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Review

All blood, No stool: enterohemorrhagic *Escherichia coli* O157:H7 infection

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Enterohemorrhagic *Escherichia coli* serotype O157:H7 is a pathotype of diarrheagenic *E. coli* that produces one or more Shiga toxins, forms a characteristic histopathology described as attaching and effacing lesions, and possesses the large virulence plasmid pO157. The bacterium is recognized worldwide, especially in developed countries, as an emerging food-borne bacterial pathogen, which causes disease in humans and in some animals. Healthy cattle are the principal and natural reservoir of *E. coli* O157:H7, and most disease outbreaks are, therefore, due to consumption of fecally contaminated bovine foods or dairy products. In this review, we provide a general overview of *E. coli* O157:H7 infection, especially focusing on the bacterial characteristics rather than on the host responses during infection.

Keywords: enterohemorrhagic, *Escherichia coli*, O157:H7

Escherichia coli

Escherichia (*E.*) *coli* was first described in 1885 by Theodore Escherich as a pure culture of slim, occasionally slightly curved, and short rods ranging in size from 1-5 μm in length and 0.3-0.4 μm in thickness [9]. As a part of the normal gut microflora, this microorganism colonizes the gastrointestinal tract of warm-blooded animals and humans within a few hours after birth and plays an important role in maintaining gut physiology [9,35]. However, some *E. coli* strains have acquired specific virulence factors by means of mobile genetic elements such as plasmids, transposons, bacteriophages, and pathogenicity islands, and have evolved into pathogenic *E. coli* [64].

Based on their common clinical features, pathogenic *E. coli* are categorized into (i) diarrheagenic *E. coli*, (ii) uropathogenic *E. coli*, (iii) meningitis/sepsis-associated *E. coli*, and (iv) avian pathogenic *E. coli* [64,93]. Diarrheagenic *E. coli* can be further categorized into six well-described pathotypes based on virulence properties, pathogenic mechanisms, clinical syndromes, and distinct serogroups/serotypes: (i) enterotoxigenic *E. coli* (ETEC), (ii) enteropathogenic *E. coli* (EPEC), (iii) enterohemorrhagic *E. coli* (EHEC), (iv) enteroaggregative *E. coli* (EAEC), (v) enteroinvasive *E. coli* (EIEC), and (vi) diffusely adherent *E. coli* (DAEC). These pathotypes of *E. coli* seem to be clonal groups that have shared O and H antigens [64,93].

The ETEC cause infantile diarrhea, traveler's diarrhea in developing countries, and diarrhea in very young animals, such as piglets, lambs, and calves [35,93]. The microorganism colonizes the surface of the small intestinal mucosa using one or more adhesive fimbriae and produces enterotoxins, heat-labile enterotoxin (LT), and/or heat-stable enterotoxin (ST) [20,93,119]. The most frequent ETEC serogroups include O6, O8, O15, O20, O25, O27, O63, O78, O85, O115, O128ac, O148, O159, and O167 [35]. People are the principal reservoir of ETEC that cause human illness [35,93].

The EPEC cause epidemic and sporadic infantile diarrhea in developing countries [35,78,93]. This microorganism produces a characteristic intestinal histopathology called attaching and effacing (A/E) lesions, in which bacteria intimately attach to the intestinal epithelial cells and rearrange the cytoskeletal actin underneath [35,78,93]. A three-stage model of pathogenesis has been proposed that includes localized adherence, signal transduction, and intimate adherence [32,33]. The most frequent EPEC serogroups implicated in human disease include O55, O86, O111ab, O119, O125ac, O126, O127, O128ab, and O142 [35]. People are the principal reservoir of EPEC that cause human illness [35,93].

The EAEC cause persistent diarrhea in children and

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adults worldwide [35,93]. By definition, this microorganism does not produce LT or ST, but has a characteristic adherence pattern on HEp-2 cells called aggregative adherence (AA), which is described as a stacked-brick configuration [92,94,114]. The plasmid-encoded aggregative adherence fimbriae I is known to mediate this AA phenotype [92,93]. A model of pathogenesis has been proposed that includes initial adherence, enhanced mucus production, and production of an EAEC cytotoxin [93]. The most frequent EAEC serogroups include O3, O15, O44, O77, O86, O92, O111, and O127 [35]. Disease outbreaks may be associated with food, but no single source has been implicated [35,93].

The EIEC cause non-bloody diarrhea and dysentery similar to that caused by *Shigella* spp. [35,93]. This microorganism is able to invade and proliferate in colonic epithelial cells and is biochemically, genetically, and patho- physiologically related to *Shigella* spp. [12,93]. Genes necessary for invasion are carried on a 140-megadalton (MDa) plasmid called pInv [93,126]. The most frequent EIEC serogroups include O28ac, O29, O112, O124, O136, O143, O144, O152, O164, and O167 [35]. Humans are the principal reservoir of EIEC, but the potential for person-to-person transmission is reduced because of a high infectious dose [53,93]. Most disease outbreaks are food- borne or water-borne [93,137].

The DAEC cause diarrhea in young children from 1 to 5 years of age, but little is known about the pathogenic features of DAEC-induced diarrhea [35,79,93]. By definition, DAEC does not produce LT or ST, does not possess the EPEC adherence factor plasmid, and does not invade epithelial cells [36,93]. However, this microorganism produces a characteristic diffusely-adherent pattern on HEp-2 cells that is distinguishable from that seen in the EAEC AA phenotype [35,36,93]. The most frequent DAEC serogroups include O1, O2, O21, and O75 [35].

The EHEC are a subset of the Shiga toxin-producing *E. coli* (STEC) that cause hemorrhagic colitis (HC) and the hemolytic uremic syndrome (HUS) in humans, produce one or more Shiga toxins (Stx), induce A/E lesions, and possess a 60-MDa plasmid called pO157 [93]. The EHEC serotypes most frequently associated with human disease include O26:H11, O103:H2, O111:H8, O145:H28, O157:H-, and O157:H7 [35,91]. Healthy cattle are the most important animal reservoir associated with human infection [52] although other healthy animals including sheep, goats, pigs, dogs, chickens, horses, deer, rats, and seagulls can also carry EHEC [10,24,35,52,73,108,144].

Enterohemorrhagic *E. coli* O157:H7

E. coli O157:H7 is one of the most important serotypes of the STEC, because it causes most of the HUS disease. It was first described in 1977 by Konowalchuk *et al.* [71]. Its association with human disease was first reported during two outbreaks of HC in 1982 [109,146] and in sporadic cases of HUS in 1983 [66]. Since then, this microorganism has been associated with many disease outbreaks in the United States and in other countries around the world [35,93].

Disease outbreaks are frequently associated with ingestion of food or water contaminated with bovine feces. Examples of these sources include undercooked ground beef, private or municipal water sources, and other food products, such as unpasteurized apple cider or milk, fresh vegetables, sprouts, and salami [8,50,134]. Visits to petting zoos, dairy farms, camping grounds where cattle have previously grazed, and recreational water sources have all resulted in infection [54,55]. Person-to-person transmission is also possible, especially in daycare centers [7,130]. Potential airborne transmission was recently reported after exposure to a contaminated building at an animal exhibit [140]. The various transmission routes may be explained by the very low infectious dose (10-100 organisms) of this microorganism. Therefore, minimal exposures can cause disease [35,93].

Human infection with *E. coli* O157:H7 has been reported in at least 30 countries on six continents [35]. In the United States, 196 outbreaks or sporadic cases were documented through 1998, and the number of reported outbreaks has increased from two cases in 1982 to 42 cases in 1998 [35]. The medical costs of human illness caused by *E. coli* O157:H7 in the United States were estimated to be $0.3-$0.7 billion per year in 1993 [18]. A more recent estimation by the Center for Disease Control and Prevention (CDC) reports that this microorganism accounts for 73,480 illnesses, 2,168 hospitalizations, and 61 deaths per year in the United States [88]. About 85% of these cases are associated with food-borne transmission [88]. According to the outbreak surveillance data from the CDC, reported infections of *E. coli* O157:H7 increased annually starting in 1994, reaching a peak of 4,744 individual patients in 1999 before decreasing to 2,544 patients in 2004 and 2,621 in 2005. Large outbreaks or sporadic cases of *E. coli* O157:H7 have also been reported in Canada, Japan, and the United Kingdom [35]. However, accumulating data indicate that non-O157 EHEC infections may be more frequent than *E. coli* O157:H7 infections in continental Europe, Australia, and Latin America, indicating the possibility of differential geographic distribution [35,115].

Patients infected with *E. coli* O157:H7 initially experience watery diarrhea; however, some individuals may be asymptomatic. Most cases are self-resolving within a week, but the disease sometimes progresses to HC (originally described as 'all blood, no stool') in one or two days, with severe abdominal cramps, and frequently no or low-grade fever in the presence of fecal leukocytes

[17,64,93]. The disease evolves to HUS in 5-10% of HC patients, especially young children and the elderly. HUS is a life-threatening sequela defined by a triad of symptoms: acute renal failure, microangiopathic hemolytic anemia, and thrombocytopenia [5,100,140]. In adults, the infection may also lead to thrombotic thrombocytopenic purpura (TTP), a variant form of HUS. The pathological features of TTP are generally the same as those of HUS, but with the addition of fever and neurological symptoms such as lethargy, severe headache, convulsions, and encephalopathy [100].

There are no common distinguishable biochemical characteristics shared by EHEC strains [93]. However, there are some biochemical characteristics of *E. coli* O157:H7 that have been used for the isolation and identification of this serotype from clinical samples. An important characteristic is a delayed D-sorbitol fermentation (> 24 h). From 75% to 94% of other *E. coli* strains ferment D-sorbitol rapidly (< 24 h) [86,93]. This serotype also has the inability to produce β-glucuronidase, a feature that can be capitalized on in the laboratory through the use of synthetic molecules such as 4-methyl-umbelliferyl-D-glucuronide, which fluoresce upon hydrolysis [133]. It has been demonstrated through the use of the MicroScan conventional Gram-negative identification system that more than 90% of the tested *E. coli* O157:H7 have one of two unique biochemical profiles that are not detected in other D-sorbitol negative *E. coli* strains [1]. However, the ID32E system showed that there were no unique profiles for *E. coli* O157:H7 [76]. Although no single biochemical profile is available for all *E. coli* O157:H7 isolates, the results indicate that *E. coli* O157:H7 possesses biochemical activities that are significantly divergent from generic *E. coli*. Examples include ornithine decarboxylase, arginine dehydrolase, urease, and 5-ketogluconate, and, to a lesser extent, fermentation of rhamnose, adonitol, D-arabitol, trehalose, and inositol [1,76]. One report shows that *E. coli* O157 strains do not ferment rhamnose on agar plates, whereas 60% of the non-sorbitol-fermenting *E. coli* belonging to other serogroups ferments rhamnose [128].

The complete genome sequences of *E. coli* O157:H7 and *E. coli* K-12 reveal that these bacteria share a 4.1-Mb backbone of common sequences [104]. However, *E. coli* O157:H7 contains an additional 1.34-Mb region of genomic DNA that is not present in the *E. coli* K-12 genome. This unique region contains approximately 1,400 novel genes scattered throughout 177 discrete regions of DNA (> 50 bp in size) called O-islands. Moreover, *E. coli* O157:H7 is missing a 0.53-Mb region of genomic DNA that is present in the *E. coli* K-12 genome [31,104]. These comparisons indicate that lateral gene transfer likely occurred during the evolution of *E. coli* O157:H7. Clonal analyses suggest that *E. coli* O157:H7 descended from the non-toxigenic and less virulent *E. coli* O55:H7 [148,149]. The current model of the emergence of *E. coli* O157:H7 from its prototype, *E. coli* O55:H7, is based on four sequential events: (i) acquisition of an *stx2*-containing bacteriophage in a single event and at a single site (probably *wrbA* encoding multimeric flavodoxin-like protein), (ii) splitting off of the clone leading to *E. coli* O157:H-, (iii) acquisition of the *stx1*-containing bacteriophage in a single site (probably *yehV*) by *E. coli* O157:H7, and (iv) loss of the ability to ferment D-sorbitol by *E. coli* O157:H7 [147].

Major virulence factors of *E. coli* O157:H7

Shiga toxins

E. coli O157:H7 produces one or more Stxs whose prototype is Stx produced by *S. dysenteriae* type I [90,93]. Stx1 is almost identical to Stx produced by *S. dysenteriae* type I, differing by only a single amino acid. Stx1 and Stx2 share approximately 56% homology in their amino acid sequences, but they are antigenetically distinct [90]. A number of Stx2 variants such as Stx2c, Stx2d, and Stx2e have been identified, and they share 84-99% amino acid sequence homology with Stx2 [90]. Stx2c and Stx2d are associated with HC and HUS in humans, whereas Stx2e is primarily associated with swine edema disease. Stx2d is known to be activated by an elastase present in human mucus [134]. Stxs belong to an AB5 toxin family consisting of one enzymatically active A subunit and five identical receptor-binding B subunits. All the genes for the Stx family are encoded as a single transcriptional unit by bacteriophages, except for the chromosomally-encoded Stx2e [90,100]. However, the Stx B-subunit gene has a stronger ribosomal binding site than that of the A-subunit gene, resulting in increased translation of B subunits [100]. This may help to maintain a 1 : 5 A/B subunit stoichiometry of the holotoxin.

To cause disease, Stxs must be translocated from the intestine to the blood stream. The underlying mechanisms are not clearly understood [35,93,100]. Patients infected with *E. coli* O157:H7 frequently shed fecal leukocytes, and although inflammation may play a role, transient epithelial damage occurs during migration of leukocytes across the intestinal epithelium to the lumen [125,134]. These eventually cause toxin uptake across the intestinal epithelium *in vitro* [57]. Furthermore, generalized intestinal inflammation caused by the infection may promote systemic Stx uptake. Stxs are associated with vascular damage in the colon, and LPS may also play a role in intestinal inflammation during *E. coli* O157:H7 infection [2,123].

The receptors for Stxs are globotriaosylceramide (Gb3) or globotetra-osylceramide (Gb4), which are both glycolipids containing a terminal [Gal-α1,4-Gal] moiety

[35,93,100]. Both Stx1 and Stx2 bind to Gb3, whereas Stx2e binds to Gb4. Gb3 is expressed on a variety of epithelial and endothelial cells in humans and animals [83,142]. After receptor-mediated endocytosis, Stxs move to the endoplasmic reticulum via the Golgi network and Golgi apparatus by retrograde transport [113]. During retrograde transport, the A subunit is cleaved by furin, a calcium-sensitive serine protease in the Golgi apparatus. In the endoplasmic reticulum, it is thought that the disulfide bond in the A subunit is reduced and the released A1 fragment is translocated into the cytoplasm [96]. The A1 fragment has RNA N-glycosidase activity that can irreversibly depurinate a specific adenine (A_{4324}) residue from the 28S rRNA of the 60S ribosome [39]. This process prevents binding of elongation factor I-dependent aminoacyl- tRNA, inhibits protein synthesis, and results in host cell death [95].

In addition to their RNA N-glycosidase activity, Stxs can induce apoptosis in HEp-2 or HeLa cells [21]. Stx1 or Stx2 treatment of HEp-2 cells results in up-regulated expression of the proapoptotic Bax protein [28]. Overexpression of Bcl-2 protein protects cells from Stx-induced apoptosis [21,61]. Interestingly, the Stx1 B-subunit itself can cause apoptosis in HEp-2 cells, although higher doses are needed compared to holotoxin [61]. This indicates that the B subunit plays a role in signal transduction, as well as in toxin binding. Recently, it was demonstrated that Stxs are able to reduce bovine leukemia virus replication *in vitro* and *in vivo* [41-44]. Virus-infected cells are able to internalize the toxins by simple diffusion through virus-induced permeable cell membranes. Although the underlying mechanism is unknown, this may explain the carriage of intestinal STEC by all cattle.

The locus of enterocyte effacement

Formation of A/E lesions is a unique characteristic of EHEC/EPEC pathogenesis [46]. The A/E lesions are characterized by the loss of microvilli, an intimate adherence of bacteria adjacent to the host cell membrane, and the generation of an organized cytoskeletal structure containing filamentous actin beneath adherent bacteria, which is called an actin pedestal [33]. The genetic element responsible for the A/E lesions is called the locus of enterocyte effacement (LEE), and it is a well-known pathogenicity island present in EPEC, EHEC, *Hafnia alvei*, *Citrobacter rodentum*, and other attaching and effacing *E. coli* that are pathogenic in animal species [46].

The complete sequence of the LEE in *E. coli* O157:H7 reveals that the O157:H7 LEE is 43,359-bp in size, which is larger than the 35,624-bp LEE in EPEC [31,38,103]. A 7.5-kb putative prophage near the *selC* end of the LEE locus is responsible for most of the size difference. The G+C content in the O157:H7 LEE is 40.9%, much lower than that of the *E. coli* K-12 genome (average 50.8%). The prophage base composition is 51.7% G+C, whereas the remainder of the LEE element is 39.6% G+C. This indicates that the O157:H7 LEE results from horizontal gene transfer from other species. The O157:H7 LEE encodes 54 open reading frames (ORFs). Thirteen ORFs are located on the putative phage, and 41 ORFs correspond to those of EPEC LEE in the order and number of genes [46,103].

The LEE region contains three segments encoding five operons [46,103]: (i) the first segment includes the LEE1, LEE2, and LEE3 operons that encode the genes for the type III secretion system (TTSS), (ii) the second segment includes the translocated intimin receptor (Tir) (LEE5) operon that encodes the genes for bacterial adhesion such as intimin and the Tir, and (iii) the third segment includes the LEE4 operon that encodes the genes for the *E. coli* secreted proteins, such as EspA, EspB, and EspD. The EspABD complex forms the translocation apparatus functioning to transfer the effector proteins of the TTSS. A recent study demonstrated that EspB itself is an effector protein of the TTSS, which are required for microvilli effacement or suppression of phagocytosis during infection [59]. Interestingly, the O157:H7 LEE does not induce the A/E lesions in the *E. coli* K-12 background, whereas the EPEC LEE does [38]. This indicates that there are some differences between the O157:H7 LEE and the EPEC LEE in terms of function and regulation. The regulatory mechanisms of the LEE genes, as well as environmental signals, are known to be complicated and are well reviewed in the reference [89].

The pO157

Initial profiling of the plasmids present in *E. coli* O157:H7 demonstrated the presence of multiple plasmids and the high prevalence of the pO157. The plasmid pO157 was found in 99% of 107, 100% of 100, and 100% of 88 clinical isolates of *E. coli* O157:H7 from humans [80,98, 107]. The subsequent epidemiological studies suggest that almost all *E. coli* O157:H7 strains possess this plasmid [93]. A pO157-like plasmid is also present in O26:H11 strains and in most STEC isolates from humans and animals [93,118]. However, its biological significance in infection is unknown.

Previous *in vivo* and *in vitro* studies have reported conflicting results on the role of the plasmid in adherence to epithelial cells [48,65,93,132,139,143]. Karch *et al.* [65] first reported that the pO157 is associated with the expression of fimbriae that enhance bacterial adherence to epithelial cells. However, supporting data have not been shown yet. Other studies have shown that the plasmid has no effect on adhesion or reduced adherence [93]. Similarly, *in vivo* studies using animal models such as mouse, rabbit, and gnobiotic piglet did not help to define the biological role of this plasmid in terms of diarrhea, intestinal

histopathology, or the fecal shedding patterns of *E. coli* O157:H7. The absence of suitable animal models may be partially responsible for these conflicting results [93]. Furthermore, a study showed that the pO157 is somehow associated with the suppression of exopolysaccharide production in *E. coli* O157:H7 [48,62].

Recently, the complete nucleotide sequence of the pO157 was determined [16]. It revealed that the plasmid is a very stable, 92-kb F-like plasmid and has a heterogeneous mosaic structure with seven insertion sequence elements located near the virulence-related segments. The pO157 contains 100 ORFs, of which only 19 have been previously characterized. These include a type II secretory system (*etpC-etpO*) [116], enterohemolysin (*hlyA-D*) [117], catalase-peroxidase (*katP*) [13], serine protease (*espP*) [14], lymphocyte inhibitory factor (*lifA/efa*) [60], a putative adhesin (*toxB*) [131], and a recently described C1 esterase inhibitor metalloprotease (*StcE*) [74]. However, the biological significance of these putative virulence factors was not demonstrated.

Pathologenesis of *E. coli* O157:H7

There are many requirements for an *E. coli* O157:H7 infection to occur, involving complex interactions between bacterial and host factors. Ingested bacteria must survive in the acidic environment of the stomach and then compete with other gut microflora to establish intestinal colonization. Once colonization has occurred, the bacteria produce Stxs in the intestinal lumen, which must be absorbed by the intestinal epithelium and must move to the blood stream. A three-stage model for EPEC and EHEC has been proposed, including (i) initial adherence, (ii) signal transduction, and (iii) intimate adherence [93,100].

Initial adherence

Although accumulating data show that intimin is clearly associated with bacterial adherence in the later stages of pathogenesis [27,35,93], it is still unclear which factors are involved in initial adherence. Nonetheless, some adherence-associated factors have been characterized.

Karch *et al.* [65] reported a fimbrial adhesin whose expression was associated with the presence of the pO157. They showed that the fimbrial adhesin mediates bacterial attachment to Henle407, but not to HEp-2 cells. Further studies have failed to support these findings, and recent sequencing data reveal that the pO157 is not predicted to encode a fimbrial gene cluster. This indicates that this fimbrial adhesin may be encoded on the chromosome. Increasing evidence indicates that lipopolysaccharide (LPS) is associated with bacterial adhesion, probably through an indirect mechanism rather than through a direct mechanism [11,26]. It has been reported that anti-LPS antibody can block bacterial adherence to Henle407 cells, whereas the pretreatment of Henle407 cells with LPS cannot block bacterial adherence [99]. Furthermore, a hyper-adherent phenotype, observed with HEp-2 cells, is an *E. coli* O157:H7 LPS-deficient mutant lacking the O-polysaccharide side chain [11,26]. Although this increased adhesion may be due to autoaggregation and rapid sedimentation of strains lacking the O-antigen [120], it seems that LPS masks adhesive structures present on the bacterial surface or that bacterial surface properties such as hydrophobicity are altered by the absence of LPS. Sherman and Soni [122] showed that outer membrane (OM) preparations from an *E. coli* O157:H7 isolate inhibit bacterial adherence to HEp-2 cells. Further analysis showed that antibodies specific for a 94-kDa OM protein inhibit adhesion and that this protein is immunologically distinct from intimin [121]. Tarr *et al.* [131] described a chromosomally encoded Iha, IrgA homologous adhesin in *E. coli* O157: H7, which is an OM protein conferring an adherence phenotype with HeLa cells. This gene shows homology to the IrgA gene of *Vibrio cholerae*, which encodes an iron-regulated protein [131]. Iha is located adjacent to the tellurite resistant loci and is therefore designated TAI (tellurite resistance- and adherence-conferring island). This region is highly conserved in distantly related pathogenic *E. coli*, but not in non-toxigenic *E. coli* O55: H7, sorbitol-fermenting STEC O157:H-, or laboratory *E. coli* strains [131].

ToxB is a large 362-kDa OM protein encoded on the pO157 [132]. This protein shares sequence similarity with the large Clostridium toxin family proteins such as the EPEC LifA protein [69] and the Efa-1 protein that has been implicated as an adhesin in non-O157 EHEC [118]. ToxB plays a role in full adherence of *E. coli* O157:H7 to Caco-2 cells by facilitating the secretion of TTSS proteins [132].

Recently, a hyper-adherent phenotype was observed in the *E. coli* O157:H7 *tdcA* mutant, which is a regulator of the *tdc* operon responsible for transport and anaerobic degradation of L-threonine [136]. Interestingly, in this mutant, an OM protein A (OmpA) was differentially expressed. OmpA has been associated with many functions, such as porin activity, mediation of conjugation, serum resistance, and bacterial invasion [136]. However, in *E. coli* O157:H7, OmpA seems to be an adherence factor that mediates bacterial adherence to Caco-2 cells and HeLa cells.

McKee and O'Brien [87] described a 'log jam' adherence phenotype in *E. coli* O157:H7, in which bacteria adhered to, and lined up at, the junction between HCT-8 cells, but not HEp-2 cells. This adherence pattern is observed in other pathogenic *E. coli*, as well as in *E. coli* O157:H7, and therefore may represent a basal adherence mechanism allowing *E. coli* to adhere to the intestinal epithelium.

A recent study has characterized an endogenous host cell receptor for intimin, called nucleolin. The data suggest that

intimin may also promote initial adherence of *E. coli* O157:H7 [124].

Signal transduction

The O157:H7 LEE encodes the TTSS required for the contact-dependent translocation of bacterial proteins into host cells [19,46,135]. The effector proteins are also encoded on the LEE and include the *E. coli* secreted proteins EspA, EspB, EspD, EspF, EspG, mitochondria-associated protein, and Tir [19,110]. EspA forms a filamentous cylindrical structure for the export of EspB and EspD [37,70]. EspB/EspD are thought to transit through the EspA filament to form a pore (or translocon) in the host membrane and also to deliver other virulence factors into the cells [58]. For example, a functional EspA filament and EspB/D translocon are required for translocation of Tir. In addition, the targeting and function of other TTSS-secreted proteins have been reviewed in detail in the reference [110].

Compared to EPEC, pedestal formation by *E. coli* O157:H7 is more complex and less well characterized [19]. However, *E. coli* O157:H7 forms pedestals independently of Nck (an adaptor protein containing src homology 2 and 3 domains), because *E. coli* O157:H7 does not recruit Nck to the site of actin polymerization, and it can form pedestals on cell lines that do not express Nck [51]. Moreover, Nck-independent actin signaling by *E. coli* O157:H7 requires translocation of one or more bacterial factors in addition to Tir [29]. The combination of Tir and other factors promotes recruitment and activation of neuronal Wiskott- Aldrich syndrome protein (N-WASP) by an unidentified mechanism. N-WASP then stimulates Arp2/3 (a heptameric actin-related protein 2/3)-based actin nucleation [19,111].

Although EPEC and EHEC contain highly conserved LEE regions and form similar actin pedestals, it seems that they induce different Tir-based signaling in the host cells. For example, the EPEC Tir is phosphorylated on a tyrosine residue (Tyr474) in its C-terminal cytoplasmic domain, whereas the EHEC Tir is not [30,67]. Similarly, mammalian adaptor proteins Grb2 and CrkII are able to localize to EPEC pedestals, but not to EHEC pedestals [49]. Localization of these proteins depends on EPEC Tir tyrosine phosphorylation. Additionally, EHEC Tir is not functional for actin signaling when expressed in EPEC [29,67]. These data indicate that the mechanism of actin signal transduction is different in EPEC and in EHEC.

Intimate adherence

The genes involved in intimate adherence are *eae* and *tir* [46]. Studies on *E. coli* O157 intimin have shown that it can act as an adhesin for bacterial adherence to HEp-2 cells [34,84]. *In vivo* studies using ruminants and gnobiotic piglets have demonstrated that intimin-deficient *E. coli* O157:H7 mutants are less virulent than the parent strain and are less able to colonize the intestines of these animals [27,34]. In addition, the *eae* gene of EPEC is functionally homologous to the EHEC *eae* gene because it restores full virulence to the O157:H7 *eae* mutant [34]. Further studies in gnobiotic piglets have shown that an *E. coli* O157:H7 intimin-negative mutant expressing the EPEC intimin adheres to different bowel sites, suggesting that the EPEC and *E. coli* O157:H7 intimins have different receptor binding specificities in this model [138].

Although of similar molecular weight, the EPEC and EHEC intimins show some differences. Overall, the two proteins are 83% homologous at the amino acid level, with the first 704 amino acids sharing 94% homology and the remaining C-terminal residues sharing only 49% homology [153]. The C-terminus is the receptor binding region, and differences in this region may explain the different tissue tropisms of the EPEC and EHEC intimins. The different tissue tropisms are dependent on the ability of intimin to bind to endogenous host cell receptors, as well as to Tir [45,47]. Recent data show that intimin can bind to both Tir and to host receptors [124]. Binding to Tir occurs through two Ig-like regions, whereas binding to the host receptors occurs through a lectin-like region.

Environmental survival of *E. coli* O157:H7

In general, Gram-negative bacteria have an OM separated from the inner membrane by a periplasmic space. This arrangement allows cells to filter and sense the extracellular environment, as well as export virulence factors to the exterior [106]. Among the structural components, the OM is very important in bacterial physiology. In enteric Gram- negative bacteria, the OM acts as a strong physical and permeability barrier that protects bacteria from host defense factors such as bile salts, digestive enzymes, and immune factors, in addition to many antibiotics [25,129, 141].

E. coli O157:H7 thrives in diverse environments－from soil, sewage, and water ecosystems to the host gastrointestinal tract. This microorganism can survive for long periods of time in water, especially at cold temperatures, and can enter a viable but non-culturable state [145]. *E. coli* O157:H7 was shown to survive for more than 8 months in a farm water trough, and the surviving cells proved to be infectious to calves [77]. *E. coli* O157:H7 can survive in raw (not composted) bovine or ovine feces for 21 months and retain its virulence traits, such as Stx production [72]. A recent study showed that *E. coli* O157:H7 survives and replicates in a common environmental protozoan, *Acanthamoeba polyphaga* [6]. Since protozoa are widely distributed in soil, water, and fecal slurry, they seem to be an important transmission vehicle of *E. coli* O157:H7 present in these environments.

E. coli O157:H7 must pass through the acidic environment of the stomach to establish an infection in the host GIT. Three systems in *E. coli* O157:H7 are involved in acid tolerance: an acid-induced oxidative system, an acid-induced arginine-dependent system, and a glutamate-dependent system [75]. The oxidative system is less effective in protecting the organism from acid stress than are the arginine- and glutamate-dependent systems [82]. The alternate sigma factor, RpoS, is required for oxidative acid tolerance, but is only partially involved with the other two systems. Once induced, acid resistance is stable during storage at 4°C (> 28 days) [35]. Stationary phase bacteria are more than 1,000 times more resistant to acid than exponentially growing organisms and do not need prior exposure to low pH to exhibit resistance. More importantly, acid resistance also increases resistance to other environmental stresses. Acid resistance-induced cells can have increased tolerance to heating, radiation, and antimicrobials [15,112]. An *E. coli* O157:H7 *rpoS* mutant is significantly less tolerant to acid, heat, and high salt conditions than is the parent strain [22,105]. In addition, a recent study suggests that the ability to produce exopolysaccharide in *E. coli* O157:H7 is associated with bacterial tolerance against heat and acid [85]. Interestingly, heat stress can induce the alteration of membrane lipid composition in *E. coli* O157:H7, which also affects virulence gene expression [154].

The tolerance of *E. coli* O157:H7 to varied environments likely requires differential gene expression. DNA topological changes have been suggested to explain differential gene expression, especially in response to various environments with extremes of temperature, pH, osmolarity, and anaerobiosis [4,40]. An example is an intrinsically curved DNA [97,101,102]. A recent study demonstrated that the pO157 *ecf* operon is thermoregulated through an intrinsically curved DNA called BNT2, which is present on its promoter upstream regulatory region [152]. Differential expression of the genes encoding in this operon has been proposed to be responsible for the structural modification of *E. coli* O157:H7 LPS, which may enhance bacterial survival in certain *ex vivo* and *in vivo* environments [63,68,81,127,151,152].

Treatment and vaccine development

Therapeutic strategies can be categorized as limiting the severity and duration of gastrointestinal symptoms and preventing systemic complications such as HUS [100]. There is controversy about the use of antibiotics. Although it was reported that early treatment with fosfomycin reduced the risk of HUS during a large outbreak in Japan in 1996, several studies have failed to recommend antibiotic treatment [93,150]. It may be possible that antibiotics allow overgrowth of *E. coli* O157:H7 by killing other gut microflora. Antibiotics may also cause bacterial cell lysis that could increase the systemic absorption of the toxin by increasing the amount of membrane-free Stx in the intestine [23]. Furthermore, antibiotics such as trimethoprim-sulfamethoxazole and ciprofloxacin (bacterial DNA synthesis inhibitors) increase the amount of free Stx in the culture medium [100]. In addition to antibiotic therapy, an alternative therapeutic strategy that has been investigated is *in vivo* binding or neutralization of Stx. This strategy may limit the severity or duration of disease, but will not reduce bacterial transmission [100]. Agents such as Synsorb-Pk, which consists of the oligosaccharide component of Gb3 covalently linked via an 8-carbon spacer to silica particles derived from diatomaceous earth, have been administrated to patients with HC in hopes of preventing the development of HUS, but have met with limited success [3]. Current treatment of renal disease includes dialysis, hemofiltration, transfusion of packed erythrocytes, and platelet infusions. Some patients that survive severe disease may still need renal transplantation [93]. There is no available vaccine to prevent infection by *E. coli* O157:H7, but current vaccine development studies using animal models have focused on three main areas: (i) vaccination against colonization factors such as intimin, (ii) vaccination against LPS, and (iii) vaccination with Stx subunits or toxoids [56,93].

Concluding remarks

E. coli O157:H7 are highly infectious to humans and animals and can tolerate diverse environments well — from nutrient-dilute water to adverse gastrointestinal tracts. The pathogenesis of this bacterium is believed to be multi-factorial, because any single functional mutation of the previously defined virulence factors is not completely attenuated. Therefore, further studies of the mechanisms by which *E. coli* O157:H7 thrives and colonizes *ex vivo* or *in vivo* during infection would provide significant opportunities for developing a new vaccine or therapeutics to prevent or ameliorate bacterial infection.

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