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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

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