Review Article

Diagnostic Methods for Shiga Toxin Producing *E. Coli*

*Lali Growther[1] and Niren Andrew S[2]
Research and Development Centre, Bharathiar University, Coimbatore.
[1]Department of Microbiology, Hindusthan College of Arts and Science, Behind NavaIndia, Coimbatore 641028
[2]Department of Microbiology, Madras Christian College, East Tambaram, Chennai-600059
*Author for Correspondence

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 pathogenecity 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 – 6days 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

Review Article

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 44-45ºC, which is the temperature commonly used to grow *E.coli* from food and water samples.

Immunoassays

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 (Dylla *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 10^2 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 (Caprioli *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 , ehx -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

Review Article

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 sprectrum 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.

Serotyping: 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 pathogenecity 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 (Xbal) 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 (RADP-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

Review Article

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.

REFERENCES

Adrienne WP and James CP (2002). Direct detection and characterization of Shiga toxigenic *Escherichia coli* by Multiplex PCR for stx1, stx2, eae, ehx A and saa. *Journal of Clinical Mirobiology* **40** (1) 271- 274.

Adwan GM and Adwan KM (2004). Isolation of shiga toxigenic *Escherichia coli*
from raw beef in Palestine *International Journal of Food Microbiol*ogy **97** 81– 84.

Al- Gallas N, Ben Aissa R, Annabi THA (2002). Isolation and characterization of Shiga toxun producing *Escherichia coli* from meat and dairy products. *Food Microbiol*ogy **19** 398- 398.

Al-Gallas N, Bahri O and Ben Aissa R (2006). Prevalence of Shiga Toxin-Producing *Escherichia coli* in a Diarrheagenic Tunisian Population, and the Report of Isolating STEC O157:H7 in Tunis. *Current Microbiol*ogy **53** 483–490.

Bastian SN, Carle J and Grimont F (1998). Comparison of 14 PCR systems for the detection and subtyping of stx genes in Shiga toxin producing *Escherichia coli. Research Microbiology* **49** 457- 472.

Beaudry M, Zhu C and Fairbrother JM (1996). Genotypic and Phenotypic characterization of *Escherichia coli* isolates from dogs manifesting attaching and effacing lesions. *Journal of Clinical Mirobiology* **34** 1023- 1031.

Beutin L and Strauch E (2007). Identification of Sequence Diversity in the *Escherichia coli fliC* Genes Encoding Flagellar Types H8 and H40 and Its Use in typing of Shiga Toxin-Producing *E. coli* O8, O22, O111, O174, and O179 Strains. *Journal of Clinical Mirobiology* **45**(2) 333–339

Beutin L, Montenegro MA, Orskov I, Prada J, Zimmermann S and Stephan R (1989). Close association of verotoxin (shiga toxin) production with enterohaemolysin production in strains of *Escherichia coli*. *Journal of Clinical Mirobiology* **27** 2559- 2564.

Beutin L, Kaulfuss S, Herold S, Oswald E and Schmidt H (2005). Genetic Analysis of

Enteropathogenic and Enterohemorrhagic *Escherichia coli* Serogroup O103 Strains by Molecular Typing of Virulence and Housekeeping Genes and Pulsed-Field Gel Electrophoresis. *Journal of Clinical Mirobiology* **43** (4)1552–1563.

Blanco JE, Blanco M, Mora A and Blanco J (1997). Production of Toxins (Enterotoxins, Verotoxins, and Necrotoxins) and Colicins by *Escherichia coli* Strains Isolated from Septicemic and Healthy Chickens: Relationship with In Vivo Pathogenicity. *Journal of Clinical Mirobiology* **35** (11) 2953–2957.

Blanco M, Blanco JE, Mora A, Dahbi G, Alonso MP, Gonza´lez EA, Berna´rdez MI and Blanco J (2004). Serotypes, Virulence Genes, and Intimin Types of Shiga Toxin (Verotoxin)-Producing *Escherichia coli* Isolates from Cattle in Spain and Identification of a New Intimin Variant Gene (*eae-_*). *Journal of Clinical Mirobiology* **42**(2) 645–651.

Boerlin P (1999). Evolution of virulence factors in shiga toxin producing *Escherichia coli*. *Cellular and Molecular Life Sciences* **56** 735- 741.

Boyce TG, Pemberton AG, Wells JG and Griffin PM (1995). Screening for *Escherichia coli* O157:H7━a nationwide survey of clinical laboratories. *Journal of Clinical Mirobiology* **33** 3275-3277.

Caprioli A, Luzzi I, Gianviti A, Russmann H and Karch, H (1995). Pheno-genotyping of verotoxin 2 (VT2)-producing Escherichia coli causing haemorrhagic colitis and haemolytic uraemic syndrome by direct analysis of patients' stools. *Journal of Medical Microbiology* **43** 348-353.

Chattopadhyay UK, Dutta S, Deb A and Pal D (2001). Verotoxin producing *Escherichia coli* – an environment-induced emerging zoonosis in and around Calcutta. *International Journal of Environmental Health Research* **1** 107–112.

Dorothea O, Katharina G, Ian F, Angelika F, Helmut T, Fleming S, Manfred PD and Reinhard W (2006). Emerging Shiga toxin producing *Escherichia coli* serotypes in Europe: O100: H- and O127: H40. *Current Microbiol*ogy **53** 428- 429.

Dutta S, Ghosh S and Dutta P (2001-2002). Search for Shiga toxin producing *Escherichia coli* (STEC) including O157:H7 strains in animals, animal products, hospitalised bloody diarrhoea cases and amongst the healthy human population in a defined community. National Institute of Cholera and Enteric Diseases (NICED) - Annual Report.

Dylla BL, Vetter EA, Hughes JG, and Cockerill FR III. (1995). Evaluation of an immunoassay for direct

Review Article

detection of *Escherichia coli* O157 in stool specimens. *Journal of Clinical Mirobiology* **33** 222-224.

Franke S, Harmsen D, Caprioli A, Pierard D, Wieler LH and Karch H (1995a). Clonal relatedness of Shiga-like toxin-producing *Escherichia coli* O101 strains of human and porcine origin. *Journal of Clinical Mirobiology* **33** 3174 - 3178.

Furst S, Scheef J, Bielaszewska M, Russmann H, Schmidt H and Karch H (2000) Identification and characterisation of *Escherichia coli* strains of O157 and non-O157 serogroups containing three distinct Shiga toxin genes. *Journal of Medical Microbiology* **49** 383 - 386.

Gannon VPJ, D'Souza S, and Graham T (1997). Use of the flagellar H7 gene as a target in multiplex PCR assays and improved specificity in identification of enterohemorrhagic *Escherichia coli* strains. *Journal of Clinical Mirobiology* **35** 656–662.

Hull AE, Achesan DW, Echeverria P, Donohue RR and Keusch GT (1993). Mitomycin immunoblot assay for detection of Shiga toxin producing *Escherichia coli* in fecal samples. Comparison with DNA Probes. *Journal of Clinical Mirobiology* **31** 1167- 1172.

Hussein HS (2007). Prevalence and pathogenicity of Shiga toxin-producing *Escherichia coli* in beef cattle and their products**.** *Journal of Animal Science* **85** E63-E72.

Jenifer L, Jaeger MD, David WK and Acheson MD (2000). Shiga Toxin–Producing *Escherichia coli*. *Current Infectious Disease Reports* **2** 61–67.

Karch H, Janetzki-Mittmann C, Aleksic S, Datz M (1996). Isolation of enterohemorrhagic Escherichia coli O157 Strains from patients with hemolytic –uremic syndrome by using immunomagnetic separation, DNA-based methods, and direct culture. *Journal of Clinical Mirobiology* **34** 516-519.

Karch H and Meyer T (1989). Evaluation of oligonucleotide probes for identification of Shiga like toxin producing *Escherichia coli*. *Journal of Clinical Mirobiology* **27** 1180- 1186.

Khan A, Yamasati S, Sata Y , Ramamurthy T, Pal A and Datta S (2003). Prevalence and genetic profiling of virulence determinants of Non-O157 shiga toxin producing *Escherichia coli* isolated from cattle, beef and humans Calcutta, India, *Emerging Infectious Diseases* **8** 54 -62.

Khan A, Das SC, Ramamurthy T, Sikdar A, Khanam J, Yamasaki S, Takeda Y and Nair GB (2002a). Antibiotic resistance, virulence gene, and molecular profiles of Shiga toxinproducing *Escherichia coli*

isolates from diverse sources in Calcutta, *Indian Journal of Clinical Microbiology* **40** 2009– 2015.

Kumar HS, Karunasagar I, Teizou T, Shima K and Yamasaki S (2004). Characterisation of Shiga toxin producing *Escherichia coli* (STEC) isolated from sea food and beef. *FEMS Microbiology Letters* **233** 173-178.

Maiden MC, Bygraves JA, Feil E (1998). Multilocus Sequence Typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proceedings of the National Academy of Sciences of the United States of America **95** 3140-3145.

March SB and Ratnam S (1986). Sorbitol-MacConkey medium for detection of Escherichia coli O157:H7 associated with hemorrhagic colitis. *Journal of Clinical Microbiology* **23** 869-879.

Martina B and Helge K (2000). Non O157:H7 shiga toxin (verocytotoxin producing) *Escherichia coli* strains: Epidemiological significance and microbiological diagnosis; *World Journal of Microbiology and Biotechnology* **16** 711 – 718.

Osek J (2002). Rapid and specific identification of shiga toxin producing *Escherichia coli* in faeces by multiplex PCR. *Letters in Applied Microbiology* **34** 304- 310.

Park C, Vandel HNM and Hixon DL (1996). Rapid immunoassay for detection of Escherichia coli O157 directly from stool specimens. *Journal of Clinical Microbiology* **34** 988-990.

Paton JC and Paton AW (1998). Pathogenesis and diagnosis of Shigatoxin producing *Escherichia coli* infections. *Clinical Microbiology Reviews* **11** 450–479.

Pierard D, Muyldermans G, Moriau L, Stevens D and Lauwers S (1998). Identification of a new verocytotoxin type 2 variant B Subunit genes in human and animal *Escherichia coli* isolates. *Journal of Clinical Microbiology* **36** 3317- 3322.

Pradel N, Livrelli V and De champs CH (2000). Prevalence and characterization of Shiga toxin producing *Escherichia coli* isolated from cattle, food and children during a one year prospective study in France. *Journal of Clinical Microbiology* **38** 1023-1031.

Ramotar K, Waldhart B, Church D, Szumski R and Louie TJ (1995). Direct detection of verotoxin-producing *Escherichia coli* in stool samples by PCR. *Journal of Clinical Microbiology* **33** 519-524.

Reid SD, Selander RK and Whittam TS (1999). Sequence diversity of flgellin (fli C) alleles in

Review Article

pathogenic *Escherichia coli. Journal of Bacteriology* **181** 153- 160.

Reymond D, Karmali MA, Clarke I, Winkler M and Petric M (1997). Comparison of the western blot assay with the neutralizing antibody and enzyme linked immunosorbent assays for measuring antibody to verocytotoxin. *Journal of Clinical Microbiology* **35** 609- 613.

Saeed S, Taghi Zahraei Salehi, Morteza Sattari, Hasan Tadjbakhsh and Mohammad Mehdi Aslani (2009). Detection of shigatoxigenic *Escherichia coli* from fecal samples of calves and cattle by molecular and serological methods. *Comparative Clinical Pathology* **18** 53–57.

Schmidt H, Beutin L, Karch H (1995). Molecular analysis of the plasmid encoded hemolysin of *Escherichia coli* O157: H7 strain EDL933. *Infection and Immunity* **63** 1055- 1061.

Smith HR and Scotland SM (1993). Isolation and identification methods for Escherichia coli O157 and other Vero cytotoxin producing strains. *Journal of Clinical Pathology* **46** 10-17.

Tarr PI, Gordon CA, Chandler WL (2005). Shiga Toxin producing Escherichia coli and haemolytic uraemic syndrome. *Lancet* **365** 1073–1086.

Thomas EG, Sloan LM, Rosenblatt JE and Patel R (2009). Rapid and sensitive detection of Shiga toxin producing *Escherichia coli* from non enriched stool specimens by real time PCR in comparison to Enzyme Immunoassay and culture. *Journal of Clinical Microbiol*ogy **47** (7) 2008- 2012.

Thompson JS, Hodge DS and Borczyk AA (1990). Rapid biochemical test to identify verocytotoxin-positive strains of Escherichia coli serotype O157. *Journal of Clinical Microbiol*ogy **28** 2165-2168.

Tramuta C, Robino P, Oswald E and Nebbia P (2008). Identification of intimin alleles in pathogenic *Escherichia coli* by PCR- Restriction Fragment Length Polymorphism analysis. *Veterinary Research Communications* **32** 1-5.

Wani SA, Pandit F, Samanta I, Bhat MA and Buchh AS (2004). Molecular epidemiology of Shiga toxinproducing *Escherichia coli* in India. *Current Science.* **87** (10) 1345- 1353.

Zhang WL, Bielaszewska M, Liesegang A, Tschape H, Schmidt H, Bitzan M and Karch H (2000). Molecular characterization and epidemiological significance of Shiga toxin producing *Escherichia coli* O26 strains. *Journal of Clinical Microbiol*ogy **38** 2134-2140.