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Shiga toxin-producing *Escherichia coli* and current trends in diagnostics

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Implications

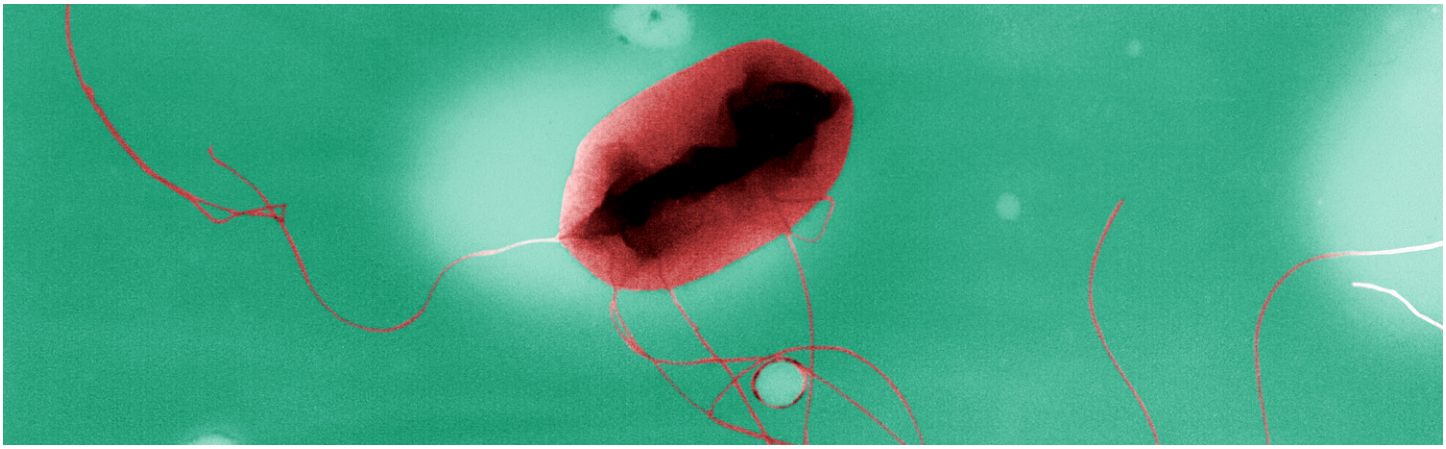
- Shiga toxin-producing *Escherichia coli* (**STEC**) are bacterial pathogens responsible for deadly foodborne outbreaks and sporadic illnesses globally. Children under five are most susceptible to severe complications and death. Seven main serogroups (O157 and top six non-O157: O26, O45, O103, O111, O121, O145) have been identified as causing the majority of STEC infections in humans.
- Beef products are one frequent source of infection, necessitating robust surveillance programs. However, detection and isolation methods for clinically relevant serogroups have several inherent limitations, making routine screening for these pathogens difficult and time consuming.
- These pathogens are constantly evolving, further allowing them to evade current detection methods. Developments in technology and genomic sequencing may improve our knowledge of these pathogens, thereby enhancing surveillance systems. With intensive beef production systems and a growing global demand for food, such advances are essential to improve food safety.

Key words: beef production, cattle, detection, foodborne outbreaks, pathogen surveillance

Introduction

Escherichia coli (*E. coli*) were first described in 1885 by the German physician Theodor Escherich in healthy human feces and originally named "Bacterium coli" (Durso, 2013). During the pre-molecular era, *E. coli* were distinguished from similar microbes based on their motility and metabolic profile such as the ability to ferment lactose. Belonging to the Enterobacteriaceae family, *E. coli* is a facultative anaerobic, rod-shaped, Gram-negative bacterium (Mathusa et al., 2010). While most *E. coli* strains are beneficial to their hosts, others are pathogenic. *E. coli* are residents of the gastrointestinal tract of warm-blooded mammals and are shed in the feces (Bettelheim, 2007). Commensal *E. coli* are unlikely to cause disease in healthy human hosts. However, several highly adapted *E. coli* clones have acquired virulence attributes, allowing them to cause a broad spectrum of disease (Croxen and Finlay, 2010).

Escherichia coli are categorized into somatic (O), flagellar (H), and capsular (K) antigen types (named for their location on the bacterial cell). Serogroup (O-type) and serotype (O- and H-type) are defined by these antigens. A total of 181 O antigens and 56 H antigens have been identified. There is a core genome among all *E. coli* strains of about 2,200 genes (Chaudhuri and Henderson, 2012). However, genomes of pathogenic *E. coli* strains are up to 1 Mb (~5,000 genes) larger than non-pathogenic strains due to the acquisition of pathogenicity islands (**PAI**) and mobile genetic



Escherichia coli cell with flagella (source: © 2009 E.H. White; P.S. Hayes; Public domain; Wikimedia Commons).

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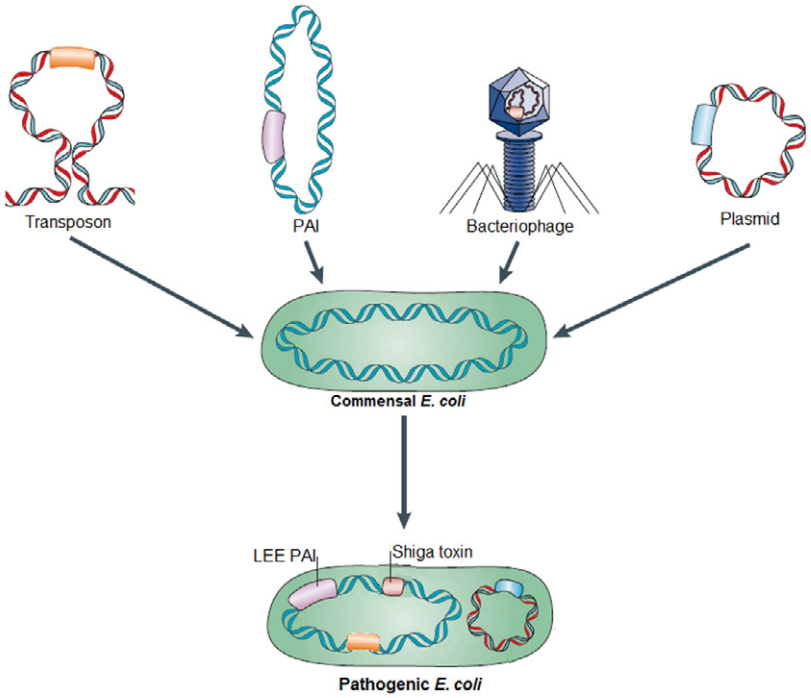

Figure 1. Virulence factors of *E. coli* can be encoded by transposons, pathogenicity islands (PAIs e.g., locus of enterocyte effacement, or LEE), bacteriophage (e.g., Shiga toxins), and plasmids (adapted from Kaper et al., 2004).

elements (Fig. 1). As a result, there is an abundance of genetic diversity and virulence genes within these strains. Disappearance of genes and acquisition of new genes has led to the emergence of novel pathogenic groups of *E. coli*. Horizontal gene transfer (**HGT**) is one mechanism *E. coli* uses to accomplish exchange of genetic information within and beyond species barriers (e.g., Shigella). Both gene loss and gain have contributed to the divergence and emergence of a diverse set of *E. coli* pathovars (Croxen and Finlay, 2010). Mobile genetic elements that are transferred through HGT include plasmids, transposons, and integrons (Fig. 1). Additionally, HGT allows for genetic exchange between *E. coli* and bacteriophages (transduction). Among other virulence factors, some bacteriophages carry toxin genes (e.g., Shiga toxins) and may be responsible for transferring genes among bacterial species such as the transfer of genes coding for Shiga toxins between Shigella spp. and *E. coli* (Fig. 1; Fogg et al., 2011).

Classification of Pathogenic *E. coli*

There are six defined groups of intestinal *E. coli* pathogens: Enteropathogenic (**EPEC**), enterohaemorrhagic (**EHEC**), enterotoxigenic (**ETEC**), enteroaggregative (**EAEC**), enteroinvasive (**EIEC**), and diffusely adherent (**DAEC**) (Kaper et al., 2004). This review will focus on STEC, a group that includes pathogenic and non-pathogenic *E. coli*.

Certain strains of *E. coli* were known for their adverse effects on Vero cells (kidney cell line from monkeys) due to the production of a harmful cytotoxin, later known as verocytotoxin or verotoxin. Protein analysis of the verotoxin showed homology to a toxin produced by Shigella spp., thereby giving rise to the term "Shiga-like toxin" or Shiga toxin (**Stx**). The strains are referred to interchangeably as Verotoxin-producing *E. coli* (**VTEC**) or Shiga toxin-producing *E. coli* (STEC). The term enterohemorrhagic *E. coli* (EHEC) refers to STEC strains with the same clinical, epidemiological, and virulence features as the pathogenic serotype O157:H7. Consequently, all EHEC are pathogenic, while some STEC are not (Mathusa et al., 2010). Furthermore, some STEC strains have only been found in animals and not in humans (Johnson et al., 2006).

The first diagnosed outbreak of EHEC O157:H7 occurred in Oregon and Michigan, USA in 1982, when the pathogen was isolated from individuals with bloody diarrhea and abdominal cramps (Riley et al., 1983). The source of infection was linked to contaminated ground beef from a restaurant chain (Pennington, 2010). Only eight O157 isolates in culture collections date back farther than this outbreak; one from the USA, one from the UK, and six from Canada (Pennington, 2010). Therefore, *E. coli* O157 is considered to be a recently emerged pathogen harboring genetic elements that had not been previously documented.

An evolutionary model for O157 has been developed from genome analyses, consisting of a stepwise evolution from a non-toxigenic sorbitol-fermenting precursor related to *E. coli* O55:H7 (Fig. 2; Wick et al., 2005). The ancestor had the locus of enterocyte effacement (**LEE**) genes, responsible for intimate attachment of the bacteria to the intestinal epithelium. The evolutionary steps leading to the pathogen consisted of acquisition of the Shiga toxin 2 gene (stx2) by process of transduction, switching of the somatic antigen from O55 to O157, and the acquisition of a large virulence plasmid (pO157). Additionally, there was a loss of the ability to metabolize sorbitol and the gene encoding for β-glucuronidase as well as acquisition of the Shiga toxin 1 gene (stx1) from bacteriophage.

Epidemiology

Worldwide, human infections with STEC have been estimated to cause 2.8 million illnesses, nearly 4,000 cases of hemolytic uremic syndrome (**HUS**), 270 cases of permanent renal disease, and 230 deaths annually (Majowicz et al., 2014). However, the amount of undiagnosed and unreported infections is likely many times higher. In the USA alone, STEC cause an estimated 265,000 illnesses, 3,600 hospitalizations, and 30 deaths annually (Scallan et al., 2011). Classified as a notifiable disease in Canada since 1990, infections due to STEC must be reported to the Public Health Agency of Canada. In the U.S., infections due to O157:H7 were classified as nationally reportable by the Centers for Disease Control and Prevention in 1995, and a number of non-O157 serogroups (including O26, O45, O103, O111, O121, and O145) were added to this list in 2000 (Brooks et al., 2005).

Several misconceptions arose following the initial O157 outbreaks, including the idea that O157 was the only serogroup responsible for large STEC outbreaks (Tarr et al., 2005). Since the majority of laboratories have screened exclusively for O157 in the past, non-O157 outbreaks have very likely been attributed to O157 due to the presence of multiple serotypes within clinical and food specimens (Bettelheim, 2007).

Reservoirs and Transmission Vehicles

Ruminants are asymptomatic carriers and are considered a main reservoir of O157 and non-O157 STEC. These bacteria do not cause disease in the host but colonize the intestinal tract and are shed in feces (Mathusa et al., 2010). Cattle feces are a principal source of contamination

for food within the farm-to-fork production chain and can lead to contamination of beef products (Fig. 3). In the USA, 41% of O157 outbreaks originate from contaminated beef trimmings and/or ground beef (Pennington, 2010). Although information regarding non-O157 cases and outbreaks related to contaminated meat are scarce, a few have confirmed red meat as the source. The first definitive non-O157 outbreak associated with beef occurred in the USA in 2010 when three people became ill after purchasing ground beef from a local grocery store (USDA, 2012). Worldwide, there have been eight confirmed non-O157 outbreaks related to consumption of ruminant meat, six of which involved beef products. Together, these outbreaks were responsible for more than 200 confirmed cases of illness, 45 cases of HUS, and three deaths (USDA, 2012).

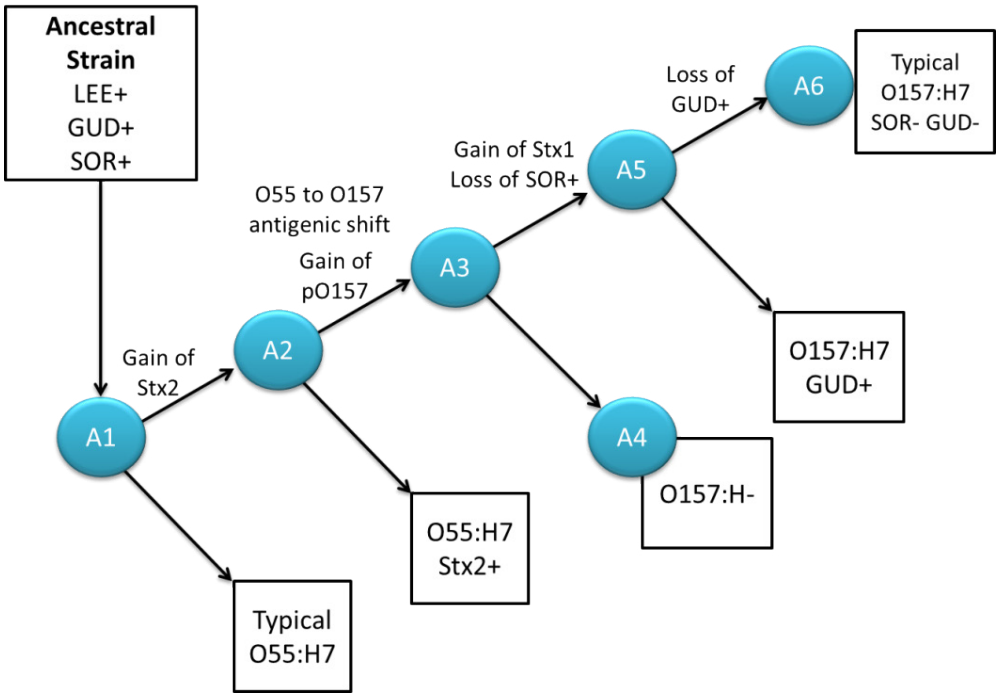

Figure 2. Evolutionary genomic changes in the emergence of *E. coli* O157:H7 (A = ancestor) (adapted from Wick et al., 2005). Positive (+) or Negative (-) SOR = sorbitol metabolism, GUD = β-glucuronidase, Stx = Shiga toxin gene, and LEE = locus of enterocyte effacement genes.

Contamination of vegetables has been increasingly associated with STEC outbreaks. Land application of manure and use of contaminated irrigation water may spread these bacteria to produce crops. Proper cooking and pasteurization procedures help to kill potential pathogens before consumption of food; however, fresh produce is often consumed raw. In 1999, a contaminated salad bar infected 56 people at a camp in Texas with STEC O111:H8 (Mathusa et al., 2010). Another outbreak was associated with *E. coli* O121:H19 on lettuce in 2006, when 73 people became ill after eating at a fast food chain (Mathusa et al., 2010). Sprouts have also been implicated in several STEC outbreaks including the high-profile O104:H4 outbreak in Germany (Beutin and Martin, 2012).

In 90 outbreaks from 1982 to 2006, food products (54%) were the most common vehicle of STEC transmission, followed by animal contact (e.g., farms, petting zoos, 8%), water (7%), and the environment (2%), with the source of infection being unidentified in 29% of outbreaks (Snedeker et al., 2009). The largest recorded outbreak due to direct animal contact occurred in England in 2009, leading to 93 infections including 17 cases of HUS (Pennington, 2010).

In livestock production systems, land application of manure and manure catchment basins can be sources of STEC, which then can enter surface and/or groundwater during heavy rainfall and flooding events. Furthermore, if allowed access, livestock may directly defecate into streams and rivers. Safety of recreational water can be subsequently compromised since STEC pathogens can survive in water for extended periods and the current can transport pathogens over long distances, increasing their potential to come in direct contact with humans (McAllister and Topp, 2012). Heavy rain has been repeatedly associated with waterborne O157 outbreaks, including the Walkerton, Canada O157 outbreak in 2000 (Pennington, 2010). This outbreak was a result of manure directly contaminating the town's water supply by flowing into an unsealed well, followed by inadequate chlorination during water treatment (McAllister and Topp, 2012).

Secondary transmission from infected persons within families, daycare facilities, and health care institutions are also recognized as sources of infection. Some of the larger reported outbreaks include 26 cases (with 10 developing HUS) of O111 infection in a school in France in 1992 and 13 cases of O111 infection in a nursing home in Australia in 2003 (Kaspar et al., 2010). There have also been numerous non-O157 outbreaks reported in nursery schools in Japan from 2005 to 2009, with more than 470 cases being linked to person-to-person transmission (Kaspar et al., 2010).

Prevalence of STEC in Cattle

Cattle carriage is dynamic, and the prevalence of STEC highly variable, with periods of high prevalence followed by long periods of apparent absence (Pennington, 2010). A survey of data collected from cattle fecal samples at slaughter found prevalence of O157:H7 and non-O157 STEC at rates ranging from 0.2 to 28% and 2 to 70%, respectively (Kaspar et al., 2010).

Nearly 200 STEC serotypes have been detected in dairy cattle and more than 260 from beef cattle (Kaspar et al., 2010). One or more of the top seven STEC serogroups were found on 44% of fecal swabs collected from individual cattle from 21 feedlots in the USA (Dargatz et al., 2013). A study of commercial feedlot cattle in the USA found all of the top seven serogroups in fecal samples, with O157 (50%), O26 (20%), and O103 (12%) being the most prevalent (Cernicchiaro et al., 2013). A study of pooled cattle fecal swabs collected from 21 feedlots found the prevalence of serogroups O157, O45, O26, O103, O121, O145, and O111 to be in the range of 0.5–20% (Dargatz et al., 2013). In a recent study (Stanford and Reuter, unpublished data), pooled fecal samples were collected from approximately 70,000 feedlot cattle over a 2-yr period at delivery to slaughter in Alberta, Canada. In this study, O103, O157, O26, and O45 were more prevalent (78.2–99.3%) than O121 (62.1%), with O111 (8.2%) and O145 (6.2%) at equal or lower prevalence than other STEC.

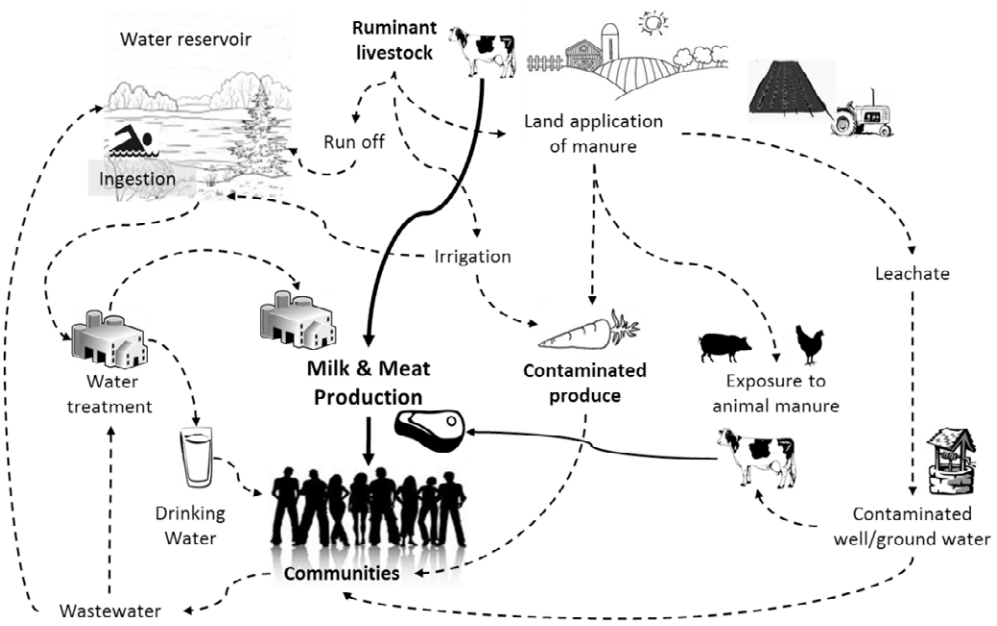

Figure 3. Flow of microbial contaminants from livestock operations to food, water, and the environment (adapted from McAllister and Topp, 2012).

Non-O157 Serotypes

Although reported before the 1982 O157:H7 outbreak, the level of awareness of non-O157 STEC was and remains low as screening for these serogroups is rare. A surveillance program was initiated by the USDA in 2011 (Bettelheim, 2007), with these pathogens being estimated to cause more than 60% of the 265,000 STEC infections each year in the United States (Lindsey et al., 2014). The majority of non-O157 STEC infections are likely undetected, making reliable estimations of the frequency of their involvement in STEC infections difficult (Bettelheim, 2007). To date, 380 STEC serotypes have been associated with human disease (Karmali et al., 2010). Of the non-O157 serogroups, six (O26, O45, O103, O111, O121, and O145) account for as much as 70% of non-O157 STEC infections (Brooks et al., 2005).

Virulence Features of STEC

The major virulence features of STEC/EHEC are the phage-encoded Shiga toxins (Stx). Among Stx, two types have been identified (Stx1 and Stx2), each of which have several subtypes (Bettelheim, 2007). Stx2 is more prevalent than Stx1 in cases of hemorrhagic colitis (**HC**) and HUS. Enterohaemorrhagic *E. coli* (EHEC) do not possess a secretory mechanism for Stx. Rather, the release of Stx has been linked to phage-mediated cell lysis, discharging the toxins into the intestinal tract of the host. Within the human host, Stx molecules attach to membrane-bound molecular receptors located on the surface of intestinal and kidney cells (Croxen and Finlay, 2010). Cattle and other ruminants lack these receptors in the intestinal tract, which may contribute to their asymptomatic STEC carriage.

Following infection with STEC/EHEC, human intestinal microvilli are effaced, also known as attaching and effacing (**A/E**) lesions. This phenotype is a result of the mobile genetic element termed LEE. The intimin encoding gene (eae) is found on the LEE, and its product (intimin) is secreted into the bacterial outer membrane. The transmembrane intimin receptor (Tir) is a LEE-encoded effector molecule translocated via the type-III secretion system, anchored to the host cell plasma membrane which binds with the intimin on the bacterial cell surface to establish a secure attachment (Croxen and Finlay, 2010).

Detection and Differentiation of STEC

Since the emergence of serotype O157:H7 and the severe disease outbreaks that followed, most research and regulatory framework has focused on this serotype (Wang et al., 2013). Methods for culture-based detection and isolation of O157 are well established, including developed media and sensitive antibody-based technologies (Durso, 2013). The unique biochemical characteristics of O157:H7 allowed for rapid development of selective and differential media; traits that are absent from non-O157 serogroups. This makes the development of similar selective media for non-O157 serogroups more problematic. Food safety regulatory agencies such as the U.S. Food and Drug Administration (FDA) have developed and validated detection methods for STEC, which are available online as Laboratory Guidebooks. Meanwhile, government and academic research is focused on improving methods for detection of STEC and other foodborne pathogens.

Enrichment Methods

Culture-based methods are commonly mandated for pathogen detection and confirmation, which primarily includes enrichment and plating on selective media. Enrichment is often used to overcome the challenges associated with pathogen isolation and detection in complex matrices. These include resuscitation of stressed or injured cells, inhibiting growth of background microflora, and diluting assay inhibitors. Since very few cells (~10 colony forming units) are sufficient to cause disease in humans (van Elsas et al., 2011) and STEC are usually present at low concentrations, enrichment is necessary to increase cell numbers to levels that can be detected and isolated in labs.

Although many have been evaluated, no enrichment media has been proven superior for all sample types (Durso, 2013). The most common enrichment media are trypticase soy broth (**TSB**), buffered peptone water (**BPW**), and *E. coli* broth (**ECB**). Frequently, these media are supplemented with antibiotics to suppress growth of background microflora. Although TSB and BPW are often reported in enrichment protocols, including the FDA procedure, there are no obvious elements in their formulation to make these broths specific for the enrichment of *E. coli* (Wang et al., 2013). In contrast, ECB contains bile salts to inhibit growth of non-Enterobacteriaceae strains and lactose, which is easily fermented by STEC.

Following enrichment, DNA is extracted and molecular detection methods can be used to identify target serogroups. Screening for STEC serogroups and/or toxin genes is typically done using polymerase chain reaction (**PCR**). Initial serogroup screening by PCR reduces the number of samples that require further processing, while also being highly sensitive. However,

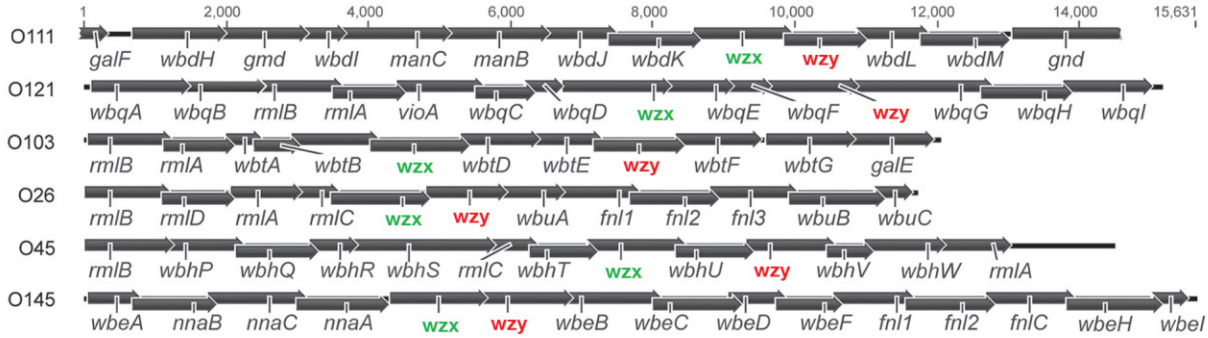

Figure 4. O-antigen gene clusters from *E. coli* serogroups O26, O45, O103, O111, O121, and O145. As an example of mobility and/or diversity of genetic elements, the location of two genes (wzy–green and wzy–red) is color coded. Base pair length is located above the gene clusters (adapted from Norman et al., 2012).

PCR does not discriminate between DNA from living vs. dead cells, nor does it determine if toxin genes and serogroup genes came from the same cell. Therefore, living cells must be obtained for further diagnostics.

Isolation of *E. coli* Serogroups

Immunomagnetic separation (**IMS**) is a technique that uses antibody-coated magnetic beads specific for the O-antigens of STEC to isolate cells of target serogroups. Most often, IMS is completed in enrichment broths. The antibodies on the beads bind their targeted antigens on the bacterial cells. Then, the IMS beads are incubated in the enrichment suspension and then removed using magnets. The bead/bacteria complexes are then rinsed and suspended in buffer before plating on agar media to obtain colonies. Using this method, target bacteria are separated from non-target cells, thereby concentrated, while at the same time, eliminating many of the background microorganisms. Consequently, IMS improves target cell recovery compared with direct plating methods. The sensitivity of primary culture methods to isolate O157 from complex matrices was significantly enhanced with the advent of O157:H7 serotype-specific IMS (Durso, 2013). Commercial IMS kits are available for O157 and the top six non-O157 serogroups, however, the sensitivity and binding capacity of IMS beads varies among serogroups. For example, commercial O111 beads rarely yield O111 cells and are often more specific to non-target serotypes like O103 (Bai et al., 2012; Conrad et al., 2014).

Selective enrichment and IMS often fail to completely eliminate background microflora. Additionally, the lack of a standard selective and differential media for non-O157 STEC makes isolation of target colonies challenging. MacConkey agar containing sorbitol (**SMAC**) rather than lactose as a carbon source is used for isolating *E. coli* O157:H7. Most strains of serotype O157:H7 cannot ferment sorbitol, so when plated on SMAC, colonies of O157:H7 appear colorless and 2–3 mm in diameter. Unlike O157:H7, most *E. coli* appear as pink colonies while growth of Gram-positive microorganisms is inhibited by the crystal violet and bile salts present in the media. Therefore, SMAC medium cannot be used to differentiate sorbitol-fermenting *E. coli*, as is the case for most non-O157 STEC, from *E. coli* O157:H7 (Karmali et al., 2010).

Several different culture media have been described to detect non-O157 STEC, for either individual or multiple serogroups. However, each has their limitations, including only being able to differentiate a few of the top six non-O157 serogroups. Additionally, colony color, size, and texture can differ as a function of incubation time, degree of colony crowding, or the matrix from which the cells were isolated (Mathusa et al., 2010). In some cases, the non-O157 serogroups can be discriminated from background microflora, but not from each other, requiring further serogroup confirmation (Kalchayanand et al., 2013).

Confirmation of Serogroups

As outlined above, the selectivity of enrichment assays, IMS, and plating procedures for the non-O157 STEC is limited. Therefore, further testing is required to confirm colonies for the type of serogroup and/or virulence. Complete antibody-based O:H serotyping are often limited to national *E. coli* reference laboratories. Full serotyping of *E. coli* isolates is laborious and expensive.

With complete sequence data for O-antigen gene clusters continually becoming available, PCR assay development and sensitivity is evolving at a fast pace. The genes most often targeted for O-serogroup identification are wzx and wzy (Fig. 4), which encode for the O-antigen flippase and O-antigen polymerase, respectively, although other genes have been used. Virulence of STEC isolates is usually based on the presence of stx1, stx2, and eae genes.

Limitations of applying PCR to environmental samples include assay inhibitors and an inability to differentiate living from dead cells. However, PCR can be applied to single colonies, and internal amplification controls can be used to address these concerns. However, the detection of virulence genes is not confirmation of the expression of pathogenic genes.

Subtyping Methods and Sequencing Applications

Subtyping methods are vital to outbreak investigations to determine genetic relationships among strains and trace their sources. Pulsed-field gel electrophoresis (**PFGE**) is considered a powerful discriminatory subtyping method (Mathusa et al., 2010). This method requires DNA to be isolated from a pure culture and digested with restriction enzymes with the resulting fragments separated by gel electrophoresis. The relationship of isolates to one another is determined by their banding pattern, and PFGE data on various *E. coli* isolates are available in online databases like PulseNet, allowing laboratories to compare newly isolated strains to those in the database (Mathusa et al., 2010).

Nucleic acid sequencing is an indispensable tool in biological research. Next-generation sequencing (**NGS**) has become more readily available with the advent of benchtop sequencers and steadily decreasing costs over the years. Whole-genome sequencing (**WGS**) technologies allow reading the entire DNA sequence of an organism and therefore provide a much

greater resolution comparing closely related genotypes and higher phylogenetic accuracy than traditional typing methods. The timeframe required to obtain data is currently a major WGS limitation, but as technologies are advancing, PFGE might be replaced as the subtyping gold standard in the future. With WGS, it would not only be possible to compare DNA sequences between strains, but it could also be used to identify serotypes and virulence genes encoded by strains, eliminating the need for other lab methods currently used for these analyses.

Traditionally, genome sequencing has been restricted to isolates from pure cultures. However, microbial communities can now be directly sequenced from environmental samples (metagenomics). Metagenomics is bypassing culture-dependent limitations while simultaneously enabling discovery of novel pathogens. However, metagenomic procedures generate a vast amount of sequence data, which is a challenge for most computing systems to store and manage. Thus, analysis of DNA sequences often represents the limitation for data interpretation. Overcoming those limitations, metagenomics might become a common approach in the future during outbreak investigations.

Summary

Human illnesses can be caused by STEC, resulting in life-threatening complications. A number of these illnesses can be linked to cattle and their edible products, which has led authorities to implement screening regulations for meat and milk products. Consequently, fast and reliable detection protocols for these pathogens are required to maintain food safety and decrease circulation of contaminated food products. Typically, recovery of viable colonies of target STEC is a requirement for food safety screening and outbreak investigations. However, no standard media exist for this diverse group of pathogens. Enrichment is an imperative screening component since these pathogens are often present at concentrations below the level of detection. A robust and credible enrichment medium for detection of multiple STEC serogroups, and isolation from different matrices (food, feces, etc.), would be valuable.

Although the aforementioned developments to STEC detection and isolation would significantly improve current methods, another restriction still remains in that the detection of individual serogroups does not indicate pathogenicity. Even with enhanced methods for detection of serogroups, there is no differentiation between pathogenic and non-pathogenic strains of the same O-type. Additionally, this diverse group of pathogens is always evolving, exchanging genetic material, and resulting in novel STEC with O-serogroup antigens not commonly associated with human infections. Therefore, solely screening for a fixed number of common O-types could result in emerging pathogens being undetected. The most significant contribution to current detection methods would be the development of a rapid, sensitive, and high-throughput approach that differentiates pathogens from non-pathogens of the same serogroup. Such novel technology would allow rapid detection of emerging pathogens regardless of serogroup. Enhanced detection of STEC can continue to maintain food safety by decreasing circulation of products compromising human health, aid in diagnostics, and support outbreak management.

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About the Authors

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Dr. Kim Stanford is a senior research scientist with Alberta Agriculture and Forestry, Lethbridge. She has been studying on-farm control of *E. coli* O157:H7 since 2002 with a focus on feedlot cattle and the occasional foray into dairies and post-harvest situations. Past studies have included prevalence of top-six non-O157 *E. coli* in western Canadian slaughter cattle, characterization of non-impacts of long- and short-haul transport on shedding of *E. coli* O157:H7 at slaughter, transmission of *E. coli* O157:H7 from super-shedders to pen mates in commercial feedlot pens, evaluations of products available for on-farm control of *E. coli* O157:H7 including direct-fed microbials (Lallemande, Nutrition Physiology) and a vaccine (Bioniche), along with characterization of bacteriophage for use in biocontrol of *E. coli* O157:H7.

Tim McAllister received an M.Sc. in animal biochemistry from the University of Alberta in 1987 and a Ph.D. in microbiology and nutrition from the University of Guelph in 1991. He has worked as a research scientist with Agriculture and Agri-Food Canada since 1996 where he now holds the position of principal research scientist in microbiology and beef cattle production. He has been studying the ecology of *E. coli* O157:H7 within beef production systems for almost 20 years with an emphasis on pre-harvest control measures. His most recent interest has been in documenting the role of "Super Shedders" in the transmission of this pathogen. His team has been the recipient of numerous societal awards for their contribution to beef cattle production in North America. McAllister is an avid biker and dreams up many of the experimental designs his team employs while riding his bike to work.

James Thomas received a Ph.D. in cell and developmental biology from McMaster University in 1980 and accepted a position at the University of Lethbridge in 1988. He was appointed full Professor in Cell and Molecular Biology in 2012. Dr. Thomas has worked as a microbiologist, looking at cause-and-effect associations in the occurrence of zoonotic, waterborne pathogens, in particular in relation to agriculture, ecology, and urban/industrial activities. His research has focused on genomic and metabolic characterization of clinical and environmental isolates of various bacterial pathogens including, *E. coli* O157:H7. Other research has involved breeding of new crops for use in arid and semi-arid environments.

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