Escherichia coli
Class Notes PHR 150

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General characteristics

First characterized by Theodor Escherich over a century ago, *E. coli* is a gram-negative rod and is a member of the family *Enterobacteriaceae*. It is the most common facultative anaerobe in the intestinal tract of humans and animals (up to 10^8 CFU/g of feces). It is a member of the coliform group of organisms that are characterized by their ability to ferment lactose with the production of acid and gas.

Table 1. General growth parameters for *E. coli*.

	Temperature (°C)	pH	Water activity
Minimum	7 – 8	4.4	0.95
Optimum	35 – 40	6 – 7	0.995
Maximum	44 – 46	9.0	–

Serology and Nomenclature

Somatic antigen O (outer-membrane protein): capsular antigen K: flagellar antigen H

173 O antigens (many unknown O groups)
103 K antigens
56 H antigens (note "NM")

Fimbrial F-antigens can also be used, but this must be done in specialized laboratories and so is not done on a routine basis.

Types of food-associated enteric *E. coli* infections.

Based on disease syndromes, laboratory characteristics, particularly their effects on tissue cultures. Six virulence groups are recognized: enteroaggregative (EAEC, EAggEC), enteropathogenic (EPEC), enterotoxigenic (ETEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), and diffusely adherent (DAEC). All cause foodborne infections although some produce active toxins once established in the host.

Table 2. Some of the O serogroups associated among five *E. coli* virulence groups. Note that some serotypes (e.g., O111 are listed under more than one virulence group). (From Jay, J. 1996. Modern Food Microbiology, Chapman & Hall, N.Y.).

EAEC	EIEC	EPEC	ETEC	EHEC
3, 4, 6, 7, 17, 44, 51, 68, 73, 75, 77, 78, 85, 111, 127, 142, 162	28, 112, 124, 135, 136, 143, 144, 147, 152, 164, 167	18, 19, 55, 86, 111, 114, 119, 125, 126, 127, 128, 142, 158	6, 8, 15, 20, 25, 27, 63, 78, 80, 85, 101, 115, 128, 139, 141, 147, 148, 149, 153, 159, 167	2, 4, 5, 6, 22, 26, 38, 45, 46, 82, 84, 88, 91, 103, 104. 111, 113, 116, 118, 145, 153, 156, 157, 163

Enteroaggregative *E. coli* (EAEC)

Some indication that EAEC can be foodborne (isolated from infant feeding bottles, in Japan associated an outbreak of school children and school lunches). They carry a 60-MDa plasmid needed for the production of fimbriae and an outer membrane protein that results in their aggregative phenotype. In the laboratory these strains adhere to HEp-2 cells in clumps.
EAEC result in a persistent diarrhea that can last for more than 2 weeks especially in children. Also associated with diarrhea and weight loss in HIV-infected individuals. Asymptomatic carriage may occur.
Model for pathogenesis involves three stages: 1) initial adherence to intestinal mucosa and mucus layer; 2) enhanced mucus production that leads to a biofilm on the mucosal surface; 3) production of toxin(s) that damage the mucosa and result in intestinal secretion.

Enteroinvasive *E. coli* (EIEC)

Similar to *Shigella* in that they enter and multiply in the colonic epithelial cells (causing cell death) and then spread to adjacent cells. Like *Shigella*, they possess a 120 – 140-MDa plasmid that codes for the production of several outer membrane polypeptides involved in invasiveness; plasmidless strains are not invasive. Profuse bloody or nonbloody diarrhea is a consequence. The incubation period is between 8 and 48 h with an average of 18 h. Foodborne illness has been documented.
They seem to be less efficient than *Shigella* in causing disease as the infective dose is thought to be quite high ($>10^6$ CFU).

The organism is atypical for *E. coli* in that most strains are nonmotile. They are unable to ferment lactose or do so only slowly, and they do not produce gas from lactose (anaerogenic), and they are unable to decarboxylate lysine.

Table 3. Disease associations of virulence types of *E. coli*. (Adapted from Bell, C., and A. Kyriakides. 1998. *E. coli* A practical approach to the organism and its control in foods, Chapman & Hall.)

Virulence type	Disease Association	Summary of *E. coli*/host interaction
Enteroaggregative (EAEC)	Persistent diarrhea in children Diarrhea watery and mucoid. Up to 30% grossly bloody stools.	EAEC bind in clumps (aggregates) to cells of the small intestine and produce toxins. Virulence factors include aggregative adherence and heat-stable enterotoxin; plasmid mediated
Enteroinvasive (EIEC)	Acute dysenteric diarrhea	EIEC invade cells in the colon and spread laterally, cell to cell. Virulence factors include cell invasion and intracellular multiplication; plasmid and chromosomally mediated.
Enteropathogenic (EPEC)	Enteritis in infants Traveler's diarrhea Acute and/or persistent profuse watery diarrhea, vomiting, fever.	EPEC attach to intestinal mucosal cells causing cell structure alterations (attaching and effacing). EPEC cells invade the mucosal cells. Virulence factors include attaching and effacing lesions, localized adherence mediated by bundle-forming pili; plasmid and chromosomally mediated
Enterotoxigenic (ETEC)	Diarrhea, vomiting and fever Traveler's diarrhea Acute watery diarrhea	ETEC adhere to the small intestinal mucosa and produce toxins that act on the mucosal cells. Virulence factors include adherence and heat-stable or heat-labile toxins; plasmid and chromosomally mediated.
Enterohemorrhagic (EHEC) "Vero cytotoxigenic (VTEC)" "Shiga toxigenic (STEC)"	*Shigella*-like dysentery (stools contain blood and mucus) Hemolytic uremic syndrome in some cases	EHEC attach to and efface mucosal cells and produce toxin(s) which cause an irreversible inhibition of protein synthesis in eukaryotic cells. A subunit shows enzymatic activity; B subunit binds to receptors on the surface of some tissues (kidney). Virulence factors include attaching and effacing adherence, Shiga toxins, hemolysin; plasmid and chromosomally mediated.
Diffusely adherent (DAEC)	Childhood diarrhea	Fimbrial and non-fimbrial adhesins identified; plasmid and chromosomally mediated.

Table 4. Characteristics of *E. coli*-related illness. (Adapted from Bell, C., and A. Kyriakides. 1998. *E. coli* A practical approach to the organism and its control in foods. Chapman & Hall.)

Virulence type	Estimated infectious dose	Time to onset of illness	Duration of illness	Range of symptoms
EIEC	10^8	8 – 24 h average 11 h	Days to weeks	Profuse diarrhea or dysentery, chills, fever, headache, muscular pain, abdominal cramps
EPEC	$10^5 – 10^{10}$	17 – 72 h average 36 h	6 h – 3 days average 24 h	Severe diarrhea, fever, vomiting and abdominal pain in infants which may persist for more than 14 days. In adults, severe watery diarrhea with prominent amounts of mucus without blood and nausea vomiting, abdominal cramps, headache, fever and chills
ETEC	$10^8 – 10^{10}$	8 – 44 h average 26 h	3 – 19 days	Watery diarrhea, low-grade fever, abdominal cramps, malaise, nausea. When severe, causes cholera-like extreme diarrhea with rice-water-like stools, leading to dehydration
EHEC	<100 (10?)	3 – 9 days average 4 days	2 – 9 days average 4 days	Hemorrhagic colitis (HC): sudden onset of severe crampy abdominal pain, grossly bloody diarrhea, vomiting, no fever. Hemolytic uremic syndrome (HUS): bloody diarrhea, acute renal failure in children, thrombocytopenia, seizures, coma, death. Thrombotic thrombocytopenic purpura (TTP): similar to HUS but also fever, central nervous system disorders, abdominal pain gastrointestinal hemorrhage, blood clots in the brain, death

Enteropathogenic *E. coli* (EPEC)

EPEC cause diarrhea in children, generally under 1 year of age. Asymptomatic carriers are known. These strains are described as diarrheagenic *E. coli* that cause illness but whose pathogenic mechanisms are not related to either heat-labile enterotoxins (LT), heat-stable enterotoxins (ST), to *Shigella*-like invasiveness, or verocytotoxigenic properties. They appear to destroy the microvilli without further invasion.

Illnesses are uncommon in temperate climates with good hygienic standards. However, they are still a common cause of disease in tropical countries, especially among the poor. Transmission: fecal/oral — food, infant formula, person-to-person, contaminated objects.

Food-related outbreaks have been rare. A coffee substitute was implicated as the vehicle in a 1961 outbreak. *E. coli* O86:K7:H34 was isolated at high levels from the coffee substitute and from the stools of patients. A waterborne outbreak was reported in 1967 near Washington, D.C. At least 170 adults became ill over a period of several weeks. *E. coli* O111:H4 was isolated from the water supply and from the fecal samples of some patients.

Immunity is thought possibly to play a role in the low incidence of this type of illness. A WHO report suggested that 50% of children possess antibodies to EPEC by 1 year of age, which may lead to immunity as an adult.

Enterotoxigenic *E. coli* (ETEC)

Major cause of infantile diarrhea in developing countries. It is the agent most frequently linked to traveller's diarrhea. Humans are the principal reservoir. Foods implicated in outbreaks include ready-to-eat items served raw or cooked but served cold.

Virulence Factors
Plasmids encode for fimbrial adhesins or colonization factors, heat-labile (LT) and heat-stable (ST) toxins. Heat-labile toxins of ETEC are inactivated at 60°C for 30 minutes. LTI closely resembles cholera toxin. LTI binds to ganglioside receptors on mammalian cell surfaces, stimulates adenylate cyclase activity ultimately leading to water and electrolyte outflow into the lumen of the small intestine which results in watery diarrhea. LTII is antigenically distinct but has similar biological properties.

ST consists of two toxic products ST_A and ST_B. It appears to act mainly as an antiabsorbent rather than in a secretory fashion. Withstands boiling for 15 min at 100°C.

Colonization factors (fimbriae/flagellar)
987P or K88 — specific for swine
K99 — specific for calves, lambs, and swine
CFA/I — specific for humans (Colonization factor antigen I)
CFA/II — specific for humans
CFA/III

Enterohemorrhagic *E. coli* (EHEC) — also VTEC/STEC

(1977 — Vero cytotoxicity phenomenon described)
1982 — Identified as cause of human illness
1986 — Foodborne illness dominant cause is recognized
1987 — Natural habitat in cattle identified

Mead et al., 1999. Estimated 62,000 cases annually (0.5% of all foodborne illness). 85% of cases presumed to be foodborne. Estimated 1843 hospitalizations (3% of all foodborne illnesses). Estimated 52 deaths (2.9% of deaths).

Course of infection/duration

Most *E. coli* O157:H7 (predominant serotype) infections are relatively mild. However, infections can result in hemorrhagic colitis (bloody inflammation of the colon). In some cases bleeding can be severe. Most cases of hemorrhagic colitis recover 6 – 8 days after onset, but patients may be hospitalized if symptoms are more severe. The symptoms may be misdiagnosed and sometimes inappropriate or unnecessary procedures may result.

Of those infected with *E. coli* O157:H7, it is estimated that 50% will not visit a physician and will fully recover. An estimated 32% are ill enough to visit a physician and fully recover, 15.4% are hospitalized and fully recover, 2.3% are hospitalized and die and 0.3% are hospitalized and develop chronic kidney failure. (From Food Safety: Information on foodborne illnesses and food safety: reducing the threat of foodborne illnesses, May 1996).

The consequences of hemolytic uremic syndrome are very severe, leading to life-threatening disease characterized by red blood cell destruction, kidney failure, and neurological complications such as seizures and strokes.

Transmission

Cattle are thought to be a major reservoir of *E. coli* O157:H7. Other ruminants are also thought to be sources. At least one outbreak has been associated with deer meat. To date, a large number of outbreaks have been associated with meat or meat products, especially undercooked comminuted meat. In other instances outbreaks there has been cross contamination from food handlers or from raw meats to cooked or ready-to-eat foods — melons, salads, mayonnaise. In some produce-associated outbreaks, contamination from the soil as a result of fertilization with manure or grazing of orchards has also been suggested as a mode of transmission. Fruit flies have also been shown to transfer *E. coli* from a contaminated source to apples.

Estimated impact

FoodNet Data: Rate per 100,000: 1996 (2.7); 1997 (2.3); 1998 (2.8); 1999 (2.1); 2000 (2.9); 2002 (1.7); 2002 (1.6); 2003 (2.3); 2004 (0.9). There are an estimated 10,000 – 20,000 cases of *E. coli* O157:H7 disease each year in the U.S. with an estimated 19 – 37 deaths. It is estimated that 80% of human illness due to *E. coli* O157:H7 is attributed to food and that the total costs of foodborne *E. coli* O157:H7 range from $0.2 – $0.6 billion annually.

Virulence factors

All strains of EHEC are capable of producing Shiga toxin 1 (Stx1)/Verotoxin 1 (VT1) and/or Shiga toxin 2 (Stx2)/Verotoxin 2 (VT2). The toxin is composed of a single A subunit of 32 kDa and five B subunits of 7.7 kDa each. The B subunits bind to specific receptors (Gb_3) on the surface of eucaryotic cells. Endothelial cells in the colon and the kidney have high levels of Gb_3. The A subunit acts as a N-glycosidase that inactivates the 28S ribosome effectively blocking protein synthesis. The presence of toxin alone is not sufficient to cause symptoms. Enterohemolysin production (an outer-membrane protein encoded by the plasmid-associated gene *eae*) is thought to be involved in attachment.

Outbreaks

More than any organism other than *Clostridium botulinum*, *E. coli* O157:H7 has had a major impact on food regulations in the U.S.

A number of important outbreaks caused by EHEC strains are listed in Table 5. The first outbreaks linked to *E. coli* O157:H7 were recorded in 1982. Both were associated with hamburgers served from the same restaurant chain in Oregon and Michigan. Retrospective studies suggest that an earlier outbreak associated with apple cider occurred in Ontario in 1980.

An outbreak in 1988 involved precooked patties purchased in the USDA school lunch program. Identification of *E. coli* in these products lead to increases in the required cook time for USDA-inspected products and ultimately to the adoption of higher cooking temperatures by the state of Washington.

Table 5. Some important reported food and waterborne outbreaks caused by EHEC.

Year	Place	Implicated food	Ill/Deaths
1980?	Ontario	Apple cider	14/0
1982	Oregon	Ground beef	26/0
	Michigan		21/0
1984	North Carolina	Person-to-person	36/0
1985	Ontario	Sandwiches	73/19
		Person-to-person	
1988	Minnesota	Ground beef (pre-cooked)	54/0
1989	Missouri	Water	243/4
1991	Massachusetts	Apple cider	23/0
1993	WA/CA/NV/ID	Ground beef	>500 (WA)/3 >200 (CA/NV/ID)
1993	Oregon	Mayonnaise	19/0
		Cantaloupe	10/0
1994	California	Fermented salami	18/0
	Washington		
1995	Australia (O111)	Fermented salami	23/1
1996	Illinois	Red leaf lettuce	27/0
	Connecticut		18/0
1996	Japan	School lunches	>10,000/10
1996	Pacific coast	Unpasteurized apple juice	66/1
1996	Scotland	Meat pies	>250/16

Case Studies

Hamburgers — 1993

Product type: Cooked hamburger patties from a fast food restaurant
Year: 1992–1993

Location: Western U.S.
Levels: Estimated 1 – 15 cells per gram, 40 – 600 cells per raw hamburger patty
Extent: >700 people affected, 195 hospitalized, 55 HUS or TTP, 4 deaths;
approximately 10% of cases secondary from person-to-person contact
Comments: Inadequate cooking of contaminated hamburger patties. While this outbreak was the largest *E. coli* O157:H7 outbreak in the U.S., many smaller outbreaks have also been associated with undercooked hamburger patties.
Control Options: Procedures in place to ensure minimal contamination of raw materials. Vendor certification. Procedure in place to ensure destruction of *E. coli* O157:H7 during cooking including defined process times and temperatures.

A 1988 outbreak involving precooked patties lead to a rapid FSIS policy change on December 27, 1988. This increased the cooking temperature from 140 to 160°F (60 to 71.1°C). Opposition to the ruling was expressed as the high temperature often resulted in a very dry unpalatable hamburger. With consideration of further comments from the industry as well as research results published in 1991, these policy changes were modified on June 5, 1990 to lower temperatures and more cooking options.

In mid-1992, the USDA study and policy change prompted Washington State to increase the required cooking temperature for hamburgers to 155°F (68.3°C). Although this is a "requirement," undercooked hamburgers can be ordered if specifically requested.

The FDA changed its policy in the Food Code during the 1993 outbreak to match the Washington State requirement.

Before the January 1993 Jack-In-The-Box outbreak in the Pacific Northwest, *E. coli* O157:H7 and related strains were considered by many to be relatively rare. During the outbreak the FDA increased its recommended cook temperature from 140°F to 155°F. Shortly after this outbreak (July 1996), changes were made to the USDA meat inspection regulations. Most controversial was the definition of *E. coli* O157:H7 as an *illegal adulterant* in raw ground meat. Although FDA did not follow suit, the USDA regulation has effectively resulted in this organism being considered an illegal adulterant if found at all in the food supply. In February 1999 the USDA approved irradiation in red meats as a means of controlling *E. coli* O157:H7 and other pathogens.

Consumers are recommended to cook hamburgers to 160°F as measured by a thermometer.

Unpasteurized Apple Cider — 1996

Product type: Unpasteurized apple juice
Year: 1996
Location: Western U.S.
Levels: Unknown, very low
Extent: 66 cases, one death
Comments: Juice not pasteurized. Source of *E. coli* O157:H7 in apple cider unclear. Earlier outbreaks suggested route was use of dropped apples with possible close proximity to cattle pastures or deer prevalent in the orchards. Supplier contracts apparently stipulated no "drops."
Control Options: At this time, pasteurization is recommended for susceptible populations. Investigations are underway to try to determine alternative approaches to achieving an acceptable reduction of *E. coli* O157:H7 (currently defined as 5-log reduction).

On July 8, 1998, FDA published the final rule for the labeling of fresh juices and extended the comment period for the HACCP proposed rule. For apple cider producers, the final ruling stated that if

the juice was not treated to achieve a 5-log reduction of the "pertinent" pathogen, it was required to have a warning label on the bottle or a warning placard at the point of sale. This option of having a warning placard in place of a warning label on the bottle itself was ONLY for 1998–1999. As of September 8, 1999, all fresh juices not treated to achieve a 5-log reduction of the "pertinent" pathogen MUST have a warning label on the bottle. The warning label must read as follows:

"WARNING: This product has not been pasteurized and, therefore, may contain harmful bacteria that can cause serious illness in children, the elderly, and persons with weakened immune systems."

The final rule for mandatory HACCP requirements for the juice industry was published in January 2001.

Survival in acidic foods. *E. coli* O157:H7 survives (but doesn't grow) in many acidic foods. Because of the low infective dose the organism does not need to multiply in the food — just survive. Outbreaks have been associated with yogurt (U.K. 1991), uncooked semi-dry fermented sausage (Australia, 1995 — O111:NM; California, 1994), and mayonnaise (Oregon, 1993).

Survival is extended at refrigerated temperatures and is known at frozen temperatures in ground meat products. Populations of *E. coli* O157:H7 did not significantly decrease in hamburger stored over 9 months at -20°C. *E. coli* is not-heat resistant and is relatively easily killed during cooking or pasteurization.

The FDA also proposed voluntary Good Agricultural Practices after a series of outbreaks associated with contaminated fruits and vegetables (*Cyclospora* from imported raspberries, 1995–1997; *E. coli* O157:H7 from lettuce, 1996; and hepatitis A from frozen strawberries, 1997). These are the first times guidelines for sanitary procedures have been provided at the farm level.

Further Reading

Bell, C., and A. Kyriakides. 1998. *E. coli*. A practical approach to the organism and its control in foods. Blackie Academic & Professional, New York.

Buchanan, R. L., and M. P. Doyle. 1997. Foodborne disease significance of *Escherichia coli* O157:H7 and other enterohemorrhagic *E. coli*. Food Technol. 51(10):69-76.

Fratamico, P. M., J. L. Smith, and R. L. Buchanan. 2002. *Escherichia coli*, p. 79–101. *In* D. O. Cliver and H. P. Riemann (ed.), Foorborne diseases, 2nd ed. Academic Press, London.

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Griffin, P. M., and R. V. Tauxe. 1991. The epidemiology of infections caused by *Escherichia coli* O157:H7, other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. Epidemiol. Rev. 13:60-97.

Slutsker, L., A. A. Ries, K. D. Green, J. G. Wells, L. Hutwanger, and P. M. Griffin. 1997. *Escherichia coli* O157:H7 diarrhea in the United States: clinical and epidemiologic features. Annals Int. Med. 126:505-513.

Web Sites of Interest (check dates)

Juice HACCP http://www.cfsan.fda.gov/~comm/haccpjui.html
FDA Bad Bug Book
ETEC: http://vm.cfsan.fda.gov/~mow/chap13.html
EPEC: http://vm.cfsan.fda.gov/~mow/chap14.html
E. coli O157:H7: http://vm.cfsan.fda.gov/~mow/chap15.html
EIEC: http://vm.cfsan.fda.gov/~mow/chap16.html
Ecomonics of *E. coli* illness
http://www.ers.usda.gov/briefing/FoodborneDisease/ecoli/index.htm
FDA BAM Manual – methods
http://www.cfsan.fda.gov/~ebam/bam-toc.html

Draft Risk Assessment:
Preliminary pathways and data for a risk assessment of *Eshcerichia coli* O157:H7 in beef

http://www.fsis.usda.gov/OPHS/ecolrisk/chapter1.pdf