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Virulence Factors and Phenotypical Traits of Verotoxin-Producing *Escherichia coli* Strains Isolated from Asymptomatic Human Carriers

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Fourteen verotoxin-producing *Escherichia coli* strains isolated from stool samples of 14 different asymptomatic human carriers were further characterized. A variety of serotypes was found, but none of the strains belonged to serogroup O157. Only one isolate carried most of the virulence genes that are associated with increased pathogenicity.

The importance of the verotoxin (VT)-producing *Escherichia coli* (VTEC) group has increased since a food-borne infection caused by enterohemorrhagic *E. coli* was first described (17). Other serogroups, like O26, O111, and O103, have also been found in affected patients, in addition to the classic serovars O157:H7 and O157:H^- (3, 5, 13). Apart from the ability to produce VT, these pathogroups may possess accessory virulence factors associated with the capacity to colonize the gut, such as intimin and a 90-kbp virulence-associated plasmid (2, 12). In patients, VTEC strains are associated with watery or bloody diarrhea, hemorrhagic colitis, and the hemolytic-uremic syndrome (7, 9). Because of the lack of a national surveillance program, the incidence of these diseases and the isolation rate of VTEC in Switzerland are not known. Cattle are considered to be the main reservoir of VTEC (9, 22). Burnens et al. (4) described a Swiss prevalence of 21%. Therefore, the source of this food-borne infection was often found to be foods of bovine origin or other fecally cross-contaminated foods. Person-to-person transmission has been reported during outbreaks and may account for a significant number of sporadic cases (7, 16). An important question to address, however, is the role of asymptomatic human carriers in food-producing companies as a source of contamination. One study of Canadian dairy farm families (a group with a high level of environmental exposure) detected carriage of VTEC in about 6% of the individuals (23). The aim of this study was to isolate VTEC strains of stool samples from staff members of food-producing companies and to compare their serotype distribution and the presence of virulence attributes.

In an ongoing study of routine stool samples from staff members of meat-processing companies, 1,730 specimens from different persons in all parts of Switzerland were examined in October and November 1997 by using PCR for detection of VT-encoding genes and by culture methods for detection of other pathogens relevant to food hygiene. All samples were collected in urban areas, and each person was tested only once. The population consisted of adults without diarrhea aged between 20 and 60 years, a quarter being female.

For the VTEC assay, samples were directly plated on sheep blood agar, and after 24 h of incubation at 37°C, the colonies were washed off in normal saline. The plate eluate obtained was then evaluated by PCR with primers based on sequences targeting a region conserved between the genes for which are VT1 and VT2 complementary to nucleotides 439 to 462 and 943 to 962 of the sequence with EMBL/GenBank accession no. M19473 for the VT1-encoding gene (20) and to nucleotides 515 to 538 and 1016 to 1035 of the sequence with EMBL/GenBank accession no. X07865 for the VT2-encoding gene (8). The sequences of primers used and the cycling conditions have been described by Burnens et al. (4). Bacterial DNA was prepared by incubating 2 μl of washed-off cultures in 42 μl of double-distilled water for 10 min at 100°C. Amplifications were performed in a total volume of 50 μl containing 200 μM deoxynucleoside triphosphates, 30 pmol of each primer, 5 μl of 10-fold-concentrated polymerase synthesis buffer, and 2.5 U of *Taq* DNA polymerase (Promega) in a Perkin-Elmer Cetus DNA cycler. The amplified products were visualized by gel electrophoresis in 0.9% agarose agar stained with ethidium bromide. *E. coli* EDL933 was used in each run as a positive control, and *E. coli* U4-41 was used as a negative control. The eluate was plated again on MacConkey agar, and at least 18 single colonies each were tested by the same PCR protocol in 27 positive samples in order to obtain VTEC isolates. Only one VT-producing strain per sample was then subjected to further typing. The strains were biochemically confirmed as *E. coli* (acid production from mannitolol, *o*-nitrophenyl-β-D-galactopyranoside [ONPG] test, H_2S and indole production, and proof of urease and lysine decarboxylase). Moreover, they were tested for sorbitol fermentation and β-D-glucuronidase

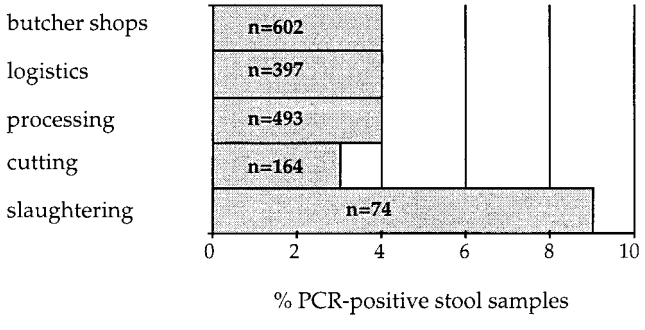

FIG. 1. Distribution frequency of PCR-positive stool samples compared to the workplaces of the employees (*n* = number of employees examined).

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TABLE 1. Characterization results of 14 VTEC strains isolated from stool samples of 14 different asymptomatic human carriers

Serotype	No. of isolates	Sex/workplace[a]	VCA[b]	Toxin type(s)	Virulence factor presence				Sorbitol/β-D-glucuronidase
					eae	60-MDa plasmid	E-*hlyA*	*astA*	
O27:H30	2	M/L	+	VT2	−	−	−	−	+
O76:H19	1	M/L	+	VT1	−	+	+	−	+
O102:H6	1	M/S	+	VT2	−	+	+	−	+
O113:H4	1	M/L	+	VT1,VT2	−	+	+	+	+
O121:H11	2	M/S	+	VT2	−	−	−	−	+
O146:H28	1	F/P	+	VT2	−	−	−	−	+
OX178:H7	1	F/BS	+	VT2	−	−	−	−	+
O rough:H2	1	M/C	+	VT1,VT2	−	+	+	−	+
O rough:H19	1	M/BS	+	VT1	−	+	+	−	+
ONT:H2	1	F/P	+	VT2	−	+	+	−	+
ONT:H21	1	M/BS	+	VT2	−	−	−	−	+
ONT:H25	1	F/P	+	VT1	+	+	+	−	+

[a] M, male; F, female; BS, butcher shop; C, cutting; L, logistics; P, processing; S, slaughtering.
[b] VCA, Vero cytotoxin production examined in the Vero cell assay.

activity on Fluorocult agar (Merck 4036) and for the hemolytic phenotype by using $CaCl_2$-washed blood agar. Production of VT by each isolated strain was confirmed by cytotoxicity tests on Vero cells (11). The genotype of the VT B subunit and the presence of the E-*hlyA*, *eae*, and *astA* genes and the 60-MDa plasmid was determined by separate PCRs. The sequences of the primers used and the cycling conditions have been previously described (6, 18, 19, 24). Serotyping of somatic and flagellar antigens was performed at the Statens Serum Institute, Copenhagen, Denmark.

The PCR product of VT-encoding genes was detected in 79 (4.6%) (61 from males, 18 from females) of the 1,730 stool samples analyzed in this study. Comparatively, we found *Salmonella* spp. in 3 (0.17%), *Campylobacter* spp. in 7 (0.4%), *Yersinia* spp. in 10 (0.69%), and *Listeria* spp. in 13 (0.75%) samples. No geographic clustering of VT gene-positive samples was found, but if the distribution frequency of PCR-positive stool samples is compared to the workplaces of the employees, the following account can be found (Fig. 1). Slaughtering stands out with 9%. Working in a slaughterhouse, where individuals would presumably encounter VTEC bacteria more frequently and in higher numbers, may put them at higher risk for carriage or excretion of these organisms. It must be considered, however, that in this area a substantially smaller number of persons ($n = 74$) could be examined. Therefore, this observation must be backed up with further examinations.

Fourteen VTEC strains isolated from stool samples of 14 different persons from all five work areas were further characterized. Serotyping yielded seven different O:H serotypes comprising seven O serogroups and seven H serogroups. Three strains were O nontypeable, and two were O rough; all strains were motile (Table 1). Compared to isolates found in patients (15), three strains (O76:H19, O113:H4, and O146:H28) isolated from asymptomatic human carriers had common serotypes, but the serotypes of these VTEC isolates are not those which have been convincingly and frequently associated with human disease. Further results of strain characterization are summarized in Table 1. Phenotypically, all 14 strains were both sorbitol and β-D-glucuronidase positive and also positive in the Vero cell cytotoxicity test. Subtyping of the VT genes of the isolated strains by using VT1- and VT2-specific primers showed that three strains possessed VT1 alone, nine strains possessed VT2 genes, and two strains possessed both VT1 and VT2. Since most patients developing the hemolytic-uremic syndrome are infected with strains that harbor the VT2 type (10, 14, 21), these findings in asymptomatic human carriers are astonishing. The *eae* gene, which is strongly correlated with symptomatic disease in humans (1), was present in only one strain of serotype ONT:H25. The 60-MDa plasmid was found in seven strains. Although E-*hlyA* was proved in seven isolates, enterohemolysin production was found in only six strains. The gene encoding EAST1, representing an additional determinant in the pathogenesis of *E. coli* diarrhea, was found in one strain. Two strains of serotypes O113:H4 and ONT:H25 harbored three of the four additional virulence factors tested.

The aim of further studies that are in progress is to isolate and characterize more strains. Moreover, asymptomatic human carriers should be tested over prolonged periods of time.

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