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Enteroaggregative, Shiga Toxin-Producing *Escherichia coli* O111:H2 Associated with an Outbreak of Hemolytic-Uremic Syndrome

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Shiga toxin-producing *Escherichia coli* O111:H2 strains from an outbreak of hemolytic-uremic syndrome showed aggregative adhesion to HEp-2 cells and harbored large plasmids which hybridized with the enteroaggregative *E. coli* probe PCVD432. These strains present a novel combination of virulence factors and might be as pathogenic to humans as the classic enterohemorrhagic *E. coli*.

Enterohemorrhagic *Escherichia coli* (EHEC) is a well-known cause of severe disease, such as hemorrhagic colitis and hemolytic-uremic syndrome (HUS) (10). The bacterium produces Shiga toxins (Stx; also known as verocytotoxins) (10), harbors large plasmids which code for production of enterohemolysin (EHEC-Hly) (24) and catalase-peroxidase (KatP) (4), and possesses the intimin-coding gene *eaeA* (27). The gene is part of a chromosomal gene cluster termed LEE, for locus of enterocyte effacement (16), which determines the production of attaching and effacing lesions on the intestinal mucosa and localized adhesion to HEp-2 cells (8, 12). Besides O157, by far the most important in human disease (10), EHEC strains belong to a restricted number of serogroups (10), among which O111 has been associated with both sporadic cases (9, 10) and outbreaks (3, 5, 20) of HUS.

During a study aimed at characterizing Stx-producing *E. coli* (STEC) O111 strains from different countries (data not shown), we found that eight strains isolated in France during an outbreak of HUS (3) showed aggregative adhesion (AA), instead of the typical localized adhesion, to HEp-2 cells (18) and possessed the genetic markers of enteroaggregative *E. coli* (EAggEC). EAggEC, defined by its aggregating pattern of adherence to Hep-2 cells (18), has been associated with protracted diarrhea in children in developing countries (6) and with cases of childhood diarrhea in Europe (9, 11, 25). AA is associated with the presence of large plasmids carrying genes coding for bundle-forming fimbriae (17) and the production of EAggEC heat-stable enterotoxin 1 (EAST1) (23). Fragments from these plasmids have been used as DNA probes (2) or PCR targets (25) for identifying EAggEC. Since AA and Stx production have never been found to be associated in *E. coli* isolates, we describe here the molecular characteristics of these unusual strains.

The *E. coli* O111:H2 strains, designated RD1 to RD8, gave negative results in the PCR analyses for the *eaeA* gene (26) and the EHEC plasmid markers *ehec-hly* (24) and *katP* (4). In addition, they did not produce hemolysin and did not hybridize with the *eaeA* (12) and EHEC (15) probes, even under low-stringency conditions. When tested in the HEp-2 cell assay (9), all these strains showed the aggregative pattern of adhesion typical of EAggEC (Fig. 1). Accordingly, they agglutinated rat erythrocytes in the presence of 0.5% mannose (17) and gave positive PCR amplification with the primer pairs which amplify a 630-bp region of the EAggEC probe (25) and the *astA* determinant of EAST1 (23). Moreover, they hybridized with the EAggEC (2) and *astA* (23) probes. The Vero cell assay (9) and the PCR analyses with Stx1- and Stx2-specific primers (21) confirmed that all the strains produced Stx2 alone. *Taq* cycle sequencing of the toxin B-subunit gene (21) showed 100% homology with the nucleotide sequence of the stx_2 B gene from the O157:H7 strain EDL933. Table 1 summarizes the characteristics of the *E. coli* O111:H2 isolates in comparison with reference EAggEC and EHEC strains used as controls in all the experiments. As previously described by Savarino and coworkers (22), the O157:H7 strain EDL933 hybridized with a probe produced by PCR amplification of the *astA* gene present in strain 17-2 (23). However, it was negative in the *astA* PCR, thus suggesting the existence of a degree of variability in the *astA* nucleotide sequences present in the different groups of *E. coli*.

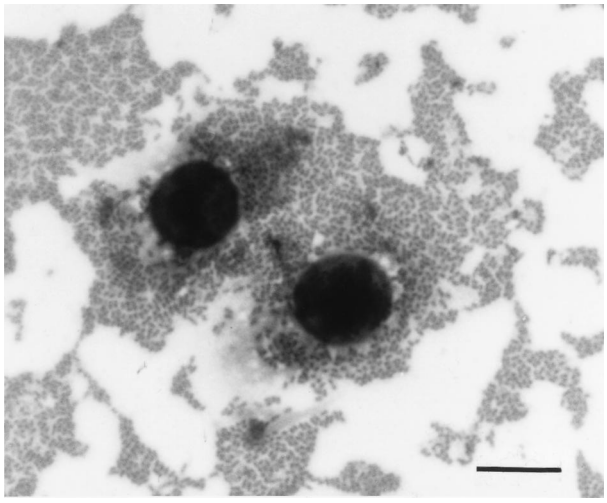

FIG. 1. Pattern of AA to HEp-2 cells of a representative STEC O111:H2 strain. Bar = 15 μm.

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TABLE 1. Virulence properties of STEC O111:H2 strains and reference EHEC and EAggEC strains

Strain	Stx production	HEp-2 cell adhesion	Positive hybridization with correlated probe				Positive PCR as defined in text			
			EAggEC probe	EAST1 gene	*eaeA* gene	EHEC probe	EAST1 gene	*eaeA* gene	EHEC-Hly gene	KatP gene
STEC O111:H2	+	Aggregative	+	+	−	−	+	−	−	−
EHEC O157 EDL933	+	Localized	−	+	+	+	−	+	+	+
EAggEC 17-2	−	Aggregative	+	+	−	−	+	−	−	−

All the *E. coli* O111:H2 strains harbored two plasmids of approximately 100 and 7 kb, respectively. Southern analysis showed that the large plasmids hybridized with the EAggEC probe but not with an stx_2 probe, which reacted only with the total cellular DNAs. These results indicated that the EAggEC gene cluster was located on the large plasmid, as in the EAggEC strain 17-2, and that the stx_2 gene was present on the chromosome, as in the EHEC strain EDL933.

Stx genes are usually phage encoded in both O157 and non-O157 EHEC (19), and a phage λ regulatory gene, designated *p*, is usually located near both the stx_1 and stx_2 genes (7). The *E. coli* O111:H2 isolates were negative in a PCR assay performed with a primer pair complementary to *p* (7) but hybridized with a probe produced by PCR amplification of the *p* gene present in strain EDL933. An attempt to induce phages was performed by UV light treatment. Lysates of the *E. coli* O111:H2 strains obtained according to the protocol described by O'Brien et al. (19) did not contain infectious phages, while strain EDL933, included as a control in all the experiments, consistently yielded lysates containing 10^4 PFU/ml. The absence of inducible phages, however, does not exclude the possibility that the stx_2 determinant is associated with a defective phage, and further work is needed to clarify this issue.

The *E. coli* O111:H2 strains described here present a combination of virulence factors found in both EHEC and EAggEC, a finding that, to the best of our knowledge, has never been described before in *E. coli* strains. These isolates can be classified as EHEC because they have been isolated from HUS patients and produce Stx. However, they do not possess the *eaeA* gene and show an aggregative pattern of adhesion to HEp-2 cells instead of the localized adhesion usually exhibited by EHEC (8, 9, 27). Stx production alone does not appear to confer human pathogenicity on STEC. In fact, most EHEC strains associated with disease in humans and cattle possess the *eaeA* determinant and EHEC plasmids detectable with the CVD419 probe (8, 9, 13, 15, 20, 27). Conversely, the *eaeA* gene and the EHEC plasmids are significantly less common among STEC strains isolated from healthy cattle (1, 13). Based on this evidence, when strains from animals or food are screened, the presence of *eaeA* is often considered to be a better predictor of the pathogenicity of STEC in humans than the Stx genes themselves. The STEC O111:H2 strains described here lack both the *eaeA* gene and the EHEC plasmid markers usually considered in diagnostic studies and should not strictly be considered EHEC. However, they have been associated with a severe outbreak of HUS (3), a typical EHEC-associated disease. In vitro these strains showed all the properties of EAggEC, and it has been reported that EAggEC cells are able to attach to human intestinal mucosa explants (14). It is therefore conceivable that the AA ability has allowed these *E. coli* O111:H2 strains to colonize the intestinal mucosa of children as efficiently as the typical *eaeA*-positive EHEC strains, and hence to cause disease.

In conclusion, *E. coli* strains possessing the novel combination of virulence factors described here, i.e., Stx production and enteroaggregative adhesion ability, might be as pathogenic to humans as the classic EHEC strains. Therefore, STEC from animal reservoirs or food should also be examined for EAggEC properties, in addition to EHEC plasmid markers and the characteristics associated with the attaching and effacing property, before excluding the possibility of their pathogenicity.

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