Review On Diagnostic Techniques And Public Health Importance Of *Escherichia Coli*

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Abstract: *Escherichia coli* are normal inhabitants of the gastrointestinal tract of animals and humans. Some strains have become highly adapted to cause diarrhoea and a range of extra-intestinal diseases. *E. coli O157:H7* is the predominant and most virulent serotype in a pathogenic subset of VTEC, designated *Enterohaemorrhagic E. coli (EHEC*). Ruminants represent the main natural host of VTEC and are generally healthy carriers of the organisms. Cattle are considered to be the main reservoir of *E. coli* O157:H7 infection for humans. The presence of VTEC in animal faeces provides the potential for these organisms to enter the food chain by fecal contamination of milk products, contamination of meat with intestinal contents during the slaughter process or contamination of fruit and vegetables by contact with infected manure. Diagnostic procedures for VTEC have been developed, primarily for *E. coli* O_{157}:H_7. Potential virulence for humans is confirmed by the demonstration of Verocytotoxin production using Vero cell assay, enzyme-linked immunosorbent assay (ELISA) or agglutination tests. Phage typing and pulsed field gel electrophoresis are widely used by reference laboratories for sub typing VTEC O157 for epidemiological purposes. Antimicrobial agents have no proven value in the treatment of *E. coli* O157:H7 infections. No vaccines are currently available for controlling VTEC infections in animals or humans, but variety of experimental vaccines are being developed.

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1. INTRODUCTION

Food borne pathogens are the leading cause of illness and death in developing countries costing billions of dollars in medical care and medical and social costs. Changes in eating habits, mass catering, complex and lengthy food supply procedures with increased international movement and poor hygiene practices are major contributing factors. Contaminated raw meat is one of the main sources of food borne illnesses. The risk of the transmission of zoonotic infections is also associated with contaminated meat. International food management agencies, especially the World Health Organization (WHO), the Food and Agriculture Organization and the International Hazard Analysis Critical Control Point (HACCP) Alliance have already provided guidelines to member countries about safe handling procedures such as HACCP and Good Manufacturing Practices (GMPs) (Nafisa *et al.,* 2010).

Despite the extensive scientific progress and technological developments achieved in recent years in developed countries, microbial food borne illness still remains a global concern. Microorganisms of concern to meat processors may originate from the faeces and skin of animals and also include environmental sources like working utensils presented for slaughter and can be transferred to the carcass during skin removal and evisceration (Elder *et al.,* 2000). Escherichia coli (*E. coli)* O157:H7) is one of the most important food borne pathogens, causing diarrhea, hemorrhagic colitis and haemolytic uremic syndrome in humans worldwide (Mersha *et al*., 2010).

E. *coli* are routinely characterised by serological identification of somatic O, flagellar H and capsular K antigens. However, while some serotypes correlate closely with certain clinical syndromes, differentiation of pathogenic strains from the normal flora depends on the identification of virulence characteristics. Since 1977, it has been recognised that some diarrheagenic strains of *E. coli* produce toxins that have an irreversible cytopathic effect on cultured Vero cells (Konowalchuk *et al.,* 1977). Such verocytotoxigenic *E. coli* (VTEC) have been shown to belong to over 100 different serotypes (Johnson *et al.,* 1996a; Strockbine *et al*., 1998). They are also described as Shiga toxin producing E. coli (STEC) due to the similarity demonstrated between verocytotoxins (VT) and Shiga toxins (Stx) of Shigella dysenteries (O'Brien and Laveck, 1983). In the past two decades, VTEC O157:H7 has increased in importance world-wide as a public health problem. *E. coli* O157:H7 is the predominant and most virulent serotype in a pathogenic subset of VTEC, designated

Enterohaemorrhagic *E. coli* (EHEC). This designation is based on their capacity to cause hemorrhagic colitis and hemolytic uremic syndrome in humans, their ability to produce VT, their ability to cause attaching and effacing lesions on epithelial cells, and their possession of a characteristic large plasmid (Nataro and Kaper, 1998).Ruminants represent the main natural host of VTEC and are generally healthy carriers of the organisms. VTEC have also been isolated from pigs, cats, dogs, chickens and wild birds; these species can be transiently colonised by the organisms (Beutin *et al*., 1993; Johnson *et al*., 1996a).

Surveys have shown that O157 strains normally represent a minority of the VTECs that colonies the intestinal tract of animals. The presence of VTEC in animal faeces provides the potential for these organisms to enter the food chain via faecal contamination of milk, contamination of meat with intestinal contents during slaughter or contamination of fruit and vegetables by contact with contaminated manure. VTEC are also transmitted through water and by direct contact with infected people, animals or animal waste. Contaminated water, used for irrigating or for washing vegetables, can also be source of infection for humans or animals. Cattle are considered to be the main reservoir of *E. coli* O157:H7 infection for humans, although the organism has been isolated from a variety of farmed animals, horses, dogs, rabbits, birds and flies. Despite its ability to cause severe disease in humans (Paton and Paton, 1998), infection in animals with *E. coli* O157:H7 is invariably subclinical. The role of pigs as subclinical carriers of STEC in the epidemiology of human disease needs further research. Because *E. coli* O157:H7 has become the predominant zoonotic VTEC, diagnostic methods have been developed to detect selectively this serotype in human clinical cases (Strockbine *et al.*, 1998) and in food sources (Vernozy and Rozand, 1997). Shiga toxin-producing *E. coli* O157:H7 (STEC O157) can cause severe enteric infections. Symptoms may include abdominal pain, bloody diarrhea, hemorrhagic colitis and haemolytic uremic syndrome (HUS) (Nataro and Kaper, 1998; Zhao *et. al.*, 1994).

Numerous sporadic infections and outbreaks caused by STECO157 have been reported in the United States and elsewhere in worldwide. The majority of STEC O157 infections are food borne; many are associated with bovine sources. STEC O157 was first linked to outbreaks of severe bloody diarrhea in 1982, and is often referred to as a "recently emerged" human pathogen (Wei *et al*., 2006).Sporadic cases and outbreaks of human diseases caused by STEC have been linked to ground beef, raw milk, meat and dairy products, vegetables, unpasteurized fruit juices and water. Infections can also be acquired by direct contact with animals and by person to person spread. The organism is destroyed in pasteurization process, but insufficient heat treatment of ground meat and raw milk forms a potential infection risk of *E. coli* O157:H7 in the clinical laboratory is dependent on distinguishing the pathogenic serotypes from normal fecal flora containing commensal strains of *E. coli* (Chapman *et al*., 2001; Battisti *et al.*, 2006).

Fortunately, *E. coli* O157:H7 has two unusual biochemical markers; delayed fermentation of D-sorbitol and lack of β-D-glucuronidase activity, which help to phenotypically separate O157:H7 isolates from nonpathogenic *E. coli* strains. One of these markers (delayed sorbitol fermentation) enables to develop several selective media (e.g., Sorbitol-MacConkey; SMAC) which aid in the initial recognition of suspicious colonies isolated from bloody stools (Bindu *et al.*, 2010). Detection of *E. coli* O157:H7 from food samples requires enrichment and isolation with selective and/or indicator media, but, lacks specificity to identify STEC. Thus, more sensitive methods are required to improve the detect ability of STEC O157:H7 from food and environmental samples. Apart from the traditional culture methods relying on biochemical characteristics, various genotypic methods have been proven useful for species identification, epidemiological typing, and determining genetic relatedness among pathogenic and non-pathogenic bacteria (Ji Yeon *et al.*, 2005).

The currently accepted methods for the isolation of O157strains consist of assays for the detection of Shiga-like toxins(SLTs), either directly or at the genomic level, coupled with direct plating on sorbitol Mac Conkey (SMAC) agar, cefixime-SMAC agar or SMAC agar supplemented with cefixime and tellurite (CT-SMAC) with subsequent stereotyping. Accurate diagnosis of EHEC O157 infections requires the isolation of the pathogen to clarify the etiology of disease and the infectiousness of patients as well as to allow sub-typing of strains for epidemiological purposes. The probability of isolating *E. coli* O157 strains from stool cultures of patients is inversely related to the interval between the onset of diarrhea and the microbiological culture (Helge *et al*.,1995).

Culture proven *E. coli* O157 diarrheal illness has been reported from a number of African countries including South Africa, Swaziland, Central African Republic, Kenya, Uganda Gabon, Nigeria and Ivory Coast (Raji *et al*., 2006). However, in the presence of all the above situations, little is known about the prevalence,

distribution and associated virulent genes of *E. coli* O157: H7 in humans, animals or in foods of animal origin in Ethiopia Furthermore, it has not been determined well to what extent abattoir and butchery house environments serve as sources of *E. coli* O157: H7 to red meat contamination (Hiko *et al.,* 2008; Mersha *et al*., 2010; Taye *et al.,* 2013).

Therefore, the objectives of this review are:

- To overview on the various diagnostic techniques of *E. coli*
- To provide an insight on the public health importance of *E. coli*

2. LITERATURE REVIEW

2.1. Historical Back Ground

Escherichia col *E. coli* first described in 1885 by Theodor Escherich. Escheria Bavarian pediatrician had performed studies on the intestinal flora of infants and had discovered a normal microbial inhabitant in healthy individuals, which he named Bacterium coli commune. *E. coli* are ubiquitous intestinal bacterial flora of animals and humans. Although comprising a small proportion of the total faecal flora they are the predominant facultative anaerobe in the human colon and presumably exist there symbiotically (Wei *et al*., 2006). Infants and post parturient animals are normally colonized shortly after parturition, acquiring their mothers' intestinal flora.

2.2. Taxonomy

E. coli is a gram negative, facultative anaerobic, non spore forming rod, which belongs to the Enterbacteriaceae family (Law, 2000). *E. coli* is a Gram negative bacterium in the Phylum Proteobacteria, class Gamma proteobacteria, order Enterobacteriales, family Enterobacteriaceae and genus Escherichia. In the family Enterobacteriaceae most important genera are *E coli,* Shigella, Salmonella, Yersinia which have some common characteristic such as Gram negative rods, non spore forming facultative anaerobic, ferment glucose, simple growth requirements, most are motile with peritrichous flagella and many produce fimbriae (pili), capsules, or both. *E. coli* are one of coli form organisms (Quinn *et al.,* 2002).

Differences between strains of *E. coli* lie in the combination of different antigens they possess. There are three types of antigens: the somatic lipopolysaccharide antigen (O), the flagellar antigens (H), and the capsular antigens (K) (Quinn *et al*., 2002). There are approximately 174 O antigens, 56 H antigens, and 103 antigens that have been identified. There are several stains of *E. coli* that have been isolated. The enteric *E. coli* are divided on the basis of virulence properties into Enterotoxigenic (ETEC), Enteropathogenic (EPEC), Enter invasive (EIEC), Verotoxigenic (VTEC), Enterohaemorrhagic (EHEC) and Enteroaggregative (EaggEC) (Frenzen and Drake, 2005).*E. coli* O157: H7 produce toxins which are toxic to Vero (African green monkey kidney) tissue culture cells and are similar to Shiga toxin of Shigella dysenteriae. They have been known as Verotoxin 1 and 2 and as Shiga-like toxin I and II. The strains of E. coli that produce these toxins have been known as verotoxin producing *E. coli* (VTEC) or as Shiga-like toxin producing *E. coli* (STEC) (Vidal *et al.,* 2004). "Stx producing *E.coli* O157" is synonymous with *E.coli* O157: H7. The term VTEC is still widely used in United Kingdom and many European scientific publications. The term STEC is used especially in American scientific papers. The term Entero haemorrhagic *E. coli* (EHEC) was originally coined to denote strains that cause HC and HUS (Duffy *et al*., 2002).

2.3. Morphology

E. coli are gram negative, Facultative anaerobic and Non sporulating organisms. The cells are about 2µ long and 0.5µ in diameter with a cell volume of 0.6 to 0.7µm3. Some strains of *E. coli* possess flagella with peritrichus arrangement (Patrick *et al.,* 2000). Most *E. coli are* motile, specially the O157: H7 strain, by means of peritrichous flagellae at opposed to polar, which are strings of protein made in the shape of a corkscrew. They are continuously replenished from inside as they may be broken off. They are attached to a hook like structure embedded in the cell wall, which rotates around changing the direction, it is reversed along the 360^{o} (Quinn *et al*., 2002).

Figure 1: Schematic presentation of *E. coli* showing its morphology (cell surface antigens)
Source: (Hirsh and Zee, 1999)

2.4. Characteristics of *E. Coli* O157: H7

E. coli can be characterized by stereotyping which is a method based on differences in antigenic structure on the bacterial surface. The serotype is defined by the bacterium's O-antigen (Ohne), a polysaccharide domain in the bacterium's lipopolysaccharide (LPS) in the outer membrane, and the H-antigen (Hauch) consisting of flagella protein. Serotyping may also include the K antigen (Kapsel) and the F-antigen (Fimbriae) (Ratnam *et al*.,1988).There are many known O, H, K and F antigens and the existing number of different serotypes is known to be very high. Serotyping is an important tool which can be used in combination with other methods to distinguish pathogenic *E. coli* strains as specific pathogencity attributes are often linked to certain serotypes (Gyles, 2007). *E. coli* O157:H7 is an enteric pathogen that can cause diseases ranging from mild diarrhea to hemolyticuremic syndrome, kidney failure, and death. The Shiga toxins, Stx1 and Stx2, are considered to be the primary *E. coli* O157:H7 virulence factors and the cells may harbour genes that express one or both of these toxins. However, the Shiga toxins alone may not be sufficient to cause disease. Additional known virulence factors include intimin and enterohemolysin, products of theeae and hlyA genes, respectively (Nataro and Kaper, 1998).

Table 1: Classification of Shiga toxin-producing *E. coli* (STEC) found in animals

Type	**STEC subsets: common designation**	**Common serotypes / Sero groups**	**Geographical Distribution**	**Animal reservoir**	**Site of isolation in animals and derived products**
Zoonotic	O157 EHEC	O157:H7	Worldwide more common unindustrialized countries	Ruminants, pigs (c),	Intestine, faeces, meat, milk, cheese
	Non-O157 EHEC	O26 (b), O111 (b),O103, O113,O145	Worldwide	Ruminants, pigs, chickens	Intestine, faeces, meat, milk, cheese
Potentially zoonotic (a)	None	O17,O56,O87,O108,O109,O130,O136, O149	Worldwide	Cattle, sheep, goats, pigs	Intestine, faeces, meat,
Animal pathogenic	EDEC	O138, O139, O141	Worldwide	Pigs	Intestine

a) not as yet associated with disease in animals or humans; few data are available on the characterization of the virulence factors associated with these strains. b) Strains of some serotypes also cause hemorrhagic enteritis in cattle c) Probably an accidental host.

Source: http://www.microbionet.com.au/vtectable.htm

2.5. Epidemiology

2.5.1.Geographical distribution

E. coli 0157:H7 infections occur worldwide; infections have been reported on every continent except Antarctica. Other EHEC are probably also widely distributed. The importance of some serotypes may vary with the geographic area (CFSPH, 2009). The most severe cases of infection typically occur in children <5 years of age, the elderly and immuno compromised persons. In severe cases of infection, individuals present with hemorrhagic colitis, haemolytic uremic syndrome (HUS) is the leading cause of acute renal failure in children, microangiopathic hemolytic anemia (fragmented erythrocytes) and thrombocytopenia (low platelet count). Long term complications of EHEC infection include irritable bowel syndrome (IBS) (Marshall *et al.*, 2010). In North America, approximately 75,000 cases of EHEC infections are reported annually, of these, 10-15% of cases develop HUS, another 5-10% result in long-term complications and 3-5% of HUS cases are fatal EHEC infections account for roughly 250 deaths in North America each year (Serna and Boedeker, 2008).

Most *E coli* O157:H7 infections and HUS occur in the summer and autumn. Non-O157:H7 STEC infections in Australia and Montana, USA, had similar seasonality but this pattern did not occur in Seattle, USA (Panos *et al*., 2006). The incidence of HUS probably increased in several regions during the 1970s and1980s, but increasing or decreasing trends have not been proven unequivocally and one population based study found stable incidence during the 1990s (Panos *et al.*, 2006). A reported lower likelihood that children of African descent would have HUS was not confirmed in recent series from Natal and North America. The risk of developing HUS relates also to consumptions and behaviours leading to acquisition of infection**,** so demographic differences in incidence might reflect demographic differences in exposure to the causative agent, rather than differences in genetic propensity to develop HUS once infected (Phillip *et al*., 2005).

2.5.2. Reservoirs of E. coli O157:H7

Understanding the epidemiology of this organism requires knowledge of where these bacteria live and grow in nature (their reservoir) and of how humans come into contacts them. Ruminants have been identified as the major reservoir of *E. coli* O157:H7, with cattle as the most important source of human infections (Calderwood *et al.,* 1996) other ruminants known to harbor these bacteria include sheep, goats, and deer. STEC bacteria are occasionally isolated from other animals but it is believed that the bacteria are present as transients and that the animals acquired these bacteria from meat, foods or water contaminated by fecal material from ruminants. STEC bacteria usually do not cause illness in animals with a few exceptions such as diarrhea in calves (Kang *et al.,* 2004).

2.5.3.Factors affecting survival and growth of E. coli O157:H7 in food

A number of factors have a significant influence on the survival and growth of *E. coli* O157:H7 in food, including temperature, pH, salt, and water activity. Studies on the thermal sensitivity of *E. coli* O157:H7 in ground beef have revealed that the pathogen has no unusual resistance to heat and that heating ground beef sufficiently to kill typical strains of Salmonella will also kill *E. coli* O157:H7. Thermal pasteurization of milk has also been determined to be an effective treatment (Dylla*et al.*, 1995).

The optimal temperature for growth of *E. coli* O157:H7 is approximately 37oC (98.6oF), and the organism will not grow at temperatures below 8°C to 10°C (46oF to 50oF) or above 44oC to 45oC. *E. coli* O157:H7 survives freezing, with some decline in the concentration of *E. coli* O157:H7 (Buchanan and Doyle, 1997). *E.coli* O157:H7 has been reported to be more acid resistant than other *E. coli*. Acid resistance enhances the survival of *E. coli* O157:H7 in mildly acidic foods and may explain its ability to survive passage through the stomach and cause infection at low doses. The ability to be acid resistant varies among strains and is influenced by growth phase and other environmental factors. Once induced, acid resistance is maintained for long periods of time during cold storage. Stationary-phase *E. coli* O157:H7 are more resistant than growing cells to acid (Meng and Doyle, 1998). The presence of other environmental stresses, such as temperature or water activity stress, will raise the minimum pH for growth *E. coli* O157:H7 survives in such foods as dry salami, apple cider, and mayonnaise, which were previously considered too acidic to support the survival of food borne pathogens. Published literature contains conflicting reports about the efficacy of acid spray washing of beef carcasses (Buchanan and Doyle 1997). A study by (Brachett *et al*., 1994) found that warm and hot acid sprays did not significantly reduce the concentration of *E. coli* O157:H7 on beef carcasses. Two recent studies have found organic acids to be effective in reducing the presence of *E. coli* O157:H7 on beef carcasses (Besser *et al*., 2003). These apparently contradictory results may reflect differences in acid resistance among strains of *E. coli* O157:H7 can survive for extended periods under conditions of reduced water activity while refrigerated; however, the organism does (Bastian *et al*., 1999).

2.5.4. Virulence factor

The Enterohaemorrhagic *E. coli* (EHEC) strain O157:H7 is a major food borne pathogen causing severe disease in humans worldwide. Healthy cattle are a reservoir of *E. coli* O157:H7. Bovine food products and fresh products contaminated with bovine waste are the most common sources for Haemorrhagic Colitis (HC) and the Haemolytic Uremic Syndrome (HUS) (Callaway *et al.,* 2009).

Three major virulence factors of *E. coli* O157:H7 have been identified including a pathogenicity island called the Locus of Enterocyte Effacement (LEE), Shiga toxins (Stx) and the plasmid (pO157) encoded gene (E-hlyA) that codes for a pore forming cytolysin. *E. coli* O157:H7 colonization of the intestinal mucosa induces a histopathologic lesion defined as an "attaching and effacing" (A/E) lesion characterized by localized destruction of brush border microvilli and intimate attachment of the bacteria to host cell plasma membranes. The Locus of Enterocyte Effacement (LEE) genetically governs adhesion and subsequent

pathology. It contains the eae gene, encoding the outer membrane protein intimin and its receptor Tir (Trans located intimin receptor). In addition, LEE encodes proteins of the type III secretion system (TTSS), which is made up of an EspA multifilament needle complex, used for insertion of the bacterial effector proteins EspB, EspD and Tir into the host cell. Injection of bacterial virulence factors via the TTSS and binding of intimin to Tir leads to a strong interaction between bacteria and host cells (Cookson and Woodward, 2003). Virulence arises also from Shiga toxin production, encoded by Shiga toxin genes (stx1 and stx2), which are the primary factors responsible for the hemorrhagic aspect of diarrhoea and systemic complications (HUS). Shiga toxins act as N-glycosidases, cleaving ribosomal RNA leading to the inhibition of host cell protein synthesis (Vilte *et al.*, 2008).

Most adults recover from *E. coli* O157:H7 infections without sequelae. Children and the elderly however, are more likely to experience complications such as HUS and even death. The use of antibiotics in treatment for *E.coli* O157:H7 infections in humans are highly controversial as antibiotics might increase the risk of HUS. Thus, treatment is largely supportive. Nonetheless, innovative therapies such as the use of probiotics, monoclonal antibodies or recombinant bacteria to neutralize or bind toxins, are currently being explored (Bavaro, 2009).

2.5.5.Source of infection and modes of transmission

E. coli O157:H7 has been isolated from the faeces or gastrointestinal tract of cattle, sheep, horses, pigs, turkeys, dogs, and a variety of wild animal species (Heuvelink *et al*. 1999); however, epidemiologic studies have found that cattle manure is the source of most human *E. coli* O157:H7 infections. *E. coli* O157:H7 has also been isolated from bodies of water (e.g., ponds, streams), wells, and water troughs and has been found to survive for months in manure and water trough sediments (Sargeant *et al.,* 2000).

E. coli O157:H7 is also present in purchased animal feeds; therefore, such feeds may be an important route by which *E. coli* O157:H7 is disseminated to farms. From the farms, *E. coli* O157:H7 contamination of meat occurs when beef carcasses come into contact with hides and faeces during the slaughter process (Hancock *et al*., 2001). Enterohaemorrhagic *E. coli* is transmitted by the faecal oral route. They can be spread between animals by direct contact or via water troughs, shared feed, contaminated pastures or other environmental sources. Birds and flies are potential vectors. In one experiment, EHEC O157:H7 was transmitted in aerosols when the distance between pigs was at least 10 feet. The organism was thought to have become aerosolized during high pressure washing of pens, but normal feeding and rooting behavior may have also contributed (Dipineto *et al.,* 2006).

EHEC O157:H7 is mainly transmitted to humans by the consumption of contaminated food and water, or by contact with animals, faeces and contaminated soil. Person-to-person transmission can contribute to disease spread during out breaks; however, humans do not appear to be a maintenance host for this organism. Most human cases have been linked to direct or indirect contact with cattle, but some have been associated with other species including sheep, goats (unpasteurized goat milk), pigs (dry fermented pork salami), deer (venison), horses, rabbits and birds. The infectious dose for humans is estimated to be under 100 organisms, and might be as few as 10 (Ateba and Bezuidenhout, 2008). EHEC O157:H7 can remain viable for long periods in many food products. It can survive for at least nine months in ground beef stored at -20°C (-4°F). It is tolerant of acidity, and remains infectious for weeks to months in acidic foods such as mayonnaise, sausage, apple cider and cheddar at refrigeration temperatures. It also resists drying (Chase-Topping *et al*., 2007).

Some human cases are caused by exposure to contaminated soil or water. EHEC are usually eliminated by municipal water treatment, but these organisms may occur in private water supplies such as wells. Swimming in contaminated water, especially lakes and streams, has been associated with some infections. Soil contamination has caused outbreaks at campgrounds and other sites, often when the site had been grazed earlier by livestock. The reported survival time for EHEC O157:H7 in contaminated soil varies from a month to more than 7 months. This organism can also survive for 2 months or longer in some freshwater sources, especially at cold temperatures, and it may remain viable for two weeks in marine water (DebRoy and Roberts, 2006).

2.6. Pathogenesis

E. coli O157:H7 can with stand the acidic environment of the human stomach and begins the arduous and complex process of infection. From the point of ingestion, the incubation period of *E. coli* O157:H7 ranges from 8 hours to 16 days, but the typical incubation period is three to four days. During this time, the bacteria progress through several phases of infection including adherence, colonization and the production and release of Stxs. First, *E. coli* O157:H7 must initially adhere to the microvilli of the host epithelial cells. The association between the bacterial and host cells consequently induces the expression of

the TTSS genes located on the LEE. Following their synthesis, the TTSS proteins are systematically assembled (Kaper *et al*., 2004).

The membrane-bound proteins first associate and form the foundation of the TTSS followed then by the proteins that form the extracellular channel and by the protein that create the pore in the host cell membrane. Once assembled, a multitude of effector proteins are shuttled through the TTSS channel and into the cytoplasm of the host cell. After the effector proteins invade the cytoplasm, they alter the host cell's normal patterns of signal transduction in order to accommodate bacterial adherence. The alterations in signal transduction are accomplished through the activities of the bacterial effector proteins, and, by selective phosphorylation, the effector proteins force actin to polymerize and the cytoskeleton to reorganize (Jores *et al.,* 2004).

2.7. Diagnosis

Food and environmental samples may also be tested to determine the source of the infection. EHEC are sometimes difficult to identify. They are a minor population in the fecal flora or food. They also closely resemble commensal *E. coli* except in verocytotoxin production. However, the verocytotoxin alone does not necessarily identify an organism as EHEC; additional virulence factors must also be present (Sass *et al.,* 2003). Many diagnostic laboratories can detect verocytotoxin producing *E. coli* (VTEC) and identify. There is no single technique that can be used to isolate all EHEC serotypes. Carrier animals are usually detected by finding EHEC in fecal samples, which are either freshly voided or taken directly from the animal. Recto anal swabs may also be used in some cases. Intestinal contents can be collected at slaughter. Repeated sampling, as well as sampling more animals, increases the chance of detection (Keen *et al.,* 2006).

2.7.1. Based on Clinical signs

In Humans

Human can be infected asymptomatically or they may develop watery diarrhea, hemorrhagic colitis and/ or hemolytic uremic syndrome. Most symptomatic cases begin with diarrhea. Some cases resolve without treatment in approximately a week; others progress to hemorrhagic colitis within a few days. Hemorrhagic colitis is characterized by diarrhea with profuse, visible blood, accompanied by abdominal tenderness, and in many cases, by severe abdominal cramps. Some patients have a low grade fever; in others, fever is absent. Nausea and vomiting may be seen, and dehydration is possible. Many cases of hemorrhagic colitis are self–limiting and resolve in approximately a week. Severe colitis may result in intestinal necrosis, perforation or the development of colonic strictures (Karch *et al.,* 2005).

Hemolytic uremic syndrome occurs in up to 16% of patients with hemorrhagic colitis. This syndrome is most common in children, the elderly and those who are immune compromised. It usually develops a week after the diarrhea begins, when the patient is improving. Occasionally, children develop HUS without prodromal diarrhea. HUS is characterized by kidney failure, haemolytic anaemia and thrombocytopenia (Radostits *et al*., 2000; Quinn *et al.,* 2002).

The relative importance of these signs varies. Some patients with HUS have haemolytic anaemia and/or thrombocytopenia with little or no renal disease, while others have significant kidney disease but no thrombocytopenia and/or minimal haemolysis. Extra renal signs including CNS involvement with lethargy, irritability and seizures are common (Garcia *et al*., 2006). In more severe cases, there may be paresis, stroke, cerebral edema or coma. Respiratory complications can include pleural effusion, fluid overload and adult respiratory distress syndrome. Elevation of pancreatic enzymes or pancreatitis may also be seen Rhabdomyolysis and myocardial involvement are rare. The form of HUS usually seen in adults, particularly the elderly, is sometimes called thrombotic thrombocytopenic purpura (TTP). In TTP, there is typically less kidney damage than in children, but neurologic signs including stroke, seizures and CNS deterioration are more common. Death occurs most often in cases with serious extra renal disease such as severe CNS signs. Approximately 65–85% of children recover from HUS without permanent damage; however, long-term renal complications including hypertension, renal insufficiency and end-stage renal failure also occur. Residual extra renal problems such as transient or permanent insulin dependent diabetes mellitus, pancreatic insufficiency, gastrointestinal complications or neurological defects such as poor fine-motor coordination are possible (Ejidokun *et al*., 2006).

In animals

E. coli cause three main kinds of disease in animals, which are urinary tract infections, neonatal meningitis and diarrheal diseases (Quinn *et al*., 2002). They are mostly associated with coli bacilosis in neonate (Radostits *et al.,* 2000; Quinn *et al*., 2002) and associated with different diseases like primary nosocomic pneumonia, wound infections and peritonitis by opportunistic. In addition to these,

EHEC O157: H7 causes more rapid and severe neurological disease in suckling neonates (Dean-nystrom *et al*., 2000), edema disease in pig, haemorrhagic entero colitis in calves and post weaning diarrhoea in pigs (Quinn *et al*., 2002).
Occasionally *E. coli* O157: H7 toxins that were first identified by their cytopathic effects on Vero cells toxins (VT1, VT2 and VT2e) that damage the vasculature in intestine and other locations. VT and endotoxins of this serotype also affect brain and cause neurological signs in animals (Dean-nystrom *et al*., 2000; Ceponis *et al*., 2005). Dogs that were experimentally inoculated with EHEC O157:H7 developed transient acute diarrhea with decreased appetite and vomiting, but recovered spontaneously without complications in 1-2 days. In the same experiment, dogs inoculated with a non O157 EHEC developed severe disease, with diarrhea and vomiting followed by lethargy and in appetence, dehydration and dramatic weight loss. These dogs also had neurological signs including seizures, cerebral infarction, blindness and coma, and died 5-6 days after the onset of clinical signs (Rasmussen and Casey, 2001).

2.7.2. Samples
In most cases, samples taken from animals for VTEC isolation will be faeces collected for surveillance purposes or as part of an epidemiological trace back exercise following an outbreak of disease in humans. Samples may be taken from the rectum or from freshly voided faeces on the farm or from intestinal contents after slaughter (Radostits *et al*., 2000; Quinn *et al*., 2002). A variety of VTEC are present in healthy animals and not all are thought to be pathogenic for humans. *E. coli* O157:H7, which is the most significant VTEC in human disease, is carried sub clinically in animals. Cattle are thought to be the most important reservoir of this serotype. In an infected herd, only a proportion of the animals will be detectably infected, the organism is usually present in carriers in low numbers and is shed intermittently in faeces. Shedding is influenced by the age of the animals, diet, stress, population density, geographical location and season (Meyer-broseta *et al*., 2001).

Some animals are thought to contribute disproportionately to transmission of infection and have been termed “super-shedders”. Isolation rates may be improved by taking faeces samples in preference to rectal swabs, by increasing the sample size, by increasing the number of individuals sampled and by repeat sampling. Use of recto anal mucosal swabs is reported to improve detection of colonised as distinct from transiently infected cattle. Precautions should be taken to avoid cross contamination of samples in transit and at the laboratory. Samples should be kept cool and cultured as soon as possible after collection (Rice *et al.,* 2003).
2.7.3. Isolation and identification methods
Clinical samples are routinely plated directly on to solid media for isolation of *E. coli*, but the number of target VTEC organisms in faeces from healthy carriers is usually low and enrichment in liquid media improves recovery. Commonly used enrichment media are buffered peptone water either un supplemented (which gives good recovery) or supplemented with 8 mg/litre vancomycin, 10 mg/litre cefsulodin and 0.05 mg/litrecefixime (BPW-VCC) to suppress the growth of Gram-positive organisms, Aeromonas spp. and Proteus spp.; modified trypticase soy broth (mTSB) supplemented with 20 mg/litre novobiocin or 10 mg/litre acriflavinto reduce the growth of Gram positive organisms; or modified E. coli broth with 20 mg/litre novobiocin (mEC+n) (Radostits et al., 2000).

EHEC E. coli grow poorly at 44°C. The optimal incubation for bovine faeces to minimize overgrowth by other organisms is 6 hours at 37°C. For meat samples, enrichment for 6 hours at 41–42°C is used and for water and dairy products, 24 hours at 41–42°C (Quinn *et al*., 2002). Non selective pre enrichment is necessary for the effective recovery of low levels of stressed *E. coli* O157. Enrichment broths should be pre warmed to prevent cold shocking the organisms and slowing their initial growth; 24 hours’ incubation may increase recovery if the organisms are stressed (Clifton hadley, 2000).

The selective medium which is commonly used for experimental and routine screening of samples for *E coli* O157:H7 is Sorbitol MacConkey (SMAC) agar. MacConkey agar containing 1% D-sorbitol instead of lactose is a useful and inexpensive medium on which non-sorbitol-fermenting *E. coli* grow as small, round grayish white colonies. Selectivity is improved by the addition of 0.5% rhamnose, and addition of 0.05 mg/litre cefixime (CR-SMAC) inhibits overgrowth by *Proteus* spp. While fewer presumptive colonies require testing on this medium, rhamnose is an expensive supplement. This medium is used for a number of reasons: i) *E coli* O157:H7 does not ferment sorbitol, and for this reason it is distinguishable as colourless colonies from other *E coli* organisms that do ferment sorbitol ii) it is inexpensive when compared to other *E coli* O157 selective media, and is thus suitable when screening large numbers of samples. However, as many as 20% of *E coli* strains may be sorbitol-negative and other species of enteric bacteria

can grow as colourless colonies on SMAC (Biolog website, 1999). SMAC is not useful in screening for other EHEC strains.

CHROM agar O157 is another non-inhibitory *E coli* O157 selective medium specially designed to differentiate *E coli* O157 from other *E coli* organisms because of its specific chromogenic properties. This medium can differentiate *E coli* O157 by its pink-mauve colony colour, from sorbitol negative background micro-organisms such as *Proteus* and *Pseudomonas* found on SMAC. CHROM agar O157 was designed to be used as plating medium after IMS with Dynabeads anti-*E coli* O157. The vast majorities of other bacterial species are inhibited or give blue or colourless colonies (CHROM agar website, 2000)

2.7.3.2. Morphology and Staining of E. coli

E. coli is Gram-negative straight rod, 1-3 µ x 0.4-0.7 µ, arranged singly or in pairs. It is motile by peritrichous flagellae, though some strains are non-motile. Spores are not formed. Capsule and fimriae are found in some strains.

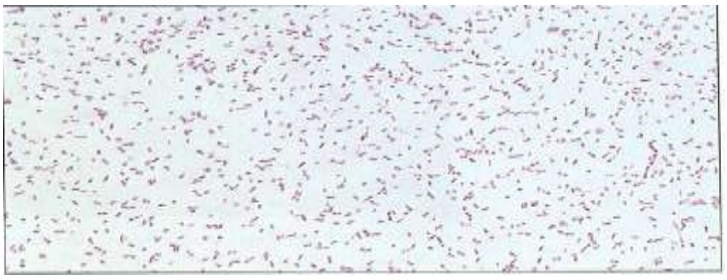

Figure 2: Appearance of E-coli under Gram stain (medium sized gram negative rods)
Source: Quinn *et al*., 2005

Cultural Characteristics of Escherichia Coli

It is an aerobe and a facultative anaerobe. The optimum growth temperature is 37°C. On Nutrient agar, colonies are large, thick, greyish white, moist, smooth, opaque or translucent discs. The smooth (s) form seen in fresh isolation is easily emulsified in saline, whereas the rough (R) formoften auto agglutinates in saline. Some strains may form "mucoid " colonies. On MacConkey agar medium, colonies are bright pink due to lactose fermentation. On selective media (Desoxycholate citrate agar-DCA; used for the isolation of salmonella, their growth is inhibited, however their colonies are pink on DCA as it contains lactose and neutral red. In broth, there is generalized turbidity and deposit which disperses on shaking. EMB – Eosin Methylene Blue Agar: selective and differential medium. Eosin differentiates between two major coliforms: *E. coli* (smaller, green-metallic sheen) and Enterobacter aerogenes (larger, rose color). Methylene blue selectively inhibits the growth of Gram+ bacteria. With this media we can also determine which bacteria are Gram-negative and which are Gram-positive, because only Gram-negative bacteria grow on this special media. The enhanced cell walls of Gram-negative bacteria protect these bacteria from the dye in the EMB plates. The dye is able to enter the cells of Gram positive bacteria and kill them.

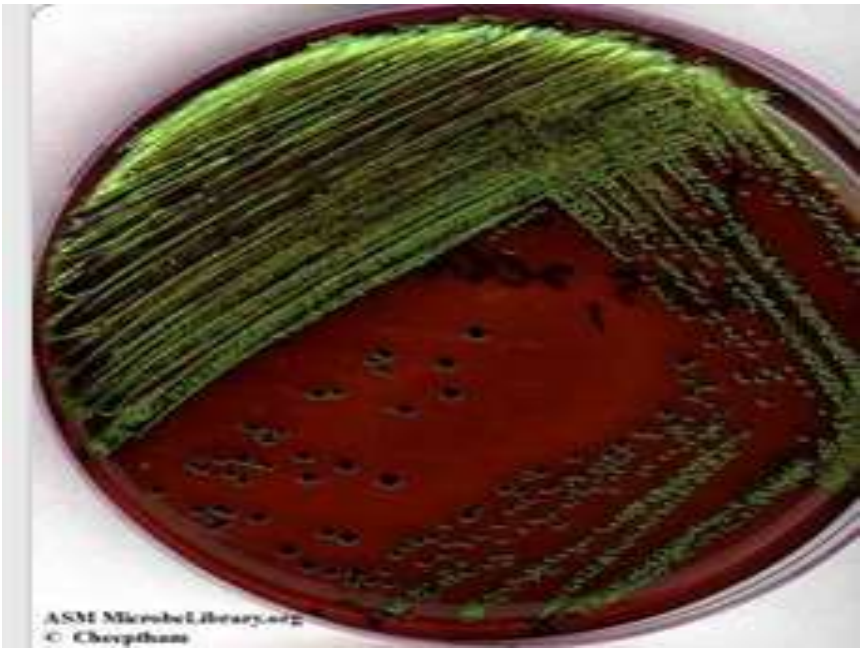

Figure 3: Preview of EMB agar plate incubated with E. coli showing good growth of dark blue-black colonies with metallic green sheen indicating vigorous fermentation of lactose and acid production which precipitates the green metallic pigment
Source: https://www.pinterest.com/pin/549931804479264745/

2.7.4. Biochemical Reaction of Escherichia Coli

Glucose, Lactose, Mannitol, maltose are fermented with acid and gas production, but sucrose is not fermented by typical strain of *E. coli*. In Triple sugar iron (TSI), acid and gas are produced. The four biochemical tests widely used for entero-bacteriaceae classification are Indole (I), Methyl Red (MR), Voges Proskauer (VP) and Citrate (C) utilisation which are referred to by the mnemonic IMViC. *E. coli* is Indole and MR positive VP and citrate negative (IMVi C++—), H_2 S is not formed and urea is not hydrolysed.

Key: a= Indole test, b=Methyl red,c=Voges proskauer,d= Simmon's citrate utilisation
Figure 1: IMViC Test of E.coli: **++–- ; Source: ASM MicrobeLibrary@Hanson**

Selective culture for E. coli O157:

There are no biochemical characteristics that distinguish the majority of VTEC from other E. coli, however, the inability of most strains of *E. coli* O157:H7 to ferment D-sorbitol rapidly and their lack of betaglucuronidase activity can be exploited in the isolation and identification of these organisms. However, the less common sorbitol fermenting and beta-glucuronidase positive *E. coli* O157: H variants (non motile due to lack of expression of the H7 antigen), will not be identified by isolation in such selective media chosen for these biochemical characteristics (Karch and Bielaszewska, 2001).MacConkey agar containing 1% D-sorbitol instead of lactose (SMAC) is a useful and inexpensive medium on which non-sorbitol-fermenting *E. coli* grow as small, round grayish white colonies. Selectivity

is improved by the addition of 0.5% rhamnose, and addition of 0.05 mg/litre cefixime (CR-SMAC) inhibits overgrowth by Proteus spp (Radostits *et al*., 2000; Quinn *et al.,* 2002).

While fewer presumptive colonies require testing on this medium, rhamnose is an expensive supplement. An alternative modification is the addition of 2.5 mg/litre potassiumtellurite in addition to cefixime (CT-SMAC), which has a greater inhibitory effect against *E. coli* non-O157 and other non sorbitol fermenters, such as Aeromonas, Plesiomonas, Morganellaand Providencia, than against *E. coli* O157. This is currently the most commonly used medium for isolating *E. coli* O157 (Lee and Choi, 2006).Media containing fluorogenic or chromogenic glucuronides are used to distinguish non-beta-glucuronidase producing *E. coli* O157:H7 from beta-glucuronidase-producing *E. Coli* (Tesh *et al.,* 1991). Sorbitol-fermenting (SF) *E. coli* O157:H7 has been isolated from patients with diarrhoea and HUS but the epidemiology of this infection is poorly understood and only rarely has the organism been isolated from animals, including cattle (Blackburn and Mccarthy, 2000).

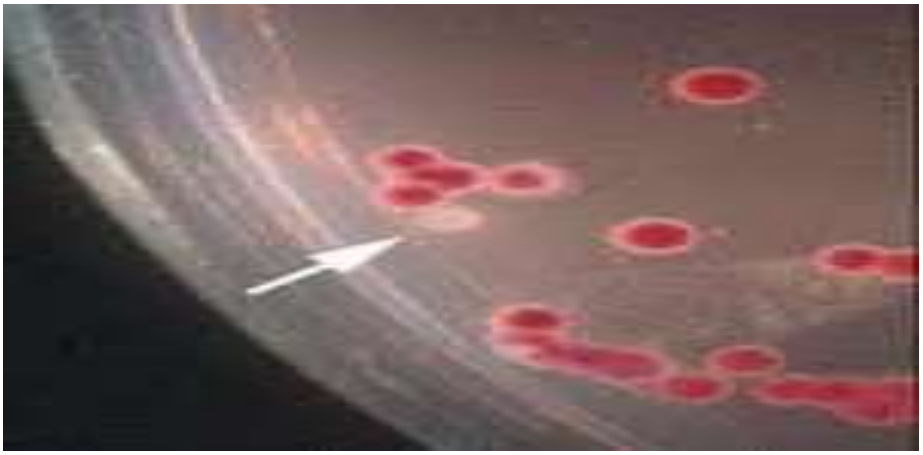

Figure 5: *E.coli O157*:H7 on Sorbitol-MacConkey agar plate. Arrow indicates distinctive colorless *E. coli* O157:H7 colony. (Source: Phillip *et al*., 2005).

2.7.5. Antimicrobial susceptibility tests of E. coli

Disk diffusion testing is one of several phenotypic assays which can be utilised to determine the antimicrobial resistance profile (antibiogramme) of an organism. Disk diffusion tests estimate in vitro susceptibility. The principle of agar diffusion is simple: Agar plates are inoculated with a standardised inoculum of the bacteria and an antimicrobial disk is placed on the inoculated agar plate. The disks used for a disk diffusion assay contain a standardised known amount of an antimicrobial agent, which diffuses into the agar when in contact with the agar surface. The plate is incubated under standardised conditions following, Clinical and Laboratory Standards Institute (CLSI) guidelines. During incubation, the antimicrobial agent diffuses into the agar and inhibits growth of the bacteria, producing a “zone of inhibition” around the disk. Following incubation, the diameter of this zone is measured and the results are interpreted as resistant, intermediate, or susceptible using standard guidelines (e.g. CLSI M100).

The size of the inhibition zone indicates the degree of resistance, and might also give important information about the resistance mechanism and the resistance genes involved. In addition, the disk diffusion method can be used for determination of MIC values provided the necessary reference curves for conversion of inhibition zones into MIC values are available.

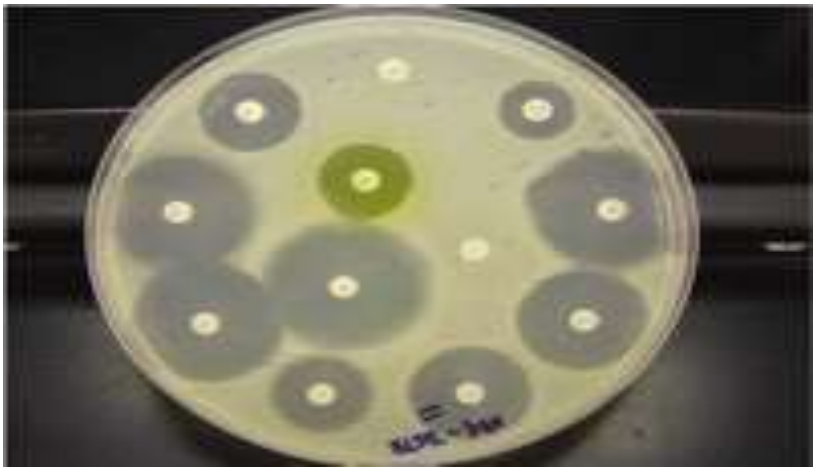

Figure 6: Antimicrobial susceptibility tests of *E. col (* **Source: CLSI, 2008)**

A disk diffusion test with an isolate of *E. coli* from a urine culture. The diameters of all zones of inhibition are measured and those values translated to categories of susceptible, intermediate, or resistant using the latest tables published by the CLSI.

2.7.5. Immunologic methods

Immunological methods are now widely used for the detection of VT. The methods utilize VT specific poly or monoclonal antibodies. There are various assay formats, several of which arecommercially available. The assay formats include enzyme linked immune sorbent assays (ELISA) and reversed passive latex agglutination (RPLA) (Beutin, 2003). Immunoassays to identify O and H antigens and VT may be used to confirm the identity of the organisms once isolated from clinical, food or environmental samples, while others, including dipstick and membrane technologies, microplate assays, colony immune blotting, immune fluorescence and ELISA, are used as rapid methods for detecting the presence of potential pathogens in samples prior to isolation, thus shortening the time for a presumptive diagnosis. Most assays for somatic and flagellar antigens are designed to detect the O157 LPS and H7 flagellar antigen. Enzyme immunoassays for O157 and VT, visual immunoassays for O157 and agglutination tests for O157, H7 and VT are available commercially as kits (Strockbine *et al.*, 1998).

Enzyme-linked immune sorbent assay (ELISA)

Immunoassays such as (ELISA) were very useful for rapid screening of *E. coli* O157:H7 and non O157 in food. ELISA assays are based upon the same reaction, with monoclonal antibody (MAb) reactive with low molecular weight outer membrane antigens of *E. coli* O157:H7, but are performed in microplates and the antibody is coupled with an enzyme that allows colorimetric screening. It has been revealed that the target antigens of the MAb are present in other serotypes of *E. coli* and that their expression and detection are influenced by culture conditions and sample preparation (Johnson *et al.*, 1995).

Latex Agglutination Test

The Plasmatic *E. coli* test kit is a latex agglutination test for the rapid identification of *E.coli* sero group O157. The test is best used in conjunction with Sorbitol MacConkey Agar. *E.coli* O157 strains cannot ferment sorbitol and will therefore give colorless colonies. The majority of other E.coli strains is capable of fermenting sorbitol and therefore, gives characteristic pink colonies. The non-sorbitol fermenting (NSF) colonies were further tested by the latex agglutination method. A positive result is indicated by agglutination with the test reagent, whilst the control reagent should appear milky and smooth (Shelton *et al.*, 2004).

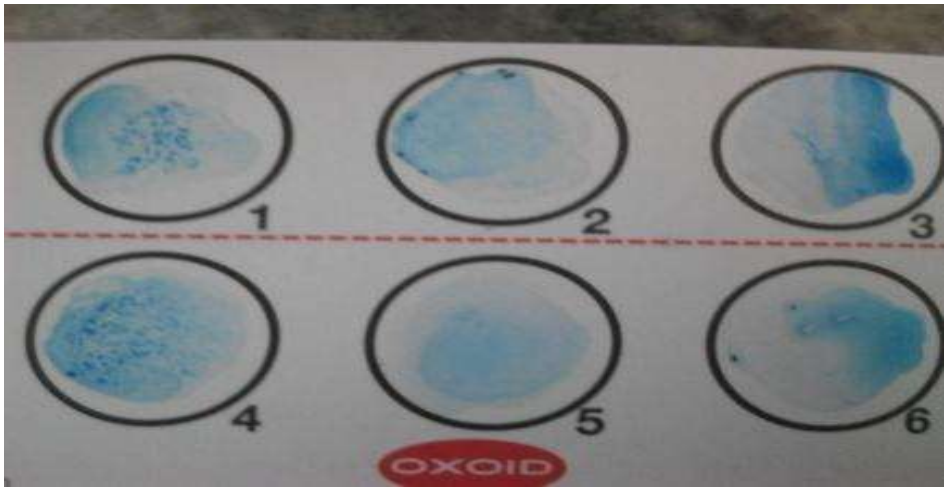

Figure7 : Latex Agglutination Test (**Source: https://www.google.com/search**)

2.7.6. Molecular Detection of Shiga Toxin-Producing E. coli

The World Health Organization (WHO) has called the rapid identification of virulent O157 STEC a public health priority (WHO, 1998). Recent recommendations from the CDC suggest that future STEC methods should include an assessment of the potential of the organisms to cause severe disease, possibly by detecting virulence factor genes. Various polymerase chain reaction (PCR) techniques are utilized for detection of virulent toxins and other virulence markers. Currently, there are a range of molecular techniques such as conventional polymerase chain reaction (PCR), multiplex PCR and Real time PCR (RT-PCR) (Nataro and Kaper, 1998).

PCR can be used on pure or mixed plate or broth cultures, and extracts from food or feces. It can also be used to detect genes in non-viable organisms. As well as its role in diagnosis, PCR has the potential to be used to screen samples for VTEC in epidemiological studies (Nataro and Kaper, 1998)

Amplification of target genes in bacterial DNA extracts from feces is less successful than from pure cultures, and careful preparation of the sample is required to improve sensitivity. Feces contain nonspecific PCR inhibitors and no single method of removing these is ideal. Sensitivity is improved by nonselective enrichment prior to testing, but remains lower than using IMS or the Vero cell cytotoxicity assay. Commercial assays are available (Paton and Paton 1998).

PCR is especially advantageous when dealing with food samples, where a relatively low number of cells are present. The occurrence of real time PCR makes it possible for simultaneous quantification and detection of *E. coli*. Recently, great efforts have been taken to design methods which can examine a strain for all known *E. coli* virulence genes to assess its virulence potential (Billips *et al*., 2007).

2.8. Public Health Importance

Escherichia coli is a bacterium that is commonly found in the gut of humans and warm blooded animals. Most strains of *E. coli* are harmless. Some strains however, such as enterohaemorrhagic *E. coli* (EHEC), can cause severe foodborne disease. It is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products, raw milk and contaminated raw vegetables and sprouts. Its significance as a public health problem was recognized in 1982, following an outbreak in the United States of America. EHEC produces toxins, known as verotoxins or Shiga-like toxins because of their similarity to the toxins produced by *Shigella dysenteriae* (FAO/WHO, 2008).

EHEC can grow in temperatures ranging from 7°C to 50°C, with an optimum temperature of 37°C. Some EHEC can grow in acidic foods, down to a pH of 4.4, and in foods with a minimum water activity (Aw) of 0.95. It is destroyed by thorough cooking of foods until all parts reach a temperature of 70°C or higher. *E. coli* O157:H7 is the most important EHEC serotype in relation to public health; however, other serotypes have frequently been involved in sporadic cases and outbreaks. Symptoms of the diseases caused by EHEC

include abdominal cramps and diarrhoea that may in some cases progress to bloody diarrhoea (haemorrhagic colitis). Fever and vomiting may also occur (WHO, 2009).

The incubation period can range from three to eight days, with a median of three to four days. Most patients recover within 10 days, but in a small proportion of patients (particularly young children and the elderly), the infection may lead to a life-threatening disease, such as haemolytic uraemic syndrome (HUS). HUS is characterized by acute renal failure, haemolytic anaemia and thrombocytopenia. It is estimated that up to 10% of patients with EHEC infection may develop HUS, with a case-fatality rate ranging from 3 to 5%. Overall, HUS is the most common cause of acute renal failure in young children. It can cause neurological complications (such as seizure, stroke and coma) in 25% of HUS patients and chronic renal sequelae, usually mild, in around 50% of survivors. Persons who experience bloody diarrhoea or severe abdominal cramps should seek medical care (WHO, 2009).

Antibiotics are not part of the treatment of patients with EHEC disease and may possibly increase the risk of subsequent HUS. Most available information on EHEC relates to serotype O157:H7, since it is easily differentiated biochemically from other *E. coli* strains. The reservoir of this pathogen appears to be mainly cattle. In addition, other ruminants such as sheep, goats, deer are considered significant reservoirs, while other mammals (pigs, horses, rabbits, dogs, cats) and birds (chickens, turkeys) have been occasionally found infected. *E. coli* O157:H7 is transmitted to humans primarily through consumption of contaminated foods, such as raw or undercooked ground meat products and raw milk. Faecal contamination of water and other foods, as well as cross-contamination during food preparation (with beef and other meat products, contaminated surfaces and kitchen utensils), will also lead to infection..Person to person contact is an important mode of transmission through the oral fecal route. An asymptomatic carrier state has been reported, where individuals show no clinical signs of disease but are capable of infecting others. The duration of excretion of EHEC is about one week or less in adults, but can be longer in children. Visiting farms and other venues where the general public might come into direct contact with farm animals has also been identified as an important risk factor for EHEC infection (WHO, 2011).

2.8.1.Economic Significance E. coli O157:H7

The CDC estimated the annual disease burden of *E. coli* O157:H7 in the United States to be more than 20,000 infections and with as many as 250 deaths (Boyce *et al*., 1995). WHO is concerned about this organism because bloody diarrhoea is a major cause of morbidity and mortality among children in developing countries in the southern hemisphere, including South Africa (WHO, 1997). The *E. coli* O157:H7 strain has tended to dominate the world literature on EHEC. Infections caused by *E. coli* O157:H7 are now recognized more frequently, which reflects increased interest in the incidence and detection of this organism (Nataro and Kaper, 1998).

According to CDC, the incidence of EHEC in humans is difficult to determine, because cases of uncomplicated diarrhea may not be tested for these organisms. In 2004, the estimated annual incidence of EHEC O157:H7 reported in Scotland, the U.S., Germany, Australia, Japan and the Republic of Korea ranged from 0.08 to 4.1 per 100,000 population, with the highest incidence in Scotland. In the U.S. estimates that EHEC O157:H7 causes approximately 73,000 illnesses, 2,000 hospitalizations, and 50-60 deaths each year.

2.9. Treatment and Prevention

Treatment*:* Antimicrobial agents have no proven value in the treatment of *E. coli* O157:H7 infections. No randomized clinical trials of the early use of antimicrobial agents in this disease have been performed (Mead and Griffin, 1998). Treatment of hemorrhagic colitis is supportive, and may include fluids and a bland diet. Antibiotics are controversial and are usually avoided: they do not seem to reduce symptoms, prevent complications or decrease shedding, and they may increase the risk of HUS (Boyce *et al.,* 1995).

The use of anti motility (anti diarrheal) agents in hemorrhagic colitis also seems to increase the risk for developing HUS. (Wilkerson *et al*. (2004) reported that some antimicrobial agents, particularly quinolones, trimethoprim, and furazolidone, were shown to induce toxin gene expression and should be avoided in treating patients with confirmed *E. coli* O157 infections. Patients with complications may require intensive care including dialysis, transfusion and/or platelet infusion. Patients who develop irreversible kidney failure may need a kidney transplant (Scheiring *et al.,* 2008).

From the recent research done by Dulo (2014) from carcass swabs taken from goast slaughtered at Dire Dawa municipal slaughter house showed the presence 100% and 83.3% resistant *E. coli* O157:H7 against Erythromycin and Ampicillin respectively. Such types of drug resistance are believed to be as a result of multiple factors like wide use drugs especially in food animals and transfer of drug resistance carrying plasmid gene among *E. coli* species.

Prevention *: c*lose cooperation and communication among clinicians, public health authorities, and clinical microbiologists are needed to help prevent *E. coli* O157:H7 infections. As with many food borne diseases, efforts to decrease contamination of foods throughout the production and distribution chain in both commercial establishment and the domestic environment are necessary to reduce the risk of infection (Besser, *et al.,* 2003). Effective prevention and control of contamination in abattoirs requires the application of good hygiene practices, the application of Hazard Analysis and Critical Control Point (HACCP) based management practices and risk-based meat inspection practices to minimize faecal contamination of carcasses. In an effort to improve quantity and quality of food, FAO is promoting good management practices in the dairy and beef sector, often in collaboration with the private sector. Examples include the preparation of manuals such as the IDF/FAO Guide to Good Dairy Farming Practice or the development of training material and capacity building interventions in relation to hygienic milk handling and processing but also testing and quality control (Sargeant and Smith, 2003).

Preventions in humans can be achieved through consumption of well cooked beef products, pasteurized milk, milk products, and juices, drinking chlorinated municipal water that has been treated with adequate levels of or other effective disinfectants are also important points (Acha and Szyfres, 2001). In general, a multiple hurdle system for reducing the probability of contamination of carcasses with bacterial pathogens helps directly and/or indirectly in the reduction of risks associated with public and animal health (Smith, 2000).

The *E. coli* O157:H7 organism is easily killed by heat. Cooking at 155°F for 0.13minutes will kill the number of organisms usually present in contaminated food products (Jay, 2000). This is easy to accomplish form eat products such as hamburger or sausage. However, for products consumed without cooking such as apple cider or lettuce it presents much more of a problem. Foods such as milk, apple cider, and apple juice should obviously be pasteurized-this is not only important for preventing infection by *E. coli* O157:H7, but also for Salmonella, Campylobacter, and other pathogens. Also, the issue of cleanliness by food handlers and food preparers is central to prevention of any food safety concern (John, 1996).

Cook all ground beef and hamburger thoroughly. Ground beef should be cooked until a thermometer inserted into the thickest part of the patty reads at least 160º F on a digital thermometer or until the patty is no longer pink inside. Drink only pasteurized milk, apple juice, or cider. Commercial juice with an extended shelf life that is sold at room temperature (e.g., juice in cardboard boxes, vacuum-sealed juice in glass containers) has been pasteurized, although this is generally not indicated on the label. Juice concentrates are also heated sufficiently to kill pathogens (SCHHSA, 2011).

There are no currently available vaccines to prevent disease due to EHEC (OIE, 2004) but a number of experimental approaches are being investigated in animals. Vaccine development has been severely hampered by the lack of an appropriate animal model, which can develop HUS after exposure to EHEC (Nataro and Kaper, 1998). A crucial antigen in any potential vaccine is the Stx. Parenteral Stx toxoid vaccines have shown protective effects in rabbits (Bielaszewska *et al*., 1994) and pigs (Bosworth *et al.*, 1996). Colonization factors, such as intimin (the intestinal adherence factor), as an edible vaccine in transgenic plants have been tried to develop as a vaccine (IFT, 2003; OIE, 2004; Edwards and Fung, 2006). A parenteral vaccine specific for O157 EHEC has been developed based on O157 polysaccharide conjugated to protein carriers (Konadu *et al*., 1994). An ideal broad-spectrum EHEC vaccine should probably engender both systemic immunity against Stx and local intestinal immunity against intimin and other intestinal colonization factors (Nataro and Kaper, 1998; IFT, 2000).

2.10. Status *of E.coli* O157:H7 in Ethiopia

In Ethiopia, there were studies conducted by some researchers to determine the occurrence and proportion of *E. coli* O157:H7 in faeces, skin swabs and carcasses of sheep, goat and cattle in different areas of the country. Based on a retrospective review of culture results of clinical sources (Dessie Regional Health Research) including urine, ear discharge, pus swab from wounds, and eye discharge, 14.2 % *E. coli* O157:H7 was isolated from 446 samples (Kibret and Abera, 2011).

One research conducted in Addis Ababa municipal abattoir showed that the prevalence of the of E. coli o157:H7 as 10.2% (13.3% Beef followed by 9.4% mutton and 7.8% goat meat). Similar findings revealed that the prevalence of *E. coli* O157:H7 as a carcass contaminant in sheep of goat and cattle slaughtered at Debre Zeit municipal abattoir and Modjo export abattoir (Hiko *et al*., 2008; Mersha *et al*., 2009).

According to the research conducted in the eastern part of Ethiopia (Dire Dawa and Haramaya), prevalence of *E. coli* O157:H7 as a contaminant of meats of ruminants was recorded from 2.55% up to 30.97% (Taye *et al*., 2013; Dulo, 2014 and Mohammed *et al* 2013). Balch *et al.* (2014) also reported that prevalence of 62.5% *E. coli* from beef samples collected from Mekele Municipal abattoir and meat retailer.

3. CONCLUSION AND RECOMMENDATIONS

E. coli has been known to cause lots of infection in animals and human beings and it is often transmitted to human beings by consumption of contaminated food or improperly cooked/boiled food items including raw or under cooked meat and meat products, raw or unpasteurized milk, vegetables, and fecal contamination of water and other foods. The disease is mainly diagnosed by isolation and identification of the *E. coli* on common and selective media and by using biochemical tests, immunological methods and detection of the toxins by molecular methods. Treatments are possibly by various antimicrobial agents however vaccines are not available so far. Prevention measures have greatest important in minimizing the occurrence the infection.

Based on the above concluding remarks, the following recommendations are forwarded:

- Prohibiting consumption of raw or under cooked food, thus the most common means transmitting of the organisms.
- Continuous training or awareness creation should be given to people to minimize the possible sources of the infection
- Effective Diagnostic methods should be chosen and implemented to properly identify the pathogen
- *In vitro* drug sensitivity testing must be frequently done to select an effective antimicrobial agent against *E. coli*
- Various molecular techniques should be used to detect the genes in shiga toxin producing strains *E. coli*

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