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Sorbitol-Negative Phenotype among Enterohemorrhagic *Escherichia coli* Strains of Different Serotypes and from Different Sources

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Enterohemorrhagic *Escherichia coli* (EHEC) strains detected with DNA probes (for virulence plasmid and Shiga-like toxins) from subjects with hemolytic-uremic syndrome ($n = 19$) or diarrhea ($n = 41$) or asymptomatic carriers ($n = 29$) were examined for sorbitol fermentability, as were enterotoxigenic ($n = 40$), enteropathogenic ($n = 40$), and enteroinvasive ($n = 40$) *E. coli* and urinary tract infection ($n = 40$) strains and normal flora *E. coli* strains ($n = 40$). Sorbitol negativity was common only in EHEC, particularly among strains from severe clinical infections. All 19 EHEC strains from patients with hemolytic-uremic syndrome, irrespective of O:H serotype or Shiga-like toxin genotype, were sorbitol negative.

In the 1980s, enterohemorrhagic *Escherichia coli* (EHEC) emerged as a recognized cause of hemorrhagic colitis and milder forms of diarrheal illness and as the major etiologic agent responsible for the hemolytic-uremic syndrome (HUS) (7, 11–13). EHEC elaborate potent phage-encoded cytotoxins called Shiga-like toxins (SLTs) (24) or verotoxins (23) harbor a ca.-90-kb virulence plasmid associated with expression of novel fimbriae (10) and produce a protein encoded by a chromosomal gene (*eae*) (27) that is involved in intimate attachment to enterocytes and effacement of microvilli of colonic mucosa in animal models. While the prototype EHEC serotype is O157:H7, a number of other fairly common EHEC serotypes are recognized as causes of HUS and hemorrhagic colitis, including O26:H11, O111:H8, O113:H21, O145:NM, O5:NM, and O128:NM, among others (1, 3, 11–13).

A well-described phenotypic characteristic of the prototype O157:H7 EHEC strain and of other early disease-associated O157:H7 isolates is their inability (or delayed capacity) to ferment sorbitol (4, 17, 20). Clinical microbiologists capitalized on this property by designing a sorbitol-MacConkey's agar medium (17, 20) and other media (4) to screen for non-sorbitol-fermenting *E. coli* isolates such as the O157:H7 clones prevalent in North America (9, 17, 19, 20, 25).

Decades before HUS emerged as an important pediatric disease in North America, it was the major cause of acute renal failure in infants and toddlers in the cone of South America, being hyperendemic in Argentina (5) and endemic in Chile (3, 4). Once the association between EHEC and HUS was demonstrated in North America and Europe, it was subsequently shown that EHEC was also the etiologic agent responsible for HUS in Argentina and Chile as well (3, 16, 22). In Chile, where more broad-based screening methods have been utilized to identify EHEC predicated upon detecting strains that carry the phage genes encoding SLTs and the 90-kb virulence plasmid, a large proportion of EHEC strains incriminated in association with cases of HUS is noted to be of well-recognized EHEC serotypes other than O157:H7 (3); O26:H11 and O111:H8 EHEC strains are particularly common. This has been even more evident among strains collected in the course of field studies of diarrheal illness (14). On the basis of these observations, we pondered how many EHEC strains might have been missed had our studies been limited to detecting strains among colonies screened for on the basis of inability to ferment sorbitol. Similarly, we wondered about the prevalence of the sorbitol-negative phenotype among *E. coli* strains that belong to distinct pathogenic categories other than EHEC.

In clinical and epidemiologic field studies of diarrheal disease and HUS that we have carried out in Chile, EHEC and other categories of diarrheagenic *E. coli* have been detected by means of DNA probes that detect virulence genes specific for one or another category of diarrheagenic *E. coli* (6, 14). EHEC hybridizes with the pCVD 419 probe detecting the 90-kb EHEC virulence plasmid (15) and with probe SLT-I or SLT-II, or both (18). Enteropathogenic *E. coli* (EPEC) hybridizes with the EPEC adherence factor probe (6, 14); enterotoxigenic *E. coli* (ETEC) hybridizes with probes for heat-labile or heat-stable enterotoxins (or both) (6, 14); enteroinvasive *E. coli* (EIEC) hybridizes with a probe for the enteroinvasive virulence plasmid (6, 14).

From our collection (stored in Dorset egg medium at room temperature), we examined strains (one colony per subject) from 89 individuals from whom EHEC strains were isolated, including patients with HUS, patients with colitis or milder

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TABLE 1. Sorbitol-negative phenotype among *Escherichia coli* strains of different categories and clinical sources

Category of *E. coli*	No. of strains	No. of strains (%) with sorbitol-negative phenotype[a]
EHEC (all sources)	89	60 (67.4)[1]
HUS	19	19 (100)[2]
Diarrheal illness	41	28 (68.3)[3]
Asymptomatic	29	13 (44.8)[4]
ETEC	40	3 (7.5)[5]
EPEC	40	5 (12.5)[6]
EIEC	40	6 (15.0)[7]
UTI	40	0 (0.0)[8]
Normal flora	40	2 (5.0)[9]

[a] Statistical values: for *2* versus *3*, $P = 0.0054$; for *2* versus *4*, $P = 0.00003$, Fisher's exact test; for *1* versus *2*, *3*, *4*, *5*, *6*, *7*, *8*, or *9*, $P = 0.001$ for each comparison.

diarrhea, and subjects with asymptomatic infection. We also examined strains from 40 subjects each whose stool cultures yielded EPEC, ETEC, or EIEC and from 40 women with *E. coli* urinary tract infections (UTI). Lastly, putative normal flora *E. coli* strains that were negative with all DNA probes and were isolated from 40 healthy control subjects were also examined.

E. coli colonies were subcultured onto Trypticase soy agar (BBL, Cockeysville, Md.) containing 1% sorbitol and examined for the presence of clear colonies (nonfermenters) after 18 h of incubation at 37°C. O:H serotyping was performed by standard tube agglutination methodology (14).

The proportion of strains within each of these categories of *E. coli* that was unable to ferment sorbitol was highest for EHEC (67%) and lowest for UTI (0%) and normal flora strains (5%) (Table 1). In the other categories of diarrheagenic *E. coli* examined (ETEC, EPEC, and EIEC), a lower proportion of the strains (from 7.5 to 15%) was sorbitol negative. Sorbitol nonfermenters were significantly more common among EHEC strains compared with all other categories of *E. coli* examined (overall chi-square test, $P < 0.001$; chi-square test or Fisher's exact test for each individual comparison of EHEC versus other categories also yields $P < 0.001$; the latter comparisons involved a Bonferroni correction for multiple comparisons).

Among the 89 EHEC strains, the sorbitol-negative phenotype was first analyzed in relation to serotype, irrespective of the origin of the strain. Twenty-five of the 89 strains fell within O serogroups previously recognized as being important in association with HUS in Chile, including O157, O26, O111, and O55 (4). The sorbitol-negative phenotype was observed in 7 of 7 (100%), 10 of 12 (83%), 3 of 5 (60%), and 1 of 1 (100%) isolates of these serotypes, respectively. Of the remaining 64 strains belonging to other O serogroups or nontypeable, 55% were sorbitol nonfermenters.

A much more revealing analysis was that of the prevalence of the sorbitol-negative phenotype of EHEC strains in relation to their clinical origin (Table 1). An interesting gradient of positivity was observed. Notably, all 19 EHEC strains derived from the 19 patients with HUS were sorbitol negative, irrespective of serotype. In contrast, only 45% of the EHEC strains from individuals with asymptomatic infection exhibited the sorbitol-negative phenotype ($P = 0.00003$ versus HUS strains); intermediate were EHEC strains isolated from subjects with diarrhea, 68% of which were sorbitol negative ($P = 0.0054$ versus HUS strains).

The genotypic and phenotypic characteristics of the 19 EHEC strains isolated from patients with HUS are shown in Table 2. Thirteen of the 19 strains fall within four O serogroups: O157 ($n = 7$), O26 ($n = 3$), O111 ($n = 2$), and O55 ($n = 1$). By definition, all strains possessed the 90-kb EHEC plasmid and carried genes for expression of at least one SLT. The two main toxin types were strains encoding both SLT-I and SLT-II ($n = 9$) or only SLT-I ($n = 9$). Within the most common serogroup, O157, the toxin type was heterogeneous among the seven Chilean strains; four strains were positive for SLT-I only, one was positive for SLT-II only, and two strains expressed both toxins.

TABLE 2. Characteristics of EHEC strains from 19 patients with HUS

Case identifier	Sorbitol phenotype	Positivity with DNA probes:			Serogroup
		Plasmid	SLT-I	SLT-II	
SA-3	Negative	+	+	+	O157
SA-5	Negative	+	+	+	O157
SA-2	Negative	+	+	−	O157
S-32	Negative	+	+	−	O157
S-9	Negative	+	+	−	O157
S-11	Negative	+	+	−	O157
S-10	Negative	+	−	+	O157
SA-1	Negative	+	+	+	O26
SA-4	Negative	+	+	+	O26
SA-7	Negative	+	+	−	O26
SA-6	Negative	+	+	+	O111
SA-8	Negative	+	+	−	O111
S-12	Negative	+	+	+	O55
S-13	Negative	+	+	−	Nontypeable
S-1	Negative	+	+	−	Nontypeable
S-5	Negative	+	+	+	Nontypeable
S-15	Negative	+	+	+	Nontypeable
S-20	Negative	+	+	+	Nontypeable
S-30	Negative	+	+	−	Nontypeable

What makes this study of sorbitol phenotype particularly interesting is that the EHEC isolates in Chile were originally detected by means of DNA probes that identify virulence properties (3, 6, 14), irrespective of serotype or fermentation characteristics. Perhaps the most intriguing observation is that EHEC isolates recovered from 19 patients with HUS were all sorbitol negative, even though they comprised a number of different serotypes of which only seven isolates were O157:H7. Lower prevalences of sorbitol-negative phenotype were observed among EHEC strains from patients with diarrhea or asymptomatic carriers. These results suggest that testing for sorbitol negativity may be a highly sensitive and predictive assay for screening *E. coli* from patients with bloody diarrhea or HUS. The observation that a small percentage of ETEC, EIEC, EPEC, and UTI strains also do not ferment sorbitol further indicates that screening with sorbitol-MacConkey's agar is likely to be much more useful among patients with distinct clinical syndromes such as bloody diarrhea or HUS. In contrast, in nonclinical surveys of animals, food products, and environmental samples, screening for sorbitol-negative coliforms may be less practical because of the higher background prevalence of such strains in those instances.

The sensitivity of sorbitol fermentation as a screening assay may vary in different geographic areas. For example, Gunzer et al. (8) used DNA probes to detect *E. coli* carrying SLT phages in stool cultures of 26 of 104 HUS patients in Germany; 14 of these patients shed SLT-II-positive O157:H strains that were found to ferment sorbitol. It was not reported whether the German strains also carried the EHEC virulence plasmid. If they did, we would conclude that in some geographic areas

screening of *E. coli* strains from patients with hemorrhagic colitis or HUS based on sorbitol fermentation may occasionally fail to detect some EHEC strains. Similarly, sorbitol-fermenting EHEC of serogroup O111 associated with HUS has recently been observed in Australia.

Nevertheless, the fact that all the EHEC strains isolated from our Chilean patients with HUS were sorbitol negative, irrespective of O:H serotype, leads us to ponder the significance of this phenotype. Is this a marker for a chromosomal locus harboring virulence genes that are in some way involved in the pathogenesis of HUS? Or rather does this phenotype denote strains of related clonal origin that carry shared plasmid, chromosomal, and phage-encoded virulence genes (26)? These would appear to be questions worthy of further study.

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