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REVIEW ARTICLE

A new health threat in Europe: Shiga toxin—producing *Escherichia coli* O104:H4 infections

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Since early May 2011, a large outbreak of enterohemorrhagic gastroenteritis and hemolytic uremic syndrome (HUS) related to infections with Shiga toxin-producing *Escherichia coli* O104:H4 (STEC O104:H4) had been reported in Germany, which marks one of the largest outbreaks ever described of the HUS worldwide. The HUS outbreak was unusual, and there were important differences between this outbreak and previous large outbreaks, primarily those of STEC O157:H7 both clinically and microbiologically. This outbreak highlights the importance of collaboration of all aspects of public health to detect the outbreak, to identify and characterize the causative agent, to find the vehicles of transmission, and to control the infection.

Since early May 2011, a large outbreak of gastroenteritis with bloody diarrhea and hemolytic uremic syndrome (HUS) related to infections with Shiga toxin—producing *Escherichia coli* O104:H4 (STEC O104:H4) had been reported in Germany.[1] As of June 30, 2011, a total of 4,137 outbreak cases have been identified globally, of which 896 cases (22%, including 33 deaths) developed the HUS, and the other 3,241 cases (78%, including 17 deaths) presented with

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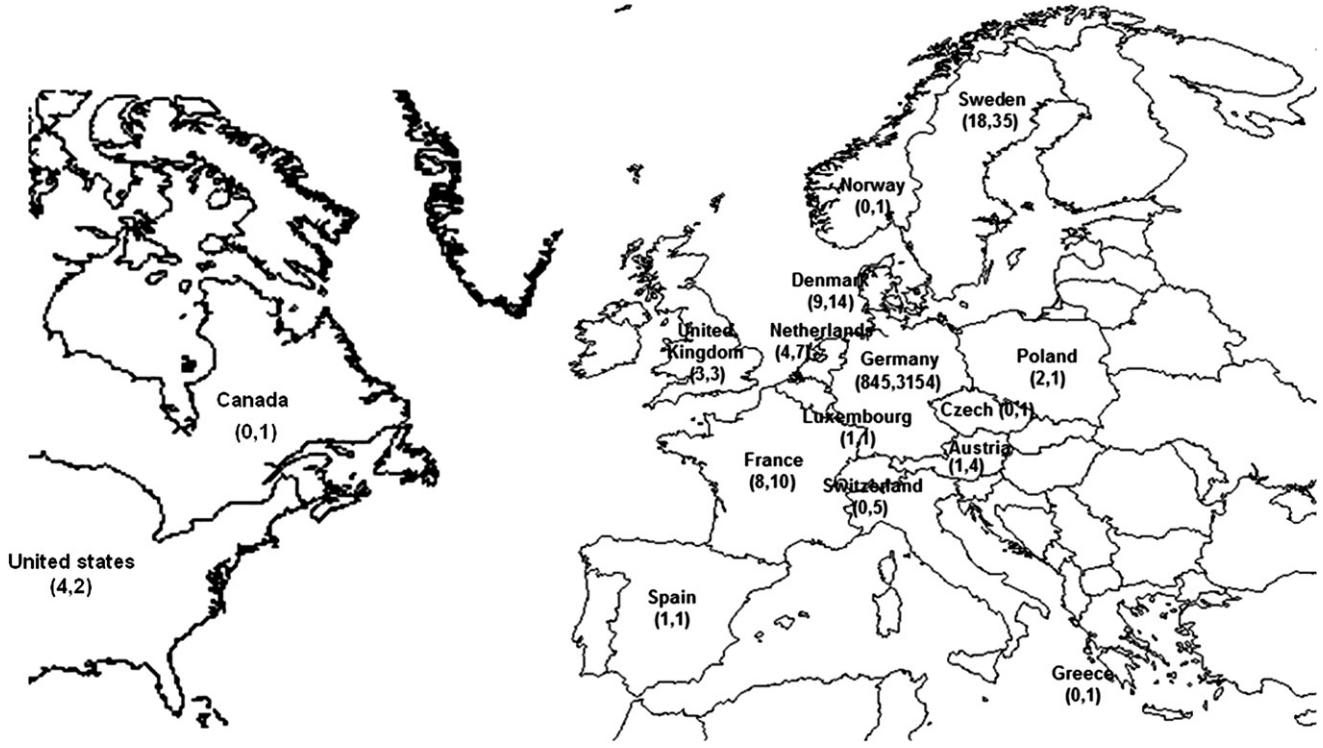

Figure 1. The case numbers of hemolytic uremic syndrome and enterohemorrhagic *Escherichia coli* gastroenteritis caused by *Escherichia coli* O104:H4, reported to the World Health Organization up to June 30, 2011, in the Europe, the United States, and Canada. The values indicate country (case number of the hemolytic uremic syndrome, case number of gastroenteritis).

enterohemorrhagic *E coli* (EHEC) gastroenteritis (Fig. 1).[2] Most of the outbreak cases have been noticed among residents in Germany (3,999 cases, 96.5%) and travelers to northern Germany in other countries.[1,2] However, Sweden and France recently reported one and four confirmed cases of *E coli* O104:H4 infection, respectively.[2] A strain *of E coli* O104:H4 from a French case was genetically related to the epidemic strain in Germany, though none of these patients in Sweden or France had traveled to Germany since May 1, 2011.[2]

The outbreak strain of the HUS was typed as an enteroaggregative STEC O104:H4.[3,4] The clinical features of infected cases were characterized by the Germany HUS Investigation Team.[3] Of all laboratory-confirmed STEC O104:H4-infected cases, 25% evolved into the HUS, and the others (75%) presented as STEC gastroenteritis. The mortality rate was 3.3% in the former and 0.5% in the latter group. The HUS, characterized by the triad of acute renal failure, hemolytic anemia, and thrombocytopenia, caused by the novel pathogen, has occurred predominantly in adults (89%), with most of the cases occurring in women (68%). The estimated median incubation period (from exposure to the onset of diarrhea) was 8 days both for cases of gastroenteritis and cases of the HUS, and the interval from the onset of diarrhea to the diagnosis of the HUS was 5 days. Bloody diarrhea (80%) and abdominal pain (78%) were the most common symptoms observed in infected cases without an apparent difference between cases of gastroenteritis and cases of the HUS, whereas vomiting (19%) and low-grade fever (7%) were less frequently reported.

The STEC O104:H4 outbreak strain has several unusual genetic factors that contribute to its ability to cause illness. Most characteristically, it simultaneously carries the virulence genes of two different diarrhea-causing *E coli* pathotypes: typical enteroaggregative *E coli* (EAEC) (*attA*, *aggR*, *aap*, *aggA*, *aggC*) and STEC (*stx2*).[3,4] The *stx2*, encoding Shiga toxin 2, was of 99% nucleotide sequence similarity to *stx2* sequence of prototypic STEC O157:H7 strain EDL933 (GenBank AE005174).[4] Aggregative adherence to intestinal epithelial cells was also demonstrated in this outbreak strain.[4] The augmented adherence to intestinal epithelium might facilitate systemic absorption of Shiga toxin in infected cases, and it explains the high frequency (25%) of progression to the HUS and the extreme consequence after infection. Because of the blended virulence profiles, it has been postulated that the outbreak strain is a typical EAEC strain that acquires the bacteriophage encoding *stx*2.[5] The other typical STEC genes, such as *stx1* (encoding Shiga toxin 1); *eae* (encoding intimin, an intestinal adherence factor); and *ehx* (encoding enterohemolysin toxin), were not present in the outbreak stain.[3]

Another distinguished microbiological feature of the STEC O104:H4 outbreak strain is the expression of extended-spectrum β-lactamase (ESBL) phenotype. Isolates in two outbreak series in Germany were resistant to penicillins (e.g. ampicillin) and third-generation cephalosporins, and all expressed the CTX-M-15-type ESBL.[3,4] Furthermore, all isolates in one series are resistant to trimethoprim-sulfamethoxazole and susceptible to fluoroquinolones (ciprofloxacin) and aminoglycosides (gentamicin or tobramycin).[4]

It is unknown when the outbreak strain acquired the gene encoding CTX-M-15-type ESBL. So far, only two STEC strains with an ESBL-producing phenotype have been reported in the literature, a CTX-M-3-type ESBL-producing STEC O26:H11 strain[6] and CTX-M-18-type ESBL-producing STEC O26:H11 strain,[7] both in 2005. Because antibiotics were not recommended in treating STEC infections,[8,9] the fact of antimicrobial resistance in the outbreak strain is not likely to impact the care of infected patients. Instead, the ESBL phenotype aids its recovery from patients' stool samples and from suspected reservoirs or vehicles of transmission by use of the culture on ESBL agars.[4]

Therefore, the clinicians should be aware of the possibility of STEC O104:H4 infections in any persons with gastrointestinal illness who has recently traveled to Germany or has contacted with ascertained cases, and send the stool specimen of suspected cases for STEC detection. The predominant cause of HUS in most parts of the world is STEC O157:H7.[8,10] In clinical laboratories, fecal STEC O157:H7 isolate is usually detected with sorbitol-MacConkey agar because of its inability to ferment sorbitol and appears colorless on sorbitol-containing agar media.[10] In contrast, the STEC O104:H4 outbreak strain, as most non-O157 STEC and human fecal *E coli* isolates, is a sorbitol fermenter[4] and appears pink on sorbitol-MacConkey agar. Therefore, to detect non-O157 STEC in suspected cases, colonies with *E coli*-like morphology should be selected on the basis of sorbitol or lactose fermentation characteristics and should be forwarded to the public health laboratory for serogroup determination by antiserum and confirmation of Shiga toxin production.[10] The presence of Shiga toxin could be detected by enzyme immunoassay for Shiga toxin production or by the polymerase chain reaction for the *stx1* and *stx2* genes.[10] Of note, the STEC O104:H4 outbreak strain harbors *stx2* but not *stx1*.[3]

To date, the HUS outbreak was unusual and there were important differences between this ongoing outbreak and previous large outbreaks, primarily those of STEC O157:H7, both clinically and microbiologically (Table 1). First, the STEC O104:H4 strain has been rarely reported in cases of the HUS. Only two STEC O104:H4 strains were reported to be associated with the HUS, one in Germany[4] and the other in Korea[11] in the past 15 years. Clinically, different from STEC O157:H7-related HUS with a preponderance of cases occurring in children,[3,8,10] cases of the HUS related to the STEC O104:H4 outbreak strain have occurred predominantly in adult women.[3] Moreover, the HUS developed in a quarter of cases infected by the STEC O104:H4 strain[3] and, in contrast, only in 8–15% of the reported cases from previous outbreaks.[8,10] A longer incubation period for the STEC O104:H4 infection was observed (8 days).[3] Microbiologically, the STEC O104:H4 strain combines the virulent properties of EAEC and STEC, exhibits the ESBL phenotype, and lacks the intestinal adherence factor intimin (encoded by *eae*), though three of them are not new but uncommon.[3,4]

In managing cases of STEC O104:H4 infection, daily laboratory testing of platelet counts, serum creatinine, and lactate dehydrogenase levels for early diagnosis of the HUS

Table 1 Summary of epidemiological, microbiological, and clinical features of two Shiga toxin–producing *Escherichia coli* strains, *E coli* O104:H4 and *E coli* O157:H7

Characteristics	*E coli* O104:H4	*E coli* O157:H7[8,10]
Areas of outbreak reported	Northern Germany, France, etc	Japan, United States, Canada, France, England, Africa, etc
Reservoirs/source of infection	Contaminated raw sprouts are suspected	Consumption of contaminated food, unpasteurized (raw) milk, water that has not been disinfected, contact with cattle, or feces of infected people
Person-to-person transmission	Minimal	Yes, in child care settings and daycare centers
Virulence genes (products)		
stx1 (Shiga toxin 1)	−	Positive in two-thirds
stx2 (Shiga toxin 2)	+	+
eae (intimin)	−	+
Sorbitol fermentation	+	−
Colony on sorbitol-MacConkey agar	Pink	Colorless
Expression of ESBL phenotype	+	−
Clinical manifestations		
Incubation period	8 d	3–4 d
Bloody diarrhea	80%	90%
Reported fever	Mostly afebrile	Mostly afebrile
Development of HUS		
Risk of infected people	25%	8–15%
Population at risk	Adult women	Children
Management	Supportive	Supportive
Antibiotic therapy	Not recommended	Not recommended
Infection control in hospitals	Contact isolation	Contact isolation

ESBL = extended-spectrum β-lactamase; HUS = hemolytic uremic syndrome.

is suggested because these laboratory values change rapidly within 24 hours and appear more sensitive than patient-reported symptoms and physical examinations.[3] Early diagnosis of STEC infection is important for proper treatment promptly. With clinical experience in the cases of O157 STEC infections, early initiation of parenteral volume expansion might decrease renal damage, prevent unnecessary procedures or treatment (e.g. surgery or corticosteroids), and improve patient outcome.[10] Antibiotic therapy might increase the risk of HUS in both children and adults infected by O157 STEC.[12,13] Based on the knowledge of management of the HUS caused by O157 STEC, the Center for Disease Control emphasizes on nonspecific supportive therapy, including hydration, and recommends current O104:H4 STEC infections not to be treated by antibiotics.[9] Antimotility agents should not be given to patients with STEC infections because these agents have been associated with an increased risk of development of the HUS.[14] Nonsteroidal anti-inflammatory agents should not be used either, which diminish renal blood flow.[8,15]

The German STEC O104:H4 infections mark one of the largest outbreaks ever described of the HUS worldwide.[1] To expeditiously find the vehicles of transmission, the German authorities conducted several consecutive epidemiological studies. They have announced that contaminated raw sprouts from one farm in Germany were the likely source of the outbreak,[16] and the sprouts produced in the farm were no longer sold. Over the past few weeks, there is a decline in the number of new EHEC infections, which may be the result of changes in the dietary consumption of cucumbers, tomatoes, and lettuce (that indirectly led to a reduced consumption of sprouts), or to a gradual disappearance of the source of infection.[16]

In Taiwan, *E coli* O157 has been found in only 0.13% of 3,062 bovine feces and in 2.56% of 78 dairy herds,[17] but no human cases of EHEC infections had been reported. As a part of the global village, the invasion of *E coli* O157 or O104 into Taiwan in the future is not surprising. Thus, this outbreak in Germany highlights the importance of collaboration of all aspects of public health to detect the outbreak, identify and characterize the causative agent, find the vehicles of transmission, and control the infection. The investigations of this outbreak and virulence analysis of the pathogen are ongoing, in hope of providing a complete picture of the outbreak and increasing our understanding of microbial pathogenesis.

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