JCM Journals.ASM.org



# Extraintestinal Pathogenic *Escherichia coli* Carrying the Shiga Toxin Gene $stx_2$

**A. L. Wester, L. T. Brandal, U. R. Dahle**

Department of Foodborne Infections, Division of Infectious Disease Control, Norwegian Institute of Public Health, Oslo, Norway

The 2011 outbreak of infections with *Escherichia coli* with characteristics of both enteroaggregative *E. coli* (EAEC) and Shiga toxin-producing *E. coli* (STEC) caused a paradigm shift with regard to the human pathogenicity of STEC (1). Diarrheagenic *E. coli* (DEC) usually does not cause extraintestinal diseases such as urinary tract infections and bacteremia (2).

The DNA of 193 *E. coli* isolates from adult bacteremic patients described previously (3) was analyzed for the presence of $stx_1$ and $stx_2$ (4). All of the strains were $stx_1$ negative, but nine (4.7%) were $stx_2$ positive. According to the Scheutz subtyping protocol (5), seven belonged to subtype $stx_{2c}$ and two belonged to $stx_{2d}$. Table 1 shows the associations between the clinical presentations of the patients and their $stx_2$ statuses.

Arbitrarily chosen, DNA from 2 of the $stx_2$-postive strains and 26 of the *stx*-negative strains was analyzed for additional DEC genes (4, 6) and extraintestinal pathogenic *E. coli* (ExPEC) genes by PCR assay as selected by B. A. Lindstedt et al. (unpublished data) (Table 2). One of the two $stx_2$-positive strains that were further analyzed for additional virulence markers carried both $stx_{2c}$ and *aggR*. Seven of the 26 *stx*-negative strains carried other DEC genes; with or without concomitant ExPEC genes.

The $stx_2$-positive strains were recultured and tested for toxin production by ImmunoCard STAT! EHEC (Meridian Bioscience, Inc.). All nine $stx_2$-positive strains tested negative for toxin production. Therefore, another isolation of DNA from the viable strains was performed and new PCR assays detecting *stx* and *aggR* were run. Surprisingly, the toxin gene was lost by all nine but *aggR* was reproduced in the relevant strain, indicating no mix-up of strains or DNA. Loss of $stx_2$-carrying bacteriophages is not a novel phenomenon (7, 8), but to our knowledge, such a frequent loss has not been described previously.

Reports of bacteremia or sepsis in patients with hemolytic-uremic syndrome (HUS) caused by STEC do exist (2, 9–11). In our study, we found that almost 5% of the strains found in adult patients with bacteremia caused by *E. coli* carried $stx_2$ subtypes associated with HUS (12–14). Not having symptoms of gastroenteritis was associated with $stx_2$-positive status, but this finding seems rather implausible. Further characterization of two $stx_{2c}$-carrying isolates showed features of STEC and EAEC, as well as ExPEC, which, to our knowledge, is a novel finding.

If reproduced, these *stx* findings may have consequences for infection control. And if an association with clinical presentation is found, differential diagnoses of bacteremia with *E. coli* should include STEC colitis, as well as HUS. This is particularly relevant for elderly patients, who may have vague symptoms and comorbidity complicating the clinical picture. Furthermore, elderly, institutionalized patients have a unique susceptibility to STEC infection and its sequelae (15). Microbiological analyses of *E. coli* blood culture isolates to detect *stx* should therefore be encouraged, and infection control measures and contact tracing should be implemented when *stx*-carrying *E. coli* bacteremia is confirmed.

**Published ahead of print** 9 October 2013

Address correspondence to Astrid Louise Wester, astrid.louise.wester@fhi.no.



doi:10.1128/JCM.01349-13

**TABLE 1** $stx_2$ statuses of *E. coli* and clinical characteristics of 193 bacteremia patients

| Characteristic | No. (%) of patients | | | *P* value |
|---|---|---|---|---|
| | All | 184 *stx* negative | 9 *stx* positive | |
| Age of ≥65 yr | 133 (68.9) | 126 (68.5) | 7 (77.8) | 0.56 |
| Male gender | 71 (36.8) | 66 (35.9) | 5 (55.6) | 0.23 |
| Symptoms of gastroenteritis | 74 (38.1) | 74 (40.4) | 0 | 0.03 |
| Clinical presentation[a] within 1 day of admission with: | | | | |
| Acute renal failure | 10 (5.2) | 9 (4.7) | 1 (12.5) | 0.35 |
| Thrombocytopenia[b] | 18 (9.5) | 18 (9.9) | 0 | 0.25 |
| One or more failing organs | 86 (45.7) | 83 (46.1) | 3 (37.5) | 0.63 |
| Death in hospital within 14 days after admission | 14 (7.3) | 13 (7.1) | 1 (12.5) | 0.65 |

[a] Data were available on acute renal failure, thrombocytopenia, and one or more failing organs for 191, 190, and 188 patients, respectively.
[b] <100,000 thrombocytes/μl.

**TABLE 2** Molecular characterization of a selection of *E. coli* bacteremia strains

| Study identification no. | O serogroup | DEC VGs[a] | ExPEC VGs[b] | | | |
|---|---|---|---|---|---|---|
| | | | Adhesins | Iron acquisition | Cytotoxins | Other |
| 11 | O? | | | *sitA*, *iroC* | | *ibeA*, *iss*, *traT*, *tsh*, *kps1* |
| 52 | O2 | | *papC* | *iutA*, *sitA*, *iucD*, *iroC* | | *iss*, *traT*, *tsh*, *etsA*, *kps1* |
| 81 | O103[c] | $stx_{2c}$ | *papC* | *iutA*, *sitA*, *iucD*, *iroC* | | *kps1*, *tsh*, *etsA*, *iss*, *traT* |
| 100 | O? | *ehaA* | | | | |
| 282 | O? | | | *iutA*, *sitA*, *iucD* | *sat* | *iss*, *traT* |
| 325 | O6 | | *sfaS* | *sitA*, *iroC* | *cnf1* | *iss*, *traT*, *tsh*, *kps1* |
| 336 | O? | *ehaA* | | *iutA*, *iucD* | | *iss*, *etsA* |
| 387 | O? | | | *iutA*, *iucD*, *iroC* | | *iss*, *traT*, *tsh*, *etsA* |
| 447 | O2 | | *papC* | *iutA*, *sitA*, *iucD*, *iroD* | | *iss*, *traT*, *tsh*, *etsA*, *kps1* |
| 585 | O2 | *cdt* | | *iutA*, *iucD*, *sitA*, *iroC* | *cnf1*, *sat* | *iss*, *kps1*, *tsh* |
| 668 | O2 | | *papC* | *iutA*, *sitA*, *iroC*, *iucD* | | *iss*, *traT*, *tsh*, *etsA* |
| 685 | O15 | $stx_{2c}$, *aggR* | | *iutA*, *sitA*, *iucD* | *sat* | *iss*, *traT* |
| 687 | O12 | | | *iutA*, *iucD*, *iroC* | | *iss*, *traT*, *tsh*, *etsA* |
| 713 | O6/O7 | | | *iutA*, *sitA*, *iucD*, *iroC* | | *iss*, *sat*, *tsh* |
| 803 | O? | | | *iutA*, *sitA*, *iucD* | *sat* | *ibeA*, *iss* |
| 804 | O6 | | | *sitA*, *iroC* | | *ibeA*, *iss*, *traT*, *tsh*, *kps1* |
| 839 | O103[c] | | *papC* | *iutA*, *sitA*, *iucD*, *iroC* | | *iss*, *traT*, *tsh*, *etsA*, *kps1* |
| 859 | O? | *ehaA* | | *sitA*, *iucD* | *sat* | *iss*, *traT* |
| 865 | O? | *ehaA* | | *iutA*, *iucD*, *icoC* | | *iss*, *traT*, *etsA* |
| 891 | O2 | | | *sitA* | | *iss*, *etsA*, *kps1* |
| 895 | O4/O12 | | | *iutA*, *sitA*, *iucD*, *iroC* | *cnf1* | *iss*, *tsh*, *kps1* |
| 915 | O75 | | | *sitA*, *iroC* | *cnf1* | *ibeA*, *iss*, *tsh*, *kps1* |
| 952 | O? | | | *sitA*, *iroC* | | *ibeA*, *iss*, *tsh*, *kps1* |
| 972 | O18 | *cdt* | *sfaS* | *iutA*, *sitA*, *iucD*, *iroC* | | *ibeA*, *iss*, *traT*, *gimB*, *tsh*, *etsA*, *kps1* |
| 1010 | O6 | | | *iutA*, *sitA*, *iucD*, *iroC* | *cnf1*, *sat* | *iss*, *traT*,*tsh* |
| 1095 | O1 | | *papC* | *sitA* | | *iss*, *traT*, *tsh*, *kps1* |
| 1121 | O103[c] | *eaeB*, *ehaA* | | | | |
| 1127 | O? | | | *sitA*, *iroC* | | *ibeA*, *iss*, *traT*, *tsh*, *kps1* |

[a] Virulence genes (VGs) associated with DEC: *ST1a*, *ST1b*, *ehxA*, *aggR*, *LT1*, $stx_1$, $stx_2$, *eaeB*, *ipaH*, *bfpB*, *saa*, *nleB*, *stcE*, *cdt*, and *subA*.
[b] VGs associated with ExPEC: adhesins (*papC* and *sfaS*), iron acquisition (*iutA*, *sitA*, *iucD*, and *iroC*), cytotoxins (*cnf1*, *cnf2*, *cnf3*, and *sat*), and others (*kps1*, *ibeA*, *iss*, *traT*, and *tsh*).
[c] Flagellar antigen H2.

## REFERENCES


1. **Beutin L, Martin A.** 2012. Outbreak of Shiga toxin-producing Escherichia coli (STEC) O104:H4 infection in Germany causes a paradigm shift with regard to human pathogenicity of STEC strains. J. Food Prot. **75:** 408–418.
2. **Lienemann T, Salo E, Rimhanen-Finne R, Ronnholm K, Taimisto M, Hirvonen JJ, Tarkka E, Kuusi M, Siitonen A.** 2012. Shiga toxin-producing Escherichia coli serotype O78:H(−) in family, Finland, 2009. Emerg. Infect. Dis. **18:**577–581.
3. **Wester AL, Dunlop O, Melby KK, Dahle UR, Wyller TB.** 2013. Age-related differences in symptoms, diagnosis and prognosis of bacteremia. BMC Infect. Dis. **13:**346. doi:10.1186/1471-2334-13-346.
4. **Brandal LT, Lindstedt BA, Aas L, Stavnes TL, Lassen J, Kapperud G.** 2007. Octaplex PCR and fluorescence-based capillary electrophoresis for identification of human diarrheagenic Escherichia coli and Shigella spp. J. Microbiol. Methods **68:**331–341.
5. **Scheutz F, Teel LD, Beutin L, Pierard D, Buvens G, Karch H, Mellmann A, Caprioli A, Tozzoli R, Morabito S, Strockbine NA, Melton-Celsa AR, Sanchez M, Persson S, O'Brien AD.** 2012. Multicenter evaluation of a sequence-based protocol for subtyping Shiga toxins and standardizing Stx nomenclature. J. Clin. Microbiol. **50:**2951–2963.
6. **Brandal LT, Sekse C, Lindstedt BA, Sunde M, Lobersli I, Urdahl AM, Kapperud G.** 2012. Norwegian sheep are an important reservoir for human-pathogenic *Escherichia coli* O26:H11. Appl. Environ. Microbiol. **78:** 4083–4091.
7. **Bielaszewska M, Prager R, Kock R, Mellmann A, Zhang W, Tschape H, Tarr PI, Karch H.** 2007. Shiga toxin gene loss and transfer in vitro and in vivo during enterohemorrhagic *Escherichia coli* O26 infection in humans. Appl. Environ. Microbiol. **73:**3144–3150.
8. **Mellmann A, Lu S, Karch H, Xu JG, Harmsen D, Schmidt MA, Bielaszewska M.** 2008. Recycling of Shiga toxin 2 genes in sorbitol-fermenting enterohemorrhagic *Escherichia coli* O157:NM. Appl. Environ. Microbiol. **74:**67–72.
9. **Buvens G, De Rauw K, Roisin S, Vanfraechem G, Denis O, Jacobs F, Scheutz F, Pierard D.** 2013. Verocytotoxin-producing *Escherichia coli* O128ab:H2 bacteremia in a 27-year-old male with hemolytic-uremic syndrome. J. Clin. Microbiol. **51:**1633–1635.
10. **Chiurchiu C, Firrincieli A, Santostefano M, Fusaroli M, Remuzzi G, Ruggenenti P.** 2003. Adult nondiarrhea hemolytic uremic syndrome associated with Shiga toxin Escherichia coli O157:H7 bacteremia and urinary tract infection. Am. J. Kidney Dis. **41:**E4. doi:10.1016/S0272-6386(03)00365-2.
11. **Manton N, Smith NM, Byard RW.** 2000. Unexpected childhood death due to hemolytic uremic syndrome. Am. J. Forensic Med. Pathol. **21:** 90–92.
12. **Friedrich AW, Bielaszewska M, Zhang WL, Pulz M, Kuczius T, Ammon A, Karch H.** 2002. Escherichia coli harboring Shiga toxin 2 gene variants: frequency and association with clinical symptoms. J. Infect. Dis. **185:**74–84.
13. **Fuller CA, Pellino CA, Flagler MJ, Strasser JE, Weiss AA.** 2011. Shiga toxin subtypes display dramatic differences in potency. Infect. Immun. **79:**1329–1337.
14. **Persson S, Olsen KE, Ethelberg S, Scheutz F.** 2007. Subtyping method for *Escherichia coli* Shiga toxin (Verocytotoxin) 2 variants and correlations to clinical manifestations. J. Clin. Microbiol. **45:**2020–2024.
15. **Reiss G, Kunz P, Koin D, Keeffe EB.** 2006. Escherichia coli O157:H7 infection in nursing homes: review of literature and report of recent outbreak. J. Am. Geriatr. Soc. **54:**680–684.