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Escherichia coli as a specialized bacterial pathogen

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ABSTRACT

The widespread species *Escherichia coli* includes a broad variety of different types, ranging from highly pathogenic strains to a virulent isolates. Pathogenicity correlates with the expression of disease-related factors that are present in pathogenic bacteria, but are generally absent from non-pathogenic species. The pathogenic *E. coli* is divided into those strains causing disease inside the intestinal tract and others capable of infection at extra-intestinal sites. Due to the ease of access of pathogens ingested with food, the human gastro-intestinal tract is susceptible to diarrhoeagenic *E. coli* infections. Several *E. coli* pathotypes have been implicated with diarrhea illness, a major public health problem worldwide. This review deals with different strategies regarding *E. coli* as a pathogen and the virulence traits of its pathotypes highlighting the species as a specialized pathogen.

Key words: Pathogenicity; Virulence; *Escherichia coli* pathotypes; Diarrhegenic *E. coli* categories; Public health

RESUMO

A espécie *Escherichia coli* inclui uma variedade de cepas muito virulentas e isolados comensais. A patogenicidade está correlacionada com a expressão de fatores relacionados à doença que estão presentes em bactérias patogênicas, mas normalmente ausentes em espécies não patogênicas. *E. coli* patogênica é dividida em cepas que podem causar infecção no trato intestinal e outras que causam doenças em sítios extra-intestinais. Devido à facilidade de acesso de patógenos ingeridos com alimentos, o trato gastrintestinal humano é susceptível à infecções ocasionadas por *E. coli* diarreiogênica. Vários patótipos de *E. coli* tem sido implicados em doença diarréica, um dos maiores problemas de saúde pública no mundo. Esta revisão descreve diferentes estratégias de *E. coli* como patógeno e os fatores de virulência de seus patótipos enfatizando esta espécie como um patógeno extremamente especializado.

Palavras-chave: Patogenicidade; Virulência; Patótipos de *Escherichia coli*; Categorias diarreiogênincas de *Escherichia coli*; Saúde Pública

1. INTRODUCTION

Escherichia coli, originally called "*Bacterium coli* commune," was first isolated from the feces of a child in 1885 by Theodor Escherich and nowadays is the best-studied bacterium. *Escherichia coli* is a common inhabitant of the gastrointestinal tract of humans and animals. There are *E. coli* strains that are harmless commensals of the intestinal tract and others that are major pathogens of humans and animals. The pathogenic *E. coli* is divided into those strains causing disease inside the intestinal tract and others capable of infection at extra-

intestinal sites (Kaper et al., 2004). *Escherichia coli* is easily cultured in the clinical laboratory, but the identification of the different pathogenic genotypes requires virulence gene detection methods. *Escherichia coli* can be found secondarily in soil and water as the result of fecal contamination. Classically, its detection has been used as an indicator of poor water and/or food quality. From biochemical, physiological and genetic perspectives, *E. coli* is one of the best understood and characterized living organisms, with laboratory studies on model strains such as *E. coli* K-12 taking place over the past sixty years.

2. *E. coli* TAXONOMY, PHYLOGENY AND CLONALITY

The comparative analysis of 5S and 16S ribosomal RNA sequences suggest that *Escherichia* and *Salmonella* diverged from a common ancestor between 120 and 160 million years ago, which coincides with the origin of mammals (Ochman & Wilson, 1987). *Escherichia* and *Shigella* have been historically separated into different genera within the *Enterobacteriaceae* family. DNA sequence analysis of their genomes reveals a high degree of sequence similarity and suggests that they should be considered a single species. Currently, the two organisms continue to be regarded as two different genera anchored in the historical perception of their disease potential and ecology.

Besides *E. coli*, there are other species within the genus, *E. adecarboxylata*, *E. blattae*, *E. fergusonii*, *E. hermanii* and *E. vulneris*. Little is known about the distribution, biology or interrelatedness of these species.

Evolutionary studies based on either DNA sequence analysis or multilocus enzyme electrophoresis has identified clonal phylogenetic groupings of *E. coli*. Phylogenetic studies have principally used the *E. coli* reference (ECOR) strain collection as a common reference for current evolutionary comparisons (Ochman & Selander, 1984). Six phylogenetic groups are generally recognized among the ECOR strains: A, B1, B2, C, D and E, (Selander et al., 1987). For the phylogenetic groups, there are some general biotype clusterings but in general, there is notably little association between host strain source and clonal designation.

3. EVOLUTION OF *E. coli* PATHOTYPES

Virulent strains of *E. coli* are differentiated clinically from one another on the basis of epidemiology, signs and symptoms of their respective diseases, microscopic observations of their interactions with host cells, and of biotypes and unique gene markers. *E. coli* colonizes the gastrointestinal tract of most warm-blooded animals within hours or a few days after birth. The bacterium is ingested in foods or water or obtained directly from other individuals handling the infant. The human bowel is usually colonized within 40 hours of birth. *E. coli* can adhere to the mucus overlying the large intestine. Once established, an *E. coli* strain may persist for months or years. Resident strains shift over a long period, and more rapidly after enteric infection or antimicrobial chemotherapy that perturbs the normal microbiota. The basis for these shifts and the ecology of *Escherichia coli* in the intestine of humans are poorly understood despite the vast amount of information on almost every other aspect of the organism's existence.

Infections due to pathogenic *E. coli* may be limited to colonization of a mucosal surface or can disseminate throughout the body and have been implicated in urinary tract infection, sepsis/meningitis and gastrointestinal infections (Nataro & Kaper, 1998). Due to the ease of access of pathogens ingested with food, the human gastro-intestinal tract is susceptible to diarrhoeagenic *E. coli* infections. Several *E. coli* pathotypes have been implicated with diarrhoeal illness, a major public health problem worldwide, with over two million deaths occurring each year (Kosek et al., 2003). The evolution of independent pathogenic types of *E. coli* is striking and to date unmatched by any other bacterial genus. How this occurred is unclear, but it is likely linked to the concomitant evolution of different mammalian hosts. Initially isolates involved in both intestinal and extra-intestinal human disease were thought to be concentrated mostly within the single, B2 ECOR phylogenetic group with a smattering of isolates found in the D group. However, some studies indicate that extensive horizontal

transmission of blocks of genes has occurred across the different phylogenetic clonal lines. Thus, with the possible exception of the enterohemorrhaghic *E. coli* O157:H7, many of the *E. coli* pathotypes apparently do not have unique evolutionary origins. The virulent *E. coli* strains have arisen independently on multiple occasions within clonal lines (Pupo et al., 1997). On the basis of the relative number of isolates identified within the general diarrheagenic and extra-intestinal pathotype groupings, the former are more frequently found in the A, B1 and D phylogenetic groups, whereas the extra-intestinal *E. coli* strains are more common to the B2 lineage (Johnson, 2002).

4. EVOLUTION OF *E. coli*

Genome sequencing of three different *E. coli* strains (laboratory K-12 strain MG1655, enterohemorrhaghic O157:H7 strain EDL933, and an uropathogenic isolate, CFT073) reveals an unambiguous conservation of nearly 40% of the core gene sequences among the three isolates (Welch et al., 2002). The synteny of the genes around the circular chromosomes is nearly intact and representative of the classic *E. coli* K-12 gene map (Berlyn, 1998). The phylogenies used to build the ECOR phylogeny is also reflected in the relatively slow divergence of the core gene sequences. *Escherichia coli*, even the pathogenic types, at some point thrives in a mammalian intestine, yet they are also capable of surviving periods in the outside environment. However, there is striking evidence that concurrent with the vertical evolutionary processes that account for the ECOR-based phylogenetic differences, horizontal genetic transfer has occurred frequently and had the greatest impact on genetic differences among strains. Comparisons of one genome with another reveal hundreds of instances where insertions, substitutions and deletions of large blocks of DNA have disrupted the order of the core genes. The majority of the unique genes are on segments that vary from 4 kb to over 100 kb. Aside from complete and partial prophages, the largest blocks of strain-specific genes share some common features. The G+C content is often lower than the typical 50–52 mol% for the core genes. The predicted codons used in the EDL933 or CFT073 strain-specific genes is skewed towards greater use of tRNAs typically less abundant than those involved in *E. coli* K-12 translation. These observations suggest that the larger gene blocks originate in and are mobilized from genera much different than any of the close relatives of *E. coli* (such as *Salmonella*). Prior to genomic sequencing, it was recognized that many of the most significant virulence genes (adhesins, extracellular protein secretion systems, and toxins) for several *E. coli* pathotypes were clustered together in these large blocks. Hacker et al. coined the term "pathogenicity-associated islands" (PAIs) to describe these (Hacker et al., 1997). Still unclear is whether much of the added, unique genetic material has anything to do with pathogenicity.

5. HABITAT

Escherichia coli are common inhabitants of the small intestine and large intestine of mammals. They are often the most abundant facultative anaerobes in this environment. The human colon maintains a microbial density approaching 10^{12} organisms per gram of feces, representing a perfectly balanced ecosystem. The commensal microbiota consists of more than 400 species and lives in perfect harmony with the human intestine (Hooper & Gordon, 2001).

They can occasionally be isolated in association with the intestinal tract of no mammalian animals and insects. The presence of *E. coli* in the environment is usually considered to reflect fecal contamination and not the ability to replicate freely outside the intestine. There is evidence however to suggest that *E. coli* may freely replicate in tropical fresh water (Bermudez & Hazen, 1988).

6. CELL STRUCTURE AND PHYSIOLOGY

Escherichia coli are Gram-negative, nonsporeforming bacilli. They are approximately 0.5 μm in diameter and 1.0–3.0 μm in length. Within the periplasm is a single layer of peptidoglycan. The peptidoglycan has a typical subunit structure where the *N*-acetylmuramic acid is linked by an amide bond

to a peptide consisting of L-alanine, D-glutamic acid, *meso*-diaminopimelic acid and D-alanine.

E. coli are commonly motile in liquid by means of peritrichous flagella. *E. coli* are commonly fimbriated. The type 1 pili are the most common and are expressed in a phase switch on or off manner that leads to piliated and nonpiliated states (Eisenstein, 1987). One of the traits commonly encoded on the larger genetic islands of the different pathotypes of *E. coli* are additional pili (chaperone-usher and type IV pili families and non-pili adhesions (Schreiber & Donnenberg, 2002).

Among *E. coli* isolates, there is considerable variation and many combinations of somatic (O and K) and flagellar (H) antigens. Among pathogenic strains, there are few patterns of these antigens and few phylogenetic groupings. For *E. coli*, there are over 150 antigenically unique O-antigens (Whitfield & Valvano, 1993). K type capsular material occurs in two or four forms on the basis of physical, biochemical and genetic criteria (Whitfield & Roberts, 1999). Over 80 serologically and chemically distinct capsular polysaccharides have been recognized. In addition, a slime layer, colonic acid extracellular polysaccharide, is common to many *E. coli* isolates and can be co-expressed with some K-type capsules. There are 53 H-antigen specificities among *E. coli.*.

E. coli is a facultative anaerobe. It is capable of reducing nitrates to nitrites. When growing fermentatively on glucose or other carbohydrates, it produces acid and gas (mainly H_2 and CO_2). By traditional clinical laboratory biochemical tests, *E. coli* is positive for indole production and the methyl red test (VM). Most strains are oxidase, citrate, urease and hydrogen sulfide negative. The classic differential test to primarily separate *E. coli* from *Shigella* and *Salmonella* is the ability of *E. coli* to ferment lactose, which the latter two genera fail to do. Aside from lactose, most *E. coli* strains can also ferment D-mannitol, D-sorbitol, and L-arabinose, maltose, D-xylose, trehalose and D-mannose. There are limited instances where pathogenic strains differ from the commensals in their metabolic abilities. For example, commensal *E. coli* strains generally use sorbitol, but *E. coli* O157:H7 does not. Most diarrheagenic strains cannot utilize D-serine as a carbon and nitrogen source, but uropathogenic and commensal fecal strains can use this enantiomer of serine (Roesch et al., 2003).

Most *E. coli* strains are capable of growing over a wide range in temperature (approximately 15–48°C). The growth rate is maximal in the narrow range of 37–42°C. *Escherichia coli* can grow within a pH range of approximately 5.5–8.0 with best growth occurring at neutrality. Some diarrheagenic *E. coli* strains have the ability to tolerate exposure to pH 2.0. Such an acid shock mimics transit through the stomach and induces expression of sets of genes involved in survival and pathogenesis.

Iron metabolism of *E. coli* is a well-studied topic (Braun & Braun, 2002). Ferric iron is brought into *E. coli* by chelating compounds such as citrate, enterobactin, aerobactin, yersinabactin and heme. These chelators each have highly specific outer membrane proteins that enable their uptake across the outer membrane where they are then brought across the cytoplasmic membrane by ATP binding cassette (ABC) transport systems. One trait that sets many of the pathogenic *E. coli* apart from the normal intestinal *E. coli* is the ability to acquire ferric iron from a wide array of chelators. The multiple gene systems enable adaptation to sites where iron might be limited by host antibacterial activities (Torres et al., 2001). These virulence-enhancing iron acquisition systems, such as aerobactin, are often encoded by plasmids or are present on pathogenicity islands.

7. *E. coli* PATHOTYPES

Although most *E. coli* are harmless commensals of the human and animal intestine, certain specific, highly-adapted *E. coli* strains are capable of causing a variety of different diseases. Infections due to pathogenic *E. coli* may be limited to colonization of a mucosal surface or can disseminate throughout the body and have been implicated in urinary tract infection, sepsis/meningitis and gastrointestinal infections (Nataro & Kaper, 1998).

One of the most notable features of *E. coli* is broad diversity of disease-causing genotypes. The diseases can encompass different symptoms and gastrointestinal tract

pathologies, but there are also diseases at extraintestinal sites. These different genotypes and their disease-causing abilities lead to categories of *E. coli* often referred to as pathotypes. There are seven intestinal and two extraintestinal pathotypes currently recognized.

8. ENTEROTOXIGENIC *E. coli* (ETEC)

ETEC (enterotoxigenic *E. coli*) strains are a major cause of secretory diarrhea in both humans and animals (Bern et al., 1992). ETEC produce toxins which are heat-labile (LT) and/or heat-stable (STa and STb) that are also causing diarrhea. A frequent cause of diarrhea in both humans and animals, enterotoxigenic *E. coli* (ETEC) are estimated to cause 600 million cases of human diarrhea and 800,000 deaths worldwide principally in children under the age of 5. Economically significant ETEC diarrheal disease in animals occurs in neonatal calves, pigs and lambs. ETEC cause watery diarrhea that can be mild in nature or in some instances can be a severe, cholera-like illness where rapid dehydration can be life-threatening. In endemic areas of ETEC-mediated diarrhea, infants and children under the age of 5 are the most commonly affected. ETEC exposure in endemic areas is one of the most common causes of traveler's diarrhea.

One of the principal virulence factors for this pathogen is the heat-labile enterotoxin (LT), which interestingly shares structural and functional similarity to the *Vibrio cholerae* cholera toxin (Spangler, 1992). LT toxin has a classic AB toxin subunit holotoxin structure. The B subunits (as a pentamer) bind to host cell surface GM1 and GD1b gangliosides and the A subunit enzymatically ADP-ribosylates the α-subunit of stimulatory G protein. This G protein regulates host cell adenylate cyclase and LT-mediated modification leads to its permanent activation and an increase in intracellular cAMP levels. This eventually leads to activation of the chloride ion channel of the intoxicated cells, increased chloride ion secretion into the intestinal lumen, and decreased sodium and chloride absorption. The overall result is to reverse the normal intestinal osmotic gradient and cause a net water loss into the gut lumen. Aside from LT, many ETEC strains also express heat-stabile enterotoxins (STs), which also contribute to the watery diarrhea. There are two structurally distinct STs, STa and STb. The STs are small polypeptides that share the common features of heat stability and multiple intramolecular disulfide bonds. The action of STa is well understood. It binds to the extracellular domain of plasma membrane-embedded guanylate cyclase. The ETEC toxins are secreted in the terminal small intestine where the ETEC adhere by expression of a complex and diverse group of surface proteins commonly referred to as "colonization factors" (Gaastra & Svennerholm, 1996).

9. ENTEROPATHOGENIC *E. coli* (EPEC)

These organisms are a significant cause of infant diarrhea in developing nations. Enteropathogenic *E. coli* (EPEC) were historically recognized on the basis of serotypes such as O55:H6 and O127:H6. EPEC (enteropathogenic *E. coli*), an established etiological agent of human infantile diarrhea, is a pathogen that subverts intestinal epithelial cell function to produce distinctive “attaching and effacing” (A/E) lesions. These lesions are characterized by localized destruction (effacement) of brush border microvilli, intimate bacterial attachment to the host-cell membrane and formation of an actin-rich cytoskeletal structure beneath intimately attached bacteria.

In developing countries, enteropathogenic *E. coli* (EPEC) is one of the most common pathogens. In Brazil, for example, EPEC can be isolated from stools of over 40% of infants with acute diarrhea and was associated with a mortality of 7% (Fagundes Neto & Scaletsky, 2000). The pathogenesis of EPEC is in some way unique for enteric bacterial pathogens since it is essentially noninvasive and produces no toxins. The attachment of EPEC to the epithelial cell, described as localized adherence, results in a so-called attaching and effacing lesion (A/E) (Celli et al., 2000). EPEC also uses its TTSS to deliver bacterial effector proteins like EspA and EspB into the host cell to alter the cytoskeleton (Knutton et al., 1998). However, the most fascinating aspect of EPEC pathogenesis is that it inserts, through the type III secretion system, its own receptor into the host cell. Rather than

searching for a receptor it provides its own receptor and uses it when needed. Thus, EPEC is able to insert the Tir receptor into the host cell membrane where it serves as the receptor for the bacterial protein intimin after it is phosphorylated on tyrosine by the host cell (Deibel et al., 1998).

EPEC disease is generally the result of growth of EPEC in the small intestine. EPEC cause a watery diarrhea that may contain mucus but typically does not have blood in it. Vomiting, fever, malaise and dehydration are also associated. The symptoms may last for a brief period of several days, although instances of long, chronic EPEC disease have been noted.

Some of the mechanisms of EPEC pathogenesis are well understood. For example, the A/E lesion is the result of a complex system of EPEC proteins that are injected into the host intestinal epithelial cell. The A/E lesion represents a dramatic rearrangement of the epithelial cytoskeleton where there is an accumulation of actin directly below the attached EPEC cell. This is described as an actin pedestal for the attached bacterial cell. There is a specific pathogenicity island, termed the "locus of enterocyte effacement" (LEE), that encodes the genes responsible for the A/E lesion. The LEE encodes a type III secretion system that provides the intimate adhesin (intimin) its receptor (which is injected into and then presented on the surface of the host cell), and the injected proteins responsible for changes in host cell signaling mechanisms, including actin pedestal formation (Jerse, 1990). Common to most EPEC strains are plasmids EAF ("EPEC adherence factor"), which encode an adherence factor, the bundle-forming pilus (bfp), (Nataro et al., 1987). Results of human volunteer studies indicate the EAF plasmid is necessary to cause disease (Levine et al., 1985). Although the A/E characteristic is critical for causing EPEC disease, probably through destruction of microvilli, the precise mechanism for the diarrhea is not completely understood and may reflect the diversity of EPEC strains.

10. ATYPICAL ENTEROPATHOGENIC *E. coli* (A-EPEC)

A-EPEC (atypical enteropathogenic *E. coli*) is EPEC that have lost the EAF (EPEC adherence factor) plasmid. Some studies (Sousa & Dubreuil, 2001; Scotland et al., 1991) had shown that, probably, A-EPEC is another EPEC category associated with diarrhea of clinical importance.

Recent attention has focused on greater understanding of atypical EPEC strains (Trabulsi et al., 2002). These strains more commonly cause diarrhea in industrialized nations than the typical EPEC strains. In addition the atypical EPEC strains have animal and human reservoirs, whereas the typical isolates are almost always associated with human fecal contamination. The atypical isolates have the ability to cause A/E lesions but lack the EAF plasmids. They often have additional virulence factors not seen among the typical strains. For example, they have significant portions of the pO157 virulence plasmid common to enterohemorrhagic *E. coli* O157:H7 strains and may have a heat stable enterotoxin (EAST-1).

11. ENTEROHAEMORRAGIC *E. coli* (EHEC)

EHEC (enterohaemorragic *E. coli*) strains are implicated in food-borne diseases principally due to ingestion of uncooked minced meat and raw milk. These strains produce shiga-like toxin 1 (stx1), shiga-like toxin 2 (stx2) and variants thereof. They are involved in episodes of diarrhea with complications. Serotype O157:H7 is the prototype of increasing importance and is associated with hemorrhagic colitis, bloody diarrhea and the hemolytic uremic syndrome (HUS). EHEC typically cause an afebrile bloody colitis and, in about 10% of patients, this infection can be followed by HUS (Pickering et al., 1994). Like EPEC, EHEC elicit an attaching and effacing lesion of the intestinal mucosa, a phenotype that requires a functional *eaeA* chromosomal gene.

These organisms share the ability to cause A/E lesions with EPEC but enterohemorrhagic *E. coli* (EHEC) are set apart from EPEC by possession of Shiga-like toxins and the clinical presentation of their disease. EHEC cause disease of the large intestine that may present as simple watery diarrhea and then progress to bloody stools with ulcerations of the bowel. In a small subset of diseased individuals

there is onset several days later of severe, life-threatening hemolytic-uremic syndrome (HUS). HUS involves a triad of hemolytic anemia, thrombocytopenia and renal failure. The transmission of EHEC disease in humans is through ingestion of contaminated beef or foods contaminated with cattle feces. In cattle, the EHEC strains are transient members of the intestinal microflora where they do not apparently cause disease. One of the remarkable features of EHEC is its low infection dose of 10–100 organisms. Clearly this microorganism has special acid-tolerance ability when compared to many other enteric bacterial pathogens. Children under the age of five are the major victims of EHEC disease, although the elderly may also exhibit bloody diarrhea and HUS. Epidemiologically in the United States, Japan, and Great Britain, a single serotype O157:H7 is the most common EHEC strain. In other parts of the world, this strain can be observed causing disease, but other serotypes (e.g., O26 and O111) cause a similar disease as well.

All factors that lead to HUS are unknown except Shiga toxin (sometimes referred to as "Shiga-like toxin" or "verotoxin"), which probably plays an important role in renal injury. Purified Stx-1 injected intravenously in baboons leads to renal disease with histopathology similar to EHEC-mediated HUS (Tailor et al., 1999). The Shiga toxin inhibits protein synthesis through cleavage of ribosomal RNA. Because EHEC do not cause bacteremia, Shiga toxin is thought to be released while the organism is growing in the large bowel, where it gets disseminated systemically to cause damage to renal endothelial cells and release of inflammatory mediators that eventually damage the kidney.

There are two evolutionarily related forms of Shiga toxin in *E. coli* (Shiga toxin 1 and Shiga toxin 2). They share approximately 55% amino acid sequence similarity. Shiga toxin 1 is only different from the Shiga toxin of *Shigella* dysenteriae by a single amino acid substitution.

There are many Shiga toxin positive *E. coli* strains (STEC) that are not associated with enterohemorrhagic colitis. It is a heterogeneous group that is occasionally associated with HUS, but their general benign nature may be due to their lack of the LEE pathogenicity island and plasmid virulence factors. The ubiquitous dissemination of the distribution of Shiga toxin genes among *E. coli* strains is due to their transmission as part of lambdoid phages. The EHEC O157:H7 strain likely originated in an O55 EPEC strain where a series of genetic events lead to acquisition of shiga toxin-encoding prophages and a large virulence plasmid, pO157 (Reid et al., 2000). The precise role of pO157 in EHEC pathogenesis is unknown but may involve some putative toxin genes and a mucin-specific zinc metalloprotease, StcE (Grys et al., 2005).

12. ENTEROINVASIVE *E. coli* (EIEC)

EIEC (enteroinvasive *E. coli*) cause a broad spectrum of human's diseases. They are biochemically, genetically and pathogenetically closely related to *Shigella* spp. Both characteristically cause an invasive inflammatory colitis, but either may also elicit a watery diarrhea syndrome indistinguishable from that caused by other *E. coli* pathogens. The pathogenesis of disease caused by EIEC and *Shigella* involves cellular invasion and spread, and requires specific chromosomal and plasmid-borne virulence genes (Nataro & Kaper, 1998).

These organisms are pathogenetically so closely related to *Shigella* species that the nomenclature distinction is questionable. There are a few biochemical traits that can be used to distinguish enteroinvasive *E. coli* (EIEC) from *Shigella*, but the principal virulence genes are shared. The diagnostic confusion between *Shigella* and EIEC is evident in that EIEC isolates are nonmotile and 70% are nonlactose fermenters (Silva et al., 1980). In addition, EIEC share with *Shigella* the inability to decarboxylate lysine, a trait common to other *E. coli*. The traits that EIEC share with *E. coli* but not *Shigella* are the ability to produce gas from glucose and fermentation of xylose.

EIEC cause invasive inflammatory colitis and dysentery with a clinical presentation (blood and mucous stools accompanied by fever and severe cramps) identical to the disease caused by *Shigella* species. EIEC/*Shigella* invade intestinal epithelium, principally in the large intestine. Once inside the cells, they lyse the phagocytic vesicle and replicate freely in the

host cell cytoplasm. The EIEC/*Shigella* cells then spread to neighboring host cells by a motility process whereby actin is nucleated on one pole of the bacillus and subsequent actin polymerization propels the bacterial cell (Goldberg & Theriot, 1995). Many of genes necessary for cellular invasion and disease are carried on a large (>200-kb) plasmid found in both EIEC and *Shigella*. A system of type III secretion genes important for delivery of modifiers of host cell signaling and membrane lysis are found on these plasmids. In addition, the plasmid encodes an outer membrane protein (IcsA) that is localized on one pole of the bacterium and directs the actin microfilament polymerization necessary for spread of bacteria to other host cells. EIEC/*Shigella* rarely invades the bloodstream, but they do invade the lamina propria immediately under the intestinal epithelium, where interaction with macrophages causes the release of pro-inflammatory mediators and even induction of apoptosis. Interestingly, the inability to decarboxylate lysine, a trait shared by EIEC and *Shigella*, is the result of mutations and gene rearrangements at the *cadC* gene. The decarboxylation of lysine results in cadverine, which acts as an inhibitor of inflammation and migration of neutrophils into the lamina propria. The lack of this function is hypothesized to be a pathoadaptive trait that enables EIEC/*Shigella* to cause disease (Casalino et al., 2003).

13. DIFUSELY ADHERENT *E. coli* (DAEC)

DAEC (diffusely adherent *E. coli*) strains are defined by the presence of the diffusely adherent pattern in the HEp-2 adherence assay, and cause a watery diarrhea syndrome in adults and children. The pathogenesis of DAEC diarrhea is not as yet elucidated, but several virulence-related characteristics have been identified (Nataro & Kaper, 1998; Servin, 2005). Most DAEC strains express a surface fimbria designated F1845 that may be encoded either by the chromosome or a plasmid. It was shown that DAEC could induce characteristic elongated projections from the surface of epithelial cells in culture.

The epidemiology and pathogenesis of the diffusely adherent *E. coli* (DAEC) are not well understood. DAEC may cause diarrhea in very young childrenl (Scaletsky et al., 2002). They are differentiated from the other diarrhegenic *E. coli* by a distinct adhesion phenotype, again on HEp-2 cells. The adhesion is brought about by F1845 fimbriae, which belong to the Dr family of adhesins (also found in some UPEC strains). The Dr adhesins recognize and bind to host cell surface decay accelerating factor (DAF). DAEC bound to cultured cells elicit a cytopathic phenotype and activation of signal-transduction pathways. The relative significance of DAEC as a pathogen and its mechanisms for causing disease await further study.

14. ENTEROAGGREGATIVE *E. coli* (EAEC)

EAEC (enteroaggregative *E. coli*) strains are defined by their distinctive adherence pattern on HEp-2 cells in culture (Nataro & Kaper, 1998). The essential element of the aggregative phenotype is the stacked brick pattern by lying side-by-side with an appreciable distinction of where one bacterium begins and another ends. The EAEC are a heterogeneous group of bacteria that display a wide array of virulence factors (Sousa & Dubreuil, 2001). EAEC are pathogens associated with persistent diarrhea in the developing world and have been implicated recently in the developed world as causes of both outbreaks and sporadic diarrhea among AIDS patients.

These organisms are defined as *E. coli* that do not possess LT enterotoxin or Shiga toxins but adhere to cultured HEp-2 cells in self-aggregates that are classically referred to as "stacked bricks. Clearly, many *E. coli* strains can mediate the "stacked brick" adhesive phenotype, but there is a subset of these that are bona fide human diarrheal pathogens. Enteroaggregative *E. coli* (EAEC) disease, as described by human volunteers, is a watery diarrhea that occurs in some cases with abdominal cramps, but no fever. There is no invasion of the bloodstream. The disease seen in natural EAEC outbreaks is often reported as a persistent, seemingly chronic watery diarrhea. These small epidemics occur in both developing as well as industrialized countries. There are no common serotypes of EAEC to aid in their

recognition in the clinical laboratory. The pathogenesis of EAEC disease is poorly understood, although several potential virulence factors are common to EAEC isolates. EAEC express a fimbrial adhesin called "aggregative adherence fimbriae" ("AAF"). EAEC isolates often produce a mucinase called "Pic" whose gene has the ability to express from its nonencoding DNA strand a smaller gene that encodes an enterotoxin (*Shigella* enterotoxin [ShET1]) first described in *Shigella* strains. EAEC strains often produce a heat stable enterotoxin EAST1 that is homologous to the ST1 of ETEC.

15. EXTRAINTESTINAL E. *coli* STRAINS

Two pathotypes of *E. coli* are generally recognized causes of extraintestinal human diseases (neonatal septicemia/meningitis *E. coli* and the urinary tract and bloodstream *E. coli*). Some isolates, *E. coli* O18:K1:H7, are recognized as having the potential to cause both invasive neonatal diseases and urinary tract infections (Johnson et al., 2001).

UPEC are a heterogeneous group of clones (Donnenbert & Welch, 1996). Within the UPEC grouping are cystitis, pyelonephritis and urosepsis isolates. These strains are the principal causes of morbidity and mortality from either community or hospital-acquired *E. coli* infections. Approximately 60% of adult women will have a UTI in their lifetimes. As much as 90% of all community-acquired UTIs and greater than 30% of the hospital-acquired UTIs are caused by *E. coli*. There have been reports of community-wide outbreaks of UTIs by multidrug resistant UPEC clones (Manges et al., 2001

UPEC strains isolated from women with pyelonephritis, but who have no underlying medical complications, often possess specific O serotypes (O1, O2, O4, O6, O7, O18 and O75 (Orskov & Orskov, 1983). What further suggests that these *E. coli* strains are extraordinary is that they are especially capable of invading the bloodstream (Johnson et al., 1994). Many of the known or putative virulence factors for these strains are not shared with common fecal *E. coli* strains. Examples of such factors are adhesins (e.g., Pap, Sfa, and Dra), hemolysin (Hly), cytotoxic necrotizing factor-1 (CNF-1), and the aerobactin (Aer) iron-sequestration systems. There are additional factors that are common to all *E. coli* that are critical for pathogenesis of extraintestinal disease. The principal factors are lipopolysaccharide, capsule production, and type 1 pili. The type 1 pili appear to play a particularly critical role in the initial colonization of the bladder (Struve & Krogfelt, 1999). It is suggested that intracellular cellular invasion leads to persistent infections of the urinary tract by successive rounds of intracellular infection, multiplication, release and reinfection of superficial, as well as deeper bladder epithelial layers (Mulvey et al., 2001). Currently no information is available about genes other than those for type 1 pili that are needed for cellular invasion.

16. FUTURE PROSPECTS FOR THE COMPREHENSION OF *E. coli*

Knowledge of the pathogenic mechanisms of *E. coli* pathotypes has led to the development of rational interventions for the treatment and prevention of *E. coli*-induced diseases.

Physiologically, *E. coli* is versatile and well adapted to its characteristic habitats. *E. coli* can respond to environmental signals such as chemicals, pH, temperature, osmolarity, and other stimulants, in a number of very remarkable ways considering it is a single-celled organism. Because of its natural habitat and its ability in subvert, circumvent and/or evade the immune defenses, the surviving of these bacteria is safeguarded in nature. The acquisition of different virulence traits, the continuous exchange of genetic elements and the expression of virulence genes generally regulated by environmental factors probably will reveal different strategies shared by *E. coli* strains.

Continuous research and investigations into *E. coli* virulence are providing us with useful insights into the origins and evolution of this versatile bacterial pathogen.

17. REFERENCES

BERLYN, M.K. Linkage map of *Escherichia coli* K-12, Edition 10: The traditional map.

Microbiology and Molecular Biology Reviews, v. 62, 1998, p.814–984.

BERMUDEZ, M., HAZEN, T.C. Phenotypic and genotypic comparison of *Escherichia coli* from pristine tropical waters. *Applied and Environmental Microbiology*, v. 54, 1988, p.979–983.

BERN, C., MARTINES, J., DE ZOYSA, I., et al. The magnitude of the global problem of diarrhoeal diseases: a ten-year update. *Bulletin WHO*, v. 70, 1992, p.705-714.

BRAUN, V., BRAUN, M. Iron transport and signaling in *Escherichia coli*. *FEBS Letters*, v. 529, 2002, p.78–85.

CASALINO, M., LATELLA, M.C., PROSSEDA, G. et al. *CadC* is the preferential target of a convergent evolution driving enteroinvasive *Escherichia coli* toward a lysine decarboxylase-defective phenotype. *Infection and Immunity,* v. 71, 2003, p.5472-5479.

CELLI, J., DENG, W., FINLAY, B.B. Enteropathogenic *Escherichia coli* (EPEC) attachment to epithelial cells: exploiting the host cytoskeleton from the outside. *Cell Microbiology*, v. 2, 2000, p.1-9.

DEIBEL, C., KRAMER, S., CHAKRABORTY, T., et al. *EspE*, a novel secreted protein of attaching and effacing bacterial, is directly translocated into effected host cells, where it appears as a tyrosine-phosphorylated 90kDa protein. *Molecular Microbiology*, v. 28, 1998, p.463-474.

DONNENBERG, M.S., WELCH, R.A. Virulence determinants of uropathogenic *E. coli.* In: H. L. a. J. W. W. Mobley (Eds.)Urinary Tract Infections: Molecular Pathogenesis and Clinical Management. ASM Press Washington, DC, 1996. p.135–174

EISENSTEIN, B.I. Fimbriae. In: Roy, C., Frederick, C., et al. (Eds.) *Escherichia coli* and *Salmonella Typhimurium*, 1st ed. ASM Press Washington, DC, 1987, p. 84–90.

FAGUNDES-NETO U., SCALETSKY, I.C. The gut at war: the consequences of enteropathogenic *Escherichia coli* infection as a factor of diarrhea and malnutrition. *Sao Paulo Medical Journal*, v. 118, 2000, p.21-29.

GAASTRA, W., SVENNERHOLM, A.M. Colonization factors of human enterotoxigenic Escherichia coli (ETEC). *Trends in Microbiology*, v. 4, 1996, p.444–452.

GOLDBERG, M.B., THERIOT, J.A. *Shigella flexneri* surface protein IcsA is sufficient to direct actin-based motility. *Proceedings of the National Academy of Science of the USA*, vol. 92, 1995, p.6572–6576.

GRYS, T.E., SIEGAL, M., LATHEM, W.L., et al. StcE protease contributes to the intimate adherence of *Escherichia coli* O157:H7 to HEp-2 cells. *Infection and Immunity*, 2005, v. 73, p.1295-1303.

HACKER, J., BLUM-OEHLER, G., MUHLDORFER, G.I., et al. Pathogenicity islands of virulent bacteria: Structure, function and impact on microbial evolution. *Molecular Microbiology*, v. 23, 1997, p.1089–1097.

HOOPER, L.V., GORDON, J.I. Commensal Host-Bacterial relationship in the gut. *Science*, v. 292, 2001, p.1115-1118.

JERSE, A.E., YU, J., TALL, B.D. et al. A genetic locus of enteropathogenic *Escherichia coli* necessary for the production of attaching and effacing lesions on tissue culture cells. *Proceedings of the National Academy of Science of the USA*, v. 87, 1990, p.7839–7843.

JOHNSON, A.T. Evolution of pathogenic *Escherichia coli*. In: Donnenberg, M.S. (Ed.). *Escherichia coli* Virulence Mechanisms of a Versatile Pathogen. Academic Press New York, NY, 2002, p.55–77.

JOHNSON, J.R., ORSKOV, I., ORSKOV, F., et al. O, K, and H antigens predict virulence factors, carboxylesterase B pattern, antimicrobial resistance, and host compromise among *Escherichia coli* strains causing

urosepsis. *Journal of Infectious Diseases*, v. 169, 1994, p.119–126.

JOHNSON, J.R. Virulence factors in *Escherichia coli* urinary tract infection. *Clinical Microbiology Reviews*, v. 4, 1991, p.80–128.

KAPER, J.B., NATARO, J.P., MOBLEY, H.L. Pathogenic *Escherichia coli. Nature Reviews Microbiology,* v. 2, 2004, p.123–140.

KNUTTON, S., ROSENSHINE, I., PALLEN, M.J., et al. A novel EspA-associated surface organelle of enteropathogenic *Escherichia* involved in protein translocation into epithelial cells. *EMBO Journal*, v.17, 1998, p.2166-2176.

KOSEK, M., BERN, C., GUERRANT, R.L. The magnitude of global burden of diarrhoeal disease from studies published 1992-2000. *Bulletin of the World Health Organization*, v. 81, 2003, p197-204.

LEVINE, M.M., NATARO, J.P., KARCH, H., et al. The diarrheal response of humans to some classic serotypes of enteropathogenic *Escherichia coli* is dependent on a plasmid encoding an enteroadhesiveness factor. *Journal of Infectious Diseases,* v. 152, 1985. p.550–559.

MANGES, A.R., JOHNSON, J.R. FOXMAN, B., et al.; Widespread distribution of urinary tract infections caused by a multidrug-resistant *Escherichia coli* clonal group. *New England Journal of Medicine*, v. 345, 2001. p.1007–1013.

MULVEY, M.A., SCHILLING, J.D., HULTGREN, S.J. Establishment of a persistent *Escherichia coli* reservoir during the acute phase of a bladder infection. *Infection and Immunity*, v. 69, 2001. p.4572–4579.

NATARO, J.P., KAPER, J.B., ROBINS-BROWNE, R. et al. Patterns of adherence of diarrheagenic *Escherichia coli* to HEp-2 cells. *Pediatric Infectious Diseases Journal,* v. 6, 1987, p.829–831.

NATARO, J.P., KAPER, J. Diarrheagenic *Escherichia coli*. Clinical Microbiology Reviews, v. 11, 1998, p.142-201.

OCHMAN, H., WILSON A.C. Evolution in bacteria: Evidence for a universal substitution rate in cellular genomes. *Journal of Molecular Evolution,* v. 26, 1987, p.74–86.

OCHMAN, H., SELANDER, R.K. Standard reference strains of *Escherichia coli* from natural populations. *Journal of Bacteriology,* v. 157, 1984. p.690–693.

ORSKOV, F., ORSKOV, I. From the National Institutes of Health: Summary of a workshop on the clone concept in the epidemiology, taxonomy, and evolution of the *Enterobacteriaceae* and other bacteria. *Journal of Infectious Diseases*, v. 148, 1983, p.346–357.

PICKERING, L.K., OBIRG, T.G., STAPLETON, F.B. Hemolytic-uremic syndrome and enterohemorrhagic *Escherichia coli. Pediatric Infectious Diseases Journal*, v. 13, 1994, p.459-476.

PUPO, G.M., KARAOLIS, D.K., LAN, R. et al. Evolutionary relationships among pathogenic and nonpathogenic *Escherichia coli* strains inferred from multilocus enzyme electrophoresis and mdh sequence studies. *Infection and Immunity*, v. 65, 1997, p.2685–2692.

REID, S.D., HERBELIN, C.J., BUMBAUGH, A.C. et al. Parallel evolution of virulence in pathogenic *Escherichia coli*. *Nature*, v. 406, 2000, p.64-67.

ROESCH, P.L., REDFORD, P., BATCHELET, S. et al. Uropathogenic *Escherichia coli* use d-serine deaminase to modulate infection of the murine urinary tract. *Molecular Microbiology*, v. 49, 2003, p.55–67.

SCALETSKY, I.C., FABBRICOTTI, S.H., CARVALHO, R.L. et al. Diffusely adherent *Escherichia coli* as a cause of acute diarrhea in young children in Northeast Brazil: A case-control study. *Journal of Clinical Microbiology*, v. 40, 2002, p.645–658.

SCHREIBER, W., DONNENBERG; M. S. Type IV pili. In: Donnenberg, M.S. (Ed.). *Escherichia coli* Virulence Mechanisms of a

Versatile Pathogen Academic Press New York, NY, 2002. p.417.

SCOTLAND, S.M., SMITH, H.R., ROWE, B. *Escherichia coli* O128 strains from infants with diarrhea commonly show localized adhesion and positivity in the fluorescent actin staining test but do no hybridize with an enteropathogenic *Escherichia coli* adherence factor probe. *Infection and Immunity*, v 59, 1991, p.1569-1571.

SELANDER, R.K., CAUGANT, D.A, WHITTAM, T.S. Genetic structure and variation in natural populations of *Escherichia coli*. In: Neidhardt F.C. (Ed.). *Escherichia coli* and *Salmonella typhimurium*. ASM Press Washington, DC. v. 2, 1987. p.1625–1648.

SERVIN, A.L. Pathogenesis of Afa/Dr diffusely adhering *Escherichia coli. Clinical Microbiology Reviews*, v. 18, 2005, p.264-292.

SILVA, R.M., TOLEDO, MR., TRABULSI; L.R. Biochemical and cultural characteristics of invasive *Escherichia coli. Journal of Clinical Microbiology,* v. 11, 1980, p.441–444.

SOUSA, C.P., DUBREUIL, J.D.; Distribution and expression of the *astA* gene (EAST1 toxin) in *Escherichia coli* and *Salmonella. International Journal of Medical Microbiology*, v, 291, 2001, p.15-20.

SPANGLER, B.D. Structure and function of cholera toxin and the related *Escherichia coli* heat-labile enterotoxin. *Microbiology Reviews*, v. 56, 1992, p.622–647.

STRUVE, C., KROGFELT, K.A. In vivo detection of *Escherichia coli* type 1 fimbrial expression and phase variation during experimental urinary tract infection. *Microbiology,* v. 145, 1999, p.2683–2690.

TAILOR, C.S., NOURI, A., LEE, C.G et al. Cloning and characterization of a cell surface receptor for xenotropic and polytropic murine leukemia viruses. *Proceedings of the National Academy of Science of the USA,* v. 96, 1999, p.927–932.

TORRES, A.G., REDFORD, P., WELCH, R.A et al. TonB-dependent systems of uropathogenic *Escherichia coli*: Aerobactin and heme transport and TonB are required for virulence in the mouse. *Infection and Immunity,* v. 69, 2001, p.6179–6185.

TRABULSI, L.R., KELLER, R., GOMES T.A.T. Typical and atypical enteropathogenic *Escherichia coli*. *Emerging Infectious Diseases*, v. 8, 2002, p.508–513.

WELCH, R.A., BURLAND, V., PLUNKETT, G. et al. Extensive mosaic structure revealed by the complete genome sequence of uropathogenic *Escherichia coli*. *Proceedings of the National Academy of Science of the USA*, v. 99, 2002, p.17020–17024.

WHITFIELD, C., ROBERTS, I.S.. Structure, assembly and regulation of expression of capsules in *Escherichia coli*. *Molecular Microbiology*, v. 31, 1999, p.1307–1319.

WHITFIELD, C., VALVANO, M.A. Biosynthesis and expression of cell-surface polysaccharides in Gram-negative bacteria. *Advanced in Microbial Physiology*, v. 35, 1993, p.135–246.

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