CHAPTER  3.3.3

The Genus *Escherichia*

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**Introduction**

*Escherichia coli*, originally called “*Bacterium coli* commune,” was first isolated from the feces of a child in 1885 by the Austrian pediatrician Theodor Escherich (Escherich, 1885). *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* are 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 not typically available in most clinical laboratories. *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 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 (extensively reviewed in *Escherichia coli* and *Salmonella: Cellular and Molecular Biology*, third edition; available online through the [http://www.asmpress.org/browse/virtual/index](http://www.asmpress.org/browse/virtual/index).DOI: 10.1007/0-387-30746-x_3). Shown in Table 1 are additional websites providing general and specific *E. coli* portals for useful genetic and metabolic information.

**Taxonomy and Phylogeny**

**General Comments**

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 and Wilson, 1987). *Escherichia* and *Shigella* have been historically separated into different genera within the Enterobacteriaceae. DNA sequence analysis of their genomes reveals a high degree of sequence similarity and suggests to many bacteriologists that they should be considered a single species (see *Shigella* genome references: Ewing et al., 1958; Kimura, 1980; Brenner, 1984; Saitou and Nei, 1987; Simmons and Romanowska, 1987; Nei and Miller, 1990; Pupo et al., 2000; Jin et al., 2002; Wei et al., 2003). Currently, the two organisms continue to be discussed 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. aderovoxylata*, *E. blattae*, *E. fergusonii*, *E. hermanii* and *E. vuln-

**Table 1. *E. coli* reference sources on the Internet.**

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<tr>
<th>Name of Web site</th>
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<tr>
<td>International <em>E. coli</em> Alliance <em>E. coli</em> Database Portal</td>
<td><a href="http://www.uni-giessen.de/~gx1052/IECA/ieca.html">http://www.uni-giessen.de/~gx1052/IECA/ieca.html</a></td>
<td>General portal site for <em>E. coli</em> information</td>
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<td>The <em>E. coli</em> Index</td>
<td><a href="http://ecoli.bham.ac.uk/">http://ecoli.bham.ac.uk/</a></td>
<td>General portal site for <em>E. coli</em> information</td>
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<tr>
<td>Center for Disease Control: Foodborne and Diarrheal Diseases Branch</td>
<td><a href="http://www.cdc.gov/ncidod/dbmd/foodborne/index.htm">http://www.cdc.gov/ncidod/dbmd/foodborne/index.htm</a></td>
<td>CDC informational site for food-borne pathogens including <em>E. coli</em></td>
</tr>
<tr>
<td>CGSC: <em>E. coli</em> Genetic Stock Center</td>
<td><a href="http://cgsc.biology.yale.edu/">http://cgsc.biology.yale.edu/</a></td>
<td>Provides database of <em>E. coli</em> genetic information includes genotypes and reference information for the strains in the CGSC collection, gene names, properties, and linkage map, gene product information and information on specific mutations</td>
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eris. Little is known about the distribution, biology or interrelatedness of these species.

Escherichia coli Clonality

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 and Selander, 1984). This collection can be acquired from Thomas Whittham’s laboratory (http://foodsafemusa.edu/whittam/ecor/). Six phylogenetic groups are generally recognized among the ECOR strains (A, B1, B2, C, D and E; Selander et al., 1987; Fig. 1). For the phylogenetic groups, there are some general biotype clusterings (e.g., raffinose fermentation is common among B1, C, D and E strains, whereas sorbose fermentation is common to B2 strains; Miller and Dancel, 1986). There are limited instances where host sources are associated with clonal types. For example, among the original B1 strains, many were commonly isolated from herbivores (Selander et al., 1987). In general, however, there is notably little association between host strain source and clonal designation.

Evolution of Pathogenic Types (Pathotypes) of E. coli

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. The specifics for each of the E. coli pathotypes will be discussed in the disease topic below. 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, recent 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 enterohemorrhagic 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

Fig. 1. Phylogenetic distribution of multicopy single-stranded DNA (msDNA)-containing E. coli strains of the E. coli reference (ECOR) collection. The tree was inferred by the neighbor-joining (NJ) algorithm applied to the genetic distances based on polymorphisms of 38 enzyme-encoding loci. Circles represent the nine ECOR strains in which msDNA was detected by the RT-labeling method.
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).

Evolution of the *E. coli* Genome

Genome sequencing of three different *E. coli* strains (laboratory K-12 strain MG1655, enterohemorrhagic 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. The general conservation of genes among the three isolates undoubtedly reflects the physiological nature of what can be termed “*E. coli ness.*” *Escherichia coli*, even the pathogenic types, at some point thrive 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. For example, the CFT073 genome is nearly 600 kbs larger than MG1655, and in one-on-one comparisons, there are approximately 1100 genes unique to K-12 and greater than 1800 genes unique to CFT073 (Fig. 2). 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. They are often located at tRNA genes (e.g., *leuX, selC, argW, pheV* and *pheU*). 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 and colleagues 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. Thus, the unique gene clusters are often simply and appropriately called “genetic islands.” The ability to build a phylogeny for some of the *E. coli* pathotypes is complicated because the horizontally acquired islands do not always share chromosomal location and genetic content (Welch et al., 2002).

Habitat

*Escherichia coli* are common inhabitants of the terminal small intestine and large intestine of mammals. They are often the most abundant facultative anaerobes in this environment. They can occasionally be isolated in association with the intestinal tract of nonmammalian 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 and Hazen, 1988).

Cell Structure

*Escherichia coli* are Gram-negative, nonspore-forming 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 finally D-alanine.

*Escherichia coli* are commonly motile in liquid by means of peritrichous flagella. Swarming behavior and differentiation into hyperflagellated and elongated bacilli typical of that seen with the *Proteus* species can be observed on some solid media (Harshey, 1994). *Escherichia coli* are commonly limbrated. The type I 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 adhesins; Bann, 2002; Schreiber and 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 (see below). For *E. coli*, there are over 150 antigenically unique O-antigens (Whitfield and Valvano, 1993). K type capsular material occurs in two or four forms on the basis of physical, biochemical and genetic criteria (Jann and Jann, 1990; Whitfield and Roberts, 1999). Over 80 serologically and chemically distinct capsular polysaccharides have been reorganized (Jann and Jann, 1992). 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 (Keenleyside et al., 1993). There are 53 H-antigen specificities among *E. coli* (Wang et al., 2003).

**Cell Physiology**

*Escherichia 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. By traditional clinical laboratory biochemical tests, *E. coli* is positive for indole production and the methyl red test. 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-xylene, 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 communal 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 (Ingraham and Marr, 1987). *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 (Waterman and Small, 1996).

There are several sources of information on genes identified as essential for growth of laboratory strain K-12. Efforts are underway to identify essential genes for drug target studies, as well as to construct a freely replicating *E. coli* strain with the smallest possible genome. The study of such an engineered strain would simplify the analyses of regulatory circuits and mechanics of fundamental processes such as cell division and chromosomal replication (Gerdes et al., 2003); information on the topic with links can be found at the [http://www.genome.wisc.edu/resources/essential.htm](http://www.genome.wisc.edu/resources/essential.htm)[University of Wisconsin Essential genes in *E. coli* Web site]. Information about profiling the *Escherichia coli* chromosome can be found at the [http://www.shigen.nig.ac.jp/ecoli/pec/index.jsp](http://www.shigen.nig.ac.jp/ecoli/pec/index.jsp)[Shigen *E. coli* Web site]).

Iron metabolism of *E. coli* is an especially well-studied topic (Braun and 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.

**Genetics**

A great deal of what is known about bacterial genetics comes from the study of the *E. coli* laboratory strain K-12. K-12 was isolated in 1922
from the stool of a convalescent diphtheria patient (Lederberg, 1951). Although K-12 strains are rough in terms of their O-antigen, physical and genetic analyses indicate that the original K-12 parent was likely to have been an O16 strain Liu and Reeves, 1994). In general, wild strains of E. coli are capable of gene transfer by conjugation and transduction, albeit at rates that are greatly reduced when compared to K-12 laboratory events. Natural competency for DNA transformation has not been observed for E. coli, although genes similar in sequence to Hemophilus influenza competence genes are present in the E. coli K-12 genome Finkel and Kolter, 2001). In the laboratory, artificial methods such as electroporation enable the uptake of DNA and its stable maintenance through recombination or replication as a plasmid or prophage. Conjugal, as well as nonconjugal, plasmids are commonly found in wild strains of E. coli.

Ecology

Escherichia coli strains are commonly carried in the mammalian intestine. Usually a predominant clone can be found at any given time, but other less abundant clones can be found simultaneously. Whittam’s lab demonstrated that in a study of healthy 3–6 year old girls the most abundant E. coli clones varied when examined on a weekly basis. In some rare instances a single clone could be found over a 4-week period, and in others an individual may have as many as 16 different clones over the same period. The average was three clones per girl over the 4-week period. Therefore, the E. coli strains found in the intestine are multiclonal and fluctuate in their predominance over time. Colonization of the periurethral area or urinary tract occurs briefly, and the strains isolated at these sites are usually not the predominant clone in the stool at the same time (Schlager et al., 2002).

Aside from adhesins and metabolic traits that would favor one E. coli strain over another in their ability to colonize particular sites, the production and immunity to bacteriocins undeniably affects the dynamics of the persistence and dominance of individual strains (Riley and Gordon, 1999). Bacteriocin production may give a special competitive advantage in nutrient-poor environments Riley and Gordon, 1999).

Disease

Escherichia coli Pathotypes

One of the most notable features of E. coli is broad diversity of disease-causing genotypes. As mentioned above, 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 six intestinal and two extraintestinal pathotypes currently recognized Nataro and Kaper, 1998a; Nataro et al., 1998b; Schreiber and Donnenberg, 2002; Kaper et al., 2004). Enterotoxigenic E. coli.

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 (World Health Organization, 1999). 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 (Sixma et al., 1991; Sixma et al., 1993; Spangler, 1992). LT 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-stable 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 and Svennerholm, 1996).

Enteropathogenic E. coli

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. They are currently defined as those diarrheagenic E. coli strains that cause attaching and effacing (A/E) lesions on intestinal epithelium but which lack Shiga toxins (verotoxins). There is a great diversity of the E. coli serotypes that possess these features. This makes the serotype classification scheme ineffective and indicates that there may be a diversity of pathogenic mechanisms and evolutionary lineages. 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 often 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 (McDaniel et al., 1995). The LEE encodes a type III secretion system that provides the intimate adhesin, 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; Kenny et al., 1997). Common to most EPEC strains are plasmids, termed “EAF” (“EPEC adherence factor”) plasmids, which encode an adherence factor, the bundle-forming pilus (bfp; Nataro et al., 1987; Donenberg and Kaper, 1992; Sohel et al., 1996). 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. For example, some but not all EPEC produce an enterotoxin, EspC (Mellies, 2001).

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).

Enterohemorrhagic E. coli

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 systematically 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; Latham et al., 2003). The precise role of pO157 in EHEC pathogenesis is unknown but may involve some putative toxin genes and a mucin-specific zinc metalloprotease, StcE (Burland et al., 1998; Latham et al., 2002; Grys et al., 2005).

**Enteroaggregative *E. coli***

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” (Nataro et al., 1987). 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 (Nataro et al., 1995). 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.

**Diffusely Adherent *E. coli***

The epidemiology and pathogenesis of the diffusely adherent *E. coli* (DAEC) are not well understood. DAEC may cause diarrhea in very young children (less than a year old; Scaletsky et al., 2002). They are differentiated from the other diarrheegenic *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.

**Enteroinvasive *E. coli***

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 and 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 invade 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 cadaverine, 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 (Murrelli et al., 1998; Fernandez et al., 2001; Casalino et al., 2003).

**Extraintestinal E. coli** Two separate pathotypes of *E. coli* are generally recognized as causes of extraintestinal human diseases (neonatal septicemia/meningitis *E. coli* [meningitis-associated *E. coli*, MAEC] and the urinary tract and bloodstream *E. coli* [uropathogenic *E. coli*, UPEC]). Some isolates, *E. coli* O18:K1:H7, are recognized as having the potential to cause both invasive neonatal diseases and urinary tract infections (UTI; Johnson et al., 2001a; Johnson et al., 2001b).

UPEC are a heterogeneous group of clones (Donnenberg and Welch, 1996). Within the UPEC grouping are cystitis, pylonephritis 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 (Kunin, 1994). As much as 90% of all community-acquired UTIs and greater than 30% of the hospital-acquired UTIs are caused by *E. coli* (Haley et al., 1985). 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 pylonephritis, but who have no underlying medical complications, often possess specific O serotypes (O1, O2, O4, O6, O7, O18 and O75; Orskov and Orskov, 1983a; Orskov and Orskov, 1983b; Orskov and Orskov, 1985; Johnson et al., 1987; Wold et al., 1992). What further suggests that these *E. coli* strains are extraordinary is that they are especially capable of invading the bloodstream (Johnson et al., 1987; Johnson et al., 1988; Johnson, 1991a; Johnson et al., 1991b; 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 Dr), hemolysin (Hly), cytotoxic necrotizing factor-1 (CNF-1), and the aerobactin (Aer) iron-sequestration systems (reviewed by Donnenberg and Welch, 1996). Recently, a member of the autotransporter protein family, Sat, has been demonstrated to be a cytotoxin of uroepithelial cells (Guyer et al., 2000). 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 (Kisielius et al., 1989; Connell et al., 1996; Langermann et al., 1997; Lim et al., 1998; Struve and Krogfelt, 1999). The type 1 pili have recently been shown to mediate cellular invasion of cultured urinary epithelial cells (Mulvey et al., 1998). 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.

The first described pathogenicity islands were found to encode the non-type 1 adhesins (e.g., pap, sfa, and dra), toxins (e.g., Hly, CNF1 and Sat), and iron-sequestration systems (e.g., aerobactin; Knapp et al., 1986; Hacker et al., 1990; Blum et al., 1994; Blum et al., 1995). The chromosomal endpoints of these UPEC islands are associated with specific tRNA loci leuX, selC, pheV and pheU. Often there are phage integrase-like genes present on one end of the islands. This has led to a common perception that nonpathogenic organisms become pathogens by the acquisition of these virulence genes en bloc via prophages that use the tRNA genes as attachment sites. Comparison of genome sequences of three different *E. coli* strains indicates in excess of 300 unique loci, which appear as insertions or substitutions along the length of these genomes. Clearly there is a subset 10–13 tRNA gene loci where genetic differences among the strains occur uniformly (e.g., selC, pheV and leuX). Therefore, phages probably participated in their acquisition and evolution. There are, however, many more sites where the genomes differ and it is unclear what genetic mechanisms lead to these differences.

**Meningitis-associated E. coli** Along with Group B streptococci, meningitis-associated *E. coli* (MAEC) are the most common causes of neonatal meningitis, a severe disease with a high mortality rate and possible long-term neurologi-
causal problems in survivors (Unhanand et al., 1993; Stoll et al., 2002). There are a limited number of \textit{E. coli} serotypes associated with this disease, but greater than 80\% of the strains express K1 capsule (Robbins et al., 1974; Sarff et al., 1975). It is generally thought that the newborn acquires the K1 strain from its mother during passage through the birth canal. The strain then progressively invades the bloodstream and subsequently crosses endothelial surfaces into the brain. The K1 capsule is a critical determinant in invasion across the blood-brain barrier. In a rat pup model, the capsule was proven to be necessary for survival in the meninges (Hoffman et al., 1999). S-fimbriae enable K1 isolates to adhere to brain microvascular endothelium (Parkkinnen et al., 1988). Several genes, \textit{ibeA}, \textit{ibeB}, \textit{ibeC}, \textit{cnf-1} and \textit{asA}, are required for endothelial cell invasion (Prasadaro et al., 1999; Badger et al., 2000a; Khan et al., 2002). Also common to most MAEC isolates is a 100-kb plasmid that confers increased virulence (Mercer et al., 1984; Badger et al., 2000b). The best-characterized MAEC isolate is RS218, which possesses that classic O18:K1:H7 serotype. Its genome sequence has been determined, although not presented in a published form as of the end of 2004 (see the http://www.genome.wisc.edu/sequencing/rs218.htm[E. coli genome project]). Preliminary studies indicate that like the other \textit{E. coli} pathotypes, the K-12, EHEC or UPEC genomes do not contain a significant number of genes unique to RS218. In a genomic comparison of RS218 to K-12, at least 500 kb of DNA are unique to RS218 (Rode et al., 1999; Bonacorsi et al., 2000). See Table 1 for \textit{E. coli} reference sources on the Internet.

\textbf{Literature Cited}


