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Review

Shiga toxin-producing *Escherichia coli* (STEC) from Farm Animals and Humans in Tropical Africa - A review

M. S. Adamu[1], I. H. Kubkomawa[1*], A. Ahmadu[2] and N. S. Abubakar[1]

[1]Department of Animal Health and Production Technology, The Federal Polytechnic, P. M. B. 35, Mubi, Adamawa State, Nigeria.
[2]Department of Animal Science, Ahmadu Bello University (ABU) Zaria, Kaduna State, Nigeria.

*Correspondent author. Email: kubkomawa@yahoo.com. Tel: 07066996221, 070580550.

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ABSTRACT: The study was designed to review the incidences and characteristics of Shiga toxin-producing *Escherichia coli* (STEC) from farm animals and humans in tropical Africa. Serotypes O157, O26, O103, O91, O45 and O111 are usually associated with public health risks, and these serotypes are most frequently isolated from food animals. The main virulent factors of STEC associated with human diseases are potent cytotoxins (shiga toxins [*stx*] stx_1 and stx_2), which are encoded by the stx_1 and stx_2 genes. Two additional markers also play a role in the pathogenesis of Hemorrhagic colitis (HC) and Hemolytic Uremic Syndrome (HUS): an outer membrane protein (intimin), encoded by the *eae* gene, and enterohaemolysin, encoded by the *ehlyA* gene. All age groups of animals and humans can be infected with STEC, but young animals and children, the elderly, and those with compromised immune systems are the most severely affected. Little is known about factors that determine susceptibility to STEC infection and the risk factors for the development of systemic complications such as HUS. The peak age incidences (infants and young children) of classical HUS is a reflection of the age incidences of STEC infection as a whole, suggesting that susceptibility to STEC-associated HUS may, like other specific infectious diseases of childhood, be due to the lack of specific immunity, possibly to ST. The increasing recognition of STEC-associated HUS in the elderly would be consistent with waning immunity in that age group. There should be sensitization and awareness regards to the incidences and distribution of STEC serogroups O157, O26, O45, O91, O103 and O111 which are associated with public health risks in livestock and humans in tropical Africa and some parts of the world.

Key words: Characteristics, *Escherichia coli,* humans, incidences, livestock, Tropical Africa.

INTRODUCTION

Shiga Toxin (*Stx*) - Producing Escherichia *coli* (STEC), also called Verocytotoxin (VT)-Producing *E. coli* (VTEC), isone of the most important groups of diarrhea causing *E. coli*that is associated with food-borne outbreaks leading to life threatening complications (Paton and Paton, 1998; Beutin et al., 2004). The incidence of STEC in camel calves diarrhea, although not studied extensively, has been reported in Eastern Sudan (Mohammed et al., 2000) and recently, Rahimi et al. (2010) reported the prevalence of STEC O157:H7 in camel carcass during processing in Iran. A restricted range of serotypes (O157, followed by O26, O103, O91, O45 and O111) are associated with public health risks and these serotypes are most frequently isolated from food or animals (Willshaw et al., 2001; Bennett and Bettelheim, 2002). Although various STEC strains have been isolated from different animals (Beutin et al., 1993; 1995; Mohammed et al, 2003; Lengacher et al., 2010; Rahimi et al., 2010), they have been shown to be more prevalent in ruminants than in other animals (Beutin et al., 1993; Caprioli et al.,

1993; Beutin et al., 1995 and Islam et al., 2008). Ruminants, mainly cattle, sheep and goats, have been established as major natural reservoirs of STEC and are known to play a significant role in the epidemiology of human infection (Hussein 2007 and Islam et al., 2007).

STEC belonging to the serotype O157 have been extensively studied and shown to be involved in many cases and outbreaks of human diseases. Most outbreaks and sporadic cases of HC and HUS have been attributed to the STEC O157 strains *(*Bell et al., 1994; Conedera et al., 1997*;* Chapman, 1997; Elder et al., 1997 and Tarr et al., 2005*).* However, infections caused by some non-O157 serotypes have also been frequently associated with severe illness in humans. In some geographic areas, STEC non-O157 strains are more commonly isolated from persons with diarrhea or HUS than STEC O157 strains (Griffin et al., 1991; Pradel et al., 2000 and Moses, 2005). Out of the 2988 cases of STEC non-O157 (O104:H4) outbreaks in Germany, 759 developed HUS (WHO, 2011).

The main virulent factors of STEC associated with human disease are potent cytotoxins (shiga toxins [*stx*] stx_1 and stx_2), which are encoded by the stx_1 and stx_2 genes. Two additional markers also play a role in the pathogenesis of HC and HUS: an outer membrane protein (intimin), encoded by the *eae* gene, and enterohaemolysin, encoded by the *ehlyA* gene (Paton and Paton1998; Karmali et al., 2010). This genetic virulent characteristics is often used in epidemiological studies to correlate between strains from various sources (Askari et al., 2010). Molecular sub typing techniques used in ten of 19 human incidents in Scotland showed that STEC O157 isolates from cattle and human cases were indistinguishable (Synge, 1997). Although STEC isolated from animals have been implicated as a cause of diarrhoea and hemorrhagic colitis in human (Gyles and Fairbrother, 2004; Radostits et al., 2007; Islam, et al., 2008 and Badouei et al., 2010).

In Nigeria, studies conducted in south-western part reported the isolation of *E. coli* 0157:H7 and other pathogenic *E. coli* strains from human patients with diarrhea (Olorunshola et al., 2000; Okeke et al., 2000a and 2003b). Similarly, the isolation of non-O157 STEC and some EPEC serotypes was reported from feces of diarrheic calves collected from various farms in Zaria, North-central Nigeria (Tekdek et al., 1995). Also in North-Eastern Nigeria, Moses, (2005) isolated STEC O157 from HIV infected patients and non-O157 from human and cattle feces. The prevalence and distribution of STEC serogroups O157, O26, O45, O91, O103 and O111 which are associated with public health risks are not popular in livestock and humans in some parts of tropical Africa in spite of the danger to public health safety. It is against this background that this study was conceived to review incidences and characteristics of Shiga toxin-producing *Escherichia coli* (STEC) from farm animals and humans in tropical Africa.

Pathogenic *E. coli*

Pathogenic forms of *E. coli* can cause a variety of diarrheal diseases in hosts due to the presence of specific colonisation factors, virulent factors and pathogenicity associated genes which are generally not present in other *E. coli*. Of the strains that cause diarrheal diseases, six pathotypes are now recognized. These pathotypes are: Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAggEC), Diffusely adherent *E. coli* (DAEC) and Verocytotoxigenic *E. coli* (VTEC) also called Shiga Toxin (*Stx*)- Producing *Escherichia coli* (STEC) (PEN, 2006)

The pioneering work that led to the discovery of the *E. coli* VTs was done by Konowalchuk and his colleagues during the late 1970s in Canada (Konowalchuk et al., 1977, 1978a and 1978b). While investigating the usefulness of Vero (African green monkey kidney) cells for detecting the heat-labile enterotoxin (LT) of *E. coli*, they observed that culture filtrates from some *E. coli* strains produced a profound irreversible cytopathic effect in Vero cells in contrast to the reversible cytotonic effect of LT. Culture filtrates from 10 of 136 *E. coli* strains from diverse sources produced a VT effect; Seven of the strains (serotypes 026, 0128: B12, 0111:B4, 018:B21, and 0126:B16) were from infants with diarrhea, One isolate (serotype 0138:K81) was from a weanling pig, and Two VT' cultures (serotypes 068:H12 and 026:K60) originated from cheese. These observations led Konowalchuk and his colleagues to speculate that VT may contribute to diarrheal disease. It should be noted that the "enterotoxic" activity of what later became known as VT had earlier been demonstrated in 1971 by Smith and Lingood (Smith and Lingood, 1971). Hardas et al. (1982) in India found that 8 of 102 *E. coli* strains from patients with diarrhea were VT'. These studies, however, failed to shed light on either the etiological significance of VTEC in diarrheal disease or the possible pathogenic significance of VT. The major breakthroughs occurred in 1983 with the publication of studies from the United States and Canada which linked VTEC infection to two conditions of previously unknown cause, hemorrhagic colitis (Riley et al., 1983) and HUS (Karmal et al., 1983). A classic epidemiologic investigation from the Centers for Disease Control, Atlanta, (Riley et al. 1983), linked two outbreaks of hemorrhagic colitis, a hitherto poorly understood bloody diarrhea condition, with what was then considered a "rare" *E. coli* serotype, 0157:H7. Shortly thereafter, the American isolates of *E. coli* 0157:H7 were subsequently confirmed by O'Brien and colleagues to be positive for a Shiga-like cytotoxin (O'Brien et al., 1983). Earlier work by O'Brien's group (O'Brien et al. 1982) had led to the important observation that the cytotoxin (VT)

from Konowalchuk's reference strain H30 was very closely related to Shiga toxin from Shigella dysenteriae type 1. Thus developed the two different nomenclatures now used for the *E. coli* cytotoxins VT and Shiga-like toxin (SLT). While the epidemiological studies on hemorrhagic colitis clearly established an association with *E. coli* 0157:H7, the potential significance of VT in this condition remained uncertain. Studies from Canada (Karmal et al., 1983 and 1985) showed a close association between VTEC infection and the classical form of HUS, which is a leading cause of acute renal failure in childhood.

Nomenclature and definitions of STEC

The terms verocytotoxins (VT) and Shiga-like toxins (SLT) are synonymous. *E. coli* strains that produce these toxins have been referred to as VT-producing *E. coli* (VTEC), SLT-producing *E. coli* (STEC), and enterohemorrhagic *E. coli* (EHEC). The term VTEC refers to all *E. coli* strains that produce VT in culture supernatants (Konowalchuk et al., 1977; 1978a and 1978b). The term EHEC refers to strains that have the same clinical, epidemiological, and pathogenic features associated with the prototype EHEC organism *E. coli* 0157:H7. Only two VTEC serotypes (0157:H7 and 026:H11) have been classified as EHEC by Levine (Levine, 1987). The original description of SLT-producing *E. coli* included three categories of strains: (i) trace-level, (ii) low-level, and (iii) high-level producers of the toxin. High-level SLT producers contained about 100- to 1,000-fold-greater amounts of toxin than the trace- and low-level producers (O'Brien et al., 1982). Furthermore, toxin was easily detectable in supernatants of high-level producer cultures, but not in culture supernatants of trace- and low-level producers. Hemorrhagic colitis and HUS have been associated only with high-level SLT producers (corresponding to VTEC), whereas the clinical relevance of trace- and low-level SLT producing strains is uncertain. Thus, use of the term SLT producing *E. coli* can be misleading unless qualified by the amount of toxin produced. Low-level SLT production has been observed in nonpathogenic strains such as *E. coli* K-12, as well as in environmental and human isolates of *Vibrio cholerae* and *Vibrio parahaemolyticus* (O'Brien et al., 1984), but there is no evidence that these groups of bacteria are associated with either HUS or hemorrhagic colitis. Shiga-like toxin quantization, using the methods described by O'Brien and others in 1982, is not practicable in clinical microbiology laboratories. On the other hand, for routine diagnostic purposes it is feasible to examine culture supernatants for cytotoxin, i.e., cultures of strains that are of known clinical relevance and that correspond to high-level, SLT-producing *E. coli*or VTEC. The term VTEC also has an ease of expression, being less cumbersome than the expression high-level SLT-producing *E. coli*.

EPIDEMIOLOGY OF STEC DISEASES

Species and serotype distribution of *stx* producers

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. Richardson et al. (1987) listed 32 O serogroups (approximately 60 distinct O:H types), and the list has grown considerably since then (Bonnet et al., 1998; Paton and Paton, 1998 and Karmali et al., 2010). Although not represented in the initial group of STEC isolates described by Konowalchuk, serotype O157:H7 was the first STEC type to be linked to outbreaks of HC and HUS (Karmali, 1989.). In many parts of the world, STEC strains belonging to this serotype as well as O157:H2 appears 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 over estimation 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). 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 (Kappeli et al., 2011). When one of the isolated types is O157, there is a (perhaps mistaken) tendency to ignore the potential etiological significance of the other(s).

Other members of the family *Enterobacteriaceae* are known to produce *stx* and to cause serious gastrointestinal disease and HUS in humans. The most notable of these is *S. dysenteriae* type 1, the causative agent of bacillary dysentery, which is frequently complicated by HUS (O'Brien and Holmes, 1987). It is the principal cause of HUS in parts of Africa and Asia (Azim et al., 1997). Disease due to *S. dysenteriae* type 1 may be particularly severe, because the organism is capable of invading the colonic mucosa, and this might result in more efficient delivery of *Stx* to the bloodstream, as well as significant endotoxemia. *Stx2-producing Citrobacter freundii* also causes diarrhea and HUS in humans, including one outbreak in a German child care centre (Schmidt et al., 1993). Haque et al. (1996) have described the production of a *Stx*1-related cytotoxin by strains of *Aeromonas hydrophila* and *A. caviae*, as judged by *stx*1-specific PCR and neutralization of Vero cytotoxicity with *Stx*1 antiserum. *Enterobacter cloacae* have also been associated with transient expression of a *stx*2-related gene, although its role in disease is unproven (Paton and Paton, 1996).

Incidence of STEC in animals

Hussein and Bollinger (2005a) reviewed published reports

in the past 3 decades and summarized the incidence of STEC in beef cattle feces and hides. In general, the incidence rates of *E. coli* O157 ranged from 0.3 to 19.7% in feedlot cattle, from 0.7 to 27.3% in cattle on irrigated pasture, and from 0.9 to 6.9% in cattle grazing rangeland forages. These observations suggest a high potential for infection and re-infection of cattle with *E. coli* O157 during grazing of the dense vegetation on pasture. On the range, however, cattle travel in large and less-dense areas seeking edible vegetation. With regard to testing for *E. coli* O157 at slaughter, the incidence rates ranged from 0.2 to 27.8%. Worldwide, the incidence rates of non-O157 STEC ranged from 4.6 to 55.9% in feedlot cattle and from 4.7 to 44.8% in grazing cattle. With regard to testing for non-O157 STEC at slaughter, the incidence rates ranged from 2.1 to 70.1%. These observations indicate that non-O157 STEC were prevalent in all beef production systems at rates as high as 70.1%. The incidence rates, however, varied widely and could be explained by the significant impact of environmental factors, by management practices on promoting or decreasing STEC incidences, or both. Cattle hides have been identified as an important source of microbial contamination of carcasses (Ridell and Korkeala, 1993; Bell, 1997 and McEvoy et al., 2000). It has been shown that O157:H7 and non-O157:H7 STEC can be easily transferred from cattle hides to the carcass (Barkocy-Gallagher et al., 2003). Because of the role that cattle hides can play in carcass contamination with STEC at slaughter, efforts (Bacon et al., 2000; Elder et al., 2000 and Barkocy-Gallagher et al., 2003) have been devoted to evaluate its significance. Testing swab samples from cattle hides at 12 US beef processing plants in the fall revealed a 3.6% incidence rate of *E. coli* O157:H7 (Bacon et al., 2000). In this study, no attempt was made to serotype the non-O157:H7 isolates. Cattle have long been regarded as the principal reservoir of STEC strains, including those belonging to serotype O157:H7. In Ibadan, Nigeria, Olatoye, (2010) isolated *E. coli* O157:H7 serotypes from 28.4% of 250 meat sampled.

Epidemiological surveys have revealed that STEC strains are also prevalent in the gastrointestinal tracts of other domestic animals, including sheep, pigs, goats, dogs, and cats (Beutin et al.,1993). The incidence of STEC in feedlot cattle had been reported to be as high as 92% (Renter et al., 2005). Estimation of the incidence of carriage of STEC is complicated by the fact that fecal shedding may be transient and is almost certainly influenced by a range of factors including diet, stress, population density, geographical region, and season (Synge et al., 1994 and Clarke et al., 1994). Serological studies have suggested that the vast majority of cattle have been exposed to STEC at some point during their lives (Pirro et al., 1995).

A study conducted to examine the incidence of STEC in seven domestic animals observed that sheep, goats and cattle were the common domestic animal reservoirs of STEC (Beutin et al, 1993). Among the animals, the incidence in sheep was the highest, followed by goats and cattle. The other domestic animals such as dogs, pigs and cats showed low prevalence of STEC. Chickens were negative for STEC. The study also indicated that 60% of the isolated bacteria were human pathogens (Beutin et al, 1993). In addition, a study has found that deer was a reservoir of STEC (Keene et al., 1997). The occurrence of *E. coli* O157 in camel has rarely been reported, in a study on camel fecal samples from the United Arab Emirates, *E. coli* O157:H7 was not identified (Moore and Mc Calmon, 2002). Studies in five east African countries on fecal and serum samples from 400 camels failed to detect STEC or anti-*Stx* antibodies (El-Sayed et al., 2008). However, Rahimi et al. (2012) reported anincidence of 2% in camel carcass in Iran.

While many domestic animals carrying STEC are asymptomatic, certain STEC strains are capable of causing diarrhea in cattle, particularly calves (Mohammed et al., 1986). STEC strains have also been detected in cats and dogs with diarrhea (Hammermueller et al., 1995 and Abaas et al., 1989). Natural and experimental infection of calves with an O111 STEC strain results in colitis with attachment and effacement of the colonic mucosa (Moxley and Francis, 1986). Other studies involving experimental infection with O157:H7 STEC showed that both adult cattle and calves could be transiently colonized but only neonatal calves developed significant intestinal lesions, therefore, STEC infects multiple hosts.

Due to the high prevalence of STEC in food animals, STEC is a common food contaminant in foods of animal origin. Samadpour et al., (2002) tested meat, poultry and seafood samples for shiga-like toxin genes and reported that 17% of the food samples were positive for shiga toxins. Due to the occurrence of STEC in meat and its impact on public health and food safety, USDA Inspection Service (FSIS) tests ground beef for *E. coli* O26, O45, O103, O111, O121, O157 and O145 (USDA, 2012b). STEC isolates from animal sources include the important human disease-causing serotypes, as well as a number of O:H types that have yet to be associated with human infections (Karmali, 1989).

Incidence of STEC in humans

In terms of seasonality, STEC commonly occurs in summer (Besser et al., 1999). The seasonality of the disease agrees with the pattern of shedding of the bacteria; STEC are shed more commonly in hot months than cold months (Chapman et al, 1997). Since cattle are the reservoir for STEC, studies have found an association between cattle population and the incidence of STEC infections in humans. The incidence of STEC

infection in humans was higher in areas with high cattle population and in areas where manure was used for agricultural practices (Frank et al., 2008). Similarly, other studies showed that the incidence was higher in rural areas where people have frequent contact with cattle (Michel et al., 1999). Mead and his colleagues estimated that about 110,220 cases of STEC infections occurred each year in the US (Mead et al., 1999). A study done in Nebraska also showed that 1.2% of stool samples collected from patients with gastroenteritis were positive for STEC (Fey et al., 2000). Moreover, STEC are causing frequent food borne outbreaks. STEC infections are also the leading cause of HUS in the U.S. (Neill et al, 1987). According to CDC foodborne outbreak online database, there were four confirmed STEC outbreaks in North Dakota from 1998 to 2009. Three of the outbreaks were caused by *E. coli* O157 while one was caused by *E. coli* O111 (CDC, 2012e). Therefore, STEC infections are significant public health problem in the U.S.

Susceptibility to STEC Infections

All age groups can be infected with STEC, but young children, the elderly, and those with compromised immune systems are the most severely affected. Little is known about factors that determine susceptibility to STEC infection and the risk factors for the development of systemic complications such as HUS. The peak age incidence (infants and young children) of classical HUS is a reflection of the age incidence of STEC infection as a whole, suggesting that susceptibility to STEC-associated HUS may, like other specific infectious diseases of childhood, be due to the lack of specific immunity, possibly to ST. The increasing recognition of STEC-associated HUS in the elderly would be consistent with waning immunity in that age group.

Seasonality of STEC Infections

Information currently available indicates that human STEC infections occur most commonly in the summer and fall (Borczyk and Lior, 1987). In a study by Pearce et al. (2004), the highest prevalence of *E. coli* O157:H7/NM was found on meat sampled in summer and fall. However, in a study of non-O157 STEC performed on sheep, the prevalence did not follow the seasonal trend previously reported for STEC O157:H7, with the highest prevalence rates (up to 26.0%) during winter and spring (Pierard, et al., 1994**)**. Kudva et al. (1999) hypothesized that changes in diet and/or environment influenced the seasonal variation in the prevalence of STEC O157:H7. A higher prevalence rate (10.7%) of *E. coli* O157 was reported when cattle hides were tested in the summer at 4 Midwestern beef processing plants (Elder et al., 2000). These different prevalence rates could be explained by sampling time (i.e., fall vs. summer). Because a large number of variables (e.g., management practices, diets fed, animal factors, and methods of STEC detection) can influence STEC prevalence, comparisons among studies should be carefully evaluated.

In a study conducted by Barkocy-Gallagher et al. (2003), they tested fecal, hide, and carcass swab samples from cattle at 3 commercial beef processing plants over 1 year. The results revealed significant seasonal differences in the prevalence rates of O157:H7 and nonO157:H7 STEC at pre-harvest (i.e., feces and hides) and post-harvest (i.e., carcasses). The prevalence rates for O157:H7 and non-O157:H7 STEC, however, varied among cattle hides (60.6 and 56.6%, respectively), feces (5.9 and 19.4%, respectively), and carcasses (26.7 and 58.0%, respectively). With regard to cattle hides, prevalence of *E. coli* O157:H7 was highest in the spring, summer, and fall (averaging 71.5%) and lowest in winter (29.4%). Prevalence of non-O157:H7 STEC, however, was lowest in the winter, spring, and summer (averaging 49.2%) and highest in the fall (77.7%).

It is important to note that quantitative fecal shedding of STEC is considered a more important factor than prevalence in influencing the risk of human exposure with these food borne pathogens (Omisakin et al., 2003 and Ogden et al., 2004). For example, prevalence of *E. coli* O157 in Scottish beef cattle at slaughter was found to be greater ($P < 0.05$) during the cooler months (11.2%) than during the warmer months (7.5%) (Ogden et al., 2004). This was the reverse of the known seasonality of human infections with STEC (WHO, 1998). Ogden et al. (2004) reported that high shedding beef cattle (i.e., excreting > 104 cfu/g of wet feces) to shed greater concentrations of *E. coli* O157 in the warmer months, which may explain increased human infections at that time. Interestingly, the high shedding cattle (9% of the cattle tested) excreted the largest amount of *E. coli* O157 (96%) produced.

Transmission of STEC

Outbreak investigators have identified three main routes of transmission: food borne infections (often associated with consumption of contaminated undercooked minced beef and unpasteurized milk), person to person spread, and direct or indirect animal contact (Parry et al., 1998). Direct and indirect zoonotic transmission through contact with animals or their faeces has been reported in several settings (Renwick et al. 1993; Synge and Hopkins, 1994). Although ingestion of undercooked food products of animal origin seems the likely route of transmission in most cases of human infection, there is increasing evidence that STEC infection can also be acquired via person-to-person transmission. In a day-care center outbreak (1987), no common food source was identified, and the sequential movement of illness from class to class was consistent with person-to-person transmission. In a large outbreak of *E. coli* 0157:H7 infection in a

Canadian nursing home (Carter et al., 1987) affecting 55 elderly residents and 18 staff members, the epidemic curve was biphasic. The first phase, which included the vast majority of the affected residents, was consistent with a point source infection presumed to be ingestion of a contaminated sandwich. The second phase, which involved many of the staff who cared for the sick patients, was indicative of person-to-person transmission.

STEC Virulent Factors

STEC is characterized by the production of one or more types of Shiga toxins (*stx*1 or *stx*2 or their variants), which inhibit the protein synthesis of host cells, leading to cell death. *stx*1 and *stx*2 are encoded by alleles in the genome of temperate, lambdoid bacteriophages that are integrated in the *E. coli* chromosome (Strockbine et al., 1986). Besides the *stx* gene(s), human pathogenic STEC strains often carry the *eae* gene, encoding the adherence factor intimin, which is an outer membrane protein (Paton and Paton, 1998). The *eae* gene is carried by a pathogenicity island in the chromosome called the locus of enterocyte effacement (LEE), which is required for intimate attachment to the host intestinal mucosa (Paton and Paton, 1998). Furthermore, human pathogenic STEC strains often harbor a large plasmid encoding possible additional virulence traits such as the enterohemorrhagic *E. coli* (EHEC) hemolysin (hly_{EHEC}) gene, which acts as a pore-forming cytolysin on eukaryotic cells (Schmidt et al., 1995).

However, many of the STEC strains found in the gastrointestinal tracts of domestic animals (the principal source of human infections) may have a low degree of virulence in humans. These strains are less likely to produce putative accessory virulence factors such as intimin (encoded by *eae*) and the plasmid-encoded enterohemolysin (encoded by enterohemorrhagic *E. coli* (EHEC) *hlyA*) (Schmidt and Karch, 1996.). Within the human disease-associated strains, those producing Shiga toxin type 2 (*stx*2, encoded by stx_2) appear to be more commonly responsible for serious complications such as HUS than those producing only Shiga toxin type 1 (*stx*1, encoded by stx_1) (Ostroff et al., 1989). Furthermore, STEC belonging to serogroup O157 and, to a lesser extent, serogroup O111 are responsible for the vast majority of HUS outbreaks (Minami, 1997; Paton et al., 1996 and Griffin, 1995). Paton and Paton in 1998 developed multiplex PCR assays for the simultaneous detection of (i) stx_1, stx_2, *eae*, and EHEC *hlyA* and (ii) portions of the *rfb* (O-antigen-encoding) regions of *E. coli* O111 and O157. These multiplex PCR assays developed to rapidly determine whether a patient is infected, or food is contaminated, with STEC belonging to serogroup O111 or O157 or whether the STEC produces virulence factors associated with more serious disease.

Clinical significance of STEC

Evidence from studies of outbreaks (Riley, et al., 1983; Carter, et al.,1987; Beutin et al., 2004; Smith-Palmer et al., 2005; Bielaszewska, 2011) and sporadic cases (Karmal et al., 1983, 1985; CDSC, 1999; Buvens, et al., 2010) of VTEC infection due in most cases to serotype 0157:H7 indicates that the spectrum of illness includes asymptomatic infection, mild uncomplicated diarrhea, hemorrhagic colitis, HUS, and thrombotic thrombocytopenic purpura (TTP) (Morrison, et al. 1985), a syndrome that is closely allied to HUS. Hemorrhagic colitis, HUS, and TTP have in the past been considered as isolated entities, although it is now clear that they span the spectrum of STEC infection, and constitute the elements by which STEC disease is recognized as a distinct clinicopathologic entity.

Hemorrhagic Colitis

Hemorrhagic colitis ("ischemic colitis") is a distinct clinical syndrome that presents typically with abdominal cramps and watery diarrhea followed by a hemorrhagic discharge resembling lower gastrointestinal bleeding. The disease is distinguished from inflammatory colitis by lack of significant fever and absence of an inflammatory exudate in the stools (Riley, 1987; Riley et al., 1983). Riley (1987) suggested that the disease was probably first recognized in 1971, in five young adults with a condition referred to as "evanescent colitis. Subsequently, several sporadic cases of the syndrome, going by names such as ischemic colitis and reversible segmental colitis, were described in the United States, Japan, and Europe (Karmali et al., 1983), although the etiology remained unclear. In 1982, workers from the Centers for Disease Control investigated two outbreaks of hemorrhagic colitis in Michigan and Oregon; they identified 47 individuals with hemorrhagic colitis, using a case definition of severe abdominal pain, grossly bloody diarrhea, and the lack of evidence of infection by recognized enteric pathogens. Case control studies showed that the illness was associated with ingestion of hamburgers at outlets of a well-known fast-food restaurant chain. *E. coli* 0157:H7 was recovered from the stools of about half the cases but from none of healthy controls. In a large surveillance study of hemorrhagic colitis in the United States (Wells et al., 1985), 103 patients were identified as meeting the case definition of hemorrhagic colitis over a 20-month period. *E. coli* 0157:H7 was identified in 28 (36%) of 76 cases in which stools were examined.

Hemorrhagic Uremic Syndrome (HUS)

Hemolytic uremic syndrome (HUS) is the most worrisome complication of EHEC infections and is characterized by

the triad of acute renal failure, microangiopathic hemolytic anemia, and thrombocytopenia, with a fatality rate between 2% and 7% (Haluk, 2008). The association between STEC and HUS was based not only on the isolation of STEC from fecal cultures, but also on the demonstration of free ST in fecal filtrates and rising levels of ST-neutralizing antibodies in patients' sera. These findings, as well as the fact that STEC isolates from these patients belonged to several different serotypes, in addition to 0157:H7, emphasized the fact that ST production was probably of direct pathogenetic significance in HUS (Schmidt et al., 1999).

Hemolytic Uremic Syndrome (HUS) has been reported in a variety of clinical and epidemiological settings, and several different agents, including drugs, chemicals, toxins, and microbes, have been postulated as potential causes **(**ECDC, 2011). The prevailing dogma for many years has been that HUS, which is probably a multi-factorial disease, being the end result of a number of different inciting events and pathogenic mechanisms. By far the most common form of the syndrome is "idiopathic" or "classical" HUS which has its highest incidence in children. Classical HUS presents typically a few days after the onset of an acute diarrheal "prodromal" illness which is often bloody and shows remarkable similarities clinicopathologically and radiologically to hemorrhagic colitis; some patients with hemorrhagic colitis have subclinical evidence of HUS (Neill et al., 1987).

HUS is a leading, and in some centers the most common, cause of acute renal failure in childhood **(**Orth et al., 2007). The syndrome was at one time associated with a very high case fatality rate of about 50% (Beutin et al., 2007). However, improvement in the treatment of renal failure and the attendant biochemical disturbances, largely through the use of peritoneal dialysis, has substantially improved the outlook. Modern management techniques have reduced the case fatality rate to 10% or less, although up to 30% of survivors may develop long-term residual disability in the form of chronic renal failure, hypertension, or a neurological deficit (Nataro, et al., 2011). Thus, even in the modern era HUS remains a disease with a significant mortality and morbidity. In Germany recently, out of 2294 cases of EHEC infection, 798 developed HUS (including 9 fatalities): 68% of cases were in females and 88% in adults aged 20 years or older, with the highest attack rates per 100 000 population in the group aged 20–49 years (WHO, 2011).

Attempts have been made to estimate the incidence of HUS in North America. Rogers et al. (1986) estimated the average incidence of HUS in Oregon during a 4-year period (January 1979 to December 1982) to be 0.97 and 2.65 cases per 100,000 children (18 and <5 years of age respectively). In another study in Sacramento, Rogers and her colleagues estimated the yearly incidence of HUS to be 0.41 case per 100,000 children <14 years during the study period of January 1979 and June 1982. The occurrence of an epidemic of HUS in Sacramento raised the yearly incidence to 11.2 cases per 100,000 children <14 years of age. In a study in King County, Washington, during a 10-year period estimated the yearly incidence of HUS to be 1.16 and 3.02 cases per 100,000 children <15 years of age and <3 years of age, respectively (Karmali et al., 1983). In South Africa, idiopathic HUS appears to be substantially more common in white than in black children. In England, the syndrome appears to be more common in rural than in urban areas. The reasons for such regional or socio-cultural differences in prevalence are yet to be established (Rivero et al., 2010**)**. The occurrence of outbreak of HUS lents support to a long-held view that the etiology of the syndrome is of infectious base (York et al., 2010; WHO, 2011). Many microbes have been implicated as etiologic agents in HUS, including *Shigella spp*., particularly, *Shigella dysenteriae* type1; *Salmonella* Typhi; *Campylobacter jejuni*;*Yersinia pseudotuberculosis; Streptococcus pneumoniae* (Wickham et al., 2006).

Thrombotic Thrombocytopenic Purpura (TTP)

First described in 1924 (Moschcowitz, 1924), TTP closely resembles HUS in its clinicopathological features, but differs in that neurological signs and fever are more prominent in TTP and the peak age incidence is in the third decade. In a review of 271 cases of TTP, it was noted that there was a rapidly progressive course, with 75% of patients dying within 90 days, and a pentad of clinical features: fever, thrombocytopenic purpura, microangiopathic hemolytic anemia, neurological manifestations which were often remittent, and renal dysfunction (Amorosi and Ultmann, 1966). Modern management techniques, in particular, plasmapheresis, have substantially improved the outlook (Black, 1993). Most cases of TTP present without an antecedent illness, whereas a prodromal diarrheal illness is an essential feature of classical HUS. However, two cases diagnosed as TTP have been associated with *E. coli* O157:H7 infection. Both cases differed from the usual form of TTP in that the patients had an antecedent bloody diarrheal illness and the disease therefore resembled classical HUS.

Pathogenesis

Production of a potent *STX* is essential for many of the pathological features as well as the life-threatening sequelae of STEC infection. However, pathogenesis is a multistep process, involving a complex interaction between a range of bacterial and host factors. Orally ingested STEC (often in very low initial doses) must initially survive the harsh environment of the stomach and

then compete with other gut microorganisms to establish intestinal colonization. STEC organisms remain in the gut, and so *STX* produced in the lumen must be first absorbed by the intestinal epithelium and then translocated to the bloodstream. This permits delivery to the specific toxin receptors on target cell surfaces inducing both local and systemic effects.

Development of Diarrhea

Based largely on evidence from animal models, three possibly mechanisms have been postulated to account for the diarrhea associated with human infection. (i) Diarrhea results from the local action of ST on the intestinal mucosa. This hypothesis has been driven by a number of experimental observations including that (a) Shiga toxin, ST1, and ST2 cause fluid accumulation in rabbit ileal loops (O'Brien and Holmes, 1987; Smith and Lingood, 1971), (b) intragastric inoculation of young rabbits with STEC O157:H7 leads to diarrhea (Pai et al., 1986), and (c) mild, nonspecific, inflammatory changes may be seen in sections of rectosigmoid biopsies from adults with *E. coli* O157:H7-associated bloody diarrhea which resemble changes in the colon of rabbits challenged with a crude ST preparation. Keenan et al. (1986) have conducted a detailed study of the histopathological changes associated with ST (SLT) and Shiga toxin in the rabbit ileal loop. They observed that both toxins appeared to act directly and selectively on the mature columnar epithelial cells of the intestinal villus, resulting in the premature expulsion of these cells from the lateral villus wall. The non-absorptive crypt epithelium underwent a rapid proliferation and maintained the epithelial integrity. It was proposed that the mechanism of fluid accumulation in the ileal loop probably involves fluid and electrolyte malabsorption by the non- absorptive crypt cells that release the sloughed-off mature absorptive columnar cells of the villas' tip (Keenan et al., 1986; O'Brien and Holmes, 1987). The histological features reported by Keenan and others are similar to those of Pai and his colleagues, except that the latter observed that the epithelium of the small intestine was spared the changes despite a high concentration of ST in the small bowel. The former observed the changes in the mucosa of small bowel loops. Possible explanations for these discrepancies have been discussed in detail by Riley in 1987. Mobassaleh et al. (1988) have provided evidence that the fluid response to Shiga toxin in the rabbit small bowel loop is age dependent and correlate with the appearance of the specific toxin-binding glycolipid receptor Gb3 in the microvillus membrane after 20 days of life. Pai et al. (1986) observed a diarrheagenic response to ST in 3-dayold rabbits; the sparing of the small bowel in these young animals would be consistent with the lack of a specific receptor in the small bowel mucosa. On the other hand, the selective appearance of lesions in the colon of these young animals suggests either that the receptor Gb3 appears earlier in the colonic mucosa than in the small bowel mucosa or the colonic lesions resulted from a non-Gb3-mediated action of the toxin or other components in the crude preparation used by these workers.

The categorical demonstration of Gb3 in the human intestinal mucosa would lend strong support to the hypothesis that the receptor-mediated toxin-induced fluid accumulation in rabbits is a valid model for ST-induced diarrhea in humans. (ii) Diarrhea is related to attaching and effacing adherence of VTEC to the intestinal cells. The development of diarrhea in animal models in association with characteristic destructive attaching and effacing lesions in the large bowel mucosa is strong suggestive evidence that this may be a mechanism by which STEC cause diarrhea in humans (Tzipori et al., 1987). (iii) Diarrhea results from the systemic effects of the toxin on the intestinal vasculature. A strong case has been made that, irrespective of a possible local mucosal diarrheagenic action, ST is probably responsible for the systemic effects of STEC-associated diseases, such as HUS, resulting from an action on the microvasculature of the bowel, kidneys, and other organs and tissues. It is possible that severe toxemia results in overt hemorrhagic colitis, whereas a mild toxemia produces bowel edema and mild diarrhea resulting from fluid malabsorption. This hypothesis is supported by the observation that the injection of ST1 into rabbits leads to cecitis and diarrhea (Richardson et al., 1987). However, other factors may also be relevant since, in contrast to the mild diarrheagenic action of ST1, injected ST2 is associated with overt hemorrhagic cecitis in the rabbit model (Head et al., 1988).

Pathogenesis of systemic manifestations of VTEC Disease

Capillary endothelial cell damage is considered to be central to the pathogenesis of HUS (Fong et al., 1984). Ultrastructural studies of capillaries in tissues from patients with HUS revealed a characteristic swelling of endothelial cells accompanied by widening of the subendothelial space. Similar appearances in rabbits challenged parenterally with ST1 (Richardson et al., 1987) supports the hypothesis that endothelial cells are primary target sites for the toxin. Additional supporting evidence for this view is that endothelial cells are susceptible to the cytotoxic action of ST1 in vitro and moreover, contain Gb3 which has also been found in large quantities in the human kidney (Lingwood et al., 1987). The pathophysiological abnormalities in HUS include not only endothelial cell damage but also a reduction in the platelet count, an increase in plasma

platelet aggregating activity and the occurrence of an abnormal factor VIII molecule in acute-phase plasma (Kaye et al., 1993).

Diagnosis

There are a number of difficulties associated with the diagnosis of STEC infection. In the early stages of infection, there may be very large numbers of STEC in feces; in many cases, the STEC constitutes more than 90% of the aerobic fecal flora (Paton and Paton, 1998). However, as disease progresses, the numbers may drop dramatically. In patients with HUS, the typical clinical signs may become apparent only a week or more following the onset of gastrointestinal symptoms, at which time the numbers of STEC may be either very small or the bacteria may have been eliminated from the gut altogether. Also, in some cases, diarrhea is no longer present and only a rectal swab is available at the time of admission to hospital, limiting the amount of specimen available for analysis. For these reasons, diagnostic tests should preferably be very sensitive and require minimal specimen volumes (Pollock et al., 2009). Also, the clinical presentation of STEC disease is sometimes confused with other conditions such as inflammatory bowel disease, appendicitis, intussusception and *Clostridium difficile* infection. Thus, rapid diagnosis is important to prevent unnecessary invasive and expensive surgical and investigative procedures or administration of antibiotic therapy which may be contraindicated for STEC infection (Chapman and Siddons, 1996).

Most VTEC studies have centered on the isolation of the single serotype, 0157:H7, largely because the latter has a phenotypic property (sorbitol negative after 24 h) that facilitates detection in mixed flora on appropriate selective media. On the other hand, HUS and hemorrhagic colitis have now been associated with a wide array of different serotypes, even though O157:H7 continues to be the predominant one. The overall diagnostic strategy, therefore, must be directed towards detecting VTEC in general, rather than detecting a single serotype (Persson et al., 2007).

Diagnostic procedures are based on detection of the presence of *STX* or *STX* in fecal extracts or fecal cultures, and/or isolation of the STEC (or other *STX*-producing organisms). Since these procedures differ in complexity, speed, sensitivity, specificity and cost, diagnostic strategies must be tailored to the clinical circumstances and the resources available (Pollock et al., 2009).

ELISA direct detection of *STX*

During the past decade, a number of enzyme-linked immunosorbent assays (ELISAs) have been developed for the direct detection of *Stx*1 and *Stx*2 in fecal cultures. Like verocytotoxicity, these play a potentially important role in diagnosis because they can detect the presence of STEC (or other *STX*-producing species) regardless of serogroup. Such assays can also be used to confirm toxin production by putative STEC isolates where tissue culture facilities are unavailable (Pulz et al*.,* 2003). Most of the published ELISA methods involve a sandwich technique with immobilized monoclonal or affinity-purified polyclonal antibodies to the toxins as capture ligands. After incubation with cultures, bound toxin is detected with a second *STX*-specific antibody followed by an appropriate anti-Ig–enzyme (usually alkaline phosphatase) conjugate (Downes et al., 1989). Tests of pure isolates show that the specificities of the various *STX* ELISAs are in close agreement with the results of verocytotoxicity assays (Ashkenazi and Cleary, 1990). Also, Law et al. (1994) reported a specificity of 99.7% when fecal cultures were tested by *STX* ELISA and the results were compared with isolation of STEC. Also false-positive reactions with several strains of *Pseudomonas aeruginosa* using the Premier EHEC kit were reported. This included one ATCC strain which had previously been tested as negative by the manufacturer (Beutin et al., 1996). The sensitivity of the various ELISAs is affected by a number of variables including the avidity of the antibodies used and the type and amount of *STX* produced by a given strain. ELISAs are generally less sensitive than the verocytotoxicity assay. Downes et al. (1989) concluded that ELISA sensitivity was inadequate to reliably detect low levels of *STX* found in direct fecal extracts. However, the amount of free *STX* present in primary fecal cultures is generally greater, particularly when broths are supplemented with polymyxin B and mitomycin to enhance the release of *Stx*1 and *Stx*2 respectively. Under such circumstances, ELISAs were reported to be capable of detecting the presence of *Stx*1-producing organisms comprising less than 1% of total flora and *Stx*2-producing organisms in less than 0.1% (Law et al., 1992). Thus, under optimal conditions, *STX* ELISAs can provide a reliable primary screen for the presence of STEC strains (including non-O157 strains) in fecal cultures as long as the specimen is obtained fairly early in the course of infection. Studies of comparative specificity and sensitivity carried out to date indicate that commercially available *stx* ELISA kits are likely to be of considerable utility for laboratories without access to more specialized diagnostic procedures, particularly for detection of non-O157 STEC strains (Bennett and Tarr, 2009). However, reports of false-positive ELISA reactions indicate that independent confirmation of *stx* production or the presence of *stx*genes would be prudent. A reverse passive agglutination test for the detection of *stx* production is also commercially available (Oxoid, Unipath Ltd., Basingstoke, United Kingdom). The test involves incubating serially diluted polymyxin B extracts of putative

STEC cultures with *Stx*1- and *Stx*2-specific antibody-coated latex particles and observing for agglutination after 24 h.

DETECTION OF *STX* GENES

Hybridization with DNA and oligonucleotide probes

The availability of cloned *stx*1 and *stx*2 genes enabled the development of DNA probes for the detection of STEC (Newland and Neill, 1988). Initially, probes labelled with 32P or 35S were used for testing large numbers of fecal *E. coli* isolates or the direct screening of colonies on primary isolation plates for the presence of *stx* genes by colony hybridization. These procedures were both highly sensitive and specific, and when stringent washing conditions were used, strains carrying *stx*1, *stx*2, or both could be differentiated. However, radioactively labelled probes had disadvantages for clinical laboratories, such as delays due to the need for long autoradiographic exposures, short probe half-life and the problems associated with handling and disposal of radioisotopes. These problems have been largely overcome by the introduction of nonradioactive labels such as digoxigenin and biotin, and *stx* probes that use these have been used for detection of STECs without loss of sensitivity or specificity (Thomas et al., 1991). The availability of nucleotide sequence data for *stx*genes has also permitted the design of synthetic oligonucleotide probes for detection of STEC (Karch et al., 1996). Some oligonucleotide probes were based on sequences which are highly conserved among the various toxin genes and hence permitted detection of all types. Other probes were directed against less highly conserved regions, which, under the appropriate hybridization and washing conditions, distinguished between *stx*1, *stx*2 and *stx*2e genes (Brown et al., 1989). Although hybridization with DNA or oligonucleotide probes is not a particularly sensitive means of screening broth cultures or fecal extracts for the presence of STEC, it is a powerful tool for distinguishing colonies containing *STX* genes from commensal organisms.

Polymerase Chain Reaction (PCR)

Access to sequence data for the various *stx* genes has also permitted the design of a variety of oligonucleotide primer sets for amplification of *stx* genes by PCR. Crude lysates or DNA extracts from single colonies, mixed broth cultures, colony sweeps, or even direct extracts of feces or foods can be used as templates for PCR. *Stx-specific* PCR products are usually detected by ethidium bromide staining after separation of the reaction mix by agarose gel electrophoresis. To date, some *stx*PCR assays have combined different primer pairs for *stx*1 and *stx*2, and in some cases *stx*2 variants, in the same reaction, thereby directing the amplification of fragments which differ in size for each gene type (Paton and Paton, 1998).

Other *stx*PCR assays uses a single pair of primers based on consensus sequences. These primers are capable of amplifying all *stx* genes with subsequent identification of the gene type requiring Southern or dot-blot hybridization with labelled oligonucleotides directed against type-specific sequences within the amplified fragment (Guion et al., 2008). Apart from increasing the sensitivity, secondary hybridization steps act as independent confirmation of the identity of the amplified product. Restriction fragment length polymorphism analysis of amplified portions of *stx*2 genes has also been used to discriminate between *stx*2 and *stx*2 variants (Bennett and Tarr, 2009).

In addition, PCR can be used for preparation of labelled DNA probes for use in hybridization reactions by amplification in the presence of, for example, digoxigenin-labelled nucleotides (Guion, et al., 2008). The use of PCR technology permits the detection of *stx* genes from samples which are microbiologically complex (such as feces or foodstuffs), including samples containing nonviable organisms. PCR assays are potentially extremely sensitive; using serially diluted DNA extracted from an STEC isolate, Brian et al. (1992) showed that amplification of less than 1,000 genomes resulted in visible *stx*1 and *stx*2 PCR products after ethidium bromide staining of agarose gels. When secondary Southern hybridization with a labelled probe was used to detect the PCR products, fewer than 10 *STX*-containing bacterial genomes per assay could be detected. In this study, the sensitivity was about 100-fold lower when the DNA template was prepared by direct extraction from feces seeded with known numbers of STEC. This was a consequence of the presence of inhibitors of *Taq* polymerase in the sample which necessitated dilution before assay. Other studies have also shown suboptimal sensitivity when PCR is carried out directly on fecal extracts (Ramotar et al*.,* 1994). Inhibitors of *Taq* polymerase are also present in meat. Begum and Jackson (1995) showed that ground beef homogenates had to be diluted 1,000-fold before assay. For both feces and food samples, the sensitivity of PCR assays is vastly increased if template DNA is extracted from broth cultures (Begum and Jackson, 1995). Broth enrichment (which can involve as little as 4 h of incubation) serves two purposes; inhibitors in the sample are diluted and bacterial growth increases the number of copies of the target sequence.

PCR for detection of other STEC markers

PCR has also been used for the detection of genes

encoding accessory virulence factors, such as *eaeA* and EHEC-*hlyA*, in STEC isolates (Schmidt et al., 1995). This information may be of significance, because there is a link between the presence of these genes and the capacity of an STEC isolate to cause serious human disease (Schmidt et al., 1995). Thus, a child presenting with acute diarrhea who is infected with a STEC isolate that is also positive for *eaeA* and EHEC-*hlyA* is likely to be at increased risk of developing complications such as HUS. Fratamico et al. (1995) combined previously described *STX*-specific and *eaeA*-specific PCR primer pairs with those specific for a portion of the 60-MDa virulence plasmid from an O157:H7 STEC in a multiplex assay. They concluded that this assay was suitable for the identification of STEC strains belonging to serogroup O157. However, the O157 virulence plasmid primers actually recognize a portion of the EHEC-*hlyA* gene, which is not confined to serogroup O157. Thus, this particular multiplex PCR will also be capable of identifying a significant proportion of potentially virulent non-O157 STEC strains. Collectively, these assays could distinguish between EPEC and STEC strains, identify STEC strains that were likely to be of increased virulence, and identify those likely to belong to serotype O157:H7 (Gannon et al., 1997).

Isolation of *stx*-Producing Bacteria

Although a substantial amount of information on the causative STEC strain can be obtained by molecular analysis of mixed cultures, isolation of the STEC strain must be considered the definitive diagnostic procedure. Apart from confirming the molecular data, isolation permits additional characterization of STEC by a variety of methods, including O:H serotyping, phage typing, restriction fragment length polymorphism, pulsed-field gel electrophoresis and amplification based DNA typing. While this characterization may have limited clinical application, it is of great importance from an epidemiological point of view, particularly in an outbreak setting.

Culture and immunological methods for O157 STEC

For many years, sorbitol-MacConkey agar culture (SMAC) has been the most commonly used method for isolation of STEC because of the predominance of O157:H7 and O157:H2 strains as etiological agents of human disease in North America and Europe. Most of these strains are unable to ferment sorbitol, which distinguishes them from the majority of fecal *E. coli* belonging to other serotypes (March and Ratnam, 1986). Sorbitol- MacConkey agar plates are inoculated with the fecal specimen and examined after 18 to 24 h of incubation for the presence of colorless, sorbitol-negative colonies. Individual colonies are tested by slide or tube agglutination with O157 and H7 antisera. Several commercial latex reagents for O157 antigen and one for H7 antigen are also available and have been shown to be both accurate and sensitive compared with standard serological tests (Persson et al., 2007). It is still necessary to confirm *STX* production in tissue culture or ELISAs since not all O157 strains are toxin producers. The sensitivity of SMAC is limited by the capacity to recognize non-fermenting colonies against the background of other organisms on the plate; this is particularly difficult when the O157 strain forms less than 1% of the flora. However, Chapman et al. (1991) improved the isolation rate of O157 STEC by supplementing SMAC with cefixime, to inhibit *Proteus* spp. and rhamnose, which is fermented by most sorbitol-negative non-O157 *E. coli*strains (O157 strains generally do not ferment rhamnose). Zadik et al. (1993) reported a further improvement in O157 isolation rates by using SMAC supplemented with cefixime and potassium tellurite (CT-SMAC). Although screening fecal cultures on SMAC is inexpensive and involves minimal labor and equipment, it will primarily detect STEC belonging to serogroup O157. Serious STEC disease has been associated with many other serogroups (Johnson et al., 1990), although, some are sorbitol negative (Ojeda et al., 1995) while most are sorbitols positive. Thus, the efficacy of SMAC will vary in accordance with the local STEC serotype prevalent. In one study, SMAC resulted in the isolation of *E. coli* O157 from 80% of fecal samples which were positive for *STX* by direct cytotoxicity (Ritchie et al., 1992), whereas in another study, SMAC was positive for only 30% of verocytotoxin-positive samples (Ramota et al., 1995).

Serology

Diagnosis of STEC-related disease can be particularly problematic when patients present late in the course of disease, because the numbers of STEC in feces may be extremely small and hence undetectable even by PCR. In such circumstances, the etiology of infection may be established by serological means. Patients with VTEC infection develop rising levels of VT-neutralizing antibodies, and this has been used to diagnose VTEC infection in patients without other evidence of infection (Karmali, 1987). Notenboom et al. (1987) reported that patients with *E. coli* O157:H7 infection develop rising antibody titers to the somatic O157 antigen and suggest that O157 serology would, be of diagnostic value in some settings and helpful in investigating epidemics. Extreme caution should be exercised in interpreting the results because the O157 antigen cross-reacts with Brucella abortus (Stuart and Corbel, 1982).

Treatment and prevention of STEC infection

The first step in the treatment of STEC is to embarked on intensive supportive therapy to maintain homeostasis (e.g., peritoneal dialysis or hemodialysis, fluid balance and treatment of hypertension). However, the availability of rapid and sensitive methods for the diagnosis of STEC infection early in the course of disease has provided an opportunity for instituting a specific therapeutic intervention. 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 (Paton and Paton, 1998).

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 diarrhea or bloody diarrhea to HUS. Such analyses have been compounded by variations in the types of antibiotics used, the timing of commencement of therapy in relation to onset of symptoms, and the possibility that the severity of disease may have influenced the decision to implement therapy. Nevertheless, the bulk of these studies suggested either that there was no significant benefit associated with administration of antibiotics or that therapy (either during or preceding infection) actually increased the risk of developing HUS (Tar et al., 2005; Ahn et al., 2008). However, in one study, HUS patients who had been given antibiotics during the diarrheal prodrome had milder illness (Martin et al., 1990).). Examination of antibiotic use in two 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 diarrhea 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. Apart from the lack of unequivocal evidence for clinical benefit, there are theoretical arguments against the use of antibiotics. First, although *STX* is extracellular, much of the toxin remains associated with the STEC cell surface. Thus, antibiotics which result in cell lysis might actually increase the amount of free *STX* in the gut lumen available for systemic absorption. Moreover, *in vitro* studies have shown that treatment of O157:H7 STEC with subinhibitory concentrations of antibiotics results in a significant increase (up to 50-fold) in the amount of free *STX* in the culture medium (Walterspiel et al., 1992). The effect was most pronounced with antibiotics such as trimethoprim-sulfamethoxazole and ciprofloxacin, which interfered with bacterial DNA synthesis and correlated with increased induction of toxin-converting bacteriophages (Wolf et al., 1997). Cordovez et al. (1992) noted a high rate of antibiotic resistance amongst STEC, and so empirical treatment with an inappropriate drug might confer a selective advantage on the STEC over other members of the gut flora and cause overgrowth. The same risk/benefit considerations are also relevant when considering whether to administer antibiotics either to asymptomatic STEC carriers to limit the spread of infection or to uninfected close contacts of patients with proven infection to prevent acquisition. Indeed, the case for prophylaxis is weakened by reports of patients becoming infected with O157:H7 STEC while undergoing therapy for an unrelated condition with an antibiotic to which the STEC was sensitive (Tarr, 1995). More extensive randomized controlled trials are required to determine whether there is a role for antibiotic prophylaxis or therapy in STEC disease. There are also sound reasons for not administering anti-motility agents to patients with STEC diarrheal disease, since these would be expected to impede the elimination of STEC from the gut and thereby extend the exposure to *STX*. Indeed, retrospective analyses have shown that administration of these agents to patients with O157:H7 infection extended the duration of bloody diarrhea and increased the risk of developing HUS and central nervous system lesions (Cimolai et al., 1994; Bell et al., 1997). At present, the risks or benefits of administration of other anti-diarrheal agents such as kaolin or bismuth are not known.

Antimicrobial Resistance of STEC

With the emergence and dissemination of antimicrobial resistance in bacteria which is well documented worldwide (Cohen, 2000), resistance to other antibiotics was detected as early as new agents were introduced for therapeutic and growth-promotant purposes (Anderson, 1968; Matthew et al., 1998). Antibiotic resistant *E. coli* has been reported for over 50 years (Adesiyun and Kaminjolo, 1992; Lambie et al., 2000).

E. coli, an important gastrointestinal flora, known to be capable of accepting and transferring plasmids and which under stress readily transfers those plasmids to other species, is therefore considered an important reservoir of transferable antibiotic resistance (Enumeration of Escherichia coli and the Coliform Bacteria, 2002). Studies in the UK found that, in the late 1950s, tetracycline resistance was already detectable in *E. coli* isolates from chickens and pigs fed rations containing less than 100 g

tetracycline/ton (Dunlop et al., 1998a and Orden et al., 1999). Anti-microbial resistant food borne pathogens are acquired primarily through consumption of contaminated foods of animal origin or water (Mead et al., 1999). Food chain, especially meat, is a major source of transmission of antimicrobial-resistant organisms to humans causing both intestinal and extra-intestinal disease (Johnson et al., 2003).

The magnitude of the public health burden due to resistant foodborne pathogens is complex and is influenced by a number of variables such as antimicrobial use practices in farming, process control at slaughter, storage and distribution systems, the availability of clean water, and proper cooking and home hygiene, among others (WHO, 2000). The major concern on the public health threat of foodborne illness is infection by antimicrobial resistant strains that lead to more intractable and severe disease (Helms et al., 2002; Martin et al., 2004). This situation is further complicated by the potential of resistant bacteria to transfer their resistance determinants to resident constituents of the human microflora and other pathogenic bacteria.

Several published data on resistance in *E. coli* originating from foods were reported from isolates cultured from retail raw meat products (Meng et al., 1988; Zhao et al., 2001 and Umolu et al., 2006). Available data from USDA-FSIS indicated that 13 million kg of ground beef and 9.5 million kg of beef trimmings were contaminated with *E. coli* O157:H7 in USA between 1999 and 2002 (Sofos, 2008). Resistance to antibiotics is highly prevalent in bacterial isolates worldwide, particularly in developing countries including Nigeria (Okeke et al., 2005 and Aibinu et al., 2007). Unhygienic butchering and floor dressing of carcasses for meat is a common practice in Nigeria resulting in carcass contamination with pathogenic microorganisms that could cause zoonotic food poisoning (Umolu et al., 2006, Ojo et al., 2009; Olatoye, 2010). Seasonal variation could affect the degree of contamination of meat from carcases dressed on the floor since waste water runoff or flooding plays an important role in contamination of food and ground water (Gay and Hunsaker, 1993).

White et al. (2004) suggested the need for continuous research on the ecology and epidemiology of major foodborne pathogens, and surveillance of retail food (including meat) products in order to characterize and mitigate food-borne bacterial resistance. Developed countries have national surveillance programs for monitoring of bacterial susceptibility to antimicrobials among zoonotic and commensal bacteria isolated from humans and animals. However, there are no national surveillance programs on the susceptibility of such bacteria from animals and products in Nigeria. Additionally, other authors reported the need for continuous exploration of risk assessment of the use of antimicrobials in the animal husbandry with regards to the potential public health consequences (Hald et al., 2004; Phillips et al., 2004). It was therefore hypothesized that the practice of indiscriminate use of antibiotics in livestock production results in shedding of resistant foodborne bacteria and the hygiene levels of meat processing thus the contamination of the meat destined for public consumption.

In a study on antibiotic susceptibility of *Escherichia coli* O157:H7 from beef in Ibadan Municipal, Olatoye (2010) reported eight different resistance patterns and all the isolates were resistant to one or multiple antibiotics. Tetracycline resistance was the highest (91.4%) among the isolates, while 72.9% of the isolates were resistant to nitrofurantoin and Chloramphenicol, 65.7% to cefuroxime, 44.3% to cotrimozole, 35.7% to nalidixic acid and 11.4% to gentamicin.

CONCLUSION AND RECOMMENDATIONS

It is therefore concluded that, the STEC recorded by researchers in Nigeria and Africa as a whole is high enough to pose a threat to the public health. Importantly, more STEC isolates came from cattle which is the main source of animal protein consumed by humans in the country. This calls for more veterinary attention in our slaughter slabs nationwide if the public safety is anything to go by. It is also signaling a strong warning on food processing hygiene and safety packages in Nigeria. It is therefore recommended that, there should be improvement in sanitary conditions in our slaughter houses to minimize the risk of human infections by the bacteria. Further systematic research be conducted on the meat of these animals to evaluate the level of contamination by STEC.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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