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Diarrheagenic *Escherichia coli*

Chan-hee, Chae
Veterinary Diagnostic Center, University of Nebraska-Lincoln, Lincoln, Nebraska 68583 USA
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설사유발성 대장균

채 찬 희
네브라스카 대학 수의진단센타, 네브라스카주 링컨
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1. Introduction

Escherichia coli is serotyped on the basis of its lipopolysaccharide (o), flagellar (H), and polysaccharide(K) antigens.[26] One hundred seventy-one O and 56 H serogroups have been identified.[26] Pathogenetically, diarrheagenic *E coli* are divided into four major groups on the basis of their O : H serogroup, their ability to produce adherence factors and toxins, their effect on enterocytes, and the clinical signs or symptoms they induce.[28] The four major groups include enterotoxigenic *E coli* (ETEC), enteroinvasive *E coli* (EIEC), enteropathogenic *E coli*(EPEC), and enterohemorrhagic *E coli* (EHEC).[28]

ETEC strains are characterized by production of certain adherence factors and of heat labile(LT), and/or heat stable toxins(ST).[29] EHEC and EPEC strains lack the ability to produce LT or ST ; instead, EHEC and some EPEC strains(i.e.026:NM, 0165 : H19, and 0165 : H25) produce Shiga-like toxin-I(SLT-I ; also called Verotoxin 1 or VT1) and/or Shiga-like toxin-Ⅱ.[6,8,37] EHEC strains typically produce high levels and EPEC low to moderate levels of SLT.[8] Since SLT is synonymous with verotoxin, EHEC and some EPEC have also been termed verotoxigenic *E coli* (VTEC).[25] EHEC and EPEC strains attach closely to the apical plasma membranes of enterocytes, resulting in disruption and distortion of the microvilli.[17,28,34] This type of colonization has been termed attaching-effacing subsequently EPEC and EHEC are also referred to as attaching and effacing *E coli* (AEEC).

2. Enterotoxigenic *Escherichia coli*

ETEC strains are a major cause of traveler's and infantile diarrhea in undeveloped countries where outbreaks are often associated with poor hygiene.[3,33] The typical symptoms of travelers' diarrhea are watery diarrhea, nausea, abdominal cramps, and mild fever.[28] ETEC strains have two distinct characteristics : (a) colonization factors which mediate adherence to specific receptors on enterocytes ; and(b) the ability to produce ST and/or LT.[28]

The colonization factors produced by ETEC are proteinaceous, filament-like structures termed pili or fimbriae that extend from the bacterial surface and interact with a specific carbohydrate receptor on the surface of the epithelial cells to promote bacterial colonization.[28] The most prominent colonization factors in strains associated with *E coli* infections in animals include F4(K88), F5(K99), F6(987P) and F41.[4,29] At least three distinct types of putative adhesion fimbriae have been detected in human ETEC pathogens, including colonization factor

antigen I(CFA/I), CFA/Ⅱ, and E8775 fimbriae.[28,29] Pili are important in the mediation of adhrence, colonization and stability against peristaltic movement of fluid and ingesta in the intestine.[28] Various animal species are susceptible to certain ETEC pilus types. Colonization with K88 and 987P strains is limited to swine[44,49], while K99 strains colonize enterocytes of calves, lambs, and pigs.[10] Resistance to colonization is mediated partially by phenotype and age of the host. Receptors for K88 are expressed as an autosomal dominant trait in swine. Swine which are homozygous recessive at this locus lack the specific receptor and are resistant to colonization by K88 strains.[55] Resistance to K99, 987P, and F41 strains is acquired with age. Age resistance in the K99 strains is mediated by the decrease or masking of epithelial receptors on enterocytes.[43]

ETEC induce diarrhea by the production of LT and/or ST.[29] LT holotoxin, consisting of an A subunit and five copies of a B subunit, is approximately 86,500 Da.[29] The pentameric B subunits bind to monosialoganglioside(GM_1) receptors in the brush border of enterocytes and allow the A subunit to be taken up into the cytoplasm.[22,23] The A subunit stimulates adenylate cyclase, which induces the cell to accumulate cyclic adenosin 5'-monophosphate(cAMP).[29] Increased intracellular cAMP activates nucleotide-dependent enzymes that phosphorylate transport proteins, resulting in secretion of fluid and electrolytes at the brush border of crypt enterocytes, and decreased water absorption by villous enterocytes.[16] Watery diarrhea results from excess fluid secretion into the intestinal lumen.[29]

Heat stable toxin(ST) is 1,000-6,000 Da, less antigenic, and chemically, more heterogeneous than LT.[2] ST can be categorized into two major groups, ST_a(STI) and ST_b(ST Ⅱ), based on chemical characteristics and host specificity. ST_a is soluble in methanol and causes diarrhea in humans and pigs ; whereas, ST_b is insoluble in methanol and affects only pigs.[5,29] ST_a is further subgrouped on the basis of species specificity and genetic composition. One subgroup, ST_aH, affects only humans ; whereas, ST_aP affects pigs and cattle. The percent DNA homology between ST_aH and ST_aP is 69%[35] ; in contrast to ST_a and ST_b, which have little DNA homology.[29] ST_a binds to a specific protein receptor in the brush border, stimulates guanylate cyclase[18,38], and induces intracellular accumulation of cyclic guanosine 5'-monophosphate(cGMP).[20] Increased cGMP induces villous enterocytes to decrease water and electrolyte absorption and crypt enterocytes to increase their secretion.[2] Compared to LT, ST_a causes less secretion by enterocytes, but is equivalent in its anit-absorptive activity.[19]

3. Enteroinvasive *Escherichia coli*

Enteroinvasive ***E coli***(EIEC) include serogroups 0128, 052, 0112, 0124, 0136, 0143, 0144, 0145 and 0147.[11,28] EIEC infections are mainly seen in Vietnam and Southeast Asia, causing diarrhea in both adults and children.[11] Affected patients are febrile and have severe abdominal cramps, malaise, and watery diarrhea followed by bloody dysentery. EIEC strains are closely related biochemically and pathogenetically to ***Shigella*** spp.[7], with the exception that EIEC strains do not produce Shiga toxin or SLT.[9] Neither ST nor LT is produced by EIEC strains.[11] Similar to ***Shigella*** spp., EIEC strains invade and replicate in colonic enterocytes and cause epithelial cell necrosis, inflammation and dysentery.[11] Cellular invasion is mediated by a large (=140 MDa) plasmid.[21] Enteroinvasiveness is correlated with the ability to cause keratoconjunctivitis in guinea pigs(the Serény test).[11]

4. Enteropathogenic *Escherichia coli*

Neter first coined the term enteropathogenic (EPEC) in reference to specific ***E coli*** serotypes that were associated with infantile diarrhea.[36] That EPEC were significant preceded the recognition of ST and LT, and their distinction from ETEC strains was later confirmed when EPEC were found to be incapable of producing ST and LT.[13] EPEC were also distinguished from EIEC, as they produced a negative Serény test[13] and they closely attached to and effaced microvilli of intestinal epithelial cells.[32,34]

The "attaching and effacing" activity of EPEC strains was first documented in photomicrographs by Staley et al. using gnotobiotic pigs as a host[50] ; however, the attaching-effacing phenomenon and its significance was largely unknown until the report by Moon et al., in which EPEC strains inoculated into gut loops of rabbits and pigs and produced characteristic lesions.[34] Ultrastructurally, bacteria were intimately associated with or "attached" to the plasma membrane of microvilli. The

attachment of bacteria caused distortion and loss("effacement") of microvilli and associated cytoskeletal elements. More recent studies have shown that the attaching-effacing phenomenon occurs in two distinct phases. The first phase, the initial association of bacteria with the cell surface, is mediated by plasmid-encoded fimbria ; whereas the intimate attachment and effacement constitute a second phase which is not pilus-mediated.[27]

EPEC strains can be classified on the basis of their adherence patterns on HEp-2 cells, a human laryngeal epidermoid carcinoma cell line ; Class I EPEC strains adhere locally, and Class Ⅱ EPEC, diffusely, to the cells.[28] Localized adherence is associated with a plasmid-encoded EPEC adherence factor(EAF).[1] Although the receptor(s) for EAF has not been determined, some Class I EPEC strains are able to bind to fibronectin.[15] Wadstrom et al. have suggested that fibronectin might be a receptor for EAF. EAF is closely associated with an outer membrane protein (OMP, 94 kDa) of the bacteria, which is thought to serve as a critical protective antigen. It is possessed by most of the clinically important EPEC serogroups, including 055, 0111, 0119, 0127, and 0142.[30]

A few EPEC strains, such as serogroups 026 : NM, 0165 : H19, and 0165 : H25 produce SLT.[25] The SLT titer in CD^{50}/mg (50% lysis of HeLa cells) is low to moderate and approximately half that typically produced by EHEC strains.[8] Since relatively small amounts of SLT are produced, it is suggested that SLT is not an improtant virulence factor for EPEC, although the significance of SLT is yet to be determined.

5. Enterohemorrhagic *Escherichia coli*

In 1982, an outbreak of hemorrhagic colitis was associated with consumption of undercooked hamburger and indicated that a rare serogroup of *E coli*, 0157 : H7, was pathogenic to humans.[41] *E coli* serogroup 0157 : H7 and others now classified as EHEC, including 0111 : H-, 0145 : H-, 045 : H2, and 04 : H-[25], do not produce heat stable toxin (ST) or heat labile toxin(LT). They are noninvasive in the Serény test, but produce attaching-effacing lesions.[28] However, EHEC do not exhibit the same adherence patterns as EPEC on HEp-2 and HeLa cells.[42] Clinically, EHEC is associated with hemorrhagic colitis in individuals of all ages ; whereas, EPEC usually is associated with infantile diarrhea.[28,40] Both EPEC and EHEC contain large(50-70 MDa) plasmids which encode for proteins that mediate adherence to certain cell lines, but not attaching-effacing adherence in gnotobiotic pigs.[52] A more recent study has shown that *E coli* 0157 : H7 has three basic plasmid profiles : profile I, characterized by 68.7 MDa and 4.2-MDa plasmids(62%) ; profile Ⅱ, characterized by 66.2 MDa and 1.8-MDa plasmids (20%) ; and profile Ⅲ, characterized by a 62.5-MDa plasmid(18%).[39]

Most strains of *E coli* 0157 : H7 adhere to Int 407 cells(a human embryonic intestinal cell line), but not HEp-2 cells. Adherence to HEp-2 cells is mediated by mannose-resistant fimbriae which are encoded by a 60 MDa plasmid.[24] Adhrence to Int 407 and HEp-2 cells is associated with presence of the EPEC adherence factor (EAF).[31] Some strains of *E coli* 0157 : H7, e.g., strain CL-49, can adhere to both HEp-2 and Int 207 cells. This particular strain can also adhere to human buccal epithelial cells, ileal enterocytes, and colonocytes.[12,47] Adherence to the latter cell types is mediated by mannose-sensitive, type 1 fimbriae, in which the receptor is an α-linked mannosyl residue.[12]

EHEC Type 1 fimbriae are much more hydrophobic than the type 1 fimbriae found on other types of *E coli*. The hydrophobicity facilitates binding to oligomanosyl derivatives in mucous secreted by goblet cells.[53] Binding of type 1 fimbriae to intestinal surface mucin of ileal enterocytes and colonocytes may enhance colonization and allow toxins to be concentrated in the underlying mucosa. Outer membrane proteins, but not lipopolysaccharide or H7 flagella, mediate binding of *E coli* 0157 : H7 to HEp-2 cell lines.[46]

Most 0157 strains found in hemorrhagic colitis contain H7 flagella which are immunogenic, composed of 66,000 Da subunits termed flagellin, and impart motility to the organism.[48] The finding that most cases of hemorrhgic colitis are associated with motile strains suggests that the flagella may be a significant virulence factor and allow the organism to overcome the peristaltic activity of the intestine and colonize the mucosa.

E coil 0157 : H7 typically produces large amounts of SLT-I and/or- Ⅱ.[51] In one field study, 54% of *E coli* 0157 : H7 isolated produced both SLT-I and -Ⅱ, 42% produced only SLT-Ⅱ, and 4% produced only SLT-I.[45]

E coli 0157 : H7 has distinct biochemical markers, namely, a negative reaction for β-glucuronidase and sorbitol (100% isolates), and a positive reaction for raffinose and dulcitol (100% isolates).[39] *E coli* 0157 : H7 is also known to produce a hemolysin, termed enterohemolysin, although its role, if any, as a virulence factor has not been determined.[2]

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