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Review Article

Isolation, genotyping and antimicrobial resistance of Shiga toxin-producing *Escherichia coli*

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Abstract Shiga toxin-producing *Escherichia coli* (STEC) is an enteric pathogen linked to outbreaks of human gastroenteritis with diverse clinical spectra. In this review, we have examined the currently methodologies and molecular characterization techniques for assessing the phenotypic, genotypic and functional characteristics of STEC O157 and non-O157. In particular, traditional culture and isolation methods, including selective enrichment and differential plating, have enabled the effective recovery of STEC. Following recovery, immunological serotyping of somatic surface antigens (O-antigens) and flagellum (H-antigens) are employed for the classification of the STEC isolates. Molecular genotyping methods, including multiple-locus variable-number tandem repeat analysis, arrays, and whole genome sequencing, can discriminate the isolate virulence profile beyond the serotype level. Virulence profiling is focused on the identification of chromosomal and plasmid genes coding for adhesins, cytotoxins, effectors, and hemolysins to better assess the pathogenic potential of the recovered STEC isolates. Important animal reservoirs are cattle and other small domestic ruminants. STEC can also be recovered from other carriers, such as mammals, birds, fish, amphibians, shellfish and insects. Finally, antimicrobial resistance in STEC is a matter of growing concern, supporting the need to monitor the use of these agents by private, public and agricultural sectors. Certain antimicrobials can induce Shiga toxin production and thus promote the onset of severe disease

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symptoms in humans. Together, this information will provide a better understanding of risks associated with STEC and will aid in the development of efficient and targeted intervention strategies.

Introduction

Shiga toxin-producing *Escherichia coli* (STEC) is an enteric pathogen that have been linked to outbreaks from foodborne and waterborne sources. STEC causes human gastrointestinal illnesses with diverse clinical spectra, ranging from watery and bloody diarrhea to hemorrhagic colitis.[1–4] In some rare cases, infection can result in the life-threatening, hemolytic uremic syndrome (HUS), and it is thought that Shiga toxins (Stx) are the key virulence factors contributing to the development of HUS.[1,3,4] Although more than 400 different serotypes of STEC have been isolated, O157:H7 is the serotype that has been most studied since it has been commonly associated with the development of severe human illness.[5] Recent epidemiological studies have revealed other STEC non-O157 serotypes, O26:H2, O45:H2, O103:H11, O111:H8, O121:H19, and O145:H28, to be highly associated with human disease.[2]

Due to the clinical importance of STEC in recent years, a number of methods have been developed to determine the diversity, virulence, and phylogenetic relationships of STEC isolates. These methods have enabled the monitoring of STEC outbreaks and traceback investigations of contamination sources.[6,7] The objective of this review article is to examine current knowledge of techniques for the phenotypic and genotypic characterization of STEC O157 and non-O157. This information will provide a better understanding of risks associated with STEC and will aid in the development of efficient and targeted intervention strategies.

Routes of transmission and mechanisms of pathogenicity in humans

STEC infections are usually acquired by ingestion of contaminated food, water, or by contact from person to person (Fig. 1).[1] A large portion of STEC infections have been attributed to the consumption of undercooked contaminated food, usually meat and dairy products.[8,9] In particular, ground meat is considered a common transmission vehicle of STEC due to the ease of cross-contamination during preparation. Also, the uneven dispersion of STEC throughout the substrate results in an inefficient killing of this pathogen in ground beef after heat exposure during cooking.[8] Certain "super-shedding" animals, which are considered main STEC reservoirs, can excrete high concentration levels of STEC in feces and are also an important source of human infections and environmental contamination (Fig. 1).[1,8,10] Consequently, the dispersed pathogen can then attach to a variety of fruits and vegetables depending on the species and specific conditions.[8] Infection can also occur from swimming, drinking or bathing with contaminated water or occupying grazing areas presumably strewn with manure. Human infections have been attributed to direct contact with dogs, sheep, horses and goats at petting zoos, open farms and animal shows (Fig. 1).[8,10] Person-to-person or secondary transmission is important in propagation of outbreaks and can account for 15–20% of the cases.

The mechanism of pathogenicity is mainly attributed to the production of Stx. Infection begins once Stx bind to the cell-surface receptor on the endothelial cells. Thereafter, the catalytic A-subunit is translocated into the cell cytosol, resulting in the inhibition of protein synthesis after inactivation of 60S ribosomal subunit of the eukaryotic cell.[4,11] STEC infections require a low infectious dose (<50 bacterial cells),[1,4,12] and the incubation period, prior to the onset of diarrhea, ranges between 2 and 12 days.[11] Typical initial symptoms include abdominal pain, diarrhea, fever and vomiting, followed by bloody diarrhea in about 90% of the cases.[11] Bacteremia is almost never found in conjunction with an enteric STEC infection. Instead, systemic complications associated with HUS arise from lesions caused by circulating Stx as soluble free Stx or by binding to blood components such as leukocytes, monocytes or red blood cells.[4,11] The rate at which severe disease symptoms result in HUS varies widely (0–15%), and death due to HUS occurs in approximately 5% of the patient.

Animal reservoirs

Studies of zoonotic STEC have shown that cattle is considered the main reservoir for STEC strains,[1,8–10] and over 430 STEC serotypes have been detected in isolates recovered from cattle.[9] Other domestic small ruminants, such as sheep and goats are also important carriers of STEC especially outside of the United States.[10,13,14] In particular, sheep and their products have been documented as reservoirs of a diverse set of non-O157 serotypes STEC (O26, O91, O115, O128, and O130), encoding key virulence factors that have been implicated in human disease and are important reservoirs in Australia and Norway.[10,13,14] Water buffalo is an important reservoir of STEC O157 in countries in Asia, South America and Europe. In Bangladesh, STEC was isolated from 38% of buffaloes sampled before slaughter, and in Vietnam 28% of the animals surveyed were STEC positive although they were not O157.[10]

STEC has also been identified in a wide variety of other carriers, including mammals, birds, amphibians, fish, shellfish and insects.[1,8,10,14,15] There is some evidence that non-ruminants may be categorized as spillover hosts; these hosts do not maintain STEC levels without continual

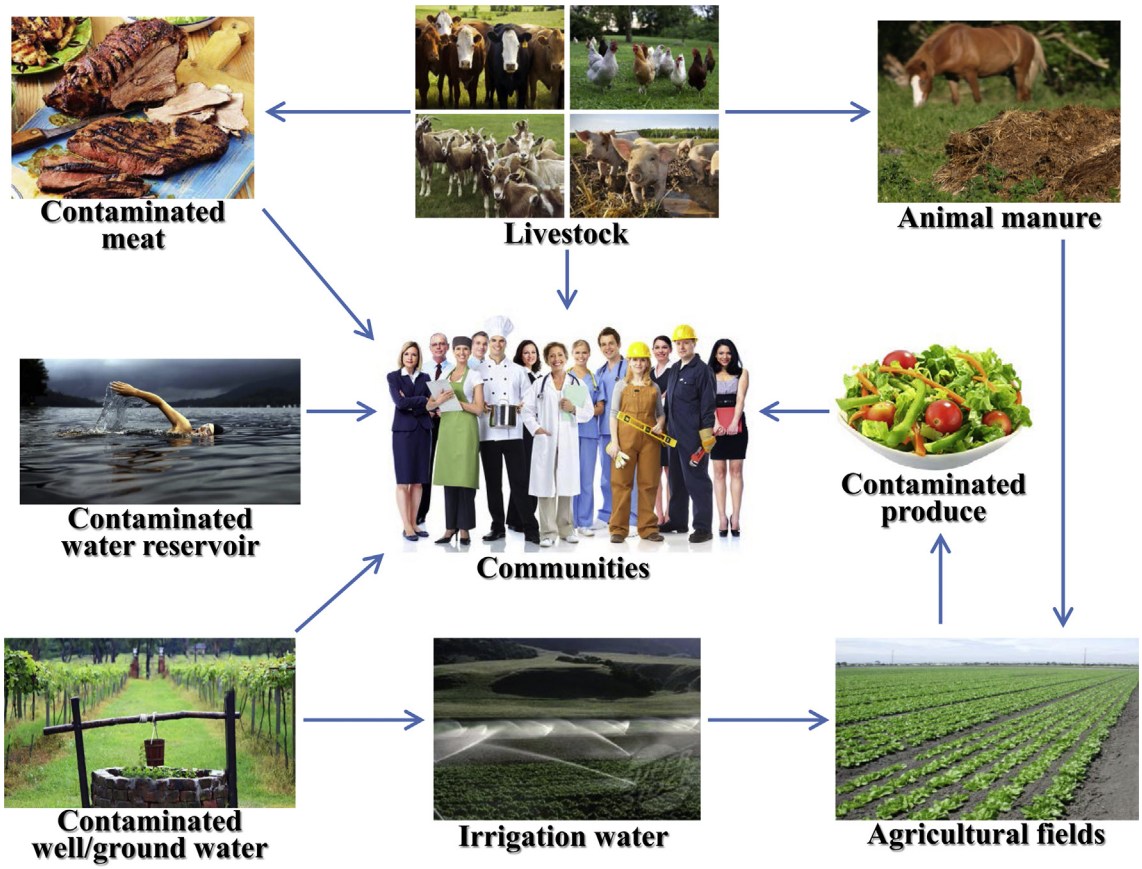

Figure 1. Transmission routes of STEC infections. STEC infections are usually acquired by ingestion of contaminated food or water. Certain animal reservoirs can excrete STEC in high concentrations which may subsequently contribute to the contamination of produce in agricultural fields, when animal manure is used as fertilizer. Contaminated water used for swimming, drinking, bathing or irrigation of agricultural fields are also relevant sources of STEC infection. Human STEC infections have also been attributed to direct contact with domestic farm animals at petting zoos, open farms and animal shows.

exposure to STEC. However, spillover hosts may still spread STEC over a wide area especially in the case of migratory birds traveling a long distance in a single day.[10] Some shellfish and other aquatic species may act as dead-end hosts for STEC since they only transmit STEC when they are consumed.[10] Most animal hosts are asymptomatic because they lack vascular receptors for Stx with pigs being a notable exception.

Isolation and culture

Culturing and isolation methods are generally regarded as the standard procedure for pathogen detection. These traditional recovery methods consist of several steps, enrichment, followed by selective and differential plating for isolation, and then by serological or molecular tests for confirmation (Fig. 2).[16,17] Enrichment facilitates the resuscitation of bacteria exposed to stress or growth inhibitors in the tested matrix and enables the recovery of isolates when present at low concentration in the tested sample.[7,12,16,17] Common growth media used for the enrichment of STEC O157:H7 and non-O157 include tryptic soy broth, *E. coli* broth and buffered peptone water (Fig. 2). Typical incubation conditions are 35–37 °C for a period of 16–24 h. To suppress a potential antagonistic activity of competing microflora, the sample can be incubated at a higher temperature of 42 °C; however, the use of this temperature may also interfere with the recovery of damaged STEC cells.[15,18] Selective enrichment broths are often supplemented with agents such as bile salts, potassium tellurite and novobiocin, but it has been reported that the addition of novobiocin can inhibit the growth of some STEC isolates.[7,12] Moreover, it has been demonstrated that avoiding the use of antibiotics in enrichment may help to increase the number of STEC recovered from a complex sample.[13,16,18,19]

Unlike commensal *E. coli*, typical *E. coli* O157:H7 do not ferment sorbitol and lack the ability to produce β-D-glucuronidase. Sorbitol-containing MacConkey agar (Difco Labs, Detroit, MI, USA), supplemented with cefixime and tellurite, has been effective in the isolation of STEC O157.[20] The use of chromogenic agars such as Rainbow® O157 Agar (Biolog, Hayward, CA, USA) or CHROMagar™ (CHROMagar, Paris, France) are suitable for screening major STEC O-antigen serogroups associated with human illness (Fig. 2).[7,15,21] Following enrichment and prior to plating on selective media, immunomagnetic separation (IMS), using antibodies for the specific recognition of *E. coli* O-antigen serogroups, is employed as a concentration step (Fig. 2).

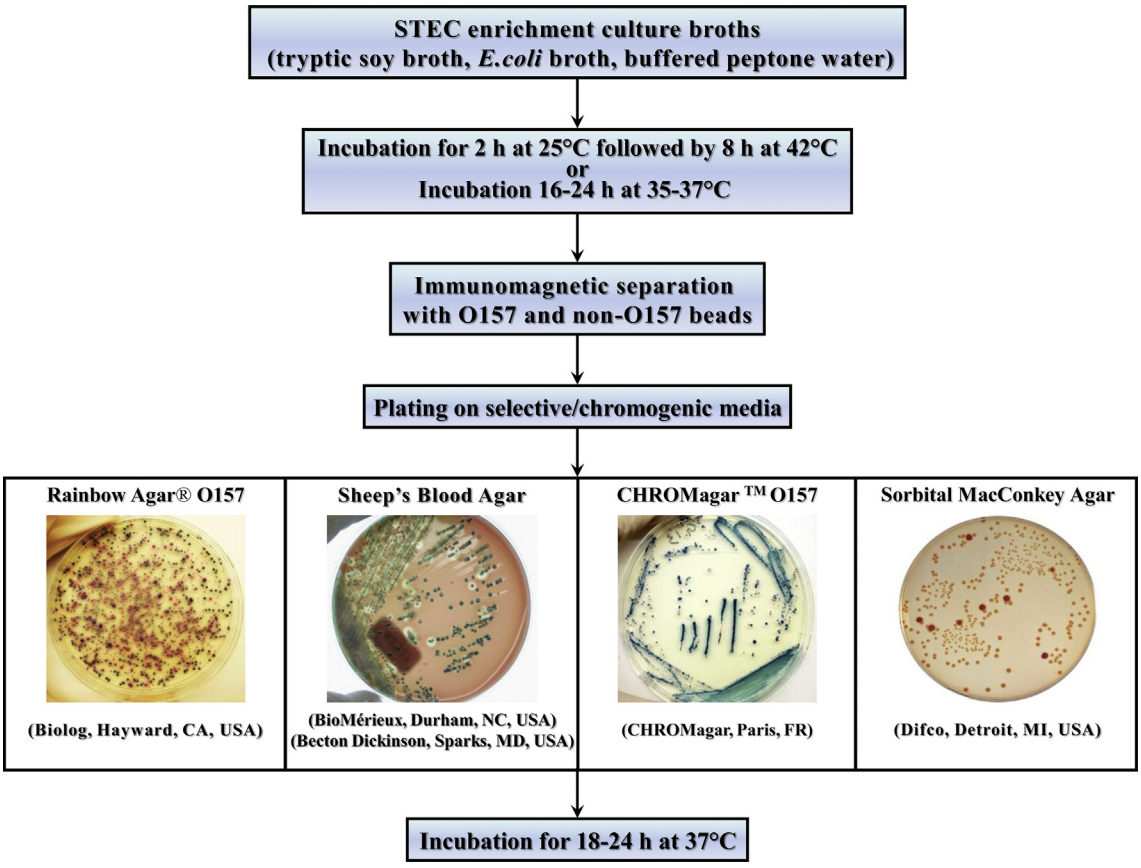

Figure 2. Culture and isolation methods for efficient STEC recovery. Recovery methods for STEC consist of several steps such as enrichment, separation, followed by the selective and differential plating for colony isolation. Common growth liquid media are used for the enrichment broth step, enabling the resuscitation of stressed or injured cells. A key step in STEC recovery involves the immunomagnetic separation prior to plating on various selective chromogenic media, resulting in the isolation of STEC colonies with a distinctive color morphology.

IMS has been demonstrated to provide a greater sensitivity and reliability in the recovery of *E. coli* O157:H7 and non-O157 from animal fecal samples.[13,15,18,22]

STEC classification schemes

One classification scheme that has been traditionally used for categorizing STEC is immunological serotyping (Table 1).[23,24] STEC serotyping is based on combinations of 174 somatic surface antigens (O-antigens) and 53 flagellum (H-antigens).[23,24] Over 400 different serotypes of STEC have been identified and several serotypes have been further classified into seropathotypes, based on reported frequency and severity of illness.[5] These designations range from seropathotype A for relatively high incidence and association with severe disease to seropathotype E for no illness in humans.[5] Serotypes belonging to seropathotype A are the most virulent and include O157:H7 and O157:NM (NM; not mobile). Seropathotype B comprises serotypes O126:H11, O103:H2, O111:NM, O121:H19, and O145:NM, which have been associated with severe disease symptoms (HUS) but less frequently than serotype O157. Seropathotype C is composed of serotypes O91:H21 and O113:H21, which are both associated with outbreaks but rarely implicated in causing HUS. Seropathotype D represents serotypes that have been implicated in sporadic cases of diarrhea; and seropathotype E contains all STEC serotypes that have not been linked to human diseases.[5]

Molecular methods have recently emerged for discriminating or fingerprinting beyond the level of the STEC serotype and also for determining the STEC isolate relatedness and attributing sources of contamination (Table 1). Each method has different advantages and limitations, and the selection of the best method to use will be dependent upon the level of the typing resolution desired by the end user. One of these STEC classification methods consists of subtyping by pulsed-field gel electrophoresis (PFGE), which has a high power of discrimination as well as reproducibility and ease of standardization.[25] PFGE uses restriction enzymes to generate DNA fragments in sizes spanning the entire genome.[25] The most-commonly used restriction enzyme for digestion of the STEC genome is *Xba*I, which recognizes a rare sequence in bacterial genomes with more than 45% of GC content. Using a second restriction enzyme leads to a better discrimination of identical PFGE patterns, when bacterial isolates are suspected to be epidemiologically linked and when investigations of large-scale outbreaks are needed.[26] Several studies suggest that combining PFGE results with data obtained with other

Table 1 Summary of methods to characterize, fingerprint and genotype STEC.

Method		Description of technique	Purpose	Advantage	Disadvantage
Immunological-based method	Serotyping	Uses specific antisera to identify O- and H-antigens.	Serotype characterization	Standard method used by laboratories for species classifications.	Antisera cross-reactivity of some can lead to false positive results; time consuming; immunological reagents can be limited in amounts.
Nucleic acid-based methods	Polymerase chain reaction (PCR)	Amplifies target using sequence-specific primers.	Detection/ virulence characterization	Simple, rapid and cost effective typing method.	Needs extra time to for results analysis on agarose gel.
	Real-time PCR	Uses fluorophore-labeled probe to detect specific amplification of target sequence.	Detection/ virulence characterization	Faster than regular PCR; quantification of target is possible.	Requires expensive instrumentation and reagents for real-time detection relative to conventional PCR.
	Pulsed-field gel electrophoresis (PFGE)	Uses restriction enzymes to fragment the entire genome and separates large size fragments on an agarose gel with pulsed-field electrophoresis, which continually changes the direction of the electrical current.	Fingerprinting	Good resolution and considered a standard method.	May not be able to discriminate among some clonal bacterial strains.
	Multilocus VNTR analysis (MLVA)	Measures number of copies of repeats at different short regions of repeated DNA sequences, known as variable number tandem repeat (VNTR).	Fingerprinting	Rapid and high throughput; allows the discrimination of certain strains not distinguished by PFGE.	Requires expensive instrumentation and fluorophore-conjugated reagents.
	DNA microarray	Detects complementary nucleotide sequences in tested bacterial isolates by measuring hybridization to DNA probes attached in an ordered fashion to a solid support.	Virulence characterization/ genotyping	Simultaneously screen for multiple markers in a large number of samples; can detect single nucleotide polymorphisms.	High cost of specialized and non-portable fluorescent array scanners. Colorimetric detection assays use unstable reagents, leading to overexposure.
	Whole genome sequencing (WGS)	Generates multiple short sequence reads across the entire genome and then assemble them based on overlapping regions among the reads.	Virulence characterization/ genotyping	Enhanced resolution with best strain discrimination; enables the detection of unknown single nucleotide polymorphisms.	Substantial cost in time and instrumentation. Requires large computational servers. In silico data comparison has to be standardized.

Table 2 Characteristics and/or associated functions of virulence genes.

Target gene	Location	Characteristics and/or associated functions
Hemolysins		
ehxA	pO157 plasmid	Enterohemolysin; produces small turbid zones of lysed red blood cells
hlyA	Chromosome	α-hemolysin; produces large clear zones of lysed red blood cells
sheA	Chromosome	induced hemolysin; found in pathogenic and non-pathogenic *E. coli*
Adhesins		
eae	LEE region	Intimin; forms attaching and effacing lesions
saa	pO113 plasmid	STEC autoagglutinating adhesin; associated with non-O157 LEE-negative
Effectors		
ent/espL2	O-Island 122	Effector; alters cytoskeleton in human cells
espK	Prophage Sp6	Effector; unknown function
espN	Prophage Sp6	Effector; unknown function
espP	pO157 plasmid	Extracellular serine protease
katP	pO157 plasmid	EHEC catalase-peroxidase
nleA	O-Island 71	Effector; dirupts protein secretion
nleB	O-Island 122	Effector; interfers with inflammatory signaling pathways
nleE	O-Island 122	Effector; interfers with inflammatory signaling pathways
nleH1-2	O-Island 71	Effector; interfers with inflammatory signaling pathways
Cytotoxins		
stx_{1a}	Chromosome	Stx1 prototype; 1000 times less cytotoxic than Stx2a, repressed by iron
stx_{1c}	Chromosome	Stx variant linked to mild symptoms in humans; common in ovine STEC
stx_{1d}	Chromosome	Stx variant not associated with a particular food source
stx_{2a}	Chromosome	Stx2 prototype; linked to severe HUS in humans
stx_{2b}	Chromosome	Stx variant linked to *eae*-negative STEC and mild disease in humans
stx_{2c}	Chromosome	Stx variant linked to diarrhea and HUS in humans
stx_{2d}	Chromosome	Stx variant found in highly virulent strains; Stx activity increased by elastase
stx_{2e}	Chromosome	Stx variant responsible for edema in pigs; rare in human disease
stx_{2f}	Chromosome	Stx variant isolated from pigeon; rare in human disease
stx_{2g}	Chromosome	Stx variant common in bovine STEC
subA	pO113 plasmid	Subtilase cytotoxin; triggers apoptosis in human cells
O-antigens		
wzy_{O26}	Chromosome	*E. coli* O26 O-antigen polymerase
wzy_{O45}	Chromosome	*E. coli* O45 O-antigen polymerase
wzx_{O91}	Chromosome	*E. coli* O91 O-antigen flippase
wzy_{O103}	Chromosome	*E. coli* O103 O-antigen polymerase
wzy_{O104}	Chromosome	*E. coli* O104 O-antigen polymerase
wzy_{O111}	Chromosome	*E. coli* O111 O-antigen polymerase
wzy_{O113}	Chromosome	*E. coli* O113 O-antigen polymerase
wzy_{O121}	Chromosome	*E. coli* O121 O-antigen polymerase
wzy_{O128}	Chromosome	*E. coli* O128 O-antigen polymerase
wzy_{O145}	Chromosome	*E. coli* O145 O-antigen polymerase
wzy_{O157}	Chromosome	*E. coli* O157 O-antigen polymerase
H-antigens		
$fliC_{H2}$	Chromosome	*E. coli* flagellar H2 antigen
$fliC_{H7}$	Chromosome	*E. coli* flagellar H7 antigen
$fliC_{H8}$	Chromosome	*E. coli* flagellar H8 antigen
$fliC_{H11}$	Chromosome	*E. coli* flagellar H11 antigen
$fliC_{H19}$	Chromosome	*E. coli* flagellar H19 antigen
$fliC_{H21}$	Chromosome	*E. coli* flagellar H21 antigen

subtyping techniques helps to increase the discriminative power of PFGE.[13,18,27,28]

Another genotyping method that has been developed to discriminate STEC from multiple sources is multiple locus variable-number tandem repeat analysis (MLVA) (Table 1).[13,18,27,28] MLVA amplifies short regions of repeated DNA sequences, known as variable number tandem repeat (VNTR), differing in size, location and number of copies.[28] When selecting VNTR loci for MLVA, the stability of the locus is important for results interpretation. The advantage of using MLVA as a typing method is that it is rapid and high throughput and allows the discrimination of certain strains by non-typeable PFGE.[13,27,28] In recent years, MLVA has become less expensive and more accessible, and has resulted in the implementation of this typing scheme for monitoring pathogen surveillance.[7]

Table 3 Summary of antimicrobial resistance in STEC, as described in previous reports.[19,43–49]

Antimicrobial class	Antimicrobial agent	Function inhibited	Domestic animal host
Aminoglycosides	Amikacin	Bacterial protein synthesis	Cattle, Sheep
	Gentamicin		Sheep
	Kanamycin		Cattle, Sheep
β-Lactamase inhibitors	Amoxicillin – Clavulanic acid	Cell wall synthesis; some β-lactamases	Cattle
Cephems (parenteral)	Cephalothin	Cell wall synthesis	Cattle, Sheep
	Cefoperazone		Cattle
	Ceftazidime		Cattle
	Ceftriaxone		Cattle
Folate pathway inhibitors	Trimethoprim-Sulfamethoxazole	Folic acid synthesis	Cattle, Chicken, Pig, Turkey
Fosfomycins	Fosfomycin	Enzymes involved in cell wall synthesis	Cattle, Pig
Lipopeptides	Colistin	Bacterial membrane permeability	Cattle, Chicken, Pig, Turkey
Macrolides	Erythromycin	Bacterial protein synthesis	Pig
Penems	Imipenem	Cell wall synthesis	Sheep
Penicillins	Ampicillin	Cell wall synthesis	Cattle, Chicken, Sheep
Phenicols	Chloramphenicol	RNA synthesis	Cattle, Sheep
Quinolones	Ciprofloxacin	DNA synthesis	Cattle, Turkey
	Nalidixic acid		Cattle, Chicken, Pig, Turkey
Tetracyclines	Tetracycline	Bacterial protein synthesis	Pig, Sheep

Polymerase chain reaction (PCR) assays are the preferred method by most research and surveillance laboratories for routine analysis since it is cost effective and simple. In particular, real-time PCR assays can yield faster results but are limited in the number of targets to be analyzed and also require expensive instrumentation and assay reagents (Table 1). Given the limitations of available detection methods, improvements in the cost-effectiveness and reliability of procedures are still required for routine high-throughput pathogen surveillance. Other molecular-based genotyping technologies, such as DNA microarrays, can simultaneously screen multiple set of specific markers for categorizing STEC in a large number of samples (Table 1).[6,29] Array-based detection is still less expensive than new sequencing technologies and is not subject to the challenging analysis of massive amounts of data when compared to sequencing.[30] Some microarray methods have been used for identifying STEC.[6,31–33] However, some of the available array methods, using fluorescent assays, can result in reduced sensitivities.[34] Alternative methods, using novel colorimetric technology, have been developed for genotyping of STEC O157 and non-O157 from livestock and wildlife in major agricultural regions in the United States.[15,31,33]

Whole genome sequencing is being used increasingly in strain typing since this method provides an enhanced resolution when compared to other genotyping methods (Table 1).[7,35] The advantage of genome sequencing is that it allows the examination of the entire genome instead of just specific genomic regions or markers and enables the detection of unknown single nucleotide polymorphisms. Sequence reads of genomic fragments are either analyzed directly or assembled into contigs to form a draft genome. Once assembled, genome comparisons lead to a better strain discrimination. Although draft-level genome sequencing still incurs a substantial cost in relation to some other typing methods, a reduction of per-sample cost is expected for next-generation sequencing. The analysis of whole genomes would consequently result in a more efficient and effective working methodology, having the potential of replacing multiple individual tests for monitoring pathogen outbreaks and emergence of hyper-virulent strains.[7,35]

Virulence factors

Virulence factors, implicated in conferring STEC an ability to cause disease in humans, can be found both on the chromosome and plasmids (Table 2).[3] The genes coding for Stx, *stx1* and *stx2*, are considered to be the primary and defining virulence factor of STEC.[4,36,37] Stxs are AB5-type toxins, which consist of a single A-subunit with enzymatic activity and five identical B-subunits with receptor binding ability. The receptors for Stx, globotriaosylceramide (Gb3) or globotetraosylceramide (Gb4), are found on the cell surface of mammalian cells. Stxs have been divided in two major groups, Stx1 and Stx2, and each group has several subtypes. In particular, the Stx1 group is composed of three subtypes, Stx1a, Stx1c and Stx1d; while the Stx2 group is more heterogeneous and diverse and consists of seven subtypes, Stx2a, Stx2b, Stx2c, Stx2d, Stx2e, Stx2f and Stx2g.

Epidemiological studies suggest that STEC strains that are Stx2-positive may be more virulent than those expressing Stx1,[4,36,37] and cytotoxicity assays revealed that these subtypes may be associated with different level of virulence.[38–40] Studies have documented that STEC expressing Stx1a subtype can potentially cause HUS, while those harboring Stx1c have been associated with mild disease or asymptomatic carriers[4,36,37] On the other hand, Stx1d may potentially cause disease in humans and very limited information about its clinical implications is

available. Moreover, molecular typing of STEC strains have shown a strong correlation between strains expressing Stx2a, Stx2b, Stx2c and Stx2d subtypes and severe illness such as bloody diarrhea and HUS. In contrast, STEC strains that are positive for Stx2e subtype have been linked with either mild disease in humans without complications or with asymptomatic carriers. Other subtypes that have had limited association with pathogenesis in humans are Stx2f and Stx2g.

Additional virulence factors present on pathogenicity islands, include the locus of enterocyte effacement (LEE) and the non-LEE effectors, implicated in host colonization and disease (Table 2).[3,41] In particular, the LEE-encoded *eae* gene is considered as a key virulence factor for the attachment to intestinal epithelial cells. An adhesin, *iha*, the iron-regulated gene A homolog adhesin, may contribute to the attachment of LEE-positive as well as LEE-negative strains. Moreover, the *nle* effectors, not encoded by the LEE region, have been implicated in altering the host cell response and have been linked to disease severity in non-O157 STEC.[3,42] Other chromosomal and plasmid virulence genes, encoding proteases (*espP*), cytotoxins (*subA*), and adhesins (*saa*), may contribute to STEC pathogenesis by allowing bacterial attachment and colonization of the human epithelium (Table 2).[3,41] The detection of these virulence genes in STEC strains would provide key information for the identification of risk factors that may potentially contribute to the development of human disease.

Antimicrobial resistance

Several published reports have recently documented an increase in antimicrobial resistance in STEC O157:H7 and non-O157:H7 strains, recovered from domestic animal reservoirs that potentially could impact food and environmental sources (Table 3).[19,43–49] In agricultural regions, the inappropriate usages of antibiotics for treating either human or plant diseases and for promoting food-animal growth are proposed to contribute to the continued increase in antimicrobial resistance as well as to the emergence of multidrug resistance profiles.[50,51] Moreover, the use of antimicrobials to treat STEC infections is highly controversial since these agents can induce Stx production and thus promoting the onset of HUS in humans.[52] For example, sub-inhibitory doses of sulfonamides, quinolones and fluoroquinolones which target DNA synthesis have resulted in an increased production of Stx.[53] However, other studies have suggested the early administration in the course of a STEC infection of certain classes of antimicrobials, belonging to macrolides, tetracyclines, fosfomycins, aminoglycosides, cephems and ansamycins. These types of antimicrobials classes target the cell wall, transcription or translation and fail to induce toxin production in STEC.[53]

In the past year, research publications have highlighted the emergence of novel antimicrobial resistance in zoonotic *E. coli*, recovered from domestic animal hosts as well as from food of animal origin (Table 3).[54] This new resistance is against colistin (polymyxin E), a cationic polypeptide antibiotic that traditionally has been approved for use in food-producing animals due to the low resistance rates reported.[54] The resistance mechanism against colistin is mediated by either the *mcr-1* and *mcr-2* genes, both transferable on conjugative plasmids.[55,56] Following the initial report on plasmid-encoded colistin resistance in pigs,[55] a series of published articles have documented transferable resistance against colistin in *E. coli* isolates from food, livestock, water, humans, and wildlife in multiple countries and five continents.[54] Moreover, the identification of plasmid-mediated colistin resistance in STEC pig isolates, which are also resistant to extended-spectrum β-lactamase, has presented new challenges to the veterinary and public health sectors.[43] The findings on transferable colistin indicate the urgent need to monitor the use of antimicrobials in animal food production in order to limit the unwanted dissemination of multidrug resistant *E. coli*.

Conclusions

STEC O157 and non-O157 represents a serious threat to public health worldwide. The potential for large-scale outbreaks and widespread prevalence in animal sources have necessitated the development and evaluation of rapid, sensitive, and specific methods for detection and surveillance for this pathogen. This review summarized the current technology trends for detection and isolation of STEC strains, including culture, isolation, phenotypic, and genotypic characterization methods.

Conflicts of interest

The authors declare no conflicts of interest with the subject matter or materials discussed in the manuscript.

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References

1. Gyles CL. Shiga toxin-producing *Escherichia coli*: an overview. *J Anim Sci* 2007;**85**:E45–62.
2. Mathusa EC, Chen Y, Enache E, Hontz L. Non-O157 Shiga toxin-producing *Escherichia coli* in foods. *J Food Prot* 2010;**73**:1721–36.
3. Bolton DJ. Verocytotoxigenic (Shiga toxin-producing) *Escherichia coli*: virulence factors and pathogenicity in the farm to fork paradigm. *Foodborne Pathog Dis* 2011;**8**:357–65.
4. Lee MS, Koo S, Jeong DG, Tesh VL. Shiga toxins as multifunctional proteins: induction of host cellular stress responses, role in pathogenesis and therapeutic applications. *Toxins* 2016;**8**.
5. Karmali MA, Mascarenhas M, Shen S, Ziebell K, Johnson S, Reid-Smith R, et al. Association of genomic O island 122 of *Escherichia coli* EDL 933 with verocytotoxin-producing *Escherichia coli* seropathotypes that are linked to epidemic and/or serious disease. *J Clin Microbiol* 2003;**41**:4930–40.
6. Law JW, Ab Mutalib NS, Chan KG, Lee LH. Rapid methods for the detection of foodborne bacterial pathogens: principles, applications, advantages and limitations. *Front Microbiol* 2015;**6**.

7. Parsons BD, Zelyas N, Berenger BM, Chui L. Detection, characterization, and typing of Shiga toxin-producing *Escherichia coli*. *Front Microbiol* 2016;**7**:478.
8. Ferens WA, Hovde CJ. *Escherichia coli* O157:H7: animal reservoir and sources of human infection. *Foodborne Pathog Dis* 2011;**8**:465—87.
9. Hussein HS. Prevalence and pathogenicity of Shiga toxin-producing *Escherichia coli* in beef cattle and their products. *J Anim Sci* 2007;**85**:E63—72.
10. Persad AK, Lejeune JT. Animal reservoirs of Shiga toxin-producing *Escherichia coli*. *Microbiol Spec* 2014;**2**.
11. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing *Escherichia coli* and haemolytic uraemic syndrome. *Lancet* 2005;**365**:1073—86.
12. Stromberg ZR, Lewis GL, Marx DB, Moxley RA. Comparison of enrichment broths for supporting growth of Shiga toxin-producing *Escherichia coli*. *Curr Microbiol* 2015;**71**:214—9.
13. Amézquita-López BA, Quiñones B, Cooley MB, León-Félix J, Castro-del Campo N, Mandrell RE, et al. Genotypic analyses of Shiga toxin-producing *Escherichia coli* O157 and non-O157 recovered from feces of domestic animals on rural farms in Mexico. *PLoS One* 2012;**7**:e51565.
14. La Ragione RM, Best A, Woodward MJ, Wales AD. *Escherichia coli* O157:H7 colonization in small domestic ruminants. *FEMS Microbiol Rev* 2009;**33**:394—410.
15. Cooley MB, Jay-Russell M, Atwill ER, Carychao D, Nguyen K, Quiñones B, et al. Development of a robust method for isolation of Shiga toxin-positive *Escherichia coli* (STEC) from fecal, plant, soil and water samples from a leafy greens production region in California. *PLoS One* 2013;**8**:e65716.
16. Hussein HS, Bollinger LM. Influence of selective media on successful detection of Shiga toxin-producing *Escherichia coli* in food, fecal, and environmental samples. *Foodborne Pathog Dis* 2008;**5**:227—44.
17. Hoefer D, Hurd S, Medus C, Cronquist A, Hanna S, Hatch J, et al. Laboratory practices for the identification of Shiga toxin-producing *Escherichia coli* in the United States, FoodNet sites, 2007. *Foodborne Pathog Dis* 2011;**8**:555—60.
18. Cooley M, Carychao D, Crawford-Miksza L, Jay MT, Myers C, Rose C, et al. Incidence and tracking of *Escherichia coli* O157:H7 in a major produce production region in California. *PLoS One* 2007;**2**:e1159.
19. Amézquita-López BA, Quiñones B, Soto-Beltran M, Lee BG, Yambao JC, Lugo-Melchor OY, et al. Antimicrobial resistance profiles of Shiga toxin-producing *Escherichia coli* O157 and non-O157 recovered from domestic farm animals in rural communities in Northwestern Mexico. *Antimicrob Resist Infect Control* 2016;**5**:1.
20. Zadik PM, Chapman PA, Siddons CA. Use of tellurite for the selection of verocytotoxigenic *Escherichia coli* O157. *J Med Microbiol* 1993;**39**:155—8.
21. Fratamico PM, Bagi LK, Cray Jr WC, Narang N, Yan X, Medina M, et al. Detection by multiplex real-time polymerase chain reaction assays and isolation of Shiga toxin-producing *Escherichia coli* serogroups O26, O45, O103, O111, O121, and O145 in ground beef. *Foodborne Pathog Dis* 2011;**8**:601—7.
22. Verstraete K, De Zutter L, Messens W, Herman L, Heyndrickx M, De Reu K. Effect of the enrichment time and immunomagnetic separation on the detection of Shiga toxin-producing *Escherichia coli* O26, O103, O111, O145 and sorbitol positive O157 from artificially inoculated cattle faeces. *Vet Microbiol* 2010;**145**:106—12.
23. Scheutz F, Strockbine NA. Genus I. *Escherichia*. In: Garrity GM, Brenner DJ, Krieg NR, JT S, editors. *Bergey's manual of systematic bacteriology*. 2nd ed. New York: Springer; 2005. p. 607—24.
24. Ørskov I, Ørskov F, Jann B, Jann K. Serology, chemistry, and genetics of O and K antigens of *Escherichia coli*. *Bacteriol Rev* 1977;**41**:667—710.
25. Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, et al. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and *Shigella* for PulseNet. *Foodborne Pathog Dis* 2006;**3**:59—67.
26. Swaminathan B, Gerner-Smidt P, Ng LK, Lukinmaa S, Kam KM, Rolando S, et al. Building PulseNet international: an interconnected system of laboratory networks to facilitate timely public health recognition and response to foodborne disease outbreaks and emerging foodborne diseases. *Foodborne Pathog Dis* 2006;**3**:36—50.
27. Lindstedt BA, Vardund T, Kapperud G. Multiple-locus variable-number tandem-repeats analysis of *Escherichia coli* O157 using PCR multiplexing and multi-colored capillary electrophoresis. *J Microbiol Methods* 2004;**58**:213—22.
28. Hyytiä-Trees E, Smole SC, Fields PA, Swaminathan B, Ribot EM. Second generation subtyping: a proposed PulseNet protocol for multiple-locus variable-number tandem repeat analysis of Shiga toxin-producing *Escherichia coli* O157 (STEC O157). *Foodborne Pathog Dis* 2006;**3**:118—31.
29. Uttamchandani M, Neo JL, Ong BN, Moochhala S. Applications of microarrays in pathogen detection and biodefence. *Trends Biotechnol* 2009;**27**:53—61.
30. Heiden L. Microarray not fade away. In: *Genet Eng News*, vol. 34. New Rochelle NY USA: Mary Ann Liebert, Inc; 2014. p. 1—3.
31. Quiñones B, Swimley MS, Narm KE, Patel RN, Cooley MB, Mandrell RE. O-antigen and virulence profiling of Shiga toxin-producing *Escherichia coli* by a rapid and cost-effective DNA microarray colorimetric method. *Front Cell Infect Microbiol* 2012;**2**:61.
32. Bugarel M, Beutin L, Martin A, Gill A, Fach P. Micro-array for the identification of Shiga toxin-producing *Escherichia coli* (STEC) seropathotypes associated with hemorrhagic colitis and hemolytic uremic syndrome in humans. *Int J Food Microbiol* 2010;**142**:318—29.
33. Quiñones B, Swimley MS, Taylor AW, Dawson ED. Identification of *Escherichia coli* O157 by using a novel colorimetric detection method with DNA microarrays. *Foodborne Pathog Dis* 2011;**8**:705—11.
34. Kuck LR, Taylor AW. Photopolymerization as an innovative detection technique for low-density microarrays. *Biotechniques* 2008;**45**:179—86.
35. Chattaway MA, Dallman TJ, Gentle A, Wright MJ, Long SE, Ashton PM, et al. Whole genome sequencing for public health surveillance of Shiga toxin-producing *Escherichia coli* other than serogroup O157. *Front Microbiol* 2016;**7**:258.
36. Scheutz F, Teel LD, Beutin L, Piérard D, Buvens G, Karch H, et al. Multicenter evaluation of a sequence-based protocol for subtyping Shiga toxins and standardizing Stx nomenclature. *J Clin Microbiol* 2012;**50**:2951—63.
37. Müthing J, Schweppe CH, Karch H, Friedrich AW. Shiga toxins, glycosphingolipid diversity, and endothelial cell injury. *Thromb Haemost* 2009;**101**:252—64.
38. Amézquita-López BA, Quiñones B, Lee BG, Chaidez C. Virulence profiling of Shiga toxin-producing *Escherichia coli* recovered from domestic farm animals in Northwestern Mexico. *Front Cell Infect Microbiol* 2014;**4**:7.
39. Fuller CA, Pellino CA, Flagler MJ, Strasser JE, Weiss AA. Shiga toxin subtypes display dramatic differences in potency. *Infect Immun* 2011;**79**:1329—37.
40. Quiñones B, Swimley MS. Use of a vero cell-based fluorescent assay to assess relative toxicities of Shiga toxin 2 subtypes from *Escherichia coli*. *Methods Mol Biol* 2011;**739**:61—71.

41. Coombes BK, Gilmour MW, Goodman CD. The evolution of virulence in non-O157 shiga toxin-producing *Escherichia coli*. *Front Microbiol* 2011;**2**:90.
42. Coombes BK, Wickham ME, Mascarenhas M, Gruenheid S, Finlay BB, Karmali MA. Molecular analysis as an aid to assess the public health risk of non-O157 Shiga toxin-producing *Escherichia coli* strains. *Appl Environ Microbiol* 2008;**74**:2153—60.
43. Bai L, Hurley D, Li J, Meng Q, Wang J, Fanning S, et al. Characterisation of multidrug-resistant Shiga toxin-producing *Escherichia coli* cultured from pigs in China: co-occurrence of extended-spectrum beta-lactamase- and *mcr*-1-encoding genes on plasmids. *Int J Antimicrob Agents* 2016:445—8.
44. Iweriebor BC, Iwu CJ, Obi LC, Nwodo UU, Okoh AI. Multiple antibiotic resistances among Shiga toxin producing *Escherichia coli* O157 in feces of dairy cattle farms in Eastern Cape of South Africa Clinical microbiology and vaccines. *BMC Microbiol* 2015; **15**.
45. Khan A, Das SC, Ramamurthy T, Sikdar A, Khanam J, Yamasaki S, et al. Antibiotic resistance, virulence gene, and molecular profiles of Shiga toxin-producing *Escherichia coli* isolates from diverse sources in Calcutta, India. *J Clin Microbiol* 2002;**40**:2009—15.
46. Sasaki Y, Usui M, Murakami M, Haruna M, Kojima A, Asai T, et al. Antimicrobial resistance in Shiga toxin-producing *Escherichia coli* O157 and O26 isolates from beef cattle. *Jpn J Infect Dis* 2012;**65**:117—21.
47. Srinivasan V, Nguyen LT, Headrick SI, Murinda SE, Oliver SP. Antimicrobial resistance patterns of Shiga toxin-producing *Escherichia coli* O157:H7 and O157:H7-from different origins. *Microb Drug Resist* 2007;**13**:44—51.
48. Uemura R, Sueyoshi M, Nagayoshi M, Nagatomo H. Antimicrobial susceptibilities of Shiga toxin-producing *Escherichia coli* isolates from pigs with edema disease in Japan. *Microbiol Immunol* 2003;**47**:57—61.
49. Schroeder CM, Meng J, Zhao S, DebRoy C, Torcolini J, Zhao C, et al. Antimicrobial resistance of *Escherichia coli* 026, 0103, 0111, 0128, and 0145 from animals and humans. *Emerg Infect Dis* 2002;**8**:1409—14.
50. Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, et al. Global trends in antimicrobial use in food animals. *Proc Natl Acad Sci USA* 2015;**112**:5649—54.
51. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012;**18**:268—81.
52. Colello R, Etcheverría AI, Di Conza JA, Gutkind GO, Padola NL. Antibiotic resistance and integrons in Shiga toxin-producing *Escherichia coli* (STEC). *Braz J Microbiol* 2015;**46**:1—5.
53. McGannon CM, Fuller CA, Weiss AA. Different classes of antibiotics differentially influence Shiga toxin production. *Antimicrob Agents Chemother* 2010;**54**:3790—8.
54. Schwarz S, Johnson AP. Transferable resistance to colistin: a new but old threat. *J Antimicrob Chemother* 2016;**71**: 2066—70.
55. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis* 2016;**16**: 161—8.
56. Xavier BB, Lammens C, Ruhal R, Malhotra-Kumar S, Butaye P, Goossens H. Identification of a novel plasmid-mediated colistin resistance gene, *mcr*-2, in *Escherichia coli*, Belgium, June 2016. *Euro Surveill* 2016;**21**.