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Virulence Profiling of Shiga Toxin-Producing *Escherichia coli* O111:NM Isolates from Cattle

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Shiga toxin-producing *Escherichia coli* (STEC) O111:NM is an important serotype that has been incriminated in disease outbreaks in the United States. This study characterized cattle STEC O111:NM for virulence factors and markers by PCR. Major conclusions are that STEC O111:NM characterized in this study lacks stx_2 and the full spectrum of *nle* gene markers, and it has an incomplete OI-122.

Shiga toxin-producing *Escherichia coli* (STEC) is annually incriminated in more than 100,000 cases of human enteric foodborne disease in the United States, and it can be complicated with severe illness in the form of hemolytic-uremic syndrome (HUS) (1, 2). Cattle are the main reservoir of STEC in North America. Human infections are acquired mainly by ingesting food or water contaminated directly or indirectly with cattle feces (3). More than 500 STEC serotypes have been recovered from various animals, foods, and environments worldwide (http://www.lugo.usc.es/ecoli/). However, only a small number of these serotypes are commonly incriminated in human disease. Depending on the severity of disease, incidence in human illness, and frequency of involvement in outbreaks, STEC have been classified into five seropathotype groups (A to E) (4). Although most cases of human STEC infections have been attributed to serotype O157:H7 (seropathotype A), there is growing evidence that non-O157 serotypes account for up to 50% of cases of human disease (5).

A number of reports have shown that non-O157 STEC strains account for more than 50% of STEC infections in the United States (5, 6, 7). One of the non-O157 serotypes that is frequently incriminated in human disease is O111:NM (seropathotype B). In 2008, STEC O111 was implicated in the largest STEC outbreak in U.S. history, in Oklahoma, affecting 341 individuals and causing more than 70 hospitalizations, 26 cases of HUS, and one death (8). In this outbreak, the largest number of HUS cases due to a non-O157 serotype in U.S. history was also recorded. Currently, there is a paucity of information on the epidemiology of non-O157 STEC, and molecular characterization of important non-O157 STEC has lagged behind considerably.

The objective of this study was to characterize STEC O111:NM isolates of cattle origin. The isolates were screened for the presence of genes that encode various virulence factors and markers. These included Shiga toxins (stx_1 and stx_2), intimin (*eae*) (1), genes located on plasmids (*ehxA*, *katP*, and *espP*) (9–11), and pathogenicity island (OI) markers for OI-43/48 (*iha*, *ureC*, and *terC*) (12–14) and OI-122 (*Z4321*, *Z4326*, *Z4332*, and *Z4333*) (4). Previously, possession of OI-43/48 and OI-122 marker genes has been used to classify STEC serotypes into seropathotypes and to distinguish STEC serotypes with a high zoonotic risk from those that present a low zoonotic risk, based on the notion that possession of these genes is significantly associated with serotypes that cause severe disease or outbreaks in humans (4). The isolates were also screened for non-locus of enterocyte effacement (LEE) effector genes (*nle*) (15). Non-LEE effector genes are genes that have been used in the recent past as molecular markers of STEC that have a high potential to cause HUS and outbreaks in humans (15). The *nle* genes that were investigated include *nleA*, *nleB*, *nleC*, *nleE*, *nleF*, *nleG2-1*, *nleG2-3*, *nleG5-2*, *nleG6-2*, *nleG9*, *nleH1-1*, and *nleH1-2* (15).

A total of 58 STEC O111:NM isolates from the collection of the *E. coli* Reference Center (University Park, PA) were screened by PCR for 25 genes that encode STEC virulence factors or markers. These isolates were of independent cattle origins and were recovered from 10 U.S. states and one province of Canada over a 23-year span, from 1976 to 1999. The boiling method was used to extract DNA from all of the strains (16). A multiplex PCR was used for the detection of stx_1, stx_2, *eae*, and *ehxA* (17). PCRs for the following genes were conducted individually: *katP* and *espP* (10, 11). The genes *Z4321*, *Z4326*, *Z4332*, and *Z4333* were examined as OI-122 markers (4), and *terC*, *iha*, and *ureC* were examined as OI-43/48 markers (12–14). Non-LEE effector gene markers were screened by PCR individually: *nleA*, *nleB*, *nleC*, *nleF*, *nleE*, *nleG2-1*, *nleG2-3*, *nleG5-2*, *nleG6-2*, *nleG9*, *nleH1-1*, and *nleH1-2* (15). PCR was performed in a 25-μl reaction mixture containing 2.5 μl of DNA, 2.5 μl of 10× PCR buffer, 1.5 or 2 mM $MgCl_2$, 200 μM each deoxynucleoside triphosphate, 2 U of *Taq* DNA polymerase, and water. STEC O157:H7 strain EDL933 was used as a positive control for all of the genes. PCR mixtures without template DNA were used as a negative control for all reactions.

Virulence profiling of 58 STEC O111:NM isolates by PCR revealed that 100% (58/58) of isolates possessed stx_1 and *eae*; OI-122 markers *Z4326*, *Z4332*, and Z4333; OI-43/48 markers *terC*, *ureC*, and *iha*; and *nle* markers *nleB*, *nleE*, *nleG2-3*, *nleG5-2*, and *nleH1-1*. The stx_2 gene was present in 18.9% of isolates, and 57% carried OI-22 marker *Z4321*. The full complement of OI-122 markers was present in 57% of the STEC O111:NM isolates. Other non-LEE effector genes were distributed as follows: *nleA*, 36%;

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nleC, 0%; *nleF*, 7%; *nleG2-1*, 71%; *nleG6-2*, 62%; *nleG9*, 95%; and *nleH1-2*, 9%. The distribution of virulence marker genes on plasmids was the following: *ehxA*, 75%; *katP*, 41%; and *espP*, 51%.

Major conclusions are that STEC O111:NM from cattle that were characterized in this study were mainly stx_1, *eae*, and *ehxA* positive but lacked stx_2, a major virulence gene that has been associated with severe disease in humans (18). The possession of stx_1, *eae*, and *ehxA* genes and lack of stx_2 is characteristic of most STEC O111:NM of cattle and human origins that have been isolated in Canada, the United States, Germany, and Brazil (6, 18–20). With regard to OI-122 and OI-43/48 and *nle* marker genes, more than 50% of isolates possessed most of these genes, except for *nleA*, *nleC*, and *nleF*, which were carried in low numbers by STEC O111:NM. STEC strains that possess stx_2, the full spectrum of *nle* marker genes, and a complete OI-122 are most likely to be incriminated in severe disease outbreaks (4, 18). Lack of stx_2 and the full spectrum of *nle* genes and an incomplete OI-122 in the majority of bovine STEC O111:NM screened may be a good reason why STEC O111:NM is infrequently incriminated in severe disease cases and outbreaks in humans compared to STEC O157:H7, a seropathotype A STEC. However, further work on a larger collection of STEC O111:NM from human and cattle is needed to validate this statement. To the best of our knowledge, this is the first study that has characterized STEC O111:NM beyond the more traditionally recognized virulence factors and markers (*stx*, *eae*, and *ehxA*). Because large-scale characterization studies on STEC O111:NM are not available, there is a possibility that some aspects of O111:NM virulence that are novel are not accounted for by examining virulence factors of enterohemorrhagic *E. coli* O157:H7, which still remains a reference STEC serotype for all of the genes examined in this study.

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