Overview of Verotoxigenic *Escherichia coli*†

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ABSTRACT

Verotoxigenic *Escherichia coli* are a recently described class of diarrheagenic *E. coli*. The most prominent member of this group, serotype O157:H7, is a well-recognized cause of hemorrhagic colitis and hemolytic uremic syndrome. This serious human pathogen has caused numerous outbreaks in the developed world and has contaminated such widely disparate foods as ground beef, apple cider, and lettuce. Serotypes other than O157:H7 have also been found to cause sporadic enteric disease and several outbreaks have been recently described. The non-O157 SLTEC are more frequently present in food animals and foods of animal origin than serotype O157:H7. Particular non-O157 serotypes (such as O26) have a definite association with HUS. Surveillance data from several regions suggests that there may be important differences in the distribution of serotypes causing HUS in different geographic areas. While more than 100 serotypes of *E. coli* have been identified as possessing one or more SLT genes, far fewer than that number have been convincingly associated with human illness. Current research needs to determine those additional virulence traits which confer pathogenicity on organisms possessing the SLT gene. Equally important will be to ascertain the relative contribution of different serotypes to human disease in order to develop sound, scientifically based, control strategies.

Key words: Enterohemorrhagic *Escherichia coli*, verotoxin, Shiga-like toxin, hemolytic uremic syndrome

Within two years of the discovery of *E. coli* serotype O157:H7, published reports appeared describing the production of SLTs by other serotypes of *E. coli*. At present, more than one hundred serotypes have been shown to produce SLTs and together with serotype O157:H7 they are designated the Shiga-like toxin producing *E. coli* (SLTEC) (4). The enterohemorrhagic *E. coli* (EHEC) are a subset of the SLTEC which is composed of those strains which cause bloody diarrhea and possess a high molecular weight plasmid. *E. coli* O157:H7 is the most common and most easily identified of the SLTEC and we have a moderate knowledge base about its molecular biology, the clinical illnesses it causes, and its epidemiology. Our understanding of these aspects of the non-O157 SLTEC is less well-developed. I will summarize what we know at present about these non-O157 SLTEC and point out their similarities and differences from *E. coli* O157:H7.

At present, more is known about the molecular biology of the SLTEC than their clinical or epidemiologic aspects. SLT production by the non-O157 SLTEC is similar to that for serotype O157, namely that the SLTs are phage-encoded and strains may produce either one or both SLT-I and SLT-II or variants of the latter. Among the non-O157 strains, toxin sequences may not be as stably integrated into the bacterial chromosome as evidenced by reports of high frequency loss of toxin production with serial subculture. The non-O157 SLTEC are serotypically heterogeneous, comprising more than one hundred serotypes. Molecular fingerprinting has shown the O157:H7 strains to be so tightly clustered genetically that they represent a pathogenic clone. In contrast, the non-O157 strains have much greater genetic diversity and do not exhibit such clonality. The non-O157 SLTEC do not have a predominant biochemical phenotype and this has made screening for them quite labor intensive. Some strains do exhibit the sorbitol negative phenotype found in nearly all *E. coli* O157:H7, but such strains are the minority. Possible virulence factors include the eae gene, important in the production of attaching and effacing lesions in the intestine and the EHEC hemolysin gene (*hly)*. Both of these sequences are found in virtually all O157 isolates but are more variably present among the non-O157 SLTEC. Although the eae and EHEC sequences appear to be more common in those non-O157 SLTEC associated with human illness and epidemiologically important, it is clear that additional virulence traits need to be identified.

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disease, they are not an absolute requirement for pathogenicity, as strains lacking these sequences have caused clinical illness.

There have been relatively few systematic studies of the clinical aspects of non-O157 SLTEC infection in humans. It is clear that some of these strains can cause diarrhea in humans. Available evidence suggests that in infection with those strains which do cause enteric disease, non-bloody diarrhea may be more common, but some serotypes more consistently produce a hemorrhagic colitis-like picture. The results from several studies have tended to suggest that the non-O157 SLTEC may be associated overall with less severe disease as measured by a smaller proportion of cases with bloody diarrhea, a lower hospitalization rate, and a lower rate of sequelae. One study has suggested that diarrhea with the non-O157 SLTEC may be longer in duration as compared with that for O157. Both O157 and the non-O157 SLTEC are associated with the hemolytic uremic syndrome (HUS) (2, 3, 6). While well documented cases with actual strain isolation and identification of the non-O157 strains are few, serotypes such as O111 and O26, have had a consistent documented association with HUS. What is not known is whether the non-O157 SLTEC overall have a lower risk of HUS or whether all of that risk is concentrated in a particular subgroup of the non-O157 SLTEC. The apparently lower rate of HUS with non-O157 serotypes is not likely the result of a lower rate of human exposure to them, as these strains are much more common in foods than serotype O157:H7. An intriguing question is whether the apparently different rates of HUS in different geographic areas may be due to differences in the circulating SLTEC serotypes.

The non-O157 SLTEC can cause both sporadic and outbreak-associated disease. Whether the apparently lower frequency of outbreaks, in comparison with O157, is real or is simply due to lack of widespread recognition of less severe disease, is unclear. Besides enteric illness and its sequelae, there have been a few other intriguing associations of SLTEC with human disease. A few cases of HUS without antecedent diarrheal illness have been described in which a non-O157 SLTEC strain was detected in stool specimens. Non-O157 SLTEC have been detected in the gastrointestinal tract of some cases of sudden infant death syndrome, and in a few persons with exacerbations of ulcerative colitis. Prospective studies will help to demonstrate whether SLTEC play a role in the pathogenesis of these diseases.

The descriptive epidemiology of the non-O157 SLTEC is fragmentary and confusing. They have been identified throughout the world. The SLTEC are commonly found in beef and dairy cattle although they are not bovine pathogens. Whether they are transient colonizers of the bovine gastrointestinal tract, as is O157, is not known. Non-O157 SLTEC have also been detected in many other species including other meat animals such as poultry and sheep, in wild animals such as deer, domestic pets such as cats, dogs, and goats, and in exotic animals in a zoo. Several studies from different parts of the world have indicated the frequent presence of SLTEC in retail meats, poultry, and seafood (7). Whether this represents contamination at the source or cross-contamination during processing or retail sale remains unclear.

There have been several recent developments which have suggested that both SLTEC and SLT itself may be moving targets. Surveillance data from Canada have shown a marked reduction in the rate of E. coli O157:H7 isolation in cases of hemorrhagic colitis, with cases decreasing by half in the 2-year period from 1989 to 1991 (9). Whether this is due to enhanced control measures such as better cooking of hamburgers or represents a secular trend in E. coli O157:H7 circulation is not clear. In Germany, the proportion of cases in which E. coli O157:H7 was identified as the cause of HUS declined from 91% in the late 1980s to 22% in 1994, and in this latter year, non-O157 serotypes were detected in the majority of HUS cases (5). There now are reports of non–E. coli species that have apparently acquired the SLT genes. An SLT-producing Citrobacter freundii caused an outbreak among school children in Germany, and in Australia an SLT-producing Enterobacter cloacae was isolated from the stool of an infant with HUS. Further epidemiologic surveillance will be required to determine if horizontal transfer of the SLT gene sequences continues to occur.

Having recognized the non-O157 SLTEC as human pathogens now leads us to confront two important questions. One question is whether clinical laboratories should screen for the non-O157 SLTEC, especially given that only half of these laboratories now screen for serotype O157:H7 (1, 8). At present, there is no clear consensus on methodology. Given that the average case of diarrhea probably lasts 5 to 7 days, physician and patient would be looking for a test with a clinically relevant turnaround time of 24 to 48 h. Antisera for only a few serotypes is commercially available which presents a problem for laboratories attempting to report results in a clinically useful time frame. Another issue is how the lab would report the results and how the clinician (and insurers) would interpret them. For example, the lab reports at 48 h that SLT was detected in the fecal filtrate and undertakes procedures to isolate a non-O157 SLTEC strain, which is then sent to a reference laboratory for serotyping. Eight weeks after the patient’s illness, the clinician receives the final report now identifying the serotype of the provisional SLTEC isolated from the patient’s stool. The patient is well, the strain is one infrequently associated with human disease and the insurer says the test was unnecessary and denies payment. Clinical laboratories are also faced with cost considerations in adding new tests. Several prospective large scale studies in different geographic areas that used comparative methodologies could answer this question in a cogent and data-based manner.

Another important question is whether a regulatory framework can be developed for the non-O157 SLTEC. This is highly problematic at present because current ways to identify these strains are very labor intensive, and a pragmatic way to classify those found as pathogens or non-pathogens is lacking. A better understanding of the epidemiology of human disease with the non-O157 SLTEC, coupled with ways to reclassify these strains at the molecular level should provide the ground work on which to base such a regulatory framework.
The remaining contributors to this symposium will present the experience with SLTEC infections in their country or geographic area. Their contributions will help to enlarge our understanding of the clinical manifestations and epidemiology of the SLTEC and illustrate important geographic differences. Ultimately their data and that of other investigators can help us to devise control strategies which are accurately focused and rationally applied.

**ADDENDUM IN PROOF**


**REFERENCES**


