

Pathogenicity, Virulence and Emerging Pathogenic *Escherichia coli*

CO-ORDINATION ACTION
FOOD-CT-2006-036256

Pathogenic *Escherichia coli* Network

Editors

D. J. Bolton, G. Duffy, C. L. Baylis, R. Tozzoli, S. Morabito, Y. Wasteson and S. Lofdahl



Co-ordination Action FOOD-CT-2006-036256

This booklet was produced as part of the co-ordination action project “Pathogenic *Escherichia coli* Network” (PEN). This project is funded by the European Commission under the Sixth Framework Programme (project number FOOD-CT-2006-036256).

2008 Ashtown Food Research Centre,
 Teagasc,
 Ashtown,
 Dublin 15,
 Ireland.

ISBN 1 84170 506 3

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means electronic, mechanical, photocopying, recording or otherwise without the prior written consent of the publisher.

No responsibility is assumed by the publisher for any injury and/or damage to persons or property as a matter of product liability, negligence or otherwise, or from any use or operation of any methods, products, instructions or ideas contained in the materials herein.

Neither the European Commission nor any person acting on behalf of the Commission is responsible for the use which might be made of the information contained in this volume. Mention of any brand or firm names does not constitute an endorsement over others of a similar nature not mentioned.

Editorial Team

Declan Bolton	Ashtown Food Research Centre Teagasc, Ashtown, Dublin 15, Ireland
Geraldine Duffy	Ashtown Food Research Centre Teagasc, Ashtown, Dublin 15, Ireland
Chris Baylis	Campden & Chorleywood Food Research Association Chipping Campden, Gloucestershire, GL55 6LD, UK
Rosangela Tozzoli	Community Reference Laboratory for <i>E. coli</i> Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy
Stefano Morabito	Community Reference Laboratory for <i>E. coli</i> Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy
Yngvild Wasteson	Norwegian School of Veterinary Science PO Box 8146, Oslo 0033, Norway
Sven Lofdahl	Swedish Institute of Infectious Disease Control (Smittskyddsinstitutet) 171 82, Nobels väg 18, Solna, Sweden

Contents

	Page
1. Introduction	1
2. Verocytotoxin-producing <i>Escherichia coli</i> (VTEC)	2
3. Enteropathogenic <i>Escherichia coli</i> (EPEC)	6
4. Enterotoxigenic <i>Escherichia coli</i> (ETEC)	9
5. Enteroinvasive <i>Escherichia coli</i> (EIEC)	11
6. Enteroaggregative <i>Escherichia coli</i> (EAaggEC)	12
7. Diffusely Adherent <i>Escherichia coli</i> (DAEC)	13
8. Bibliography	15
9. Further Reading	15

1. Introduction

The Evolution and Emergence of Pathogenic Escherichia coli

Escherichia coli are facultative Gram-negative rods within the family *Enterobacteriaceae* and are found in the natural gastro-intestinal flora of humans and warm-blooded animals. Although most *E. coli* are harmless commensal organisms, there are many pathogenic strains which can cause a variety of illness in humans and animals. Comparative analysis of genome sequences would suggest that all *E. coli* share a common genetic backbone which contains approximately 2,800 open reading frames (ORFs). Pathogenic strains are highly diverse because of the insertion and deletion of genes over time. Diversification may have started 9 million years ago with the acquisition of islands of DNA from other bacteria by horizontal gene transfer (e.g. via bacteriophages, transposons and plasmids) a process that continues today. Thus the evolution of *E. coli* is associated with the continuous generation of novel genetic variants.

Bacteria must survive under continuously changing environmental conditions necessitating adaptation. In addition to an immediate response to changing conditions at the gene expression level, bacteria rely on the development of genetic diversity. Thus mutation and the capture of new genetic material by horizontal gene transfer mechanisms generate genome flexibility and increased ability to adapt and survive. Often, these genes are clustered on large mobile genomic islands enabling bacteria to rapidly develop new bacterial variants and adapt to specific ecological niches. Successful new genotypes tend to stabilize allowing for the further genetic variation and ultimately the development of new genera and new species. This process is the same for both pathogenic and non-pathogenic organisms. The former acquire genes encoding virulence factors such as capsules, adhesins and toxins which enable them to survive and multiply in a particular host resulting in disease.

Unknown prior to the late 1970s, Verocytotoxin-producing *E. coli* (VTEC) are a good example of the evolution and emergence of pathogenic *E. coli*. These bacteria comprise over 100 serotypes of *E. coli* (Scheutz and Strockbine, 2005) and some strains are responsible for a wide range of clinical manifestations. Some VTEC strains are a major cause of haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) worldwide. The Verocytotoxin they produce has two antigenic forms each of which has a number of allelic variants. These toxins are encoded on bacteriophages that integrate into the chromosome of the host *E. coli*. There is some evidence to suggest that VTEC evolved from Enteropathogenic *E. coli* (EPEC) through a series of genetic events, most notably the acquisition of bacteriophages encoding the Verocytotoxins. It is probable, for example, that VTEC O157:H7 evolved from EPEC O55:H7. However, this may not hold true for all VTEC as many have different phylogenetic lineages including locus of enterocyte effacement (LEE) negative HUS causing strains.

Furthermore, the emergence of VTEC was almost certainly not a single event. Evidence that VTEC have emerged on several different occasions can be

found in the fact that genetic insertions into the genome, such as the pathogenicity island (PAI) LEE, occur at several different locations suggesting that this PAIO was acquired more than once. Within VTEC, serogroup O157 is a significant pathogen worldwide. Other VTEC serogroups are less often implicated in human illness, possibly because they represent less stable clones.

Bacterial evolution is an ongoing process and there is little doubt that new, more virulent pathogenic clones of *E. coli* will emerge in the future. This booklet describes the pathogenicity and virulence characteristics of well established and emerging pathogenic *E. coli*.

2. Verocytotoxin-producing *Escherichia coli* (VTEC)

2.1 Pathogenicity factors

Verocytotoxins

The definition of VTEC is based upon the production of a family of toxins with cytotoxic activity against Vero cells *in vitro*. As Verocytotoxins (VT) are structurally and functionally similar to the Shiga toxin produced by *Shigella dysenteriae* type 1, a parallel nomenclature system exists, the Shiga toxin (Stx) family.

The VTs consists of two main groups of closely related toxins, VT1 and VT2 of which the former includes the Shiga toxin produced by *Shigella dysenteriae* type 1. The VT1 and VT2 groups could be further divided into subtypes based on sequence analysis. Attempts to develop a consensus nomenclature have been made (Scheutz *et al.* 2001).

Several studies have demonstrated a correlation between *E. coli* seropathotypes, severity of clinical symptoms and toxin subtypes. *In vitro* studies in Vero cells have demonstrated a higher toxicity of VT1 than VT2. On the other hand the up-regulation of genes encoding proinflammatory molecules involved in the pathogenicity of HUS is much more efficient when induced by VT2 than triggered by VT1. Moreover, VTEC producing VT2 only generally cause more severe disease than those that produce VT1 or both VT1 and VT2. The more significant association between severe disease and VT2 seems to be particularly linked to subtypes VT2 and VT2c. Other subtypes are associated with milder disease in humans or only associated with disease in animals.

VTs have an A-B subunit structure consisting of one enzymatically active A subunit and five receptor-binding B subunits. The host cell receptor for the B subunit is a membrane glycolipid called Gb3 for VT1 and VT2 and Gb4 for VT2e. Following binding to target cells, the toxin is internalized by endocytosis. It is then transported via early endosomes, and the Golgi apparatus to the endoplasmic reticulum, from where it retrotranslocates to its

final destination, the cytosol. The toxic effect of VT is to inactivate ribosomes and thus inhibit protein synthesis leading to apoptosis and cell death.

The VT encoding genes (*vtx*) are located on lysogenic lambdoid bacteriophages (VT-phages), or remnants thereof. *vtx* are encoded in the late gene region of the phage, a region whose expression is enhanced when the phage is replicating during the lytic cycle. Toxin production and subsequent release of toxins seems to be coupled to the induction of the phage to enter the lytic cycle. Nonetheless, it has been observed that VT2 and VT2c variant coding genes have a basal level of transcription in the absence of induction of the lytic cycle (de Sablet *et al.* 2008). Phage induction can be triggered after exposure to DNA-damaging agents or certain antibiotics. Another result of the induction process is lysis of the bacterial host cells and the release of free phage particles that can infect other bacteria.

After release of VTs in the intestinal lumen, the toxins are transported from the gut epithelium to the renal endothelium and other target organs. The mechanism for this transport and the interaction between polymorphonuclear leukocytes and VTs are still a matter of debate. Probably, the VTs bind to the circulating leukocytes through a low affinity, currently unknown, receptor.

Host-pathogen interactions

Besides VT production, colonisation of the host intestinal mucosa is a key factor for full virulence of VTEC, and several factors involved in the process have now been characterised.

VTEC included in the seropathotypes A and B colonise the intestinal mucosa inducing a characteristic histopathologic lesion, defined as "attaching and effacing"(A/E). The A/E lesion is characterised by effacement of microvilli and intimate adherence between the bacteria and the epithelial cell membrane. The ability to trigger the A/E mechanism of adhesion is governed by the LEE locus which encodes a type III secretion system (TTSS), some of the secreted effector molecules, the adhesin "intimin" and its translocated receptor (Tir), which is delivered directly into the host cell plasma membrane by the TTSS.

LEE-positive VTEC strains inject several effector proteins directly into the eukaryotic cell via the TTSS. These effectors subvert the normal cellular functions to allow a more efficient adhesion of the bacterium to the host cell membrane. Some of the translocated effectors are encoded by the LEE itself, while other effectors are encoded outside the LEE by genes harboured by lambdoid prophages or by putative PAIs. One of the effector proteins, EspF is required for inhibition of bacterial phagocytosis while yet another, EspJ, inhibits opsono-phagocytosis. The EspA filament, a part of the TTSS apparatus, has been reported to be involved in the attachment to salad leaves.

Another important colonisation factor is encoded by the PAI OI-122. This PAI carries *efa1/lifA*, a large gene involved in both the repression of host lymphocyte activation response and in the adhesion to cultured cells. Moreover, *in vivo* experiments have shown that the product of this gene is

associated with the ability of colonise the intestinal tract of cattle and of inducing diarrhoea in young calves.

Seropathotype C strains are usually LEE-negative and may use different mechanisms of adhesion to the host enterocyte. O113:H21 strains, for example, produce an autoagglutinating adhesin encoded by a genetic locus, termed *saa*, located on a large virulence plasmid which seems to have a role in the colonization of the host intestinal mucosa.

Recently, four fimbria-encoding genomic islands of VTEC O157:H7 EDL 933 strain (O-I 1, O-I 47, O-I 141 and O-I 154) have been specifically associated with seropathotype A strains and this may explain their apparently greater ability to cause HUS outbreaks than the strains belonging to the other seropathotypes.

Colonisation of the host is a complex multifactorial process involving a wide array of adhesion determinants. Moreover, expression of genes involved in the colonisation mechanism seems to be modulated according to the needs of the bacterial cells.

Plasmid-encoded virulence factors

VTEC O157 possess a large virulence plasmid of approximately 90 Kb termed pO157. It encodes 35 proteins, some of which are presumably involved in the pathogenesis of infections. The *hly* operon, which encodes genes involved in the biosynthesis and transportation of the enterohaemolysin, is considered to be the hallmark of the presence of pO157 and is also present in the large plasmids detected in most of the strains belonging to seropathotypes A. Large plasmids resembling pO157 can be found in most of the non-O157 VTEC strains belonging to seropathotype B. These plasmids usually carry the *hly* operon, while other markers like the operon coding for a type II secretion system, *katP* and *espP* genes can be found in less than 50% of the isolates.

2.2 Clinical symptoms

Human illness

In terms of human infection, the most important VTEC are a sub-set called the Enterohaemorrhagic *E. coli* (EHEC). EHEC are characterised by their ability to produce highly potent VT(s) in addition to their ability to adhere to the human large intestine forming a characteristic attaching and effacing (A/E) lesion. In practice the terms EHEC and VTEC are used interchangeably, but in the context of this booklet VTEC refers only to human disease causing strains. There are a number of serogroups of *E. coli* within the VTEC group, of which the five deemed most important in terms of clinical infection are *E. coli* O157, O26, O111, O103, and O145. A recent European Food Safety Authority (EFSA) scientific opinion added *E. coli* O91 to this list. However, this list is not exhaustive and many other VTEC serogroups have also caused infection.

While some cases of VTEC infection present with uncomplicated non-bloody diarrhoea, in most cases that come to medical attention the diarrhoea

becomes bloody on the second or third day of illness, leading to HC or bloody diarrhoea which persists for up to one week (5 to 7 days). While most cases resolve at this stage, in approximately 20% of cases, life-threatening complications then occur, of which HUS is the most common. HUS is characterised by a lack of urine formation and acute kidney failure. Approximately half of all HUS patients require renal dialysis. HUS occurs most often in children under the age of ten years. The number of VTEC required to cause illness is very low and while the precise infectious dose is not known it has been reported to be as low as 10 colony forming units (CFU).

Animal illness

Several different animals have been shown to be healthy carriers of VTEC. Some VTEC serogroups however have caused intestinal disease and diarrhoea in newborn calves and other young ruminants. The most common serotypes associated with diarrhoea in calves are O5:NM, O8:H8, O20:H19, O26:H11, O103:H2, O111:H8/H11/NM, O118:H16 and O145:H+. In pigs, VTEC can cause oedema typically involving serogroups O138, O139 and O141.

2.3 Pathogenomics of VTEC/Evolution of VTEC clones

VTEC strains belonging to serotype O157:H7 are responsible for the most of the HUS cases and outbreaks worldwide, nonetheless non-O157 VTEC are increasingly reported as causative agents of human illness. VTEC serotypes seem to have different pathogenic potential, in fact O26:H11, O103:H2 O111:NM, O121:H19 and O145:NM are associated with both outbreaks and sporadic cases of HUS, but less commonly than serotype O157:H7, while other serotypes (e.g. O113:H21 and O91:H21) are only rarely associated with outbreaks but are still capable of causing sporadic episodes of HUS. Many other serotypes are isolated from patients with diarrhoea but have never been associated with severe disease. A large number of VTEC serotypes are commonly isolated from natural and animal reservoirs, but have never been linked to human disease.

An important objective of several studies conducted in recent years on virulence and pathogenicity has been to define the combination of virulence genes and the mechanisms that make a VTEC fully pathogenic to humans. The production of VT is the main virulence feature of VTEC but cannot be solely responsible for full pathogenicity. As a matter of fact, VTEC associated with severe human disease are usually capable of colonizing the intestinal mucosa with a characteristic “attaching and effacing” mechanism, genetically governed by the LEE locus, and possess other mobile genetic elements carrying additional virulence genes such as plasmids, phages and pathogenicity islands (PAI) (e.g. O-112).

Seropathotype is an emerging concept that classifies VTEC into five main groups (A to E) based on the incidence of the serogroup in human disease, association with outbreaks versus sporadic infection, their capacity to cause HUS or HC, and the presence of virulence markers (Karmali, 2003; Wickham *et al.*, 2006). This approach attempts to combine these inputs to better

understand the apparent differences in virulence of VTEC. Seropathotype A strains (VTEC O157) have a high relative incidence, commonly cause outbreaks and are associated with HUS. Seropathotype B includes O26:H11, O103:H2/NM, O111:NM and O145:NM together with O121:H19 as they have a moderate incidence and are uncommon in outbreaks but are associated with HUS. Seropathotype C includes O91, O104 and O113 strains all of H-type 21 and associated with HUS, but these strains are of low incidence and rarely cause outbreaks. Seropathotypes D and E are not HUS-associated, are uncommon in humans or are found only in non-human sources. This concept is likely to be further refined and will provide a valuable tool in the future for the assessment of the human pathogenic potential of different VTEC serotypes.

2.4 Emerging issues

Despite the huge amount of data collected after the sequencing of the full genome of VTEC O157, the virulence and the evolution of different VTEC seropathotypes have only been partially unravelled. A greater understanding of the factors governing the development of severe disease in humans and colonisation of animal hosts must be achieved before effective intervention strategies aimed at reducing the burden of infection can be developed. In this respect it is remarkable that within the seropathotype A strains, VTEC O157, it is possible to identify subpopulations of isolates, which seem to be more virulent or better transmitted to human hosts. Some molecular characterisation studies of VTEC O157 isolates suggested the existence of separate lineages: it has been observed that strains isolated from human cases of disease clustered in different groups as compared to those from the bovine reservoir. This hypothesis is strengthened by the observation that about 70% of human cases of VTEC O157 infections reported in Europe in the last four years have been caused by strains belonging to a restricted number of phage types (PTs) including PT8, PT2, PT21/28, and PT14. In contrast, the strains isolated from the bovine reservoir in the same period mainly belonged to different PTs such as PT21, which have never been reported in human infections.

In conclusion, defining all the factors characterising a fully pathogenic VTEC strain will be crucial to improve the efficacy of the diagnosis of human infections, the surveillance of animal reservoirs and the assessment of public health risks.

3. Enteropathogenic *Escherichia coli* (EPEC)

3.1 Pathogenicity factors

Enteropathogenic *E. coli* (EPEC) are described as typical (t) and atypical (a) depending on their complement of virulence factors with typical EPEC having a fuller complement. They also differ in genetic characteristics and serotypes.

Host-pathogen interaction

The typical intestinal histopathology associated with EPEC infections is the A/E lesion, which is shared also by VTEC strains belonging to the seropathotypes A and B (see VTEC above). As in VTEC infections, the bacteria intimately attach to intestinal epithelial cells and cause subversion of host cell function leading to cytoskeletal changes, including the accumulation of polymerized actin directly beneath the adherent bacteria. The microvilli of the intestine are effaced and pedestal-like structures are formed.

As in VTEC, the ability to induce the A/E lesion is encoded by a 35-kb PAI termed the locus of enterocyte effacement (LEE). The EPEC LEE also encodes the 94-kDa outer-membrane protein intimin, which mediates the intimate attachment of EPEC to epithelial cells and its translocated receptor Tir which is directly inserted in the host cell membrane. Additional EPEC virulence factors are encoded outside the LEE. Another factor, which is also shared with some VTEC seropathotypes, is a large protein called lymphostatin (LifA) referred to as Efa1 in VTEC strains. It inhibits lymphocyte activation and also has adhesive properties.

Plasmid-encoded virulence factors

Typical EPEC strains possess a large virulence plasmid of 70–100 kb called EAF (EPEC adherence factor). It encodes a type IV pilus termed the bundle-forming pilus (BFP), which seems to mediate either inter-bacterial adherence or adhesion to epithelial cells. The EAF plasmid also contains the *per* locus (plasmid-encoded regulator), whose product regulates the *bfp* operon and most of the genes carried by the LEE upon interaction with the LEE-encoded regulator (Ler). Atypical EPEC contain the LEE but not the EAF plasmid.

Model of pathogenesis

The model of EPEC pathogenesis is complex and has not been completely unravelled yet. The emerging model consists of a first stage of infection in which EPEC adhere to epithelial cells upon activation of an adhesin. The type III secretion system is then activated and various effector proteins are translocated into the host cell. EPEC intimately binds through the interaction of intimin/Tir inserted in the membrane and numerous cytoskeletal proteins accumulate underneath the attached bacteria. Several host factors are then activated leading to several downstream effects, including increased permeability due to loosening of tight junctions and induction of an inflammatory response. Diarrhoea probably results from multiple mechanisms, including active ion secretion, increased intestinal permeability, intestinal inflammation and loss of absorptive surface area resulting from microvilli effacement.

3.2 Clinical symptoms

Human illness

EPEC were the first group of *E. coli* recognised as a causative agent of diarrhoeal illness in humans. Symptoms in general appear about 12-36 hours after ingestion and include vomiting and diarrhoea accompanied by low grade fever. Stools are rarely bloody. In infants the disease can be severe lasting longer than 2 weeks. The major O groups within this group which are linked to human illness include O55, O86, O111, O119, O126, O127, O128 and O142. Through volunteer feeding studies, the infectious dose of EPEC in healthy adults has been estimated to be 10^6 organisms.

Typical EPEC is a leading cause of infantile diarrhea in developing countries but is rare in industrialized countries, where atypical EPEC seems to be a more important cause of diarrhea. Investigators have reported a high prevalence of aEPEC in both infants and older children in contrast to a stronger tendency for infants to be infected with tEPEC. Patients infected with aEPEC are far more likely to experience diarrhea which lasts more than 14 days, the point long recognized as a clinical watershed that heralds increased risk of illness and death.

Animal illness

EPEC is associated with disease in some animals (cattle, rabbits, pigs and dogs) but the serogroups involved differ from those causing human illness. Additionally while for typical EPEC, the only reservoir is humans; for atypical EPEC, both animals and humans can be reservoirs. An association between serotype O26:H11 and calves is well known and serotype O128:H2 is rather frequent in rabbits and dogs and is EAF negative. More studies of the prevalence of atypical EPEC serotypes in animals are needed, but available data strongly suggests that the primary reservoir for these organisms is different animal species.

3.3 Pathogenomics of EPEC

EPEC are diarrhoeagenic *E. coli* producing a characteristic histopathological A/E lesion on intestinal cells. EPEC share with VTEC strains pathogenic to humans the capability of inducing the A/E lesion but do not produce VTs. On the basis of different genetic characteristics, EPEC are divided into two distinct groups: typical and atypical EPEC (see above). Twelve EPEC serogroups were recognized by the World Health Organization in 1987: O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, and O158. These include both typical and atypical EPEC strains, however they can also be distinguished by the fact that they belong to distinct classes of serotypes although some of them are not easily classified. These include VT-producing strains, such as O26:H- and H11, O111ac:[H8] and O128:H2, that cannot be considered as true atypical EPEC or VTEC serotypes but rather heterogeneous serotypes including different clones or genetic lineages. The main genetic difference between typical and atypical EPEC is the presence of

the EAF plasmid in the first group.

3.4 Emerging issues

In industrialised countries, atypical EPEC are more frequently isolated from diarrhoeal cases than typical EPEC are, while the latter dominate in developing countries. Atypical EPEC have also caused large outbreaks of diarrhoeal disease involving both children and adults in industrialized countries. They are similar to VTEC in genetic characteristics, serotypes, reservoir, and some aspects of their epidemiology including the presence of animal reservoirs. Atypical EPEC can be considered as emerging pathogens and require further study to clarify their virulence mechanisms, the pathogenesis of the infection as well as their epidemiological significance.

4. Enterotoxigenic *Escherichia coli* (ETEC)

4.1 Pathogenicity factors

Enterotoxins

Enterotoxigenic *E. coli* (ETEC) isolates produce at least one of the two main types of enterotoxins; the heat-labile enterotoxins (LT) and the heat-stable enterotoxins (ST).

The LT toxins are AB₅ subunit toxins structurally and functionally comparable to the cholerae toxin, consisting of one enzymatically active A subunit and five receptor-binding B subunits. Following binding of the B moieties to specific receptors (GM1) at the intestinal epithelial cell surfaces, the toxin-receptor complex is endocytosed and transported in a retrograde manner through the Golgi apparatus and the endoplasmic reticulum. The A1 peptide is then released into the cytosol, and its enzymatic effect elicits a cascade that results in the formation of cyclic AMP (cAMP). The increased level of cAMP results in water efflux from the gut through a Cl⁻ secretory response, causing characteristic watery diarrhoea. The LT toxins can be classified into LTI and LTII, similar in both structure and mode of action, but they do not cross-react immunologically. LTI are plasmid-encoded proteins designated LTh and LTp after their initial discovery in humans and pigs, respectively, and important virulence factors in both human and porcine diarrhoea.

The ST toxins are also classified into two distinct types; STa (STI) and STb (STII) that differ in structure and mode of action. Their encoding genes are usually found on different plasmids. The STa toxin is a small toxin (18-19 amino acids) which binds to the extracellular domain of the enzyme guanylate cyclase. The subsequent increase in cyclic GMP results in fluid secretion from the intestinal epithelial cells, resulting in watery diarrhoea. Similar to LTI, STa consists of two variants designated STh and STp after their initial discovery in humans and pigs, respectively. However, both variants can be found in human ETEC isolates. STb is primarily associated with ETEC isolates from pigs, where they cause disease by inducing damage in the intestinal epithelium, resulting in loss of the villus epithelial cells and partial villus atrophy.

ETEC may also produce other enterotoxins like the enteroaggregative heat-stable toxin-1 (EAST-1) first described in EAaggEC, and *Shigella* enterotoxin (ShET1) first described in *Shigella flexneri* 2a.

Colonisation factors

In spite of the production of similar toxins from ETEC of human or animal origin, ETEC are not regarded as zoonotic pathogens due to their dependence on host specific receptors for adhesion to and colonisation of the gut epithelium, a prerequisite for disease to occur. ETEC express a range of different colonisation factors (CFAs) that are filamentous bacterial surface structures and that are different from human and animal strains. Like the toxin encoding genes, the colonisation factor genes are also located on plasmids, and often in coexistence with the toxin genes.

Human ETEC strains express one or more of over 20 different described CFAs, a minority of which has been unequivocally incriminated in pathogenesis. Some of these gene clusters are also fully sequenced. The different CFAs may be fimbrial, nonfimbrial or fibrillar structures and they have been grouped into four big families (CFA/I-IV) according to the homology in their N-terminal amino acid sequences. CFA/III is less common than the three other families. CFA/I is the archetype of a family of eight ETEC fimbriae that share genetic and biochemical features designated class 5 fimbriae. This family includes coli surface antigen 1 (CS1), CS2, CS4, CS14, CS17, CS19, and putative CF O71. These organelles comprise a rigid stalk of polymerized major subunits and a tip-localized minor adhesive subunit.

In animals, piglets are the most susceptible host to ETEC infections, but calves, lambs and dogs are also affected. The colonisation factor F5 (k99) has, for example, been detected in ETEC isolates from all these animal species, while the F4 (k88) antigen is regarded as specific for porcine isolates.

4.2 Clinical symptoms

Human illness

Illness caused by ETEC usually occurs between 12 and 36 h after ingestion and symptoms can range from mild diarrhoea to a severe cholera like illness with diarrhoea characterised by watery stools accompanied by vomiting and severe stomach pains. The illness normally persists for 2 to 3 days. Serotypes which cause illness in humans include O6, O8, O15, O25, O78, O148, O159 and O167. The infective dose of ETEC for adults has been estimated to be at least 10^8 cells; but the young, the elderly and the infirm may be susceptible to lower levels. ETEC is a common cause of travellers diarrhoea.

Animal illness

In animals ETEC is a major disease causing pathotype mainly causing illness in newborn animals with symptoms including severe diarrhoea, dehydration and death. The most common serogroups in calves and lambs are O8, O9, O20 and O101 while in piglets, serogroups O8, O138, O139 and O141 are

most common. ETEC, which are pathogenic in animals, do not appear to be pathogenic to humans due to differences in virulence factors and host susceptibility.

4.3 Pathogenomics of ETEC

Most of the pathogenicity factors in ETEC are encoded by plasmids, but the complete annotated sequences of these plasmids are not yet available. This information will probably reveal additional genes with functions associated with virulence. Relatively little is known about the ETEC chromosome. While full genome comparisons between the laboratory *E. coli* K-12 strain MG1655 and VTEC strains have shown a high level of diversity due to horizontal gene transfer events, the ETEC chromosome from one ETEC strain has been shown to be highly homologous to that of strain MG1655. Thus, the main event in the derivation of ETEC from *E. coli* occurred with the acquisition of virulence plasmids. ETEC seems to have undergone fewer chromosomal changes compared to some of the other pathogenic *E. coli*.

4.4 Emerging issues

ETEC are not regarded as emerging pathogens. Although there is evidence in the scientific literature that ETEC are responsible for more cases of human diarrhoea than previously anticipated. ETEC O169:H41 has been implicated in the rise in cases in the USA. This requires further investigation.

5. Enteroinvasive *Escherichia coli* (EIEC)

5.1 Pathogenicity factors

Enteroinvasive *E. coli* (EIEC) are invasive organisms and do not produce toxins. They also lack fimbrial adhesions but possess a specific adhesin that is thought to be an outer membrane protein. Unlike other *E. coli*, EIEC are non motile, lysine decarboxylase negative and 70% of strains do not ferment lactose. Typical serotypes include O159:H2 and O143:H-. The invasive phenotype is encoded on a high molecular weight (120-140 MDa) plasmid (pINV). Several virulence factors are encoded by pINV genes including the ability to invade and multiply within epithelial cells, the ability to spread infection intracellularly and between adjacent cells and the synthesis of contact mediated hemolysin. pINV genes encode a range of invasion antigens (invasion plasmid antigen genes) (*lpaA* to *lpaD*). In the colonic mucosa, EIEC adhere to the epithelial layer using an adhesin (*lpaD*) and cross this layer by invading M cells overlying the lymphoid follicles. Entry to the epithelial cells involves *lpaB* and *lpaC* which effect a rearrangement of the cell cytoskeleton leading to membrane ruffling and engulfment of the bacterium within a vacuole. This vacuole containing the bacterium is then internalised in the epithelial cell, the bacterium lyses the membrane of the entry vacuole and multiplies within the cytoplasm. *lpaA* and the products of

lcsA facilitate movement of the EIEC within the invaded cell resulting in the formation of protrusions into adjacent cells that are engulfed by these neighbouring cells resulting in dissemination of the EIEC.

The expression of the virulence phenotype is regulated by temperature, a trait that ensures that virulence genes are transcribed only when within a suitable host. pINVs can integrate into the host chromosome but in this state the organisms are usually non pathogenic.

5.2 Clinical symptoms

Human illness

EIEC are biochemically, genetically and pathogenically related to *Shigella* spp. Infection with EIEC results in an illness with the classical symptoms of invasive Bacillary dysentery similar to that caused by *Shigella* species. Onset of human illness occurs about 24 hours post ingestion and clinical features include fever, severe abdominal pains and watery diarrhoea followed by the passage of bloody stools. The infective dose appears to be substantially higher than for *Shigella* and this may be related to the greater sensitivity of EIEC to gastric acidity. Volunteer feeding studies showed that at least 10^6 EIEC organisms are required to cause illness in healthy adults. There are at least 32 different EIEC strains of 12 different serotypes. Serotypes implicated in human illness include O6, O15, O25, O78, O148 and O159. There are no known animal reservoirs of EIEC and the primary source is considered to be infected humans with transmission via the faecal-oral route. Not surprisingly therefore EIEC are more important in developing countries where sanitation and hygiene levels are poor.

Animal illness

EIEC are not found in animals and it is generally accepted that the EIEC only cause illness in humans.

5.3 Pathogenomics of EIEC/Evolution of EIEC clones

The evolutionary history of EIEC is debatable, but recent data supports the theory of transmission of multiple forms of ancestral virulence plasmids into many different *E. coli* during the creation of EIEC (and the closely related *Shigella* spp.). As EIEC, in contrast to the other *E. coli*, are intracellular organisms, this lifestyle has probably selected for a particular pattern of gene expression. It is hypothesized that gene inactivation in EIEC (and *Shigella* spp.) has generated polymorphism in gene expression that have served as a basis for further selection.

5.4 Emerging issues

Although the *Shigella* pINV is nonconjugative, genetic analysis of pINV from three EIEC strains show that these form a single pINV cluster and also harbour a full length transfer region. It is therefore possible that transferable

pINV are still around, with the potential of creating new strains of EIEC (and *Shigella*).

6. Enteroaggregative *Escherichia coli* (EAggEC)

6.1 Pathogenicity of EAggEC

Enteroaggregative *E. coli* (EAggEC) are defined as *E. coli* strains that do not secrete LT or ST enterotoxins and which adhere to Hep-2 cells in a stacked-brick like fashion. The latter is associated with a 60 MDa plasmid. These *E. coli* produce a low molecular weight heat stable toxin known as enteroaggregative heat stable enterotoxin (EAST-1) and a heat labile toxin termed plasmid encoding toxin (Pet), although this is not present in the majority of strains. East-1 is also found in ETEC, EPEC and EHEC strains and is structurally similar to STI and is composed of a 38 amino acid polypeptide with four cysteine residues. East-1 is encoded on a 60 MDa plasmid which also carries genes encoding the aggregative adherence fimbriae AAF/I and AAF/II and the transcriptional regulator AggR (involved in the regulation of the virulence genes). Some strains are reported to express nonfimbrial surface-exposed proteins which also facilitate adhesion.

Pathogenesis which involves adherence and colonisation of the intestinal mucosa is mediated by AAF/I and AAF/II. Colonisation results in increased mucus production which may promote persistent colonisation. Toxins are then released damaging the host cell.

6.2 Clinical symptoms

Human illness

EAggEC is associated with acute or persistent diarrhoea in humans, especially in children in developing countries. Infection is typically followed by a watery, mucoid, diarrhoeal illness with little to no fever and an absence of vomiting.

6.3 Emerging issues

VTEC O111:H2 strains isolated from an outbreak of HUS in France were shown to display aggregative adhesion to HEp-2 cells and to possess the genetic markers of enteroaggregative *E. coli*. Although strains possessing such a novel combination of virulence factors have been reported only once so far, they might be as pathogenic to humans as the classic EHEC strains.

7. Diffusely Adherent *Escherichia coli* (DAEC)

7.1 Pathogenicity of DAEC

This *E. coli* pathotype is characterised by their adherence to HEp-2 cells in a diffuse pattern, the presence of the Afa/Dr family or the absence of virulence genes typical of other *E. coli* pathotypes. Our understanding of the pathogenicity of disease caused by DAEC remains rudimentary. The adherence pattern, also found in some EPEC strains, is mediated by fimbrial structures including the surface fimbria F1845 encoded by the *daaC* gene. Some strains of DAEC may also carry a type III secretion system similar to those found in EPEC and EHEC. Genes encoding the EAST toxin found in EPEC and EAaggEC and the enterotoxins found in *Shigella* spp. have also been reported.

7.2 Clinical symptoms

Human illness

There is a dearth of information about the clinical characteristics of DAEC but it is known that they are capable of causing watery diarrhoea without blood and patients often experience vomiting. DAEC also induce finger like projections on the surface of infected HEp-2 and Caco-2 cells.

8. Bibliography

Caprioli A, Morabito S, Brugère H, Oswald E. 2005. Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. *Vet Res.* 2005. 36(3):289-311.

Chen Q, Savarino SJ, Venkatesan MM. 2006. Subtractive hybridization and optical mapping of the enterotoxigenic *Escherichia coli* H10407 chromosome: isolation of unique sequences and demonstration of significant similarity to the chromosome of *E. coli* K-12. *Microbiology.* Apr;152(Pt 4):1041-54.

Cheryl L. Tarr, Adam M. Nelson, Lothar Beutin, Katharina E. P. Olsen, and Thomas S. Whittam. 2008. Molecular Characterization Reveals Similar Virulence Gene Content in Unrelated Clonal Groups of *Escherichia coli* of Serogroup O174 (OX3) *J. Bacteriol.* 2008 190: 1344-1349.

Devasia RA, Jones TF, Ward J, Stafford L, Hardin H, Bopp C, Beatty M, Mintz E, Schaffner W. 2006. Endemically acquired foodborne outbreak of enterotoxin-producing *Escherichia coli* serotype O169:H41. *Am J Med.* 2006 Feb;119(2):168.e7-10.

de Sablet T, Bertin Y, Vareille M, Girardeau JP, Garrivier A, Gobert AP, Martin C. 2008. Differential expression of *stx2* variants in Shiga toxin-producing *Escherichia coli* belonging to seropathotypes A and C. *Microbiology.* 154:176-86.

EFSA (2007). Monitoring of verotoxigenic *Escherichia coli* (VTEC) and identification of human pathogenic VTEC types. Scientific Opinion of the Panel on BIOLOGICAL HAZARDS (Question No EFSA-Q-2007-036) The EFSA Journal

Karmali, M. A. (2003). The medical significance of Shiga toxin-producing *Escherichia coli* infections. An overview. *Methods Mol. Med.* 73: 1-7

Le Gall T, Darlu P, Escobar-Páramo P, Picard B, Denamur E. 2005. Selection-driven transcriptome polymorphism in *Escherichia coli*/*Shigella* species. *Genome Res.* Feb;15(2):260-8.

Morabito S, Karch H, Mariani-Kurkdjian P, Schmidt H, Minelli F, Bingen E, Caprioli A. 1998. Enterohaemorrhagic, Shiga toxin-producing *Escherichia coli* O111:H2 associated with an outbreak of hemolytic-uremic syndrome. *J Clin Microbiol.* 1998. 36(3):840-2.

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

Scheutz, F., Beutin, L., Pierard, D. and Smith, H. R. (2001). Nomenclature of Vero cytotoxins. In *Verocytotoxigenic E. coli*. G. Duffy, P. Garvey and D. A. McDowell Eds. Trumbull, Connecticut, Food and Nutrition Press Inc: 447-452.

Scheutz, F. and Strockbine, N. A. (2005). *Escherichia*. In Bergey's manual of systematic bacteriology. G. M. Garrity, D. J. Brenner, N. R. Krieg and J. T. Staley Eds. New York, NY, Springer, : 607-624.

Wickham, M. E., Lupp, C., Mascarenhas, M., Vazquez, A., Coombes, B. K., Brown, N. F., Coburn, B. A., Deng, W., Puente, J. L., Karmali, M. A. and Finlay, B. B. 2006. Bacterial genetic determinants of non-O157 STEC outbreaks and hemolytic-uremic syndrome after infection. *J. Infect. Dis.* 194 (6): 819-827

Welch RA, Burland V, Plunkett G 3rd, Redford P, Roesch P, Rasko D, Buckles EL, Liou SR, Boutin A, Hackett J, Stroud D, Mayhew GF, Rose DJ, Zhou S, Schwartz DC, Perna NT, Mobley HL, Sonnenberg MS, Blattner FR. 2002. Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*. *Proc Natl Acad Sci U S A.* Dec 24;99(26):17020-4.

Wick LM, Qi W, Lacher DW, Whittam TS. 2005. Evolution of genomic content in the stepwise emergence of *Escherichia coli* O157:H7. *J Bacteriol.* Mar;187(5):1783-91.

Yang J, Nie H, Chen L, Zhang X, Yang F, Xu X, Zhu Y, Yu J, Jin Q. 2007. Revisiting the molecular evolutionary history of *Shigella* spp. *J Mol Evol.* Jan;64(1):71-9.

9. Further Reading

Baylis, C. L., Penn, C. W., Thielman, N. M., Guerrant, R. L., Jenkins, C. and Gillespie, S. H. (2006). *Escherichia coli* and *Shigella* spp. In Principles and Practice of Clinical Bacteriology. Second Edition. S. H. Gillespie and P. M. Hawkey Eds., John Wiley & Sons Ltd, London: p 347-365.

Donnenberg, M. S. (2002). *Escherichia coli* virulence mechanisms of a versatile pathogen, London, Academic Press.

Duffy, G., Garvey, P. and McDowell, D. A. (2001). Verocytotoxigenic *E. coli*, Trumbull, Connecticut, Food & Nutrition Press Inc.

Kaper, J. B. and O'Brien, A. D. (1998). *Escherichia coli* O157:H7 and other Shiga toxin-producing *E. coli* strains, Washington D.C, ASM Press.

Scheutz, F. and Strockbine, N. A. (2005). *Escherichia*. In Bergey's manual of systematic bacteriology. G. M. Garrity, D. J. Brenner, N. R. Krieg and J. T. Staley Eds. New York, NY, Springer, : 607-624.

Sussman, M. (1997). *Escherichia coli* Mechanisms of virulence, Cambridge, Cambridge University Press.