Sahel J. Vet. Sci. Vol. 8, No. 2, pp. 79 - 87 (2009)

1605-8954/09/$25.00 + 00

Sahel Journal of Veterinary Science

Evolution, Clonality and Some Virulence Characteristics of Enterohaemorrhagic *Escherichia coli* (EHEC): An Update

J. A. Musa*, H. M. Kazeem, M. A. Raji and N. M. Useh

Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine,
Ahmadu Bello University, Zaria, Nigeria

ABSTRACT

The evolution of *Escherichia coli* O157 was thought probably to be due to its ability to produce verotoxin. Thus, it appears that non-O157:H7 *E. coli* strains producing *stx* have been around for several decades but it was only with the emergence in the early 1980s of the O157:H7 clone that this pathogenic class of *E. coli* was recognized. The major and the most essentially suggested cardinal feature of EHEC strains is the production of shiga toxins (stx_1 and stx_2), which comprise a family of structurally related cytotoxins with similar biological activity and distinct antigenic structures. The colonization of the intestinal mucosa by most of the EHEC is associated with a mechanism that subverts the function of the epithelial cells. The effect of this interaction is the inducement of a characteristic "attaching and effacing" (A/E) lesion, a complex mechanism genetically controlled by a locus of large pathogenicity island (PAI) called the "locus of enterocyte effacement (LEE)". Intimin mediates the intimate attachment of EHEC by binding to β1-integrins and to cell-surface localized nucleolin. All these changes together with other factors which may be genetic in origin have resulted in evolution, the existence of clones and the occurrence and acquisition of virulence characteristics of enterohaemorrhagic *Escherichia coli*.

Keywords: Evolution, clonality, enterohaemorrhagic *E. coli*, virulence characteristics.

INTRODUCTION

The term 'enterohaemorrhagic *Escherichia coli* ' (EHEC) was originally used to describe strains that cause haemorrhagic colitis (HC) and haemolytic-uremic syndrome (HUS), express shiga-toxins (*stx*), cause attaching and effacing (A/E) lesions on epithelial cells and possess large plasmid (Nataro and Kaper, 1998).

Escherichia coli are found in the environment and occur as commensals in the mammalian gut. Lateral gene transfer events have allowed the transition of some *E. coli* strains from commensals to pathogens (Jores *et al.,* 2004; Eklund, 2005). Studies on several areas such as evolution, clonality, pathogenesis and epidemiology were aided by the carriage of EHEC- associated virulence (Jores *et al.,* 2004; Kelly *et al*., 2009b).

The presence of specific virulence factors associated with specific forms of pathogenesis may be due to lateral transfer of properties on plasmids and bacteriophages (Whittam *et al.,* 1993; Sooaka *et al.*, 2004).

The evolution of *Escherichia coli* O157 was thought probably to be due to its ability to produce verotoxin (Feng *et al.,* 1998). However, the acquisition of the new serogroups O157 antigen encoding the genetic region, 6-phosphogluconate dehydrogenase locus with the gene for the O chain of lipopolyssacharide (*gnd-rfb*), integration of the stx_2-phages, and the large virulence plasmid, designated as pO157 are regarded as the principal events (Kim *et al*., 2001). Two highly phylogenetic gene clusters stx_1 and stx_2 have provided an example of the rapid exchange of genetic cassettes between different *E. coli* strains and other bacteria (Brüssow *et al*., 2004). This is due to the fact that they separated long ago and underwent sequence evolution outside *Escherichia coli*. This may likely be the reason why the organism accumulated several mutations and was able to move to another level by transduction (Whittam, 1998).

Several virulence charcteristics for EHEC bacteria have been recognized. They are either chromosomally encoded or plasmid mediated (Ludwig *et al*., 2004). An increasing number of additional virulence characteristics like enterohaemolysin (Ehly or *ehx*), type II secretion system (ETP), that are usually carried by mobile genetic elements have been described (Hacker *et al.,* 1997; Friedrich *et al*., 2004).

*Author for correspondence

Evolution

The main focus on the evolutional studies of EHEC have been the locus of enterocyte effacement (LEE) which is chromosomally located, encoding the phenotype (attachment and effacement adherence) and shiga toxin-phage (*stx*-phage) integrated chromosomal *stx* loci (Feng, *et al*., 1998; Caprioli *et al*., 2005).

EHEC have probably been present in the environment for many years and have eluded recognition as aetiological agents of human morbidity and mortality, such as the outbreak caused by *E. coli* O111 variants in United States of America in the 1950s (Belnap and O'Donnell, 1955; Tschäpe and Fruth, 2001).

Genes encoding virulence determinants are transferred between species in many different environments. Examples of the setting for these transfer events include: the GI tract, the rumen, the oral cavity, and in food matrixes, where the flux of virulence factors from *E. coli* O157:H7 is described as an example of gene flow in the environment (Kelly *et al*., 2009a).

The emergence of verotoxin- producing *E. coli* was probably in late 1970s (Ørskov *et al*., 1987; Feng *et al.*, 1998). Some strains of *E. coli* were known earlier to produce toxins on Vero cells of African green monkey (Konowalchuk *et al*., 1977), occurred 25 years ago. These were later known as verocytotoxigenic *E. coli* (VTEC) or Shiga toxin producing *E. coli* (STEC) as human pathogens causing severe diseases such as diarrhoea, haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) (Bettelheim, 2003; Bettelheim and Beutin, 2003; Ramamurthy, 2008).

Escherichia coli O157:H7 is a serotype that has long been associated with worldwide outbreaks of infant diarrhea, and EHEC and EPEC share many intestinal adherence and other virulence factors. Thus, it appears that non-O157:H7 *E. coli* strains producing *stx* have been around for several decades but it was only with the emergence in the early 1980s of the O157:H7 clone that this pathogenic class of *E. coli* was recognized (Whittam *et al*., 1993). An outbreak with O157:H7 serotype resulting from the consumption of cookie dough was recently reported in the United States of America (CDC, 2009).

Escherichia coli O157:H7 strains are closely related to *stx*-negative O55:H7 EPEC strains and its pathogenic lineage has arisen from an enteropathogenic *E. coli* (EPEC) ancestor of serotype O55:H7 inhabiting the LEE on the basis of a step-wise evolutionary model (Fig. 1) during the past 50 years (Whittam *et al*., 1988; Feng *et al*., 1998; Dannenberg and Whittam, 2001; Wick *et al*., 2005). The evolution of O157 was probably due to its ability to produce verotoxin (Feng *et al.,* 1998). However, the acquisition of the new serogroups O157 antigen encoding *gnd-rfb* region, integration of the stx_2-phages, and the large virulence plasmid, designated as pO157 are the principal events (Kim *et al*., 2001). In subsequent steps, lysogenization of EHEC O157 strains by stx_1 phage, loss of ability to ferment sorbitol, inactivation of the *uidA* gene, with resultant loss of glucuronidase activity have occurred. Additionally, two separate groups of O157 have emanated: the non-sorbitol fermenting (NSF) EHEC O157:H7, and sorbitol fermenting (SF) non-motile O157:H^- strains (Feng, *et al*., 1998; Kim *et al*., 2001; Monday *et al*., 2004; Wick *et al*., 2005).

Clonality

In the evolutionary studies, two highly divergent phylogenetic gene clusters (stx_1 and stx_2) were also described, which separated long ago and underwent most of their sequence evolution outside *E. coli* (Whittam, 1998). Of these, stx_1 has been highly conserved, and it has been postulated that this gene region first moved from *Shigella dysenteriae* into the ancestral bacteria of EHEC2 group, accumulated several mutations, and then moved by transduction recently into EHEC1 O157:H7 (Whittam, 1998). Instead, the stx_2 genes have expressed several variants, and in addition to *E. coli*, have moved among divergent species like *Citrobacter freundii* and *Enterobacter cloacae* (Whittam, 1998). In general, both stx_1 and stx_2 have provided an example of the rapid exchange of genetic cassettes between different *E. coli* strains (Brüssow *et al*., 2004) and other bacteria.

Bacteria use various ways to transfer genetic information. These methods include: conjugation, which requires cell to cell contact between cells, transduction, which is bacteriophage-facilitated transfer of genetic information, and transformation, which is the uptake of free DNA from the environment. Usually the genes to be transferred lie on mobile genetic elements, pieces of DNA that encode proteins important to facilitate movement of DNA within or between genomes. This highlights the transfer methods and the role of the assorted mobile genetic elements in the evolution of food borne bacterial pathogens such as *Escherichia coli* O157:H7 (Kelly *et al*., 2009b).

Molecular typing method(s) should also be considered to identify the nature and spread of different clones across the country (Dhanashree and Mallya, 2008).

The clonality and epidemiological relationships of the EHEC O157 (Liesegang *et al*., 2000, Karch and Bielaszewska, 2001; Kim *et al*., 2001, Beutin *et al*., 2002) and various non-O157 strains (Schmidt *et al*., 1999a), such as O103, O118 or O145 (Wieler *et al*., 2000; Prager *et al*., 2002; Sonntag *et al*., 2004) were exploited in studies of outbreaks or sporadic infections.

Virulence characteristics

Several virulence characteristics of EHEC have been recognized. These characteristics are either chromo-

somally encoded or plasmid mediated (Friedrich *et al*., 2004; Ludwig *et al*., 2004).

An increasing number of additional virulence characteristics have been described, and they are usually carried by mobile genetic elements like plasmids and pathogenicity islands (PAI), large genetic elements carrying virulence genes and inserted in chromosomal loci encoding tRNA (Hacker *et al.,* 1997).

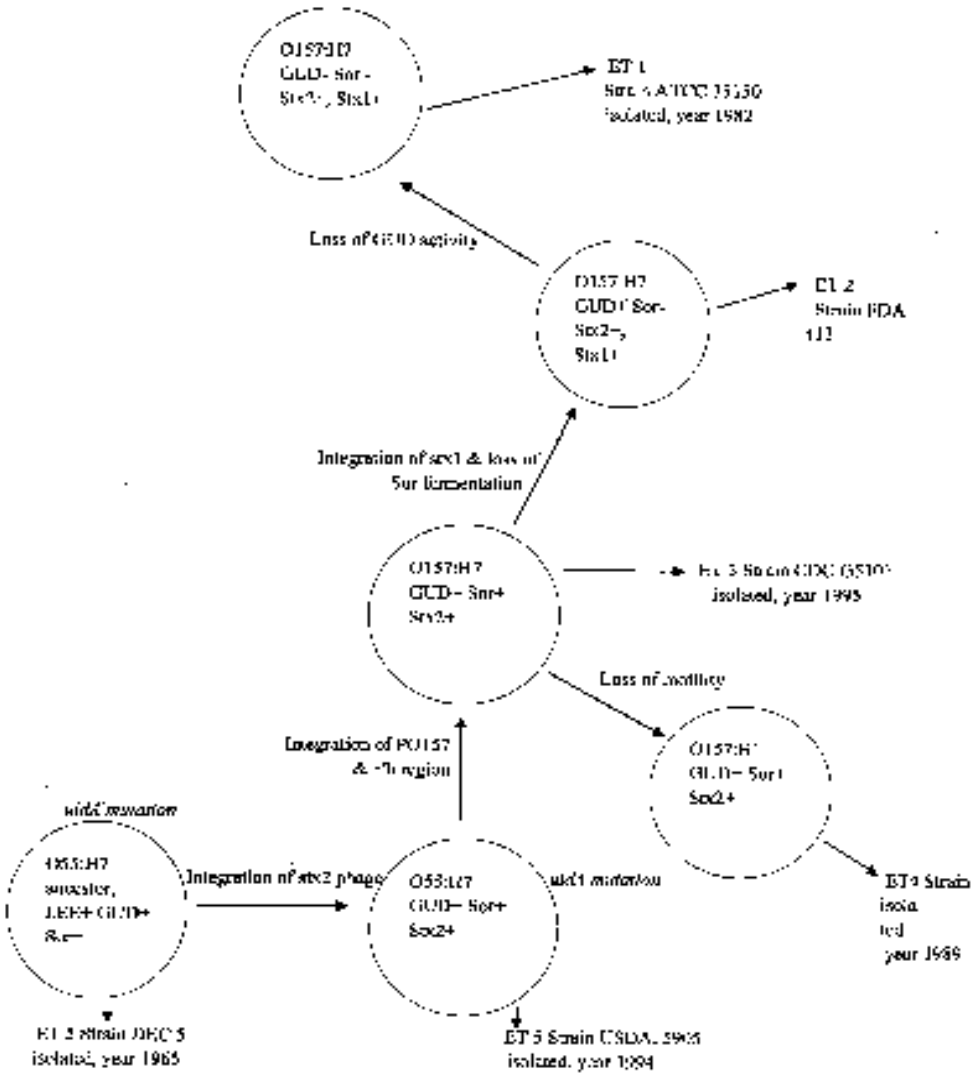

Fig. 1. Evolution of *E. coli* O157 lineage (Feng *et al.,* 1998; Wick *et al.,* 2005) with modification in the directional flow of arrows. **Abbreviations:** *uidA* = gene for β-glucoronidase activity; LEE = locus of entrocyte effacement; GUD = β-glucoronidase; Sor = sorbitol; *stx* = genes for Shiga toxin; pO157 = plasmid pO157; *rfb* = gene for LPS synthesis; ET = electrophoretic type

Chromosomally encoded virulence characteristics

Shiga toxin (stx) and stx variants

The major and the most essentially suggested cardinal feature of EHEC strains is the production of shiga toxins, which comprise a family of structurally related cytotoxins with similar biological activity and distinct antigenic structures (Melton-Celsa and O'Brien, 1998). These toxins are different proteins encoded by different genes (Blanco *et al*., 2003). The two main groups consist of stx_1 is relatively homogeneous, and nearly identical to the toxin of *S. dysenteriae* type 1, and stx_2, which shares less than 60% amino acid sequence with stx_1. However, stx_2 toxins are more heterogeneous and serologically distinct from stx_1 (Tyler *et al*., 1991; Melton-Celsa and O'Brien, 1998). The genetic information for the production of stx_1 and stx_2, e.g. (O26:H11 O157:H7) is located in the genome of lambdoid prophages integrated in the STEC chromosome (Melton-Celsa and O'Brien, 1998; Herold *et al*., 2004), whereas stx_1 shows only little sequence variations (Zhang *et al*.,2002). Several variants of stx_2 with altered antigenic or biological characteristics have been described and were termed as stx_{2c}, stx_{2d}, stx_{2e} and stx_{2f} (Schmidt *et al*., 2000; Friedrich *et al*., 2002b).

It has been shown that stx_2 is more critical in the development of haemolytic uraemic syndrome than stx_1.This

is due to the fact that strains producing stx_2 were more frequently associated with cases of HUS as compared to those expressing stx_1 (Griffin and Tauxe, 1991; Nataro and Kaper, 1998).

The shiga toxin producing *Escherichia coli* variant (stx_{2c}) was found to be less associated with diarrhoea and haemolytic uraemic syndrome (HUS) than stx_2 (Bettelheim and Beutin, 2003). Toxin types such as stx_{2d} and stx_1-ox$_3$ (also called stx_{1c}) were associated with sheep as an animal reservoir (Brett *et al*., 2003). They were also found in STEC from diseased humans but were more frequently associated with uncomplicated diarrhoea. Shiga toxin variant (stx_{2e}) causes oedema disease in pigs and stx_{2f} (also called vtev), which is found to be associated with avian species are rarely found in STEC from diseased humans and are regarded as less pathogenic of the strains for humans (Schmidt *et al*., 2000).

The locus of enterocyte effacement (LEE)

The colonization of intestinal mucosa by most of the EHEC is associated with a mechanism that subverts the function of the epithelial cells. The effect of this interaction is the inducement of a characteristic "attaching and effacing" (A/E) lesion, a complex mechanism genetically controlled by a large pathogenicity island (PAI) called the locus of enterocyte effacement (LEE) (Nataro and Kaper, 1998; Frankel *et al*., 1998; Caprioli *et al*., 2005). The A/E lesion is due to marked cytoskeletal changes and is characterized by effacement of microvilli and intimate adherence between the bacteria and the epithelial cell membrane, with accumulation of polymerized actin directly beneath the adherent bacteria (Nataro and Kaper, 1998; Garmendia *et al*., 2006). Epidemiological studies have shown that LEE-positive strains are highly associated with severe human disease (Nataro and Kaper, 1998; Brunder *et al*., 1999). Locus of enterocyte effacement (LEE) is consist of three functionally different modules. The first encodes a type III secretion system (TTSS), that secrets, delivers or translocates a set of bacterial proteins or virulence determinants into the host cell cytoplasm or apical membrane (Frankel *et al*.,1998), that are homologous to those produced by EPEC (Kaper *et al*., 2004). Some of these subvert pathways that regulate the host cell cytoskeleton and bring about pedestal formation (Frankel *et al*., 1998). The second encodes the secreted proteins espA, B, and D, which function as part of the type III secretion apparatus associated with the intimate adherence to intestinal epithelial cells, initiation of host signal transduction pathways, and the formation of A/E lesions (Schmidt and Hensel, 2004; Torres *et al*., 2005). The third encodes the adhesin "intimin" and the "translocated intimin receptor" (Tir), which is translocated into the host, cell plasma membrane by the TTSS (Delahay *et al*., 2001).

Intimin (*eae*)

The intimin coding genes (*eae*) bind to the enterocytes and Tir resulting in some corresponding changes within the amino acid sequence, reflecting an occurrence of antigenic variations (Frankel *et al*., 1998; Hartland *et al*., 1999). Intimin mediates the intimate attachment of EHEC by binding to β1-integrins (Frankel *et al*., 1996) and to cell-surface localized nucleolin, although, the significance of this in intestinal colonization has not been clearly elucidated. Distinct types of intimin alleles (17 in number) have been identified and classified on the basis of sequence and antigenic differences (Oswald *et al*., 2000; Garrido *et al*., 2006), which are responsible for different host tissue tropism. The commonly prevalent types of intimin alleles are termed α, β, ε and γ (Oswald *et al*., 2000), in accordance with Greek alphabet. Intimin α is generally found in EPEC, while γ and ε types are in close association with EHEC O157 and non-O157 serogroups: intimin γ is produced by serogroups O157, O111, and O145, while intimin ε by serogroups O103 and O121. Intimin β can be found in both EPEC and EHEC, the most important EHEC serogroup producing intimin β being O26. Several other less frequent *eae* gene variants have been described (Zhang *et al*., 2002).

Identification of intimin alleles is also considered as one of the epidemiological markers. Polymerase chain reaction (PCR) and restriction fragment length polymorphism on intimin allele identifications have recently been developed (Tramuta *et al*., 2008).

Plasmid mediated virulence characteristics

Various plasmid-mediated determinants have been reported to be carried by enterohaemorrhagic *Escherichia coli* (Kim *et al*., 2001; Beutin *et al*., 2002). These determinants include; enterohaemolysin (Ehly or *ehx*), type II secretion system (ETP), haemolytic protein (cytolysin A) and esterase inhibitor-specific metalloprotease (Schmidt *et al*., 2001; Lathem *et al*., 2003; Ludwig *et al*., 2004). Enterohaemorrhagic *E. coli* (EHEC) O157 sorbitol fermenting isolates does not seem to harbour catalase peroxidase (KatP, *katP*) and serine protease (EspP, *espP*) (Karch and Bielaszewska, 2001). However, sorbitol fermenting O157:H$^-$ has been shown as the only strain that possessed a gene cluster (*sfp*) specifically responsible for the expression of fimbriae (Brunder *et al*., 2001; Friedrich *et al*., 2004)

Enterohaemolysin (*ehly*)

Enterohaemolysin (*ehly*) production is regarded as a potentially useful virulence marker for EHEC (OIE, 2008).

Enterohaemorrhagic *E. coli* strains have in common a 60 MDa plasmid that carries four open reading frames (ORFs) responsible for the enterohaemolytic phenotype (Whittam, 1998; Karch *et al*., 1998). It is a member of pore-forming RTX (repeats in toxin) family of toxins and could contribute to the occurrence of disease through lysis of erythrocytes and release of haemoglobin as a potential source of iron for the bacteria (Beutin *et al*., 1994, Kaper *et al*., 2004). These genes encoding the *ehly* protein have been termed, EHEC-*hlyA,* EHEC-*hlyB* EHEC-*hlyC* and EHEC-*hlyD,* and to a higher degree they are related to the genes of the *E. coli* -haemolysin, named with respect to the genes of the *E. coli* –haemolysin operon (Karch *et al*., 1998). Therefore loss or reduction of the enterohaemolytic phenotype has been shown to be due to mutations of the open reading frames (ORFs) of the EHEC-*hly* gene, which has membrane damaging effect and is capable of inducing proinflammatory cytokines. Several molecular studies have provided an evidence that STEC having *ehxA* genes (typical STEC) are considered epidemiologically important due to the association of virulence genes with diarrhoeal/HUS outbreaks in different parts of the world (OIE, 2008). Among EHEC strains, the carriage of EHEC *hlyA* and production of Ehly have been typical of the O157:H7 strains. Instead, of the SF O157:H strains, the strains have typically possessed the EHEC-*hlyA* but have not clearly shown enterohaemolytic activity (Karch and Bielaszewska, 2001).

Putative type II secretion system (ETP)

Deoxyribonucleic acid (DNA) analysis of EHEC plasmids has shown that ETP system of EHEC strains includes 13 open reading frames with respective genes called *etpC* to *etpO* (Schmidt *et al*., 2001). Although this system has often been involved in the transportation of pathogenic factors in gram-negative bacteria outside their cells, their overall function and specificity has not yet been elucidated (Brunder *et al*., 2001; Friedrich *et al*. 2004; Caprioli *et al*., 2005).

Secreted proteins and type III secretion system

Type III secretion system is encoded by the LEE and is homologous to those found in other pathogenic organisms. It secretes and translocates proteins associated with pathogenicity and is involved in direct transfer of virulence factors into the host cells from STEC (Hueck, 1998). *Escherichia coli* secreted proteins (Esps) are responsible for signal transduction events seen in the A/E lesion. Mutation of the genes encoding EspA, EspB or EspD proteins can abolish the signal transduction in epithelial cells and the A/E histopathology (Kaper *et al*., 1998).

Formation of the filamentous structures bridging to the host cells surface occur on the bacterial surface. Secreted protein EspB is delivered primarily into the host cell membrane where it becomes an integral membrane protein (Wolff *et al*., 1998). Consequently, small fraction of this protein is delivered into the host cytosol. EspB and EspD probably forms a pore structure as a means for other bacterial effectors (such as Tir) to gain access to the host cell (DeVinney *et al*., 1999). Insights into the expression of type III secretion system, secretion of EspA in the pathogenesis of disease attributable to STEC intestinal colonization may require knowledge on the regulation and function of the LEE genes.

Katalase peroxidase (*katP*) and serine protease (*espP*)

This is a bacterial catalase peroxidase and is bi-functional in nature. Possession of amino terminal signal peptide reveled that *katP* is transported through the cytoplasmic membrane. Genes associated with *katP* have been found to be present mainly in non-sorbitol fermenting O157 strains, but absent in sorbitol fermenting O157 isolates (Schmidt *et al*., 2001).

Secreted serine protease (*espP*) is a plasmid mediated virulence characteristics unlike other Esp proteins, such as *espA, espB, espD*. The human coagulation factor V has been cleaved by *espP in vitro* and might act as an accessory virulence factor in exacerbating haemorrhagic colitis (HC). Isolates of O157 sorbitol fermenting *E. coli* have typically lacked *espP* genes (Brunder *et al*., 1999; Karch and Bielaszewska, 2001; Schmidt *et al*., 2001).

O157 plasmid (pO157)

All strains of O157:H7 contain a highly conserved plasmid, designated as pO157 (Schmidt *et al*., 1994) which varies in size from 93.6 to 104 kb (Schmidt *et al*., 1996). Also, most of the SF O157:H^- strains have possessed a chromosomal gene cluster encoding a novel type of cytolethal distending toxin (CDT) designated CDT-V, which has rarely been found in *E. coli* O157:H7 (Janka *et al*., 2003).This plasmid is also present in O26:H11 strains and in most of the strains of *stx*-producing *E. coli* strains isolated from humans (Levine *et al*., 1987; Beutin *et al*., 1994). A 3.4-kb fragment of this plasmid, was subsequently shown to encode enterohaemolysin (Schmidt *et al*., 1995) and catalase-peroxidase, but the function of the later is unknown (Brunder *et al*., 1996). A possible role of this plasmid in the suppression of production of an exopolysaccharide has been suggested (Fratamico *et al*., 1993). Among newly recognized plasmid pO157 mediated toxins, ToxB belonging to a large clostridia toxin family has been characterized (Schmidt *et al*., 2001). Furthermore, 94- to 104-kb pO157 plasmid, a number of other plasmids ranging in size from 2 to 87 kb have been found in strains of *E. coli* O157:H7 (Willshaw *et al*., 1992). However, correlation of this plasmid with the clinical disease has not been seen. Contrary to the high level of the plasmid in

human isolates, only a minority of *stx*-positive strains of non-O157:H7 serotypes isolated from cattle is said to possess this plasmid (Barret *et al*., 1992).

CONCLUSION

The different clones of enterohaemorrhagic *Escherichia coli* that are known have emerged basically by means of lateral gene transfer resulting from accumulated mutation outside *Escherichia coli*. This was probably due to acquisition of shiga toxin stx_1 and stx_2. These organisms have persisted in the environment as commensals haven't eluded recognition as pathogens, but were later known to cause disease with the aid of their virulence factors in addition to other changes that had occurred in the environment and within the organism(s). All these changes together with other factors which may be genetic in origin have resulted in evolution, the existence of clones and the occurrence and acquisition of virulence characteristics of enterohaemorrhagic *Escherichia coli*.

REFERENCES

Barrett, T. J., Kaper, J. B., Jerse, A. E. and Wachsmuth, I. K. (1992). Virulence factors in shiga-like toxin-producing *Escherichia coli* isolated from humans and cattle. *J. Infec. Dis.* 165: 979-980.

Belnap, W. D. and O'Donnell, J. J. (1955). Epidemic gastroenteritis due to *Escherichia coli* O-111. In: Bettelheim, K.A. *Supplement. Non-o157 Verotoxin-producing Escherichia coli: A Problem, Paradox, and Paradigm. Exp. Biol. Med.* 228: 333-344.

Bettelheim, K. A. (2003). Supplement: Non-O157 verotoxin-producing *Escherichia coli:* A problem, paradox, and paradigm.*Exp. Biol. Med.* 228: 333-344.

Bettelheim, K. A. and Beutin, L. (2003). A Review: Rapid laboratory identification and characterization of verocytotoxigenic (Shiga toxin producing) *Escherichia coli* (VTEC/STEC). *J. Appl. Microbiol.* 95: 205-217.

Beutin, L., Aleksic, S., Zimmermann, S. and Gleier, K. (1994). Virulence factors and phenotypic traits of verotoxigenic strains of *Escherichia coli* isolated from human patients in Germany. *Med. Microbiol. Immunol.* (Berlin) 183: 13-21.

Beutin, L., Kaulfuss, S., Cheasty T., Brandenburg, B., Zimmermann, S., Gleier, K., Willshaw, G. A., and Smith, H. R. (2002). Characteristics and association with disease of two major subclones of Shiga toxin (Verocytotoxin)-producing strains of *Escherichia coli* (STEC) O157 that is present among isolates from patients in Germany. *Diag. Microbiol. Infec. Dis.* 44: 337-346.

Blanco, J., Blanco, M., Blanco, J. E., Mora, A., González, E. A., Bernárdez, M. I., Alonso, M. P., Coira, A., Rodríguez, A., Rey, J., Alonso, J. M., and Usera, M. A. (2003). Verotoxin-producing *Escherichia coli* in Spain: Prevalence, serotypes, and virulence genes of O157:H7 and non-O157 VTEC in ruminants, raw beef products, and humans. *Exp. Biol. Med.* 228: 345-351.

Brett, K. N., Ramachandran, V., Hornitzky, M. A., Bettelheim, K. A., Walker, M. J. and Djordjevic, S. P. (2003). Stx1c is the most common shiga toxin 1 subtype among shiga toxin *Escherichia coli* isolates from sheep but not among isolates from cattle. *J. Clin. Microbiol.* 41: 926-936.

Brunder, W., Schmidt, H. and Karch. H. (1996). KatP, a novel catalase-peroxidase encoded by the large plasmid of enterohaemorrhagic *Escherichia coli* O157:H7. *Microbiol.* 142: 3305-3315.

Brunder, W., Schmidt, H., Frosch, M. and Karch, H. (1999). The large plasmids of Shiga-toxin producing *Escherichia coli* (STEC) are highly variable genetic elements. *Microbiol.* 145: 1005–1014.

Brunder, W., Khan, A. S., Hacker, J. and Karch, H. (2001). Novel type of fimbriae encoded by the large plasmid of sorbitol-fermenting enterohaemorrhagic *Escherichia coli* O157:H. *Infec. Immun.* 69: 4447-4457.

Brüssow, H., Canchaya, C. and Hardt, W. D. (2004). Phages and the evolution of bacterial pathogens: From genomic rearrangements to lysogenic conversion. *Microbiol. Mol. Biol. Rev.* 68: 560-602.

Caprioli, A., Morabito, S., Brugère, H. and Oswald, E. (2005). Enterohaemorrhagic *Escherichia coli*: Emerging issues on virulence and modes of transmission. *Vet. Res.* 36: 289–311.

Centers for Disease Control and Prevention (CDC) (2009). Multistate outbreaks of *E. coli* O157:H7 infections linked to eating raw refrigerated, prepackaged cookie dough. 800-CDC Info, Atlanta, USA. March 1 to June 18.

Delahay R.M., Frankel G., Knutton S.*et al.* (2001). Intimate interactions of enteropathogenic *Escherichia coli* at the host cell surface. *Cur. Opinions Infec. Dis.* 14: 559-565.

DeVinney, R., Stein, M., Reinscheid, D., Abe, A., Ruschkowski, S. and Finlay, B. B. (1999). Enterohemorrhagic *Escherichia coli* O157:H7 produces Tir, which is translocated to the host cell membrane but is not tyrosine phosphorylated. *Infec. Immun.* 67: 2389-2398.

Dhanashree, B. and Mallya, P. S. (2008). Detection of shiga-toxigenic *Escherichia coli* (STEC) in diarrhoeagenic stool and meat samples in Mangalore, India. *Indian J. Med. Res.* 128: 271-277.

Donnenberg, M. S. and Whittam, T. S. (2001). Pathogenesis and evolution of virulence in enteropathogic and enterohaemorrhagic *Escherichia coli. J. Clin. Invest.* 107: 539-548.

Eklund, M. (2005). Enterohaemorrhagic *Escherichia coli* (EHEC) findings from humans in Finland. Publication of the National Public Health Institute, Helsinki. KTLA 23. pp. 1-98.

Feng, P., Lampel, K. A., Karch, H. and Whittam, T. S. (1998). Genotypic and phenotypic changes in the emergence of *Escherichia coli* O157:H7. *J. Infec. Dis.* 177: 1750-1753.

Frankel, G., Lider, O., Hershkoviz, R., Mould, A. P., Kachalsky, S. G., Candy, D. C. A., Cahalon, L., Humphries, M. J. and Dougan, G. (1996). The cell-binding domain of intimin from enteropathogenic *Escherichia coli* binds to beta1 integrins. *J. Biol Chem*, 271, 20359-20364.

Frankel, G., Phillips, A. D., Rosenshine, I., Dougan, G., Kaper, J. B. and Knutton, S. (1998). Enteropathogenic and enterohaemorrhagic *Escherichia coli*: more subversive elements. *Mol. Microbiol.* 30: 911–921.

Fratamico, P. M., Bhaduri, S. and Buchanan. R. L. (1993). Studies on *Escherichia coli* serotype O157:H7 strains containing a 60-MDa plasmid and on 60-MDa plasmid-cured derivatives. *J. Med. Microbiol.* 39: 371-381.

Friedrich, A.W., Bielaszewska, M., Zhang, W. L., Pulz, M., Kuczius, T., Ammon, A. and Karch, H. (2002b) *Escherichia coli* harbouring shiga toxin 2 gene variants: frequency and association with clinical symptoms. *J Infec. Dis.* 185: 74–84.

Friedrich, A. W., Nierhoff, K. V., Bielaszewska, M., Mellmann, A. and Karch, H. (2004). Phylogeny, clinical associations, and diagnostic utility of the pilin subunit gene (*sfpA*) of sorbitol-fermenting, enterohaemorrhagic *Escherichia coli* O157:H. *J. Clin. Microbiol.* 42: 4697-4701.

Garmendia, J., Carlier, M. F., Egile, C., Didry, D. and Frankel, G. (2006). Characterization of tccp-mediated n-wasp activation during enterohaemorrhagic *Escherichia coli* infection. *Cell. Microbiol.* 8(9): 1444-1455.

Garrido, P., Blanco, M., Moreno-Paz, M., Briones, C., Dahbi, G., Blanco, J. E., Blanco, J. and Parro, V. (2006). STEC-EPEC oligonucleotide microarray: a new tool for typing genetic variants of the LEE pathogenicity island of human and animal Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) strains. *J. Clin. Chem.* 52: 192-201.

Griffin, P. M., and Tauxe, R. V. (1991). The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohaemorrhagic *E. coli*, and the associated haemolytic uremic syndrome. *Epidemiol. Rev.* 13: 60-98.

Hacker, J., Blum-Oehler, G., Muhldorfer, I. and Tschape, H. (1997). Pathogenicity islands of virulent bacteria: structure, function and impact on microbial evolution. *Mol. Microbiol.* 23: 1089–1097.

Hartland, E. L., Batchelor, M., Delahay, R. M., Hale, C., Matthews, S., Dougan, G., Knutton, S., Connerton, I. and Frankel G. (1999). Binding of intimin from enteropathogenic *Escherichia coli* to Tir and to host cells. *Mol. Microbiol.* 32: 151–158.

Herold, S., Karch, H. and Schmidt, H. (2004). Shiga toxin-encoding bacteriophages–genomes in motion. *Int. J. Med. Microbiol.* 294: 115-121.

Hueck, C. J. (1998). Type III protein secretion systems in bacterial pathogens of animals and plants. *Microbiol. Mol. Biol. Rev.* 62: 379-433.

Janka, A., Bielaszewska, M., Dobrindt, U., Greune, L., Schmidt, M. A. and Karch, H. (2003). Cytolethal distending toxin gene cluster in enterohaemorrhagic *Escherichia coli* O157:H and O157:H7: Characterization and evolutionary considerations. *Infec. Immun.* 71: 3634-3638.

Jores, J., Rumer, L. and Wieler, L. H. (2004). Impact of the locus of enterocyte effacement pathogenicity island on the evolution of pathogenic *Escherichia coli. Int. J. Med. Microbiol.* 294: 103-113.

Kaper, J. B., Elliott, S., Sperandio, V., Perna, N. T., Mayhew, G. F. and Blattner, F. R. (1998). Attaching and effacing intestinal histopathology and the locus of enterocyte effacement. In: Kaper J. B. and O'Brien, A. D. (eds.) *Escherichia coli* O157*:H7 and other Shiga toxin-producing E. coli strains*. ASM, Washington D.C., pp. 121-128.

Kaper, J. B., Nataro, J. P. and Mobley, H. L. T. (2004). Pathogenic *Escherichia coli*. *Nature Rev.* 2: 123-140.

Karch, H. and Bielaszewska, M. (2001). Sorbitol-fermenting shiga toxin-producing *Escherichia coli* O157:H$^-$ strains: Epidemiology, phenotypic and molecular characteristics and microbiological diagnosis. *J. Clin. Microbiol.* 39: 2043-2049.

Karch, H., Schmidt, H. and Brunder, W. (1998). Plasmid-encoded determinants of *Escherichia coli* O157:H7. In: Kaper, J. B., and O'Brien, A. D. (eds.) *Escherichia coli O157:H7 and other Shiga toxin-producing E. coli strains*. ASM, Washington D.C., pp.183-193.

Kelly, B. G., Vespermann, A. and Bolton, D. J. (2009a). The role of horizontal gene transfer in the evolution of selected foodborne bacterial pathogens. *Food Chem. Toxicol.* 47(5): 951-968.

Kelly, B. G., Vespermann, A. and Bolton, D. J. (2009b). Gene transfer events and their occurrence in selected environments. *Food Chem. Toxicol.* 47(5): 978-983.

Kim, J., Nietfeldt, J., Ju, J., Wise, J., Fegan, N., Desmarchelier, P. and Benson, A. K. (2001). Ancestral divergence, genome diversification, and phylogeographic variation in subpopulations of sorbitol-negative, -glucoronidase-negative enterohaemorrhagic *Escherichia coli* O157. *J. Bacteriol.* 183: 6885-6897.

Konowolchuk, J., Speirs, J. L. and Stavric, S. (1977). Vero response to a cytotoxin of *Escherichia coli. Infec. Immun.* 18: 775-779.

Lathem, W. W., Bergsbaken, T., Witowski, S. E., Perna, N. T. and Welch, R. A. (2003). Acquisition of *stcE*, a C1 esterase inhibitor-specific metalloprotease, during the evolution of *Escherichia coli* O157:H7. *J. Infec. Dis.* 187: 1907-1914.

Levine, M. M., Xu, J., Kaper, J. B., Lior, H., Prado, V., Tall, B., Nataro, J., Karch, H. and Wachsmuth, K. (1987). A DNA probe to identify enterohemorrhagic *Escherichia coli* of O157:H7 and other serotypes that cause hemorrhagic colitis and hemolytic uremic syndrome. *J. Infec. Dis.* 156: 175-182.

Liesegang, A., Sachse, U., Prager, R., Claus, H., Steinruck, H., Aleksic, S., Rabsch, W., Voigt, W., Fruth, A., Karch, H., Bockemühl, J., and Tschäpe, H. (2000). Clonal diversity of Shigatoxin producing *Escherichia coli* O157:H7/H- in Germany-A ten-year study. *Int. J. Med. Microbiol.* 290: 269-278.

Ludwig, A., von Rhein, C., Bauer, S., Hüttinger, C., and Goebel, W. (2004). Molecular analysis of cytolysin A (ClyA) in pathogenic *Escherichia coli* strains. *J. Bacteriol.* 186: 5311-5320.

Melton-Celsa, A. R. and O'Brien, A. (1998). Structure, biology, and relative toxicity of shiga toxin family members for cells and animals. *In*: Kaper J.B., O'Brien A.D. (Eds.), *Escherichia Coli O157:h7 And Other Shiga Toxin Producing E. Coli Strains*, American Society for Microbiology, Washington, DC. pp. 121–128.

Monday, S. R., Minnich, S. A., and Feng, P. C. H. (2004). A 12-base-pair deletion in the flagellar master control gene *flhC* causes non-motility of the pathogenic German sorbitol-fermenting *Escherichia coli* O157:H strains. *J. Bacteriol.* 186: 2319-2327.

Nataro, J. P. and Kaper, J. B. (1998). Diarrhoeagenic *Escherichia coli*. *Clin. Microbiol. Rev.* 11(1): 142-201.

Office International des Epizootics Terrestrial Manual (OIE) (2008). Verocyto-toxigenic *Escherichia coli*. 1294-1304.

Ørskov, F., Ørskov, I. and Villar, J. A. (1987). Cattle as a reservoir of verotoxin producing *Escherichia coli* O157:H7. *Lancet,* 2: 276.

Oswald, E., Schmidt, H., Morabito, S., Karch, H., Marches, O. and Caprioli, A. (2000). Typing of intimin genes in human and animal enterohaemorrhagic and enteropathogenic *Escherichia coli*: characterization of a new intimin variant. *Infec. Immun.* 68: 64–71.

Prager, R., Liesegang, A., Voigt, W., Rabsch, W., Fruth, A. and Tschäpe, H. (2002). Clonal diversity of Shiga toxin-producing *Escherichia coli* O103:H2/H$^-$ in Germany. *Infec. Genetic Evol.* 1: 265-275.

Ramamurthy, T. (2008). Commentary ; Shiga toxin-producing *Escherichia coli* (STEC): the bug in our backyard. *Indian J. Med. Res.* 128: 233-236.

Schmidt, H., Beutin, L. and Karch, H. **(**1995). Molecular analysis of the plasmid-encoded hemolysin of *Escherichia coli* O157:H7 strain EDL 933. *Infec. Immun.* 63: 1055-1061

Schmidt, H. and Karch, H. (1996). Enterohaemolytic phenotypes and genotypes of Shiga toxin producing *Escherichia coli* O111 strains from patients with diarrhea and haemolytic-uremic syndrome. *J. Clin. Microbiol.* 34: 2364-2367.

Schmidt, H., Karch, H. and Beutin, L. (1994). The large-sized plasmids of enterohaemorrhagic *Escherichia coli* O157 strains encode haemolysins which are presumably members of the *E. coli* á-hemolysin family. *FEMS Microbiol. Lett.* 117: 189-196

Schmidt, H., Geitz, C., Tarr, P. I., Frosch, M. and Karch, H. (1999a). Non-O157:H7 Pathogenetic Shiga toxin-*Escherichia coli*: phenotypic and genetic Profiling of virulence traits and evidence for clonality. *J. Infec. Dis.* 179: 115-123.

Schmidt, H., Scheef, J., Morabito, S., Caprioli, A., Weiler, L. H. and Karch, H.(2000). A new shiga toxin variant (Stx2f) from *Escerichia coli* isolated from pigeons. *Appl. Environ. Microbiol.* 66: 1205-1208.

Schmidt, H., Karch, H. and Bitzan, M. (2001). Pathogenic aspects of STEC infections in humans. In: Philpott, D., and Ebel, F. (eds) *Methods in Molecular Medicine, E. coli Shiga toxin Methods and Protocols*. Humana Press Inc. New Jersey. pp. 241-261.

Schmidt, H. and Hensel, M. (2004). Pathogenicity islands in bacterial pathogenesis. *Clin. Microbiol. Rev.* 17: 14-56.

Sinclair, J. F. and O'Brien, A. D. (2002). Cell surface-localized nucleolin is a eukaryotic receptor for the adhesin intimin-gamma of enterohaemorrhagic *Escherichia coli* O157:H7. *J. Biol. Chem.* 277: 2876-2885.

Sonntag, A-K., Prager, R., Bielaszewska, M., Zhang, W., Fruth, A., Tschäpe, H. and Karch, H. (2004). Phenotypic and genotypic analyses of enterohaemorrhagic *Escherichia coli* O145 strains from patients in Germany. *J. Clin. Microbiol.* 42: 945-962.

Sooka, A., Plessis, M. and Keddy, K. (2004). Review Article: Enterovirulent *Escherichia coli*. *S. Afr. J. Epidemiol. Infec.* 19 (1): 23-33

Torres, A. G., Zhou, X. and Kaper, J. B. (2005). Adherence of diarrhoeagenic *Escherichia coli* strains to epithelial cells. *Infec. Immun.* 73: 18-29.

Tramuta, C., Robino, P., Oswald, E. and Nebbia, P. (2008). Identification of intimin alleles in pathogenic *Escherichia coli* by PCR restriction fragment length polymorphism analysis. *Vet. Res.* Commun. 32: 1-5.

Tschäpe, H. and Fruth, A. (2001). Enterohaemorrhagic *E. coli. Emerging Bacteriol. Pathol.* 8: 1-11.

Tyler, S. D., Johnson, W. M., Lior, H., Wang, G. and Rozze, K. R. (1991). Identification of verotoxin type 2 variant B-subunit genes in *Escherichia coli* by the polymerase chain reaction and restriction fragment length polymorphism analysis. *J. Clin. Microbiol.* 29: 1339-1343.

Whittam, T. S. (1998). Evolution of *Escherichia coli* O157:H7 and other shiga toxin-producing *E. coli* strain. In: Kaper, J. B., and O'Brien, A. D. (eds.) *Escherichia coli* O157:H7 and other shiga toxin-producing *E. coli*

strains. ASM Press, Washington, DC. pp. 195-209.

Whittam, T. S., Wachsmuth, I. K. and Wilson, R. A. (1988). Genetic evidence of clonal descent of *Escherichia coli O157:H7 associated with haemorrhagic colitis and haemolytic uremic syndrome. J. Infec. Dis.* 157: 1124-1133.

Whittam, T. S., Wolfe, M. L., Wachsmuth, I. K., Orskov, F., Orskov, I. and Wilson, R. A. (1993). Clonal relationships among *Escherichia coli* strains that cause hemorrhagic colitis and infantile diarrhea. *Infec. Immun.* 61: 1619-1629.

Wick, L. M., Qi, W., Lacher, D. W. and Whittam, T. S. (2005). Evolution of genomic content in the stepwise emergence of *Escherichia coli* O157:H7. *J. Bacteriol.* 187: 1783-1791.

Wieler, L. H., Busse, B., Steinrück, Beutin, L., Weber, A., Karch, H. and Baljer, G. (2000).Enterohaemorrhagic *Escherichia coli* (EHEC) strain of serogroup O118 display three distinctive clonal groups of EHEC pathogens. *J. Clin. Microbiol.* 38: 2162-2169.

Willshaw, G. A., Scotland, S. M., Smith, H. R. and Rowe, B. **(**1992). Properties of vero cytotoxin-producing *Escherichia coli* of human origin of O serogroups other than O157. *J. Infec. Dis.* 166: 797-802.

Wolff, C., Nisan, I., Hanski, E., Frankel, G. and Rosenshine, I. (1998). Protein translocation into host epithelial cells by infecting enteropathogenic *Escherichia coli*. *Mol. Microbiol.* 28: 143-55.

Zhang, W., Bielaszewska, M., Kuczius, T. and Karch, H. (2002). Identification, characterization, and distribution of a shiga toxin 1 gene variant (*stx* (1c)) in *Escherichia coli* strains isolated from humans. *J. Clin. Microbiol.* 40: 1441-1446.