stx antigen in bacterial cultures, food samples and fresh or frozen stool samples.

An immuno magnetic separation (IMS) with commercially available magnetic beads coated with antibody against E.coli.O157 has been used by various authors (Karch et al., 1996; Wani et al., 2004) These authors concluded that IMS is the most sensitive of all detection techniques, even more sensitive than PCR, which required approximately 10^5 CFU O157 organisms per gram of stool to yield a positive result (compared to 10^9 for IMS).

**Vero cell Assay**

Shiga toxins produced by Shiga toxin producing E.coli are capable of producing cytopathic effects on vero cell lines. Hence this also can be used as an assay to confirm these strains (Al-Gallas et al.,2002; Martin and Helge, 2000; Blanco et al., 1997). The assay involves the treatment of veromonolayers with sterile extracts of filtrates of test materials and examining cells for cytopathic effects after 48- 72 hrs of incubation (Wani et al., 2004). This method remains the “gold standard” for confirmation of putative stx producing isolates (Paton and Paton, 1998).

**Free faecal cytotoxic Assay**

Free faecal cytotoxic activity testing on vero cell line for stx activity in stool samples is also an extremely sensitive method to detect current or recent presence of stx producing organisms (Capiroli et al., 1995), but this method is time consuming and impractical for most clinical microbiology laboratories.

**Molecular Methods**

**Detection of stx genes (stx genotyping):** The PCR technique has been extensively used to detect stx genes(Bastian et al, 1998) either in stx -only techniques or in multiplex PCR techniques incorporating primers for eae -attaching and effacing gene (Beaudry et al., 1996), uid A -β-glucuronidase , ech -hly-hemolysis A gene (Pradel et al., 2000), flic -H7 antigen, saa- STEC autoagglutinating adhesin or 60MDa plasmid -CVD 419 probe (Khan et al., 2003; Adrienne and James, 2002; Gannon et al., 1997). Numerous primers and PCR protocols have been designed to amplify these genes (Khan et al., 2003; Martina and Helge, 2000; Jenifer and David, 2000; Reid et al., 1999, Osek, 2002). The various PCR techniques are highly sensitive and specific when used with bacterial colonies or cultures, but the use of PCR for direct analysis of stool samples suffers from the same problems with background and inhibitory factors that are seen with other applications of PCR to stool samples. According to Ramotar et al., (1995), PCR technique was more sensitive than SMAC agar for the detection of E.coli. O157:H7 but was less sensitive than were cell culture assays for free fecal cytotoxin. STEC
non O157 strains can harbor a variety of stx variants (Furst et al., 2000; Pierard et al., 1998, Franke et al., 1995a). Hence a broad spectrum of primers that could identify these variants must be used. Thomas et al., 2009, had developed a real time PCR that detects STEC directly from stool with100% sensitivity and specificity.

**Strain Sub typing:** *E.coli* O157:H7 strains form a highly conserved clone that shows low genetic diversity. This clone is only distantly related to other serotypes of stx – producing *E.coli*. Because the O157:H7 clone is so highly conserved, a variety of techniques have been used to differentiate strains of this serotype for epidemiological studies.

The methods available for the sub typing of STEC vary in the speed, technical complexity, cost and ability to reliably discriminate between strains targeting the genome. No single typing method is individually superior; hence ideally a combination of techniques should be employed depending on the level of discrimination.

**Phage typing:** Phage types are determined by the lysis pattern obtained when a test isolate is subjected to a panel of established standard lytic phages. Phage typing can separate O157:H7 strains into different phage types, but this technique is available only in reference centers that possess the typing phage.

**Plasmid profiling:** Plasmid profiling has been used as a means of sub typing STEC strains on the basis of profiles produces when intact plasmids are size separated by gel electrophoresis. Further discrimination may be obtained by restriction enzyme analysis.

Plasmid profiles are only useful as epidemiology markers in fresh bacterial isolates that carry a number of different plasmids. In the case of plasmid profiling, problems can occur in interpretation when plasmid are lost, gained or transferred, conformational changes of the plasmid or strand nicking. In addition, plasmid of the same molecular weight may not share the same DNA sequence. Thus plasmid profiling is not a particularly discriminatory subtyping technique.

**Sero typing:** Identification of O antigens and H antigens is used for classifying into serotypes. This also helps in epidemiological investigation. The relationship of different serogroups in pathogenicity has been proved by several authors (Blanco et al., 1997; Boerlin, 1999; Hussein, 2007; Blanco et al., 2004).

**Pulsed field gel electrophoresis (PFGE):** Pulsed field gel electrophoresis is a modification of conventional agarose DNA electrophoresis to enable large DNA molecules to be separated. Macro restriction analysis of the STEC genome carried out using an infrequent cutting restriction enzyme (XbaI) has proved most discriminatory for subtyping STEC and cuts genome into 10–20 fragments ranging in size from 20–100 kb. PFGE has been successfully applied to STEC to characterize the genome & there are several documented cases of its application in epidemiological investigation (Al- Gallas et al., 2006; Al-Gallas et al., 2002, Zhang et al., 2000; Beutin et al., 2005; Dorothea et al., 2006).

In USA, PFGE is a standard technique for subtyping STEC in public health reference laboratories due to it accuracy & reproducibility between laboratories. In 1995, the centres for disease control & prevention (CDC) set up “Pulsenet” a national network of public health laboratories in the United States which perform PFGE using O157 strains for rapid comparison of the fingerprints with an electronic database at the CDC. Through this network system it is now easy to identify the clones & trace the spread of O157, its sources etc., soon after its isolation.

The use of Multilocus sequence typing (MLST), which assigns alleles at each locus directly by nucleotide sequencing has been proposed by Maiden et al., 1998.

**Other Methods**

Ribotyping has also been applied to this pathogen, but this technique was unable to differentiate among O157:H7 strains. Ribotyping comprises restriction enzyme digestion of the genomic DNA, which is probed using a plasmid containing the *E.coli* rRNA operon. This detects DNA polymorphisms in or flanking the genes encoding the ribosomal RNA, studies by Khan et al., 2003 show that the rRNA genes are too conserved to allow discrimination of *E.coli* O157 strains (Tramuta et al., 2008; Beutin and Strauch, 2007)

Random amplified polymorphic DNA PCR (RAPD-PCR), also called arbitrarily primed PCR has been successfully used to discriminate among O157:H7 strains. This technique uses low stringency PCR amplification with arbitrarily chosen oligonucleotide primers and allows any laboratory with a PCR machine to distinguish strains of this serotype.

Screening for antibiotic resistance patterns may also provide useful sub typing data, but because the use of antibiotic is not indicated for treatment, such information is useful only for epidemiological purposes (Adwan and Adwan, 2004; Khan et al., 2002a; Al-Gallas, 2006). However Chattopadhyay et al., 2001, had observed a uniform antibiotic sensitivity pattern to the common antibiotics in STEC strains.

Other methods found to be rapid, sensitive, and specific— particularly at low levels of bacteria—include filtration capture combined with immunoelectrochemical
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detection and Colony hybridization for stx1 and stx2 genes (Kumar et al., 2004; Hull et al., 1993; Karch and Meyer, 1989). In particular, the diagnosis of EHEC has to be made within 5 days after inoculation or within 2 days after initiation of diarrhea, a challenge that cannot be achieved routinely with current techniques. However, new diagnostic tools are under development. Therefore, research is necessary, and international preventive strategies are mandatory.

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