14 *Escherichia coli*

Introduction

Theodor Escherich, a German–Austrian pediatrician and a professor at universities in Graz and Vienna, Austria, reported, in 1885, the isolation of a bacterium called *Bacterium coli* from a fecal sample. Later, in 1919, the bacterium was renamed *Escherichia coli*. *E. coli* is a Gram-negative, short motile rod that inhabits the intestinal tract of animals and humans since birth. *E. coli* has been used extensively as a model organism to study bacterial physiology, metabolism, genetic regulation, signal transduction, and the cell wall structure and function. The bacterium is one of the natural microflora of human and animal gut microbial community. Hence, fecal shedding and contamination of water and food with *E. coli* or coliforms are common and are often used as indicators for hygiene monitoring. A majority of *E. coli* strains are nonpathogenic; however, only a small subset is pathogenic and causes a variety of diseases in humans and animals. The diseases include gastroenteritis, dysentery, hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS), urinary tract infection (UTI), septicemia, pneumonia, and meningitis. In recent years, however, the major concern has been the increasing numbers of foodborne outbreaks, caused by pathogenic *E. coli* in the industrialized countries due to consumption of contaminated meat, fruits, and vegetables.

Biology

Escherichia coli is a member of the genus *Escherichia* and family *Enterobacteriaceae*. Other lesser-known species in the genus *Escherichia* are *E. albertii*, *E. blattae*, *E. hermannii*, *E. vulneris*, and *E. fergusonii*. The *Enterobacteriaceae* family consists of 40 genera and about 180 species, and they are either primary pathogens, opportunistic pathogens, or commensals (Fig. 14.1). The primary pathogens are also recognized as enteric pathogens, and they include *Escherichia coli*, *Salmonella enterica*, *Shigella* species, and *Yersinia* species. The opportunistic pathogens are *Proteus*, *Klebsiella*, *Enterobacter*, *Edwardsiella*, *Morganella*, *Hafnia*, *Serratia*, and so forth. The members of *Enterobacteriaceae* family ferment glucose and lactose, are oxidase negative and catalase positive, and reduce nitrate to nitrite. The members are resistant to bile salts; therefore, they can be readily isolated using violet red bile glucose agar (VRBGA), brilliant green bile agar (BGBA), and MacConkey agar (MAC) media.

Escherichia coli is a Gram-negative, short-rod (1–2 μm in length), facultative anaerobe and is motile. It expresses peritrichous flagella, fimbriae or pili, and curli. Some strains express capsules. The optimum growth temperature is 35–37 °C. They are lactose fermenter and produce pink colonies when grown on MacConkey agar plate.

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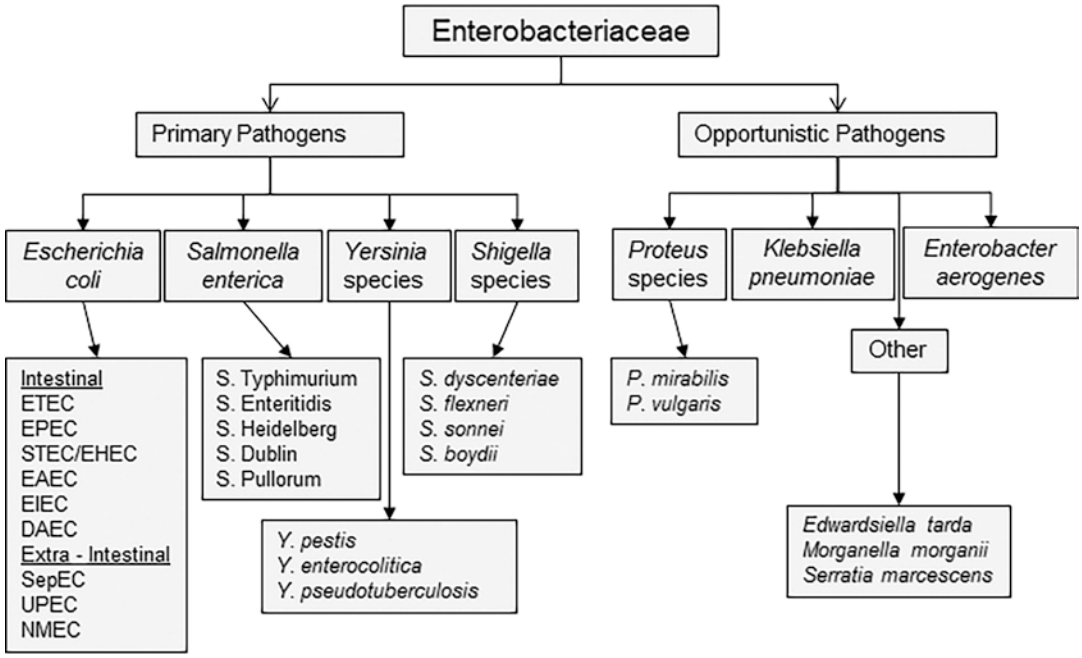

Fig. 14.1 *Enterobacteriaceae* family and its major pathogenic members (genus and species)

Some pathogenic strains are also acid tolerant. The genome size of a pathogenic strain could be 1 Mb larger than the nonpathogenic strains because of the presence of virulence genes. Note: the genome size of nonpathogenic *E. coli* K12 strain is 4.6 Mb. In pathogenic strains, the virulence genes are clustered in pathogenicity islands (PAIs), plasmids, or prophages. The PAIs are flanked by mobile genetic elements – bacteriophages, insertion sequences, or transposons. Horizontal gene transfer facilitates host adaptation and emergence of new pathogenic strains such as enterohemorrhagic *E. coli* (EHEC), Shiga-toxin producing *E. coli* (STEC), and enteroaggregative *E. coli* (EAEC) pathovars (Fig. 14.2).

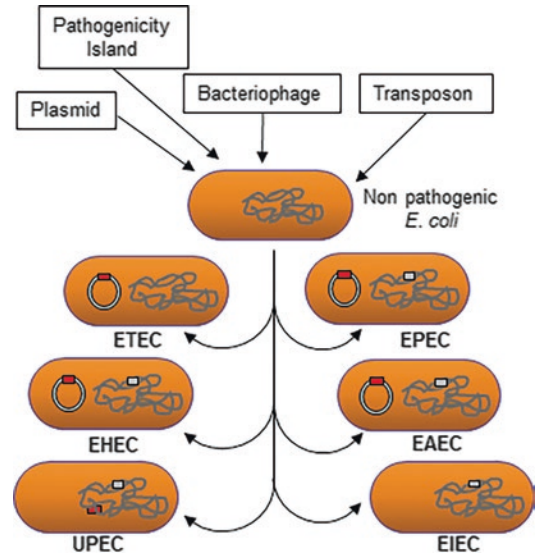

Fig. 14.2 Evolution of pathogenic *Escherichia coli* through horizontal gene transfer

Sources

Escherichia coli is a member of the intestinal microflora of humans, animals, and birds (Fig. 14.3). The bacterium is routinely shed into the environment through feces, and it can contaminate drinking water, irrigation water, and soil, consequently fresh fruits and vegetables, especially if untreated manures are used as fertilizers. Contaminated fresh produce may even

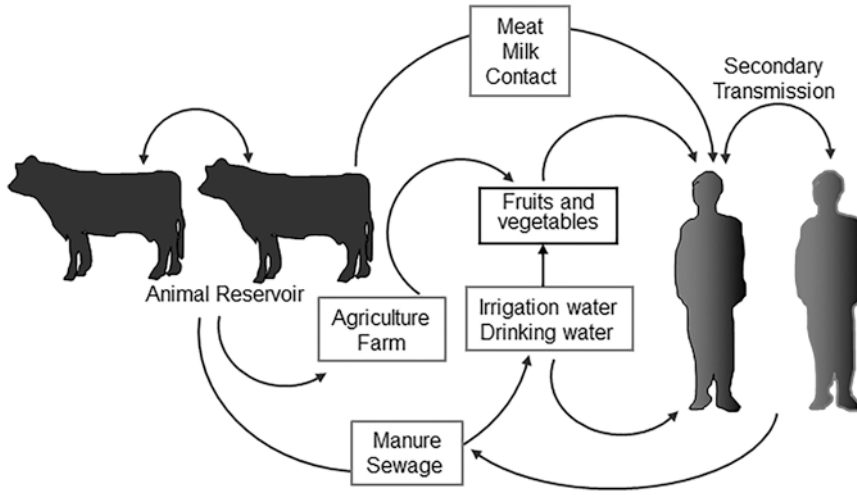

Fig. 14.3 Mode of transmission of *Escherichia coli* to humans. The primary vehicle of transmission is meat, but the bacteria can be transported via animal-to-person contact, milk, contaminated water, and vegetables contaminated with cow manure. Bacteria can move from cow-to-cow and also from wild animals such as deer, caribou, and domestic sheep. Person-to-person transmission occurs directly or via contaminated water such as in the swimming pool

harbor some bacteria inside the plant tissues. Some *E. coli* pathotypes such as enterohemorrhagic *E. coli* (EHEC) can be transferred through meat, which may be acquired during slaughter through fecal and hide contact. EHEC outbreaks have been associated with meat (especially ground beef), dairy products, mayonnaise, apple cider, sprouts, lettuce, and spinach. Outbreaks are also associated with various establishments including swimming pools, nursing homes, restaurants, petting zoo, and day-care facility. Travelers in the endemic region may experience *E. coli*-mediated diarrhea and dysentery. Human-to-human transmission can also happen.

Classification

Serotypes

Somatic "O" antigens are one class of *E. coli* serogroup determinants, consisting of lipopolysaccharide (LPS), and there are 174 O antigens, numbered 1–181, with numbers 31, 47, 67, 72, 93, 94, and 122 omitted. In addition, there are 53 serotypes of "H" or flagellar antigens (H1–H53). Strains that lack flagella are nonmotile (NM). *E. coli* isolates can have a variety of antigen combinations. Thirty serovars are reported to be responsible for diarrheal diseases, and the first serogroup identified was O111 and was isolated from a child. The "O" antigen identifies serogroup, while the "H" identifies serotype. For example, two strains with same O antigens but different H antigens such as O111:H4 and O111:H12 have same serogroup but different serotype. There are also 80 capsular antigens or "K" antigens.

Virotypes

Virotype or pathotype classification is based on the presence of certain virulence factors and their interaction with the mammalian cells and cell signaling events for effective adhesion to and/or invasion of mammalian cells and toxin production (Fig. 14.4). Pathogenic *E. coli* are classified broadly into two groups: gastrointestinal tract-infecting *E. coli* that cause diarrhea and extraintestinal tract-infecting *E. coli* that affect the kidney, urinary tract, brain, and circulatory system leading to septicemia (Table 14.1). These *E. coli* strains are designated septicemic *E. coli*

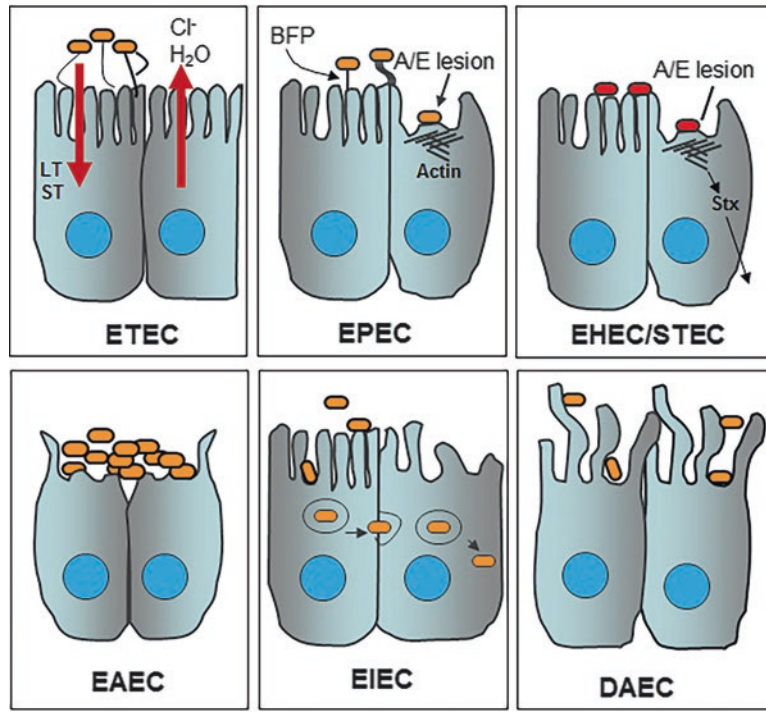

Fig. 14.4 Virotype classifications of *Escherichia coli* based on their interaction with intestinal villous epithelial cells: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adhering *E. coli* (DAEC). *LT* Heat-labile toxin, *ST* heat-stable toxin, *bfp* bundle-forming pili, *A/E lesion* attachment/effacement lesion showing actin accumulation, *Stx* Shiga toxin

Table 14.1 Colonization sites of pathogenic *E. coli* in the human body

Organs and tissues affected	Pathotypes
Small intestine	ETEC, EPEC, DAEC, EAEC
Large intestine	EHEC (STEC), EIEC, EAEC
Kidney	UPEC
Bladder	UPEC
Brain	NMEC
Bloodstream	UPEC, NMEC, SepEC

(SepEC), uropathogenic *E. coli* (UPEC), and neonatal meningitis-causing *E. coli* (NMEC). Diarrheagenic *E. coli* are again divided into six pathotypes (Fig. 14.4): (1) enterotoxigenic *E. coli* (ETEC), (2) enteropathogenic *E. coli* (EPEC), (3) Shiga toxin-producing *E. coli* (STEC) and their most pathogenic subset enterohemorrhagic *E. coli* (EHEC), (4) enteroaggregative *E. coli* (EAEC), (5) enteroinvasive *E. coli* (EIEC), and (6) diffusely adhering *E. coli* (DAEC).

1. Enterotoxigenic *E. coli* (ETEC) adhere to epithelial cells and produce several toxins including heat-labile (LT) and/or heat-stable toxins (ST), but they do not invade epithelial cells. The predominant serogroups are O6, O8, O11, O15, O20, O25, O27, O78, O128, O148, O149, O159, and O173.
2. Enteropathogenic *E. coli* (EPEC) adhere to epithelial cells intimately, produce attachment/effacement (A/E) lesion, and are noninvasive. They do not produce any heat-labile (LT), heat-stable (ST), or Shiga toxins (Stx). The notable serogroups are O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, and O158.
3. Shiga toxin-producing *E. coli* (STEC) and their most pathogenic subset enterohemorrhagic *E. coli* (EHEC): EHEC bind strongly to the epithelial cells, produce attachment/effacement lesions, and produce Stx. The serogroups are O4, O5, O16, O26, O45, O55, O91, O103, O111ab, O113, O121, O117, O145, O157, O172, O176, O177, O178, O180, and O181.
4. Enteroaggregative *E. coli* (EAEC) adhere to epithelial cells; form aggregates, appearing like "stacked brick;" and produce toxin but do

not invade. The serogroups in this virotype are O3, O15, O44, O86, O77, O104, O111, and O127.

5. Enteroinvasive *E. coli* (EIEC) cells also adhere, invade cells, and move from cell to cell but do not produce toxin. The pathogenicity of EIEC resembles infection caused by *Shigella* spp., and the predominant symptom is dysentery. The EIEC serogroups are O28, O29, O112, O124, O136, O143, O144, O152, O159, O164, and O167.
6. The diffusely adhering *E. coli* (DAEC) adhere to epithelial cells, but they neither invade nor produce toxin. Details of each virotype are described below.

Enterotoxigenic *E. coli*

Characteristics

Enterotoxigenic *E. coli* (ETEC) cause toxicoinfection. ETEC were first reported to cause cholera-like illness in adults and children in Calcutta (India) in 1956. In general, ETEC commonly cause infectious diarrhea in people living in the tropical climate, and children under the age of 2 are highly susceptible. Infants in many developing countries suffer from ETEC due to poor sanitary conditions. ETEC also affect travelers since the water and food consumed by them may be contaminated with ETEC, and native people may be resistant because of their frequent exposure to the organism. Thus, ETEC-mediated diarrhea is also known as "traveler's diarrhea" because of the bacterial association with international travel. The same disease has gained regional names such as "Montezuma's revenge," in travelers who travel to South American countries, especially Mexico, and "Delhi belly" for those who acquire the disease while traveling to India. Globally, about 200 million cases and approximately 380,000 deaths are associated with ETEC.

ETEC cells adhere to the mucosal epithelial cells and produce heat-labile toxin (LT) and/or heat-stable diarrheal toxins (ST). LT resembles cholera toxin (CT) and produces symptoms similar to the *Vibrio cholerae* infection.

Virulence Factors and Pathogenesis

Adhesion Factors

ETEC express at least 25 colonization factors (CFs) that target the intestinal mucosa, and the genes (*cfa*) for CFs are located mostly on the plasmid. CFs are proteinaceous fimbrial and afimbrial (fibrillar) structures (lengths, 1–20 μm) that allow bacteria to attach to the intestinal mucosa. Fimbrial colonization factors are also known as colonization factor antigens or colonization fimbrial antigen (CFA), and three types are reported: CFA/I, CFA/II, and CFA/III. CFA/I is rigid rod shaped; CFA/II is flexible fimbriae present alone or in association with other rod-shaped fimbriae; and CFA/III is a bundle-forming flexible pilus (BFP), called longus, because of its unusual length (about 40 μm long). One afimbrial colonization factor, CFA/IV, is present in ETEC. The tip of CFA is hydrophobic and promotes ETEC adhesion to epithelial cells.

Other non-fimbrial adhesion factors include TibA and Tia. TibA is a 104 kDa afimbrial adhesin, encoded on the chromosome. TibA aids in bacterial aggregation on epithelial cells and promotes biofilm formation. Tia, a 25 kDa outer membrane protein encoded on a 46 kb pathogenicity island, is involved in adhesion. A Tia homolog has been found in other *E. coli* (EPEC and EAEC), suggesting it has a broader role in pathogenesis.

ETEC express peritrichous flagella and each of which is comprised of 20,000 individual flagellin proteins. Flagella contribute significantly to intestinal adhesion and colonization.

Toxins

ETEC produce two types of toxins: (i) heat-labile toxin (LT; LT-I and LT-II) and (ii) heat-stable toxin (ST; STa and STb).

Heat-Labile Toxins

Heat-labile toxin (LT) is of two types, LT-I and LTII, and they are encoded by the *eltAB* operon. The genetic organization of LT-I is similar to the cholera toxin. LT-I is expressed in *E. coli* that induce disease in both humans and animals, while LT-II is found mainly in animal isolates. LT-II has same basic structure and mechanism of

action as LT-I. LT-I is an A–B-type toxin with a molecular mass of 87.5 kDa. The A subunit is 27 kDa, while the B subunit consists of five identical subunits of 11.7 kDa (pentameric form), and the subunits are arranged in a ring. The A subunit consists of two domains: A1 and A2. A1 domain is the active toxin molecule, and the A2 anchors to B pentamer subunit via disulfide bridge. This $A–B_5$ complex is also called holotoxin, because of covalent association of A and B subunits, and the amino acid sequence is similar to that of the cholera toxin.

After synthesis, the toxin is transported across the outer membrane by a two-step process using SecA2 and the type II secretion system (T2SS). SecA2 translocates LT across the cytoplasmic membrane, and the T2SS helps translocate across the outer membrane (OM). The secreted toxin may remain associated with the LPS in the OM. During infection of the host cell, the B subunit binds a GTP-binding protein and ganglioside (GM1) receptor on the epithelial cells and triggers endocytosis of the holotoxin. [Note: cholera toxin interacts with the same receptor and acts in the same manner; see Chap. 18.] The A subunit then causes ADP-ribosylation of ganglioside (Gs) protein and activates adenylate cyclase resulting in an increase in cyclic adenosine monophosphate (cAMP) level in the cytoplasm. cAMP then activates cAMP-dependent protein kinase A (PKA), which in turn causes phosphorylation of cystic fibrosis transmembrane regulator (CFTR), a chloride ion transporter protein, increases Cl^- secretion in the crypt cells, and decreases absorption of Na^+ and Cl^- by absorptive cells (Fig. 14.5).

In addition, the A subunit is involved in arachidonic acid metabolism leading to the formation of prostaglandin E_2 (PGE_2) and 5-hydroxytryptamine (5-HT), which promote electrolyte and water release from intestinal cells. The LT-II action is similar to LT-I with an exception where it binds to ganglioside GD1 receptor instead of GM1.

Heat-Stable Toxin

Heat-stable toxin (ST) consists of a family of small peptide toxin of 2 kDa, which is stable at 100 °C for 30 min. Two major types of ST, (i) STa (STh), a methanol-soluble toxin isolated from human (h), and (ii) STb (STp), a methanol insoluble toxin isolated from pig (p), are also found in human isolates. ST is produced as a 72-amino acid-long precursor protein, which is stored in the periplasm. After N-terminal 19 amino acids are removed, a 54-amino acid-long peptide is secreted to the periplasm by SecA2 and through outer membrane by TolC protein exporter. The peptide is further cleaved to a 17–19-amino acid-long active peptide with a molecular mass of approximately 2 kDa. STa (STh) binds to the membrane guanylyl cyclase C (GC-C) receptor on the brush border of epithelial cells of the small intestine and colon. STa binding to GC-C activates protein kinase G (PKG) and protein kinase C (PKC), increasing inositol 1,4,5-trisphosphate (IP_3)-mediated Ca^{2+} to increase intracellular cyclic guanidine monophosphate (cGMP). Increased cGMP activates calcium ion channel, CFTR, and increases the concentration of Cl^- ions in the extracellular space. As a result, electrolyte balance in the bowel is disrupted, which leads to fluid accumulation within the lumen of the intestine resulting in diarrhea (Fig. 14.5).

STb is a 48 amino acid containing a peptide, found primarily in swine isolates but also in human ETEC isolates. STb has no sequence homology with STa, and the receptor for STb is sulfatide, a widely distributed glycosphingolipid. After endocytosis of STb, it activates GTP-binding regulatory protein, which increases the efflux of Ca^{2+}, opens ion channels, and activates protein kinase C. Increased Ca^{2+} levels regulate phospholipases (A2 and C) that release arachidonic acid from membrane phospholipids, leading to the formation of prostaglandin E_2 (PGE_2) and 5-hydroxytryptamine (5-HT), which mediate electrolyte and water release from the intestinal cells. Unlike STa, STb also stimulates the secretion of bicarbonate from epithelial cells. STb also reported to forming a multimeric structure on epithelial membrane forming pores and increased membrane permeability.

Other Toxins

ETEC also secretes 38 amino acid containing enteroaggregative heat-stable toxin 1 (EAST1), which has been isolated from ETEC strains of

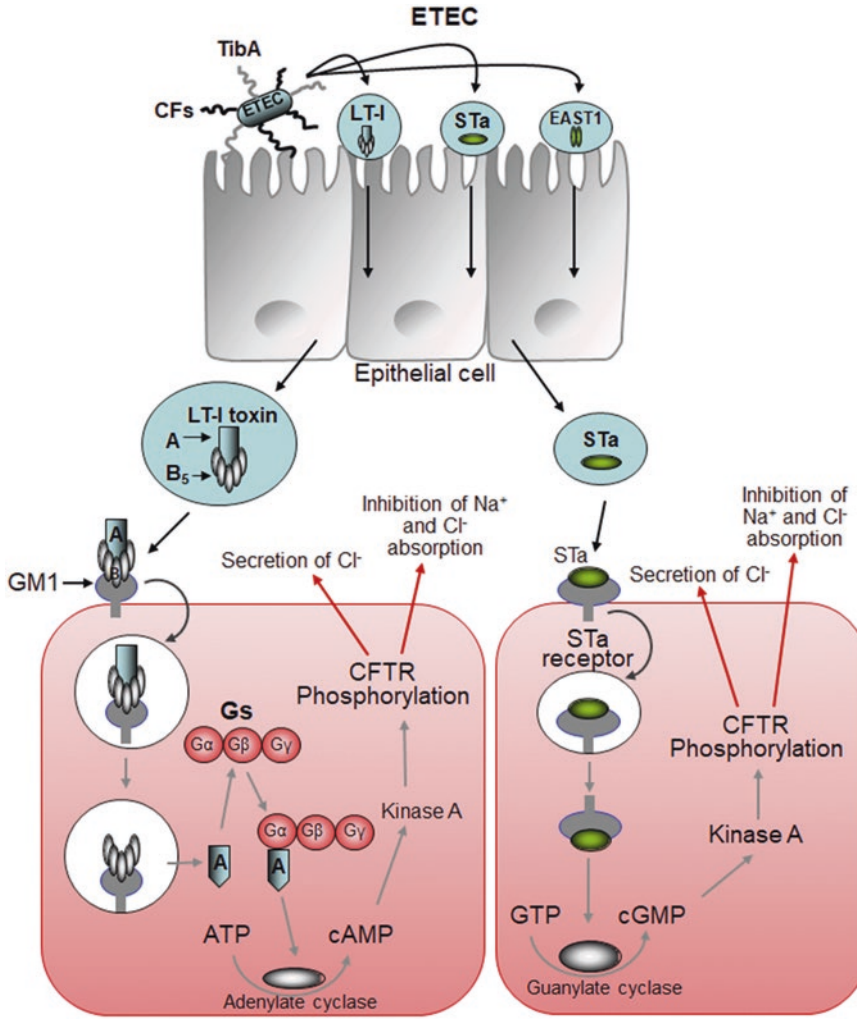

Fig. 14.5 Mechanism of action for enterotoxigenic *Escherichia coli* (ETEC)-mediated diarrhea. After arrival in the intestine, ETEC binds to the epithelial cells using colonization factors (CFs) and/or TibA and produces several toxins including LT-I, LT-II, STa, STb, EAST1, and ClyA. Mechanism of action of LT-I and STa is presented. LT increases cAMP level, while ST increases cGMP, and both mediate phosphorylation of CFTR (cystic fibrosis transmembrane conductance regulator), a chloride ion transporter protein, which increases Cl^- secretion in crypt cells and decreases absorption of Na^+ and Cl^- by absorptive cells

human and animal origin. The toxin activates cGMP, induces fluid accumulation, and possibly plays a role in the onset of diarrhea. In addition, ETEC also produces EatA, a serine protease autotransporter, which plays a role in pathogenesis by damaging the epithelial cell surface. Some strains of ETEC may secrete ClyA, a cholesterol-dependent cytolysin (CDC), and form pore in the cell membrane. It binds to cholesterol on the membrane and shows lytic activity against erythrocytes, macrophages, and HeLa cells.

Symptoms

ETEC infection does not show any apparent histological changes in the mucosal layer, and there is little or no inflammation in the intestine. The symptoms may include watery diarrhea, vomiting, sunken eyes, massive dehydration, and a collapse of the circulatory system. Diarrhea lasts for 3–4 days and is self-limited. Diarrhea may be lethal in young children and infants, with a mortality rate of less than 1%.

Enteropathogenic *E. coli*

Characteristics

Enteropathogenic *E. coli* (EPEC) was the first virotype of *E. coli* to be described, and the bacterium primarily causes fatal diarrhea in children under the age of 5. EPEC is also pathogenic to calves, pigs, rabbits, and dogs. EPEC expresses bundle-forming pilus (BFP) or EPEC adherence factor (EAF) encoded by *bfpA* gene located on a plasmid, for adhesion and colonization. EPEC also expresses intimin encoded by the *eae* gene located on 35 kb pathogenicity island designated locus of enterocyte effacement (LEE) to produce attachment and effacement (A/E) lesion. EPEC does not express Shiga toxin (Stx). Based on the virulence gene distribution, EPEC is classified into two groups: typical EPEC (tEPEC) and atypical EPEC (aEPEC). tEPEC is $eae^{+}bfpA^{+}stx^{-}$ and produces localized adherence (LA) phenotype, while aEPEC is $eae^{+}bfpA^{-}stx^{-}$ and produces localized-like (LAL) diffuse adherence phenotype. tEPEC adheres intimately to epithelial cells and exhibits a "patchy" pattern of adherence. Adherence promotes a dramatic change in the ultrastructure of epithelial cells, resulting in the formation of an attaching and effacing lesion, which is characterized by the formation of a "cuplike" or "pedestal" structure due to extensive cellular actin rearrangements in the architecture. Microvilli structures gradually disappear and the epithelium lose the ability to absorb nutrients. EPEC strains are highly invasive and cause an inflammatory response and potentially fatal diarrhea in children and infants.

Virulence Factors and Pathogenesis of EPEC

EPEC interaction with the host cells occurs in four stages: (1) expression of adhesion factors, (2) initial localized adherence, (3) signal transduction and intimate contact, and (4) cytoskeletal rearrangement and pedestal formation (Fig. 14.6).

Expression of Adhesion Factors

Initially, the bacterium binds to epithelial cells but the adhesion is non-intimate. The adhesion is mediated by the type IV adhesion fimbriae, BFP or EAF, which is similar to Tcp pilus of *V. cholerae*. BFP is a ropelike structure, which interacts with other bacterial cells to form microcolonies for localized adherence and with N-acetyllactosamine-containing receptors on epithelial surface. In addition, DsbA, a periplasmic enzyme, encoded by the *dsbA* gene, facilitates the disulfide bond formation between proteins that are involved in localized adherence. EPEC also produces intimin and a short, surface-associated filament, EspA.

Localized Adherence

EPEC adheres to epithelial cells using BFP, intimin, and EspA and the type III secretion system (T3SS, a molecular syringe). T3SS injects translocated intimin receptor (Tir, 78 kDa) and several other effector molecules (EspB, EspD, EspG, EspF, EspH) directly into the host cell. The effector molecules activate cell signaling pathways, allowing actin polymerization and depolymerization to alter the cytoskeletal structure. Tir is then phosphorylated by protein kinases and inserted into the host cell membrane to facilitate binding with bacterial intimin (Figs. 14.6 and 14.7).

Signal Transduction and Intimate Contact

The attachment of EPEC to mammalian cells triggers host cell signal transduction pathways and activates host cell tyrosine kinase, which causes the release of two signaling molecules: inositol triphosphate (IP_3) and intracellular Ca^{2+}. These activate calcium-dependent actin depolymerization enzyme and trigger phosphorylation of host cell proteins–myosin light chain (MLC) and the 90 kDa epithelial Hp90 protein. Intracellular Ca^{2+} can inhibit Na^{+} and Cl^{-} absorption and stimulate Cl^{-} secretion.

Intimate contact is mediated by adhesion of intimin (Eae), a 95 kDa outer membrane protein, to the Tir receptor on the host cell membrane. Intimin protein has 27 variants (eae alleles) based on the heterogeneity in the C-terminal sequence

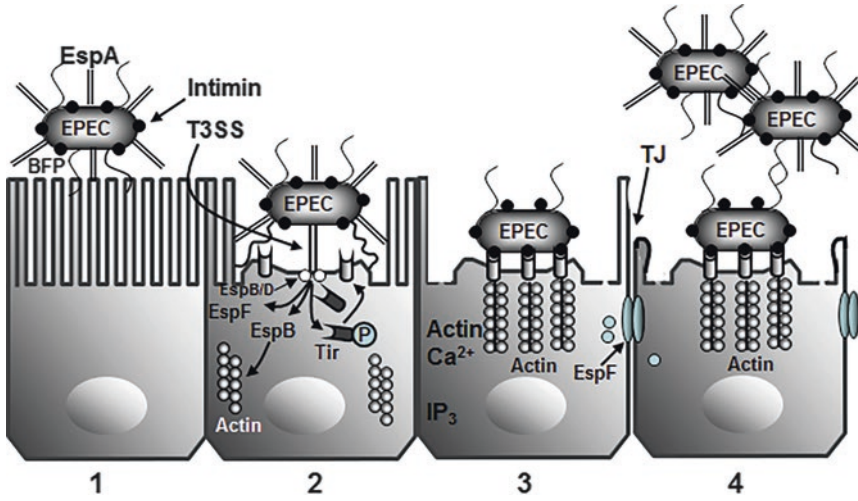

Fig. 14.6 Schematic diagram showing the sequence of events for enteropathogenic *Escherichia coli* (EPEC) on enterocytes during infection. The pathogenic event can be grouped into four stages (**1**) expression of adhesion factors, (**2**) initial localized adherence, (**3**) signal transduction and intimate contact, and (**4**) cytoskeletal rearrangement and pedestal formation. *Esp E. coli* secreted protein, *BFP* bundle-forming pilus, *T3SS* type III secretion system, *Tir* translocated intimin receptor, *TJ* tight junction

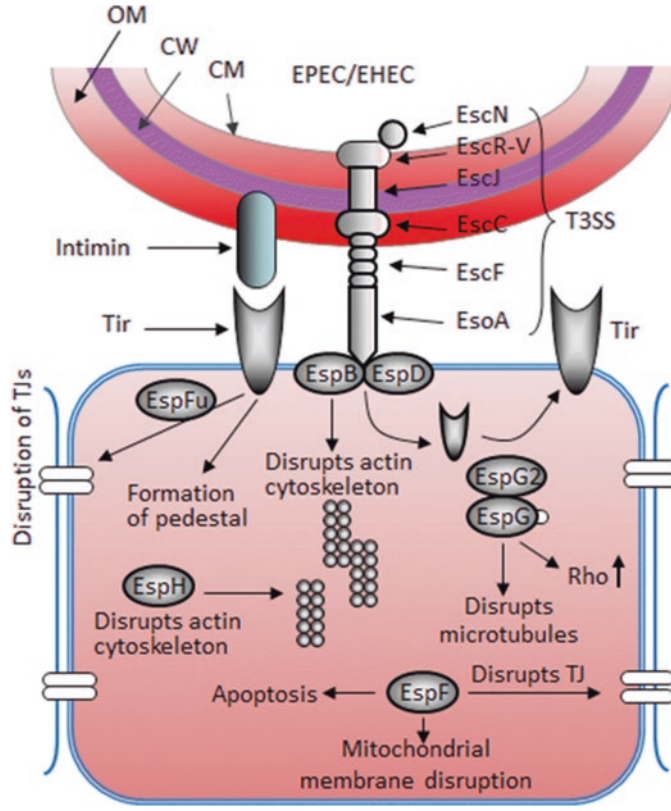

Fig. 14.7 Delivery of virulence effectors of enterohemorrhagic *Escherichia coli* (EHEC) and enteropathogenic *E. coli* (EPEC) to the host cell by the type III secretion system (T3SS) (Redrawn from Hayward et al. 2006. Nat. Rev. Microbiol. 4:358–370)

of the protein. The intimin subtypes α-, β-, and γ-type are most relevant clinically. EHEC also expresses intimin and has 83% amino acid sequence homology with intimin from EPEC. EPEC uses T3SS to inject Tir into the host cytosol, which is initiated by Ca^{2+} sensing, and Tir is expressed on the surface to interact with intimin. Tir is then phosphorylated by the host cell kinases, which recruit Nck to the site of attachment and activate neural Wiskott–Aldrich syndrome protein (N-WASP) and the actin-related protein 2/3 (Arp2/Arp3) complex to ochestrate actin rearrangements and pedestal formation.

Extensive rearrangement of actin causes abnormalities in cytoskeletal structure, resulting in the formation of the characteristic attaching and effacing (A/E) lesions. Effacement refers to the loss of microvilli. The epithelial cell membrane beneath the bacterium forms a pedestal, a pseudopod-like structure, due to massive cellular cytoskeletal protein rearrangements, which include actin filament and its cross-linker, talin, ezrin, α-actinin, and MLC. In this phase, bacteria lose EspA filaments from the surface (Figs. 14.6 and 14.7).

Cytoskeletal Rearrangement and Pedestal Formation

As mentioned above, intimate contact results in the formation of a "pedestal-like" structure and attachment and effacement lesion due to massive accumulation of actin filaments. The lesion is further characterized by deformation and loss of microvilli due to depolymerization of the actin filament in microvilli. The effector protein EspF translocated through the T3SS also affects tight junction (TJ) proteins and mitochondrial function and increases epithelial membrane permeability. As a result, there is malabsorption of nutrients and ions, cell death, and the onset of osmotic diarrhea. EspF and EspB also inhibit phagocytosis.

LEE and Regulation of Virulence Genes

Virulence factors for EPEC pathogenesis are located primarily on the 35 kb LEE pathogenicity island and on non-LEE (Nle)-encoded genes. LEE is integrated into the chromosome near tRNA gene, *selC*. LEE has five polycistronic operons (LEE1–LEE5), which contain genes for A/E lesion (*map*, *espF*, *espG*, *espZ*, *espH*, and *espB*), intimin (*eae*), Tir (*espE*), the T3SS (*espA*, *espB*, and *espD*), and the exported proteins. LEE is also present in EHEC and the organization of genes is similar to EPEC, but the size and the number of genes may vary between EHEC and EPEC.

Nle effector proteins are involved in dampening the host immune response. NleB, NleC, NleD, NleE, and NleH have all been shown to inhibit NF-κB activation. Nle effectors such as EspJ have antiphagocytic activity, while NleA alters host protein secretion and tight junction integrity.

EPEC virulence genes are also located on a large plasmid, EAF (*E. coli* adherence factor). Two important operons are present: *bfp* and *per* (plasmid-encoded regulator). *bfp* encodes for BFP, while Per is the transcriptional activator, which regulates manifestation of A/E activity and BFP.

Symptoms

EPEC causes diarrhea in children under the age of 5 and is associated with high mortality. Diarrhea may be acute or persistent, and the latter type is the most common form of clinical presentation. In severe cases, diarrhea may be bloody and the infection may persist for several days.

Enterohemorrhagic *E. coli*

Characteristics

Enterohemorrhagic *E. coli* (EHEC) is a subset of a broadly classified Shiga toxin-producing *E. coli* (STEC) that inflict bloody diarrhea or hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) and is prevalent in industrialized countries. Broadly, any *E. coli* strain that produces Stx is called STEC. Not all STEC strains

are pathogenic to humans; however, the EHEC strains that express Stx and Eae are the most virulent pathotypes to humans. EHEC, a subset of STEC, carries the LEE pathogenicity island and displays attaching and effacing lesion. The STEC strains that lack the LEE are less virulent. EHEC does not express bundle-forming pili (BFP); instead, the EHEC plasmid carries a homolog of the *lifA* gene encoding lymphostatin, as well as the genes encoding T3SS, catalase, peroxidase, a serine protease, and hemolysin.

In 1977, Konowalchuk and his colleagues demonstrated that Shiga toxin infects Vero cells derived from the kidney; thus, this toxin is also referred to as verotoxin (VT), and the verotoxin-producing *E. coli* is called VTEC. Both VTEC and STEC terminologies have been used interchangeably. Karmali and his colleagues in 2003 divided STEC strains into five seropathotypes (A–E), based on the type of outbreaks and the severity of infection. Pathotype A (O157:H7, O157:NM) is the common outbreak group responsible for severe HUS and HC, and pathotype B (O26:H11, O103:H2, O111:NM, O121:H19, O145:NM) is responsible for occasional outbreak and can cause HUS and HC. Pathotypes C and D rarely cause outbreaks and may cause HUS and HC, while pathotype E is not implicated in outbreaks.

The principal serotype associated with EHEC group is *E. coli* O157:H7 and nonmotile serovar, O157:NM, and the first outbreak of O157:H7 was reported in 1982–1983. Other important non-O157 EHEC serovars include O26, O45, O103, O111, O121, and O145 all of which express Stx and Eae. The USDA-FSIS (Food Safety Inspection Service) has imposed a zero tolerance for these six serovars plus O157:H7 in ground beef and meat trimmings and considered these serovars to be adulterant if present in meat. Likewise, the European Food Safety Authority (EFSA) has also placed greater emphasis on a slightly modified list of serovars that include O157:H7, O26, O103, O145, O111, and O91.

As opposed to other commensal strains, *E. coli* O157:H7 generally does not ferment sorbitol and does not have β-glucuronidase activity (GUD). It grows rapidly at 30–42 °C, grows poorly at 44–45 °C, and does not grow at 10 °C or below. Strains resistant to pH 4.5 or below (pH 3.6–3.9) have been identified, and acid resistance is mediated by RpoS, a sigma factor. The organism is destroyed by pasteurization (at 64.3 °C in 9.6 sec), but the cells survive well in food at −20 °C.

Several selective and chromogenic media are available for isolation of EHEC especially the serovar, O157:H7: sorbitol MacConkey agar (SMAC) supplemented with cefixime–tellurite, Rainbow® Agar, CHROMagar™, and R&F® *E. coli* (Fig. 14.8). The US Food and Drug Administration (FDA)'s *Bacteriological Analytical Manual* (BAM) provides details of diarrheagenic *E. coli* detection (https://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm2006949.htm).

Food Association and Outbreaks

Cattle are the natural reservoir of STEC. STEC is generally present in the intestine of animals without causing disease. STEC also have been isolated from the feces of chickens, goats, sheep, pigs, dogs, cats, and sea gulls. Foods of animal origin, especially ground beef, have been implicated in many outbreaks in the USA, Europe, and Canada; however, in late 2006, a major outbreak of O157:H7 involving 26 states was associated with spinach. The affected people (199 with 3 deaths) were found to have consumed spinach. In the 1993 outbreak, affecting over 500 people and causing 4 deaths, consumption of undercooked hamburgers served by a fast-food chain in Washington, Nevada, Oregon, and California was implicated. In addition to ground beef, other foods, such as uncooked sausages, fermented hard salami, raw milk, yogurt, mayonnaise, raw milk cheese, apple cider, fruits, sprouts, and salad, have been implicated in epidemic and sporadic outbreaks. STEC has been routinely isolated from many different types of foods of animal origin, such as ground beef, pork, poultry, lamb, and raw milk (Fig. 14.3). The organism was also isolated in low frequencies from dairy cows, calves, and chickens. However, in some

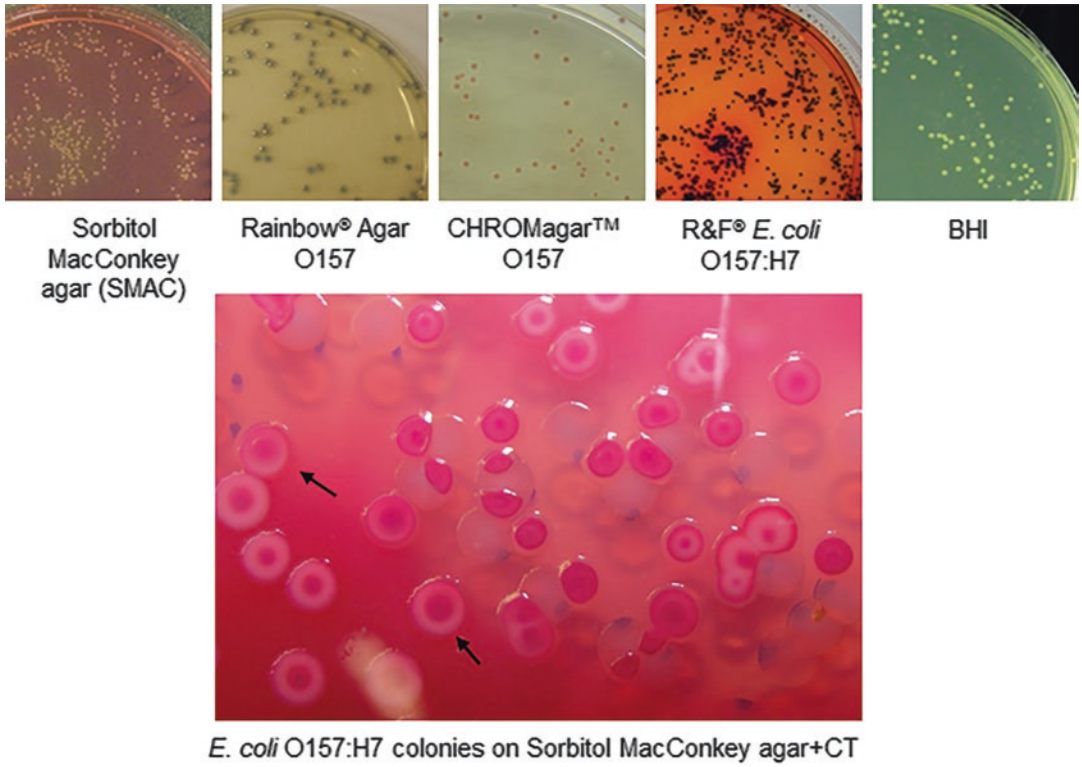

Fig. 14.8 Colonies of *E. coli* O157:H7 on various selective agar media. *BHI* brain heart infusion, *CT* cefixime–tellurite

cases, a high percent of feedlot cattle shed *E. coli* O157:H7, some of which are persistent shedders. Interestingly, calves after weaning shed in higher frequency than before weaning.

EHEC Pathogenesis

EHEC reaches the intestine from contaminated food or water and colonizes the intestine. The prototype EHEC strain, *E. coli* O157:H7, is acid resistant; it can pass through the stomach unharmed and reaches the small intestine. A small infectious dose of 50–100 cells is sufficient to cause infection. In addition, preexposure of cells to mild acid, as with acidic foods, such as apple cider or fermented hard salami, the bacterium becomes more resistant to low pH and ensures better survival during transit through the stomach.

Attachment and Effacement

EHEC causes characteristic attaching and effacing lesion similar to EPEC and occurs in three stages: localized adherence, signaling event, and intimate contact. In the first stage, adhesion of bacteria to the microvilli of the intestinal epithelial cells is mediated by fimbriae (encoded on the 60 MDa plasmid), type IV pilus called the hemorrhagic coli pilus, and flagella. Unlike EPEC, EHEC does not express BFP. In the second stage, a signal is transmitted to the host cell via T3SS (Fig. 14.7), and phosphorylation of eukaryotic protein occurs, leading to actin polymerization, cytoskeletal rearrangement, and effacement of microvilli. In the third stage, intimate contact is mediated by intimin protein encoded by the *eae* gene located on the LEE pathogenicity island, similar to EPEC. There are 27 intimin variants, based on the heterogeneity in the

C-terminal sequence of the protein, and γ-subtype intimin is associated with EHEC *E. coli* O157:H7 and EPEC O55:H- and O55:H7 strains. Intimin binds to the Tir receptor, and subsequent signaling events amplify the cytoskeletal rearrangement of proteins beneath the adherent bacteria. Increased actin filament accumulations are mediated by Arp2/Arp3 complex, which is regulated by N-WASP, and form pedestal with the loss of microvilli structure. The A/E pathology causes enterocyte sloughing, inflammation, and possibly diarrhea, which may result from the inhibition of sodium and chloride absorption, activation of the chloride channel, loosening of tight junction, increased paracellular permeability, inflammatory response, and cytokine production.

T3SS and Delivery of Effector Proteins

T3SS plays a crucial role during EHEC pathogenesis, and it delivers virulence effector proteins directly inside the host cell cytoplasm that are responsible for A/E lesion (Fig. 14.7). The T3SS needle complex is composed of several Esc proteins (EscN, EscR-V, Esc J, EscC, EscF, and EspA), which spans from the bacterial cytoplasmic membrane (CM) to outer membrane (OM). The T3SS injects effector proteins known as Esp (*E. coli* secreted proteins), which perform various functions: EspB and EspD form a plasma membrane translocon for effective delivery of effector proteins. EspB also affects cytoskeletal structure by disrupting the actin cytoskeleton. Another effector protein, EspH, also promotes disruption of the actin cytoskeleton. EspG and EspG2 disrupt microtubule and activate a small GTPase protein, Rho. EspF causes membrane disruption in the mitochondria, disrupts tight junction proteins, and causes increased membrane permeability. Bacteria also inject Tir (also known as EspE in EHEC) which binds to the intimin. After translocation into the host cell, unlike EPEC, Tir is not tyrosine phosphorylated, and pedestal formation is independent of Nck protein.

Shiga Toxin

STEC produces two types of Stx, Stx1 and Stx2, and the genes are located on bacteriophage (prophage). The Stx1 sequence is highly conserved and exhibits a high sequence similarity to Stx produced by *Shigella dysenteriae* type 1 (see Chap. 19). The antibody developed against the Stx from *S. dysenteriae* type 1 can neutralize Stx1 from STEC but not the Stx2. Stx1 has three subtypes: Stx1a, Stx1c, and Stx1d. Stx2 has seven subtypes: Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f, and Stx2g (Table 14.2). STEC strains do not have a secretory system for Stx; thus, secretion is dependent on the cell lysis mediated by the lytic bacteriophages. Stx2 is highly toxic, and if the isolate also has *eae*, there are greater risk of causing hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) than Stx1. Strains of STEC with *eae* and producing Stx2 cause more severe disease than the strains producing only Stx1 or both Stx1 and Stx2.

As mentioned above, the gene for Stx production is encoded in a temperate bacteriophage (*stx* phage) related to the classic λ phage, which integrates into the chromosome and maintains in the lysogenic state. The genome size of *stx* phage ranges from 29.7 to 68.7 kb, mostly above 60 kb. The *stx* phages can be induced to enter from lysogenic to lytic cycle, and the resulting free phages can transfer *stx* genes horizontally to *E. coli* or other members of the *Enterobacteriaceae* family. Most lysogens stably maintain *stx* phage; however, only a small subpopulation is induced spontaneously. Induction of *stx* prophages is controlled by RecA, a regulator of the SOS bacterial response during DNA damage. RecA-dependent UV irradiation and mitomycin C can induce *stx* phases, while RecA-independent ethylenediamine tetra acetic acid (EDTA) can induce stx_2 phages. Other factors that regulate the lysogeny switch and lysis include H_2O_2, sodium citrate, high temperature plus UV, amino acid starvation, phenethyl isothiocyanate, colicins, sodium chloride, nitric oxide, gamma irradiation, and antibiotics (azithromycin,

Table 14.2 Shiga toxin types associated with STEC

Shiga toxin types	Receptor types	Description
Stx1	GB3	STEC and identical to Stx from *Shigella dysenteriae*
Stx1a	GB3	Linked to serious human disease
Stx1c	GB3	Found in *eae*-negative STEC, common in sheep STEC isolates, mild diarrhea in humans
Stx1d	GB3	Mild diarrhea or asymptomatic in humans
Stx2a	GB3	High virulence and HUS in humans
Stx2b	GB3	Not involved in a serious disease
Stx2c	GB3	Diarrhea and HUS in humans, common in ovine STEC, less toxic to Vero cells
Stx2d	GB3	It can be activated by mucus and cause severe diarrhea and HUS in humans even in the absence of Eae. It is less toxic to Vero cells
Stx2e	GB3 and GB4	Edema disease in pigs, rare in humans, low pathogenicity
Stx2f	GB4	Pigeon isolates, rare in humans, pathogenicity – uncertain
Stx2g	GB3	Low pathogenicity in humans

ciprofloxacin, fosfomycin, imipenem, gentamicin, norfloxacin, and rifampicin).

The Stx molecules are A–B_5 heterohexamer toxins of 70 kDa, in which the A subunit (StxA) is about 32 kDa and the B subunit (StxB) is 7.7 kDa each. StxA is an enzyme and StxB interacts with the receptor, and both are secreted into the bacterial periplasm and assemble via a noncovalent bond, hence called holotoxin. A single enzymatic A subunit remains associated with a pentamer of B subunits. StxB interacts with the host cell receptor, globotriaosylceramide (Gb3), a glycolipid consisting of galactose α (1–4), galactose β (1–4), glucose ceramide, or globotetraosylceramide (Gb4), and is abundant in the endothelium and the kidney tubule.

Following the binding of the StxB subunit to the receptor (GB_3 or GB_4), the StxA subunit is internalized by receptor-mediated endocytosis and transported to the Golgi and then to the endoplasmic reticulum (ER) (Fig. 14.9). The StxA1 subunit is activated after cleavage of the 4 kDa C-terminal A2 peptide, which remains associated with the StxB subunit. The resulting active A1 portion has *N*-glycosidase activity and cleaves a purine residue (adenine base) in VI domain of 28S ribosomal RNA of host ribosome, altering the function of ribosome such that it no longer can interact with the elongation factors EF-1 and EF-2 required for chain elongation thus inhibiting protein synthesis. A lack of protein synthesis leads to cell death. The severity of infection and damage to the tissues depend on the number of receptors present.

Both Stx1 and Stx2 toxins bind to Gb3. Stx1 causes localized damage to the colonic epithelium because of high binding affinity to Gb3, whereas Stx2 has low affinity for Gb3. Stx1 and Stx2 can reach the circulatory system and the kidneys. Human kidney tubules have high Gb3 and are the major target for toxin-induced damage resulting in hemolytic uremic syndrome. Stx subtypes, Stx2e and Stx2f, use Gb4 as the preferred receptor (Table 14.2). Stx2 has significantly higher toxicity than Stx1. Stx2 together with intimin (Eae) pose the highest risk of developing HUS. The crystal structure of toxin reveals a greater accessibility of the active site of Stx2 than Stx1, and this possibly contributes to the enhanced cytotoxicity.

Stx has been shown to possess nephrotoxic, cytotoxic, enterotoxic, and neurotoxic effects. Stx causes nephrotoxicity following enteric infection, resulting in massive damage to the kidney tubules, bloody urine, and the hemorrhagic uremic syndrome. Since Stx causes chronic kidney damage, there is a need for dialysis and kidney transplant. HUS is also characterized by thrombocytopenia and hemolytic anemia. Though calves are susceptible to EHEC infection, they do not develop HUS because they lack the receptors in the endothelial cells of blood vessels. Stx also acts as a neurotoxin, causing a neurological disorder called thrombotic thrombocytopenic purpura (TTP), which is characterized by hemolysis, thrombocytopenia, renal failure, and fluctuating fever. Stx is also reported to have enterotoxin activity resulting in fluid accumulation and diarrhea. Stx is cytotoxic, inhibits protein synthesis, and induces pro-

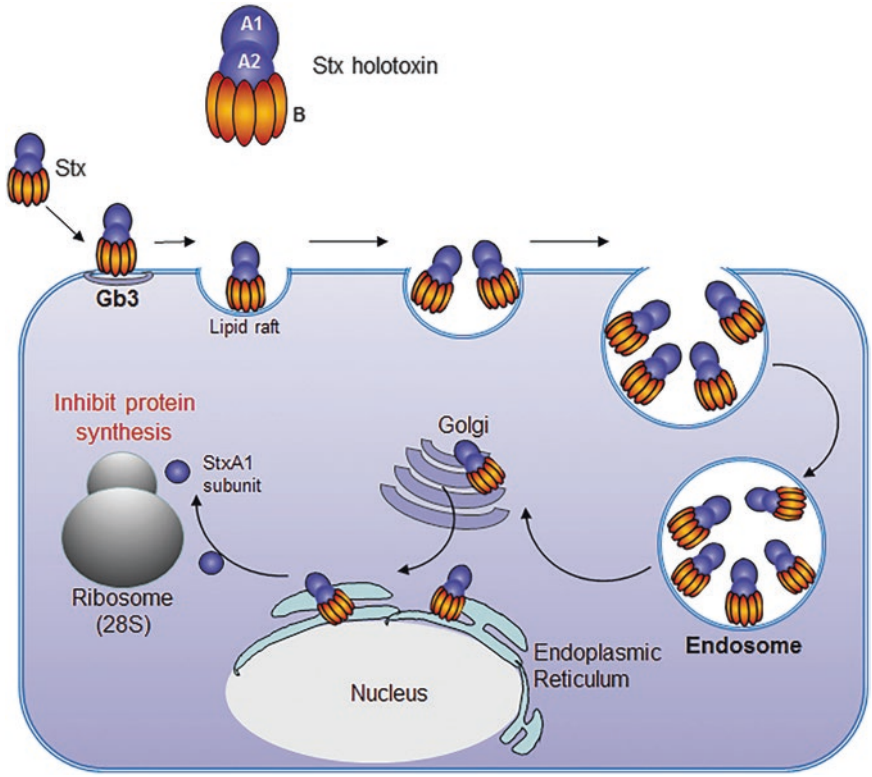

Fig. 14.9 Mechanism of Shiga toxin (Stx) pathogenesis

grammed cell death. Stx subtypes (Stx2e, Stx2f) are also found in pigs, humans, or pigeons and can cause edema disease, bloody diarrhea, HC, and HUS.

Inflammation

Inflammation is very prominent in the intestine during infection with *E. coli* O157:H7. Flagellin (H7) is thought to be responsible for the inflammatory response. It binds to the toll-like receptor 5 (TLR-5) on epithelial cells; activates p38, ERK (extracellular signal-regulated kinase)–MAP (mitogen-activated protein kinase) kinase, and NF-κB; and increases expression of proinflammatory cytokine, IL-8. The inflammation likely disrupts epithelial barrier function and facilitates Stx passage from the lumen to the submucosal layer. LPS (O157 antigen) activates platelets and together with Stx may cause endothelial cell injury and may contribute to the thrombocytopenia observed in HUS. An LPS-mediated release of cytokines IL-1 and TNF-α from activated macrophages can cause vascular damage during the renal failure in the HUS patients.

Enterohemolysin

Enterohemolysin (Ehly or Ehx) has been isolated from EHEC, and it belongs to the family of RTX (repeats in toxin). It is a monomeric pore-forming toxin and its role in pathogenesis is unclear. It is encoded by four genes (*ehxC*, *ehxA*, *ehxB*, and *ehxD*) and is located on the 60 MDa plasmid. Ehly is secreted by type I secretion system (T1SS). RTX may cause localized lesions or affect cells of renal tubules, but its involvement in pathogenesis is undetermined.

Other Virulence Factors

EHEC O157:H7 genome sequence also revealed the presence of several putative virulence factors: fimbrial adhesins such as Lpf and SfpA, non-fimbrial adhesins (EfaI, Iha, OmpA, and ToxB), toxins (cytolethal distending toxin; CDT), proteases (EpeA, EspP/PssA), and urease.

Regulation of Virulence Genes

Regulation of genes located on LEE is complex and involves non-LEE-encoded (Nle) and LEE-encoded genes. The transcriptional regulators, Ler (LEE-encoded regulator) and Grl (global regulator of LEE activator), positively regulate the genes on LEE. EHEC uses a quorum sensing regulatory system to recognize the intestinal environment and activate genes that are required for colonization in the gut. Autoinducers, like epinephrine and norepinephrine, also regulate genes for flagella and motility, which allow bacteria to find a suitable niche in the gut.

The *stx* genes are located in the lysogenic lambdoid phage and are highly expressed when the lytic cascade of the phage is activated. *Stx* gene expression is regulated by iron concentration where higher concentration suppresses expression.

Symptoms

Symptoms of EHEC/STEC infection appear 3–9 days after ingestion of contaminated food and generally last for 4–10 days. The colitis symptoms include a sudden onset of abdominal cramps, watery diarrhea (which in 35–75% of cases turns bloody), and vomiting. Damage to the blood vessels in the colon is responsible for bloody diarrhea and hemorrhagic colitis. Stx may damage endothelial cells in the kidney and hemolytic uremic syndrome that develops in 5–10% of STEC-infected patients. The HUS is characterized by acute renal failure, hypertension, microangiopathic hemolytic anemia, and thrombocytopenia. EHEC infection can be fatal, particularly in children under the age of 5 and the elderly. About 1–2% of patients die during the acute phase of the disease, and about 30% of patients exhibit long-term renal damage. Though the kidney is the primary target organ, other organs such as the lungs, central nervous system, pancreas, and heart are also affected. Thrombotic thrombocytopenic purpura (TTP) may result from the blood clot in the brain, eliciting seizures, coma, and death. The most severely affected patients require blood transfusion and dialysis therapy.

Enteroaggregative *E. coli*

Characteristics

Enteroaggregative *E. coli* (EAEC) causes persistent diarrhea, lasting more than 14 days, in children and adults, and is prevalent in developing countries. It is also increasingly responsible for persistent diarrhea in HIV-infected persons in developed countries. The bacterium causes mostly sporadic cases, but recent data show it also causes outbreaks and traveler's diarrhea. EAEC is a highly heterogeneous group, and 40 different O types have been identified. Persistent diarrhea in children is similar to ETEC, characterized by mild but significant mucosal damage. EAEC also possesses pathogenicity islands that carry genes for enterotoxin and mucinase activity.

A Stx-producing EAEC serovar O104:H4 was involved in a large outbreak in Germany in 2011. Fenugreek sprout was the vehicle, and the seeds were imported from Egypt. About 3800 people were infected, of which 2987 suffered from gastroenteritis and bloody diarrhea, and 855 people exhibited hemolytic uremic syndrome with severe kidney damage and 54 deaths.

Virulence Factors and Pathogenesis

EAEC pathogenesis involves three major steps: (1) adhesion to the mucosal surface, (2) biofilm formation, and (3) signal transduction and toxin production.

Adhesion

In the first stage of pathogenesis, EAEC strains express aggregative adherence fimbriae (AAF), and there are four variants with the distinct structure of pilin subunits: AAF/I, AAF/II, AAF/III, and AAF/IV. Fimbriae bind to intestinal epithelial cell matrix proteins such as laminin, collagen, cytokeratin, and fibronectin. In addition, EAEC strains express the surface protein dispersin, which is encoded by *aap* gene. The bacterium also expresses 18 and 30 kDa outer membrane adhesin proteins. The 18 kDa adhesin protein is a thin filamentous (fibrillar) structure and is called GVVPQ fimbria (G, glycine; V, valine; P, proline; and Q, glutamine). This sequence is located near the N-terminal end and may be responsible for "clumping" of cells or adherence to each other promoting autoaggregation, rather than facilitating bacterial attachment to the host cell. Aggregated EAEC cell adhesion appears as a characteristic "stacked brick." The genes for adhesion are encoded on the 60 MDa plasmid. The virulence genes located on the plasmid or the chromosome are regulated by *aggR* located on the plasmid.

Biofilm Formation

In the second stage of pathogenesis, EAEC forms a thick aggregating biofilm on the mucosal layer. This helps persistent colonization with prolonged diarrhea. Biofilm production is regulated by AggR, and it requires other gene products that are involved in biofilm formation. Fis, a DNA-binding protein, is involved in bacterial growth regulation, and YafK (28 kDa) and Shf (32.8 kDa) proteins are both involved in biofilm formation.

Toxins

The third stage of pathogenesis involves the production of toxins by EAEC, which elicit an inflammatory response, mucosal toxicity, and intestinal secretion. EAEC produces several toxins:

1. Heat-stable, ST-like toxin, also called EAST (enteroaggregative ST), encoded by *astA* gene, is responsible for fluid loss similar to STa of ETEC.
2. The plasmid-encoded toxin (Pet), a serine protease autotransporter that cleaves spectrin protein within the cytoskeleton of the epithelium, resulting in cell elongation and exfoliation.
3. Sat (secreted autotransporter toxin) affects cellular tight junctions in the kidney cells and vacuolation in both the kidney cells and the bladder cells.
4. Pic (protein involved in intestinal colonization), a mucinase that interferes with the integrity of the mucus membrane.
5. *Shigella* enterotoxin I (ShET1) similar to *Shigella* enterotoxin that induces intestinal cAMP- and cGMP-mediated secretion, hemorrhagic necrosis and shortening of villi, enlarged crypt openings, and formation of crypt abscesses.

Mechanism of Pathogenesis

EAEC adhere to the enterocytes forming aggregates, and adherence is characterized by a "stacked brick." EAEC also enhance mucus secretion from the goblet cells and trap themselves in mucus-forming biofilms. EAEC do not invade epithelial cells, but the toxins are responsible for the histopathological damage. The toxins induce shortening of villi and hemorrhagic necrosis of villous tips, increased epithelial cell extrusion, and inflammation that is characterized by the infiltration of mononuclear cells to the submucosa. Virulence factors induce levels of fecal cytokines and inflammatory markers, such as TNF-α, IL-1, IL-6, IL-8, IFN-γ, lactoferrin, fecal leukocytes, and occult blood. IL-8 also recruits neutrophils to the epithelial mucosa without mucosal injury and facilitates intestinal fluid secretion. Flagellin (*fliC*) binding to TLR-5 on monocytic cells initiates a signaling cascade through p38, MAPK, and NF-κB to induce the production of IL-8. Infection results in mucoid stool and persistent diarrhea. EAEC also infect immunocompromised hosts, and bacteria are isolated frequently from HIV–AIDS patients stool.

The Shiga toxin-producing EAEC serovar O104:H4 produces Stx2a, which may have

acquired the *stx* gene through a bacteriophage and considered a hybrid strain of EAEC and STEC strain. This strain also expresses aggregative adherence fimbriae (AAF) for attachment and carries several virulence genes (*aggA*, *aggR*, *set1*, *pic*, *aap*, and *stx2*) that are encoded on the phage and on a plasmid. The colonization factor and virulence genes are common in both EHEC and EAEC thus explains its increased virulence properties. The pathogenesis of O104:H4 is attributed to increased adhesion, colonization, and damage of the intestinal epithelial cells followed by increased dissemination of Stx to blood circulation and the kidney resulting in high numbers of HUS patients that was associated with the outbreak. This strain is sensitive to antibiotics carbapenems but resistant to penicillin and cephalosporin.

Symptoms

The EAEC-mediated disease could be acute or chronic (>14 days) in nature. Symptoms of EAEC infection include watery, mucoid secretory diarrhea, abdominal pain, nausea, vomiting, and low-grade fever. Some patients show grossly bloody stools. HUS, kidney failure, and death can occur due to infection with the newly emerged EAEC O104:H4 strain.

Enteroinvasive *E. coli*

Characteristics

Enteroinvasive *E. coli* (EIEC) is a facultative intracellular pathogen and causes bacillary dysentery similar to *Shigella* species (see Chap. 19). *Shigella* was discovered by a Japanese physician and bacteriologist, Kiyoshi Shiga, in 1897 from an epidemic in Japan, where the bacterium infected more than 91,000 people and greater than 20% mortality. EIEC was discovered 50 years later, and it is genetically, biochemically, and pathogenetically related to *Shigella* spp. and produces watery diarrhea and dysentery. EIEC strains that are generally lysine decarboxylase negative, nonmotile, and lactose negative can be misidentified as *Shigella*. Sporadic outbreaks are common; however, occasional foodborne outbreaks may occur. An outbreak in the USA, as early as 1971, was recognized from the consumption of imported camembert cheese contaminated with serotype O124:H17. An outbreak was also reported in a restaurant in Texas involving 370 people.

Disease and Symptoms

Ingestion of as many as 10^6 EIEC cells may be necessary for an individual to develop the disease. Mechanism of infection is similar to *Shigella*, but the infective dose of shigellosis is 10–100 cells (see Chap. 19), and EIEC disease is less severe. In colonic mucosa, EIEC first binds and invades epithelial cells, lyses the endocytic vesicle, multiplies in the cytoplasm, moves inside the cytoplasm directionally, and projects toward adjacent cells to spread from cell-to-cell. The genes responsible for invasion are encoded in a 140 MDa plasmid, pInv. A toxin of 63 kDa, encoded by *sen* gene located on the plasmid, has been linked to causing watery diarrhea. Extensive cell damage due to invasion and cell-to-cell spread elicits a strong inflammatory response and bloody mucoid diarrhea, similar to bacillary dysentery caused by *Shigella*. Human carriers, directly or indirectly, also spread the disease.

The symptoms appear as abdominal cramp, profuse diarrhea, headache, chills, and fever. Some patients may develop dysentery. A large number of pathogens are excreted in the feces. The symptoms can last for 7–12 days, but a person may be a carrier and shed the pathogens in feces for a prolonged period.

Diffusely Adhering *E. coli*

Diffusely adhering *E. coli* (DAEC) causes infantile diarrhea and produces a diffuse adherence (DA) to cultured HEp-2 cell lines, which is mediated by a fimbrial adhesin, designated F1845. The genes encoding the fimbriae are located on

the chromosome or on a plasmid. DAEC also expresses afimbrial adhesins (Afa) belonging to the Afa/Dr. family of adhesins, which include AfaE-I, AfaE-II, AfaE-III, AfaE-V, Dr., Dr.-II, F1845, and NFA-I adhesins. Adhesion leads to cytoskeleton rearrangement, destroying or partially rearranging microvilli structure. Adhesion also affects paracellular permeability by rearranging occludin and ZO-1 proteins in the TJ. During adhesion, flagellin interaction with TLR-5 stimulates mitogen-activated protein kinases, p38, ERK1/2 (extracellular signal-regulated kinases), and Jun-C kinase and activates NF-κB to produce proinflammatory cytokine, IL-8. Locus of enterocyte effacement (LEE) has been isolated from DAEC, and it possibly carries genes required for the attachment/effacement lesions and signaling events similar to EPEC.

DAEC causes watery diarrhea in children without blood or fecal leukocytes. The DAEC-induced diarrhea is age related and increases with age from 1 year to 4–5 years. The older adults become the asymptomatic carrier of DAEC.

Animal and Cell Culture Model Used for Diagnosis of *E. coli*

A ligated rabbit ileal loop (RIL) assay has been used for detection of diarrheal toxins produced by different virotypes/serotypes. For diagnosis of ETEC, calves and piglets are used since no small animal models are available. ETEC causes diarrhea in gnotobiotic pig (e.g., specific pathogen-free pig), while EPEC causes attaching and effacing lesions in the piglet intestine.

Tissue culture models have been used extensively to study specific traits. For example, attachment and effacement phenomenon of EPEC has been studied using HEp-2 (laryngeal cells) and HeLa (cervical cancer) cell lines. Caco-2 cells and HT-29 (colon cancer cells) are used to study ETEC attachment. Interestingly, ETEC does not adhere to HEp-2 cells. HEp-2 cells are also used to study diffuse adherence phenotype of DAEC. Vero cells (African green monkey kidney) have been used to study cytotoxicity (Fig. 5.1) of EHEC/STEC and HEp-2 for attaching/effacing (A/E) assay. The HEp-2 adherence assay is the gold standard for identification of EAEC, although a PCR assay has been developed to detect pathogenic *E. coli* isolates.

Prevention, Control, and Treatment

Fatalities from diarrheal diseases are due to the extensive dehydration and electrolyte imbalance (loss). Oral hydration and the electrolyte replenishment are the most important therapy. Antibiotics can shorten the duration of infection. Antibiotic therapy is less effective and is not recommended for STEC; however, for ETEC and EAEC infection, fluoroquinolones (e.g., ciprofloxacin, norfloxacin, and ofloxacin) and rifaximin are recommended for treatment. As a preventive measure, travelers can use doxycycline, rifaximin, and trimethoprim–sulfamethoxazole before a scheduled trip to the endemic region. Concerns of antibiotic resistance discourage the use of antibiotics as a prophylactic measure; therefore, travelers are advised to avoid potential hazardous food and water. Water should be boiled and food should be properly cooked to prevent infection. The antidiarrheal drug, Imodium, is effective against diarrhea.

Proper sanitation, cooking or heating at appropriate temperatures, proper refrigeration, and prevention of cross-contamination should be practiced in order to control the presence of EHEC *E. coli* O157:H7 in a ready-to-eat food. EHEC is a heat-sensitive organism and is inactivated at 62.8 °C for 0.3 min in ground beef. The USDA-FSIS has provided several guidelines to control EHEC−/STEC-related foodborne illnesses: Use only pasteurized milk; quickly refrigerate or freeze perishable foods; never thaw a food at room temperature or keep a refrigerated food at room temperature over 2 h; wash hands, utensils, and work areas with hot soapy water after contact with raw meat and meat patties; cook meat or patties until the center is gray or brown or internal temperature reaches to 68.3 °C (155 °F); and prevent fecal–oral contamination through proper personal hygiene. Routine sur-

veillance of cattle for the presence of EHEC should be carried out, and cattle should be tested for pathogen presence before slaughter. HACCP (hazard analysis critical control points) principles should be incorporated into the slaughtering and processing operations. Consumers should be educated for safe handling of raw meats and should avoid cross-contamination of cooked products.

Summary

Most *Escherichia coli* are a harmless inhabitant of the intestinal tract, and only a small percentage of strains are considered pathogenic. However, a recent surge in the enterohemorrhagic *E. coli* (EHEC), a highly virulent subset of Shiga toxin-producing *E. coli* (STEC) outbreaks, suggests a possible increased horizontal or vertical transfer of pathogenic genes among bacterial species. There are six virotypes of *E. coli* (EHEC, EPEC, ETEC, EIEC, DAEC, and EAEC), of which EHEC, EPEC, and ETEC are known to cause severe disease worldwide. Increased insight into their genetic and phenotypic properties of virulence factors and their pathogenic mechanisms should help in formulating appropriate preventive or therapeutic measures. The common themes shared by all *E. coli* virotypes include the following: they adhere to the epithelial cells and cause damage to the cells by initiating signaling events that lead to blockage of protein synthesis, alter the cytoskeletal structure leading to attachment and effacement lesion, affect ion pumps, increase fluid loss, or cause cell death. In recent years, however, the research focus is geared more toward EHEC group because of their continued association with serious foodborne outbreaks from a wide variety of foods, including fruits, vegetables, meats, and dairy products. Analysis of recent outbreak strains indicates association of Stx2 and Eae to be the most important virulence factor of EHEC/STEC, causing hemorrhagic colitis (HC), severe hemolytic uremic syndrome (HUS), and kidney damage. Association of this pathogen with fresh vegetables presents a serious problem because these products are minimally processed and, apparently, the processing conditions are inadequate for complete removal or inactivation. Furthermore, these organisms probably have developed strategies to utilize nutrients from plants for prolonged survival inside the plant tissues, and they are resistant to washing and disinfections. Diarrheal diseases are preventable by adopting proper sanitary condition during the preparation of food, by thorough cooking, and by avoiding foods that might be the potential source of the organism. Dehydration and electrolyte loss result from diarrhea, which can be fatal; thus, hydration is the most important therapy against diarrheal diseases. The most severely affected patients suffering from EHEC/STEC require blood transfusion and dialysis therapy.

Further Readings

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