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A Survey of Stool Culturing Practices for *Vibrio* Species at Clinical Laboratories in Gulf Coast States

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Non-cholera *Vibrio* infections are an important public health problem. Non-cholera *Vibrio* species usually cause sporadic infections, often in coastal states, and have also caused several recent nationwide outbreaks of gastroenteritis in the United States. We report a survey of laboratory stool culturing practices for *Vibrio* among randomly selected clinical laboratories in Gulf Coast states (Alabama, Florida, Louisiana, Mississippi, and Texas). Interviews conducted with the microbiology supervisors of 201 clinical laboratories found that 164 (82%) received stool specimens for culture. Of these, 102 (62%) of 164 processed stool specimens on site, and 20 (20%) of these 102 laboratories cultured all stool specimens for *Vibrio*, indicating that at least 34,463 (22%) of 152,797 stool specimens were cultured for *Vibrio*. This survey suggests that despite an increased incidence of non-cholera *Vibrio* infections in Gulf Coast states, a low percentage of clinical laboratories routinely screen all stool specimens, and fewer than 25% of stool specimens collected are routinely screened for non-cholera *Vibrio*.

Vibrio organisms are free-living, widely distributed inhabitants of coastal waters worldwide (3) and are associated with gastroenteritis, wound infections, and septicemia (2). *Vibrio* infections are more frequently reported in coastal states, apparently because of greater consumption of shellfish and frequent contact with marine waters by residents and visitors to these states; these infections also occur more commonly in the warmer months (10). Although toxigenic *Vibrio cholerae* O1 and O139, the causes of epidemic cholera, have been of greatest interest to physicians and public health officials, other *Vibrio* species, including *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Vibrio alginolyticus*, *Vibrio mimicus*, and non-toxigenic *V. cholerae*, are also pathogenic to humans (8, 12, 17). *Vibrio* infections are associated with significant morbidity and mortality, causing an estimated 7,974 infections and 57 deaths each year in the United States (15). In the United States, *Vibrio* species usually cause sporadic illness (11), although several recent outbreaks of *V. parahaemolyticus* infection (1, 6, 7) have been reported.

This report focuses on laboratory stool culturing practices for the variety of vibrios that cause gastroenteritis, particularly *V. parahaemolyticus*. We did not address the specific tests needed to identify toxigenic *V. cholerae* O1 or O139. Laboratory diagnosis of *Vibrio* infections that cause gastroenteritis usually requires isolation of the organism from the stool specimen of a patient with diarrhea. However, many cases of gastroenteritis caused by *Vibrio* spp. are undetected by clinical laboratories because vibrios are not easily identified on routine enteric media (2). Isolation of vibrios from stool is greatly enhanced through the use of a selective medium, particularly thiosulfate-citrate-bile salts-sucrose agar (TCBS). Although their reliability has not been demonstrated for many *Vibrio* strains (14), commercial biochemical identification systems are also used for the identification of *Vibrio* species (18).

In 1989, in response to the increased incidence of *Vibrio* infections in the states along the Gulf Coast, four Gulf Coast states (Alabama, Florida, Louisiana, and Texas) began systematically reporting infections with *Vibrio* species to the Centers for Disease Control and Prevention (CDC) (13). To facilitate surveillance for vibrios, the use of TCBS agar for a stool culture was recommended if a patient presented with a compatible diarrheal illness and a history of eating raw seafood (13). However, to our knowledge, the use of TCBS has never been evaluated. In the summer of 1998, during an outbreak of *V. parahaemolyticus* infections associated with the ingestion of raw oysters in Galveston, Tex. (CDC, unpublished data), we assessed laboratory stool culturing practices for *Vibrio* species in five Gulf Coast states, where *Vibrio* infection is reportable. As part of the outbreak investigation, objectives were to ascertain the role of the clinical laboratory in performing surveillance for *Vibrio* by determining the proportion of clinical laboratories in the Gulf Coast states that routinely cultured stools for *Vibrio* and the perceived barriers to culturing for *Vibrio* with TCBS agar.

MATERIALS AND METHODS

Between 13 and 27 July 1998, we surveyed a sample of clinical laboratories in Alabama, Florida, Louisiana, Mississippi, and Texas to determine stool culturing practices for *Vibrio*. We randomly selected 231 (10%) of the 2,302 laboratories in these five states that applied for certification in 1998 to perform highly or moderately complex tests, as part of the Clinical Laboratory Improvement

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Amendment. We contacted the selected laboratories as many as three times by telephone to interview the microbiology supervisor. The interview was conducted using a structured questionnaire, which required approximately 10 min to administer. Questions focused on types of media routinely used, procedures for isolating and identifying vibrios, knowledge of reportability of *Vibrio*, perceived barriers to testing for *Vibrio*, and changes in laboratory procedures since 1 January 1998 that would affect surveillance for *Vibrio*. Supervisors were also asked if their laboratory had isolated any *Vibrio* species since 1 June 1998. If *Vibrio* species had been isolated, the supervisors were asked for the species identification and method of identification. In Texas, for those cases in which the *Vibrio* isolate had been submitted to the Texas Department of Health Laboratory (TDHL), we compared the *Vibrio* species identification performed at TDHL with species identification obtained by the clinical laboratories. Data were analyzed by Epi-Info 6.04 (CDC, Atlanta, Ga.). The chi-square test was used to determine if there was a significant difference between two or more groups. P values of <0.05 were considered statistically significant. All P values were two-tailed.

RESULTS

We contacted the microbiology supervisors of 201 (87%) of 231 selected clinical laboratories; 164 (82%) of these laboratories received stool specimens for culture. These laboratories received an estimated 162,559 stools for culture in 1997. Among laboratories that received stools for culture, 127 (77%) were hospital based and 37 (23%) were independent. The median annual number of stool specimens received for culture was 245 (range, 6 to 18,000) for hospital-based laboratories and 813 (range, 18 to 12,000) for independent laboratories.

Among those that received stool specimens for culture, 102 (62%) of 164 laboratories performed at least some stool cultures on-site. Hospital-based laboratories were significantly more likely than independent laboratories to culture at least some stools on-site (86% versus 14%; odds ratio, 3.7; 95% confidence interval, 1.6 to 8.6). Because hospital-based laboratories received more stool specimens and performed more on-site testing than did independent laboratories, 94% (152,797 of 162,559) of all stools received by laboratories in this survey were cultured on-site; the remainder (6%) were sent by the primary clinical laboratory to a reference laboratory. These 102 laboratories reported receiving 88% of stool samples as whole stools and 12% as rectal swabs. Among samples received as whole stools, 18% were submitted with transport media and 82% were submitted without transport media. Among samples received as rectal swabs, 88% were submitted with transport media and 12% were submitted without transport media. The median length of time from stool sample collection to receipt by the laboratory was 2 h. Among 102 supervisors from laboratories that performed on-site stool cultures, 78 (76%) knew that *Vibrio* infections were reportable in their state. The laboratories managed by these supervisors processed 72% of the stools cultured on-site. Forty-eight (47%) of 102 laboratories that performed on-site stool cultures (representing 63% of stools cultured on-site) routinely sent *Vibrio* isolates to the state public health laboratory. When asked about the type of patient information to which laboratories routinely had access, only 13 (13%) of 102 laboratories that performed stool cultures reported routinely receiving a patient's food history from the ordering physician. None of the 102 laboratories that processed stool cultures reported changes in their stool culturing practices since 1 January 1998.

Of the 102 laboratories that processed stool cultures, 48 (47%) reported that they used TCBS agar on some or all stool cultures (Table 1). These 48 laboratories processed 85% of the stools that were cultured on-site. Twenty (20%) of 102 laboratories that performed on-site stool cultures used TCBS agar routinely on all stool cultures (which represented 22% of stools cultured in this survey), and 28 (27%) of 102 used TCBS agar on at least some stool cultures (which represented 63% of stools cultured in this survey). One (2%) of 48 laboratories that used TCBS agar reported using TCBS seasonally (May to October). When asked how they confirmed growth of *Vibrio* on TCBS agar, 40 (83%) of the 48 laboratories that used TCBS agar on some or all stool specimens reported using a commercial biochemical identification system. The 14 Texas laboratories that used TCBS agar on some, but not all, stool cultures were asked what triggered their decision to use TCBS agar. Seven (50%) of the laboratories reported that they used TCBS agar only upon physician request, while the other seven reported using TCBS agar when colony morphology on other enteric agars was suggestive of *Vibrio*.

TABLE 1. Screening practices for *Vibrio* spp. of 102 Gulf Coast laboratories surveyed and number of all stool samples (n = 152,797) cultured, by laboratory practice

TCBS screening practices, by state (no. of laboratories)	No. of laboratories with each practice (%)	No. of stools cultured (%)
Alabama (11)		
Do not use TCBS	9 (82)	4,006 (69)
Used TCBS on some	1 (9)	720 (12)
Used TCBS on all	1 (9)	1,080 (19)
Florida (22)		
Do not use TCBS	9 (41)	2,399 (8)
Used TCBS on some	8 (36)	21,334 (68)
Used TCBS on all	5 (23)	7,782 (24)
Louisiana (13)		
Do not use TCBS	3 (23)	1,340 (15)
Used TCBS on some	3 (23)	2,700 (27)
Used TCBS on all	7 (54)	5,740 (58)
Mississippi (10)		
Do not use TCBS	7 (70)	3,205 (37)
Used TCBS on some	2 (20)	5,520 (56)
Used TCBS on all	1 (10)	600 (7)
Texas (46)		
Do not use TCBS	26 (57)	11,673 (12)
Used TCBS on some	14 (30)	65,437 (68)
Used TCBS on all	6 (13)	19,261 (20)
All states (102)		
Do not use TCBS	54 (53)	22,623 (15)
Used TCBS on some	28 (27)	95,711 (63)
Used TCBS on all	20 (20)	34,463 (22)

The 54 laboratories that did not use TCBS agar on any stool cultures were asked how they screened stool cultures for *Vibrio*. Thirty-two (59%) of these laboratories reported that they did not screen for vibrios, 20 (37%) used only routine enteric media and a commercial biochemical identification system to identify *Vibrio*, and 2 (4%) performed a Gram stain. Laboratories that did not use TCBS agar were asked for reasons why they did not use TCBS. Thirty-seven (69%) believed that the local incidence of *Vibrio* was too low, 23 (43%) thought the cost of testing was too high, and 3 (6%) reported that testing with TCBS agar took too much time.

During the Galveston Bay outbreak in the summer of 1998, 12 (26%) of the 46 selected laboratories that processed stool specimens in Texas identified a *Vibrio* species from a stool culture and forwarded the isolate to the TDHL for confirmation. At the clinical laboratories, 8 (67%) of the 12 specimens were processed using TCBS agar (with the use of commercial systems for species identification) and 4 (33%) were identified by using other agars (with the use of commercial systems for

species identification). Among the eight isolates identified by clinical laboratories that used TCBS agar, seven (88%) were correctly identified; the remaining isolate was correctly identified to genus, but not species level. Among the four isolates identified by clinical laboratories that used other agars, two (50%) were correctly identified. Of the two remaining isolates, the species was incorrectly identified in one, and the remaining isolate was not a *Vibrio* sp.

DISCUSSION

Clinical microbiology laboratories have a critical role in the surveillance of *Vibrio* infections and the early detection of *Vibrio* outbreaks. In our survey of Gulf Coast laboratories, the majority (85%) of stools were cultured in laboratories where TCBS agar was available, but only a small percentage (22%) of stools in these laboratories were screened for *Vibrio* using TCBS agar. Furthermore, there was no evidence of seasonal use of TCBS agar, even though *Vibrio* infections are more common during the warmer months (10). *Vibrio* infections are reportable in each of the five states in this survey; however, 24% of microbiology supervisors in laboratories that performed stool cultures did not know that isolation of *Vibrio* was reportable in their state. These findings suggest that a marked burden of *Vibrio* illness (sporadic illness as well as outbreaks) may go undetected in those states.

Cost was perceived by 43% of laboratories to be a barrier to the routine use of TCBS agar in screening for *Vibrio* spp. Cost per positive culture has been used as a determinant of the practicality of routine laboratory screening for enteric pathogens, such as *Escherichia coli* O157:H7 (4). Cost per positive culture in this survey was estimated by taking the number of stool samples routinely screened using TCBS during the month of June 1998 in laboratories that met the following criteria: (i) screening all stools for *Vibrio* with TCBS agar and (ii) isolating a *Vibrio* organism from TCBS agar. To approximate screening for sporadic *Vibrio* infections, Texas laboratories were excluded from this cost estimate. The number of stool samples screened during June was multiplied by the cost per TCBS agar plate ($1.50) and then divided by the number of culture-confirmed *Vibrio* infections identified by these laboratories. Using this formula, cost per positive culture was $72.00. The average treatment cost per culture-positive *V. parahaemolyticus* infection is $1,000.00 (9). If laboratories were to focus their surveillance for *Vibrio* by routinely screening all stool samples with TCBS agar during the summer months, more *Vibrio* infections would be detected, thus reducing cost per positive culture.

Alternatively, laboratories could reduce the cost and make more efficient use of TCBS agar by having access to patient's food histories, particularly during the warmer months. Only 13% of laboratories reported obtaining a food history from the physician. Physicians' knowledge of risk factors associated with various enteric infections is an important clinical diagnostic tool (5, 19). Increased awareness among physicians regarding *Vibrio* infections may increase the likelihood of obtaining a food history, as well as increase the number of requests for stool cultures to be screened for *Vibrio*. A food history indicating recent seafood exposure provided to the microbiologist may help those in the laboratory choose which stool specimens to test with TCBS agar, even if a specific request was not made.

The use of commercial systems for the identification of *Vibrio* species needs further evaluation. Commercial biochemical identification systems such as Vitek (Vitek Systems, Inc., Hazelwood, Mo.) originated in the 1960's. These systems are capable of identifying gram-negative bacteria, gram-positive bacteria, yeasts, and anaerobic organisms. In 1989, the gram-negative identification test kit for the Vitek system was expanded to include members of the *Vibrio* species (18). In one study, the Vitek system was evaluated for its ability to correctly identify 212 *Vibrio* isolates, compared with conventional biochemicals and another commercial system, the API20E (Analytab Products, Plainview, N.Y.) (S. Farnham, N. Moss, and J. Scott, Abstr. 89th Annu. Meet. Am. Soc. Microbiol. 1989, abstr. C-263, 1989). The overall correlation of Vitek with the reference identification systems was 95%. Another study used 60 *Vibrio* isolates to compare three different commercial systems against the use of standard biochemicals; only one, the API20E, was considered to be a valid system for use in the identification of the more commonly isolated members of the family *Vibrionaceae* (16). Our limited survey indicated that using other enteric agars in conjunction with commercial biochemical identification systems to identify *Vibrio* spp. yielded accurate results only 50% of the time. However, when TCBS agar was used in conjunction with a commercial system, 88% of isolates were correctly identified. This finding suggests that until further evaluation of a large number of *Vibrio* strains is completed, TCBS agar should be used for primary laboratory isolation of *Vibrio* species, with the use of commercial systems for species identification.

Unfortunately, even though *Vibrio* infections continue to occur in coastal areas (6, 7), the percentage of Gulf Coast laboratories routinely screening stools for *Vibrio* with TCBS agar was significantly lower (20% versus 30%) than that found in one recent national laboratory survey (20) and approximately the same as that from another national laboratory survey (T. J. Van Gilder, D. Christensen, S. Shallow, T. R. Fiorentino, S. Desai, M. Pass, J. Wicklund, C. Stone, and M. Cassidy, Abstr. 99th Gen. Meet. Am. Soc. Microbiol., abstr. C-419, 1999). Furthermore, the majority (53%) of Gulf Coast laboratories that processed stool specimens on-site never used TCBS agar. Finally, there was no evidence of seasonal use of TCBS agar in our survey. Taken together, these data indicate that TCBS agar is underused in Gulf Coast states.

Surveillance data and recent outbreaks of *V. parahaemolyticus* infections (6, 7) indicate that *Vibrio* infections continue to be an important cause of gastroenteritis in coastal areas of the United States; therefore, clinicians, laboratories, and health authorities should remain vigilant in their diagnosis, detection, and surveillance of these organisms. Clinicians should consider requesting a stool culture for *Vibrio* when a patient presents with gastroenteritis and a recent history of raw seafood consumption, and they should make this patient's history available to the clinical laboratory. Clinical laboratories should provide clinicians with a specimen submission form that requests food history information, as a guide to clinical laboratory diagnosis. To enhance detection of vibrios, clinical laboratories should encourage the appropriate use of transport media for sample submission, consider screening all stools with TCBS agar during the warmer months (May to October), when *Vibrio* infections are most likely to occur, and use TCBS agar in conjunction with commercial biochemical identification systems for *Vibrio* species identification. Any *V. cholerae* isolates identified should be referred to the state public health laboratory for confirmation, serotyping, and toxin testing. State health authorities in Gulf Coast states should remind clinical laboratories and physicians that *Vibrio* infections are reportable and should encourage increased stool culturing for *Vibrio* during the warmer months in laboratories where TCBS agar use is not routine. Taken together, these recommendations would enhance surveillance for *Vibrio* infections, resulting in earlier detection and recognition of sporadic cases and outbreaks.

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