The Worst of Both Worlds: Examining the Hypervirulence of the Shigatoxigenic/Enteroaggregative Escherichia coli O104:H4

Theodore S. Steiner
University of British Columbia, Vancouver, Canada

(See the major article by Boisen et al on pages 1909–19.)

Keywords: hemorrhagic colitis; diarrheal infections; bacterial pathogenesis.

Infectious diarrhea remains one of the most common causes of human illness. While by far the greatest burden of diarrhea is in young children in developing areas, morbidity and mortality from diarrheal pathogens can occur in anyone, anywhere, at any time. Nowhere is this broad vulnerability more evident than in large outbreaks of food-borne or water-borne diarrhea. Some of the most frightening of these outbreaks have been due to enterohemorrhagic Escherichia coli (EHEC), including the notorious O157:H7 serotype, which causes hemorrhagic colitis and its potentially lethal complication, hemolytic-uremic syndrome (HUS).

In 2011, a large, unusual, and devastating outbreak of hemorrhagic colitis due to E. coli occurred in northern Europe, affecting around 4000 people [1]. The responsible organism was not O157:H7 but rather belonged to a rarely reported serotype, O104:H4. The origin of the outbreak was ultimately traced to fenugreek sprouts imported from African seeds and produced at a farm in Germany, and the majority of cases and deaths occurred in that country. What was particularly worrisome in this outbreak was the unusually high rate of HUS, estimated at 22% and resulting in 54 fatalities. In contrast, historical HUS rates with O157:H7 are estimated at 5%–10%.

The investigation into the 2011 outbreak was unprecedented in its pace and sophistication. Somewhat surprisingly, while the responsible isolate carried the Stx2 phage (expressing Shiga-like toxin 2 [SLT2]) characteristic of EHEC, it did not possess other EHEC virulence traits such as the locus of enterocyte effacement, which allows for the characteristic epithelial adherence that EHEC shares with enteropathogenic E. coli. Instead, O104:H4 carries genes characteristic of a completely different pathotype, enteroaggregative E. coli (EAEC), including aggregative adherence fimbriae type 1 and several serine protease autotransporters (SPATEs) frequently found in EAEC [2]. The confirmation of the outbreak strain as EAEC came with whole-genome sequencing of the responsible organism and other O104:H4 isolates, confirming them as a unique clade [3]. The conclusion from these studies was that E. coli O104:H4 was an EAEC that acquired the Stx2 phage, rather than an EHEC that lost its locus of enterocytes effacement and gained EAEC virulence traits. Thus, it could rightly be called a “shigatoxigenic enteroaggregative E. coli,” or ST/EAEc, although this nomenclature is not standard.

EAEC (formerly known as “EAggEC,” to distinguish it from the now-obsolete term “enteroadherent E. coli”) was first identified as a unique E. coli pathotype in 1987, as a cause of childhood diarrhea in developing areas [4]. Since that time, EAEC has been established as a common cause of both endemic and sporadic diarrheal illness throughout the world and in multiple populations, where it has an unusual predilection for prolonged illness, particularly in vulnerable people, such as those with HIV infection, with or without progression to AIDS, and individuals with chronic malnutrition [5]. EAEC possesses a unique complement of plasmid-borne and chromosomal virulence traits, mostly under control of the AggR master regulator. The large pAA plasmid of EAEC expresses one of 4 distinct types of aggregative adherence fimbriae (AAF) along with its assembly machinery, dispersin (an antiaggregative protein that assists in AAF function), and, in some cases, a SPATE toxin known as “Pic.” Chromosomal virulence traits include another SPATE, called “Pia,” which cleaves mucin, and a type VI secretion system.
One question that has remained unanswered is why ST/EAEC O104:H4 had such a strong predilection to cause HUS. EHEC O157:H7 clearly expresses virulence traits that allow it to adhere to epithelial cells, alter their cytoskeletal and secretory physiology, and release SLTs to cause further damage. Is EAEC even more damaging? It has been difficult to answer these questions because both EHEC and EAEC are human-specific pathogens, lacking an animal infection model that mimics human disease. While EAEC will colonize some strains of mice, particularly underfed neonatal mice, the resulting illness does not strongly resemble human disease [6]. Moreover, EAEC disease in humans is somewhat heterogeneous and can produce asymptomatic colonization (albeit associated with poor growth in children), nonspecific watery diarrhea, or a more severe inflammatory diarrhea with blood and mucus. However, one encouraging recent publication addressed the virulence of O104:H4 in mouse and rabbit models, particularly when competing intestinal bacteria were reduced with ampicillin; in this study, SLT2 was, not surprisingly, the major virulence factor associated with weight loss, renal impairment, and death [7].

As a result of these limitations, a large body of work has used ex vivo and in vitro studies to create a model of how EAEC likely causes disease [8]. After ingestion and passage through the acidic gastric environment, EAEC adheres to intestinal mucosa within a mucus-containing biofilm, which can easily be demonstrated even in broth culture. Formation of the biofilm requires AAF; adherence is assisted by the Pic mucinase and dispersin. The AAF also induce an inflammatory response in intestinal epithelial cells, as does the EAEC flagellin, which activates Toll-like receptor 5 [9, 10]. This inflammatory response can be measured as increased cytokine production from intestinal epithelial cells in vitro, as well as elevated fecal cytokine levels in patients with EAEC diarrhea. In addition, some EAEC strains express enterotoxins (e.g., ShET-1) that can act as secretagogues, as well as other SPATE toxins (such as Pet) that cause cytotoxicity due to alteration of cytoskeletal elements.

In this issue, Boisen et al examine the pathogenesis of ST/EAEC O104:H4 with an eye toward explaining its hypervirulent behavior. Using an isolate from the 2011 outbreak (C227-11), they deleted and reintroduced key virulence traits, measuring relevant phenotypes. This standard approach provided some interesting and unexpected results.

Not surprisingly, deletion of the pAA plasmid abrogated the ability of C227-11 to adhere to cynomolgus monkey intestinal explants; the AA plasmid was also required for mucus loss and crypt dilatation. As C227-11 expresses AAF/I, which are less well characterized than AAF/II of prototye EAEC O42, the role of AAF/I on biofilm production and epithelial cell adherence were also measured. As expected, expression of the AggA fimbrial adhesins was necessary and sufficient for adherence and biofilm formation.

Next, the authors examined alteration of host cell physiology by EAEC virulence traits, using polarized T84 colon carcinoma cells as a model. Disruption of the actin cytoskeleton and transepithelial resistance were both dependent on AggR and AggA but not on the Stx2 phage. Indeed, a prototype O157:H7 failed to decrease transepithelial resistance, supporting the notion that it is the AAF or AAF-dependent factors rather than the Shiga toxins that are responsible for epithelial barrier loss. Infected monolayers also released the chemokine interleukin 8 (CXCL8) into basolateral media, and this release was dependent on plasmid virulence traits as well, as has been previously reported for EAEC O42.

The drop in epithelial resistance caused by AAF-expressing EAEC suggested a possible mechanism by which SLTs could penetrate the epithelial barrier. Indeed, C227-11 caused translocation of verotoxic activity (reflecting the presence of SLT2) across T84 monolayers, and the AA plasmid, AggR, and AAF were required for this full activity. Interestingly, O157:H7 was unable to translocate SLT2 in this same assay, but addition of the aag operon expressing AAF to non-pathogenic E. coli K12 allowed for SLT translocation (when recombinant SLT was added to the top of the monolayer). Together, these results provide strong evidence that the unique virulence factors of EAEC can contribute to epithelial damage and SLT translocation in vitro.

If these in vitro results are reflective of human infection, they may go a long way toward explaining why the O104:H4 outbreak was associated with unusually high rates of HUS. Interactions with AAF and the epithelial monolayer could facilitate delivery of SLT to the lamina propria, where it would have increased access to intestinal vasculature and, ultimately, to the target organs that express high levels of the SLT receptor, Gb3 (particularly, kidneys and brain). In support of this hypothesis is the observation that a proportion of O104:H4 isolates in the outbreak had lost the pAA plasmid, and patients whose initial isolate expressed the plasmid were significantly more likely to develop HUS than those infected with a pAA-negative strain [11].

Many unanswered questions remain regarding ST/EAEC O104. Is the acquisition of the Stx2 phage by EAEC a rare event or something likely to occur more frequently? Can EAEC virulence traits be exploited to develop a new treatment or vaccine for ST/EAEC? Do antibiotics affect SLT2 production in O104:H4? This latter question is particularly relevant clinically, since the role of antibiotics in the management of hemorrhagic colitis and HUS remains controversial even for the more well established O157:H7 and related EHEC organisms. While rapid eradication of the organism would be predicted to improve illness and reduce secondary transmission, certain antibiotics increase SLT production by the organism, and some case-control studies suggested a higher rate of HUS in patients with O157:H7 infection who received antibiotics, although a meta-analysis failed to demonstrate a pooled increased risk [12, 13].
One particular challenge with the O104:H4 outbreak strain is that it expressed an extended-spectrum β-lactamase, as well as trimethoprim-sulfamethoxazole resistance, limiting antibiotic choices, even if these were deemed beneficial. However, the outbreak isolates remained susceptible to fluoroquinolones, carbapenems, and azithromycin, and antibiotics were used in certain centers. Retrospective case-control studies suggested that ciprofloxacin treatment was associated with reduced rates of HUS [14] and that the use of combination antibiotics was associated with reduced risk of seizures and death in patients admitted to the intensive care unit with HUS [15]. An additional study found that azithromycin treatment was associated with a reduced duration of shedding [16]. However, these studies are limited by the potential bias of different routine practices in different treatment centers. Moreover, in vitro studies measuring toxin production after antibiotic exposure have yielded conflicting results, although they suggest decreased toxin induction in O104:H4, compared with O157:H7 [17, 18]. Unfortunately, prospective randomized studies will be the only way to know how best to treat hemorrhagic colitis due to ST/EAEC, to prevent HUS and its complications. We can only hope that opportunities for such a study will be very few and far between.

**Note**

*Potential conflicts of interest.* T. S. has received honoraria and research funding from Cubist Pharmaceuticals and research funding from Merck and Sanofi-Pasteur.

The author has submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**