The Relentless Evolution of Pathogenic *Escherichia coli*

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(See the article by Mellmann and Bielaszewska et al. on pages 785–92)

Shiga toxin (Stx)—producing strains of *Escherichia coli* (STEC) are important emerging pathogens. Unknown before the late 1970s, these bacteria are now recognized as a leading cause of sporadic cases and outbreaks of afebrile bloody diarrhea (“hemorrhagic colitis”) in industrialized countries and are the major cause of diarrheal-associated hemolytic uremic syndrome (HUS) worldwide [1]. STEC strains that are associated with these serious conditions are often referred to as enterohemorrhagic *E. coli* (EHEC) [2]. Although EHEC strains that cause HUS generally carry a number of virulence-associated determinants, the sine qua non of these bacteria is the ability to produce Stx. This toxin (also known as verotoxin, verocytotoxin, and Shiga-like toxin) occurs in 2 antigenic forms, Stx1 and Stx2, each of which has a number of allelic variants [3]. Stx1 and Stx2 are encoded by genes carried by 2 distinct λ-like bacteriophages that are integrated into the chromosome of their *E. coli* hosts [4]. The mechanisms of action of Stx1 and Stx2 are identical to each other and similar to that of ricin [3]. This mechanism of action involves inhibition of protein synthesis in susceptible cells, which, in the case of Stx, are predominantly endothelial cells lining small blood vessels. The damage these toxins inflict on these cells triggers intravascular coagulation, which leads to the major manifestations of HUS—namely, thrombocytopenia, microangiopathic hemolytic anemia with RBC fragmentation, and uremia, which is aggravated by the direct action of Stx on renal glomerular and tubule cells [2].

HUS is commonly caused by EHEC strains of serotype O157:H7, although several other serotypes of EHEC, such as O26:H11, have also been implicated in this condition. Among the accessory virulence determinants of O157:H7 and O26:H11 EHEC is a pathogenicity island known as the locus for enterocyte effacement (LEE) [5]. This pathogenicity island consists of a number of virulence-associated genes, including *eae*, which encodes an outer membrane protein adhesin and is used as a target in some diagnostic assays [6]. The LEE was first identified in enteropathogenic *E. coli* (EPEC) [7], a distinct category of pathogenic *E. coli* that causes nonspecific diarrhea in infants and young children but does not produce Stx or cause HUS [8]. The observations that EPEC appear to have predated EHEC [9], that EHEC and EPEC share a closely related LEE (which is absent from all other varieties of *E. coli*) [5], and that some EPEC strains belong to the same phylogenetic lineages as EHEC [10], have led to the suggestion that EHEC strains that carry the LEE originated from EPEC. For example, Feng et al. [11] have provided evidence that EHEC O157:H7 evolved from EPEC O55:H7 through a series of genetic events that included acquisition of the bacteriophages encoding Stx1 and Stx2 and modification of the gene encoding the O antigen. Similar events involving EPEC strains of other serotypes may have led to the emergence of other EHEC strains.

In this issue of *Clinical Infectious Diseases*, Mellmann, Bielaszewska, and colleagues [12] report that some varieties of EHEC may lose the Stx-encoding bacteriophage during the course of infection in patients with HUS. For their study, these investigators collected 2 sequential stool samples from 210 patients with HUS. The first was collected 5–14 days after the onset of diarrhea, and the second was collected 3–16 days later. Comparison of the first and second samples from each patient showed a marked reduction in the proportion of samples that yielded *stx*- and *eae*-positive (*stx+eae+*) strains, from 137 (65.2%) of the patients to 12 (5.7%). This finding confirms previous reports demonstrating the need to collect fecal samples as early in the illness as possible to establish an etiological diagnosis of HUS [13]. The novel aspect of this report, however, is that 7 patients who were originally infected with *stx+/eae+* strains of *E. coli* were found to have *stx*-negative, *eae*-positive...
(stx−/eae+) strains of the same serotype when a follow-up culture was performed several days later. Comprehensive molecular analysis of the 7 pairs of isolates suggested that, in 6 cases, the stx− strain was derived from the stx+ strain that was isolated initially. Five of these 6 strains were serogroup O26:H11, and 1 was O157:NM (nonmotile). Of interest, none of the 61 O157:H7 EHEC strains recovered in the first sample became stx−, compared with 6 of 21 O26:H11/MN isolates. This suggests that the stx-encoding phage may be less stable in O26 EHEC than in other EHEC strains.

The findings of this study are noteworthy for 3 reasons. First, the results serve as a cautionary warning to microbiology laboratories undertaking epidemiological investigations of EHEC and using diagnostic tests that rely entirely on biology laboratories undertaking epidemiological investigations of EHEC and using diagnostic tests that rely entirely on the demonstration of Stx or stx, because these tests may have negative results if the bacteria are present in a modified form. Second, the study raises the issue of whether these stx−/eae+ derivatives of EHEC are pathogens in their own right. Although both EHEC O157:H7 and O26:H11 may have evolved from EPEC strains, which by definition are stx negative and eae positive, EHEC strains differ from their purported progenitors in a number of respects, including their plasmid content. Given the important contribution that plasmid-encoded factors make to the virulence of EPEC [14], it is likely that the loss of these plasmids will have a profound influence on their ability to cause disease.

The third and most significant implication of this report is what it reveals about the evolution of pathogenic strains of E. coli. The fact that many different serotypes of EHEC of different phylogenetic lineages, including some LEE-negative strains, have been implicated as causes of HUS suggests that a variety of strains of E. coli acquired the ability to produce Stx at different times. Further evidence that EHEC have emerged on more than 1 occasion stems from the observation that LEE has inserted into the E. coli chromosome at several different locations, suggesting that it was acquired more than once [15]. Some E. coli strains that acquired the Stx phages—notably, O157:H7—have been particularly successful, as evidenced by their worldwide spread as a relatively stable clone [16]. Others, such as O26:H11, appear to be still evolving, possibly because the Stx-encoding phage in this strain is more easily converted to a virulent phage that lyses its host, thus selecting against strains that carry it.

Comparative analysis of the fully sequenced genomes of a nonpathogenic E. coli strain and that of EHEC O157:H7 has shown that these bacteria share a common genetic backbone with interspersed islands of DNA that imbue each strain with its distinctive features [17]. Many of these islands show evidence of having been acquired from other bacteria by horizontal gene transfer (e.g., via bacteriophages, transposons, and plasmids) [18]. The report by Mellmann and Bielaszewska et al. [12] reminds us that bacterial evolution is an ongoing process that undoubtedly will lead to the emergence of other successful pathogenic clones of E. coli in future.

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References