IJFANS INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES
ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 3, 2022

CLINICAL IMPLICATIONS OF ESCHERICHIA COLI O157 AND ITS O157 PLASMID: A REVIEW

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Abstract:

Enterohemorrhagic *Escherichia coli* O157 is a widespread foodborne pathogen that causes severe illness in humans globally. In the United States, the most common sources of disease outbreaks are bovine food products and fresh produce contaminated with bovine feces, as healthy cattle serve as a reservoir for E. coli O157. This bacterium is also resilient in the environment, capable of surviving in diverse conditions. To cause human illness, colonize the bovine gastrointestinal system, and persist in the environment, E. coli O157 must adapt to various environments. Important virulence factors of E. coli O157 include Shiga toxins, products of the pathogenicity island known as the locus of enterocyte effacement, and products of the F-like plasmid pO157. Among these, pO157 plays a significant role, though it is the least understood of the virulence factors. This article provides a general overview of E. coli O157, with a particular emphasis on pO157.

Keywords E. coli O157:H7, pO157

Introduction:

Escherichia coli O157

Escherichia coli (E. coli) is a Gram-negative, rod-shaped, facultative anaerobic bacterium first characterized by Theodor Escherich in 1885. Most E. coli strains naturally inhabit the gastrointestinal tracts of humans and animals. However, some strains have evolved into pathogenic E. coli due to virulence factors acquired through plasmids, transposons, bacteriophages, and/or pathogenicity islands. Pathogenic E. coli can be classified based on serogroups, pathogenicity mechanisms, clinical symptoms, and virulence factors. Enterohemorrhagic E. coli (EHEC) is a type of pathogenic E. coli that produces Shiga toxins (Stxs), leading to hemorrhagic colitis (HC) and potentially fatal hemolytic uremic syndrome (HUS) in humans. Several EHEC serotypes, including O26, O91, O111, O157, and O157, are associated with human illnesses. This study focuses on E. coli O157, the most frequently isolated EHEC serotype from infected individuals in the United States, Japan, and the United Kingdom.

History

In 1982, the EHEC serotype O157 was first identified as a human pathogen during outbreaks of bloody diarrhea in Oregon and Michigan, USA, and in rare cases of HUS in 1983. Since then, numerous EHEC outbreaks have been recorded in the United States, establishing E. coli O157 as one of the most dangerous foodborne pathogens[1].

Prevalence and Economic Cost

The Centers for Disease Control and Prevention (CDC) estimate that E. coli O157 infections cause approximately 73,000 illnesses, 2,200 hospitalizations, and 60 deaths annually in the United States. While CDC data show a decline in E. coli O157 infections since a peak in 1999, significant outbreaks and isolated cases continue to occur. The economic burden of E. coli O157 infections amounts to $405 million per year in lost productivity, medical expenses, and premature deaths. Due to the high cost of this illness, increased efforts are needed to combat this infection[2].

Isolation and Identification

E. coli O157 expresses both somatic (O) antigen 157 and flagella (H) antigen 7. It is characterized by its inability to produce β-glucuronidase, an enzyme that can hydrolyze the synthetic compound 4-methylumbelliferyl-β-D-glucuronide (MUG), and by its delayed fermentation of D-sorbitol (taking longer than 24 hours). For detecting E. coli O157 , Sorbitol MacConkey (SMAC) agar supplemented with MUG is used. To enhance the selectivity for E. coli O157 and suppress other Gram-negative flora, cefixime, potassium tellurite, and vancomycin are added to SMAC agar plates. Confirmation of serotypes O157 and H7 can be performed using a commercially available latex agglutination assay[3].

Genomic Organization

E. coli O157 has a chromosomal size of 5.5 Mb, which includes a 4.1 Mb backbone sequence found in all E. coli strains. The remaining genomic content is unique to E. coli O157. Comparative genomic studies reveal that E. coli O157 lacks 0.53 Mb of DNA present in the nonpathogenic E. coli K12, indicating that genomic reduction played a role in the evolution of E. coli O157[4]. The E. coli O157 genome also contains 1.4 Mb of horizontally transferred foreign DNA, including prophage and prophage-like elements. E. coli O157 has 463 phage-associated genes, compared to only 29 in E. coli K-12. Research by Putonti et al. estimates that at least 53 distinct species contributed to these unique sequences, with changes in G+C content serving as indicators of horizontal gene transfer. Sequencing of two E. coli O157 strains shows that their virulence-associated genes are almost identical (99%). Both DNA acquisition and loss have been crucial in the development of E. coli O157pathogenesis.

Evolution

Comparative and epidemiological research suggests that E. coli O157 may have evolved from the non-toxigenic and less virulent strain E. coli O55. The emergence of E. coli O157 involved four sequential events: (i) acquisition of a stx2-containing bacteriophage, (ii) acquisition of pO157 and the rfb region, (iii) acquisition of a stx1-containing bacteriophage,

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 3, 2022

and (iv) loss of the ability to ferment D-sorbitol and loss of beta-glucuronidase (GUD) activity[5].

Animal Reservoir

Cattle are the primary reservoir for E. coli O157 , typically remaining asymptomatic when infected. However, this serotype has occasionally caused diarrheal illness in young calves. The proportion of cattle shedding E. coli O157varies over time. Additionally, E. coli O157 has been found in the feces of sheep, goats, pigs, and turkeys[6].

Molecular Subtyping

To better understand the epidemiology of E. coli O157 outbreaks, various molecular subtyping methods have been developed. These include pulsed-field gel electrophoresis (PFGE), restriction fragment length polymorphisms (RFLP), amplified fragment-length polymorphisms (AFLP), and phage typing. The CDC standardized the PFGE technique, which has been effectively used to differentiate outbreak-associated, sporadic, or unrelated cases since 1993[7].

Infection

E. coli O157 infection is a significant public health concern in North America, Europe, and other parts of the world. Although the overall number of E. coli O157 infections is lower than those caused by other enteric pathogens such as Salmonella or Campylobacter spp., the illnesses caused by E. coli O157 have substantially higher hospitalization and mortality rates. Human infection with E. coli O157 can range from asymptomatic to fatal. Most cases start with non-bloody diarrhea and resolve without complications. However, within 1-3 days, some individuals develop bloody diarrhea or hemorrhagic colitis (HC). In 5-10% of HC patients, the condition can progress to life-threatening complications such as hemolytic uremic syndrome (HUS) or thrombocytopenic purpura (TTP). In the United States, E. coli O157 is the most common cause of HUS, with severe clinical signs more frequently observed in children and the elderly[7].

Acid Resistance of E. coli O157

Acid resistance (AR) is the ability of bacteria to survive in extremely low pH environments (pH 3.0). One of the primary host defenses against foodborne enteric infections is the acidic environment of the stomach (pH 1.5 to 3.0). Bacteria that can withstand the stomach's acidity have a higher likelihood of colonizing the intestines and causing illness. AR is associated with a decrease in the infectious doses of enteric pathogens. A well-known characteristic of E. coli O157 is its low infectious dose, which makes it highly contagious. Numerous studies have documented the AR of E. coli O157 strains. These studies identified three effective AR systems[8].

The first AR system requires RpoS, an alternate sigma factor, and glucose repression. In experimentally infected mice and calves, the rpoS mutant of E. coli O157was shed in reduced quantities. The second AR system requires the addition of arginine after acidic exposure and involves arginine decarboxylase (adiA) and its regulator (cysB). The third AR system relies on glutamate for protection in low pH conditions and involves two glutamate decarboxylase

isozymes (gadA and gadB), and a suspected glutamate, gamma-aminobutyric acid antiporter (gadC). At pH 2.5, only one of the two glutamate decarboxylase isozymes is necessary for protection, but at pH 2.0, both are required. Previous research has shown that glutamate-dependent AR provides the most efficient protection in complex media at pH 2.0. While the three AR systems overlap, each system has distinct controls and requirements for AR activity. In addition to these three AR systems, several other proteins involved in E. coli O157 AR have been identified, including chaperone HdeA, RNA polymerase-associated protein SspA, and DNA-binding protein Dps. Additionally, changes in the cell wall membrane or colonic acid production are linked to the success of AR[9].

Environmental Survival

E. coli O157can survive and persist in various environments, including soil, water, food, and animal reservoirs. It has been shown to remain viable for a year in manure-treated soil and for 21 months in raw, uncomposted manure. Composting manure can effectively kill E. coli O157 if the temperature is maintained above 50°C for 6 days[10].

The bacterium can also persist in water for extended periods, especially at low temperatures. Water trough sediments contaminated with bovine excrement can serve as long-term reservoirs (>8 months) for E. coli O157 , and bacteria surviving in these troughs can cause illness.Barker et al. demonstrated that E. coli O157 an live and replicate within Acanthamoeba polyphaga, a common environmental protozoan found in soil, water, and feces, potentially serving as an effective transmission vehicle. To thrive in various environments, E. coli O157 must adapt to fluctuations in temperature, pH, and osmolarity. For example, exopolysaccharide (EPS) production in E. coli O157 is linked to heat and acid tolerance, and heat stress causes changes in membrane lipid content. These adaptations are crucial for the bacterium's survival and spread on farms, increasing transmission from cattle to cattle. Moreover, the ability to survive outside the host reservoir raises the risk of crop contamination through bovine dung pollution, irrigation with contaminated water, or direct contact with infected animals.

Major Virulence Factors

Research has focused on identifying the virulence factors and mechanisms of E. coli O157 pathogenesis. Although Shiga toxins (Stxs) are considered important, they are not solely responsible for the disease. Additionally, E. coli O157 linked to severe human disease must colonize the intestinal mucosa, and the presence of the pO157 plasmid is associated with the ability to cause illness. Each of these characteristics is discussed in detail below.

Shiga Toxins (Stxs)

Shiga toxins (Stxs) are highly potent cytotoxins encoded by bacteriophages. Stx is a single transcriptional unit that can cause damage to various cell types. Stxs are classified into two groups: Stx1 and Stx2, which do not produce cross-reactive antibodies due to only 56% amino acid sequence similarity. The only difference between Stx1 and the Stx from Shigella dysenteriae I is a single amino acid. Virulent E. coli O157 strains can express Stx1 exclusively, Stx2 exclusively, or both toxins. Strains producing Stx2 are more hazardous than

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 3, 2022

those producing Stx1 and are more frequently associated with hemorrhagic colitis (HC) or hemolytic uremic syndrome (HUS) in human infections.

The structure of Stx consists of one enzymatically active A subunit (A1) and five identical receptor-binding B subunits (B5). The B5 subunit binds to globotriaosylceramide (Gb3) or globotetraosylceramide (Gb4) receptors on host cells. After binding to the host cell, the A subunit is internalized into the cytoplasm. The A1 subunit inhibits protein synthesis by removing a single adenine residue from the 28S rRNA of the 60S ribosomal subunit. The exact mechanisms of Stx translocation to different tissues are not fully understood.

The Locus of Enterocyte Effacement

Attaching and effacing (A/E) lesions are histopathological lesions caused by E. coli O157 colonization of the intestinal mucosa. These lesions are characterized by microvilli effacement and bacterial adhesion to the epithelial cell membrane. Attached bacteria increase actin polymerization in the host cell, forming a higher attachment pedestal. The genes responsible for A/E lesions are located in a 13-region genetic locus called the locus of enterocyte effacement (LEE). The LEE is also found in enteropathogenic E. coli (EPEC) and is associated with pathogenicity. The LEE of E. coli O157 is 43 kb in size and includes an additional 7.5 kb prophage sequence, whose function is unclear.

The LEE consists of at least 41 genes organized into three major regions: (i) a type III secretion system (TTSS) that exports effector molecules; (ii) an adhesion molecule called intimin and its translocated receptor, Tir, which is inserted into the host cell membrane by the TTSS; and (iii) several secreted proteins (Esp) as part of the TTSS, which play crucial roles in modifying host cell signal transduction during the formation of A/E lesions. Recently, non-LEE encoded effectors have been discovered, and understanding their functions will enhance our knowledge of the pathogenic processes in E. coli O157 infections.

Plasmid O157 (pO157)

A plasmid is an extrachromosomal DNA element that can replicate independently of chromosomal DNA. Plasmids are mobile genetic elements that confer various advantages to their host organisms, including antibiotic and heavy metal resistance, production of toxins and other virulence factors, hydrocarbon biotransformation capabilities, and symbiotic nitrogen fixation. In many enteropathogenic bacteria, such as Shigella, Yersinia, Salmonella, and Escherichia coli, plasmid-encoded genes are essential for full pathogenicity.

pO157

The plasmid pO157 is a highly conserved extrachromosomal element found in E. coli O157. It is a nonconjugative F-like plasmid, ranging from 92 to 104 kb in size. The complete sequencing of pO157 has been achieved in two separate epidemic isolates. pO157 has a dynamic structure and contains a variety of mobile genetic elements, including transposons, prophages, insertion sequences (IS), and fragments from other plasmids. The functional zones of pO157 are delimited by its heterogeneous composition. IS or IS remnants are typically associated with virulence-related regions, similar to the components of Shigella spp.'s virulence plasmid. This suggests that pO157 was formed through the integration of fragments

from evolutionarily distinct species into an F-like plasmid, with virulence factors or potential virulence factors on various segments originating from different sources.

The entire pO157 sequence contains 100 open reading frames (ORFs). Among these, 43 ORFs showed sufficient similarity to known proteins to suggest their functions, while 22 ORFs had no credible match to any known proteins. Thirty-five proteins are thought to be involved in the pathogenesis of E. coli O157 infections, but only 19 genes have been identified, including a hemolysin (ehxA), a catalase-peroxidase (katP), a type II secretion system apparatus (etp), a serine protease (espP), and a putative adhesin. Despite these findings, the full biological importance of pO157 in the pathogenesis of E. coli O157 infections remains unclear.

pO157-Like Plasmids in EHEC

Many non-O157 enterohemorrhagic E. coli (EHEC) isolates possess large plasmids similar to pO157, ranging in size from 70 to 200 kb, although not all human isolates exhibit this feature. These plasmids often carry the hemolysin operon (ehx), although the presence of genes such as etpC-O, katP, and espP is observed in less than half of these isolates. Some of these EHEC-hemolysin plasmids have been associated with adhesion, while others have not shown such links. Epidemiological studies suggest that the presence of these EHEC-hemolysin plasmids correlates more strongly with the development of hemolytic uremic syndrome (HUS) rather than diarrhoea.In addition to pO157 or EHEC-hemolysin plasmids, various other plasmids ranging in size from 2 to 87 kb have been identified in E. coli O157 isolates. However, no clear association has been established between the presence of any specific plasmids and clinical illness.

In vitro

Year	Target	Pathogenesis	Effect
1987	Whole plasmid	Expression of fimbriae Adherence to epithelial cells	Yes
1990	Whole plasmid	Adherence to epithelial cells	Yes
1993	Whole plasmid	Production of pilli Adherence to epithelial cells	No
2001	toxB gene on pO157	Adherence to epithelial cells	Yes
2005	stcE gene on pO157	Adherence to epithelial cells	Yes
2007	espP gene on pO157	Adherence to bovine primary intestinal epithelial cells	Yes

In vivo

Year	Target	Pathogenesis	Effect
1987	Whole plasmid	Attaching and effacing lesion in gnotobiotic piglets	No
1990	Whole plasmid	Colonization of mouse	No
1993	Whole plasmid	Clinical symptoms in rabbit	No
2006	Whole plasmid	Colonization of cattle	Yes
2007	Whole plasmid	Colonization of cattle	Yes
2007	espP gene on pO157	Colonization of calves	Yes

ISSN PRINT 2319 1775 Online 2320 7876
 UGC CARE Listed (Group -I) Journal Volume 11, Iss 3, 2022

CONCLUSION

This article centers on E. coli O157 and its 92-kb plasmid. E. coli O157 is a bacterium that causes significant human illness worldwide. Three key virulence factors include Shiga toxins, products of the pathogenicity island known as the locus of enterocyte effacement, and products of the F-like plasmid pO157. From its reservoir in healthy cattle to various agricultural environments, this pathogen thrives in diverse conditions. Genes expressed on pO157 influence bacterial adhesion to eukaryotic cells, colonization of cattle, and acid resistance. Further research into the etiology and persistence of E. coli O157 in the environment will contribute to more effective strategies to prevent human illness.

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