JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 2009, p. 3058–3059 Vol. 47, No. 9
0095-1137/09/$08.00+0 doi:10.1128/JCM.00706-09

Prevalence of the Operon Encoding Subtilase Cytotoxin in Non-O157 Shiga Toxin-Producing *Escherichia coli* Isolated from Humans in the United States[▽]

Shiga toxin-producing *Escherichia coli* (STEC) causes diarrhea and can lead to hemolytic uremic syndrome (HUS). In addition to Shiga toxin (Stx), gene products encoded by the *l*ocus of *e*nterocyte *e*ffacement (LEE) pathogenicity island are well-known virulence factors. This gene cluster is present in certain STEC strains that have caused large-scale outbreaks, such as O157:H7. STEC strains lacking LEE can also cause HUS, suggesting the importance of other virulence factors (4). A novel toxin called subtilase cytotoxin (SubAB) was discovered in a non-O157, LEE-negative Stx2-producing strain that caused a small HUS outbreak (9). The role of SubAB in HUS pathogenesis is unclear, but in a murine model, SubAB injected intraperitoneally causes widespread microangiopathic changes (similar to the pathology in humans with HUS) (10). Given that the gene for SubAB has not been detected in O157 STEC and non-O157 STEC sometimes lack a key STEC virulence factor (LEE), our objective was to estimate the prevalence of the *subA* gene among non-O157 STEC isolated from humans in the United States.

Other studies demonstrate the presence of *subA* in STEC from various sources in Brazil, Canada, and the United States (3, 5, 6). We previously identified *subA* in several non-O157 North American STEC strains, but the prevalence of *subA* in U.S. human STEC disease isolates is unknown (6). In Australia, approximately 10% of stool samples from humans with STEC disease contain *subA* (8).

A study conducted by the Michigan Department of Community Health examined stool samples by enzyme immunoassay for Stx's between April 2003 and October 2005 (7). Similarly, the Connecticut Department of Public Health reported on non-O157 STEC strains from Connecticut between 2000 and 2005 (1). We used multiplex PCR as previously described to genotype the non-O157 strains collected from these studies (6, 8). We identified the presence of *subA* and several other known virulence factors—*eae*, *hly*, stx_1, and stx_2.

Of the 153 non-O157 strains included for analysis, 21 lack LEE, and 3/21 are *subA* positive (Table 1). These three strains have virulence pattern profiles similar to those of most of the known *subA*-containing STEC strains (6, 8). They lack *eae*, the gene encoding intimin that is present in the LEE pathogenicity island, but contain *hly* (hemolysin) and stx_2. One strain also has stx_1.

TABLE 1. *subA*-positive and *eae*-negative strains

Location	No. (%) of:		
	subA-positive strains	*eae*-negative strains	*subA*-positive of *eae*-negative strains
Michigan	1/40 (2.5)[a]	7/40 (17.5)	1/7 (14.3)
Connecticut	2/113 (1.8)[b]	14/113 (12.4)	2/14 (14.3)
Total	3/153 (2)	21/153 (13.7)	3/21 (14.3)

[a] Patient was an elderly female who presented to an outpatient facility. No clinical information is available. The strain was serotype O110:H28 and *hly* positive, *eae* negative, stx_1 negative, and stx_2 positive.

[b] One of these two patients was a female in her twenties with bloody diarrhea and cramps, but no HUS. The strain was serotype O163:H19 and *hly* positive, *eae* negative, stx_1 negative, and stx_2 positive. The other patient was an asymptomatic teenager under evaluation for worms in stool. Strain was O91:H14 and *hly* positive, *eae* negative, stx_1 positive, and stx_2 positive.

Because of the low prevalence of strains with *subA* in this study, we are unable to form conclusions about SubAB's role in non-O157 STEC virulence. To assess the clinical significance of SubAB and gain understanding of its contribution to disease pathogenesis, large population-based epidemiologic studies should be performed where there is a higher prevalence of SubAB. Increased surveillance for both O157 and non-O157 STEC with enzyme immunoassay or PCR, as recommended by the Centers for Disease Control and Prevention, will allow future studies that can further characterize the burden of disease caused by non-O157 STEC and SubAB-producing STEC (2). Although *subA*-positive strains currently account for a small percentage of recognized STEC strains affecting humans in the United States, distribution of these strains could change over time. If LEE-positive STEC strains are successfully removed from the food chain, more LEE-negative strains may emerge. In the meantime, animal models and tissue culture studies may be helpful to expand our knowledge of the possible contribution of SubAB to STEC pathogenesis.

REFERENCES

1. **CDC.** 2007. Laboratory-confirmed non-O157 Shiga toxin-producing *Escherichia coli*—Connecticut, 2000–2005. MMWR Morb. Mortal. Wkly. Rep. **56**:29–31.
2. **CDC.** 2006. Importance of culture confirmation of Shiga toxin-producing *Escherichia coli* infection as illustrated by outbreaks of gastroenteritis—New York and North Carolina, 2005. MMWR Morb. Mortal. Wkly. Rep. **55**: 1042–1045.
3. **Cergole-Novella, M. C., L. S. Nishimura, L. F. Dos Santos, K. Irino, T. M. I. Vaz, A. M. M. Bergamini, and B. E. C. Guth.** 2007. Distribution of virulence profiles related to new toxins and putative adhesins in Shiga toxin-producing *Escherichia coli* isolated from diverse sources in Brazil. FEMS Microbiol. Lett. **274:**329–334.
4. **Johnson, K. E., C. M. Thorpe, and C. L. Sears.** 2006. The emerging clinical importance of non-O157 Shiga toxin-producing *Escherichia coli*. Clin. Infect. Dis. **43:**1587–1595.
5. **Karama, M., R. P. Johnson, R. Holtslander, S. A. McEwen, and C. L. Gyles.** 2008. Prevalence and characterization of verotoxin-producing *Escherichia coli* (VTEC) in cattle from an Ontario abattoir. Can. J. Vet. Res. **72:**297–302.
6. **Khaitan, A., D. M. Jandhyala, C. M. Thorpe, J. M. Ritchie, and A. W. Paton.** 2007. The operon encoding SubAB, a novel cytotoxin, is present in shiga toxin-producing *Escherichia coli* isolates from the United States. J. Clin. Microbiol. **45:**1374–1375.
7. **Manning, S. D., R. T. Madera, W. Schneider, and S. E. Dietrich, W. Khalife, W. Brown, T. S. Whittam, P. Somsel, and J. T. Rudrik.** 2007. Surveillance for Shiga toxin-producing *Escherichia coli*, Michigan, 2001–2005. Emerg. Infect. Dis. **13**:318–321.
8. **Paton, A. W., and J. C. Paton.** 2005. Multiplex PCR for direct detection of Shiga toxigenic *Escherichia coli* strains producing the novel subtilase cytotoxin. J. Clin. Microbiol. **43:**2944–2947.

9. **Paton, A. W., P. Srimanote, U. M. Talbot, H. Wang, and J. C. Paton.** 2004. A new family of potent AB_5 cytotoxins produced by Shiga toxigenic *Escherichia coli*. J. Exp. Med. **200:**35–46.

10. **Wang, H., J. C. Paton, and A. W. Paton.** 2007. Pathologic changes in mice induced by subtilase cytotoxin, a potent new *Escherichia coli* AB_5 toxin that targets the endoplasmic reticulum. J. Infect. Dis. **196:**1093–1101.

Jennifer J. Wolfson*
Dakshina M. Jandhyala
Lynn A. Gorczyca
Zulekha Qadeer
Tufts Medical Center
800 Washington Street Box 41
Boston, Massachusetts 02111

Shannon D. Manning
National Food Safety & Toxicology Center, and Department of Pediatric and Human Development
Michigan State University
East Lansing, Michigan

James Hadler
Connecticut Department of Public Health
Hartford, Connecticut

James T. Rudrik
Michigan Department of Community Health
Lansing, Michigan

Cheleste M. Thorpe
Tufts Medical Center
800 Washington Street Box 41
Boston, Massachusetts 02111

*Phone: (617) 636-8419
Fax: (617) 636-3198
E-mail: jwolfson@tuftsmedicalcenter.org

▽ Published ahead of print on 1 July 2009.