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Toxin Genotypes and Plasmid Profiles as Determinants of Systemic Sequelae in *Escherichia coli* O157:H7 Infections

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In 1987, 93 *Escherichia coli* O157:H7 isolates were collected during routine surveillance for this pathogen in the state of Washington. Toxin genotypes and plasmid profiles were correlated with the clinical sequelae of illness in 88 of the 93 patients from whom these strains were isolated. Thirteen plasmid patterns were observed among the 88 tested isolates; four patterns accounted for 82% of the isolates. Genetic probing for Shiga-like toxins (SLT) I and II demonstrated the presence of both genes in 67 (76%), SLT I alone in three (3%), and SLT II alone in 18 (20%). The hemolytic uremic syndrome or thrombotic thrombocytopenic purpura developed in seven (39%) of 18 patients infected with isolates having only the SLT II gene, while these complications occurred in only four (6%) of 70 patients infected with isolates having the other two genotypes (relative risk, 6.8; 95% confidence interval, 1.9, 26.4). This study shows that *E. coli* O157:H7 isolates systematically collected from a single geographic region over a defined time period exhibit considerable diversity in plasmid content and toxin genotype and that the toxin genotype of the infecting strain may influence the risk of developing microangiopathic sequelae.

Enteric infection with *Escherichia coli* O157:H7, a cytotoxin-producing organism first recognized as a human pathogen in 1982 [1], leads to a spectrum of illnesses that can include nonbloody diarrhea, hemorrhagic colitis, the hemolytic uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (TTP) [2]. The incidence of HUS and TTP was reported to be low in two series of sporadic cases of cytotoxic *E. coli* infections [3, 4], but in outbreaks the proportion of patients developing these complications has ranged from none [1] to 53% [5]. The reasons for this varying complication rate are unclear. *E. coli* O157:H7–related HUS and TTP are more common at the extremes of age [2], but even in reported outbreaks involving elderly patients the rates of HUS and TTP have ranged from 3% [6] to 22% [7] of cases. Factors such as host response, inoculum size, treatment, and possibly others may contribute to the highly variable rate of complications [2, 5], but strains of *E. coli* O157:H7 may also vary in their ability to produce HUS and TTP. Although *E. coli* O157:H7 isolates from multiple geographic areas and different time periods appear to be highly interrelated [8], they vary in plasmid content [9] and produce two antigenically distinct toxins known as Shiga-like toxins (SLT) I and II, also termed verotoxins I and II [10].

To assess the relationship between strain variability characteristics and clinical illness, we performed plasmid analysis and genetic probing for SLT genotype on patient isolates referred to the Washington State Public Health Laboratory as part of the first year of notifiable disease surveillance of *E. coli* O157:H7 infections [11]. Because clinical status was known, we could compare isolates from patients with and without HUS and TTP.

Methods

During 1987, bacterial isolates referred to the Washington State Public Health Laboratory from 93 different patients were confirmed as *E. coli* O157:H7. All submitted isolates were plated on MacConkey-sorbitol culture medium [12], and sorbitol-neg-

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ative *E. coli* colonies were serotyped using tube agglutination for both the O157 and H7 antigens. Isolates confirmed as *E. coli* O157:H7 were stored at room temperature on brain-heart infusion agar slants.

Clinical and epidemiologic data were collected in a standardized manner from case-patients (or their parents) with confirmed *E. coli* O157:H7 infections and from their attending physician. Hemorrhagic colitis was defined as acute grossly bloody or blood-streaked diarrhea (≥3 loose or watery stools over a 24-h period). Patients diagnosed with either HUS or TTP had the triad of acute microangiopathic anemia (hematocrit <30%) with evidence of red cell fragmentation on a peripheral smear, a platelet count of <150,000/mm^3, and serum creatinine >2.0 mg/dl.

Plasmids were analyzed by the method of Portnoy et al. [13], except that isolates were grown in "L" broth instead of brain-heart infusion broth and there were minor modifications in plasmid drying methods.

SLT I and SLT II probes consisting of radiolabeled internal fragments of the respective toxin structural genes were used to determine SLT genotype [14]. Sample and control isolates grown on nutrient agar were transferred to Whatman 541 paper, steam denatured, neutralized, and dried as described in detail elsewhere [15]. Isolates were probed by P. I. T., who was blinded to the clinical data that accompanied the strains.

Calculation of relative risks and confidence intervals was performed using the methods described by Thomas [16]. Stratified analysis, including the evaluation of interaction between variables, was done by the same methods.

Results

Eighty-eight (95%) of the 93 isolates of *E. coli* O157:H7 were available for testing; the remaining 5 had become nonviable during storage. The spectrum of illness represented by these isolates included nonbloody diarrhea in 3 (3%) patients, hemorrhagic colitis (HC) in 74 (84%), HC followed by HUS in 9 (10%), and HC followed by TTP in 2 (2%). The 5 nonviable isolates were from patients with uncomplicated HC. All isolates came from sporadic cases of illness, except for four pairs of isolates that came from members of the same household. In each of these pairs the isolates had similar plasmid profiles and toxin genotypes.

Plasmid profiles. On plasmid analysis, a plasmid of ~98 kilobase pairs (kbp) was present in all 88 isolates (table 1). In 13 (15%) isolates, no other plasmids were detected, while the remaining 75 had 1–4 additional plasmid bands. These bands ranged from 2 kbp to 87 kbp. A total of 13 plasmid patterns were observed, although four patterns accounted for 82% of the isolates. Table 1 lists the plasmid profiles of isolates from patients by disease category. No individual plasmid or combination of plasmids was significantly associated with cases of HUS or TTP.

Toxin genotype analysis. Each of the 88 *E. coli* O157:H7 isolates tested contained the gene sequence of at least one of the Shiga-like toxins. Of the isolates, 3 had only SLT I present, 18 (20%) had only SLT II, and the rest (67, 76%) had both SLT I and SLT II sequences.

The age distribution of patients by SLT genotype is shown in figure 1. While the mean and median ages of patients with isolates containing the three genotypes were similar, patients with only the SLT II sequence were more likely to be at the extremes of age. Fourteen (78%) of 18 patients whose isolates had the SLT II sequence alone were <10 y or >60 y old compared with 32 (46%) of the 70 patients whose isolates had the other two genotypes (rate ratio [RR], 1.7; 95% confidence interval [CI], 1.1, 2.2).

Isolates of *E. coli* O157:H7 containing solely the SLT II gene caused illness that progressed to HUS

Table 1. Plasmid profiles of 88 *Escherichia coli* O157:H7 isolates from patients in the state of Washington, by clinical outcome, 1987.

Plasmid profile (kbp)	HUS-TTP	Non-HUS-TTP	Total (%)
98,3	4	33	37 (42)
98,6	1	14	15 (17)
98	4	9	13 (15)
98,60,3	1	6	7 (8)
98,6,3	0	3	3 (3)
98,33,6,2	0	3	3 (3)
98,33,6	0	2	2 (2)
98,60	1	1	2 (2)
98,8,6,5	0	2	2 (2)
98,33,3	0	1	1 (1)
98,45	0	1	1 (1)
98,87,3	0	1	1 (1)
98,87,11,3	0	1	1 (1)
Total	11	77	88 (100)

NOTE. kbp = kilobase pairs, HUS = hemolytic uremic syndrome, TTP = thrombotic thrombocytopenic purpura.

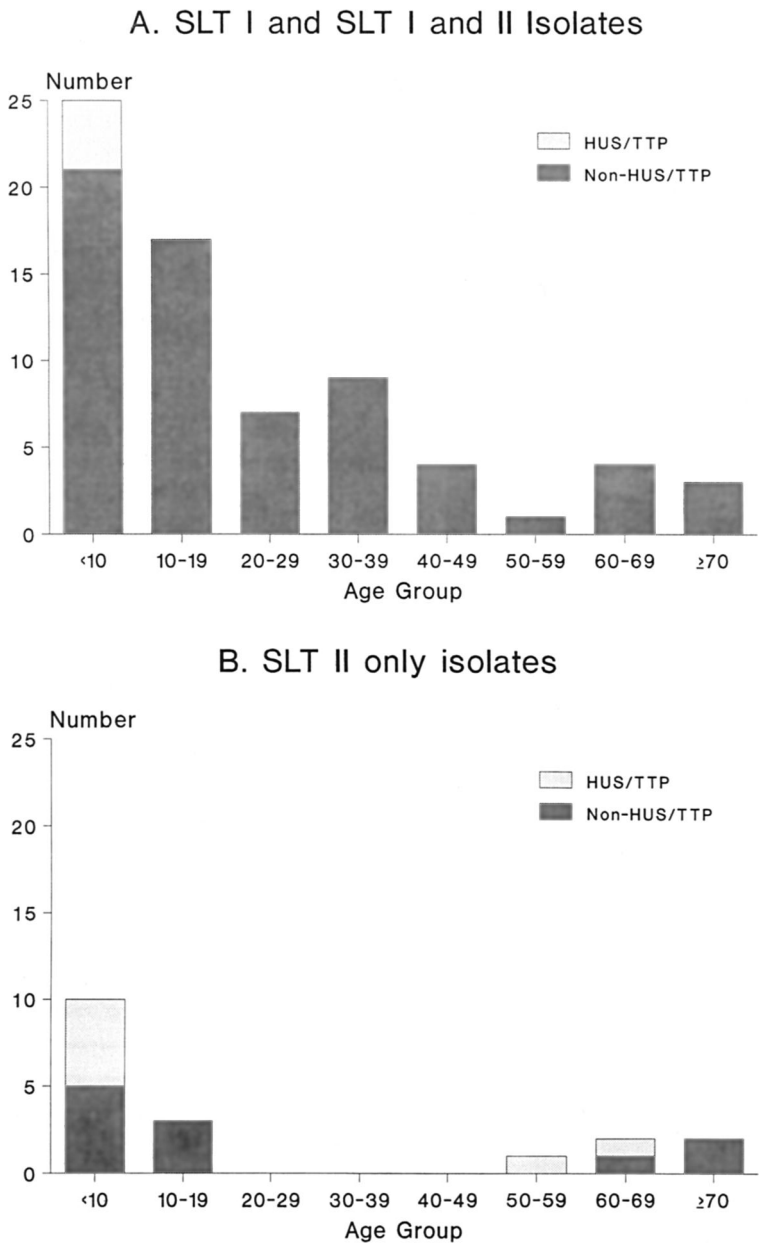

Figure 1. Age distribution of patients with *Escherichia coli* O157:H7 infections by (*A*) Shiga-like toxins (SLT) I and I and II and (*B*) SLT II only. HUS = hemolytic uremic syndrome, TTP = thrombotic thrombocytopenic purpura.

or TTP in 7 (39%) of 18 patients. In contrast, only 4 (6%) of the 70 isolates with the other genotypes were from patients with these complications (RR, 6.8; CI, 1.9, 26.4). When the analysis was restricted to patients at the age extremes indicated above (potentially the group at highest risk for HUS and TTP), a similar trend was seen; 43% (6/14) of the SLT II-only isolates were from HUS-TTP patients compared with 13% (4/32) of isolates with other genotypes (RR, 3.4; CI, 0.9, 12.8).

Since age was associated with toxin genotype and toxin genotype was associated with systemic sequelae, we examined the relationship between these two variables using the method of Thomas [16]. We controlled for one of these two variables while examining the other with systemic sequelae being the outcome of interest for both. When controlling for toxin genotype, age was not a significant risk factor for systemic sequelae (P = .08, two-tailed), but when controlling for age, a significant association

between toxin genotype and HUS and TTP was present ($P = .01$).

Antimicrobial treatment history was known in 78 of the 88 patients whose isolates were probed. Of the cases with known treatment status, 34 (44%) received at least one antimicrobial while 44 (56%) did not; 9 (53%) of 17 patients infected with isolates containing only SLT II were treated compared with 25 (41%) of 61 patients infected with the other genotypes. In both treated and untreated persons, those infected only with an SLT II organism had a higher risk of HUS and TTP. In the treated group, 4 (44%) of 9 SLT II-only isolates were from HUS-TTP patients compared with 1 (4%) of 25 isolates with the other genotypes (RR, 11.1; CI, 1.3, 440.8). In the untreated group, 3 (38%) of 8 SLT II-only isolates were from HUS-TTP patients compared with 2 (6%) of 36 isolates with the other genotypes (RR, 6.8; CI, 0.9, 58.9). Because the number of cases receiving individual agents was small, the data were not analyzed by individual antimicrobial agent.

Discussion

During the 12-mo study period, a significant association between toxin genotype and clinical outcome was found. *E. coli* O157:H7 strains with the SLT II gene alone were more likely than the other genotypes to be isolated from patients with HUS or TTP. This finding was independent of age, which is an important risk factor for these complications, and antimicrobial use. Similar trends have been noted elsewhere. As part of a study of *E. coli* O157 isolates in Great Britain, Scotland et al. [17] noted that although most isolates in their series produced SLT II alone, these isolates were most common in cases of HUS. Tarr et al. [15], in a study of *E. coli* O157:H7 isolates collected in the state of Washington during 1984–1987 (including 16 patients reported here), also noted that organisms containing solely SLT II genes were more likely to cause a diarrheal syndrome progressing to HUS or TTP than were organisms with other genotypes (79% of genotypic SLT II organisms were from HUS-TTP patients compared with 47% of organisms with the other genotypes). However, in that series, the findings did not reach statistical significance and the isolates were not collected systematically.

We observed considerable strain variation among *E. coli* O157:H7 clinical isolates drawn from a well-defined geographic area. At least 13 different plasmid profiles and three Shiga-like toxin genotype combinations were detected. Our findings probably underestimate the actual strain variation in Washington, since screening for *E. coli* O157:H7 is not a standard procedure in most clinical laboratories and the isolates referred were likely to be from more severely ill patients. Many laboratories screened for *E. coli* O157:H7 only if the stool specimen appeared bloody [11]; some investigators [3, 4] have reported that nonbloody diarrhea in sporadic *E. coli* O157:H7 infection is unusual. Thus, if clinical manifestations are strain-related, failure to detect patients with mild or atypical illness might underestimate strain variation, but as *E. coli* O157:H7 appears to be rare in cases of nonbloody diarrhea, it is unlikely that many cases were missed.

It is unclear why *E. coli* O157:H7 isolates with only the SLT II gene should be more likely to produce HUS and TTP. One possible explanation is that isolates with only the SLT II gene produce greater amounts of SLT II in vivo than do isolates with both toxin genes. Another possibility is that this SLT genotype acts as a marker for other as yet unrecognized virulence characteristics. Evidence indicates that Shiga-like toxin plays a crucial role in the pathogenesis of both the intestinal and extraintestinal manifestations of *E. coli* O157:H7 infection [18], but other factors may also be important. Lastly, differences in immunologic response to the infecting strain may play a role. Ashkenazi et al. [19] have demonstrated antibodies to SLT I, but not SLT II, in pooled commercial immunoglobulin. A brisk anamnestic response to strains producing SLT I may neutralize part or all of the systemic toxicity of the related, but distinct, SLT II. All of these hypotheses remain speculative until adequate animal models for hemorrhagic colitis with subsequent systemic microangiopathy are developed, allowing these hypotheses to be tested in a controlled fashion.

The age-related differences noted between *E. coli* O157:H7 strains could result from these strains becoming host- or food-adapted. If so, certain strains would preferentially infect certain segments of the population. An example would be a milk-specific strain associated with illness in children. Since food sources are difficult to implicate in sporadic cases, such relationships could not be assessed in our study. The absence of genotypic SLT II-only isolates in middle-aged patients is striking and may support the concept that strains are host adapted. No SLT II-only isolates were detected in patients between the

ages of 17 and 56 y, a group in which isolates with the SLT I gene were frequently found.

Five isolates had become nonviable during storage. These isolates were from patients (four adults and one child) with HC and were not geographically or temporally related. Even in the unlikely event that all of these isolates possessed only the SLT II gene, the relationship we found between this genotype and HUS-TTP would be little changed. Although storage could have altered plasmid profiles or toxin genotype, several isolates were subjected to repeated plasmid analysis over a 1-y period with no change in the profiles. Also, it is doubtful that strains from patients with HUS or TTP would lose the SLT I gene more often than isolates from other patients.

In summary, it appears that in Washington the relationship between *E. coli* O157:H7 and the extraintestinal manifestations of infection is in part determined by characteristics of the infecting strain. It is likely that bacterial and host factors both play a role in determining the sequelae of hemorrhagic colitis, since multiple clinical outcomes from a single strain of *E. coli* O157:H7 have been observed in the outbreak setting. However, our study shows that the genetic diversity of these organisms needs to be addressed in assessing the spectrum of illness in persons with *E. coli* O157:H7 infections. Further studies are needed to determine if this is also true in areas other than Washington.

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