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Importance of Testing Stool Specimens for Shiga Toxins

Shiga toxins (ST) I and II, elaborated by serotypes of *Escherichia coli* O157:H7 (ECO157), are well established as the major cause of hemolytic uremic syndrome (HUS) and hemorrhagic colitis. According to the Centers for Disease Control and Prevention (CDC), at least 200 deaths and 20,000 illnesses in the United States are annually attributed to ST-producing *E. coli* (STEC) organisms belonging to the O157:H7 serotype. However, it is indisputable that serotypes other than O157 also cause clinical illness identical to that of STO157, and more than 50 STECs are believed to exist. The STECs other than O157 (STNO157) are widely prevalent outside the United States (5, 7, 10) but are believed to be at very low prevalence in this country. For this reason, the majority of the laboratories in this country do not search for STNO157. The delayed advent of commercially available assays for ST may also have contributed to a lack of testing for STNO157. It is appropriate to quote Acheson and Keusch (2) in this regard: "We cannot let ourselves be complacent in thinking that *E. coli* O157:H7 is the only Shiga-toxin producing microorganism that can cause problems." At Inova Fairfax Hospital, we began to test for ST in June 1995. The purpose of this paper is to show that there is a much greater incidence of STNO157 than was previously suspected.

All stool specimens submitted for bacterial culture were inoculated onto sorbitol MacConkey agar (SMAC) in addition to media for isolation of the usual enteric pathogens. The ST assays were performed only on specimens exhibiting one of the following characteristics: liquid, semiliquid, mucous, or bloody. Additionally, specimens for ST testing were inoculated into MacConkey broth and incubated overnight. Early in this study, STO157 was identified by biochemical tests, latex agglutination, and fluorescence antibody stain, but the ImmunoCard STAT *E. coli* O157:H7 (Meridian Diagnostics, Cincinnati, Ohio) later replaced the fluorescence antibody staining procedure. The ST assay was performed by using the Premier EHEC kit (Meridian Diagnostics) following the manufacturer's instructions. The test kit contains microwell test strips coated with monoclonal anti-ST I and II. The toxin assay was performed directly from fresh stools and from MacConkey broth cultures. Isolates of STO157 and STNO157 were submitted to the Commonwealth of Virginia, Division of Consolidated Laboratory Services (DCLS) for confirmation of STO157 or STNO157. The STNO157 isolates were referred to CDC by DCLS for serotyping. In the past six and a half years, the following enteric pathogens (with numbers of cases in parentheses) were isolated from our 660-bed community tertiary care hospital: *Salmonella* (125), STEC (65), *Shigella* (58), *Campylobacter* (46), and *Vibrio parahemolyticus* (4). All of the STEC isolates referred for confirmation were correctly identified as either STO157 or STNO157. Among the 65 patients with STEC, 45 had *E. coli* serotypes belonging to STO157 whereas 20 (31%) had isolates that were found to be STNO157 with the following distribution (serotypes in parentheses): 4 (O45:H2), 3 (O26:H12), 3 (O103:H2), 3 (O111:NM), 3 (O153:H2), 2 (O88:H25), 1 (O145:NM), 1 (O96:H9). There were four HUS cases (three children and one adult) with no fatality, and all were caused by STO157. The majority of STO157 patients (84%) exhibited bloody stools, while only 45% of STNO157 patients produced bloody stools (Table 1). The SMAC plates missed four STO157 specimens, but subculturing the broth enabled the isolation of STO157. There was only one specimen from which broth culture failed to yield a positive ST reaction; however, the SMAC showed a few sorbitol-negative STO157 colonies. The ST assay from direct stools and broth cultures yielded 42 positives of 65 samples (65%) and 64 positives of 65 samples (98%), respectively (Table 1).

In Europe and South America, more than 50 serotypes of STNO157 are known to be associated with outbreaks of HUS and hemorrhagic colitis. In a Belgian study involving 10,242 stool samples, the isolation rate of STEC was 1%, of which 38% were ECO157 and 62% were STNO157 (2). In addition, STEC was isolated from 13 patients among 468 gastroenteritis cases in a German pediatric hospital (5) in which 2 were identified as ECO157 and 11 as STNO157, whereas in France (7) 6 of 69 HUS cases were caused by STNO157.

In the United States, the publicity of STNO157 cases (1, 4, 7, 8) has been limited, contributing to an underestimation of its clinical importance. Moreover, the CDC did not underscore its importance until last year at the 101st Annual Meeting of the American Society for Microbiology. In 1994 the U.S. Department of Agriculture's Food Safety and Inspection Service (FSIS) introduced a ground-beef testing program for *E. coli* O157:H7. Interestingly, in January 2002, the American Society for Microbiology's Public and Scientific Affairs Board Committee on Agriculture and Food Microbiology submitted a recommendation to FSIS to include the testing for STNO157.

We do not know the incidence of STNO157 in this country due to a lack of consistent testing. However, our data (Table 2) from northern Virginia show that 31% (20 of 65) of STECs were attributed to STNO157 and 45% (9 of 20) of those patients produced bloody diarrhea. Without the ST assay, the diagnoses of 25 patients (5 with STO157 and 20 with STNO157) would have been missed.

In our institution, physicians are immediately notified once ST is detected (3 to 30 h) directly from stools or broth cultures. In such cases, patients are often discharged in 1 to 2 days without antibiotic treatment or invasive procedures unless complications such as HUS and dehydration occur. In 1997, a liver transplant patient presented with a severe hemorrhagic colitis in the late evening. The direct stool ST assay performed the next morning due to the bloody appearance of the stool was strongly positive, establishing the etiology in 10 h. This isolate was identified as STNO157 and was later confirmed as O26:H11. Our rapid diagnosis prevented further exploratory procedures and longer hospitalization.

Not every laboratory has sufficient resources to incorporate ST assays in addition to the use of SMAC plates. However, an attempt should at least be made to test for ST when bloody stools or suspected cases do not result in the isolation of O157:H7 on SMAC. Since STNO157 serotypes are sorbitol fermenters and have no unique phenotypic markers, the toxin assay is the only method of detection available to clinical laboratories.

Another reason for which we are advocating the use of toxin in addition to the use of SMAC is the relatively low sensitivity of SMAC for the detection of STO157. Visible sorbitol-negative colonies do not always prevail over the sorbitol-positive enteric flora. The presence of only a few colonies is often

TABLE 1. Profiles of patients and Shiga toxin-producing *E. coli*

E. coli serotype	No. of patients (%) with:				
	Bloody stools	HUS	Positive assay result		
			Growth on SMAC	Shiga toxin	
				Stools	Broth
O157:H7 ($n = 45$)	38 (84)	4	40 (89)	24 (53)	44 (98)
Non-O157 ($n = 20$)	9 (45)	0		18 (90)	20 (100)

obscured by the overwhelming number of sorbitol fermenters. During this study, 5 of 45 (11%) specimens did not exhibit sorbitol-negative colonies on SMAC, but the isolation of STO157 colonies was possible by subculturing the ST-positive broth. The sensitivity of SMAC plating reported by investigators ranges from 50 to 82.5% (6, 9, 11). Since most laboratories are using only SMAC as the primary plate medium, one would not recognize its limitation unless another method, such as the toxin assay or PCR, is employed concomitantly. Generally, the use of SMAC is perceived as the "gold standard" for the isolation of STO157, although our results as well as other findings demonstrate otherwise.

The existence and prevalence of the sorbitol-positive STEC have not been identified in this country, for no one has actively searched for it. In the Czech Republic (3), however, there were two cases of HUS caused by sorbitol-positive O157:H7, and this strain is also believed to be widespread in Germany, with cattle being a possible reservoir. Again, the toxin assay is currently the only method of detecting such strains due to the lack of phenotypic markers. In conclusion, our data clearly demonstrate that STNO157 is more prevalent than we were aware of, leading us to believe that the use of SMAC alone for detection may be insufficient.

TABLE 2. Annual incidence of Shiga toxin-producing *E. coli*

Yr	No. of patients	
	O157:H7	Non-O157
1995 (7 mos)	5	6
1996	6	1
1997	4	4
1998	11	2
1999	3	2
2000	10	2
2001	5	2
2002 (3 mos)	1	1
Total	45	20

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