Chapter 3: REVIEW OF LITERATURE

Shiga toxin producing \textit{Escherichia coli} (STEC) is a newly emerging pathogen that has been the focus of immense international research effort driven by its recognition as a major cause of large scale epidemics and thousands of sporadic cases of gastrointestinal illness. It produces severe bloody diarrhoea that is clinically distinct from other types of diarrhoeal diseases caused by other enteric pathogens.

Konowalchuk and co-workers (1977) identified a distinct group of \textit{E. coli} named as Verocytotoxigenic/Verotoxic \textit{E. coli}, which had the ability to produce a toxin with profound and irreversible effect on vero cells. The toxin differed from heat labile (LT) and heat stable (ST) enterotoxins of \textit{E. coli} and was cytotoxic only for vero cells. Later on O’Brien and La Veck (1983) purified and characterized the cytotoxin produced by one of the Konowalchuk’s isolates (serotype O26:H11) and found that it had similar structure and biological activity like shiga toxin (Stx), produced by \textit{Shigella dysenteriae} type 1. Moreover, it could be neutralized by anti-Stx. Therefore, the term shiga-like toxin (SLT) was also applied to verotoxin (VT). Therefore, SLT and VT nomenclature systems have been used interchangeably. Thus, verotoxin-producing \textit{E. coli} is also termed as shiga-like toxin producing \textit{E. coli} (SLTEC) or shiga-toxin producing \textit{E. coli} (STEC).

The presence of multiple verotoxin type was initially understood from the fact that anti-Stx could not neutralize cytotoxic effect of some VTEC strains (Scotland \textit{et al}., 1985, Strockbrine \textit{et al}., 1986). On the other hand crude antisera raised against non-neutralizable strains didn’t neutralize Stx. This demonstrated that some VTEC strains produced only anti-Stx neutralizable toxin (i.e. VT1/SLT1/Stx-1), others produced only the non-neutralizable toxin (VT2/SLT-II/Stx-2) while another group produced both VT1 and VT2. VT1 was essentially identical to shiga-toxin (Stx) and was neutralized by anti-Stx but it was not true for VT2 (O’Brien and Holmes, 1987). One of the most important areas of current exploration concerns how STEC enters our food chain, an investigational avenue that begins with the ecology of STEC in animals and in the environment. A
variety of foods have been identified as vehicles of STEC-associated illness and this makes the organism one of the most serious threats to the food industry in recent years.

3.1. Species and serotype distribution of STEC

It has been recognized for a number of years that STEC strains causing human disease belong to a very broad range of O:H serotypes. Karmali (1989) listed 32 O serogroups (approximately 60 distinct O:H types), and the list has grown considerably since then (Goldwater and Bettelheim, 1994; Kudoh et al., 1994; Pierard et al., 1994). Although not represented in the initial group of STEC isolates described by Konowalchuk (1977), serotype O157:H7 was the first STEC type to be linked to outbreaks of Haemorrhagic colitis (HC) and Hemolytic Uremic Syndrome (HUS) (Karmali, 1989; Riley, 1987). In many parts of the world, STEC strains belonging to this serotype (as well as O157:H2) appear to be the most common causes of human disease. However, the relative ease of isolation of this serotype on the basis of its inability to ferment sorbitol may be contributing to an overestimation of its prevalence with respect to other STEC serotypes. Other common STEC serogroups include O26, O91, O103, and O111, and in several studies, non-O157 STEC serotypes such as these have been the predominant cause of human disease (Bielaszewska et al., 1996; Goldwater and Bettelheim, 1994; Pierard et al., 1994). There have been several reports of multiple STEC serotypes being isolated from a single patient, and in such circumstances, the contribution of each type to the pathogenesis of disease is difficult to ascertain (Bielaszewska et al., 1996; Paton et al., 1996; Thomas et al., 1994). When one of the isolated types is O157, there is a tendency to ignore the potential etiological significance of the other(s).

3.2. Non-O157 STEC strains

Non-O157 STEC strains belonging to non-O157 serogroups have been recognized as causes of human disease since the beginning of the 1980s. In the first study that established the association between HUS and STEC infection, only 3 out of 12 STEC strains (25%) isolated from HUS patients in Canada were STEC O157:H7 while 9
isolates (75%) belonged to 5 different non-O157 serogroups (Karmali et al., 1985). During 1985 to 1990, non-O157 STEC strains were identified as a cause of 10% to 41% of sporadic cases of HUS in several European countries including Germany (Bitzan et al., 1991), Italy (Caprioli & Tozzi 1998), and the Czech Republic (Bielaszewska et al., 1994). Moreover, studies searching for non-O157 STEC in patients with diarrhoea identified such strains in 6% of patients with bloody diarrhoea in the Czech Republic (Bielaszewska et al., 1994) and in 1.1% and 0.5% of patients with watery diarrhoea in Germany (Karch et al., 1997) and the Czech Republic (Bielaszewska et al., 1994), respectively. Although cases of HUS and bloody and watery diarrhoea associated with non-O157 STEC strains have been reported from USA (Bokete et al., 1993; BeAguea et al., 1994; Acheson & Keusch 1995; Acheson et al., 1997), Canada (Karmali et al., 1985; Pai et al., 1988), South America (Lopez et al., 1989), Australia (Goldwater & Bettelheim, 1995a, b) and Japan (Kudoh et al., 1994), prospective studies that would allow determination of the significance of these pathogens in the etiology of human disease have not been performed in all of these geographic areas.

In a prospective study from Alberta, Canada, non-O157 STEC strains were isolated from 29 out of 5,415 (0.5%) patients with diarrhoea and two of the patients infected with non-O157 STEC strains developed HUS (Pai et al., 1988). The non-O157 STEC isolates belonged to a broad spectrum of serotypes including O26:H11, O103:H2, O111:H and O145: H. More recent studies from Canada suggest that non-O157 STEC can be the cause of at least 7% (Rowe et al., 1993) but possibly up to 20% (Johnson et al., 1996) of HUS cases. In USA, non-O157 STEC strains belonging to 5 different serotypes including O26:H were isolated from 1.1% of 445 stools investigated (Bokete et al., 1993). Studies using immunological techniques to detect Stx suggest that the incidence of non-O157 STEC infections could be up to 50% (Park et al., 1996). Among STEC characterized so far in Australia, O111:H, O6:H31, O48:H21 and O98:H are the most common ones(Goldwater & Bettelheim 1995a, b). Although the results of the above studies suggest that non-O157 STEC strains have been emerging as human pathogens also outside Europe, the real significance of these pathogens in the etiology of human disease in the other geographic areas remains to be established. Several outbreaks caused
by non-O157 STEC strains have been identified to date in different parts of the world. In contrast to outbreaks caused by STEC O157:H7, outbreaks caused by non-O157 STEC strains have never been associated with consumption of hamburgers. In almost half of the cases, the mode of transmission could not be determined (Martina and Helge, 2000).

Interestingly, the majority of these outbreaks, including the largest non-O157 STEC outbreak that occurred in Australia in 1995 (Paton et al., 1996), were caused by STEC serogroup O111. The Australian STEC O111 outbreak clearly evidenced the pathogenic potential and public health importance of non-O157 STEC. More than 200 persons were affected, most of them presenting with watery or bloody diarrhoea. However, 22 children developed HUS and 4 adults were diagnosed with thrombotic thrombocytopenic purpura. The infection was traced to uncooked, semidry fermented sausages (Paton et al., 1996). The infectious dose determined for STEC O111 in this outbreak was fewer than one E. coli O111 bacterium per 10 g of the fermented sausage (Paton et al., 1996), being as low as that determined previously for STEC O157:H7.

3.3. STEC in the food industry

The recent and apparently sudden emergence of STEC and its association with an increasingly wide range of foods have resulted in this group of organisms being a major focus for the food industry. Ground beef, so far, has been found to be major vehicle for STEC transmission. Although, there is much focus on O157:H7, the pathogenic roles of other serovars are gradually being recognized. Non-O157 STEC serotypes have been found to be associated with 15 per cent beef samples in US (Acheson, 2000). If all STEC positive meat was to be excluded, then up to 25 per cent of the meat supply would be condemned. STEC strains possess a number of characteristics that combine to make them one of the most serious threats of food safety in recent years, although other members of this species, such as EPEC and ETEC have been associated with food–borne diseases for many years. The low infective dose for STEC (fewer than 100 cells) than EPEC (>105), ETEC (>108), EIEC (>108) (Tilden et al., 1996; Bolten et al., 1996) and its tolerance to acidic environments (pH<2.5 for more than 2 h)(Waterman and Small, 1996; Benjamin
and Datta, 1995) are believed to play a key role in food-borne illness. One of the most important control measures for STEC contamination in food is heating (Stringer et al., 2000).

3.4. Modes of transmission

3.4.1. Food-borne transmission

Investigations revealed that ground beef is the most common vehicle for STEC transmission (Bell et al., 1994) although other food items such as mayonnaise (Griffin, 1995), unpasteurized apple juice (Besser et al., 1993; Mc Carthy, 1996; Hilborn et al., 2000), fermented hard hamburger (CDC, 1993), cow's milk (Martin et al., 1986; Wells et al., 1991), cheese (Deschenes et al., 1996), yoghurt (Morgan et al., 1993), raw potatoes (Morgan et al., 1988), vegetables (Hillborn et al., 1999), cooked maize (Isaacson et al., 1993), fish (Terajima et al., 1999) and raw vegetables have been incriminated in several outbreaks. Particularly, in Japan consumption of STEC-infected raw lettuce resulted in over 9,000 STEC infected cases (Morgan et al., 1988).

3.4.2. Transmission through water

During 1982 to 1994, four water-borne outbreaks of STEC infection were reported in the United States (Challness et al., 2000; Swerdlow et al., 1992) where two each were associated with swimming pool water and contaminated drinking water, respectively. Drinking water, probably contaminated with bovine faeces, has been implicated in outbreaks in Scotland (Dev et al., 1991) and southern Africa (Isaacson, 1993), and subsequently with contaminated well water in Japan (Akashi et al., 1994).

3.4.3. Person to person transmission

Person to person transmission of STEC is well documented during outbreaks and may also account for a significant proportion of sporadic cases (Griffin and Tauxe, 1991; Reida et al., 1994). In a study of patients with O157 infection, the median duration of faecal shedding of STEC was 2 to 3 weeks, but 13 per cent of patients shed O157 for more than one month (the maximum was 124 days) and were clinically asymptomatic
during the later stages (Karch et al., 1992). Thus, there is ample scope for secondary transmission, which may involve direct hand to hand contact (e.g. among children in day care centres) or indirect contact (e.g., via contaminated water used for swimming).

3.4.4. Zoonoses

Zoonoses are "those diseases and infections, which are naturally transmitted between the vertebrate animals and humans". STEC is a serious human pathogen but except in greyhounds and in some very young animals, it has not yet been recognized as a significant animal pathogen. However it has enormous veterinary public health significance as many reports indicate that livestock are the reservoir of STEC (Synge, 2000). Apparently direct transmission of STEC from bovines to humans has been documented in two instances: one in Canada (Renwick et al., 1993) and another in Scotland both in 1992(Syne et al., 1993). The evidence for zoonotic transmission from cattle to humans was found in two other cases in Germany (Beutin et al., 2000) while in another study, sorbitol fermenting (SF) O157:H− strains isolated from humans showed a clonal relatedness to SF O157:H− strains from bovine isolates(Bielaszewska et al., 2000) Contact with animal faeces(Evans et al., 2000) or direct contact with animals such as calves(Renwick et al., 1993; Synge et al., 1993) or lambing ewes(Allison et al., 1997) is well-established risk factor for STEC acquisition. It is difficult to explain why only few humans are affected when the prevalence of STEC is very high among cattle. One of the possible explanation is the existence of two distinct lineages of STEC as detected by an octamer-based genome scanning system that identifies a population of STEC found in cattle but not found in man(Kim et al., 1999).

3.5. BACTERIAL VIRULENCE FACTORS

3.5.1. Structure and type of Shiga toxins (Stx)

The cardinal virulence factor, a defining characteristic of STEC, is Shiga toxin (Stx) that leads to HC and HUS in patients infected with STEC(O'Brien and Holmes,
The Stx family contains two major, immunologically non-cross reactive groups called Stx1 and Stx2. Single STEC strain may either express only Stx1, or only Stx2 or both. Stx1 is identical to Shiga toxin from *S. dysenteriae*1 (O'Brien and Holmes, 1987). Members of the Stx family are compound toxins (the holotoxin is approximately 70 kDa), comprising a single catalytic 32 kDa A subunit and pentameric B subunit (7.7 kDa monomers). The basic A-B subunit structure is conserved across all the members of the Shiga toxin family (O'Brien *et al*., 1992). A subunit is proteolytically cleaved to yield a 28 kDa peptide (A1) and a 4 kDa peptide (A2); these peptides remain linked by a disulphide bond. The A1 peptide contains the enzymatic activity and the A2 peptide serves to bind the A subunit to a pentamer of five identical B subunits (5x7.7 kDa). Crystallographic analysis of the Stx holotoxin demonstrated that the B subunits from a pentameric ring, encircle a helix at the C terminus of the single A subunit. These residues form an alpha helix that penetrates the pore in the center of the B pentamer, flanking charged residues appear to stabilize this interaction (O'Brien *et al*., 1992).

Despite minor differences between the A subunit amino acid sequences of the Stx variants, all appear to exhibit identical enzymatic activity. However, differences in the B subunit amino acid sequences are important in determining the receptor to which the Stx will bind and thus the target cell type. The B pentamer binds the toxin to a specific glycolipid receptor, globotriaosylceramide or Gb3, which is present on the surface of eukaryotic cells (Waddell *et al*., 1988; Lingwood *et al*., 1987). While Gb3 is the main receptor for Stx, the Stx2e variant associated with pig oedema disease uses Gb4 as the receptor (DeGrandis *et al*., 1989; Samuel *et al*., 1990).

A number of variants of Stx1 and Stx2 have been reported. In the case of Stx1, till now 4 Stx1 variants have been identified which include PH, 94C, CB168 and Stx1c (Paton *et al*., 1993; Paton *et al*., 1995; Zhang *et al*., 2002). Stx1c differed from Stx1 and other variants by nine and three amino acid residues in the A and B subunits, respectively while sequence variation is very low between Stx1 and its other variants (Zhang *et al*., 2002). Several Stx2 variants have also been identified on the basis of sequence homology.
and immunological cross reactivity. Variations in the Stx2 amino acid sequence have a direct impact on the capacity of a given STEC to cause disease in mice, suggesting that variation may result in Shiga-like toxins with different properties. To date, at least 12 different Stx2 variants produced by STEC strains from patients and animals have been described (Bertin et al., 2001).

3.5.2. Organization of Stx Genes

The nucleotide sequences of the genes encoding Stx from _S. dysenteriae_, as well as Stx1 and Stx2 from _E. coli_, were determined in the late 1980s (DeGrandis et al., 1987; Jackson et al., 1987; Kozlov et al., 1988; Strockbine et al., 1988, Calderwood et al., 1996). The operons had a common structure consisting of a single transcriptional unit, encoding first the A subunit followed by the B subunit. The _stx_ B-subunit gene has a stronger ribosome binding site than that of the A-subunit gene, resulting in increased translation of B subunits, thereby satisfying the 1:5 A/B-subunit stoichiometry of the holotoxin (Habib and Jackson, 1993). The predicted amino acid sequences were 315, 315, and 318 amino acids long for the A subunits of Stx, Stx1, and Stx2, respectively, and 89 amino acids for the B subunits of all three toxins. Both A and B subunits had hydrophobic N-terminal signal sequences characteristic of secreted proteins. Comparison of the deduced amino acid sequences indicated that Stx and Stx1 were virtually identical (there was a single amino acid difference in the A subunit) whereas Stx2 had only 56% identity to the other toxins for both the A and B subunits (Jackson et al., 1987).

In 1988, the sequence of an operon encoding the variant toxin Stx2e, associated with piglet edema disease, was reported (Gyles et al., 1988; Weinstein et al., 1988). The deduced amino acid sequence of the A subunit of Stx2e was 1 amino acid longer than that of Stx2 and exhibited 94% homology to it. The B subunit of Stx2e was 2 amino acids shorter than that of Stx2, and there was only 87% homology. A number of other variant forms of Stx2 and also Stx1 have since been reported for human STEC isolates, illustrating the diversity of the Stx family (Gannon et al., 1990; Ito et al., 1990; Lin et al., 1993; Meyer et al., 1992; Oku et al., 1989; Paton et al., 1995; Paton et al., 1993; Paton et
One particular subgroup of Stx2 variants contains specific B-subunit amino acid differences with respect to classical Stx2 (Asp163Asn and Asp243Ala), which correlate with a lower binding affinity for the receptor Gb3 and reduced in vitro cytotoxicity for Vero cells (Lindgren et al., 1994). In view of this functional distinction, these toxins are now considered as a separate subgroup and have been designated Stx2c.

### 3.5.3. Role of Stx in Pathogenesis

Studies with rabbits have shown that stx has direct enterotoxic properties which result from selective targeting of Gb3-containing absorptive villus epithelial cells in the ileum (Mobassaleh et al., 1988; Mobassaleh et al., 1994). It is possible that the diarrhoea seen in human STEC infections is due at least in part to direct exposure of enterocytes to Stx in the gut lumen. Since STEC strains appear to be unable to invade gut epithelial cells to any significant extent, the generation of systemic sequelae must presumably involve translocation of Stx produced by colonizing bacteria from the gut lumen to underlying tissues and the bloodstream. One possible route might be through lesions in the mucosal barrier caused either by the direct effects of Stx or other factors such as intimin or perhaps through gaps between adjacent epithelial cells.

An alternative route from gut lumen to tissues might be through intact epithelial cells. This possibility has been examined by using polarized human colonic carcinoma cells (CaCo-2A and T84) grown on collagen-coated polycarbonate membranes (Acheson et al., 1996). When grown for extended periods, these cells form tight junctions and the monolayers exhibit high transepithelial electrical resistance. For both cell lines, a significant proportion of active Stx1 added to the culture medium on the apical side was translocated to the medium on the basolateral side over 24 hours. During this time, there was no toxin-induced damage to the epithelial barrier as judged by electrical resistance. The total amount of Stx1 that could be translocated appeared to be saturable, suggesting the involvement of a cellular receptor. Thus Stx is capable of translocation across
intestinal epithelial cells without apparent cellular disruption via a transcellular pathway (Acheson et al., 1996).

3.5.4. Interaction of Stx with its glycolipid receptor

Once having crossed the epithelial barrier and presumably entered the bloodstream, Stx targets tissues expressing the appropriate glycolipid receptor. Stx1 exhibits optimum binding to Gb3 with a fatty acyl chain length of 20 to 22 carbons, while for Stx2c the optimum chain length is 18. Three distinct sites on the Stx1 B subunit have now been postulated to interact with Gb3 (Bast et al., 1997; Clark et al., 1996; Lingwood, 1996; Nyholm et al., 1995; Nyholm et al., 1996). Thus, if the lipid moiety of Gb3 influences the conformation of the oligosaccharide component, this could in turn alter the binding affinity for one site on the B subunit relative to the others. Moreover, variations in the B subunit among members of the Stx family may result in a preference for one receptor binding site over another, which would then result in differential specificity for Gb3 receptor subsets on the basis of the lipid moiety (Lingwood, 1996). This may result in different specific activities for Stx toxins against given cell types, as well as in vivo variations in tissue specificity, affecting the pathological findings and the 50% lethal dose (LD50).

3.5.5. Pathogenicity Island - Locus of Enterocyte Effacement (LEE)

The genes encoding the attaching and effacing (A/E) phenotype are situated on a 43 kb pathogenicity island –PI (Elliot et al., 1998), located on the Escherichia coli chromosome which is termed as the Locus of Enterocyte Effacement(LEE). LEE fulfills most of the criteria proposed for a PI. In most STEC, it is inserted at min 82 of the chromosome, just downstream of the selC locus (encoding the tRNA for selenocysteins)(Mc Daniel et al., 1995) while in some, it is inserted at min 94 on chromosome at the pheU gene also encoding a tRNA(Benkel et al., 1997). The overall G+C content (39%) of LEE is lower than that found in the rest of the chromosome (51%), suggesting that it might be acquired by horizontal gene transfer from another species.
LEE is not present in the normal flora of *Escherichia coli*, ETEC and *Escherichia coli* K–12 strain but is found in EPEC strains (Kaper *et al.*, 1998).

Studies have shown that the LEE of STEC is much larger than that of EPEC (35 kb), which can be explained by the presence of a 7.5 kb putative prophage, with homology to the P4 family, at the right end of LEE in STEC strains which is absent in the LEE of EPEC (Kaper *et al.*, 1998). It has been suggested that the prophage is inserted into LEE after acquiring the PI in the chromosome and is unlikely to encode any known virulence function (Jerse and Kaper, 1991).

### 3.5.6. Virulence factors encoded in the LEE

A total of 54 Open reading frames (ORF’s) have been identified in LEE of which 13 fall within the putative prophage and 41 correspond to the rest of the PI (Perna *et al.*, 1998). The majority of the LEE genes are in five major polycistronic operons named LEE1 through LEE4 and *tir*. Operons LEE1, LEE2 and LEE3 encode type III secretion system while *tir* operon encodes an intestinal adherence factor (intimin) and its receptor (Tir). The LEE4 encodes some secreted proteins such as EspA, EspB and EspD, involved in host signal transduction pathways.

### 3.5.7. Intimin

The best characterized of the LEE proteins is intimin, a 94 to 97 kDa outer membrane protein that is required for intimate adherence to epithelial cells characteristic of attaching and effacing enteropathogens and for full virulence of STEC in humans (Donnenberg *et al.*, 1993; McKee *et al.*, 1995). Intimin is encoded by *Escherichia coli* attaching and effacing gene (*eae*). The sequences of intimin proteins from different strains of EPEC and STEC and from several animal pathogens show a pattern of strong conservation on the central and N-terminal portions and more divergence in the C-terminal region (Elliot *et al.*, 1998). The cell binding activity is known to reside within the C-terminal 192 amino acid of the intimin and the C-terminal variability between the
corresponding intimin might indicate that the strains bind different host cells (Elliot et al., 1998). This could explain why EPEC is a small bowel pathogen and STEC a large bowel pathogen (Yu and Kaper, 1992). On the basis of antigenic variation, RFLP-PCR and intimin type specific (TS) PCR, intimin can be divided into ten distinct types α, β, δ, γ, ε, ζ, η, φ, τ and κ (Zhang et al., 2002; Adu-Bobie et al., 1998; Oswald et al., 2000) and these intimin types were found to be preferentially associated with defined STEC and EPEC serotypes (Phillips et al., 2000). The γ intimin is divided into two subtypes namely γ1 (O145:H− and O157:H) and γ2 (O86:H40 and O111:H) (Oswald et al., 2000). Further, the single representative of the intimin β group (EPEC O86:H34) as reported by Adu-Bobie et al. (1998) was reclassified as intimin β2 based on the similarity of the nucleotide sequence of this intimin type to that of intimin β1.

3.5.8. Tir

The Tir protein is encoded in the LEE upstream from the eae gene. It is produced in the bacteria as a 78 kDa protein that is translocated via type III secretion pathway into the eukaryotic cell membrane where it serves as intimin receptor (Rosenshine et al., 1996). In both STEC and EPEC, Tir is predicted to be an integral membrane protein that contains two trans-membrane domains. DeVinney et al. (1999) proposed a "hairpin" model for Tir topology (Devinney et al., 1999). In this model, the amino and carboxy termini are intracellular, and the intimin-binding domain is predicted to be the extracellular loop. Both the amino and carboxyl termini are predicted to reside within the host cell and the intimin is thought to bind to a putative extracellular loop. Tir protein showed a high degree of amino acid identity, particularly in its amino terminal region, but is most divergent in its carboxy terminal domains (Devinney et al., 1999). This region of the protein contains tyrosine residues that in EPEC Tir proteins are potential substrates for phosphorylation. STEC Tir, however, lacks one of these residues and is not tyrosine phosphorylated, but still plays a key role in both bacterial adherence to epithelial cells and pedestal formation (Devinney et al., 1999). In EPEC, Tir translocation is evident 2 to 3 hours post infection, while in STEC, Tir translocation is not observed until 5 to 6 hours following infection. These results are most likely due to differences in the
regulation of Tir expression and translocation in STEC and EPEC (Rosenshine et al., 1996).

3.5.9. Secreted proteins and type III secretion system

Growth of STEC strains having LEE in tissue culture media results in the secretion of several Extracellular serine proteases (EspS) (Ebel et al., 1996; Jarvis and Kaper, 1996). These proteins are responsible for signal transduction events seen in the A/E lesion. Their apparent molecular masses on sodium dodecyl sulphate polyacrylamide gel electrophoresis are EspA (25.5 kDa), EspB (37 kDa), and EspD (40 kDa). Mutation of the genes encoding any of these proteins abolishes the signal transduction in epithelial cells and the A/E histopathology (Kaper et al., 1998). The EspA, EspB and EspD proteins are translated without a conventional N-terminal signal peptide (leader sequence), which is cleaved during sep (Secretion of E.coli proteins) -dependent secretion through the periplasmic membrane (Jarvis et al., 1995; Jarvis and Kaper, 1996). The LEE also encodes a type III secretion system, homologous to those found in other pathogens, dedicated to the secretion and translocation of pathogenicity associated proteins.

The type III secretion system delivers virulence determinants directly into host cells (Hueck, 1998). These factors include EspA, which form filamentous structures on the bacterial surface bridging to the host cells surface (Knutton et al., 1998). These structures may deliver other virulence factors directly into the host cells from STEC. EspB is delivered primarily into the host cell membrane, where it becomes an integral membrane protein (Wolff et al., 1998; Elliot et al., 1999). EspB, along with EspD, forms a pore structure through which other bacterial effectors, such as Tir, gain access to the host cell. Additionally, a small fraction of EspB is delivered into the host cytosol. Once translocated into the host cells Tir functions as the receptor for intimin (DeVinney et al., 1999). Tir intimin binding attaches STEC to the intestinal cell surface and triggers actin cytoskeletal rearrangements beneath adherent STEC, resulting in pedestal formation. Elliott et al. (1998) reported that transcription of the STEC LEE2, LEE3 and tir operons are regulated by the product of the first ORF in the LEE1 operon, Ler. Sperandio et al. (1999) reported that an additional level of virulence gene regulation for STEC whereby
the majority of genes encoded on the LEE was also regulated by quorum sensing although it was likely that additional genes were regulated by this mechanism given the widespread distribution of the luxS gene. Sperandio et al., (1999) also revealed that the LEE1 and LEE2 operons were directly activated by quorum sensing whereas the LEE3 and tir operons were indirectly activated via the Ler regulator.

3.5.10. Role of the 60-MDa plasmid in STEC adherence

The involvement of the 60-MDa STEC plasmid, referred to as pO157, in the adherence of O157:H7 STEC was initially suggested by Karch et al., (1987). These investigators found that the presence of the plasmid correlated with expression of fimbriae and adherence to Henle 407 but not HEp-2 cells. However, subsequent studies have produced conflicting results (Fratamico et al., 1993; Junkins and Boyle, 1989; Toth et al., 1990), and there is no consistent in vitro evidence for a role for pO157 in STEC adherence. Tzipori et al. (1987) found that the presence or absence of the plasmid had no effect on the capacity of STEC strains to colonize the colon or to cause A/E lesions in gnotobiotic piglets. On the other hand, Wadolkowski et al. (1990) demonstrated that both O157:H7 strain 933 and its plasmid-cured derivative 933cu could individually colonize the gut of streptomycin-treated mice but that 933cu could not establish colonization when used together with 933. Although the same strains were used in the above piglet experiments, competitive colonization studies were not performed. Therefore, it is not possible to determine whether the apparent contribution of pO157 is influenced by host species.

3.5.11. Haemolysin production

Oswald et al. (1991) tested haemolysin production in 43 isolates of E. coli obtained from diarrhoeic calves and found that 4 (9%) produced α-haemolysin. Joon and Kaura (1993) isolated E. coli strains from diarrhoeic and non-diarrhoeic cow and buffalo calves and found that 4 each of 28 strains of E. coli from cow-calves and buffalo calves, caused haemolysis of heterologous erythrocytes. However 2 and 4 E. coli strains each
from cow and buffalo calves respectively, haemolysed homologous RBCs. Patil et al. (1999) carried out a study for testing drug resistance and virulence characters viz. haemolysin and entero toxin production in *E. coli* isolated from diarrhoeic calves. Eight (44.4%) of 18 *E. coli* isolates were found to produce haemolysin and among them 6 (75%) were entero toxigenic (ETEC). Khan et al. (2002) investigated prevalence of shiga toxin producing *E. coli* in diarrhoeic patients, healthy domestic cattle and raw beef samples and studied haemolysin production. Fourteen of 30 STEC isolates showed α-haemolytic activity. However three STEC strains did not produce haemolysin. Hussain et al. (2003) screened 101 *E. coli* strains isolated from diarrhoeic calves for virulence characteristics and they found that eight (7.92%) of 101 strains of *E. coli* belonging to five serogroups (O22, O98, O123 and O164) and one untypable strain produced haemolysin. Salvadori et al., (2003) screened 205 *E. coli* strains isolated from calves with diarrhoea for the presence of virulence factors associated with bovine colibacillosis, like shigatoxins, α-haemolysin, enterohaemolysin, CNF (Cytotoxic Necrotizing Factor) type 1 and LT and ST enterotoxin production. One hundred and two (49.8%) of the *E. coli* strains produced toxins: like Shiga toxins, α-hemolysin, enterohemolysin, Cytotoxic Necrotizing Factors type 1, and type 2, enterotoxins LT-II and STa. No strain produced enterotoxin LT-I. Fimbrial adhesins F5 and F17 were produced by 7.3% and 4.8% of the strains, respectively, and none expressed F41. Seven strains (3.4%) possessed the gene *eae* and belonged to serotypes O26:H-; O111:H- and O118:H16. Of the 205 *E. coli* strains 20 were α-haemolysin positive. Two (1%) of α- haemolysin strains were CNF producing strains. None of the strains producing haemolysin was associated with enterotoxins.

### 3.5.12. Serine protease (EspP)

Another putative virulence factor also encoded on pO157 is the recently described extracellular serine protease that is plasmid encoded (EspP) (Brunder et al., 1997). The *espP* gene encodes a 1,300-amino-acid protein, which is subsequently subjected to N- and C-terminal processing during the secretion process. The mature form has an apparent size of 104 kDa. The amino acid sequence of the N terminus of the mature protein...
indicated that EspP may be the same as a 104-kDa secreted protein found in an O26:H2 STEC strain by Ebel et al. (1996). The deduced amino acid sequence of the complete O26 protein was subsequently shown to be virtually identical to the sequence of EspP (Djafari et al., 1997). EspP has significant homology (approximately 70% overall) to EspC, a 110-kDa EPEC secreted protein, and to a lesser degree to immunoglobulin A1 (IgA1) proteases of *Haemophilus influenzae* and *Neisseria* spp. The region of homology includes the serine-containing proteolytic active site. EspP did not exhibit IgA1 protease activity in vitro but was capable of cleaving pepsin, an activity which was inhibited by the serine protease inhibitor phenylmethylsulfonyl fluoride. EspP was also able to cleave human coagulation factor V. Thus, Brunder et al. (1997) have suggested that secretion of EspP by STEC colonizing the gut could result in exacerbation of hemorrhagic disease. Djafari et al. (1997) also reported that EspP is cytotoxic for Vero cells. A role for EspP in pathogenesis is also consistent with the presence of antibodies to the protease in sera from five of six children with STEC infection but not in sera from age-matched controls. Nevertheless, EspP was produced by only four of six O157 and one of two O26 isolates but by none of two O103 STEC clinical isolates tested, indicating that it is not a universal virulence factor of STEC (Brunder et al., 1997).

3.5.13. Heat-stable enterotoxin

A final virulence factor that might contribute to the pathogenesis of the watery diarrhoea often seen during the early stages of STEC infection is the enterotoxin EAST1 (encoded by *astA*). This is a 39-amino-acid enterotoxin that was initially recognized in certain strains of enteroaggregative *E. coli* (EAggEC) and is distinct from the heat-stable toxins produced by ETEC. Savarino et al. (1996) demonstrated that an *astA*-specific probe hybridized with all 75 O157:H7 STEC strains tested, as well as with 8 of 9 O26:H11 strains and 12 of 23 STEC strains of other serotypes. This probe also hybridized with 41, 41, and 22% of EAggEC, ETEC, and EPEC strains, respectively. Enterotoxicity for rabbit ileal tissue was confirmed by testing culture ultrafiltrates of three of the *astA*1 O157:H7 strains in Ussing chambers. In these three strains, there were two copies of *astA*, which were located on the chromosome.
3.5.14. Acid resistance of STEC

An important feature of STEC strains that may impact upon their capacity to colonize the human gut, particularly at low infectious doses, is resistance to the acidity of the stomach. It is now known that exposure of certain enteric bacteria, including *E. coli*, to low pH induces an acid tolerance response (Goodsun and Rowbury, 1989), and this has been shown to increase the survival of O157:H7 STEC in mildly acidic foods (Leyer *et al.*, 1995). A distinct phenotype referred to as acid resistance has also been described and is mediated by *rpoS*, which encodes a stationary-phase sigma factor. This factor regulates genes enabling stationary-phase *E. coli* organisms to survive for extended periods below pH 2.5 (Gordon and Small, 1993).

3.6. Clinicopathological Features of STEC Disease

It is now recognized that there is a very broad spectrum of human diseases associated with Stx-producing organisms. STEC-related disease may involve either sporadic cases or large outbreaks involving a common contaminated food source. Some individuals infected with STEC may be completely asymptomatic, in spite of the presence of large numbers of organisms as well as free toxin in the feces (Edelman *et al.*, 1988; Brian *et al.*, 1992;). Very little is known of the true incidence of asymptomatic carriage. Many STEC-infected patients initially suffer watery diarrhoea, but in some this progresses within 1 or 2 days to bloody diarrhoea and Haemorrhagic colitis (HC) (O’Brien *et al.*, 1983; Riley *et al.*, 1983; Riley, 1987). Severe abdominal pain is also frequently reported. In a proportion of patients, STEC infection progresses to Haemolytic Uremic Syndrome (HUS), a life-threatening sequela characterized by a triad of acute renal failure, microangiopathic hemolytic anemia, and thrombocytopenia (Karmali *et al.*, 1983; Karmali *et al.*, 1985). Some individuals with HUS experience neurological symptoms including lethargy, severe headache, convulsions, and encephalopathy (Tesh and O’Brien, 1991). Although HUS occurs in all age groups, its incidence is higher in infants, young children, and the elderly. Improved clinical management and pediatric
renal dialysis techniques have reduced the mortality associated with HUS from about 50% to less than 10% over the last two to three decades (Karmali, 1989). STEC infection can also result in a variant form of HUS, sometimes referred to as thrombotic thrombocytopenic purpura (TTP). This “diarrhoea associated TTP” is more common in adults than in children. The pathological features are essentially the same, but it differs from the typical form of HUS in that patients are more often febrile and have marked neurological involvement (Morrison et al., 1985; Karmali, 1989). Whether STEC-associated diarrhoeal disease progresses to life-threatening complications, depends upon interplay between bacterial and host factors. Characteristics of individual STEC strains will also have a major impact.

3.7. Studies on STEC- International Status

During 1997-1998, 649 pigeons were trapped and examined in three different squares of Rome. Stool samples were collected from each bird and enrichment cultures were examined for the presence of Stx by the vero cell assay. Stx-producing *E. coli* (STEC) were isolated from the positive cultures and characterized by serotyping and PCR analysis of *stx* and other virulence-related genes. Stx was detected in 10.8% of the stool enrichment cultures. The percentage of positive birds did not differ significantly for the three flocks considered and the season of sample collection. Conversely, STEC carriage was significantly more frequent in young than in adult birds (17.9 versus 8.2%). None of the birds examined showed signs of disease. STEC strains were isolated from 30 of 42 Stx-positive cultures examined. All the strains produced Stx2f, and most of them possessed genes encoding for intimin and the cytolethal distending toxin (CLDT) (Morabito et al., 2001).

Between July 1999 and December 2000, the prevalence of Shiga toxin-producing *Escherichia coli* (STEC) was established in 200 Argentine healthy young beef steers (14-16 months old) grown under local production systems with a feed grain period of 3-4 months, and the STEC strains isolated were examined in regard to their phenotypic and genotypic characteristics (Meichtri et al., 2004). Stool samples (n = 70) and rectal swabs
(n = 130) were taken at the slaughterhouse level. By polymerase chain reaction (PCR), Shiga toxin (stx) gene sequences were detected in 69% of the samples. Eighty-six STEC strains were isolated from 39% of the animals. Subtyping of stx2 variants showed the prevalence of stx2vh-b (25.6%) and stx2vh-a type (24.4%), and revealed the presence of an atypical stx2-v. Only 7.0% of STEC strains carried eae, and 33.7% harbored EHEC-hlyA gene. The full virulent genotype (stx/eae/EHEC-hlyA) was found to be present in 4 of the 86 (4.7%) STEC strains isolated. They indicate that young steers from the main beef-producing area of Argentina are an important reservoir of STEC strains.

An outbreak of Shiga toxin-producing *Escherichia coli* (STEC) O148 infection occurred among wedding attendees in France in June 2002 (Espie *et al.*, 2006). A retrospective cohort study was performed and ten cases were identified, including two adults with haemolytic uraemic syndrome (HUS). The analytical study revealed that >80% of affected individuals had eaten lightly roasted mutton and poultry pate, but only the consumption of pate tended to be associated with illness.

By using sequence analysis of Shiga toxin 1 (Stx 1) genes from human and ovine Stx-producing *Escherichia coli* (STEC) strains, Zhang *et al.* (2002) identified an Stx1 variant in STEC of human origin that was identical to the Stx1 variant from ovine STEC, but demonstrated only 97.1 and 96.6% amino acid sequence identity in its A and B subunits, respectively, to the Stx1 encoded by bacteriophage 933J. All 36 stx(1c)-positive STEC strains were eae negative and belonged to 10 different serogroups, none of which was O157, O26, O103, O111, or O145. Stx1c was produced by all stx(1c)-containing STEC strains, but reacted weakly with a commercial immunoassay. They conclude that STEC strains harboring the stx(1c) variant account for a significant proportion of human STEC isolates. In a study by Gomez *et al.* (2002), in Argentina, the prevalence of STEC in 95 samples of frozen hamburgers and in 114 samples of soft cheese was established in 8.4% and 0.9%, respectively.

Fecal swabs obtained from 1,300 healthy lambs in 93 flocks in Spain in 1997 were examined for Shiga toxin-producing *Escherichia coli* (STEC) (Blanco *et al.*, 2003).
STEC O157:H7 strains were isolated from 5 (0.4%) animals in 4 flocks, and non-O157 STEC strains were isolated from 462 (36%) lambs in 63 flocks. A total of 384 ovine STEC strains were characterized in this study. PCR showed that 213 (55%) strains carried the stx (1) gene, 10 (3%) possessed the stx (2) gene, and 161 (42%) carried both the stx(1) and the stx(2) genes. Enterohemolysin (ehxA) and intimin (eae) virulence genes were detected in 106 (28%) and 23 (6%) of the STEC strains, respectively. The majority (82%) of ovine STEC strains belonged to serotypes previously found to be associated with human STEC strains, and 51% belonged to serotypes associated with STEC strains isolated from patients with hemolytic-uremic syndrome. They confirm that healthy sheep are a major reservoir of STEC strains pathogenic for humans.

The pulsed-field gel electrophoresis (PFGE) patterns of 46 Shiga toxin-producing Escherichia coli (STEC) strains isolated in Sao Paulo, Brazil, during the period from 1976 to 2003 were compared with those found among 30 non-STEC strains that carried eae and that belonged to the same serogroups as the STEC strains (Vaz et al., 2006). Multiple PFGE patterns were found among STEC strains of distinct serotypes. Moreover, the PFGE restriction patterns of STEC strains differed substantially from those observed among non-STEC strains of the same serogroup except serotype O26 strains.

Werber et al. (2003), investigated the association between Shiga toxin-producing Escherichia coli (STEC) O157 and the simultaneous presence of the virulence genes stx2 and eae in STEC from patients with gastroenteritis in Germany. They conclude that independent of serogroup, the virulence genes stx2 and eae have been associated with severe disease.

Ritchie et al. (2003) investigated whether toxin production differed between HUS- and bovine-associated STEC strains. Based on their study, basal production of Stx by HUS-associated STEC exceeded that of bovine-associated STEC. In addition, following mitomycin C treatment, Stx2 production by HUS-associated STEC was significantly greater than that by bovine-associated STEC. Unexpectedly, mitomycin C treatment had a minimal effect on Stx1 production by both HUS- and bovine-associated
STEC. However, Stx1 production was induced by growth in low-iron medium, and induction was more marked for HUS-associated STEC than for bovine-associated STEC. Their observations reveal that disease-associated and bovine-associated STEC bacteria differ in their basal and inducible Stx production characteristics.

Pulz et al. (2003) examined 295 stool specimens for the presence of Stx-producing *E. coli* by three different methods: an Stx enzyme-linked immunosorbent assay, a conventional PCR assay, and a LightCycler PCR (LC-PCR) assay protocol recently developed by the laboratory at the Institute of Medical Microbiology at Hannover Medical School. They concluded that LC-PCR was a very useful tool for the rapid and safe detection of STEC in clinical samples.

Friedrich et al. (2003) identified stx (1c) in 76 (54.3%) of 140 eae-negative, but in none of 155 eae-positive, human STEC isolates (P < 0.000001). The 76 stx (1c)-harboring *E. coli* isolates belonged to 22 serotypes and each produced Stx1c as demonstrated by latex agglutination. Characterization of putative virulence factors demonstrated the presence of the locus of proteolysis activity (LPA) and the high-pathogenicity island in 65.8 and 21.1%, respectively, of the 76 Stx1c-producing *E. coli* isolates. Moreover, all but three of these strains contained saa, the gene encoding an STEC autoagglutinating adhesin. The virulence profiles of Stx1c-producing *E. coli* isolates were mostly serotype independent and heterogeneous.

Phenotypic and genetic markers of Shiga toxin-producing *Escherichia coli* (STEC) O26 from North America were used to develop serotype-specific protocols for detection of this pathogen by Murinda et al. (2004). Carbohydrate fermentation profiles and prevalence of gene sequences associated with STEC O26 (n = 20) were examined. Non-STEC O26 (n = 17), *E. coli* O157 (n = 20), *E. coli* O111 (n = 22), and generic *E. coli* (n = 21) were used as comparison strains. Of the 26 carbohydrates tested, only rhamnose had diagnostic value. Rhamnose non-fermenters included STEC O26 (100%), non-STEC O26 (40%), generic *E. coli* (29%), *E. coli* O111 (23%), and *E. coli* O157 (0%). All STEC O26 were MUG-fluorescent, while STEC O157 (n = 18) were non-
fluorescent. *E. coli* O111 and generic *E. coli* strains were either MUG-positive or negative. Serotype-specific detection of PFGE indicated the presence of one major cluster of *E. coli* O26 with 72-100% DNA fragment-length digest similarity among test strains.

Murinda et al. (2004) again characterized *Escherichia coli* isolates from dairy cows/feedlots, calves, mastitis, pigs, dogs, parrots, iguana, human diseases, and food products for prevalence of Shiga toxin-producing *E. coli* (STEC) virulence markers. Multiplex polymerase chain reaction (PCR) was used to detect the presence of genes encoding Shiga toxin 1 and 2 (stx1 and stx2), H7 flagella (flicC), enterohemolysin (hly) and intimin (eaeA) in *E. coli* isolates (n = 400). Shiga toxin-producing isolates were tested for production of Shiga toxins Stx1 and Stx2 and enterohemolysin. Of the *E. coli* O157:H7/H- strains, 150 of 164 (mostly human, cattle, and food) isolates were stx positive. Sixty-five percent of O157 STEC produced both Stx1 and Stx2; 32% and 0.7% produced Stx2 or Stx1, respectively. Ninety-eight percent of O157 STEC had sequences for genes encoding intimin and enterohemolysin. Five of 20 *E. coli* O111, 4 of 14 O128 and 4 of 10 O26 were Stx positive. Five of 6 Stx positive O26 and O111 produced Stx1, however, Stx positive O128 were Stx-1 negative. Acid resistance (93.3%) and tellurite resistance (87.3%) were common attributes of O157 STEC, whereas, non-O157 Stx positive strains exhibited 38.5% and 30.8% of resistance respectively. Stx-positive isolates were mostly associated with humans and cattle, whereas, all isolates from mastitis (n = 105), and pigs, dogs, parrots and iguanas (n = 48) were Stx-negative.

The distribution of eight putative adhesins that are not encoded in the locus for enterocyte effacement (LEE) in 139 Shiga toxin-producing *Escherichia coli* (STEC) of different serotypes was investigated by PCR. Five of the adhesins (Iha, Efa1, LPF(O157/OI-141), LPF(O157/OI-154), and LPF(O113)) are encoded in regions corresponding to genomic O islands of *E. coli* EDL933, while the other three adhesins have been reported to be encoded in the STEC megaplasmid of various serotypes (ToxB [O157:H7], Saa [O113:H21], and Sfp [O157:NM]). The most prevalent adhesin was encoded by the iha gene (91%; 127 of 139 strains). Two fimbrial genes, lpfA(O157/OI-141) and lpfA(O157/OI-154), were strongly associated with seropathotype A. The wide
distribution of iha in STEC strains suggested that it could be a candidate for vaccine development (Toma et al., 2004).

Beutin et al. (2004), have investigated 677 Shiga toxin-producing Escherichia coli (STEC) strains from humans to determine their serotypes, virulence genes, and clinical signs in patients. Six different Shiga toxin types (1, 1c, 2, 2c, 2d, and 2e) were found in the STEC strains. Intimin (eae) genes were present in 62.6% of the strains and subtyped into intimins alpha1, beta1, gamma1, epsilon, theta, and eta. Shiga toxin types 1c and 2d were present only in eae-negative STEC strains, and type 2 was significantly (P < 0.001) more frequent in eae-positive STEC strains. Enterohemorrhagic E. coli hemolysin was associated with 96.2% of the eae-positive strains and with 65.2% of the eae-negative strains. Clinical signs in the patients were abdominal pain (8.7%), nonbloody diarrhoea (59.2%), bloody diarrhoea (14.3%), and haemolytic-uremic syndrome (HUS) (3.5%), and 14.3% of the patients had no signs of gastrointestinal disease or HUS. Infections with eae-positive STEC were significantly (P < 0.001) more frequent in children under 6 years of age than in other age groups, whereas eae-negative STEC infections dominated in adults. The STEC strains were grouped into 74 O:H types by serotyping and by PCR typing of the flagellar (fliC) genes in 221 nonmotile STEC strains. Eleven serotypes (O157:H7, O26:H11, O103:H2, O91:H14, O111:H8, O145:H28, O128:H2, O113:H4, O146:H21, O118:H16, and O76:H19) accounted for 69% of all STEC strains.

A total of 722 Shiga toxin-producing Escherichia coli (STEC) isolates recovered from humans, cattle, bovines and food materials during the period from 1992 to 1999 in Spain were examined to determine antimicrobial resistance profiles and their association with serotypes, phage types and virulence genes. Fifty-eight (41%) out of 141 STEC O157:H7 strains and 240 (41%) out of 581 non-O157 STEC strains showed resistance to at least one of the 26 antimicrobial agents tested. STEC O157:H7 showed a higher percentage of resistance by the strains recovered from bovine (53%) and beef meat (57%) than from human (23%) and bovine (20%) sources, whereas the highest prevalence of antimicrobial resistance in non-O157 STEC was found among isolates recovered from
beef meat (55%) and human patients (47%). Most strains showing resistance to five or more antimicrobial agents belonged to serotypes O4:H4 (4 strains), O8:H21 (3 strains), O20:H19 (6 strains), O26:H11 (8 strains eae-beta1), O111:H- (3 strains eae-gamma2), O118:H- (2 strains eae-beta1), O118:H16 (5 strains eae-beta1), O128:H- (2 strains), O145:H8 or O145:H- (2 strains eae-gamma1), O157:H7 (10 strains eae-gamma1), O171:H25 (3 strains), O177:H11 (5 strains eae-beta1), ONT:H- (3 strains/1 eae-beta1) and ONT:H21 (2 strains). Interestingly, most of these serotypes, were found among human STEC strains isolated from patients with hemolytic uremic syndrome (HUS) reported in previous studies. They also detected, among non-O157 strains, an association between a higher level of multiple resistance to antibiotics and the presence of the virulence genes eae and stx (1) (Mora et al., 2005).

The CDC(2000-2005) findings indicated that a total of 403 STEC infections were reported by clinical laboratories in Connecticut, including 207 identified as STEC by Stx Enzyme Immuno Assay (EIA) testing alone, and that the use of Stx EIA increased from 2000 to 2005. Use of Stx EIA without prompt culture confirmation can delay or prevent serotyping and subtyping of isolates and detection of both O157 and non-O157 STEC outbreaks. The report added that all the Public health authorities in all states should ensure that clinical laboratories forward Stx EIA-positive specimens to the state laboratory for isolation and identification of STEC, as recommended by the Association of Public Health Laboratories and CDC.

The associations of virulent factors (VFs) with phylogenetic background were assessed among the strains in comparison with the different seropathotypes by Girardeau et al. 2005. Although certain Virulent factors (VFs) (eae, stx(2-EDL933), stx(2-vha), and stx(2-vhb)) were concentrated in seropathotypes associated with disease, others (astA, HPI, stx(1c), and stx(2-NV206)) were concentrated in seropathotypes that are not associated with disease.

A total of 42 Shiga toxin-producing (STEC) strains from slaughtered healthy cattle in Switzerland were characterized by phenotypic and genotypic traits. The 42
sorbitol-positive, non-O157 STEC strains belonged to 26 O:H serotypes (including eight new serotypes) with four serotypes (O103:H2, O113:H4, O116:H-, ONT:H-) accounting for 38.1% of strains. Out of 16 serotypes previously found in human STEC (71% of strains), nine serotypes (38% of strains) were serotypes that have been associated with hemolytic-uremic syndrome (HUS). Polymerase chain reaction (PCR) analysis showed that 18 (43%) strains carried the stx1 gene, 20 strains (48%) had the stx2 gene, and four (9%) strains had both stx1 and stx2 genes. Of strains encoding for stx2 variants, 63% were positive for stx2 subtype (Zweifel et al., 2005).

Shiga toxin-producing Escherichia coli (STEC) and enteropathogenic E. coli (EPEC) strains were isolated from 191 fecal samples from cattle with gastrointestinal infections (diagnostic samples) collected in New South Wales and Australia (Hornitzky et al., 2005). By using a multiplex PCR, E. coli strains possessing combinations of stx1, stx2, eae, and ehxA were detected by a combination of direct culture and enrichment in E. coli (EC) (modified) broth followed by plating on vancomycin-cefixime-cefsulodin blood (BVCC) agar for the presence of enterohemolytic colonies and on sorbitol MacConkey agar for the presence of non-sorbitol-fermenting colonies. Forty-seven STEC serotypes were identified, including O5:H-, O8:H19, O26:H-, O26:H11, O113:H21, O157:H7, O157:H- and Ont:H- which were known to cause severe disease in humans and 23 previously unreported STEC serotypes. Serotypes Ont:H- and O113:H21 represented the two most frequently isolated STEC isolates and were cultured from nine (4.7%) and seven (3.7%) animals, respectively. Fifteen eae-positive E. coli serotypes, considered to represent atypical EPEC, were identified, with O111:H- representing the most prevalent. Using both techniques, STEC strains were cultured from 69 (36.1%) samples and EPEC strains were cultured from 30 (15.7%) samples, including 9 (4.7%) samples which yielded both STEC and EPEC. Culture on BVCC agar following enrichment in EC (modified) broth was the most successful method for the isolation of STEC (24.1% of samples), and direct culture on BVCC agar was the most successful method for the isolation of EPEC (14.1% samples). These studies show that diarrhoeogenic calves and cattle represent important reservoirs of eae-positive E. coli.
153 fecal samples of cattle randomly selected from six dairy farms in Sao Paulo State, Brazil, were examined for Shiga toxin (Stx) production by the Vero cell assay. Isolates presenting cytotoxic activity were submitted to colony hybridization assays with specific DNA probes for stx1, stx2, eae, Ehly and astA genes. The isolation rate of STEC ranged from 3.8 to 84.6% depending on the farm analysed. STEC was identified in 25.5% of the animals, and most of them (64.1%) carried a single STEC serotype. A total of 202 STEC isolates were recovered from the animals, and except for the 2 O157:H7 isolates all the others expressed cytotoxic activity (Irino et al., 2005).

Between 1983 and 2002, 43 state public health laboratories submitted 940 human non-O157 STEC isolates from persons with sporadic illnesses to the Centers for Diseases Control and Prevention reference laboratory for confirmation and serotyping. The most common serogroups were O26 (22%), O111 (16%), O103 (12%), O121 (8%), O45 (7%), and O145 (5%). Non-O157 STEC infections were most frequent during the summer and among young persons (median age, 12 years; interquartile range, 3-37 years). Virulence gene profiles were as follows: 61% stx (1) but not stx (2); 22% stx (2) but not stx (1); 17% both stx (1) and stx (2); 84% intimin (eae); and 86% enterohemolysin (E-hly). Stx (2) was strongly associated with an increased risk of HUS, and eae was strongly associated with an increased risk of bloody diarrhoea. STEC O111 accounted for most cases of HUS and was also the cause of 3 of 7 non-O157 STEC outbreaks reported in the United States (Brooks et al., 2005).

The importance of obtaining isolates to identify the source of STEC infections was illustrated by CDC (CDC, 2006), after the outbreak of STEC O157 infections associated with eating fresh spinach. This report describes findings from outbreaks of gastroenteritis in 2005 in New York and North Carolina in which clinical diagnostic laboratories initially used only non-culture methods to detect Shiga toxin (Stx). The findings highlight the importance of confirmation of Stx-positive stool specimens by bacterial culture. An important part of that identification is determining the serotype of all STEC isolates and the subtype of STEC O157 strains so that outbreaks can be detected and traced back to sources.
According to Rivas et al. (2006), Argentina has a high incidence of hemolytic uremic syndrome (HUS). They studied 103 STEC strains isolated from 99 children. The 103 STEC strains belonged to 18 different serotypes, and 59% were of serotype O157:H7. Stx2 was identified in 90.3%, and stx1 in 9.7%. Among the 61 STEC O157 strains, 93.4% harbored the stx2/stx2vh-a genes; PT4 (39.3%) and PT2 (29.5%) were the predominant phage types. Using Pulse Field Gel Electrophoresis (PFGE) with the enzyme XbaI, a total of 41 patterns with at least 80% similarity were identified, and seven clusters with identical profiles were established. STEC strains with different genotypes and belonging to diverse serotypes were isolated in Argentina. Some STEC O157 strains could not be distinguished by applying subtyping techniques such as PFGE and phage typing.

Fremaux et al. (2006) determined the dissemination and persistence of STEC on 13 dairy farms in France, which were selected out of 151 randomized dairy farms. A total of 1309 samples were collected, including 415 faecal samples from cattle and 894 samples from the farm environment. The samples were cultured and screened for Shiga toxin (stx) genes by polymerase chain reaction (PCR). STEC isolates were recovered from stx-positive samples after colony blotting, and characterized for their virulence genes, serotypes and XbaI digestion patterns of total DNA separated by pulsed-field gel electrophoresis (PFGE). Stx genes were detected in 145 faecal samples (35%) and 179 (20%) from environmental samples, and a total of 118 STEC isolates were recovered. Forty-six percent of the STEC isolates were positive for stx1, 86% for stx2, 29% for intimin (eae-gene) and 92% for enterohemolysin (ehx), of which 16% of the STEC strains carried these four virulence factors in combination. PFGE profiles indicated genetic diversity of the STEC strains and some of them persisted in the farm environment for up to 12 months.

31 Shiga toxin-producing *E. coli* (STEC) strains harboring stx2e were characterized by phenotypic and genotypic traits by Zweifel et al. 2006, in Switzerland. Nine of the thirty-one sorbitol-positive non-O157 STEC (stx2e) isolated from healthy
pigs belonged to serotypes found in STEC isolated from humans, including two serotypes (O9:H-, O26:H-) reported in association with hemolytic-uremic syndrome.

A total of 922 STEC isolates obtained from patients with hemolytic uremic syndrome or bloody or nonbloody diarrhoea or from asymptomatic carriers were tested for the gene encoding Stx2d (activatable) by PCR and PstI restriction analysis. The toxin activatability by human and mouse intestinal mucus and by an elastase was determined by quantifying the cytotoxicity using the Vero cell assay. The stx(2d-activatable) gene was identified in 60 (6.5%) of 922 STEC strains; in 31 of these strains, it was the sole stx gene. Thirty of these 31 strains produced Stx2d (activatable). All of them lacked the intimin-encoding eae gene. Among eae-negative STEC, which typically cause mild diarrhoea or asymptomatic infection, production of Stx2d(activatable) was significantly associated with the ability to cause severe disease, including bloody diarrhoea (P<.001), and with systemic complications, such as hemolytic uremic syndrome (P<.001) (Bielaszewska et al., 2006).

Shiga toxin-producing Escherichia coli (STEC) strains isolated from healthy cattle (O111:NM, seven strains; O111:H8, three strains) in Brazil were studied and compared to previously characterized human strains in regard to their phenotypic and genotypic characteristics to evaluate their pathogenic potential (Tristao et al., 2007). Most bovine STEC O111 strains were isolated from dairy calves, and strains with genotypes stx1 alone and stx1/stx2 (variant stx2) occurred in different regions. Irrespective of the stx genotype, all strains were positive for eae theta, alpha variants of tir, espA and espB, and for ler, qseA, iha, astA and efa1 genes. Only one strain was negative for EHEC-hlyA and all strains were negative for iha, saa and espP genes and for EAF and bfpA, genetic markers of EPEC. They also concluded that healthy cattle may be a potential source of infection for humans in Brazil.

To provide a global assessment of the STEC problem, published reports on beef and beef carcasses in the past 3 decades were evaluated by Hussein (2007). The prevalence rates of E. coli O157 ranged from 0.1 to 54.2% in ground beef, from 0.1 to
4.4% in sausage, from 1.1 to 36.0% in various retail cuts, and from 0.01 to 43.4% in whole carcasses. The corresponding prevalence rates of non-O157 STEC were 2.4 to 30.0%, 17.0 to 49.2%, 11.4 to 49.6%, and 1.7 to 58.0%, respectively.

Mora et al. (2007) detected STEC in 95 (12%) of the 785 minced beef samples tested. STEC O157:H7 was isolated from eight (1.0%) samples and non-O157 STEC from 90 (11%) samples. Ninety-six STEC isolates were further characterized by PCR and serotyping. PCR showed that 28 (29%) isolates carried stx1 genes, 49 (51%) possessed stx2 genes, and 19 (20%) both stx1 and stx2. Enterohemolysin (ehxA) and intimin (eae) virulence genes were detected in 43 (45%) and in 25 (26%) of the isolates, respectively. Typing of the eae variants detected four types: gamma1 (nine isolates), beta1 (eight isolates), epsilon1 (three isolates), and theta (two isolates). The majority (68%) of STEC isolates belonged to serotypes previously detected in human STEC and 38% to serotypes associated with STEC isolated from patients with HUS. Ten new serotypes not previously described in raw beef products were also detected. The highly virulent seropathotypes O26:H11 stx1 eae-beta1, O157:H7 stx1 stx2 eae-gamma1 and O157:H7 stx2 eae-gamma1, which are the most frequently observed among STEC causing human infections in Spain, were detected in 10 of the 96 STEC isolates.

546 fecal samples from 264 diarrhoeic calves and 282 healthy calves in beef farms in São Paulo, Brazil, were screened by PCR. STEC and EPEC were isolated in 10% and 2.7% of the 546 animals, respectively. Although Immunomagnetic Separation (IMS) test was used, the STEC serotype O157:H7 was not detected. The most frequent serotypes among STEC strains were O7:H10, O22:H16, O111:H(−), O119:H(−) and O174:H21, whereas O26:H11, O123:H11 and O177:H11 were the most prevalent among EPEC strains. In this study, serotypes not previously reported were found among STEC strains: O7:H7, O7:H10, O48:H7, O111:H19, O123:H2, O132:H51, O173:H(−), and O175:H49. The eae gene was detected in 25% of the STEC and 100% of EPEC strains. The intimin type theta/gamma2 was the most frequent among STEC, whereas the intimin beta1 was the most frequent intimin type among EPEC strains (Aidar Ugrinovich et al., 2007).
Beutin et al. (2007) examined 219 Shiga toxin-producing Escherichia coli (STEC) strains from meat, milk, and cheese samples collected in Germany between 2005 and 2006. All strains were investigated for their serotypes and for genetic variants of Shiga toxins 1 and 2 (Stx1 and Stx2). Stx (1) or variant genes were detected in 88 (40.2%) strains and stx(2) and variants in 177 (80.8%) strains. Typing of stx genes was performed by stx-specific PCRs and by analysis of restriction fragment length polymorphisms (RFLP) of PCR products. Major genotypes of the Stx1 (stx (1), stx (1c), and stx (1d)) and the Stx2 (stx (2), stx (2d), stx (2-O118), stx (2e), and stx (2g)) families were detected, and multiple types of stx genes coexisted frequently in STEC strains. Only 1.8% of the STEC strains from food belonged to the classical enterohemorrhagic E. coli (EHEC) types O26:H11, O103:H2 and O157:H7 and only 5.0% of the STEC strains from food were positive for the eae gene, which is a virulence trait of classical EHEC. In contrast, 95 (43.4%) of the food-borne STEC strains carried stx (2) and/or mucus-activatable stx (2d) genes, an indicator for potential high virulence of STEC for humans. Most of these strains belonged to serotypes associated with severe illness in humans, such as O22:H8, O91:H21, O113:H21, O174:H2, and O174:H21. Stx (2) and stx (2d) STEC strains were found frequently in milk and beef products. Other stx types were associated more frequently with pork (stx (2e)), lamb, and wildlife meat (stx (1c)).

The prevalence of Shiga toxin-producing Escherichia coli (STEC) and its characteristics were determined among hospitalized patients with diarrhoea and children with diarrhoea in an urban slum community of Dhaka city using sensitive culture and PCR methods by Aminul Islam et al.(2007). Stool samples were collected from 410 patients with diarrhoea enrolled in the 2% surveillance system (every 50th patient attending the hospital with diarrhoeal disease is included) at the ICDDR,B hospital and from 160 children of 2–5 years of age with diarrhoea living in an urban slum in Dhaka, between September 2004 and April 2005. Shiga toxin genes (stx) were detected by multiplex PCR in the enrichment broth of nine samples (2.2%) from hospitalized patients and 11 samples (6.9%) from the community patients. STEC was isolated from five stool samples with positive PCR results using a colony patch technique. All five isolates were
positive in the Vero cell assay and PCR fragments of stx genes were confirmed by sequencing. Two isolates were positive for the E. coli attaching-and-effacing (eae) gene and four were positive for the enterohaemolysin (hly\_EHEC) gene and enterohaemolysin production. The five isolates belonged to five different serotypes: O32:H25, O2:H45, O76:H19, ONT: H25 and ONT: H19. It can be concluded that STEC is not a common pathogen in Bangladesh among hospitalized patients with diarrhea or among mild cases of diarrhea in the community.

Bielaszewska et al. (2008) compared stx-/EAF- Attaching and effacing Escherichia coli (AEEC) recovered from patients (mostly children) with bloody and nonbloody diarrhea in Germany, during the period 1995-2007. Stx-/EAF- AEEC were isolated as the only bacterial pathogens from stool specimens obtained from 18 (15.3%) of 118 patients with bloody diarrhea and from 141 (1.3%) of 10,550 patients with nonbloody diarrhea (P<.001). All but 1 of 18 strains recovered from patients with bloody diarrhea resembled enterohemorrhagic E. coli (EHEC) on the basis of serotypes, non-Stx virulence profiles, and multilocus sequence types. In contrast, most (75.9%) of 141 Stx-/EAF- AEEC recovered from patients with nonbloody diarrhea belonged to other serotypes and differed from the former strains phylogenetically and with regard to virulence genes. Three of 18 patients with bloody diarrhea and none of 141 patients with nonbloody diarrhea who shedded Stx-/EAF- AEEC developed hemolytic uremic syndrome. They conclude that most Stx-/EAF- AEEC associated with bloody diarrhea are plausibly EHEC that lost Shiga toxin during infection (EHEC-LST) and Multilocus sequence typing has potential to distinguish EHEC-LST from less pathogenic Stx-/EAF- AEEC.

Shelton et al. (2008) evaluated the use of a multiple protocol approach to improve diagnosis, isolation, and characterization of STEC strains. Among 18 presumptive STEC-positive stool samples received by the INOVA Fairfax Hospital, Falls Church, VA, in 2006, 16 were Shiga toxin positive. From these 16 stool samples, 8 O157:H7 and five non-O157 STEC were isolated by plating onto sorbitol Mac Conkey (SMAC) agar. The remaining 5 stool samples that did not yield colonies on SMAC agar plates were
enriched. All enriched samples were Shiga toxin positive, and 2 O157:H7 and 1 non-O157 STEC were subsequently isolated. The 2 remaining enriched samples did not yield isolates; however, based on polymerase chain reaction (PCR) analysis, both samples contained STEC genes. Based on PCR analysis of non-O157 strains, 3 strain types were identified. Samples from 3 patients, received within 2 days of one another, had a similar gene profile-eae and stx (1) negative and stx (2) positive-suggesting that these patients were likely infected with the same strain. Their results indicate that a multiple protocol approach is necessary to reliably diagnose and isolate STEC strains, and that PCR profiling of strains could allow for more rapid identification of outbreaks.

Seventy-three O157 STEC strains, isolated from HUS (36 numbers), Bloody diarrhoea (BD) (20 numbers), Non Bloody Diarrhoea (NBD) (10 numbers), or unspecified conditions (7 numbers) in Argentina, Australia and New Zealand, were analysed by Leotta et al. (2008). The strains were confirmed to be E. coli O157 by biochemical tests and serotyping. A multiplex polymerase chain reaction (PCR) was used to amplify the stx1, stx2 and rfbO157 genes and a genotyping method based on PCR-RFLP was used to determine stx1 and stx2 variants. This analysis revealed that the most frequent stx genotypes were stx2/stx2c (vh-a) (91%) in Argentina, stx2 (89%) in New Zealand, and stx1/stx2 (30%) in Australia. No stx1-positive strains were identified in Argentina or New Zealand. All strains harboured the eae gene and 72 strains produced enterohaemolysin (EHEC-Hly). The clonal relatedness of strains was investigated by phage typing and pulsed-field gel electrophoresis (PFGE). The most frequent phage types (PT) identified in Argentinian, Australian, and New Zealand strains were PT49 (n = 12), PT14 (n = 9), and PT2 (n = 15), respectively. Forty-six different patterns were obtained by XbaI-PFGE; 37 strains were grouped in 10 clusters and 36 strains showed unique patterns.

Coombes et al. (2008) reported the identification of three genomic islands encoding non-LEE effector (nle) genes and 14 individual nle genes in non-O157 STEC strains that correlate independently with outbreak and HUS potential in humans. Their results and methods offer a molecular risk assessment strategy to rapidly recognize and
respond to non-O157 STEC strains from environmental and animal sources that might pose serious public health risks to humans.

Hedican et al. (2009) identified two hundred and six STEC isolates. One hundred and eight (52%) were non-O157 serotypes, and 98 (48%) were O157. Of the non-O157 cases, 54% were involved in bloody diarrhoea, and 8% were responsible for hospitalization. Non-O157 isolates with at least stx2 were not more likely to cause severe illness (bloody diarrhoea, hospitalization, or HUS) than were non-O157 isolates with only stx1. O157 cases were more likely than non-O157 cases to involve bloody diarrhoea (78% vs 54%; P < .001), hospitalization (34% vs 8%; P < .001 and HUS (7% vs 0%; P = .005). When including only isolates with at least stx2, O157 cases were still more likely to involve bloody diarrhoea (78% vs 56%; P = .02) and hospitalization (33% vs 12%; P = .01) than non-O157 cases.

Guidelines for laboratory identification of STEC infections by clinical laboratories were published in 2006 (Gould et al., 2009). This report provides comprehensive and detailed recommendations for STEC testing by clinical laboratories, including the recommendation that all stools submitted for routine testing from patients with acute community-acquired diarrhoea (regardless of patient age, season of the year, or presence or absence of blood in the stool) be simultaneously cultured for E. coli O157:H7 (O157 STEC) and tested with an assay that detects Shiga toxins to detect non-O157 STEC.

3.8. Studies on STEC- National Status

Khan et al. (2002) investigated the prevalence of Shiga toxin-producing Escherichia coli (STEC) in hospitalized diarrhoea patients in Kolkata, India, as well as in healthy domestic cattle and raw beef samples collected from the city's abattoir. Multiplex polymerase chain reaction using primers specific for stx1 and stx2 detected STEC in 18% of cow stool samples, 50% of raw beef samples, and 1.4% and 0.6% of bloody and watery stool samples, respectively, from hospitalized diarrhoea patients. Various
virulence genes in the STEC isolates indicated that stx1 allele predominated. Plasmid-borne markers, namely, hlyA, katP, espP, and etpD, were also identified. Bead enzyme-linked immunosorbent assay and Vero cell assay were performed to detect and evaluate the cytotoxic effect of the Shiga toxins produced by the strains. STEC is not an important cause of diarrhoea in India; however, its presence in domestic cattle and beef samples suggests that this enteropathogen may become a major public health problem in the future.

Chattopadhyay et al. (2003), attempted to isolate and characterize Shiga toxin producing E. coli (STEC) from animals handlers, animal products and admitted diarrhoeic children in and around Kolkata. A total of 415 samples were processed for detection of STEC by PCR and colony hybridization techniques. 4.81% samples were found to be positive for STEC. Diarrhoeic cattle accounted for maximum (22.1%) isolation. The study showed PCR to be more sensitive than hybridization technique for detection of STEC.

Wani et al. (2003), determined the prevalence and molecular characteristics of Shiga toxin-producing Escherichia coli (STEC) and enteropathogenic E. coli (EPEC) in calves and lambs with diarrhoea in India. Faecal samples originating from 391 calves and 101 lambs which had diarrhoea were screened for presence of E. coli. A total number of 309 (249 bovine and 60 ovine) E. coli strains were isolated. A total of 113 bovine and 15 ovine strains were subjected to multiplex polymerase chain reaction (m-PCR) for detection of stx1, stx2, eaeA and EHEC hlyA genes. STEC and EPEC belonging to different serogroups were detected in 9.73% of calves studied. Six per cent and 26.66% of lambs studied were carrying STEC and EPEC, respectively. Majority of the STEC serogroups isolated in this study did not belong to those which have been identified earlier to be associated mainly with diarrhoea and enteritis in cattle and sheep outside India. The most frequent serogroup among bovine and ovine EPEC was O26 (40%). One of the most important STEC serogroups O157, known for certain life-threatening infections in humans, was isolated from both bovine and ovine faecal samples. They concluded that a high percentage of STEC and EPEC belonging to different serogroups
Shiga toxin-producing *Escherichia coli* (STEC) strains isolated in Mangalore, India, were characterised by bead-enzyme-linked immunosorbent assay (bead-ELISA), Vero cell cytotoxicity assay, PCR and colony hybridisation for the detection of stx1 and stx2 genes. Four strains from seafood, six from beef and one from a clinical case of bloody diarrhoea were positive for Shiga toxins Stx1 and Stx2 and also for stx1 and stx2 genes. The seafood isolates produced either Stx2 alone or both Stx1 and Stx2, while the beef isolates produced Stx1 alone. The stx1 gene of all the beef STEC was found to be of recently reported stx1c type. All STEC strains and one non-STEC strain isolated from clam harboured EHEC-hlyA. Interestingly, though all STEC strains were negative for eae gene, two STEC strains isolated from seafood and one from a patient with bloody diarrhoea possessed STEC autoagglutinating adhesion (saa) gene, recently identified as a gene encoding a novel autoagglutinating adhesion (Kumar *et al.*, 2004).

Wani *et al.* (2004) investigated the presence or absence of shiga toxin-producing *Escherichia coli* (STEC) in avian species in India. Faecal samples originating from 500 chicken and 25 free flying pigeons were screened for the presence of *E. coli*. A total of 426 (chicken, 401; pigeons, 25) *E. coli* strains were isolated. Of 426 *E. coli* strains, 387 were grouped into 77 serogroups, while 70 and 59 strains were untypable and rough, respectively. All isolates were subjected to multiplex polymerase chain reaction (m-PCR) for the detection of stx(1), stx(2), eaeA, hlyA and saa genes. None of the *E. coli* strains studied showed the presence of stx(1), stx(2) or their variants and saa genes. Overall 11 (2.74%) and seven (1.74%) strains from chicken possessed eaeA and hlyA genes respectively, while as only six (1.49%) strains from chicken possessed both eaeA and hlyA genes. O9, O8, O60 and O25 serogroups were most predominant of which there were 24 (5.63%), 23 (5.39%), 23 (5.39%) and 20 (4.69%) strains, respectively. None of the isolates from pigeons showed the presence of any of the virulence genes studied. He
concluded that STEC were absent in chicken and pigeons. This study was the first attempt to investigate STEC in chicken and free flying pigeons in India.

Shiga Toxin Producing *Escherichia coli* (STEC) strains were isolated from diarrhoeal samples, stool samples from cattle, beef, mutton samples, urine, water and sewage samples from different places in Gulbarga region of Karnataka, India. The highest number of STEC was found in the Sewage sample (14.84%) whereas urine sample did not contain any STEC. Among the 2109 samples 65 isolates were confirmed as STEC. The highest (73%) incidence of resistance was recorded against Ampicillin, closely followed by streptomycin (70.77%) and cephalxin (69.23%). While only two antibiotics, chloramphenicol (21.54%) and gentamicin (12.3%) recorded comparatively lower incidence of resistance. (Kesava Naidu *et al.*, 2007).

Wani *et al.* (2007) determined the subtypes of stx and eae genes of Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *Escherichia coli* (EPEC) from calves. One hundred and eighty-seven faecal samples from 134 diarrhoeic and 53 healthy calves were investigated for the presence of stx, eae and ehxA virulence genes by polymerase chain reaction and enzyme-linked immunosorbent assay. Subtype analysis of stx(1) exhibited stx(1c) in 13 (31.70%) isolates, while that of stx(2) revealed stx(2c) in eight (24.24%) and stx(2d) in two (6.06%) isolates. Subtyping of eae gene showed the presence of eae-beta, eae-eta and eae-zeta in two, three and four isolates respectively. None of the *E. coli* isolates possessed stx (2e), stx (2f), eae-alpha, eae-delta, eae-epsilon and eae-xi. All EPEC isolates were atypical. They concluded that stx (1), stx (1c), stx (2), stx (2c), stx (2d), eae-beta, eae-eta and eae-zeta subtypes are prevalent in STEC and EPEC isolates in India. This is the first subtype analysis of stx (2) and eae genes of animal *E. coli* isolates in India and emphasizes the need to investigate their transmission to humans.

Rajkhowa *et al.* (2010) have studied the faecal samples obtained from 190 healthy mithuns. Total one-hundred and five *E. coli* isolates were obtained from these samples, which belonged to 55 different serogroups. These isolates were subjected to multiplex
polymerase chain reaction (m-PCR) for detection of stx1, stx2, eaeA and hlyA genes. Twenty-three (21.90%) E. coli isolates belonging to 14 serogroups revealed the presence of at least one virulence gene when examined by m-PCR. Nineteen percent and 2.85% of the mithuns were found to carry Shiga toxin-producing E. coli (STEC) and enteropathogenic E. coli, respectively. Stxl and stx2 genes were found to be prevalent in 7 (6.67%) and 18 (17.14%) of the isolates respectively, whereas eaeA and hlyA genes were found to be carried by three (2.85% each) isolates. Interestingly, none of the STEC isolates belonged to serogroup O157.

Lanjewar et al. (2010) studied the prevalence of diarrhoegenic Escherichia coli (DEC) in dysentery cases with special reference to Shiga-like toxin producing Escherichia coli (STEC) in Mumbai. During a two-year period, 1066 stool samples were collected from hospitalized patients with diarrhoea and dysentery. Of the 100 E.coli strains isolated in pure culture and sent for sero typing to Central Research Institute (CRI), Kasauli, 43% were found to be Diarrhoegenic Escherichia coli (DEC), giving an isolation rate of 4.03%. Results of serotyping showed 37.21% STEC which were more common in children. Abdominal pain and stool with mucus flakes were statistically significant parameters (p less than 0.01) in patients with dysentery due to E.coli strains. Though E.coli O157 was not encountered, it was seen that 25% of STEC did not ferment sorbitol. The DEC strains showed maximum in vitro sensitivity to amikacin (83.72%) and all strains were resistant to nalidixic acid. Though Enterohemorrhagic E.coli (EHEC) O157:H7 was not encountered in this study, STEC caused by E.coli non O157 was reported.

An outbreak of acute diarrhoea in poultry birds at Aizawl, Mizoram was investigated for detection and characterization of STEC and enteropathogenic E. coli (EPEC) (Dutta et al., 2011). E. coli was isolated and identified from rectal swabs, intestinal contents, heart blood and spleen of 19 poultry birds that died due to acute diarrhoea during the outbreak. Phenotypic characterization was done by standard bacteriological and biochemical techniques. All the isolates were serotyped based on their somatic antigens. Virulence genes (stx1, stx2, eaeA and hlyA) were detected by multiplex
PCR assay. A total of 42 *E. coli* isolates were obtained, of which 24 belonged to 3 serogroups (O64, O89 and O91) and the remaining 18 were untypeable (UT). Altogether, 14 (33.33%) isolates carried at least 1 virulence gene, of which 10 (23.81%) and 4 (9.52%) were recorded as STEC and EPEC, respectively. Of the 10 STEC isolates, one carried only *stx*2; one carried *stx*2 and *hly*A, four carried *stx*1, *stx*2 and *hly*A, two carried *stx*1, *eae*A and *hly*A genes and two carried *stx*1 and *eae*A. Of the four EPEC isolates, two carried *eae*A and *hly*A, one carried only *eae*A gene and 1 carried only *hly*A gene. This was the first report on the involvement of STEC in poultry in India.

Kesava Naidu *et al.* (2011) investigated the presence of Shiga toxin producing *E. coli* (STEC) in diarrhoea patients (885 numbers) attending various health centers in Gulbarga as well as in fecal samples from diarrhoeic farm animals (n=158), ground beef (n=205), meat (n=157), sewage (n=182) and water (n=247) from ponds and bore wells. STEC was detected in 65 samples, in which detection of Stx1 & Stx2 genes was performed by polymerase chain reaction. Stx1 (n=56; 86.2%) was found to be present more frequently than Stx2 (n=7; 12.5%) and the presence of both was noted in only two human stool samples (3.1%). Plasmid profiling and protein profiling by SDS-PAGE were performed on 30 and 18 of the isolated STEC strains respectively. A total of 9 different sized plasmids ranging from 1kb to 90 kb have been detected, forming about 12 different plasmid profiles in 30 SETC isolates. Plasmid profile was subjected for cluster analysis and the dendrogram developed indicated seven different clusters, the major cluster including as many as 12 isolates. Whole cell protein profiles were done for 18 STEC isolates. Seven distinguishing protein bands ranging in size from < 20 kDa to > 97.4 kDa were detected. The 66 kDa protein was found in only one isolate, while the protein of slightly less than < 97.4 kDa (n=10) was detected in maximum number of isolates. A large number of protein profiles were observed indicating a quite diversified nature of the STEC isolates. The dendrogram prepared using cluster analysis of the distinguishing proteins indicated the existence of only two closely related clusters of human and sewage isolates.
A total of 107 faecal samples were collected from diarrhoeic lambs of high altitude terrains (2,000 to 5,000 meters above the mean sea level) of Tawang and West Kameng districts of Arunachal Pradesh, India (Bandyopadhyay et al., 2011). A total of 234 *Escherichia coli* were isolated and further subjected to PCR for the study of virulence repertoire characteristics of Shiga toxin-producing *E. coli* (STEC) and enterotoxigenic *E. coli* (ETEC). Out of the 234 isolated *E. coli*, 32% were found positive for STEC, and 9% were carrying virulence gene for ETEC. The isolated STEC serogroups were O159, O127, O120, O113, O60, O30, O25, O8 and O2. Of all the 74 STEC strains, PCR showed that 18% isolates carried stx (1), 26% possessed stx (2) and 47% produced positive amplicon for both. Other virulent attributes like intimin (eaeA), enterohaemolysin (ehxA) and STEC auto-agglutinating adhesin (saa) were present in 18%, 43% and 44% of the isolates, respectively.

### 3.9. Prospects for treatment and prevention of STEC Infection

#### 3.9.1. Antibiotics

The objectives of therapeutic strategies would be threefold: (i) to limit the severity and/or duration of gastrointestinal symptoms, (ii) to prevent life-threatening systemic complications such as HUS, and (iii) to prevent the spread of infection to close contacts.

Antibiotic therapy might be expected to satisfy all three of the above goals. However, doubts have been raised as a consequence of retrospective studies of its efficacy in preventing the progression of STEC infection from diarrhoea or bloody diarrhoea to HUS. However, in one study, HUS patients who had been given antibiotics during the diarrhoeal prodrome had milder illness (Martin et al., 1990). Examination of antibiotic use in two recent large O157:H7 STEC outbreaks in Scotland and Japan have also produced conflicting findings. Stewart et al. (1997) found a significant association between prior antibiotic usage and subsequent development of HUS. On the other hand, Takeda et al. (1997) found that the proportion of patients who progressed from bloody diarrhoea to HUS was significantly lower when antibiotics had been administered within 3 days of the onset of symptoms, compared with untreated patients or those given...
antibiotics later in the course of infection. Very few prospective studies have been performed, but Proulx et al. (1992) found that administration of trimethoprim-sulfamethoxazole to patients infected with O157 STEC (albeit late in the course of infection) did not prevent progression to HUS.

3.9.2. Therapeutic strategies directed against Stx

Stx is principally responsible for the clinical manifestations of STEC infection, particularly the serious systemic complications. Thus, in vivo binding or neutralization of Stx is an attractive therapeutic alternative, particularly in view of the possible risks associated with the use of antibiotics. Such strategies have the potential to limit the severity or duration of disease but would not, of course, be expected to reduce STEC transmission. Therapeutic strategies exploiting the high degree of specificity and the strength of the interaction between Stx and its glycolipid receptor are currently being developed. One particularly promising agent is Synsorb-Pk (Chemiomed, Edmonton, Canada), which consists of the oligosaccharide component of Gb3 covalently linked via an 8-carbon spacer to silica particles derived from diatomaceous earth (Armstrong et al., 1991). Synsorb-Pk was capable of binding and neutralizing Stx1 and Stx2 in polymyxin B extracts of STEC cultures and in feces from patients with HC and/or HUS. A phase I clinical trial did not detect any adverse effects associated with oral administration, and Synsorb-Pk retained its Stx binding capacity even after passage through the human gastrointestinal tract (Armstrong et al., 1995). Boyd et al. (1997) synthesized a galabiosyl analog dimerized via the meta position of benzene, which is capable of blocking both Gb3 binding sites on the Stx1 B subunit. The analog also protected Vero cells against Stx1 cytotoxicity and to a lesser extent against Stx2.

3.9.3. Vaccines based on STX

Vaccines based on Stx are likely to be effective in preventing HUS in persons infected with STEC and may also have a significant impact on the severity of gastrointestinal symptoms. This confidence is underpinned by the results of studies of the
protective efficacy of vaccines based on Stx2e for protection of pigs from edema disease. MacLeod and Gyles (1991) demonstrated that immunization with Stx2e toxoid with glutaraldehyde protected piglets from intravenous challenge with an otherwise lethal dose of purified toxin. Gordon et al. (1992) found that Stx2e toxoid with formaldehyde was immunogenic in piglets but that there appeared to be residual in vivo toxicity. However, adverse reactions were not observed when piglets were immunized with an immunogenic derivative of Stx2e with a defined amino acid substitution (Glu1673Gln) in the active-site region of the A subunit. Immunization with this “genetic toxoid” has recently been shown to protect piglets from edema disease after oral challenge with a Stx2e-producing strain of E. coli (Bosworth et al., 1996).

An alternative means of circumventing the problem of residual toxicity is to use just the B subunit as an immunogen. Several B subunit-specific Stx/Stx1-neutralizing monoclonal antibodies have been prepared (Strockbine et al., 1985), and neutralizing polyclonal antibodies were isolated from hyperimmune sera by affinity chromatography with immobilized B subunit (Donohue-Rolfe et al., 1984). Boyd et al., (1991) demonstrated that immunization of rabbits with purified Stx1 B subunit confers protection against challenge with an otherwise lethal intravenous dose of purified Stx1.

3.9.4. Vaccines to prevent gut colonization by STEC

While vaccines based on Stx would be expected to prevent the serious systemic complications of STEC disease, they would not be expected to prevent colonization of the gut by STEC and so would have little effect on the transmission of disease in the community. Vaccines directed against colonization factors would be expected to be more effective, but at present our knowledge of these factors is incomplete. STEC intimin (EaeA) may be an appropriate vaccination target, at least for the majority of human pathogenic STEC strains which produce it. Indeed, Butterton et al., (1997) constructed a V. cholerae vaccine strain carrying a chromosomally integrated copy of eaeA which expressed intimin on its surface. Oral immunization of rabbits with this strain elicited a serum antibody response to intimin. However, the C-terminal portion of intimin exhibits
marked amino acid sequence heterogeneity among different STEC strains (Louie et al., 1994), and it remains to be seen whether this will limit cross-protection. Vaccines directed against LPS may also be effective against STEC, since experience with other enteric pathogens suggests that LPS-specific serum IgG is likely to leak into the gut lumen in quantities sufficient to block colonization. Phase I clinical trials of O157 O antigen conjugated to a protein carrier (to enhance immunogenicity) have shown that such vaccines are safe and immunogenic (Szu, 1997).

3.10. Medicinal plants effective against STEC

Yoshikazu et al. (2001) investigated the inhibitory effect of several plant extracts on the production of verotoxin by enterohemorrhagic Escherichia coli O157 : H7 (EHEC). The extracts from four plant species, Limonium californicum (Boiss.) A. Heller, Cupressus lustianica Miller, Salvia urica Epling and Jussiaea peruviana L., were effective on the inhibition of verotoxin production (31.3–125 μg/ml). The inhibition against verotoxin production was observed at a concentration lower than the minimal inhibitory concentration (MIC) of each extract of test plants (1000 μg/ml), indicating that these plant extracts would preferentially prevent the production of verotoxin rather than bactericidal effect on EHEC. These findings suggest that the administration of any appropriate plant extract might prevent the production of verotoxin by EHEC in the human intestines.

Fifty-eight preparations of aqueous and ethanolic extracts of 38 medicinal plant species commonly used in Thailand (Voravuthikunchai et al., 2004) to cure gastrointestinal infections were tested for their antibacterial activity against different strains of Escherichia coli, including 6 strains of Escherichia coli O157:H7, Escherichia coli O26:H11, Escherichia coli O111: NM, Escherichia coli O22; 5 strains of Escherichia coli isolated from bovine; and Escherichia coli ATCC 25922. Inhibition of growth was primarily tested by the paper disc agar diffusion method. Among the medicinal plants tested, only 8 species (21.05%) exhibited antimicrobial activity against Escherichia coli O157:H7. Acacia catechu(Karungali), Holarrhena antidysenterica
(Kudasapalai), *Peltophorum pterocarpum*(Perunkonrai), *Psidium guajava*(Koiyaa), *
*Punica granatum*(madulai), *Quercus infectoria*(Maasikaai), *Uncaria gambir*(Otalaimaram/ Kampirceti), and *Walsura robusta*(Cattuvakku/ Valacura) demonstrated antibacterial activity with inhibition zones ranging from 7 to 17 mm. The greatest inhibition zone against *Escherichia coli* O157:H7 (RIMD 05091083) was produced from the ethanolic extract of *Quercus infectoria*. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined by the agar microdilution method and agar dilution method in petri dishes with millipore filter. Both aqueous and ethanolic extracts of *Quercus infectoria* and aqueous extract of *Punica granatum* were highly effective against *Escherichia coli* O157:H7 with the best MIC and MBC values of 0.09, 0.78, and 0.19, 0.39 mg/ml, respectively. These plant species may provide alternative but bioactive medicines for the treatment of *Escherichia coli* O157:H7 infection.

Voravuthikunchai et al. (2005), studied the antibacterial activity of *Punica granatum* pericarp extracts against different strains of Enterohaemorrhagic *Escherichia coli* O157:H7 and Non O157:H7 strains. The ethanolic extract was found to be the most effective and the Minimum Inhibitory Concentration was 0.09mg/ml.

Duraipandian et al. (2006), evaluated the antimicrobial activity of 18 medicinal plant extracts against nine bacterial strains (*Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Enterococcus faecalis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Ervinia sp, Proteus vulgaris*) and one fungal strain (*Candida albicans*). The collected ethnomedicinal plants were used in folk medicine in the treatment of skin diseases, venereal diseases, respiratory problems and nervous disorders. Among the plants tested, *Acalypha fruticosa*(Kuppaimeini), *Peltophorum pterocarpum*(Perunkonrai), *Toddalia asiatica*(Milakaranai), *Cassia auriculata*(Avarai), *Punica granatum*(madulai) and *Syzygium lineare*(campai) were most active. The highest antifungal activity was exhibited by methanol extract of *Peltophorum pterocarpum* and *Punica granatum* against *Candida albicans*. 

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Ethanolic extracts of eight Thai medicinal plants (representing five families) that are used as traditional remedies for treating diarrhoea were examined with a salt aggregation test for their ability to modulate cell surface hydrophobicity of enterohemorrhagic \textit{Escherichia coli} strains, including \textit{E. coli} O157:H7. Four of these medicinal plants, \textit{Acacia catechu}, \textit{Peltophorum pterocarpum}, \textit{Punica granatum}, and \textit{Quercus infectoria}, have high bacteriostatic and bactericidal activities. The ethanolic extract of \textit{Q. infectoria} was the most effective against all strains of \textit{E. coli}, with Minimum Inhibitory Concentrations (MICs) of 0.12 to 0.98 mg/ml and Minimum Bactericidal Concentrations (MBCs) of 0.98 to 3.91 mg/ml. The ethanolic extract of \textit{P. granatum} had MICs of 0.49 to 1.95 mg/ml and MBCs of 1.95 to 3.91 mg/ml. Ethanolic extracts of \textit{Q. infectoria}, \textit{P. pterocarpum}, and \textit{P. granatum} were among the most effective extracts against the two strains of \textit{E. coli} O157:H7. The other four plants, \textit{Andrographis paniculata} (Nilavembu), \textit{Pluchia indica} (phaar-hindi), \textit{Tamarindus indica} (cukkilaimaram), and \textit{Walsura robusta} (Cattuvakku/ Valacura), did not have high bacteriostatic and bactericidal activities but were able to affect hydrophobicity characteristics on bacterial outermost surface. All plants except \textit{Q. infectoria} had some ability to increase bacterial cell surface hydrophobicity (Voravuthikunchai and Limsuwan, 2006).

Sharon \textit{et al.} (2006) developed prototypic orally delivered, plant-based vaccine against Stx2, an AB5 toxin. The genetically inactivated Stx2 active A subunit genes were transformed in the \textit{Nicotiana tabacum} (tobacco) cell line NT-1 by \textit{Agrobacterium tumefaciens}- mediated transformation. Toxoid expression was detected in NT-1 cell extracts, and the assembly of the holotoxoid was confirmed. Finally, mice were immunized by feeding with the toxoid-expressing NT-1 cells or by parenteral immunization followed by oral vaccination (prime–boost strategy). The immunized mice produced Stx2-specific mucosal IgA and Stx2-neutralizing serum IgG. The protective efficacy of these responses was assessed by challenging the immunized mice with \textit{E. coli} O91:H21 strain B2F1, an isolate that produced an activatable variant of Stx2 (Stx2d) and was lethal to mice. The oral immunization fully protected mice from the challenge.
Results of their study demonstrated that a plant-based oral vaccine can confer protection against lethal systemic intoxication.

Aboaba et al. (2006) screened sixteen preparation of the ethanol and aqueous extracts of four edible plants, *Entada Africana* (bark), *Terminalia avicennoides* (bark), *Mitragyna stipulosa* (bark) *Lannea acida* (stem bark) to test their inhibitory effects on ten strains of *E coli 0157:H7* (EHEC) using the agar diffusion method. It was found that ethanol extracts of *Entanda africana* inhibited all the ten strains used, some extracts showed variable antibacterial activities while some others could not cause any inhibition. The minimum inhibitory concentration (MIC) of the potent extracts ranged from 1.56mg/ml to 50.00mg/ml while the minimum bacteriocidal concentration (MBC) was between 6.25mg/ml to 25.00mg/ml. Pytochemical screening of the extracts revealed that all contained saponin. Some showed the presence of tannins and glycosides while alkaloid was not detected in all samples.

Voravuthikunchai et al. (2008) reported that *Quercus infectoria* was effective against a broad range of bacteria of clinical importance including *Acinetobacter baumannii*, *Bacillus cereus*, *Enterobacter faecalis*, *Escherichia coli*, *Helicobacter pylori*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Shigella flexneri*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Streptococcus pyogenes*. Ethanol extracts of *Quercus infectoria* showed a broad spectrum of activity against all bacterial species tested. They also studied the activity of *Quercus infectoria* on clinical isolates of pathogens commonly presenting problems with the use of antibiotics, including enterohemorrhagic *Escherichia coli* (EHEC), methicillin-resistant *Staphylococcus aureus* (MRSA), multidrug-resistant *Klebsiella pneumoniae*, multidrug-resistant *Helicobacter pylori* and *Salmonella* spp. The extracts of *Quercus infectoria* displayed remarkable activity against MRSA with MICs ranging from 0.02 to 0.4 mg/mL, and MBCs ranging from 0.4 to 1.6 mg/mL. More importantly, this plant species could exhibit strong antibacterial activity against all Gram-Negative organisms. Its significant activity was shown with EHEC, with MICs of 0.05 to 0.1 mg/mL and MBCs of 0.8 to 1.6 mg/mL. The results from this study indicate that *Quercus infectoria* is
potentially a good source of antibacterial substance with broad spectrum of activities against antibiotic-resistant bacteria.

A natural compound that has potent antimicrobial properties is citrus peel, which contains a variety of essential oils that inhibit the growth or kill pathogenic bacteria. In a study by Ramakrishna et al. (2008), seven citrus-based natural antimicrobials were evaluated for their ability to inhibit the growth of the pathogen *Escherichia coli* O157:H7. Zones of inhibition of *E. coli* O157:H7 by the citrus-derived fraction were determined by a disk-diffusion assay on Sorbitol-MacConkey agar. Inhibition zones were observed after 48 hours lawn growth of *E. coli* O157:H7 cells at 37°C. Two citrus-based fractions, orange CP VAL terpeneless FAB 968611 and Limonene 1_Dist FAB 955430, inhibited *E. coli* O157:H7 with inhibition zones of approx. 11–24mm diameter.

Antibacterial actions against STEC O157:H7 were investigated by observing cell viability as well as morphological and ultrastructural changes (Suwalak and Voravuthikunchai, 2009). Ethanolic extract of *Q. infectoria* showed inhibitory and bactericidal effects on all of the strains tested with minimal inhibition concentrations (MICs) at 0.78-1.56 mg ml(-1) and minimal bactericidal concentrations (MBCs) at 1.56-3.12 mg ml(-1). At MIC (0.78 mg ml(-1)), there was some evidence that the cytoplasmic membranes of the treated *E. coli* were bulging and/or ruptured, and the cells appeared to be discharging intracellular materials. At another concentration, the outer membrane of the treated *E. coli* which was attached to the cell wall became separated from the wall. Disruption in the outer wall and cytoplasmic membranes, especially at the polar regions of the cells occurred and some vacuolization appeared. At yet another concentration, the damage to *E. coli* cells was extensive, and there was loss to their cellular integrity.

In a study by Masibo and He (2009), Chinese mango leaves were subjected to different extraction regimes namely decoction (50 °C for 48 hours and boiling for 3 hours) and solvent extraction (50% ethanolic solution at 62 °C for 1 hour). The extract solutions were lyophilized and 24–26% powdered extracts were used for the antimicrobial tests against four pathogenic (*Salmonella typhi, Escherichia coli*,
Staphylococcus aureus, Bacillus cereus) and two non pathogenic (Streptococcus thermophilus, and Lactobacillus acidophilus) microbial strains. Antimicrobial activity was assessed by inhibition zones which were found to range from 9–15.5 mm for the three extracts against all the bacterial strains tested and inhibition growth curves showed the extracts as first acting within the first 20 minutes after which a steady state (bacteriostatic) was attained and maintained throughout the testing time (360 minutes) with a significant difference (p<0.05) from the negative control. The minimum inhibitory concentrations ranged from 36.2–18.2 mg/mL against all the strains. The extracts were then subjected to reversed-phase HPLC to identify and quantify the polyphenol mangiferin which was found to be the abundant compound in the extracts at 100 μg/mL.

John Hwa et al. (2011), studied the antibacterial activity of a herbal combination composed of Mume Fructus, Coptidis Rhizoma, and Schizandrae Fructus extracts on enterohemorrhagic Escherichia coli (EHEC). The combination demonstrated antibacterial activity against all EHEC strains tested in this study, including those resistant to multiple antibiotics; minimum inhibitory concentration values ranged from 0.49 to 31.25mg/mL. In in vivo antibacterial activity assay, the herbal combination was administered to mice after initial E. coli O157 infection and had significant effects on mouse mortality. The effects of the herbal combination on Shiga toxin release from EHEC O26, EHEC O111, and EHEC O157 strains containing the stxl and stx2 genes were assessed by the reversed passive latex agglutination method, and there was no increased Shiga toxin release in the strain cultures containing the herbal combination. These results suggested that the herbal combination may be a safe and effective remedy for EHEC inhibition.

Doughari et al. (2011) characterized verotoxic non O157: H7 Escherichia coli strains from different samples. Isolates were tested for virulence factors such as verotoxin, haemolysin, gelatinase, extended spectrum beta lactamases (ESBLs), cell surface hydrophobicity and bacterial serum resistance, as well as susceptibility (using disc diffusion method) to stem bark extracts of Curtisia dentata. Results showed the presence of different serotypes of E. coli (69 isolates in all) including O26: H11, 055, O111: NM, O126, O44, O124, O96:H9, O103:H2, O145: NM and O145:H2. Over 60%
of the isolates exhibited serum resistance, haemolysin and gelatinase production, 81% exhibited cell surface hydrophobicity and over 52% produced ESBLs. Sixty percent of the isolates showed various levels of resistance to different antibiotics [ampicillin (10 μg), cefuroxime, cephalaxin, ceftazidime and tetracycline (30 μg in each case) (multidrug resistance index (MDRI) values 4.20 to 5.60%), but only 28% were resistant to ethanol stem bark extracts of C. dentata (MIC, 70 to 150 mg/ml). They conclude that C. dentata has a potential for sourcing novel antibiotic substances for chemotherapy against these resistant pathogenic strains of E. coli.

3.11. Novel methods to find active anti shiga toxin agents

Adhesion is the critical early phase in all diarrheal infections caused by pathogenic Escherichia coli strains. It is important, therefore, to fully understand the mechanisms underlying E. coli adhesion and in that way to be able to develop methods of maintaining the intestinal normal microflora and to prevent pathogenic E. coli from initiating an infectious process.

The two E. coli pathotypes, EPEC and EHEC are distinct from other pathogenic E. coli strains because they produce a distinct histopathological lesion on intestinal epithelial cells known as the attaching and effacing (A/E) lesion. A/E lesions are marked by localized degeneration of the intestinal brush border surface, loss of epithelial microvilli, and assembly of highly organized pedestal-like actin structures in the epithelial cells at the sites of bacterial attachment. All the proteins associated with the formation of A/E lesions are encoded on a chromosomal pathogenicity island known as the locus of enterocyte effacement (LEE). The LEE contains the eae (stands for E. coli attaching and effacing) gene, encoding the outer membrane protein intimin. This protein mediates intimate adherence to target eukaryotic cells upon interaction with its translocated receptor Tir (stands for translocated intimin receptor), a protein encoded upstream of the eae gene on the LEE (Alfredo Torres et al., 2005).

The concept of how EPEC and EHEC strains causing A/E lesions adhere to mammalian cells has been enhanced by the description of the crystal structure of intimin coupled to the Tir receptor. That study resulted in a model that indicates that, once translocated, the Tir protein spans the host cell membrane, adopting a hairpin
loop structure featuring both its N and C termini in the host cytoplasm and a central extracellular domain that binds intimin. In addition to serving as a receptor for intimin, the Tir protein is capable of interacting with host cytoskeletal and signaling components using its N- and C-terminal domains located in the host cell cytoplasm. Further, it has been shown that intimin has an affinity for the eukaryotic proteins nucleolin and β1 integrin, and they serve as potential receptors for intimin during STEC infection. The numerous host proteins that accumulate in the A/E lesion around Tir and the mechanisms by which Tir exploits the host cell signaling networks results in actin cytoskeletal rearrangements (Fig.3.1) (Alfredo Torres et al., 2005).

**Fig.3.1. Mode of action of intimin and shiga toxin**

Stx is principally responsible for the clinical manifestations of STEC infection, particularly the serious systemic complications. Thus, *in vivo* binding or neutralization of Stx is another attractive therapeutic alternative, particularly in view of the possible risks associated with the use of antibiotics. Such strategies have the potential to limit the severity or duration of disease but would not, of course, be expected to reduce STEC transmission. Therapeutic strategies exploiting the high degree of specificity and the strength of the interaction between Stx and its glycolipid receptor are currently being developed. In our study the phytochemicals selected from the drug bank were docked against the intimin receptor and the shiga toxin.