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Diarrheagenic *Escherichia coli* types and their pathogenesis

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Abstract:

Most *Escherichia coli* strains live harmlessly in the intestines and rarely cause disease in healthy individuals. Nonetheless, a number of pathogenic strains can cause diarrhea or extraintestinal diseases both in healthy and immunocompromised individuals. Diarrheal illnesses are a severe public health problem and a major cause of morbidity and mortality in infants and young children, especially in developing countries. *E. coli* strains that cause diarrhea have evolved by acquiring, through horizontal gene transfer, a particular set of characteristics that have successfully persisted in the host. According to the group of virulence determinants acquired, specific combinations were formed determining the currently known *E. coli* pathotypes, which are collectively known as diarrheagenic *E. coli*. In this review, we have gathered information on current definitions, serotypes, lineages, virulence mechanisms, epidemiology, and diagnosis of the major diarrheagenic *E. coli* pathotypes.

Key words: *Escherichia coli*, Enterobacteriaceae, diarrheal illness, pathogenesis, infections, virulence

Introduction:

The genus *Escherichia,* which was named after the German pediatrician Theodor Escherich, consists of facultative anaerobic Gram-negative bacilli that belong to the family Enterobacteriaceae [1]. The genus type species *Escherichia coli* is widely distributed, where it is the major facultative anaerobe inhabiting the large intestine of humans and warm-blooded animals [2]. Although most *E. coli* strains live harmlessly in the colon and seldom cause disease in healthy individuals as number of pathogenic strains can cause intestinal and extraintestinal diseases both in healthy and immunocompromised individuals [3]. Diarrheal illnesses are a severe public health problem and a major cause of morbidity and mortality in infants and young children [4]. Low- and middle-income countries in Africa, Asia and Latin America are the most affected regions with diarrheal diseases occurring more often with lethal outcomes mainly due to poor living conditions [5]. *E. coli* strains involved in diarrheal diseases are one of the most important of the various etiological agents of diarrhea, where strains have evolved by the acquisition, through horizontal gene transfer, of a particular set of characteristics that have successfully persisted in the host [3,5,6]. According to the group of virulence determinants acquired, specific combinations were formed determining the currently known *E. coli* pathotypes, which are collectively known as diarrheagenic *E. coli* (DEC) [6]. The DEC pathotypes differ regarding their preferential host colonization sites, virulence mechanisms, and the ensuing clinical symptoms and consequences, and are classified as enteropathogenic *E. coli* (EPEC), enterohemorrhagic (Shiga toxin-producing) *E. coli* (EHEC/STEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli* (ETEC), and enter invasive *E. coli* (EIEC). Each of these pathotypes represents a group of clones that share specific virulence factors. Nevertheless, it should be pointed out that the plasticity of the *E. coli* genome has hindered the identification of certain *E. coli* isolates as a pathotype, because some isolates combine the main virulence characteristics of different pathotypes and are thus considered potentially more virulent hybrid pathogenic strains [5]. Another less well-defined pathotype has been described, that is, the diffusely-adherent *E. coli* (DAEC) pathotype, which comprises strains that adhere to epithelial cells in a diffused distribution [6]. Despite their classification as a group distinct from the other pathotypes, the designation of DAEC as a different DEC pathotype requires further epidemiological studies, which have been hampered by the difficulties in its identification and classification. Furthermore, certain *E. coli* strains that have been classified as the adherent invasive *E. coli* (AIEC) pathotype, comprise one of the potential agents for Crohn's disease (CD).

CD is an inflammatory bowel disease (IBD), which is thought to be caused by a combination of factors like genetics, the intestinal microbiota, environmental factors, and enteric pathogens [7,8]. Diarrheal episodes due to DEC infections are an important public health issue among children and adults in developing countries, because of their association with morbidity and mortality of children less than five years of age. Typical and atypical enteropathogenic *E. coli* the term enteropathogenic *E. coli* (EPEC) was first used in 1995 by Neter [9] to describe a number of *E. coli* strains epidemiologically related to a series of outbreaks of infantile diarrhea in the 1940s and 1950s [10,11]. Originally identified by serotype, EPEC are now defined as those *E. coli* strains having the ability to cause diarrhea, to produce a histopathology on the intestinal epithelium known as the attaching and effacing (AE) lesion, and the inability to produce Shiga toxins and heat-labile (LT) or heat-stable (ST) enterotoxins. Improvements in techniques allowing a better understanding of the genome and virulence mechanisms among EPEC strains over the years have led to the sub-classification of EPEC into typical EPEC (tEPEC) and atypical EPEC (aEPEC) [3,12]. Typical EPEC strains causing human infectious diarrhea possess a large virulence plasmid known as the EPEC adherence factor (EAF) plasmid (pEAF), which encodes the type IV fimbriae called the bundle-forming pilus (BFP), while aEPEC do not possess this plasmid [6,12]. The majority of tEPEC strains fall into well-recognized O serotypes. Classical EPEC O serogroups include O55, O86, O111, O114, O119, O127, and O142. The most common H antigens associated with EPEC are the H6 and H2 antigens [12–15]. A less common EPEC type is H34, and a number of tEPEC strains are classified as non-motile (H-) in conventional tests. Typical EPEC strains belonging to non-classical serotypes have also been reported [12,16]. Based on multilocus enzyme electrophoresis analysis (MLEE) of allelic differences between housekeeping genes, tEPEC strains have been subtyped into two major lineages, previously designated EPEC1 and EPEC2 [13,14]. EPEC1 includes widespread serotypes such as O55:H6 and O119:H6, whereas EPEC2 consists of serotypes with more limited occurrence such as O111:H2 and O114:H2. Based on a whole-genome phylogeny and analysis of type III secretion system (T3SS) effectors, tEPEC strains have been demonstrated to cluster in three main lineages, designated EPEC1, EPEC2, and EPEC4, which probably acquired the locus of enterocyte effacement (LEE) region and pEAF independently [17]. In turn, aEPEC belong to a large diversity of classical and non-classical serotypes [12,16,18]. Over 20% of strains of non-classical EPEC serotypes are O non-typeable and the O-typeable strains belong to more than 4200 different serotypes, with many non-motile and H non-typeable strains

[12,18]. Interestingly, it has been found that 35% of the aEPEC strains also belong to the tEPEC lineages. Thus, it has been hypothesized that at least some aEPEC may have originated from tEPEC strains that lost pEAF in the host or in the environment [17,19,20].

Epidemiology:

The prevalence of EPEC infections varies between epidemiological studies on the basis of differences in study populations, age distributions, and methods including serotyping, adherence patterns, and presence of the *eae* or conserved LEE genes used for detection and diagnosis [21]. In addition, differences in geographic regions, periods of time and socioeconomic class may also contribute to differences in the epidemiology of EPEC induced diarrheal disease [22]. Lack of discrimination between tEPEC and aEPEC in some studies also makes such analysis difficult. Diarrhea due to tEPEC decreases with age, and infections in adults are rarely reported. This apparent resistance in adults and older children has been attributed to the loss of specific receptors with age or development of immunity [6]. For many decades, studies conducted worldwide have shown that tEPEC serotypes are strongly associated with diarrhea in children less than 5 years of age from seven sites in Africa and Asia, tEPEC was significantly associated with moderate to severe diarrhea in children under 2 years of age in Kenya, whereas aEPEC was not associated with this type of diarrhea [23]. Transmission of tEPEC follows a fecal-oral process through contaminated surfaces, weaning fluids, and human carriers [24]. Although rare, outbreaks among adults seem to occur through the ingestion of contaminated food and water; however, no specific environmental reservoir has been identified. Humans are the only known reservoir for tEPEC, with symptomatic and asymptomatic children and asymptomatic adults being the most likely source [6]. In contrast to tEPEC, aEPEC have been found in diarrheic patients of all ages and in adults with HIV-AIDS [82,126]. The role of aEPEC in diarrhea is not clear because of its detection at similar rates in both diarrheic and non-diarrheic patients in various geographical areas [18,25,26]. In studies conducted in the last five years, aEPEC have been found at rates varying from ∼0.05 to ∼12% in diarrheic versus 0 to ∼14% in non-diarrheic patients [27]. Some recent studies have also implicated aEPEC as the cause of persistent and bloody diarrhea [18,28]. The tEPEC, which are seldom found in animals,12 many aEPEC strains have been found in both diarrheic and healthy animals [18,27]. Interestingly, animal aEPEC serogroups associated with human diarrhea have been identified (e.g., O26, O103, O119, O128, O142 and O157) [18,29,30]. In addition, foods

including raw meat, pasteurized milk and vegetables and water have also been implicated as vehicles of aEPEC in human infections [27]. aEPEC strains comprise a very assorted group with various additional virulence mechanisms that altogether can modulate the disease outcome or their occurrence in asymptomatic persons. There have been continuous advances in our knowledge of the genetic background and pathogenicity of aEPEC as well as in the information gathered from epidemiological studies, and may contribute to the discrimination between strains that cause diarrhea and those that cause asymptomatic infections.

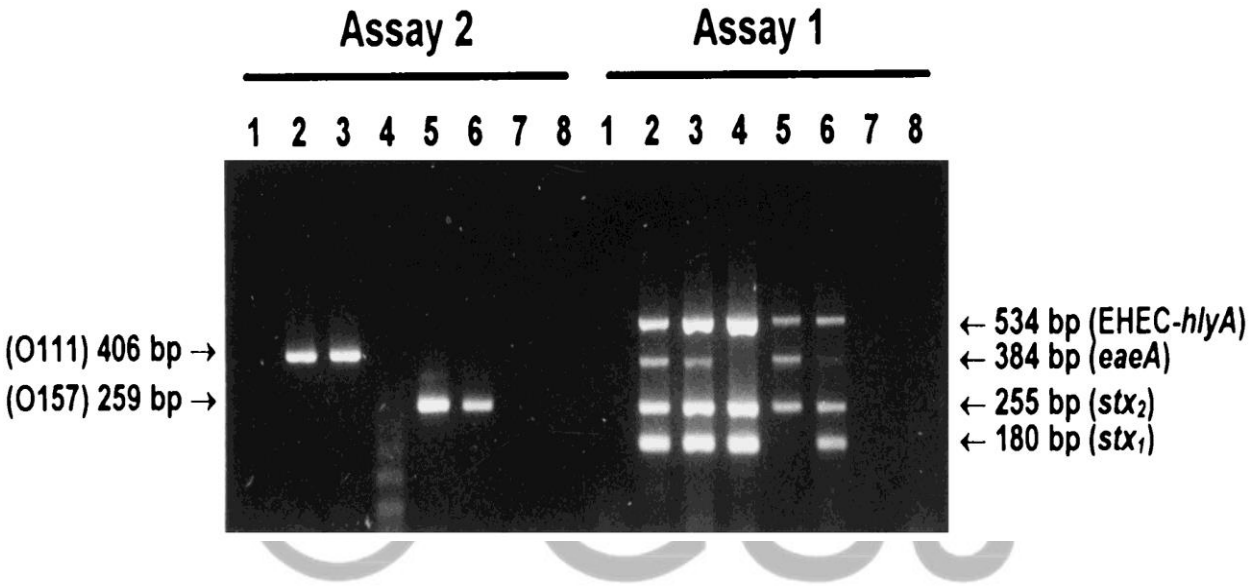

Figure 1: Multiplex PCR analysis of primary fecal cultures. Crude DNA extracts of primary fecal cultures were analyzed by multiplex PCR assay 1 or assay 2, as indicated. Lanes: 1, negative control; 2, patient 1 (HUS); 3, patient 2 (HUS); 4, patient 3 (HUS); 5, patient 4 (HUS); 6, patient 5 (bloody diarrhea); 7, patient 6 (control); 8, patient 7 (control). The expected mobilities for the various specific PCR products are also indicated. https://doi.org/10.1128/JCM.36.2.598-602.1998.

Typical and Atypical Enteropathogenic *E. coli*:

The term enteropathogenic *E. coli* (EPEC) was first used in 1995 to describe a number of *E. coli* strains epidemiologically related to a series of outbreaks of infantile diarrhea in the 1940s and 1950s [10,11]. Originally identified by serotype, EPEC are now defined as those *E. coli* strains having the ability to cause diarrhea, to produce a histopathology on the intestinal epithelium known as the attaching and effacing (AE) lesion, and the inability to produce Shiga toxins and heat-labile (LT) or heat-stable (ST) enterotoxins [6]. Improvements in techniques allowing a better

understanding of the genome and virulence mechanisms among EPEC strains over the years have led to the sub-classification of EPEC into typical EPEC (tEPEC) and atypical EPEC (aEPEC) [3,12]. Typical EPEC strains causing human infectious diarrhea possess a large virulence plasmid known as the EPEC adherence factor (EAF) plasmid (pEAF), which encodes the type IV fimbriae called the bundle-forming pilus (BFP), while aEPEC do not possess this plasmid [6,12]. The majority of tEPEC strains fall into well-recognized O serotypes. Classical EPEC O serogroups include O55, O86, O111, O114, O119, O127, and O142. The most common H antigens associated with EPEC are the H6 and H2 antigens [12–15]. A less common EPEC type is H34, and a number of tEPEC strains are classified as non-motile (H-) in conventional tests. Typical EPEC strains belonging to non-classical serotypes have also been reported [12,16]. Based on multilocus enzyme electrophoresis analysis (MLEE) of allelic differences between housekeeping genes, tEPEC strains have been subtyped into two major lineages, previously designated EPEC1 and EPEC2 [13,14]. EPEC1 includes widespread serotypes such as O55:H6 and O119:H6, whereas EPEC2 consists of serotypes with more limited occurrence such as O111:H2 and O114:H2. Based on a whole-genome phylogeny and analysis of type III secretion system (T3SS) effectors, tEPEC strains have been demonstrated to cluster in three main lineages, designated EPEC1, EPEC2, and EPEC4, which probably acquired the locus of enterocyte effacement (LEE) region and pEAF independently [17]. In turn, aEPEC belong to a large diversity of classical and non-classical serotypes [12,16,18]. Over 20% of strains of non-classical EPEC serotypes are O non-typeable and the O typeable strains belong to more than 4200 different serotypes, with many non-motile and H non-typeable strains [12,18]. Interestingly, it has been found that 35% of the aEPEC strains also belong to the tEPEC lineages [17]. Thus, it has been hypothesized that at least some aEPEC may have originated from tEPEC strains that lost pEAF in the host or in the environment [17,19,20].

Intimin types	Typical	Atypical
Alpha	O55:[H6],[a] O127:H6, O142:H6, O142:H34	O111:[H9], O125ac:H6
Beta	O111:[H2], O114:H2, O119:[H6]	O26:H[11], O119:H2, O128:H2
Gamma		O55:[H7], O111ac:[H8]
Delta	O86:H34	

[a] Brackets denote the frequent occurrence of nonmotile strains.

Figure 2: Frequently isolated enteropathogenic *Escherichia coli* (EPEC) serotypes, including typical and atypical strains. http://dx.doi.org/10.3201/eid0805.010385

Virulence factors and pathogenesis:

Typical EPEC strains adhere to HeLa, HEp-2, and other cell lines and to organ cultures in vitro in a distinctive pattern of three-dimensional microcolonies, a so-called localized adherence (LA) pattern [6,29]. A similar adherence pattern has been seen in tissue biopsies of EPEC-infected humans [30]. The LA phenotype is mediated by the BFP,23 which also contributes to antigenicity, auto aggregation, and biofilm formation [30,31,32,33,34,35]. An operon of 14 genes contained on the pEAF is necessary for BFP expression, with bfpA encoding the major structural subunit 28 and being highly conserved among EPEC1 and EPEC2 strains. The self-transmissible pEAF pMAR2 is found among strains of the EPEC1 lineage and contains an intact transfer region, unlike pB171, which is more common among EPEC2 strains [36,37]. Besides the bfp gene cluster, encoding BFP, the pEAF carries the per locus, encoding the transcriptional activator called plasmid-encoded regulator [36]. Recent comparative genomics of the EAF plasmids from diverse EPEC phylogenomic lineages demonstrated significant plasmid diversity even among isolates within the same phylogenomic lineage [38]. Typical EPEC have the ability to form tight, spherical, bacterial auto aggregates when grown in liquid culture. Like LA, auto aggregation requires BFP. Typical EPEC also form biofilms on abiotic surfaces under static conditions, or in a flow through continuous culture system, and a model of EPEC biofilm formation has been proposed [26]. Mutagenesis analysis has identified adhesive structures such as the common type 1 pilus (T1P),

antigen, BFP and the EspA filament as participants in bacterial aggregation during biofilm formation on abiotic surfaces [39].

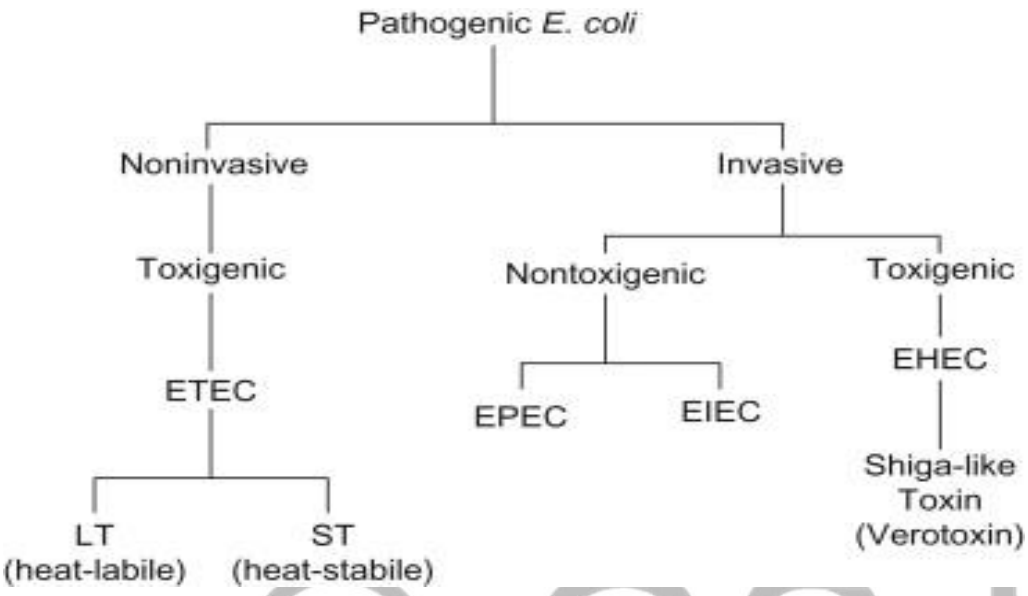

Figure 3: Mechanisms of pathogenesis of *Escherichia coli* strain

(Charles P. Gerba et al., 2009)

Shiga toxin-producing *E. coli*:

EHEC/STEC represent a well-known group of foodborne pathogens distributed worldwide. The ability to produce one or more of the Shiga toxins (Stx) family cytotoxins [40] constitutes the main virulence attribute of this pathogroup of *E. coli.* A wide array of infections from mild and almost unapparent diarrhea to more serious manifestations such as hemorrhagic colitis (HC) and the development of a life-threatening syndrome known as hemolytic uremic syndrome (HUS) are caused by EHEC/STEC. Infants and children are the main affected patients, and although the incidence of infection varies in different regions, the impact and importance of EHEC/STEC infections in public health is immense, being the main cause of acute renal failure in children in many countries. E. coli O157:H7 serotype was the first to be linked to HC and HUS cases in the

early 1980s, and has been since then responsible for numerous outbreaks and sporadic cases of severe diseases all over the world, therefore considered to be the prototype of this pathogenic group of bacteria [41]. It is well known that hundreds of other *E. coli* serotypes can harbor the stx genes, but epidemiological studies carried out worldwide have proven that only some of them have been responsible for causing human diseases. Some serogroups including O26, O45, O103, O111, O121 and O145 can be highlighted among those most commonly related to human infections [42]. Moreover, in recent years the emergence of some particular clones such as the hybrid O104:H4 enteroaggregative *E. coli* carrying Stx2 genes, responsible for a severe outbreak of HUS starting in Germany in 2011 [43] the spread of a new O26:H11 clone in Europe [44].

Enterotoxigenic *E. coli*:

ETEC strains are characterized by the production of colonization factors (CFs) and at least one of two enterotoxins: LT and ST. ETEC represent one of the most common causes of diarrhea in children in developing countries and in travelers to these regions. ETEC is also an economic burden to farmers and industry, where itis an important pathogen for broilers, swine, cattle and other farm animals. The group represents a highly diverse pathovar of diarrhoeagenic *E. coli*, harboring mobile genetic elements such as plasmids and phages. ETEC heterogeneity was first demonstrated by phenotypic traits including the large diversity of lipopolysaccharide (LPS) and flagellin composition and the expression of different CFs and toxin types [45.46]. Serological typing of ETEC strains have relied on the composition of outer membrane proteins and, mainly, in the somatic LPS (O) and flagellar (H) antigens [46,47,48]. ETEC comprise more than 100 somatic serogroups (O) and at least 34 flagellar types (H), combined in an unpredicted number of O:H serotypes, but only a limited number of serotypes are associated with infectious diseases, such as O8:H9, O6:H16, O78:H12 and O25:H42, and are therefore of major clinical relevance [46,49]. The genetic diversity of ETEC has also been evaluated by molecular approaches including random amplification of polymorphic DNA (RAPD), MLEE, PFGE, multilocus sequence type (MLST) and whole-genome sequencing [50,51]. More recently, 362 human-derived strains were subjected to next generation whole-genome sequencing; 21 genotypes could be identified, and ETEC strains could be classified into 5 major phylogroups (A, B1, B2, D and E) [330]. Genetically distinct ETEC strains, frequently found among asymptomatic subjects show high antigen heterogeneity with regard to virulence traits and serotypes [333].

Conclusions:

The genomic plasticity of *E. coli* strains is noteworthy, as can be seen by the variety of strains ranging from commensal residents of the gastrointestinal tract to assorted pathogens that are able to promote intestinal or extraintestinal illnesses with different clinical consequences. It is thus important to note that the continuous evolution of the *E. coli* genome has hindered the classification of certain *E. coli* isolates into a pathotype, because some isolates combine the main virulence characteristics of different pathotypes and are thus considered hybrid pathotypes with the potential of allowing the rise of new and more virulent pathogenic *E. coli* hybrids. Whole-genome sequencing has provided a great amount of useful information on the genome of pathogenic *E. coli*, which will help improve diagnosis, typing, disease management, epidemiology and outbreak investigations as well as helping to monitor the spread of pathogens [5]. Despite the recent advances in our knowledge of the genetic background and pathogenicity of strains of different DEC pathotypes, various novel genes encoding unknown functions are yet to be characterized to further our understanding of the interactions of these pathogens with their hosts.

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