Profile of *Escherichia coli* O157:H7 Pathogen Responsible for Hamburger-Borne Outbreak of Hemorrhagic Colitis and Hemolytic Uremic Syndrome in Washington

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We analyzed *Escherichia coli* O157:H7 isolates from stool samples of five patients who had bloody diarrhea and were infected during a large food-borne outbreak of hemorrhagic colitis in Washington state. The isolates were assessed for Shiga-like toxin profile, adherence and plasmid traits, mouse virulence, capsule, and enterohemolysin production. The profiles of the five isolates were indistinguishable from each other and similar to that of *E. coli* O157:H7 strain EDL933, an organism responsible for a similar hamburger-associated food poisoning episode in 1982.

In January 1993, a hamburger-borne outbreak of hemorrhagic colitis (bloody diarrhea) associated with ingestion of undercooked fast-food hamburgers occurred in the Seattle-Tacoma, Wash., area (2). Some of the infected individuals also developed the hemolytic uremic syndrome (HUS; 2). As of late February 1993, the Centers for Disease Control reported 477 cases of culture-confirmed *Escherichia coli* O157:H7 infection or the HUS (2). Five of the culture-positive individuals were seen at the Madigan Army Medical Center in Tacoma, Wash. All five of the patients had bloody diarrhea and had eaten hamburgers from a particular fast-food chain. The goals of this study were to develop a profile of the isolates and to compare the resultant portrait with that of enterohemorrhagic *E. coli* (EHEC) O157:H7 strain EDL933. That strain was isolated in 1982 from hamburger incriminated in the first documented EHEC fast-food chain outbreak in the United States (13, 18).

The characteristics of all of the EHEC O157:H7 strains reported to date include production of one or more types of Shiga-like toxins (SLTs; also called verotoxins; 5, 11) and reactivity with specific probes for both an attaching-and-effacing gene (*eae*; 19) and an EHEC large plasmid (8). Additional features that have been described for some EHEC strains of the O157:H7 serotype and other serotypes include capsule formation at 25°C (6) and enterohemolysin production (1). We have also found that some EHEC strains, particularly those that make SLT-II- or SLT-II-related toxins, kill orally infected, streptomycin-treated mice (9, 17). We sought to categorize the five isolates by a number of parameters to determine whether the large size of the Washington outbreak might be related to some unique attribute of the infecting strain. The results of our study are summarized in Table 1.

Isolates from five patients were shipped from the Madigan Army Medical Center to our laboratory in Bethesda, Md. They were confirmed as nonfermenters on sorbitol MacConkey agar and as members of the O157:H7 serotype by slide agglutination with antisera generously supplied by Nancy

Strockbine of the Centers for Disease Control. Several sets of experiments were then done to classify the isolates. For all of the studies, EHEC strain EDL933 was used as a control (13). Three other strains were used as controls in some of the experiments: (i) EHEC O157:H7 strain 86-24, which was isolated in Seattle in 1986 from a patient with hemorrhagic colitis (4) and kindly provided by Phillip Tarr, (ii) EHEC O91:H21 strain B2F1, which was isolated in about 1983 from a patient in Canada with the HUS and kindly supplied by Mohamed Karmali, and (iii) strain 0023, a sorbitol-nonfermenting *E. coli* isolate from a stool sample of an adult. The latter, non-O157:H7 strain was obtained at the Madigan Army Medical Center during the Washington outbreak but had no characteristics of EHEC.

The first set of experiments was designed to ascertain which types of SLTs were produced by the five isolates and whether there was more than one copy of a particular SLT gene. Cytotoxicity and neutralization studies were done as described previously (14), with the following modifications: sonically disrupted culture filtrates were serially diluted and incubated with an equal volume of polyclonal serum (diluted 1:25). The specificity of the antisera tested was α -SLT-I (F45 antitoxin), α -SLT-II (AJ65 antitoxin), or a combination of α -SLT-I and α -SLT-II. Normal rabbit serum was used as a control. Cytotoxic titers in the presence and absence of antitoxin were compared. The 50% cytotoxic dose per milliliter of the clinical isolates was $\sim 10^5$ for Vero cells. The non-O157:H7 strain produced little or no toxin (~ 40 50% cytotoxic doses per ml). The cytotoxicity was neutralized, i.e., decreased to ~ 320 50% cytotoxic doses per ml, only when both antisera were present. Therefore, all five clinical isolates produce both SLT-I and SLT-II. The control toxins were neutralized by homologous antiserum to the same degree as the lysates of the clinical isolates.

Total DNA from each clinical isolate was hybridized with probes specific for *slt-I* or *slt-II* (14). EDL933 and the non-O157:H7 strain were used as positive and negative controls, respectively. All five clinical strains showed patterns identical to that of EDL933, which contains the *slt-I* and *slt-II* genes on *EcoRI* fragments of approximately 7.5 and 4.5 kb, respectively (Fig. 1). Hybridization patterns

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TABLE 1. Characteristics of sorbitol-negative *E. coli* isolates from patients with bloody diarrhea who had eaten fast-food hamburgers in Tacoma, Wash.

Strain (patient sex, ^a age [yr])	Serotype	SLT profile	Hybridization		Mouse lethality (no. of deaths/total)	Capsule	Enterohemolysin	Adherence
			<i>eae</i> probe	EHEC plasmid probe				
1 (F, 7)	O157:H7	I, II	+	+	0/5	+	+	+
2 (F, 16)	O157:H7	I, II	+	+	ND	+	+	+
3 (M, 10)	O157:H7	I, II	+	+	0/6	+	+	+
4 (F, 40)	O157:H7	I, II	+	+	2/5	+	+	+
5 (F, 36)	O157:H7	I, II	+	+	ND	+	+	+
Controls								
EDL933	O157:H7	I, II	+	+	0/6	+	+	±
B2F1	O91:H21	IIc	—	+	5/5	+	+	—
86-24	O157:H7	II	+	+	ND	+	+	+
0023	Non-O157	None	—	—	0/5	+	—	—

^a F, female; M, male.^b ND, not determined.

were also identical to that of EDL933 when *Hind*III fragments were probed (data not shown). Therefore, each of the isolates contain one copy of the *slt*-I and *slt*-II operons. The non-O157:H7 strain (0023) showed no hybridization with the toxin probes.

The objectives of the second group of studies were to determine whether the five isolates reacted with an *eae* probe or a probe for the EHEC plasmid and to compare the plasmid migration pattern of the strains with the established pattern of an *E. coli* O157:H7 control. The *eae* probe was derived from EHEC O157:H7 strain 86-24 and was comparable to the 567-bp *Pst*I-*Sal*I internal fragment of *eae* in EDL933. The EHEC plasmid probe was the 3.4-kb *Hind*III fragment of the large plasmid (8). Total DNAs from the five isolates were digested with *Bgl*III and hybridized with the *eae* probe, while plasmid DNA was hybridized with the EHEC probe. The DNA from the clinical isolates hybridized with both probes, as did DNA from the *E. coli* O157:H7 control. *E. coli* O91:H21 strain B2F1 was probe positive for the EHEC plasmid but not for *eae*. As anticipated, DNA from the non-O157:H7 isolate did not react with either probe. Lastly, all five clinical isolates contained a large plasmid that comigrated with the large plasmid of EDL933 but none of the isolates appeared to contain a smaller (~2.5-kb) plasmid observed in the EDL933 plasmid profile.

In the third series of experiments, we assessed whether or

not the five isolates were virulent in the streptomycin-treated mouse model and whether they were able to adhere to HEP-2 cells in vitro. The experiments reported here were conducted in accordance with the principles set forth in the *Guide for the Care and Use of Laboratory Animals* (10). Three of the O157:H7 isolates and the non-O157:H7 (strain 0023) isolate were individually fed at a dose of approximately 10¹⁰ CFU to groups of five or six streptomycin-treated mice as described previously (9, 16). B2F1 and EDL933 were used as positive and negative controls, respectively. Three of the Washington isolates were fed to a total of 16 streptomycin-treated mice, and only two deaths were observed. Although both of these deaths occurred with the same isolate, this strain was indistinguishable from the other isolates in all of the other assays. In contrast, B2F1 killed all five mice. These results are consistent with previous studies in which EHEC isolates that produce SLT-I and SLT-II are essentially avirulent in mice while the SLT-IIc producer, B2F1, is virulent (9).

Isolates 1 to 5 and the controls were allowed to interact with HEP-2 cells for 6 h in a standard adherence assay (3). The cells were stained with Giemsa or fluorescein isothiocyanate-conjugated phalloidin (7) to assess the level of adherence and the fluorescence actin staining phenotype, respectively. The Washington outbreak strains adhered to HEP-2 cells and formed microcolonies like those of the positive control, EHEC strain 86-24. The Washington strains were fluorescence actin staining positive, which indicates that these EHEC strains can cause the attaching-and-effacing lesion. Strain EDL933 adhered only to the edges of HEP-2 cells and was fluorescence actin staining negative; however, previous studies have shown that this strain can cause attaching-and-effacing lesions in vivo in the gnotobiotic piglet model (16).

Finally, we tested the isolates for capsule formation and enterohemolysin production. All of the isolates, including the non-O157:H7 strain, produced a capsule when grown at 25°C on Maloney agar (6). Enterohemolysin production was detected in all of the EHEC isolates grown on tryptose blood agar plates supplemented with 5% defibrinated sheep blood and 10 mM CaCl₂ (1). In contrast, the non-O157:H7 isolate did not produce enterohemolysin.

In conclusion, the virulence attributes of the EHEC O157:H7 strain responsible for the Washington state out-

FIG. 1. Total DNA was digested with *Eco*RI and hybridized with an *slt*-I-specific probe (A) or an *slt*-II-specific probe (B). Lanes: 1, EDL933; 2, strain 1; 3, strain 2; 4, strain 3; 5, strain 4; 6, strain 0023; 7, strain 5. The numbers to the right of each panel are molecular sizes in kilobases.

break closely resemble those of the earlier outbreak strain, EDL933. That the 1993 isolates and strain EDL933 appear to have similar pathogenic traits rules against an increase in virulence of the 1993 EHEC strain as an explanation for the magnitude of the outbreak (477 cases compared with 47 patients in 1982; 13). Therefore, other factors, such as the degree of the EHEC contamination of hamburger and/or the temperature at which the hamburgers were cooked, are probably responsible for the size of the 1993 outbreak. Additionally, increased surveillance for O157:H7 infection since the 1982 outbreak has probably resulted in recognition of more cases than would otherwise have been identified. Finally, approximately 6% of the infected individuals developed the HUS or thrombotic thrombocytopenic purpura (the adult version of the HUS) in the 1993 outbreak (2). This percentage is in keeping with the 3 to 6% reported by Ostroff and colleagues (12) for development of systemic sequelae in patients infected with EHEC O157:H7 strains that make SLT-I alone or SLT-I and SLT-II but significantly below that reported for infection with SLT-II-only-producing strains (39%). The reason(s) for this differential expression of systemic sequelae is unclear but may reflect our findings that SLT-II is about 400-fold more toxic than SLT-I for mice (15) and that the expression of SLT-I in conjunction with SLT-II appears to diminish the mouse virulence of an infecting EHEC strain (9). Whatever the explanation, the one fortunate aspect of the 1993 outbreak may be that the incriminated EHEC strain produced both SLT-I and SLT-II rather than SLT-II only.

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