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Screening for *Escherichia coli* O157:H7—a Nationwide Survey of Clinical Laboratories

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The Association of State and Territorial Public Health Laboratory Directors has recommended that clinical laboratories screen at least all bloody stools for *Escherichia coli* O157:H7. We contacted the microbiology supervisors of 230 randomly selected clinical laboratories in the United States and administered a standardized questionnaire regarding stool culture practices. Of the 129 laboratories that performed stool cultures, only 70 (54%) reported screening either all stools or all bloody stools submitted for culture for *E. coli* O157:H7.

Escherichia coli O157:H7 was first identified as a human pathogen in 1982, when it caused two outbreaks of diarrheal illness that were linked to eating undercooked ground beef from the same fast-food restaurant chain (25). Since then, *E. coli* O157:H7 has emerged as a common cause of diarrhea in North America. In studies comparing the isolation rate of *E. coli* O157:H7 with those of other bacterial enteric pathogens, *E. coli* O157:H7 has commonly been isolated more frequently than *Shigella* spp. (4, 8, 13, 16, 19, 23). It is isolated at an especially high rate if the diarrhea is bloody. Preliminary data from a U.S. multicenter study showed that *E. coli* O157:H7 was isolated more frequently from stool specimens with visible blood than was any other pathogen (24). In addition, *E. coli* O157:H7 infection is responsible for most cases of hemolytic uremic syndrome, a major cause of acute renal failure in children (2, 3, 21, 26–28, 30).

In June 1993, the Association of State and Territorial Public Health Laboratory Directors, in a joint resolution with the Council of State and Territorial Epidemiologists, recommended that clinical laboratories screen at least all bloody stool specimens for *E. coli* O157:H7 with sorbitol-MacConkey medium (12). However, the proportion of clinical laboratories in the United States that routinely screen at least all bloody stools for *E. coli* O157:H7 has not been determined.

MATERIALS AND METHODS

In December 1994 and January 1995, we surveyed a sample of U.S. laboratories to determine their screening practices for *E. coli* O157:H7 for the years 1985 to 1994. We randomly selected 230 (2%) of the 11,548 U.S. laboratories that have applied for certification to perform highly or moderately complex tests as defined by the Clinical Laboratory Improvement Amendment of 1988. We conducted telephone interviews with the microbiology supervisor at each laboratory. For analysis, states were divided into 4 standard regions as follows: Northeast (Maine, N.H., Vt., Mass., R.I., Conn., N.Y., N.J., and Pa.); Midwest (Ohio, Ind., Ill., Mich., Wis., Minn., Iowa, Mo., N.Dak., S.Dak., Nebr., and Kans.); South (Del., Md., D.C., Va., W.Va., N.C., S.C., Ga., Fla., Ky., Tenn., Ala., Miss., Ark., La., Okla., and Tex.); and West (Mont., Idaho, Wyo., Colo., N.Mex., Ariz., Utah, Nev., Wash., Oreg., Calif., Alaska, and Hawaii). Data were analyzed by using Epi Info 5.01. The χ^2 test was used to determine if there was a significant difference between two groups. For continuous variables, analysis of variance was used to compare the means. All *P* values are two tailed.

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RESULTS

We were able to contact 229 of the 230 selected laboratories. Of these, 129 (56%) performed stool cultures. One hundred seventeen (91%) were hospital based and 12 (9%) were independent laboratories. The median annual volume of all tests performed in these laboratories was 187,765 (range, 700 to 5,204,646). Thirty-six states were represented.

Thirty-eight (29%) laboratories reported screening all stools, and an additional 32 (25%) reported screening all bloody stools for *E. coli* O157:H7 in 1994 (Table 1). Thus, 70 (54%) laboratories were screening at least all bloody stools for *E. coli* O157:H7 in 1994. Since 1985, the percentage of laboratories screening for *E. coli* O157:H7 has increased every year, with a marked increase in the last 3 years. From 1992 to 1994, the percentage of laboratories screening all stools for *E. coli* O157:H7 increased from 6 to 29% ($P < 0.001$), and the percentage of laboratories screening at least all bloody stools increased from 15 to 54% ($P < 0.001$) (Fig. 1).

Hospital-based laboratories were significantly more likely than independent laboratories to screen at least some stools for *E. coli* O157:H7 (77 versus 33%; $P = 0.003$). Laboratory size (as measured by annual test volume) was not predictive of screening for *E. coli* O157:H7. However, region of the country was associated with screening. In 1994, the percentages of laboratories screening all stools, by region, were as follows: Northeast, 42%; West, 50%; Midwest, 26%; and South, 13% ($P = 0.003$). The percentages of laboratories screening at least all bloody stools, by region, were as follows: Northeast, 74%; West, 73%; Midwest, 47%; and South, 39% ($P = 0.007$).

Among the laboratories that used bloody stools as a criterion for screening, all 32 reported using gross inspection to determine the presence of blood; in addition, 2 (6%) used microscopic inspection, 1 (3%) performed a test for occult blood, and 1 (3%) used a patient report of bloody stools as an indication that the stool was bloody.

Among the 94 laboratories that reported screening at least some stools for *E. coli* O157:H7, 80 (85%) used sorbitol-MacConkey agar and 14 (15%) used MacConkey or other agar and then checked colonies typical of *E. coli* for sorbitol fermentation. After sorbitol-negative colonies were detected, the next step varied by laboratory: 27 (29%) of laboratories performed a test to detect the O157 antigen, 45 (48%) performed biochemical tests to confirm the isolate as *E. coli*, and 20 (21%) sent the isolate to a reference laboratory for further testing. Of the 41 laboratories that performed both O157 antigen testing and biochemical tests to confirm the isolate as *E. coli*, 24 (58%)

TABLE 1. Indications for screening for *E. coli* O157:H7 among 129 laboratories that perform stool microbiology

Indication for screening for *E. coli* O157:H7	No. (%) of laboratories
Screen all stools	38 (29)
Screen some stools[a]	56 (43)
All bloody stools	32 (25)
Upon physician request	47 (36)
Patients with HUS[b]	6 (5)
Other indication[c]	11 (9)
Never screen stools	35 (27)

[a] Percentages in this category add up to more than 43% because some laboratories reported more than one indication.
[b] HUS, hemolytic uremic syndrome.
[c] Specimens from stools of children (four laboratories), liquid stools (three laboratories), stools with mucus (one laboratory), bloody stools during warm months (one laboratory), stools received in Cary-Blair transport medium (one laboratory), and stools testing positive for *E. coli* (one laboratory).

performed antigen testing first. Of the 44 laboratories that reported performing O157 antigen testing, 6 (14%) did their own H-typing. In addition, 43 (98%) of those 44 laboratories indicated that they reported positive isolates to the local or state health department.

For the 59 laboratories that did not screen at least all bloody stools for *E. coli* O157:H7, we asked the microbiology supervisor for the reasons why their laboratory did not do so. Thirty-nine (66%) believed that the local incidence of *E. coli* O157:H7 infection was too low. Twenty-four (41%) thought that the cost of testing was too high, 8 (14%) said that testing took too much time, and 17 (29%) cited other reasons, including lack of physician requests for testing for the organism, low volume of stool culture requests received, and lack of familiarity with the screening method.

DISCUSSION

E. coli O157:H7 is a common cause of both sporadic and outbreak-associated diarrheal illness in the United States, causing an estimated 21,000 infections and 250 deaths each year (5). In 1993 and 1994, 46 outbreaks of *E. coli* O157:H7 infection were reported to the Centers for Disease Control and Prevention (11). Detection of *E. coli* O157:H7 infection by clinical microbiologists is often the first step in recognizing outbreaks. By January 1995, *E. coli* O157:H7 infection was a reportable disease in 32 states (11). However, unless clinical laboratories routinely screen stool specimens for *E. coli* O157:H7, outbreaks are likely to go undetected, even in states in which the infection is reportable (10).

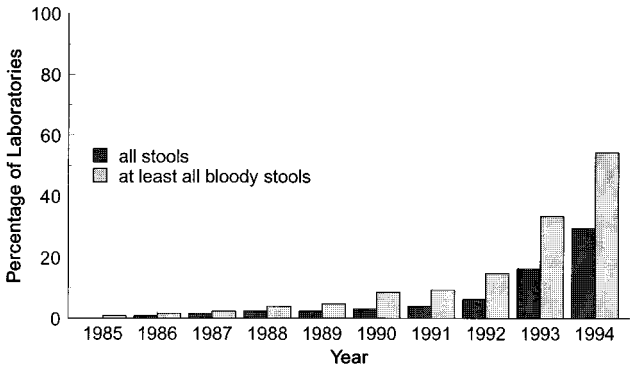

FIG. 1. Percentage of laboratories (n = 129) screening all stools and at least all bloody stools for *E. coli* O157:H7, by year.

The results of this study indicate that 18 months after the recommendation by the Association of State and Territorial Public Health Laboratory Directors that clinical laboratories screen at least all bloody stools for *E. coli* O157:H7 (12), only 54% of laboratories were doing so. The most common reason cited for not screening at least all bloody stools was the impression that the local incidence was too low. For laboratories that are unsure of the local isolation rate, we recommend performing a trial in which all stools submitted for culture during the warmer months are screened for *E. coli* O157:H7. The minimum number of stools needed to be screened depends on the point estimate of the isolation rate and on the volume of specimens received in that laboratory. Data from a U.S. multicenter study demonstrated an isolation rate for *E. coli* O157:H7 of 0.4% (24). On the basis of a point estimate of 0.4% (range, 0.1 to 0.7%), a laboratory that receives 1,000 stool specimens annually would need to screen approximately 600 stools to estimate the local isolation rate with 95% confidence; a laboratory that receives 10,000 stool specimens each year would need to screen about 1,400 stools. Depending on the local isolation rate, some laboratories may find it best to culture all stools for *E. coli* O157:H7 during the warmer months, when these infections are most common (22, 23), and to culture all bloody stools for *E. coli* O157:H7 the rest of the year.

In our study, the second most common reason for not screening at least all bloody stools for *E. coli* O157:H7 was an impression that the cost of testing was too high. A study in Washington State estimated the cost of culturing for *E. coli* O157:H7 at $1.10 per culture, or $183 per positive culture if all stools are cultured for *E. coli* O157:H7 (7). To decrease the cost of testing, some laboratories culture only grossly bloody stools for *E. coli* O157:H7, which has been estimated to reduce the cost of screening to $40 per positive culture (7). Patients infected with *E. coli* O157:H7 may develop diarrhea that is nonbloody, minimally blood streaked, or "all blood and no stool" (5). In reported outbreaks, the percentage of patients with *E. coli* O157:H7 infection who report bloody diarrhea has ranged from 35 to 90% (1, 29). Defining the term "bloody diarrhea" is important, since the percentage of stool specimens that appear grossly bloody in the microbiology laboratory is lower than the percentage of patients reporting bloody stools or the percentage of stools testing positive for the presence of occult blood (24).

In the United States, both sporadic cases and outbreaks of *E. coli* O157:H7 infection are more commonly reported from northern than southern states (14). In our study, laboratories from southern states were significantly less likely to screen stools for *E. coli* O157:H7. This suggests that laboratory practices may contribute to the apparent regional differences in the incidence of *E. coli* O157:H7 infections.

The recommended method (9) for screening for *E. coli* O157:H7 is relatively simple and is based on the fact that *E. coli* O157:H7 does not ferment sorbitol rapidly (17). Stool is plated on sorbitol-MacConkey agar, which contains 1% sorbitol instead of lactose. After 18 to 24 h of incubation, organisms that ferment sorbitol appear as pink colonies. Non-sorbitol-fermenting organisms, including *E. coli* O157:H7, are colorless. If there are no sorbitol-negative colonies, the specimen needs no further testing.

If the sorbitol-MacConkey plate contains sorbitol-negative colonies, these are selected and assayed for the O157 antigen with commercially available antiserum (18). Because of possible cross-reactions with other organisms, sorbitol-negative or-

ganisms that agglutinate in O157 antisera should then be biochemically identified as *E. coli*. Performing the O157 antigen testing before biochemical identification will save time, because antigen testing is the quicker procedure; isolates testing negative for the O157 antigen need not be biochemically identified as *E. coli.*

After biochemical identification of an O157-positive isolate as *E. coli*, a preliminary report should be issued to the clinician while the strain is forwarded to the state public health laboratory for identification or confirmation of the H7 flagellar antigen. For laboratories that perform their own H7 antigen testing, strains testing negative for the H7 antigen and nonmotile strains should also be forwarded to the state public health laboratory for toxin testing.

Screening for *E. coli* O157:H7 has value for clinical decision making as well as for public health action. In searching for a cause of bloody diarrhea, physicians have performed unnecessary exploratory surgery, hemicolectomies, colonoscopies, barium enemas, and appendectomies on patients subsequently shown to be infected with this pathogen (15, 20). Public health intervention in outbreaks is crucial to prevent ongoing transmission and person-to-person spread. In the large January 1993 outbreak affecting persons in the western United States, early detection of *E. coli* O157:H7 infections by clinical microbiologists enabled a rapid recall of contaminated ground beef and prevented an estimated 800 infections (1). The increasing percentage of laboratories in the United States screening all stools for *E. coli* O157:H7 is encouraging but is still less than 30%. Because many patients infected with the organism do not develop bloody diarrhea, we recommend that clinical laboratories screen all stools submitted for culture for *E. coli* O157:H7. This is consistent with recent recommendations from multidisciplinary consensus panels in both the United States (6) and the United Kingdom (31). For laboratories in areas with a low incidence of *E. coli* O157:H7 infections, at least all bloody stools should be cultured for this organism.

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REFERENCES

1. **Bell, B. P., M. Goldoft, P. M. Griffin, M. A. Davis, D. C. Gordon, P. I. Tarr, C. A. Bartleson, J. H. Lewis, T. J. Barrett, J. G. Wells, R. Baron, and J. Kobayashi.** 1994. A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers: the Washington experience. JAMA **272:**1349–1353.
2. **Bitzan, M., K. Ludwig, M. Klemt, H. Konig, J. Buren, and D. E. Muller-Wiefel.** 1993. The role of *Escherichia coli* O157 infections in the classical (enteropathic) haemolytic uraemic syndrome: results of a Central European, multicentre study. Epidemiol. Infect. **110:**183–196.
3. **Bitzan, M., E. Moebius, K. Ludwig, D. E. Muller-Wiefel, J. Heesemann, and H. Karch.** 1991. High incidence of serum antibodies to *Escherichia coli* O157 lipopolysaccharide in children with hemolytic-uremic syndrome. J. Pediatr. **119:**380–385.
4. **Bokete, T. N., C. M. O'Callahan, C. R. Clausen, N. M. Tang, N. Tran, S. L. Moseley, T. R. Fritsche, and P. I. Tarr.** 1993. Shiga-like toxin-producing *Escherichia coli* in Seattle children: a prospective study. Gastroenterology **105:**1724–1731.
5. **Boyce, T. G., D. L. Swerdlow, and P. M. Griffin.** 1995. *Escherichia coli* O157:H7 and the hemolytic-uremic syndrome. N. Engl. J. Med. **333:**364–368.
6. **Brotman, M., R. A. Giannella, P. F. Alm, H. Bauman, A. R. Bennett, R. E. Black, C. M. Bruhn, M. B. Cohen, S. L. Gorbach, J. B. Kaper, M. R. Roberts, J. L. Staneck, S. Taylor, and H. F. Troutt.** 1995. Consensus conference statement: *Escherichia coli* O157:H7 infections—an emerging national health crisis, July 11–13, 1994. Gastroenterology **108:**1923–1934.
7. **Bujak, J., P. Bauer, D. Campbell, J. Claridge, J. Lewis, A. Ries, P. Stepak, J. Turner, N. Turner, and D. Anderson.** 1993. Avoidance of economic entropy in culturing *E. coli* O157:H7, abstr. C445. *In* Abstracts of the 93rd General Meeting of the American Society for Microbiology 1993. American Society for Microbiology, Washington, D.C.
8. **Cahoon, F. E., and J. S. Thompson.** 1987. Frequency of *Escherichia coli* O157:H7 isolation from stool specimens. J. Infect. Dis. **33:**914–915.
9. **Centers for Disease Control and Prevention.** 1994. *E. coli* O157:H7—what the clinical microbiologist should know. Centers for Disease Control and Prevention, Atlanta. (Video.)
10. **Centers for Disease Control and Prevention.** 1994. Laboratory screening for *Escherichia coli* O157:H7—Connecticut, 1993. Morbid. Mortal. Weekly Rep. **43:**192–194.
11. **Centers for Disease Control and Prevention.** 1995. *Escherichia coli* O157:H7 outbreak linked to commercially distributed dry-cured salami—Washington and California, 1994. Morbid. Mortal. Weekly Rep. **44:**157–160.
12. **Council of State and Territorial Epidemiologists.** 1993. CSTE position statement #4: national surveillance of *Escherichia coli* O157:H7. Council of State and Territorial Epidemiologists, Atlanta.
13. **Gransden, W. R., M. A. Damm, J. D. Anderson, J. E. Carter, and H. Lior.** 1986. Further evidence associating hemolytic uremic syndrome with infection by verotoxin-producing *Escherichia coli* O157:H7. J. Infect. Dis. **154:**522–524.
14. **Griffin, P. M.** 1995. *Escherichia coli* O157:H7 and other enterohemorrhagic *Escherichia coli*, p. 739–761. *In* M. J. Blaser, P. D. Smith, J. I. Ravdin, H. B. Greenberg, and R. L. Guerrant (ed.), Infections of the gastrointestinal tract. Raven Press, Ltd., New York.
15. **Griffin, P. M., S. M. Ostroff, R. V. Tauxe, K. D. Greene, J. G. Wells, J. H. Lewis, and P. A. Blake.** 1988. Illnesses associated with *Escherichia coli* O157:H7 infections: a broad clinical spectrum. Ann. Intern. Med. **109:**705–712.
16. **MacDonald, K. L., M. J. O'Leary, M. L. Cohen, P. Norris, J. G. Wells, E. Noll, J. M. Kobayashi, and P. A. Blake.** 1988. *Escherichia coli* O157:H7, an emerging gastrointestinal pathogen: results of a one-year, prospective, population-based study. JAMA **259:**3567–3570.
17. **March, S. B., and S. Ratnam.** 1986. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J. Clin. Microbiol. **23:**869–872.
18. **March, S. B., and S. Ratnam.** 1989. Latex agglutination test for detection of *Escherichia coli* serotype O157. J. Clin. Microbiol. **27:**1675–1677.
19. **Marshall, W. F., C. A. McLimans, R. E. Van Scoy, and J. P. Anhalt.** 1990. Results of a 6-month survey of stool cultures for *Escherichia coli* O157:H7. Mayo Clin. Proc. **65:**787–792.
20. **Martin, D. L., K. L. MacDonald, K. E. White, J. T. Soler, and M. T. Osterholm.** 1990. The epidemiology and clinical aspects of the hemolytic uremic syndrome in Minnesota. N. Engl. J. Med. **323:**1161–1167.
21. **Neill, M. A., P. I. Tarr, C. R. Clausen, D. L. Christie, and R. O. Hickman.** 1987. *Escherichia coli* O157:H7 as the predominant pathogen associated with the hemolytic uremic syndrome: a prospective study in the Pacific Northwest. Pediatrics **80:**37–40.
22. **Ostroff, S. M., J. M. Kobayashi, and J. H. Lewis.** 1989. Infections with *Escherichia coli* O157:H7 in Washington State: the first year of statewide disease surveillance. JAMA **262:**355–359.
23. **Pai, C. H., N. Ahmed, H. Lior, W. M. Johnson, H. V. Sims, and D. E. Woods.** 1988. Epidemiology of sporadic diarrhea due to verocytotoxin-producing *Escherichia coli*: a two-year prospective study. J. Infect. Dis. **157:**1054–1057.
24. **Ries, A., P. Griffin, K. Greene, and the *Escherichia coli* O157:H7 Study Group.** 1993. *Escherichia coli* O157:H7 diarrhea in the United States: a 10 center surveillance study, abstr. 1454. *In* Program and abstracts of the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
25. **Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Hebert, E. S. Olcott, L. M. Johnson, N. T. Hargrett, P. A. Blake, and M. L. Cohen.** 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N. Engl. J. Med. **308:**681–685.
26. **Rowe, P. C., E. Orrbine, H. Lior, G. A. Wells, P. N. McLaine, and Canadian Pediatric Kidney Disease Reference Centre co-investigators.** 1993. A prospective study of exposure to verotoxin-producing *Escherichia coli* among Canadian children with haemolytic uraemic syndrome. Epidemiol. Infect. **110:**1–7.
27. **Rowe, P. C., E. Orrbine, G. A. Wells, P. N. McLaine, and members of the Canadian Pediatric Kidney Disease Reference Centre.** 1991. Epidemiology of hemolytic-uremic syndrome in Canadian children from 1986 to 1988. J. Pediatr. **119:**218–224.
28. **Siegler, R. L., A. T. Pavia, R. D. Christofferson, and M. K. Milligan.** 1994. A 20-year population-based study of postdiarrheal hemolytic uremic syndrome in Utah. Pediatrics **94:**35–40.
29. **Swerdlow, D. L., B. A. Woodruff, R. C. Brady, P. M. Griffin, S. Tippen, H. D. Donnel, E. Geldreich, B. J. Payne, A. Meyer, J. G. Wells, K. D. Greene, M. Bright, N. H. Bean, and P. A. Blake.** 1992. A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhea and death. Ann. Intern. Med. **117:**812–819.
30. **Tarr, P. I., M. A. Neill, C. R. Clausen, S. L. Watkins, D. L. Christie, and R. O. Hickman.** 1990. *Escherichia coli* O157:H7 and the hemolytic uremic syndrome: importance of early cultures in establishing the etiology. J. Infect. Dis. **162:**553–556.
31. **Working Group of the Advisory Committee on the Microbiological Safety of Food.** 1995. Report on Vero cytotoxin producing *Escherichia coli*. Her Majesty's Stationery Office, London.