JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 2005, p. 452–454 Vol. 43, No. 1
0095-1137/05/$08.00+0 doi:10.1128/JCM.43.1.452–454.2005

Phenotypic and Molecular Analysis of Tellurite Resistance among Enterohemorrhagic *Escherichia coli* O157:H7 and Sorbitol-Fermenting O157:NM Clinical Isolates

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Received 21 July 2004/Returned for modification 2 September 2004/Accepted 13 September 2004

A total of 66 (98.5%) of 67 enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 strains had increased potassium tellurite (Te) MICs (32 to 1,024 μg/ml), grew on Te-containing media, and possessed Te resistance (*ter*) genes, whereas 83 (96.5%) of 86 sorbitol-fermenting (SF) EHEC O157:NM strains had Te MICs of ≤4 μg/ml, did not grow on Te-containing media, and lacked *ter* genes. Optimal detection of SF EHEC O157:NM strains requires Te-independent strategies.

Tellurite (Te)-resistant (Te^r) non-sorbitol-fermenting enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 strains cause diarrhea and hemolytic-uremic syndrome (HUS) worldwide (17), but sorbitol-fermenting (SF) EHEC O157:NM (nonmotile) strains have emerged as pathogens only in Europe (1, 6, 8, 12) and Australia (3) so far. SF EHEC O157:NM strains are not distinguishable from commensal *E. coli* strains on sorbitol MacConkey agar (SMAC), and do not grow (11) on cefixime-Te (CT)-SMAC (22), which is frequently used for selective isolation of EHEC O157:H7 strains from feces, foods, and the environment (2, 4, 5, 9, 13, 20, 21). Te^r in EHEC O157:H7 is associated with the *ter* (*terZABCDEF*) gene cluster (19), duplicated in strain EDL933 in O islands OI 43 and OI 48 (14). One of these islands was originally identified in strain 86-24 (16) as the Te^r and adherence-conferring island (16). Te^r in SF EHEC O157:NM strains has not been investigated. Because Te susceptibility (Te^s) could thwart the detection of such strains on media containing Te, we investigated Te^r and the presence of *ter* genes in a large collection of SF EHEC O157:NM clinical isolates. We compared these characteristics with those of EHEC O157:H7.

Isolation and characterization of strains. A total of 67 EHEC O157:H7 and 86 SF EHEC O157:NM strains were isolated between 1987 and 2003 from patients with HUS (n = 118) or bloody (n = 11) or watery (n = 19) diarrhea and from asymptomatic carriers (n = 5). To avoid biases from strains selected by their Te^r, only EHEC O157 strains isolated on Te-free media by methods described previously (6, 10, 11) were included in this study, and they comprise a subset of the 572 *E. coli* O157 strains recovered during this interval. The 67 EHEC O157:H7 strains belonged to Shiga toxin (Stx) genotypes stx_1 (2 strains), stx_2 (28 strains), $stx_1 + stx_2$ (6 strains), stx_{2c} (8 strains), $stx_1 + stx_{2c}$ (4 strains), and $stx_2 + stx_{2c}$ (19 strains). All 86 SF EHEC O157:NM strains contained stx_2 only. Te MICs (the lowest Te concentrations which inhibited growth) were determined using a microdilution broth method (15). Each strain was tested in duplicate and in two independent experiments using 4×10^4 to 5×10^4 CFU/well and serial twofold concentrations (1 to 1,024 μg/ml) of potassium Te (Sigma, Taufkirchen, Germany) in 100 μl of Luria-Bertani (LB) broth. The ability of EHEC O157 to grow on solid media containing the Te concentration routinely used for *E. coli* O157:H7 selective isolation was tested by inoculating 10^5 CFU from overnight LB broth cultures on CT-SMAC (potassium Te 2.5, μg/ml; cefixime, 0.05 μg/ml [Oxoid, Basingstoke, United Kingdom]) and LB agar plus 2.5 μg of potassium Te per ml (LB-Te agar). The presence of *ter* genes was determined by PCRs using primer pairs TerZ1 plus TerZ2 (*terZ*), TerA1 plus TerA2 (*terA*), TerB1 plus TerB2 (*terB*), TerC1 plus TerC2 (*terC*), TerD1 plus TerD2 (*terD*), TerE1 plus TerE2 (*terE*), and TerF1 plus TerF2 (*terF*) (19), with strains EDL933 and C600 as positive and negative controls, respectively. Genomic DNA was digested (BamHI and PstI; New England Biolabs, Frankfurt, Germany), separated in 0.6% agarose, and probed under stringent conditions with digoxigenin-labeled *terC* (19) (DIG DNA labeling and detection kit; Roche Molecular Biochemicals, Mannheim, Germany).

Te^r and *ter* presence among EHEC O157. Of 67 EHEC O157:H7 strains, 50 (74.6%) and 16 (23.9%) had high (256 to 1,024 μg/ml) or intermediate (32 to 128 μg/ml) Te MICs, respectively (Table 1). All 66 Te^r strains grew well on CT-SMAC and LB-Te agar and contained *terZ, terA, terB, terC, terD*, *terE*, and *terF* (Table 1). One EHEC O157:H7 strain (5288/91) had a Te MIC of <1 μg/ml, failed to grow on CT-SMAC and LB-Te agar, and lacked all *ter* genes (Table 1). In contrast, 83 (96.5%) of 86 SF EHEC O157:NM strains were susceptible to Te (Te MICs of ≤4 μg/ml) (Table 1). Of these 83 strains, 70 (84.3%) failed to grow on CT-SMAC and LB-Te agar and 13 strains (15.7%) were strongly inhibited on both media (<10 colonies grew after overnight incubation). All 83 Te^s SF EHEC O157:NM strains lacked *ter* genes (Table 1).

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TABLE 1. Te^r and the presence of *ter* genes among EHEC O157:H7 and SF EHEC O157:NM strains

Serotype	Total no. of strains	SF[b]	No. (%) of strains with a characteristic									
			Te MIC (μg/ml) in range of:				Robust growth[c] on:		No or strongly reduced growth[d] on:		*ter* genes[e]	
			256–1024	32–128	2–4	<1	CT-SMAC	LB-Te agar	CT-SMAC	LB-Te agar	Present	Absent
O157:H7	67	0	50 (74.6)[f]	16 (23.9)[f]	0 (0)	1 (1.5)	66 (98.5)	66 (98.5)	1 (1.5)	1 (1.5)	66 (98.5)[g]	1 (1.5)
O157:NM[a]	86	86	1 (1.2)[f]	2 (2.3)[f]	40 (46.5)	43 (50.0)	3 (3.5)	3 (3.5)	83 (96.5)	83 (96.5)	2[h] (2.3)[g]	84[i] (97.7)

[a] NM, nonmotile.
[b] SF, sorbitol fermentation as determined after overnight incubation on SMAC agar.
[c] Robust growth, >1,000 colonies of normal size per plate.
[d] No growth, no colonies grown; strongly reduced growth, <10 colonies reduced in size present after overnight incubation.
[e] As determined by PCRs targeting *terZ*, *terA*, *terB*, *terC*, *terD*, *terE*, and *terF* (19); present, all genes investigated were present; absent, all genes investigated were absent.
[f] Tellurite resistance (Te^r): EHEC O157:H7 versus SF EHEC O157:NM, $P < 0.000001$ by the χ^2 test.
[g] Presence of *ter* genes: EHEC O157:H7 versus SF EHEC O157:NM, $P < 0.000001$ by the χ^2 test.
[h] Two of three Te^r strains; the third was negative for *ter* genes.
[i] 83 Te^s strains and 1 Te^r strain.

Two of three Te^r SF EHEC O157:NM isolates (3226/98 and 3323/98) had Te MICs of 128 μg/ml, grew well on CT-SMAC and LB-Te agar, and contained all *ter* genes; the remaining Te^r (MIC, 256 μg/ml) SF EHEC O157:NM strain (4180/97) had no *ter* genes (Table 1).

Southern hybridization. *terC* was carried on a ca. 9-kb DNA fragment in *ter*$^+$ SF EHEC O157:NM strains 3226/98 and 3323/98 (Fig. 1, lanes 6 and 7), on a ca. 6.3-kb DNA fragment in strain EDL933 (lane 1), and on no DNA fragments in strains 5288/91, 4180/97, and *ter*-negative SF EHEC O157:NM strain 493/89 (lanes 3 to 5).

Effect of Te^r on detection of EHEC strains. Our study provides for the first time a basis for the inability of SF EHEC O157:NM to grow on CT-SMAC (11), which was until now only speculated to be caused by their Te^s (12, 16). In contrast to EHEC O157:H7, almost all SF EHEC O157:NM strains lack *ter* genes and are Te^s. Low Te MICs for SF EHEC O157 strains and the comparable growth inhibition of these isolates on CT-SMAC and LB-Te agar suggest that Te, and not cefixime, is the growth-inhibiting component in CT-SMAC. The Te^r and Te^s correlated with the presence and absence, respectively, of *ter* genes in all but one of the 153 EHEC O157 strains investigated. The single Te^r, *ter*-negative SF EHEC O157:NM strain (4180/97) is currently being investigated for other presently known mechanisms of Te^r (18). Interestingly, Southern hybridization suggests that the genomic positions of *terC* differ in the two *ter*$^+$ SF EHEC O157:NM isolates and EHEC O157:H7 strain EDL933 (Fig. 1). Studies are under way to determine if the *ter* genes in these SF EHEC O157:NM strains are clustered, similar to those in EDL933 (14), and to determine the genomic location of the *ter* cluster as well as its copy number. Also, further studies should clarify the reasons for the substantially lower frequency of Te^s found among central European EHEC O157:H7 isolates (1.5%) than among North American *E. coli* O157:H7 (20%) (19). Taken together, our data demonstrate a significant difference between EHEC O157:H7 and SF EHEC O157:NM in the frequency of Te^r and *ter* genes, demonstrate a diversity among SF EHEC O157:NM strains as far as the presence of *ter* genes is concerned, and suggest that other mechanisms of Te^r exist in a minority of such strains. However, most importantly, our data clearly indicate that, because of their Te^s, most SF EHEC O157:NM strains are missed by strategies currently used for the isolation of EHEC O157:H7 strains in many clinical laboratories. A similar observation has been reported for a subset of other Te^s EHEC strains (5). The selectivity offered by incorporating Te into agar media, while appropriate for isolating *E. coli* O157:H7 (the most important EHEC serotype worldwide), hinders the assessment of the geographic distribution, medical significance, and epidemiology of SF EHEC O157:NM strains, which in Germany are the second most common cause of HUS (6). Detection opportunities that do not select against SF EHEC O157:NM and, optimally, specifically target these organisms (7) are needed to answer the question about the relative significance of both EHEC O157 pathogens in human diseases.

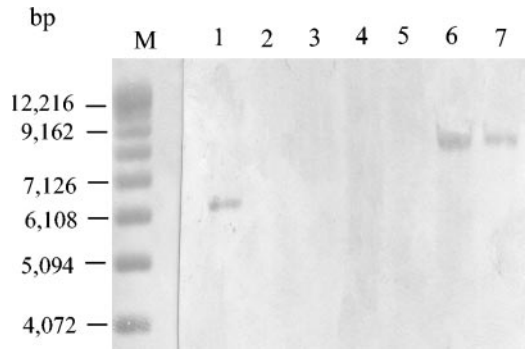

FIG. 1. Hybridization of BamHI-PstI-digested genomic DNA from EHEC O157 strains and controls with the *terC* probe. M, molecular weight marker (1-kb DNA ladder; Gibco-BRL). In lanes 1 to 7, the following strains are displayed (serotype, Te-MIC in micrograms per milliliter, and the presence of *ter* genes as detected by PCR are given in parentheses): lane 1, EDL933 (O157:H7, 128, *ter*$^+$); lane 2, C600 (not available; <1; *ter* negative) (negative control); lane 3, 5288/91 (O157:H7; <1; *ter* negative); lane 4, 4180/97 (SF O157:NM; 256; *ter* negative); lane 5, 493/89 (SF O157:NM; <1; *ter* negative); lane 6, 3226/98 (SF O157:NM; 128; *ter*$^+$); lane 7, 3323/98 (SF O157:NM; 128; *ter*$^+$). Two *terC* copies detected in EDL933 after DNA separation by pulsed-field gel electrophoresis (19) were not distinguishable by conventional gel electrophoresis.

This study was supported by grant from the Bundesministerium für Bildung und Forschung (BMBF) Project Network of Competence Pathogenomics Alliance "Functional genomic research on enterohaemorrhagic *Escherichia coli*" (BD no. 119523) and by NIH grant R01 AI47499.

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