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Diarrheal Disease and DRGs

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Current regulations, by which reimbursement is based on diagnosis related groups (DRGs), are changing clinical laboratories from revenue producing centers to cost centers (25). This transition is happening at the same time as a dramatic increase in our knowledge of infectious diseases, including new understanding of the etiology of diarrheal disease. Ten years ago, the etiology of enteric disease in many patients was unknown because stool specimens were routinely screened only for Salmonella spp., Shigella spp., and perhaps intestinal parasites such as Giardia lamblia. At present, many laboratories also routinely examine stool specimens for Campylobacter spp., Yersinia enterocolitica, Clostridium difficile, Cryptosporidium, and rotavirus. Aeromonas spp. (1), Vibrio spp. (4, 5), strains of Escherichia coli (6, 17–19), Norwalk agent, enteric adenoviruses, coronavirus, and caliciviruses (8) have also been suggested as causes of diarrheal disease; food poisoning agents such as Staphylococcus aureus and Bacillus cereus must also be considered. Faced with declining resources and expanding knowledge, what approaches should be taken to provide efficient, yet comprehensive, diagnostic services to patients with diarrheal disease?

To adequately address this question, retrospective review of laboratory results is needed to determine which agents are the ones most likely to cause diarrheal disease in a particular patient population. Table 1 lists data on the frequency with which we detected enteric pathogens during the past 3 yr at our 600-bed hospital. These data were obtained by culturing approximately 10,000 patients for Campylobacter spp., Salmonella spp., and Shigella spp.; examining approximately 4000 patients for intestinal parasites; 1500 patients for C. difficile toxin, and 160 children for rotavirus. C. difficile was the enteric pathogen detected most frequently, probably because our tertiary care facility admits...
Table 1
Detection Frequency of Enteric Pathogens at the North Carolina Memorial Hospital (January 1, 1982–December 31, 1984)

| Agent                        | No. of Patients |
|------------------------------|-----------------|
| Clostridium difficile toxin   | 197             |
| Salmonella spp.              | 129             |
| Campylobacter spp.           | 104             |
| Giardia lamblia              | 90              |
| Rotavirus                    | 45              |
| Trematodes and Nematodes     | 39              |
| Shigella spp.                | 25              |
| Yersinia enterocolitica      | 13              |
| Aeromonas                    | 10              |
| Vibrio spp.                  | 2               |

many patients who have complicated clinical courses that require antimicrobial or cancer chemotherapy, treatments that predispose to this disease (3, 7). On the other hand, patients with Salmonella, Campylobacter, and rotavirus would more likely be seen at a children’s hospital than in our institution.

Selective Stool Examination
Not all stool specimens should be examined routinely for all agents listed in Table 1. For the most efficient use of laboratory facilities, as well as maximum benefit to the patient, a selective examination process must be followed based on factors such as patient age and history, as well as the clinical microbiologist’s understanding of the pathogenesis and local epidemiology of diarrheal disease. Figure 1 presents an algorithm that can be used as a guideline to determine how to examine stool specimens from different patient populations. This algorithm divides patients according to age, whether those older than 3 yr are inpatients or outpatients, and whether they have received antimicrobial agents or cancer chemotherapeutics. Stools from patients who have currently or recently received antimicrobial agents or cancer chemotherapeutics should be examined only for C. difficile toxin because studies have shown that, in such patients, other enteric pathogens are rarely, if ever, present (9). Tests for detecting C. difficile toxin must be done instead of culture because up to 20% of people receiving antimicrobial agents are asymptomatic carriers of the organism (24). On the other hand, only 2% of patients have toxin in the absence of symptoms. Toxin is detected in the feces of approximately 30% of patients with antimicrobial agent-associated diarrhea and essentially all patients with pseudomembranous colitis (3). Tissue culture assay remains the standard test for toxin detection. Alternative methods such as counterimmunoelectrophoresis (12) and latex agglutination (21) lack sufficient sensitivity and specificity; ELISA tests (13), although accurate, are not yet widely available.

Inpatients
Stools from inpatients who are not receiving antimicrobial therapy usually are negative for enteric pathogens. Because nosocomial outbreaks of Salmonella and Shigella have occurred (10, 16), stool specimens may be screened for these agents. In certain areas of the United States where Giardia is endemic, e.g., Colorado (11), stools from patients with appropriate signs and symptoms may be examined for this protozoan.

AIDS Patients
Patients with acquired immune deficiency syndrome (AIDS) present a special challenge because diarrhea can be a frequent, potentially life-threatening illness. Cryptosporidium, Salmonella, Shigella, and Giardia are commonly associated with this syndrome (10). The diagnosis of C. difficile enterocolitis must also be considered because these patients may receive a variety of antimicrobial agents to treat their opportunistic infections.

Outpatients
Outpatients with diarrheal disease present a greater challenge than inpatients because they can be infected by a large variety of microbial agents.

Rational strategies for evaluating these patients can be developed by carefully noting their travel and food history. Stool examinations should not be performed unless the patient has been symptomatic for at least 3 days. In this way, persons with self-limited disease will spare the expense of the culture, an especially important factor in health maintenance organizations (HMOs). Stools from patients with persistent symptoms should be cultured for Salmonella, Shigella, and Campylobacter, as well as examined for intestinal parasites, which may be found almost as commonly as bacterial pathogens (Table 1).

Specimen Examination
The complete stool work-up should include two cultures for bacteria and three examinations for parasites. Specimen collection should be spaced so that the examinations can be completed before another stool analysis is performed. If the tests are negative and the patient remains symptomatic, a search for “unusual” agents of diarrheal disease should be made, e.g., in patients with bloody diarrhea. Strains of E. coli that cause hemorrhagic colitis (17) are usually identified in reference laboratories by a combination of biochemical tests, serotyping, and ability to produce a Shiga-like toxin (15). State health laboratories should be contacted about their ability to process specimens to detect these, as well as other, pathogenic E. coli.

The stools of patients who have either a history of travel to undeveloped countries or of ingestion of raw seafoods, should be examined for Vibrio spp. and enterotoxigenic E. coli. Vibrios can be cultured on TCBS agar, but specimens for enterotoxigenic E. coli should be sent to a reference labo-
Figure 1. Guidelines for stool examinations. *Fecal screen: culture for Salmonella, Shigella, Campylobacter, and stool for intestinal parasites. † History of antimicrobial agent or cancer chemotherapy.

Neonates and Infants

Most enteric pathogens are detected in neonates and infants up to 3-yr old. The epidemiology of disease in this patient population greatly influences the diagnostic approach. In temperate zones of the Northern Hemisphere, from December to March, rotavirus is the most important cause of diarrheal disease in young children (8). For diagnosis, a rotavirus EIISA test is usually the first step because the results are often available within 24 hr. The specimen can be refrigerated and further tests deferred until the rotavirus test result is known. If the test is negative, the fecal screen outlined previously can be performed. If symptoms persist for more than 5 days in rotavirus-positive patients, a stool specimen should be examined for other pathogens. Pathogens such as calicivirus and coronavirus produce disease with a clinical course similar to that of rotavirus. These viral infections, however, are diagnosed by electron microscopy which is too expensive for routine diagnostic work.

When routine fecal screens are negative and diarrhea persists, stools should be examined for hemorrhagic colitis-inducing E. coli (bloody stools), and duodenal aspirates or biopsies for Giardia (chronic diarrhea), and enteroadherent E. coli. The latter E. coli can be isolated by culturing duodenal tissue or fluid on MacConkey agar and it can be identified in a reference laboratory by its adherence to HEP-2 cells (6). Enteroadherent E. coli are associated with failure-to-thrive syndrome and nursery outbreaks of diarrheal disease (18).

The role of C. difficile diarrheal disease in children less than 3-yr old is unclear. Some investigators believe that this organism is normal flora in neonates and that toxin can be present in feces without accompanying disease. Others, however, have associated C. difficile with chronic diarrhea and failure-to-thrive syndrome (23).

The age at which children can develop antimicrobial agent-induced diarrhea and colitis requires clarification. Stark and Lee (22) suggest that the organism is normal flora for at least 9 months. In children younger than 3-yr old, the diagnosis of C. difficile-associated disease should be based on strong evidence such as a positive sigmoidoscopic examination and a positive toxin test.

Y. enterocolitica is frequently isolated from children. Although selective media and special culture conditions may be used to enhance its recovery, personal experience has shown that special measures are not necessary for recovering this organism from clinical material. During 1 yr, we used both cold enrichment and CIN agar (20) in parallel with our routine culture media (XLD, MacConkey, and 5% sheep blood agar) and recovered 15 Y. enterocolitica. Fourteen of the 15 strains were initially isolated on MacConkey medium incubated at 35°C. Only one isolate, which was considered clinically insignificant, was recovered by cold enrichment. Y. enterocolitica appears as pinpoint, lactose-nonfermenting organisms on MacConkey agar incubated at 35°C for 24 to 48 hr. Alert laboratory technologists can recognize Y. enterocolitica on routine media without resorting to
expensive enrichment and selective culture methods.

Food-Borne Outbreaks

The possibility of a food-borne outbreak should be considered when diagnosing diarrheal disease. In most instances outbreaks are limited to small numbers of people and the disease is mild and self-limited. Some outbreaks, however, are widespread, affect hundreds to thousands of individuals, and have potentially high morbidity and mortality. Clinical microbiologists, in conjunction with their infectious disease colleagues, should evaluate unusual patterns of recovery of enteric pathogens. For example, this past summer one of our pediatric infectious disease physicians, working with our pediatric infectious disease physicians, helped to uncover an outbreak of shigellosis which occurred after a family reunion. Nine family members had stool cultures positive for *Shigella*. By working with public health officials, we were able to contain the outbreak to this single family.

Summary

This article has presented a rational protocol for examining stools for enteric pathogens. When modified according to a laboratory’s geographical location and patient population, this approach should allow efficient, comprehensive diagnosis of diarrheal disease. Laboratories should be able to examine stools routinely for *Campylobacter* spp., *Salmonella* spp., *Shigella* spp., *Y. enterocolitica*, intestinal parasites [including *Cryptosporidium* (14)], rotavirus, and *C. difficile* toxin. When unusual organisms such as pathogenic *E. coli* are suspected, or tissue culture facilities for *C. difficile* toxin assays are unavailable, the use of reference laboratories is strongly encouraged.