PATHOGENICITY, VIRULENCE AND EMERGING PATHOGENIC STRAINS OF *ESCHERICHIA COLI* TRANSMITTED THROUGH FOOD AND WATER

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Contents

Summary

Escherichia coli is an important part of the intestinal flora of man and many animals. In man it is part of the commensal flora and constitutes about 0.1 % of the total flora. In a

healthy host these bacteria is a useful part of the intestinal flora. However, through microevolution several different pathotypes of *E. coli* have developed relatively recently. Six (maybe seven) different pathotypes can cause disease through the intestinal tract: Enteropathogenic (EPEC), enterotoxic (ETEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), enteroaggregative (EAEC) and diffusely adherent (DAEC) *E. coli*. The large outbreak in Germany in the spring-summer of 2011 may become a seventh group: enteroaggregative hemorrhagic (EAHEC) *E. coli*. Apart from EHEC which has its natural reservoir mainly in ruminants the reservoir is man. All types of *E. coli* enteric infections can be transmitted directly for person to person, but can also (at least in large outbreaks) be transferred through water and food. For EHEC the majority of cases are transmitted through foods. It has been estimated that about 1.5-2 million deaths per year are due to diarrheal diseases in the developing world, specifically hitting children under two years of age, and the different types of *E. coli* are responsible for approximately 20 % of the cases. The *E. coli* enteric diseases range from relatively mild diarrheal (ETEC) to severe systemic diseases involving kidney failure (EHEC). The majority of the fatal cases in the developing world are most probably due to dehydration.

1. Introduction

Escherichia coli was first described in 1885 by the German pediatrician Theodor Escherich. He isolated the bacterium from stools of children with enteritis. Since then we have learned that *E. coli* is a commensal bacterium in the intestine of man and many mammals. In man only about 0.1 % of the total number of bacteria is accounted for by *E. coli* giving about 10^8-10^9 in one gram of feces. Being present in the normal intestinal flora, *E. coli* is used as indicator organisms for fecal contamination of foods and water. Although the majority of the *E. coli* is non-pathogenic, several different pathogenic variants have evolved. As virulence mechanisms, epidemiology and characteristics of pathogenic *E. coli* vary tremendously, pathogenic *E. coli* are categorized into *E. coli* pathotypes on the basis of markers and typical characteristics. Each pathotype includes strains that cause a common disease using a common set of virulence factors. The pathotypes enclose both intestinal and extraintestinal pathogenic *E. coli*, and while some of the pathotypes are well defined and recognizable, others are not. The lack of a clear definition is reflected in the corresponding lack of a consistent number of pathotypes referred to in the literature where the number varies from six to nine. The diarrhogenic pathotypes are however quite well-described, and form six distinct categories: the enteropathogenic *E. coli* (EPEC), enterotoxic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), shigatoxin producing *E. coli* (STEC), enteroaggregative *E. coli* (EAEC) and diffusely adherent *E. coli* (DAEC) (Figure 1). The term enterohemorrhagic *E. coli* (EHEC) is used for a subgroup of strains within the STEC pathogroup that is able to cause disease in humans. *Shigella* species are closely related to *E. coli* and phylogenetic analysis have revealed that Shigella species are positioned interspersed among *E. coli* clones, and should evolutionary be regarded as *E. coli*. Shigella bacteria are however kept as a separate genus for practical reasons, i.e. *Shigella* causes a distinct set of disease syndromes, and is also quite easily distinguished from *E. coli* primarily by its inability to ferment lactose and by being nonmotile. *Shigella* will not be treated in this chapter, since they cause specific diseases that are described elsewhere.

Of the pathogenic *E. coli* causing diarrhoea EPEC was the first to be described in the 1940s where a specific type was discovered after nursery epidemics. In 1956 ETEC was describe from India, where rabbit loop tests were positive after injection with living *E. coli* form patients with a cholera-like illness. First 12 years later the disease was shown to be due to an enterotoxin which we now know is almost identical to the cholera toxin. This toxin is referred to as *E. coli* heat labile enterotoxin (LT). Later also the heat stable toxins (ST) from ETEC have been described. More recently the EIEC, EAEC, DAEC and EHEC have been added to the list of intestinal pathogenic *E. coli*. The latter type (EHEC) is by far the most important type of *E. coli* transmitted through food in the industrialized world, and is also causing the most severe type of disease. Although all the enteric pathotypes can probably be transmitted through food and water, the infections are probably more frequently through other routes, i.e. person to person contact, maybe apart for ETEC in the developing world. The spring of 2011 brought a new type of *E. coli* to our attention when a large outbreak of *E. coli* illness characterized by bloody diarrhoea and with a high frequency of serious complications appeared. This was due to an EAEC that had acquired genes for Shiga toxin production, and the outbreak appeared more or less like an EHEC outbreak. The strain causing the outbreak is an example of crossover pathotypes.

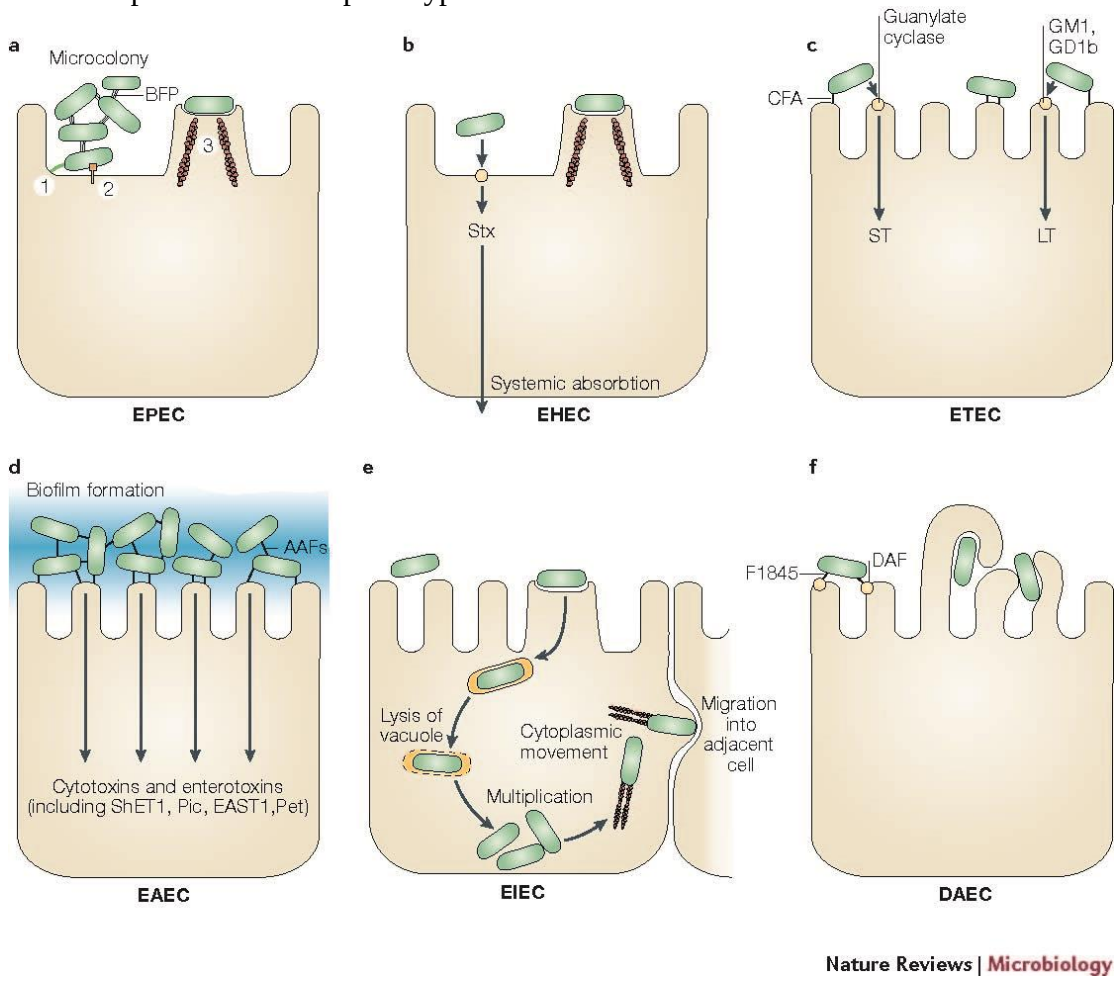

Figure 1. The six pathotypes of diarrheagenic *E. coli*

It has been estimated that as many at 1.5-2 million deaths per year are due to diarrheal diseases in the developing world, specifically hitting children under two years of age, reflecting lack of immunity in this age group. Different types of *E. coli* are responsible for a high percentage of these cases (approximately 20 %). Although many of the types of pathogenic *E. coli* are transmitted directly between humans, the exception is EHEC which has an animal reservoir and is zoonotic.

2. Taxonomy

E. coli is a Gram negative rod belonging to the family *Enterobacteriaceae* and the genus *Escherichia.* They are 1-1.5 x 2-6 µm in size and are motile by peritrichous flagella. They have an optimal growth temperature of about 37 °C, but grow in the range from 10 to 45 °C. They are quite resistant to heat and can survive 50 °C for as long as 15 minutes. Most *E. coli* are lactose fermenters with acid production, and this biochemical trait is utilized in selective media to recognize possible *E. coli* colonies. *E. coli* is facultative anerobic and ferment glucose with acid and gas production. It is indole positive (with few exceptions) and is mostly positive in the methyl red test and negative in the Voges-Proskauer test and citrate test. *E. coli* do rarely hydrolyze urea or produce hydrogen sulfide in the TSI (triple sugar iron) medium.
Serotyping of *E. coli* is important in categorizing strains after food- and waterborne outbreaks, and is mostly based on somatic O antigens (lipopolysaccharide, LPS) and the flagellar H antigens. Capsular K antigens are also used to some extent. Serotyping is a complex classification system, and presently 173 O antigens, 103 K antigens and 56 H antigens have been identified, giving rise to more than 50,000 serotypes. Serogrouping can be used both to identify particularly virulent strains (e.g., EHEC O157:H7), and the serogroup is also utilized in the isolation by immunomagnetic separation (IMS, magnetic beads coated with an antibody to the O antigen).

2.1. Serotyping classification

E. coli is serologically divided in serogroups and serotypes on the basis of major surface antigens of the bacterial cell, i.e. O (somatic, lipopolysaccharide), H (flagellar), and K (polysaccharide capsules) antigens. The presence of the O antigen defines the serogroups (e.g. serogroup O157), while the additional identification of the H antigen defines the serotypes (e.g. serotype O157:H7). The K antigen is only occasionally used in serotyping. Serotyping is a complex classification system, owing to the high number of antigen variants (173 O antigens, 56 H antigens, and 80 K antigens), and is not a subject for this chapter. We refer the reader to specialized literature for this topic.

Serotyping alone is not enough to identify an organism as a pathogenic *E. coli*, specific virulence genes or features characteristic of each pathotype must also be present. Traditionally, specific serotypes were associated with certain pathotypes and/or disease, but the picture has been shown to be much more complex, and serotyping has a more limited value in diagnosis of *E. coli* infections today. However, the serogroup may be utilized in the isolation of certain major serogroups, particularly in the EHEC pathotype. As these pathogens reside along with the commensal *E. coli* of the intestinal flora, but often as a minor part of the population, they can be difficult to isolate. To enrich the number of pathogens, IMS is used prior to plating.

3. Reservoir

Since *E. coli* survives well in many environments including food and water (specifically if cold) and is easily grown, it is used as indicator for fecal contamination. It has its main reservoir in feces of man and warm blooded animals, including slaughter animal, and is therefore highly suitable for the purpose. However, the use of *E. coli* as indicator organism for fecal contamination has been questioned from some sources, at least in areas (specifically for water) where environmental strains are abundant in soil and water, as in some tropical areas. There is, however, at present no other bacterial species more suited as indicator for fecal contamination than *E. coli*. Although many animal species carry *E. coli* the majority of the human pathogenic *E. coli* has man as the main reservoir.

4. Pathogenic *E. coli*

Even if the vast majority of the total numbers of *E. coli* are non-pathogenic commensal members of the intestinal flora of man and warm blooded animals, pathogenic strains have evolved through microevolution. More than 60 complete genomic sequences of *Escherichia* and *Shigella* species are publicly available. Comparison of these sequences shows a surprisingly high diversity and only about 20 % of each genome represents sequences that are present in all of the isolates. Although the individual genomes contain between 4,000 and 5,500 genes the total number of different genes among all of the sequenced *E. coli* strains (the pan-genome) now exceeds 16,000. This very large variety is believed to arrive through the process of horizontal gene transfer from other species. Since the majority of the *E. coli* strains co-exist with several hundred of other species which constitute the dense, complex microbiota in the intestinal tract of man and many animals, this is not surprising. Although nobody knows exactly when and how the pathogenic strains first appeared it is not unexpected that new variants are continuously made and that some have become pathogenic. An overview of the different pathotypes and virulence factors are given in Figure 1 and Table 1.

Pathotype	Predilection site	Invasive	Main attachment mechanism	Virulence characteristics	Accessory virulence genes
ETEC	Small intestine	No	Fimbrial adhesin	LT, ST	
tEPEC	Small intestine	No	A/E lesion, LA	Intimin, bfp, TTSS	
aEPEC	Small intestine	No	A/E lesion	Intimin, EAST1, TTSS,	
EHEC	Colon	No, but Stx has systemic effect	A/E lesion	Intimin, Stx, TTSS	EhxA, lifA/Efa, subAB,
EIEC	Colon	Yes	Adhesin, outer membrane protein	Ipa gene, *Shigella* enterotoxin 2	
EAEC	Small and large intestine	No	Biofilm, AAF	Pet, EAST1, ShET1	AggR (regulator)
DAEC	Small intestine	No	Adhesin F1845	Unclear	

LA, localized adherence
A/E, attaching and effacing
AAF, aggregative adherence fimbriae
TTSS, type III secretion system
Bfp, bundle forming pili
LT, heat labile toxin
ST, heat stabile toxin
Stx, Shiga toxin
EAST1, enteroaggregative *E. coli* ST1
ShET1, *Shigella* enterotoxin 1
Pet: plasmid encoded toxin

Table 1. Overview of central factors and mechanism involved in pathogenesis of diarrhogenic *E. coli*

4.1. EPEC

Enteropathogenic *E. coli* was the first of the *E. coli* pathotypes to be described. In large outbreaks of infant diarrhoea in England in the 1940s, serologically distinct *E. coli* strains were isolated and described. The term EPEC was introduced in 1955 at a time when the pathogen was responsible for large outbreaks of infant diarrhoea, and mortality rates could be > 50 %. EPEC is no longer associated with large outbreaks in developed countries, but remains so in developing countries. However, EPEC may be an underestimated cause of childhood diarrhoea also in developed countries. The identification of pathogenic *E. coli* from cases of diarrhoea is challenging, because it inhabits the same environment as commensal strains, and is probably underreported from diagnostic laboratories. Diagnosis of EPEC is now mainly using molecular approach, and the prevalence has increased.

EPEC is a non-invasive pathogen of the small intestine (Figure 1). The pathogenicity exhibited by EPEC is first and foremost an ability to deliver effector proteins directly into the enterocyte and to generate an intimate attachment to these cells. The intimate attachment is called an attaching and effacing (A/E) lesion and is a hallmark for EPEC. This attachment is similar to the attachment mechanism seen in enterohemorrhagic *E. coli* (EHEC), but in contrast to EHEC, the EPEC do not produce Shiga toxin. In the A/E lesion, the bacteria attach tightly to the enterocytes, and this attachment is a multistage strategy mediated by the pathogen. Initially, the bacteria display a nonintimate localized adherence in form of discrete microcolonies associated with the enterocytes. EPEC then injects effector molecules into the host cell through a Type III secretion system (TTSS). These effector molecules induce cytoskeleton restructuring and the formation of pedestals occurs beneath the bacteria adherence site, and the A/E lesion is finalized. The A/E lesion disrupts the microvilli, and the absorptive surface of the enterocyte is reduced.

The genes needed to display this intimate attachment are mainly located on the approximately 35 kb pathogenicity island locus of enterocyte effacement (LEE). Among factors encoded by LEE is the TTSS which is fundamental for delivering effector molecules into the host cell. Through the TTSS the bacteria inject the translocated

intimin receptor (Tir) into the enterocytes where it is inserted in the cell membrane. Tir functions as a receptor for intimin which is an outer membrane protein in the bacteria. The gene encoding intimin, *eae*, is also located in LEE. At least 27 distinct types or subtypes of the intimin gene *eae* have been demonstrated. The C-terminal of intimin is responsible for receptor binding and the difference subtypes may have implications for tissue cell tropism. In addition to Tir and intimin, several other effector proteins and chaperones are necessary for the development of the intimate attachment.

The EPEC pathotype is further divided into two subtypes, the typical EPEC (tEPEC) and the atypical EPEC (aEPEC). The definition of the two groups was accepted by the scientific community in 1995. The classification of the two subtypes is based on the presence of a 50-70 MDa *E. coli* adherence factor (EAF) plasmid, where the tEPEC contain the plasmid and the aEPEC do not. The EAF plasmid encodes bundle forming pili (*bfp*), which mediates the localized adherence and formation of microcolonies in the first stage of the attachment. While the tEPEC display this localized adherence, the aEPEC demonstrate a diffuse adherence. The aEPEC is a more heterogeneous group and the prevalence of aEPEC is higher than tEPEC in both developing and developed countries.

The pathogenic potential of EPEC is controversial. EPEC is an important and emerging pathogen of young children, associated with acute diarrhoea; occasionally vomiting and low grade fever (Table 2). EPEC may also cause persistent diarrhoea, and dehydration is not uncommon. It is an important pathogen for children, high prevalence in the community and a central cause of persistent diarrhoea. Diagnosis is now based mainly on molecular approach and the more accurate has resulted in an increased prevalence.

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Bibliography

Bielaszewska M, Mellmann A, Zhang W, Kock R, Fruth A, Bauwens A, Peters G, Karch H (2011) Characterisation of the *Escherichia coli* strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study. *Lancet Infect Dis* 11: 671-676. [This article gives a thorough description of the hybrid EAEC-EHEC isolates of *E. coli* O104:H4 involved in the large European outbreak of *E. coli* in 2011]

Brzuszkiewicz E, Thurmer A, Schuldes J, Leimbach A, Liesegang H, Meyer FD, Boelter J, Petersen H, Gottschalk G, Daniel R (2011) Genome sequence analyses of two isolates from the recent *Escherichia coli* outbreak in Germany reveal the emergence of a new pathotype: Entero-Aggregative-Haemorrhagic *Escherichia coli* (EAHEC). Arch *Microbiol* 193: 883-891. [An analysis of the genomes of two *E. coli* O104:H4 isolates from the European outbreak in 2011 compared to the genome of other *E. coli* genomes. The authors suggest the new pathotype Entero-aggregative-haemorrhagic *E. coli* (EAHEC)]

Hernandes RT, Elias WP, Vieira MA, Gomes TA (2009) An overview of atypical enteropathogenic *Escherichia coli*. FEMS *Microbiol Lett* 297: 137-149. [The minireview gives a thorough overview of the atypical EPEC regarding serotypes, virulence properties, epidemiology, reservoir and diagnosis].

Kaper JB, Nataro JP, Mobley HL (2004) Pathogenic *Escherichia coli*. *Nat Rev Microbiol* 2: 123-140. [A very informative and educational presented overview of the six pathotypes of diarrhoeagenic *E. coli*. Figure 1 in this chapter is derived from this article].

Karch H, 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. [Although not a very recent publication, this minireview gives a good introduction to SF E. coli O157, the characteristics and the corresponding challenges].

Kaur P, Chakraborti A, Asea A (2010) Enteroaggregative *Escherichia coli*: An Emerging Enteric Food Borne Pathogen. Interdiscip Perspect Infect Dis 2010: 254159. [A comprehensive updated review of the current knowledge of epidemiology, detection, virulence properties and treatment of EAEC]

L'Abée-Lund TM, Jørgensen HJ, O'Sullivan K, Bolin J, Ligård G, Granum PE, Lindbäck T (2012) The highly virulent 2006 Norwegian EHEC O103:H25 outbreak strain is related to the 2011 German O104:H4 outbreak strain. *Plos One* 7: e31413. [An article which further highlights the epidemiology of the Shigatoxin phage in the hybrid EAEC-EHEC from the European outbreak in 2011].

Scheutz F, Teel LD, Beutin L, Pierard D, Buvens G, Karch H, Mellmann A, Caprioli A, Tozzoli R, Morabito S, Strockbine NA, Melton-Celsa AR, Sanchez M, Persson S, O'Brien AD (2012) Multicenter evaluation of a sequence-based protocol for subtyping Shiga toxins and standardizing Stx nomenclature. *J Clin Microbiol* 50: 2951-2963. [A presentation of a universal Shiga toxin nomenclature, including a protocol for subtyping of the toxins].

Schmidt MA (2010) LEEways: tales of EPEC, ATEC and EHEC. *Cell Microbiol* 12: 1544-1552. [A minireivew of pathogenicity island 'locus of enterocyte effacement (LEE)' involved in the capability of the characteristic A/E lesions of EHEC and EPEC].

Servin AL (2005) Pathogenesis of Afa/Dr diffusely adhering *Escherichia coli*. *Clin Microbiol Rev* 18: 264-292. [The review addresses the virulence aspects of the diffusely adhering *E. coli*].

Vieira N, Bates SJ, Solberg OD, Ponce K, Howsmon R, Cevallos W, Trueba G, Riley L, Eisenberg JN (2007) High prevalence of enteroinvasive *Escherichia coli* isolated in a remote region of northern coastal Ecuador. *Am J Trop Med Hyg* 76: 528-533. [The review addresses the virulence aspects of the diffusely adhering *E. coli*].

Biographical Sketches

Trine L'Abée-Lund is a veterinarian and graduated from the Norwegian School of Veterinary Science (NSVS) in 1996. She received her Ph.D. degree from NSVS in 2002 and has been Associate Professor in the same place since 2003. Her research area has mainly been antimicrobial resistance and pathogenicity in Gram negative bacteria, primarily in fish pathogenic bacteria like *Aeromonas salmonicida* and *Moritella viscosa*, but also in *E. coli*. In warm blooded animals she has studied the development of antibiotic resistance and the disturbance in the intestinal microbiota during antibiotic treatment of animals.

Per Einar Granum received his Ph.D. degree from the University of Oslo in 1982. After more than 10 years at the Norwegian Food research Institute he came to the Norwegian School of Veterinary Science in 1988 as Associate Professor, and was appointed Professor in food safety in 1993. He has worked mainly with food poisoning organisms and their toxins throughout his career. The main emphasis has been on the spore forming bacteria *Clostridium perfringens* and *Bacillus cereus*, but during the last six years also *E. coli* (EHEC) has been in focus. He has worked several years abroad at University of California (Davis), University of Cambridge (UK) and at University of Oxford (UK). Professor Granum was elected member of Norwegian Academy of Science and Letters in 2006.