CHAPTER 2

REVIEW OF LITERATURE
between 1985 and 1995 but represented 12 (18.2%) of 66 of the non-O157 STEC isolated during 1996.

**Morphological and biochemical characteristic:**

*E.coli* is a Gram negative, straight, rod measuring 1-3 x 0.4-0.7 μm arranged singly or in pairs. It is motile by peritrichate flagella, though some strains may be nonmotile. Capsules and fimbriae are found in some strains. Spores are not formed. It is an aerobe and facultative anaerobe. Many pathogenic isolates have polysaccharides capsules. Some strains may occur in the ‘mucoid’ form. Many strains, especially those isolated from pathogenic conditions, are haemolytic on blood agar. On MacConkey’s medium, colonies are bright pink due to lactose fermentation. Colonies show greenish metallic sheen on EMB agar plates. Growth is largely inhibited on selective media such as DCA or SS Agar used for the isolation of salmonellae and shigellae. In broth, growth occurs as general turbidity and a heavy deposit, which disperses completely on shaking.

Glucose, lactose, mannitol, maltose and many other sugars are fermented with the production of acid and gas. Typical strains do not ferment sucrose. The four biochemical tests widely employed in the classification of enterobacteria are the indole, methyl red (MR), Voges-Proskauer (VP) and citrate utilization tests, generally referred to by the mnemonic’ IMViC’.

*E.coli* is indole and MR positive, and VP and citrate negative(IMViC++--). Gelatin is not liquefied, H$_2$S is not formed, urea is not split and growth does not occur in KCN medium. In addition, there are several commercially available systems that allow strains to be biochemically characterized.

**Antigenic structure:**

*E. coli* is a genetically and phenotypically diverse species whose strains are identified on the basis of ‘O’, ‘H’ and sometimes ‘K’ antigens, which together constitute the serotype. Serotyping of *E.coli* is based on three antigens- the somatic antigen O, the capsular antigen K and the flagellar antigen H. So far some 170 types of O antigen, 100 K antigens and 75 H antigens have been recognised. The antigenic pattern of a strain is recorded as the number of the particular antigen it carries, as for example O111:K58:H2.
The K antigen is the acidic polysaccharides antigen located in the ‘envelop’ or microcapsule. (K for Kapsel, German for capsule). It encloses the O antigen and renders the strains inagglutinable by the O antiserum. It may also contribute to virulence by inhibiting phagocytosis. Formerly K antigens were subdivided into three kinds- the thermolabile L antigens, the thermostable A antigens and the B antigen found on enteropathogenic strains associated with infantile diarrhoea. Later it was shown that the B antigen was not a separate entity. K antigens are therefore currently classified into two groups, I and II, generally corresponding to the former A and L antigens.

**Epidemiology:**

**World-**

Diarrheal diseases are leading causes of morbidity and mortality, especially in Africa, Asia, and Latin America (Kaper, *et al.*, 2004). STEC has been increasingly recognized as an important human diarrheal pathogen and as the predominant cause of hemorrhagic colitis and hemolytic uremic syndrome (HUS). A smaller number of outbreaks (6 total) of human illnesses was attributed to infections with nonO157 STEC strains from contaminated beef. These infections involved 8 STEC serogroups (O1, O2, O15, O25, O75, O86, O111, and O160) and 3 serotypes (O26:H11, O111:H7, and O111:H−). These outbreaks were reported in Argentina, Australia, Germany, and Italy and were traced to consumption of undercooked ground beef or its sausage. Two of these outbreaks involved large numbers of affected people (161 and 433) as shown in Australia (CDC, 1995) and Argentina (Lopez, *et al.*, 1997), respectively. The significance of nonO157 STEC infections through contaminated beef was illustrated in the incidence of HUS cases in 5 out of 6 reported outbreaks. Several recent outbreaks have been attributed to non- O157 STEC (Johnson *et al.*, 1996). Non-O157 STEC is more often isolated from foods and animal feces (Montenegro *et al.*, 1990, Smith, *et al*, 1991). However, not all pathogenic serotypes have been reported to be associated with severe human disease. As far as *E.coli* infections are concerned, these have great prevalence in US and beef is main source of infection. In addition to beef (CDC, 1993; Lopez *et al.*, 1997; CDC, 2003), human infections were traced to vegetables (Cieslak *et al.*, 1993), raw milk (Martin *et al.*, 1986; Herriott *et al.*, 1994; Lahti *et al.*, 2002), dairy products (Morgan et
al., 1993; Reid, 2001), and drinking water (Yatsuyanagi et al., 2002) containing STEC. Other infection routes included person to person (Reida et al., 1994) and animal to person (Synge et al., 1993; Crump et al., 2002). The infection caused human illnesses (Griffin et al., 1991; Paton et al., 2000) ranging from mild diarrhea to hemolytic uremic syndrome (HUS) that could lead to death (Pavia, et al., 1990; CDC, 1993; Cowden, et al., 1997). This is critically important because recent evidence (Hussein and Bollinger, 2005; Hussein and Sakuma, 2005) illustrated a large number of pathogenic STEC serotypes to derive from bovine origin. This review is intended to provide a global assessment of the cattle role in human infection with STEC. 50% of VT-producing E coli (VTEC) in human diarrheal stool samples in these areas are non-O157 serogroups (Spika, et al., 1998). This pattern fits with data from Europe and Australia, where non-O157 VTEC predominates. In these geographical locales, non-O157 VTEC, like O157 VTEC in North America, has been associated with large outbreaks and severe sequelae, such as hemorrhagic colitis and hemolytic uremic syndrome (HUS). In general, VTEC infections share several common epidemiological patterns. They typically originate from food, water and zoonotic sources or by human-to-human transmission. These bacteria have several factors implicated in pathogenesis, including Shiga toxins (Stx) and a pathogenicity island called LEE that encodes proteins, such as intimin (eaeA), involved in attaching effacement (Tesh, et al. 1992; McDaniel, et al. 1995). Evolutionarily, the O157:H7 serotype marks a distinct clone that is only distantly related to other Stx-producing enterohemorrhagic E. coli (EHEC) and is most closely related to an enteropathogenic E. coli (EPEC) clone serotype O55:H7, a non-Stx-producing strain associated with infantile diarrhea (Whittam, et al., 1993).

**India-**

In India, there is paucity of information on pathogenic E.coli. It has not been identified as a significant etiologic agent of diarrhoea for humans in India. However, isolation of O157 and non-O157 serogroups of E. coli that exhibited the cytotoxic activity in vero cells has been reported from human patients with diarrhoea in India, but these strains have not been well characterized, and their origin is uncertain (Gupta et al.,1992). Similarly, there are not many reports of isolation of STEC(Shiga toxin producing E.coli) from various animal species in
India. (Pal, et al., 1999) were first to report the isolation of STEC from non-diarrhoeic animal sources in India. These workers collected faecal samples from 67 healthy cattle in a semi-urban community near Calcutta (now Kolkata) and examined these for STEC by multiplex PCR and culturing on SMA. STEC was isolated from the faeces of seven (10.5%) animals. The eight strains isolated belonged to eight serotypes, viz. O146 : H1, O149 : HNT, ONT : H19, O88 : HN, ONT : H2, O82 : H8 and O28ac : H21. Bead enzyme-linked immunosorbent assay showed that three strains produced Shiga toxin 1, one produced Shiga toxin 2 and four produced both. Subsequently, (Chattopadhyay, et al., 2001) isolated and characterized STEC strains from animal, human and food products. A total of 876 samples (330 animal, 184 human, 362 food samples) were screened for the presence of STEC by conventional as well as PCR techniques. Seventeen STEC strains (12, 1 and 4 from animal, human and food samples respectively) were isolated. The isolation rate was higher in diarrhoeic animals (6.02%), followed by diarrhoeic handler (3.12%) and raw beef (1.78%) samples. Though the STEC strains were isolated from different sources of animal and human origin, they showed a uniform antibiogram. This suggested a zoonotic association. (Kumar, et al., 2001) investigated the occurrence of STEC in fresh fish, shellfish and meat sold in open markets in Mangalore, India. Two out of 60 fish samples and two out of the 48 clam samples were positive for stx and hlyA genes by PCR. STEC strains belonged to non- O157 serogroups. They concluded that the seafood could be a vehicle for transmission of STEC even in tropical countries. Again in Kolkata, (Khan, et al., 2002) investigated the prevalence of STEC in hospitalized patients with diarrhoea, as well as in healthy cattle and raw beef samples collected from the city’s abattoir. Multiplex PCR using primers specific for stxl and stx2 genes detected STEC in 18% of cow stool samples, 50% of raw beef samples and 1.4% of bloody and 0.6% of watery stool samples from hospitalized patients. Various virulence genes in the STEC isolates indicated that stxl allele predominated. Plasmid-borne markers, namely hlyA, KatP, espP and etpD were also identified. However, they concluded that STEC is not an important cause of diarrhoea in Kolkata, but its presence in cattle and beef samples suggests that this enteropathogen may become a major public health problem in the future. The first systematic study on strains of STEC in India was carried out by (Khan, et al., 2002).
In this study antibiotic resistance, virulence gene and other molecular profiles of STEC strains isolated from human stool samples, cow stool samples and beef samples over a period of two years in Kolkata, were determined. (Gupta & Gupta., 2006) determined age and sex specific attack rate. They also hypothesized it as a food borne beef meat outbreak. Resistance to one or more antibiotics was observed in 49.2% of the STEC strains, with some strains exhibiting multidrug resistance. The dominant combinations of virulence genes present in the strains studied were stx1 and stx2 (44.5% of strains) and stx2 and hlyA (19% of strains). Only 6.4% of the STEC strains harboured eae. The diversity of STEC strains from various sources was assessed by random amplification of polymorphic DNA (RAPD). STEC strains that gave identical or nearly similar DNA fingerprints in RAPD–PCR and possessed similar virulence genotypes were further characterized by pulsedfield gel electrophoresis (PFGE). Identical RAPD and PFGE profiles were observed in four sets of strains, with each set comprising two strains. There was no match in the RAPD and PFGE profiles between strains of STEC isolated from cows and those isolated from humans. It appears that the clones present in bovine sources are not transmitted to humans in Kolkata, although these strains showed evolutionary relatedness. May be for this reason, STEC has still not become a major problem in India. (Wani, et al., 2003) reported for the first time the isolation and characterization of STEC serogroups associated with diarrhoea in calves and lambs in India. Many STEC and EPEC isolates belonged to serogroups known for certain lifethreatening diseases in humans. About 249 bovine and 60 ovine E. coli strains were recovered from faecal samples of 391 calves and 101 lambs. Then 130 bovine and 15 ovine strains were subjected to multiplex PCR for detection of stx1, stx2, eaeA and EHEC hlyA genes. STEC strains belonging to different serogroups were detected in 9.73% of calves and 6% of lambs studied. Majority of the STEC isolates in this study did not belong to those serogroups which had been identified earlier to be mainly associated with diarrhoea and enteritis in cattle and sheep outside India. One of the most important serogroups, O157 known to cause certain life-threatening infections in humans, was isolated from both bovine and ovine faecal samples. Similarly, (Wani, et al., 2004) reported an outbreak of bloody diarrhoea in 1–16-week-old crossbred calves in an organized dairy farm in Kashmir. Seven out of ten were
affected. Affected calves were diarrhoeic for 4–5 days and voided blood tinged malodorous faeces. O116 was recovered from five calves with diarrhoea. The virulence gene profile revealed stxl, eaeA and hlyA genes. In another study, association of STEC O4 : NM serotype with an outbreak of diarrhoea in 4–7-week-old calves was also demonstrated by (Wani et al. 2005). Nine calves with diarrhoea revealed the presence of O4 : NM serotype. Six E. coli O4 : NM strains carried eaeA and EHEC hlyA genes and three possessed stxl in addition to eaeA and EHEC hlyA genes. Recently, we screened 426 E. coli strains isolated from 500 chicken and 25 freeflying pigeons. None of these strains possessed Shiga toxin genes. However, six and seven E. coli isolates revealed the presence of eaeA and hlyA genes respectively (Wani, et.al, 2004). Likewise 136 E. coli isolates from rabbits with and without diarrhoea did not reveal the presence of STEC. However, eaeA gene was found in three isolates while another isolate showed the presence of hlyA gene (unpublished data). Again, (Kumar, et al., 2004) characterized STEC strains isolated from seafood and beef in Mangalore by bead-ELISA, vero cell cytotoxicity assay, PCR and colony hybridization for the detection of stxl and stx2 genes.

Four strains from seafood, six from beef and one from a clinical case of bloody diarrhoea were found to carry Shiga toxins. The seafood isolated carried stxl and or stx2 in combination or alone, while the beef isolates produced stxl alone. stxl gene of beef STEC was found to be stxlc subtype. Interestingly, though all STEC strains were negative for eae gene, two STEC strains isolated from seafood and one from a patient with bloody diarrhoea carried STEC autoagglutinating adhesin gene, recently identified as the gene encoding a novel autoagglutinating adhesin.

**Common Themes In E. Coli Virulence**

Like most mucosal pathogens, E. coli can be said to follow a requisite strategy of infection: (i) colonization of a mucosal site, (ii) evasion of host defenses, (iii) multiplication, and (iv) host damage. The most highly conserved feature of pathogenic E. coli strains is their ability to colonize the intestinal mucosal surface despite peristalsis and competition for nutrients by the indigenous flora of the gut (including other E. Coli strains). The presence of surface adherence fimbriae is a property of virtually all E. coli strains, including non
pathogenic varieties. However, pathogenic *E. coli* strains possess specific fimbrial antigens that enhance their intestinal colonizing ability and allow adherence to the small bowel mucosa, a site that is not normally colonized.

**A) Enteropathogenic *E. Coli***

The hallmark of infections due to EPEC is the attaching-and-effacing (A/E) histopathology, EPEC strains which tended to aggregate and form bundles, thereby suggesting the name “bundle-forming pilus” (BFP). EPEC causes primarily acute diarrhea, although many cases of protracted EPEC diarrhea have also been reported (Levine, *et al.*, 1984). In outbreaks in the United States and the United Kingdom in the mid-20th century, mortality rates of 25 to 50% were reported, and in recent outbreaks from developing countries, 30% mortality was reported (Nataro *et al.*, 1985).

**B) Enterohemorrhagic *E. Coli* –**

The major virulence factor, and a defining characteristic of EHEC, is Stx. This potent cytotoxin is the factor that leads to death and many other symptoms in patients infected with EHEC. Stx in the blood of HUS patients. This possibility is supported by the fact that patients with bloody diarrhea due to *E. coli* O157:H7 are more likely to develop HUS than are those with non-bloody diarrhea (Griffin *et al.* 1991). Stx-producing *E. coli* can be found in the fecal flora of a wide variety of animals including cattle, sheep, goats, pigs, cats, dogs, chickens, and gulls (Beutin, *et al.* 2003, Griffin, *et al.* 1990; Johnson, *et al.* 1990; Wallace, *et al.* 1997). However, the great majority of these strains are of serotypes other than O157:H7 and are of questionable pathogenicity. The most important animal species in terms of human infection is cattle. The most common non-O157:H7 serotypes associated with human disease include O26:H11, O103:H2, O111:NM, and O113:H21 (Griffin, *et al.* 1990). About 150 non-O157 serotypes have now been attributed to sporadic and epidemic human infections. PCR techniques to detect the gene (*flIC*) encoding the H7 antigen have been developed. The *flIC* primer pair has also been combined with primers for *stx* and *eae* in a multiplex PCR to allow the specific identification of *E. coli* O157:H7, O157:NM, and other EHEC strains (Gannon, *et al.*, 1997).
C) Enterotoxigenic *E. Coli* -

ETEC is defined as containing the *E. coli* strains that elaborate at least one member of two defined groups of enterotoxins: ST and LT. The LTs of *E. coli* are oligomeric toxins. The B subunits are arranged in a ring or “doughnut” and bind strongly to the ganglioside GM1 and weakly to GD1b and some intestinal glycoproteins. The A subunit is responsible for the enzymatic activity of the toxin. The STs are small, monomeric toxins that contain multiple cysteine residues, whose disulfide bonds account for the heat stability of these toxins. Human ETEC strains possess their own array of colonization fimbriae, the CFAs.

**FIG. 2.1.** Pathogenic schemes of pathogenic *E. coli*. The six recognized categories of pathogenic *E. coli* each have unique features in their interaction with eukaryotic cells. Here, the interaction of each category with a typical target cell is schematically represented. It should be noted that these descriptions are largely the result of in vitro studies and may not completely reflect the phenomena occurring in infected humans. Three major morphologic varieties exist: rigid rods, bundle-forming flexible rods, and thin flexible wiry structures. CFA/I, the prototype rigid rod-shaped fimbria, CFA/III is a bundle-forming pilus. CFA/I, CFA/II, or CFA/IV is expressed by approximately 75% of human
ETEC strains worldwide (Wolf et al.). Travelers to the developing world should also be counselled on the need to maintain hydration when they experience diarrhea. In addition, bismuth subsalicylate or loperamide is effective in decreasing the severity of diarrhea (Arduino, et al., 1993).

D) Enteroaggregative *E. Coli*

Scaletsky et al. and Nataro et al. examined collections of *E. coli* from studies of diarrhoea in the developing world and found, like (Cravioto, et al., 1979). Enteropathogenic *E. coli* (EPEC), a term more recently used as a synonym for attaching and effacing *E. coli* (AEEC), is an emerging cause of diarrhoea in humans and animals (Wani, et al., 2004). (Benjamin, et al., 1995) have suggested that some EAEC strains may invade intestinal epithelial cells in vitro. However, human intestinal explants infected with EAEC strains do not reveal internalization of the bacteria, and clinical evidence for a role for invasiveness is as yet lacking. The association of EAEC with diarrheal disease appears to be geographic. On the Indian subcontinent, five separate studies have been published which demonstrate the importance of EAEC in pediatric diarrhoea. Studies in India also suggest that the illness is most likely be manifested by watery, secretory diarrhea in the absence of fever and without gross blood (Bilge, et al., 1989).

E) Enteroinvasive *E. Coli*

EIEC strains were first shown to be capable of causing diarrhea in volunteer studies conducted by DuPont et al. in 1971 (DuPont, et al., 1971) Documented EIEC outbreaks are usually foodborne or waterborne (Lanyi et al., 1959; Marier et al., 1973; Snyder et al., 1984; Tulloch et al., 1973) although person-to-person transmission does occur.

F) Diffusely Adherent *E. Coli*

The term “diffusely adherent *E. coli*” was initially used to refer to any HEp-2-adherent E. coli strain that did not form EPEC-like microcolonies. With the discovery of EAEC, most authors now recognize DAEC as an independent category of potentially diarrheagenic *E. coli*. Several recent studies have implicated DAEC strains as agents of diarrhea, while other
studies have not recovered DAEC strains more frequently from diarrheal patients than from asymptomatic controls. In one study, the majority of patients infected with DAEC had watery diarrhea without blood or fecal leukocytes.

Other Categories of *E. Coli* which are potentially pathogenic -

The six categories of *E. coli* that are described in the above sections have each been implicated in several diarrhoea studies and are now generally accepted as diarrhoeagenic categories. However, some studies have suggested that there may be still other categories of diarrhoeagenic *E. coli* which are quite distinct from those described above. These will be briefly considered. (Gunzberg, *et al.*, 1993) reported a study of Australian aborigine children with diarrhoea in which a significant association was found between diarrheal illness and the presence of a cytotoxic phenotype on HEp-2 cells in the HEp-2 assay. This phenotype, typified by detachment of the cells from the glass within 3 h of incubation with bacteria, was found in organisms regardless of their adherence pattern. This pattern led the investigators to propose that cell-detaching *E. coli* (CDEC) may be a new category of diarrhoeagenic *E. coli*. (Elliott *et al.*, 1994) have characterized CDEC strains further and have shown that the detaching phenotype appears to be closely associated with the production of the *E. coli* hemolysin and that these organisms frequently secrete the cytotoxic necrotizing factor (CNF) but do not belong to any of the recognized categories above. Moreover, these investigators have shown that CDEC strains are able to elicit diarrhoea and destructive histopathology in the rabbit model. The role of CDEC in human diarrhea has yet to be determined. Two forms of CNF have been described, CNF1 and CNF2.. Most CNF-producing *E. coli* strains isolated from diarrheal stools have been isolated from animals rather than humans; most human isolates of CNF-producing *E. coli* have been from extraintestinal infection. There is a clear need for case-control studies of CNF-producing *E. coli* to definitely establish whether these organisms are true human pathogens. (Scott, *et al.*, 1994) and (Pickett, *et al.*, 1994) have cloned and characterized a gene from *E. coli* which encodes a cytolethal distending toxin (CDT).
*E. coli* has undergone a remarkable transformation in recent decades and undoubtedly will continue to evolve. Once dismissed as a harmless inhabitant of the intestinal tract, *E. coli* is now seen as a pathogenic species with remarkable versatility in its ability to cause disease in humans and animals. Outbreaks of disease due to *E. coli* can affect thousands of individuals and can engender national and international headlines. Pathogen-specific virulence factors have been discovered that adversely affect a wide range of eukaryotic cell processes including protein synthesis, cell division, ion secretion, and transcription. These factors are encoded on a variety of mobile genetic elements such as plasmids, bacteriophages, transposons and pathogenicity islands; this genomic plasticity implies ongoing reassortment of virulence factors that complicates our efforts to categorize the various subgroups into sharply delineated pathotypes. This dynamism promises to present new challenges in the diagnosis, treatment, and prevention of *E. coli* infections. Hence the nature of diseases on farms can be easily assessed, allowing farmers and public health officials to evaluate the risk of infection to animals or humans. Outbreak investigations, especially for emerging pathogens such as pathogenic *E. coli*, are critical for better understanding these pathogens’ epidemiology, which affect policy and behavior changes.

**Diagnosis of Pathogenic *E. coli***

Currently, three main methods based on cytotoxicity, serology and gene detection in conjunction with isolation are being followed for identification of pathogenic *E. coli*.

**Cytotoxic assays**

The profound cytotoxicity of Stx to vero cells remains the ‘gold standard’ for confirmation of putative Stx-producing isolates (Paton, *et al.*, 1998). The assay involves treatment of vero monolayers (usually in 96-well trays) with sterile extracts or filtrates of test materials and examining cells for cytopathic effects after 48–72 h of incubation. The specificity of cell-culture cytotoxicity tests can be considerably improved by employment of Stx1- and Stx2-specific neutralizing antisera45. Since vero cells have a high concentration of Gb3 and Gb4 receptors in their plasma membranes, they can be used to detect all known Stx variants.
HeLa cells have also been used, but this cell line lacks Gb4 moiety and, therefore, is less sensitive to Stx2e (Paton, et al., 1998).

**Serological assays**
Several immunoassays have been developed to detect Stx in culture supernatants, in bacterial extracts, or directly in faecal samples: sandwich and other ELISA techniques with immune sera, affinity-purified polyclonal antibodies, immobilized monoclonal antibodies or a Gb3 receptor as a capture system, and reverse passive latex agglutination (Paton, et al., 1998). Over the years, a number of other immunological methods like colony blot and passive agglutination assays have also been developed.

The major advantage of these immunological methods is ease and flexibility of use. Most of the ELISA methods involve a sandwich technique with immobilized monoclonal or affinity-purified polyclonal antibodies to the toxins as capture ligands. (Reymond et al., 1997) described a Western immunoblot assay for Stx1 antibodies, which was more specific and sensitive than either ELISA or cytotoxicity neutralization methods and proposed this as a more useful tool for seroepidemiological studies. Several commercial kits are available for STEC diagnosis.

**DNA-based assays**
The DNA-based assays are colony hybridization and polymerase chain reaction (PCR) assays. The colony hybridization assay with polynucleotidic probes was the first one to be used (Newland, et al., 1988) 47. Non-radioactive labels such as digoxigenin and biotin have been used for detection of STECs without loss of sensitivity or specificity (Thomas, et al., 1991) 48. PCR-based techniques are used to determine if there is STEC in a sample at all, without having to purify the organisms or isolate separate colonies. Crude lysates or DNA extracts from single colonies, mixed broth cultures, colony sweeps, or even direct extracts of faeces 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. A simple multiplex-PCR assay to detect stx1, stx2 and their variants was initially developed by (Paton, et al., 1998). There are certain disadvantages of this PCR-based diagnostic method. (1) The examination of faecal samples by direct PCR can be a
problem because substances inhibitory to PCR could be present in faeces or number of target organisms present in the test sample could be small, resulting in false negative or unclear results. (2) False positive results can be obtained if cryptic target gene sequences are present (free stx encoding phages or defective stx genes in bacteria) (Beutin, et al., 1997). Subsequently, a rapid and specific multiplex PCR, which detected rfbO157 gene (involved in the biosynthesis of O157 E. coli antigen) and main virulence genes (stx1, stx2 and eaeA) of STEC in the faeces were developed by (Osek, et al., 2002). Direct PCR (DPCR) has also been described as a simple and rapid approach for the detection of STEC because the untreated environmental sample is used directly as a template in PCR, eliminating the steps of cell recovery or DNA extraction (Fode-Vaughan, et al., 2003). The ability of DPCR to detect dead cells or free DNA could be advantageous in some situations. The recent development of real-time PCR has added newer dimension of quantitation to the conventional PCR assays (Bellin, et al., 2001). The technique has been applied to detect a number of pathogens, including STEC with the use of modified probes or oligonucleotides that are coupled with reporter and quencher dyes at the 5¢ and 3¢ ends respectively. In an intact probe, the quencher dyes suppress the fluorescence emission of the reporter dye. The continuous measurement of incremental fluorescence of each PCR cycle provides an accurate estimate of the number of cells of a bacterial pathogen present in contaminated food and faecal samples even up to levels of 1–10 CFU/g of food or faeces (Sharma, V.K., 2002). The optimization of the reverse transcriptase PCR designed to detect viable STEC as recently developed should become a useful tool (Mcingvale, et al. 2002). (Ge, et al., 2002) combined PCR with ELISA to develop a sensitive and specific detection method for STEC in foods. The principle of the method is incorporation during the PCR amplification process, of digoxigenic labelled dtp and a biotin-labelled primer specific for the VT genes. The labelled PCR products, bound to streptavidincoated wells of a microtitre tray through the biotin, are then detected by an ELISA technique (Bettelheim, et al., 2003).

**Treatment-**

**Antibiotic therapy and resistance**-Bacterial infections are usually treated with antibiotics. However, the antibiotic sensitivities of different strains of *E.coli* vary widely.
As Gram-negative organisms, *E. coli* are resistant to many antibiotics that are effective against Gram-positive organisms. Antibiotics which may be used to treat *E. coli* infection include amoxicillin, as well as other semisynthetic penicillins, many cephalosporins, carbapenems, aztreonam, trimethoprim-sulfamethoxazole, ciprofloxacin, nitrofurantoin and the aminoglycosides.

Antibiotic resistance is a growing problem. Some of this is due to overuse of antibiotics in humans, but some of it is probably due to the use of antibiotics as growth promoters in animal feeds (Johnson, *et al.*, 2006). A study published in the journal *Science* in August 2007 found the rate of adaptative mutations in *E. coli* is "on the order of $10^{-5}$ per genome per generation, which is 1,000 times as high as previous estimates," a finding which may have significance for the study and management of bacterial antibiotic resistance (Perfeito, *et al.*, 2007).

Antibiotic-resistant *E. coli* may also pass on the genes responsible for antibiotic resistance to other species of bacteria, such as *Staphylococcus aureus*, through a process called horizontal gene transfer. *E. coli* bacteria often carry multiple drug-resistance plasmids, and under stress, readily transfer those plasmids to other species. Indeed, *E. coli* is a frequent member of biofilms, where many species of bacteria exist in close proximity to each other. This mixing of species allows *E. coli* strains that are piliated to accept and transfer plasmids from and to other bacteria. Thus, *E. coli* and the other enterobacteria are important reservoirs of transferable antibiotic resistance (Salyers, *et al.*, 2004).

**Beta-lactamase strains**

Resistance to beta-lactam antibiotics has become a particular problem in recent decades, as strains of bacteria that produce extended-spectrum beta-lactamases have become more common (Paterson, *et al.*, 2005). These beta-lactamase enzymes make many, if not all, of the penicillins and cephalosporins ineffective as therapy. Extended-spectrum beta-lactamase–producing *E. coli* are highly resistant to an array of antibiotics, and infections by these strains are difficult to treat. In many instances, only two oral antibiotics and a very limited group of intravenous antibiotics remain effective.
In 2009, a gene called New Delhi metallo-beta-lactamase (shortened NDM-1) that even gives resistance to intravenous antibiotic carbapenem, were discovered in India and Pakistan on E. coli bacteria.

Increased concern about the prevalence of this form of "superbug" in the United Kingdom has led to calls for further monitoring and a UK-wide strategy to deal with infections and the deaths. Susceptibility testing should guide treatment in all infections in which the organism can be isolated for culture.

**Phage Therapy**

Phage therapy—viruses that specifically target pathogenic bacteria—has been developed over the last 80 years, primarily in the former Soviet Union, where it was used to prevent diarrhoea caused by E. coli. (Polish Academy of Sciences) Presently, phage therapy for humans is available only at the Phage Therapy Center in the Republic of Georgia and in Poland (http://www.phagetherapycenter.com). However, on January 2, 2007, the United States FDA gave Omnilytics approval to apply its E. coli O157:H7 killing phage in a mist, spray or wash on live animals that will be slaughtered for human consumption. The enterobacteria phage T4, a highly studied phage, targets E. coli for infection.

**Vaccination**

Researchers have actively been working to develop safe, effective vaccines to lower the worldwide incidence of E. coli infection (Girard, et al., 2006). In March 2006, a vaccine eliciting an immune response against the E. coli O157:H7 O-specific polysaccharide conjugated to recombinant exotoxin A of Pseudomonas aeruginosa (O157-rEPA) was reported to be safe in children two to five years old. Previous work had already indicated it was safe for adults (Ahmed et al., 2006). A phase III clinical trial to verify the large-scale efficacy of the treatment is planned (Ahmed, et al., 2006).

In 2006, Fort Dodge Animal Health (Wyeth) introduced an effective, live, attenuated vaccine to control airsacculitis and peritonitis in chickens. The vaccine is a genetically modified
avirulent vaccine that has demonstrated protection against O78 and untypeable strains (Pearson, et al., 2007). In January 2007, the Canadian biopharmaceutical company Bioniche announced it has developed a cattle vaccine which reduces the number of O157:H7 shed in manure by a factor of 1000, to about 1000 pathogenic bacteria per gram of manure (Pearson et al., 2007).

In April 2009, a Michigan State University researcher announced he had developed a working vaccine for a strain of E. coli. Mahdi Saeed, professor of epidemiology and infectious disease in MSU’s colleges of Veterinary Medicine and Human Medicine, has applied for a patent for his discovery and has made contact with pharmaceutical companies for commercial production.

Present Scenario-
In 1999 it was estimated that about 73,000 people in the U.S. got sick each year from E. coli. About 60 died. It’s believed that the number of illnesses and deaths has been dropping since then. In October 6, 2006, 199 persons were infected with the outbreak strain of E. coli O157:H7 had reported by CDC from 26 states, Multistate outbreak of E. coli O157 Infections Linked to Topp's Brand Ground Beef Patties were reported in October 26, 2007; Investigation of Outbreak was done for Human Infections Caused by E. coli O157:H7 November 1, 2007; Then investigation of Multistate Outbreak of E. coli O157:H7 Infections July 18, 2008; Widespread outbreak of E. coli O157:H7 Infections Linked to Eating Raw Refrigerated, Prepackaged Cookie Dough June 30, 2009; Epidemic caused by Outbreak of E. coli O157:H7 Infections Associated with Beef from Fairbank Farms November 24, 2009; another outbreak of E. coli O157:H7 Infections Associated with Cheese November 24, 2010; Outbreak of Shiga toxin-producing E. coli O104 (STEC O104:H4) Infections Associated with Travel to Germany July 8, 2011; Recently E. coli O157:H7 Infections linked to Romaine Lettuce was reported December 7, 2011