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PRACTICAL GUIDANCE FOR CLINICAL MICROBIOLOGY (FORMERLY *CUMITECHS*)

Laboratory Diagnosis of Bacterial Gastroenteritis

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SUMMARY

Bacterial gastroenteritis is a disease that is pervasive in both the developing and developed worlds. While for the most part bacterial gastroenteritis is self-limiting, identification of an etiological agent by bacterial stool culture is required for the management of patients with severe or prolonged diarrhea, symptoms consistent with invasive disease, or a history that may predict a complicated course of disease. Importantly, characterization of bacterial enteropathogens from stool cultures in clinical laboratories is one of the primary means by which public health officials identify and track outbreaks of bacterial gastroenteritis. This article provides guidance for clinical microbiology laboratories that perform stool cultures. The general characteristics, epidemiology, and clinical manifestations of key bacterial enteropathogens are summarized. Information regarding optimal specimen collection, transport, and processing and current diagnostic tests and testing algorithms is provided. This article is an update of *Cumitech 12A* (P. H. Gilligan, J. M. Janda, M. A. Karmali, and J. M. Miller, *Cumitech 12A*, *Laboratory diagnosis of bacterial diarrhea*, 1992).

INTRODUCTION

Over 1.7 billion global cases of diarrheal disease are reported annually (http://www.who.int/mediacentre/factsheets/fs330/en/index.html) and are associated with an estimated 2.2 million deaths. The burden of diarrheal disease is most critical in developing countries, facilitated by unsafe water supplies, poor sanitation, and nutritional deficiencies. Diarrheal disease in children aged <5 years in these countries is devastating, where repeated diarrheal episodes contribute to malnutrition, which in turn puts these children at heightened risk of acquiring infectious diarrhea

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TABLE 1 Types of bacterial gastroenteritis[a]

Parameter	Secretory gastroenteritis	Inflammatory gastroenteritis	Invasive gastroenteritis
Location	Proximal small intestine	Colon	Distal small intestine
Type of illness	Watery diarrhea	Dysentery	Enteric fever
Stool examination	No fecal leukocytes	Fecal polymorphonuclear leukocytes	Fecal mononuclear leukocytes (if patient has diarrhea)
Mechanism	Enterotoxin or bacterial adherence/invasion causes a shift in water and electrolyte excretion/adsorption	Bacterial invasion or cytotoxins cause mucosal damage that leads to inflammation	Bacteria penetrate the mucosa and invade the reticuloendothelial system
Classic pathogens	*Vibrio cholerae*, ETEC, *Clostridium perfringens*, *Bacillus cereus*, *Staphylococcus aureus*	*Shigella*, STEC, *Salmonella* (not *Salmonella* Typhi/Paratyphi), *Vibrio parahaemolyticus*, *Clostridium difficile*, *Campylobacter*	*Salmonella* Typhi/Paratyphi, *Yersinia enterocolitica*

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and is associated with stunting and impaired cognitive development (1, 2). While less common in high-income countries, diarrheal diseases remain a significant health concern. There are an estimated 211 to 375 million episodes of diarrheal illnesses each year in the United States, with 1.8 million hospitalizations and 3,100 deaths (3). Many of these cases are foodborne. The Foodborne Diseases Active Surveillance Network (FoodNet) at the Centers for Disease Control and Prevention (CDC) reported 1,000 foodborne outbreaks that resulted in 48 million illnesses, 128,000 hospitalizations, and 3,000 deaths from 10 sites in the United States over a 15-year period (4). It is important to note that many cases of foodborne diarrheal illness are not part of a recognized outbreak and thus are not captured by the FoodNet data (5).

Diarrhea is defined by the Infectious Diseases Society of America (IDSA) and the American College of Gastroenterology (ACG) as the passage of three or more loose or liquid stools per day. It can be further be classified by the duration of symptoms (3, 6). Patients with acute diarrhea have symptoms lasting less than 14 days. Those with diarrhea for >14 days, but <1 month are said to have persistent diarrhea. Those experiencing diarrhea for longer than 30 days are said to have chronic diarrhea. Diarrhea may be infectious, i.e., caused by bacteria, viruses, or parasites, but with increasing frequency in high-income nations, the etiology of diarrhea is noninfectious. In these cases, diarrhea is caused by food intolerances, reactions to medication, intestinal disorders such as irritable bowel syndrome, or intestinal diseases, including Crohn's disease, ulcerative colitis, and celiac disease. In these instances, laboratory tests for infectious etiologies, including a bacterial stool culture, are useful for diagnosis by either ruling out or ruling in a common infectious process (3).

The primary mechanisms for bacterial gastroenteritis are (i) excessive secretion of fluids in the proximal small intestine induced by the action of luminal toxins expressed by enteropathogens or by minimally invasive bacteria, (ii) inflammatory or cytotoxic damage of the ileal or colonic mucosa which may produce blood and pus, or (iii) penetration of the bacterium through the mucosa to the reticuloendothelial system, as is the case with typhoid fever. Classic examples of bacteria that cause these various syndromes are presented in Table 1. Regardless of mechanism, most cases of bacterial gastroenteritis are self-limiting, and, with a few exceptions, neither empirical antimicrobial therapy nor bacterial stool culture is indicated (3). For most patients who present with acute diarrhea, symptoms have resolved by the time bacterial culture results are available, and these generally do not change patient management (7). Rather, the primary goal for the patient with acute diarrhea is symptomatic relief, rehydration (or prevention of dehydration), and potentially preventing transmission of the infection. In contrast, a bacterial stool culture is indicated for patients with severe or prolonged diarrhea, those with symptoms consistent with invasive disease, or those with a medical history predictive of complications associated with their gastrointestinal disease (3, 6, 8–10). For example, the American College of Gastroenterology recommends a routine stool culture for a patient who presents with any of the following symptoms: severe or persistent diarrhea, temperature of >38.5°C, bloody diarrhea, or the presence of stool leukocytes, lactoferrin, or occult blood (6). The IDSA similarly recommends that stool cultures be performed for a patient with diarrhea for >1 day, fever, dehydration, systemic illness, bloody stools, or a clinical history that would include bacterial pathogens in the differential diagnosis (3).

In addition to the value for patient care, a bacterial stool culture is an important tool for public health. Isolates recovered from stool cultures performed by clinical laboratories are used to identify and track outbreaks at the local, national, and international levels. The dilemma with some of the newer test methods, including molecular assays, is the lack of organism recovery, which is currently needed for public health investigations.

The objectives for this practical guideline are to discuss the more common bacterial organisms associated with diarrheal disease, briefly describe emerging bacterial pathogens associated with diarrheal disease, describe stool specimen collection, transport, and processing, and discuss test methods used to identify these bacterial agents and antimicrobial susceptibility testing.

BACTERIAL PATHOGENS

Aeromonas Species

Over 26 different species of *Aeromonas* have been described to date, but the vast majority of these are of limited clinical or public health significance. *Aeromonas* spp. are ubiquitous in aquatic habitats, and concentrations peak when water temperatures rise substantially during the summer months. Consumable products such as poultry, lamb, veal, pork, and ground beef can harbor *Aeromonas* spp. Consumption of contaminated foods or potable water or accidental ingestion of untreated water during recreation are the most common sources of infection. In humans, *Aeromonas* spp. are not considered to be normal gastrointestinal flora, and the

estimated human intestinal carrier/colonization rate is extremely low in healthy persons.

Most authoritative documents list *Aeromonas* spp. as accepted enteropathogens, although there still are no bona fide outbreaks of gastroenteritis attributable to this genus (11, 12). The incidence of *Aeromonas*-associated gastroenteritis on a global basis varies dramatically in association with geographic and socioeconomic factors. In developing countries where sanitary conditions are substandard, the reported incidence of *Aeromonas* diarrhea can be high, ranging from approximately 4% to 22% (13–16). In industrialized countries, regardless of patient population and sample size, *Aeromonas*-associated gastroenteritis has been reported at frequencies of 0% to 10% (17, 18).

Aeromonas diarrhea presents as either an acute watery diarrhea (enteritis) or as a more invasive bloody form resembling dysentery or enterocolitis (17). The secretory form is much more common than the dysenteric variety. A third, extremely rare variation of *Aeromonas* gastroenteritis presents as a cholera-like illness with profound watery diarrhea. Most intestinal infections associated with *Aeromonas* spp. are self-limiting, although chronic diarrhea exceeding for 1 year has been described (19, 20).

Several potential serious complications can result secondary to *Aeromonas* gastroenteritis, including ulcerative colitis, pan colitis, segmental colitis, or inflammatory bowel disease (17). In a few instances, cases of hemolytic-uremic syndrome (HUS) associated with *Aeromonas hydrophila* or *Aeromonas veronii* biovar sobria have been reported in infants and adults (21). Some *Aeromonas* spp. have been shown to carry the Shiga toxin (Stx) genes 1 and 2 (22), and development of HUS in patients infected with *Aeromonas* spp. may be attributable to this virulence factor. The most serious complication of *Aeromonas* gastroenteritis is translocation from the gut into the circulatory system, producing frank septicemia (23). This situation typically exists in persons with underlying conditions, including hepatic cirrhosis or malignancies of the circulatory systems. Attributable fatality rates due to *Aeromonas* sepsis range from 32% to 45% (17).

Bacillus cereus

B. cereus is ubiquitous in the environment, being found in decaying organic matter, soil, freshwater and salt water, vegetables, and the intestinal tracts of invertebrates (24). The spores are resistant to heat, freezing, and drying and can survive gamma radiation and pasteurization processes (25). Hydrophobic in nature, the spores can adhere to cooking and food surfaces (26, 27). *B. cereus* spores can germinate in foods that are not promptly cooled and refrigerated after meals or in food heated for prolonged periods at temperatures below 60°C. Outbreak surveillance data from 2009 and 2010 documented 427 illnesses associated with 25 outbreaks in the United States due to *B. cereus* (28).

There are two distinct syndromes associated with *Bacillus cereus* food poisoning: an emetic syndrome and a diarrheal syndrome. The emetic syndrome is due to intoxication by a preformed toxin ingested in food. The emetic toxin, called cereulide, is a plasmid-encoded peptide that is resistant to heat, proteolysis, and acid. As such, the toxin is not destroyed by gastric acids or proteolytic enzymes in the intestinal tract or by food reheating (29). Cereulide is responsible for symptoms of nausea and vomiting (25, 29, 30), which appear within 1/2 to 6 h after ingestion (30). These symptoms are similar to those seen with *Staphylococcus aureus* enterotoxins. Symptoms usually resolve within 6 to 24 h, but rare case reports have documented fulminant hepatic failure and death associated with emetic *B. cereus* (31–34). The emetic toxin is most often found in starchy foods, such as fried rice, pastry, and noodles (35).

The diarrheal syndrome is characterized by abdominal cramps, pain, and watery diarrhea within 8 to 16 h of ingestion of food that contains viable vegetative cells or spores of *B. cereus*. The symptoms of this diarrheal illness are similar to those seen with *Clostridium perfringens* food poisoning (35). Symptoms typically resolve with 12 to 24 h (35). Although rare, fatalities have occurred with *B. cereus* diarrheal disease (29). In the diarrhea syndrome, 3 pore-forming enterotoxins are expressed by the vegetative cells in the small intestine, which damage the ileal epithelial cell membranes. The 3 enterotoxins are hemolysin BL (HBL), nonhemolytic enterotoxin (NHE), and cytotoxin K (25, 35).

Individuals at increased risk of *B. cereus* diarrheal disease include those with lowered stomach acidity, such as is seen in patients with achlorydria or the elderly (36). *B. cereus* has been isolated from the stools of 0 to 43% healthy children and adults, at various concentrations. However, these cases represent transient colonization, most likely obtained from low-level exposure from the environment (24, 37, 38).

Campylobacter Species

Campylobacter is one of the leading causes of bacterial diarrhea worldwide (39). FoodNet estimates that 1.3 million persons in the United States are affected each year by *Campylobacter* infections (40). The true incidence may be up to 35 times higher due to undiagnosed or unreported cases (41). Geographic variation in rates of campylobacteriosis has been consistently observed in the United States between 1996 and 2006, with the mean annual rate of culture-confirmed campylobacteriosis being 5-fold higher in California (34 cases per 100,000 population) than in other states (42). The reason for this difference is unclear, but does not appear to be associated with increased physician visits, laboratory test ordering, or exposure to risk factors among patients in California compared to other states.

Campylobacter inhabits the intestinal tracts of food animals, such as poultry, cattle, swine, and sheep, and domestic pets, including cats and dogs. The organism rarely causes disease in animals but is shed in the feces. Meat typically becomes contaminated with animal feces harboring *Campylobacter* spp. during slaughtering. Transmission of the organism is typically foodborne, by ingestion of undercooked contaminated meat and meat products or contaminated dairy products. In addition, waterborne infections occur, via consumption of contaminated water and ice. Contact with infected animals, particularly cats and puppies, has also been shown to be a route of transmission. The typical incubation period for *Campylobacter* is 2 to 5 days, but it may be up to 10 days (43). Most cases of *Campylobacter* enteritis are sporadic, but the incidence increases starting in March and throughout the summer months. Outbreaks associated with *Campylobacter* have been due to consumption of raw milk or well water contaminated with effluent from livestock operations (44–46). Higher rates of *Campylobacter* enteritis are seen in those <4 years of age and 15 to 44 years of age (47). Travelers to developing countries are also at increased risk of *Campylobacter* enteritis.

Campylobacter jejuni subsp. *jejuni* and *Campylobacter coli* are the most common *Campylobacter* species associated with diarrheal illness. *C. jejuni* is responsible for >90% of cases (43, 48).

Campylobacter upsaliensis, which was first isolated from dogs with diarrhea, has also been shown to cause human disease. The incidence of *C. upsaliensis* among patients with diarrhea may be underappreciated, as the organism cannot grow on the selective media typically used to recover *Campylobacter* in clinical laboratories (49–51). Other *Campylobacter* spp. associated with gastroenteritis include *Campylobacter fetus* subsp. *fetus*, *Campylobacter lari*, *Campylobacter concisus*, *Campylobacter jejuni* subsp. *doylei*, and *Campylobacter hyointestinalis* (48, 52).

C. jejuni and *C. coli* cause indistinguishable infections (48). Before the onset of diarrhea, a febrile period with malaise, abdominal pain, and myalgia occurs in about 50% of symptomatic patients (43). Diarrhea is characterized by loose watery stools, with or without blood. Blood and fecal white cells may be present. Abdominal cramping can mimic pain associated with acute appendicitis. In most cases, the diarrhea is self-limited, resolving within a week without antimicrobial therapy. However, relapse occurs in 5 to 10% of untreated patients (43). Extraintestinal *Campylobacter* infections such as bacteremia, urinary tract infections, cholecystitis, hepatitis, pancreatitis, nephritis, meningitis, abortion, and neonatal sepsis have also been reported (53). *Campylobacter* bacteremia is typically uncommon, but it occurs more frequently in patients with HIV infection, malignancy, and liver disease (54). Bacteremia and extraintestinal infections are also more common in neonates and the elderly (55).

Autoimmune complications, such as reactive arthritis and Guillain-Barré syndrome (GBS), can occur post-*Campylobacter* infection (56). Reactive arthritis affects 2 to 4% of patients postcampylobacteriosis and is characterized by pain and joint swelling that lasts for several weeks to a year (48). In 5% of cases, arthritis is chronic or relapsing (57). Symptoms typically begin 3 to 40 days postdiarrhea and most commonly affect the knees (58). GBS is an acute paralytic disease of the peripheral nervous system and is seen in approximately 0.1% of *Campylobacter* cases. Lipooligosaccharides of *C. jejuni*, which mimic human ganglioside, elicit autoantibodies that then react with peripheral nerve targets (56). The onset of GBS usually occurs within 2 to 21 days of the diarrheal illness (59).

Clostridium difficile

Clostridium difficile is an obligately anaerobic, spore-forming Gram-positive rod. The spores of *C. difficile* are resistant to stomach acid, heat, and many commercial disinfectants used in hospitals (60). Following ingestion, exposure of the spores to bile salts in the small intestine triggers germination (61). Pathogenic strains of *C. difficile* harbor a pathogenicity locus (PaLoc) that encodes the organism's two main virulence factors: toxin A, an enterotoxin (encoded by *tcdA*), and toxin B, a highly potent cytotoxin (encoded by *tcdB*) (62). The individual role of these two toxins in disease are controversial. Clinical isolates of *C. difficile* that do not express toxin A have been isolated from symptomatic patients (63, 64), albeit rarely, whereas toxin B-deficient strains have not. Both toxin A- and toxin B-deficient mutants remain capable of causing disease in hamsters, although both are attenuated compared to the wild-type strain (65).

C. difficile can readily be found in soil and the intestinal tracts of animals and humans. *C. difficile* colonization rates are as high as 50% in healthy infants and children <1 year of age (66, 67), whereas 3% to 5% of healthy adults are colonized (67). Much higher rates of colonization, 10 to 50%, are seen in high-risk populations, such as hospitalized patients and long-term-care facility residents. Previous antimicrobial use and previous *C. difficile* infection (CDI) are predictors of colonization in these populations (68–70). *C. difficile* is acquired through the ingestion of spores via the fecal-oral route or through exposure to spores in the environment. A recent study demonstrated that only a third of CDI cases could be linked by whole-genome sequencing of isolates to a symptomatic patient, whereas the remainder of cases were attributed to exposure from the environment or asymptomatic carriers (71).

C. difficile is the primary pathogen associated with antibiotic-associated colitis (72, 73). In the United States, the rate of CDI increased 4-fold between 1993 and 2009 but leveled off at 110 per 100,000 hospital stays in 2009 (74). By far the highest rate of CDI is among patients aged 65 and older, with over 1,000 cases per 100,000 hospitalizations in 2009 reported for this age group (74).

In 2005, the NAP1/027/B1 strain emerged in Canada, Europe, and the United States, concomitant with a significant rise in morbidity and mortality associated with CDI over those in previous years (75, 76). At the time, this change in severity of CDI was attributed to the "hypervirulent" nature of the NAP1/027/B1 strain. NAP1/027/B1 has since become the predominant strain in many locations, and it continues to be associated with high mortality and relapse rates (77). Early studies pointed to heightened toxin expression (78), more efficient sporulation (79, 80), expression of the binary toxin, and fluoroquinolone resistance (75) as reasons for the epidemiological success of this strain. However, some studies questioned the relevance of the NAP1/027/B1 strain type in disease severity (81, 82), and it has since been confirmed that not all NAP1/027/B1 strains express larger quantities of toxin than historical strains (83).

The range of symptoms associated with infection with toxigenic *C. difficile* includes asymptomatic carriage, mild to moderate diarrhea, and pseudomembranous colitis (PMC). Patients may present with a brief, self-limiting diarrhea or with profuse watery diarrhea similar to that in cholera (84). Fever, abdominal cramping, and leukocytosis can be seen in individuals with more severe diarrhea. Persons with PMC present with abdominal pain, fever, marked leukocytosis, and severe diarrhea that may be bloody. Poor prognostic indicators include a rapid increase in the peripheral white blood count with an increase in band forms and a sudden absence of diarrhea (85).

The most common conditions associated with CDI are dehydration and electrolyte disorders, which may affect up to 92% of patients. Less frequent conditions associated with CDI include septicemia, hypoalbuminemia, renal failure, septic shock, ascites, and peritonitis. The more severe complications of CDI include intestinal perforation and toxic megacolon. While these severe complications are only observed in 0.1% to 3% of all CDI cases (74, 86, 87), the mortality associated with toxic megacolon is high, ranging from 38% to 80% (86, 88).

Recurrence of CDI is seen in 10% to 20% of cases after initial symptom resolution (89). Recurrent infections are attributable to both relapse (i.e., spores that are not killed by antimicrobial therapy, which can then germinate once therapy is completed) and reinfection with a new strain (90–93). However, it is important to note that patients who are asymptomatically colonized with *C. difficile* are at decreased risk for CDI, although the reason for this remains unclear (94).

Exposure to antimicrobial agents and exposure to health care

facilities are hallmark risk factors for CDI. While almost all antimicrobial agents have been associated with CDI, the most common are penicillins, second- and third-generation cephalosporins, clindamycin, and fluoroquinolones (84, 95). As stated previously, advanced aged (>65 years) is also an important risk factor for CDI; this age group has over 10-fold the number of CDI hospitalizations than the general population in the United States (74). Other, less well-defined risk factors for CDI include use of gastric acid suppressors, stool softeners, laxatives, and/or enemas, chemotherapy, and gastrointestinal surgery (96).

Clostridium perfringens

Clostridium perfringens is ubiquitous in the environment and can be found in the feces of humans and animals. Food poisoning with *C. perfringens* requires ingestion of a high burden of vegetative cells, usually 10^8. The typical mechanism for this is food contaminated with *C. perfringens* that is improperly cooked, stored, and reheated. Spores that survived the initial heating processes germinate and proliferate during a slow cooling of food or when the food is insufficiently reheated. Following ingestion, the organism sporulates upon entry into the small intestine, which is concomitant with expression of an enterotoxin that is responsible for patient symptoms. *C. perfringens* serotype A is the most common serotype associated with food poisoning and diarrhea (97, 98).

From 2009 to 2010, there were 60 confirmed *C. perfringens* foodborne outbreaks and 3,225 reported illnesses, making *C. perfringens* the second most common cause of bacterial foodborne disease in the United States in this time period (28). Symptoms most often associated with *C. perfringens* food poisoning are watery diarrhea, severe abdominal cramping and pain, and vomiting. The onset of symptoms ranges from 8 to 24 h after the ingestion of contaminated food. The illness is self-limiting, and symptoms resolve within 24 h.

A rare type of food poisoning called enteritis necroticans or "pig-bel" is associated with the ingestion of food, usually pork, heavily contaminated with *C. perfringens* serotype C. This organism produces a beta toxin that causes intestinal wall necrosis. Pig-bel has a mortality rate of 40% and primarily affects malnourished persons, especially children (99). *C. perfringens* has also been linked to antibiotic-associated diarrhea that does not cause pseudomembranous colitis (73, 100).

Escherichia coli

Escherichia coli was initially considered to only be a commensal residing in the gastrointestinal tract. However, several pathogenic variants (pathotypes) are now recognized and associated with diarrheal diseases. Although *E. coli* is easy to identify to species level, it is extremely difficult to recognize strains belonging to different pathotypes of diarrheagenic *E. coli*, as these are defined by the expression of one or more group-specific virulence factors. The six major diarrheagenic pathotypes described to date are enteropathogenic *E. coli*, Shiga toxin-producing *E. coli* (STEC), enteroinvasive *E. coli* (EIEC), enterotoxigenic *E. coli*, enteroaggregative *E. coli*, and adherent invasive *E. coli* (101). Of these, only STEC is routinely identified by most clinical and public health laboratories, and it will be the focus of the discussion here. STEC is defined by the presence of a Shiga toxin 1 (Stx1) and/or Shiga toxin 2 (Stx2) gene. Historically, these isolates were called enterohemorrhagic *E. coli* (EHEC) or verocytotoxin-producing *E. coli* (VTEC). STEC includes both O157 and non-O157 serotypes of *E. coli*.

Ruminants, such as cattle, are the major reservoir for STEC. Poor sanitation, fecal runoff into rivers and streams, and inadequate control measures in the meat and food processing industries have all led to the recovery of STEC from virtually any consumable product. Infection with STEC occurs following consumption of these contaminated products. Infections occur predominantly in the summer months but can be observed year round (102).

The incidence of STEC infections in the United States is monitored by FoodNet. In 2012, the incidence of O157 STEC was 1.12 per 100,000 population, and the incidence of non-O157 STEC was 1.16 per 100,000 (103). Among the non-O157 STEC strains, O26, O103, O111, O121, O45, and O145 are the most common serotypes isolated in the United States (104). The incidence of STEC in other developed countries varies; it is as low as 0.4 per 100,000 in Australia (105) and as high as 5.33 per 100,000 in Ireland (106). The incidence of STEC is much higher in developing countries such as Argentina and India, but formal surveillance data are not available for these countries.

STEC disease presents as enteritis that may quickly progress to hemorrhagic colitis (107). The chief symptoms included bloody diarrhea, abdominal pain, nausea, and vomiting (108). Importantly, not all STEC infections are associated with bloody diarrhea (109, 110), and so laboratory algorithms that only test bloody specimens for STEC are no longer considered standard of care. The most common and serious complication of STEC infection is the development of HUS, which typically presents 5 to 13 days after the onset of diarrhea (11). HUS is life-threatening and consists of the triad of renal failure, microangiopathic hemolytic anemia, and thrombocytopenia. The mortality rate connected with HUS is 3% to 5% (111). It has been estimated that 61% of all HUS cases are related to STEC infection (111). HUS has been observed more frequently in O157 (11% of cases) versus non-O157 (1% of cases) STEC infections (104). Approximately 15% of children <10 years of age develop HUS following STEC infection. However, in the recent outbreak of O104 STEC in Germany, 22% of children developed HUS (112–114). It should be noted that this outbreak was caused by an atypical STEC strain that harbored enteroaggregative *E. coli* virulence factors in addition to the Shiga toxins. HUS occurs much less frequently among adults and is associated predominantly with advanced age (>75 years) (115). Increased rates of HUS have been more frequently associated with Stx2-expressing STEC strains. Exposure to antibiotics also increases the risk of HUS in children (114). However, recent data demonstrated that treatment with ciprofloxacin reduced the risk of HUS in patients infected with the 2011 German O104 STEC strain (116). These data are supported by a recent meta-analysis of studies between 1980 and 2011 (117). Despite this, the decision to treat a patient with STEC infection with antimicrobials remains controversial. In addition, use of antimotility agents has been associated with longer duration of bloody diarrhea, as well as progression to HUS (118).

Listeria monocytogenes

The genus *Listeria* is composed of six species, of which *Listeria monocytogenes* is the common human pathogen, causing intestinal as well as extraintestinal infections. *L. monocytogenes* is a common environmental inhabitant of soil, vegetation, and animals (119). Because *Listeria* spp. can survive under acidic and salt-enhanced conditions in foods and can grow at refrigeration temperatures (4°C), they have the capacity to survive and multiply in

large numbers in a variety of refrigerated foods (119, 120). A high percentage (32%) of foods recalled by the FDA involve *L. monocytogenes* (121). The major risk factor associated with *L. monocytogenes* gastroenteritis is the consumption of foods heavily contaminated (10^7 to 10^9 CFU/g or ml) with *L. monocytogenes* (122).

The incidence of *L. monocytogenes* gastroenteritis is unknown. Surveillance data from the CDC and other sources, including FoodNet, have focused on invasive listeriosis (bacteremia and central nervous system infection) as a consequence of foodborne infection. In 2011, the incidence of invasive listeriosis was 0.31 per 100,000 population. Many patients with invasive listeriosis have a history of gastrointestinal symptoms that consist of diarrhea, nausea, vomiting, and fever. This, coupled with reports of *L. monocytogenes* outbreaks of gastroenteritis (122, 123), suggests that *L. monocytogenes* may be an infrequent cause of gastroenteritis in patients with negative bacterial stool cultures. One 2005 Canadian study found the maximum incidence of *L. monocytogenes*-associated diarrhea to vary from 0.2% to 0.5%, depending upon the population studied (123). On rare occasions, *Listeria ivanovii* has been reported to cause diarrhea in severely immunosuppressed individuals (124).

The typical incubation period for gastrointestinal infection is 24 h; however, it can range from 6 h to as long as 10 days (120). Once symptoms begin, diarrhea lasts for 1 to 3 days (122). In a study of cases of gastroenteritis linked to outbreaks, attack rates ranged from 50% to 90% and the median number of stools/day was 12 (range, 3 to 50) (122). The syndrome is typically characterized by a febrile illness with diarrhea, headache, and arthralgia/myalgia. Other, less frequently encountered complications include abdominal pain, nausea, vomiting, dizziness, lymphadenopathy, and presence of a rash (12, 122). Fever, which occurs in 60% to 100% of infected persons, is a cardinal feature associated with *L. monocytogenes* diarrhea.

The most serious complication of listeriosis is invasive disease, including septicemia and meningitis. *L. monocytogenes* has tropism for the brain and as a result can cause encephalitis, rhombencephalitis (brain stem encephalitis), and brain abscess. The case fatality rate for most cases of listeriosis with comorbidities has been reported to be between 20% and 40% (125).

Reputed risk factors associated with acquiring *L. monocytogenes* gastroenteritis include gastric acidity, use of antacids, use of H_2 receptor antagonists, and use of laxatives (119, 122, 126). In addition, those with inflammatory bowel disease (IBD) and Crohn's disease may have a more frequent incidence of *Listeria* diarrhea (as opposed to *Campylobacter* or *Salmonella*) (123, 126).

Plesiomonas shigelloides

Plesiomonas shigelloides is the sole oxidase-positive member of the *Enterobacteriaceae* family. While *P. shigelloides* has been associated with diarrheal disease in numerous reports, a definitive causal relationship with *P. shigelloides* has yet to be established through volunteer or animal studies (127).

P. shigelloides is found in aquatic environments and has been isolated from both cold-blooded and warm-blooded animals. In humans, there has been a reported prevalence rate of 0.01% to 5.5% in asymptomatic individuals (128, 129). Transmission occurs primarily through the consumption of seafood, such as oysters and shellfish, or water that has been contaminated with sewage. Most cases of *P. shigelloides* diarrheal illness are sporadic; however, there have been reported outbreaks associated with the organism (130–132). Coinfection with *P. shigelloides* and other enteropathogens has been reported (132, 133), and some evidence suggests that *P. shigelloides* causes diarrhea only as a coinfecting pathogen, rather than on its own (133). Both secretory and dysentery-type diarrhea have been reported with *P. shigelloides* infections (130, 134). Most infections are characterized by self-limiting diarrhea with blood or mucus, abdominal cramps, vomiting, and fever (130). While most diarrheal episodes are described as acute, there have been reported chronic cases lasting over 2 weeks (135).

Salmonella Species

Salmonella, a member of the family *Enterobacteriaceae*, is a facultatively anaerobic Gram-negative rod. *Salmonella* taxonomy is a complicated matter, with two species in the genus: *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* has six subspecies (*S. enterica* subsp. *enterica*, *S. enterica* subsp. *salamae*, *S. enterica* subsp. *arizonae*, *S. enterica* subsp. *diarizonae*, *S. enterica* subsp. *indica*, and *S. enterica* subsp. *houtenae*) that can be further serotyped using the Kauffmann-White-Le Minor scheme, based on the properties of their somatic (O), flagellar (H), and capsular polysaccharide (Vi) antigens (136, 137). There are over 2,500 serotypes of *S. enterica* (136, 137). Because of the diversity of the genus, several isolates may be difficult to identify due to atypical biochemical reactions.

Salmonella colonizes the intestinal tracts of vertebrates. Some serotypes, including *Salmonella enterica* subsp. *enterica* serotype Typhi (*Salmonella* Typhi), are only found in human hosts. The majority of *Salmonella* cases occur as the result of ingesting contaminated food or water. *Salmonella* can also be acquired by contact with domestic animals and their food products, farm animals or animals in petting zoo, and exotic pets like turtles, hedgehogs, and iguanas (138–142). *Salmonella* can also be transmitted from person to person via the oral-fecal route.

The incidence of *Salmonella* infections in the United States in 2011 was 1,645 per 100,000 population (143), with higher rates in late summer and early fall. Worldwide, there are an estimated 94 million cases of nontyphoidal *Salmonella* gastroenteritis and about 155,000 deaths (144). In developing countries, and the Indian subcontinent in particular, typhoidal isolates cause the majority of disease and are associated with an estimated 21.6 million annual cases and 216,500 deaths (145). In sub-Saharan Africa, nontyphoidal *Salmonella*, predominantly the *Salmonella* Typhimurium ST313 strain, are a significant cause of bloodstream infections in both children and adults (146, 147). In the United States, the most common serotypes reported are *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Salmonella* Newport (143).

Nontyphoidal salmonellosis consists of diarrhea, nausea, headache, and abdominal cramps, which last for 4 to 7 days. Fever may be present and usually resolves in 24 to 48 h. The disease is typically limited to the lamina propria of the small intestine, and antimicrobial therapy is not indicated. Extraintestinal manifestations, such as bacteremia, septic arthritis, urinary tract infections, and osteomyelitis, are seen in 5% of cases (148–153). Some individuals may become asymptomatic carriers of the organism, and shedding occurs for several weeks to a few months.

Typhoid fever is caused by *Salmonella* Typhi, and a similar syndrome is caused by *Salmonella* Paratyphi A, *Salmonella* Paratyphi C, and tartrate-negative variants of *Salmonella* Paratyphi B. In typhoid, the organism disseminates from the lamina propria to

the reticuloendothelial system in infected phagocytes via lymphatic and hematogenous routes. Fever, malaise, anorexia, headaches, and vomiting are common symptoms of typhoid and typically start 1 to 3 weeks after infection. Patients may have diarrhea following ingestion of the organism, but many do not. Rose spots, which are blanching maculopapular lesions 2 to 4 mm in diameter, are seen in 5 to 30% of cases. A complication of untreated typhoid fever is the erosion of the blood vessels in the Peyer's patches, which can lead to intestinal hemorrhage (145). The organism persists in the mesenteric lymph nodes, gallbladder, and bone marrow for years. Five to 10 percent of patients will have a relapse of infection, typically 2 to 3 weeks following resolution of symptoms (154). Up to 10% of asymptomatic patients will become carriers, and 1 to 4% of these will shed for more than 1 year (154).

The severity of *Salmonella* disease depends on the inoculating dose (155), infecting serotype (151), and predisposing host factors. Children under 1 year of age have the highest incidence of *Salmonella* in the United States (143). Because *Salmonella* must survive the gastric acid barrier in order to gain access to the small intestine where it causes disease, patients with decreased gastric acid production, from advanced age, gastrectomy, or H_2 receptor antagonists, are at increased risk of infection. Individuals with impaired cellular immunity (e.g., AIDS) or altered phagocyte function (e.g., sickle cell anemia) are at increased risk for both invasive nontyphoid *Salmonella* infections and typhoid (156, 157). However, these individuals do not appear to have more severe typhoid infections should they become infected (158, 159). In the United States, nearly all cases of typhoid and paratyphoid fever are in returning travelers and immigrants (160).

Shigella Species

Shigella species are host adapted to humans but have been documented in rare instances from dogs and primates (161). They can be acquired from ingestion of a variety of foods or water contaminated with human feces, sexually during oral-anal sex, or by laboratory workers. The four species of *Shigella* are *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii*, and *Shigella sonnei*. Transmission by person-to-person contact is common for *Shigella* spp. because of a low infectious dose of 10 to 100 organisms (161). Between 2009 and 2010, *Shigella* accounted for 508/8,523 (2%) of reported illnesses associated with foodborne outbreaks (28). The incidence of *Shigella* infections reported by FoodNet in the United States in 2011 was 3.24 per 100,000 and ranged from 0.99 to 6.78 per 100,000, depending on the region (143).

Shigellosis and dysentery are diseases associated primarily with poor hygiene and lack of access to medical care. Approximately 150 million cases are reported annually in developing countries, in contrast to 1.5 million cases in industrialized nations. Of importance, one multicenter study found that half of patients with culture-negative, bloody stools were positive by PCR for *Shigella*, suggesting that the actual incidence of *Shigella* is grossly underestimated (162). Shigellosis symptoms range from watery diarrhea to mucoid and/or bloody stools, which can be accompanied by fever, malaise, and abdominal pain. In one study of 1,114 culture-confirmed patients followed for 14 days or longer, 29% (241) reported diarrhea persisting for ≥14 days (162). Factors associated with persistence were age, fever, mucoid diarrhea, vomiting, and abdominal pain. Headache and nuchal rigidity are common, with 95% and 39% of patients reporting these symptoms, respectively (161). *S. dysenteriae* type 1 is responsible for classic dysentery, which is manifested by fever, abdominal cramping, and bloody stool. Sepsis occurs primarily in malnourished pediatric patients in developing countries and is most commonly caused by *S. flexneri* (163). Long-term carriage (>1 year) occurs but is rare (164).

Meningitis, pneumonia, and urinary tract infections (UTIs) are rare complications of shigellosis and are most commonly seen with *S. flexneri* and *S. sonnei* (165–167). Notably, 40% of UTIs are asymptomatic and 35% are culture negative (167). Reactive arthritis has been reported in 1 to 3% of cases from outbreak data (161). The onset of reactive arthritis occurs within 3 weeks of gastrointestinal symptoms, with the duration of symptoms ranging from a few days to a few months; only *S. flexneri* has been associated with reactive arthritis.

HUS is the most serious complication of shigellosis. HUS occurs in ~13% of cases of *S. dysenteriae* type 1 shigellosis and is attributable to the expression of Stx1 by this organism (168). However, in rare cases, non-*S. dysenteriae* species of *Shigella* have been isolated from children with HUS (168, 169). *S. dysenteriae* type 1 HUS is seen mainly in children <5 years old in Asia and Africa.

Staphylococcus aureus

S. aureus food poisoning is an intoxication caused by the ingestion of preformed, heat-stable enterotoxin. There are 21 known staphylococcal enterotoxins, but phage-encoded staphylococcal enterotoxin A is the most frequently reported cause of *S. aureus* food poisoning worldwide (170–172). Coagulase-negative staphylococci (CoNS) can also acquire enterotoxins, but the reported cases or outbreaks of CoNS food poisoning have been limited (173, 174).

S. aureus is ubiquitous in the environment and colonizes the skin and mucous membranes of many mammals and birds (175). In humans, the anterior nares is the most commonly colonized site, and the organism is shed on to healthy skin (176). The rate of persistent carriage of *S. aureus* is reported to be 10 to 35%, and the rate of intermittent colonization ranges from 20 to 75% (176, 177). For those individuals harboring *S. aureus*, the organism can be transferred from their hands while preparing food. *S. aureus* is most commonly found in foods such as cream-filled pastries, cream pies, and sandwich fillings. However, food products involved in *S. aureus* food poisoning differ widely from one country to another (175). The CDC estimates that there are approximately 241,000 cases of foodborne illnesses in the United States caused by *S. aureus* annually.

A rapid onset of symptoms is characteristic of *S. aureus* food poisoning. General malaise, nausea, vomiting, stomach cramps, and diarrhea can occur within 30 min of ingestion of the contaminated food. The typical incubation period is 2 to 7 h, with symptoms resolving in about 12 h (11). Patients with staphylococcal food poisoning are not febrile. In most cases, medical treatment is not required. However, hospitalization for the severity of symptoms may be seen in 10% of those with *S. aureus* food poisoning (178). Severe dehydration may be seen in young children and elderly patients (178).

S. aureus food poisoning requires consumption of food or beverages harboring the staphylococcal enterotoxins. Unsafe food handling practices, including neglecting to wash hands prior to handling food and to promptly refrigerate prepared foods, are the primary reason for intoxication.

Vibrio and *Vibrio*-Like Species

The genus *Vibrio* is currently comprised of over 60 species. A number of other species traditionally associated with this genus have been recently reclassified into phylogenetically related neighboring clades, including *Grimontia hollisae* (*Vibrio hollisae*). Of the more than 60 *Vibrio* or *Vibrio*-like species that have been described, only a few these taxa have been consistently associated with bacterial gastroenteritis, with the two major species being *Vibrio cholerae* and *Vibrio parahaemolyticus*. Less frequent, but still of concern, are *Vibrio mimicus*, *Vibrio fluvialis*, *Vibrio vulnificus*, and *G. hollisae*.

Vibrio and vibrio-related bacteria are widely distributed in saltwater environments with salt concentrations of 17 to 37 ppt. Freshwater habitats with low salt concentrations (<0.5 ppt) can harbor nonhalophilic *Vibrio* spp. such as *V. cholerae* and *V. mimicus*. Because of their intimate association with the marine environment, *Vibrio* spp. can be found in many inhabitants of this macroecosystem, including shellfish such as oysters, clams, shrimp, and scallops.

The preeminent pathogen of this group is *V. cholerae*, which can cause sporadic, epidemic, and pandemic cholera. The WHO estimates that over 1.4 billion persons worldwide are at risk of developing cholera each year, with an estimated 2.8 million cases occurring annually and with over 130,000 deaths (179, 180). Today, the highest incidence of cholera is found in Africa and the southern regions of Asia. Two serogroups of *V. cholerae*, O1 (El Tor biotype) and O139, are responsible for the ongoing pandemic of cholera disease.

Cholera is not common in the United States, but the incidence of vibriosis (*V. parahaemolyticus*, *V. vulnificus*, and *V. alginolyticus*) is increasing. There are an estimated 80,000 illnesses with 500 hospitalizations and 100 deaths each year due to *Vibrio* illnesses in the United States, based upon data submitted through the Cholera and Other *Vibrio* Illness Surveillance (COVIS) system and FoodNet (181, 182). These cases include not only patients with diarrhea but also those with primary septicemia, wound infections, and otitis externa caused by *Vibrio* spp. The annual incidence of vibriosis in the United States has increased from 0.09 to 0.15 per 100,000 population in 1996 to 0.28 to 0.42 per 100,000 in 2010, with the highest incidence in coastal areas (181).

V. parahaemolyticus is responsible for many outbreaks of food-associated gastroenteritis worldwide. In Japan, it has been one of the most important causes of foodborne diarrhea since the 1960s (183). This species has also been responsible for the global spread of a pandemic clone, O3:K6, causing gastroenteritis in such diverse locales as North, Central, and South America, the Indian subcontinent, parts of Africa and Europe, and Indonesia from 1996 through 2004 (184). Other clonal strains, such as O4:K12, have caused more restricted outbreaks of disease, such as on the west coast of the United States (185). *V. mimicus* has been reported to cause at least two outbreaks of diarrheal disease (186, 187). The number of studies and case reports worldwide describing gastrointestinal infections cause by *V. fluvialis* seems to be increasing as well (188, 189). In the United States, *V. fluvialis* is typically the third most common *Vibrio* species associated with gastroenteritis, following *V. parahaemolyticus* and non-O1, non-O139 *V. cholerae*.

The chief clinical features of cholera are an afebrile, painless, watery diarrhea associated with *V. cholerae* O1 El Tor infection, accompanied by multiple bowel movements over a short period of time. Incubation periods for cholera typically span from 18 h to 5 days (190). Asymptomatic colonization is relatively common in areas of endemicity due to constant exposure to the infecting agent under unsanitary conditions. For symptomatic persons, clinical presentations of cholera range from a mild to moderate diarrhea to a more fulminant form termed cholera gravis (190). Cholera gravis is characterized by the release of large volumes of water (500 to 1,000 ml/h), which rapidly leads to severe dehydration, shock, and death over a short period of time if left untreated. The more severe forms of cholera are associated with pandemic strains bearing the O1 serogroup that carry a series of virulence genes, the two most important of which are those for cholera toxin and toxin-coregulated pilus (191). Cholera toxin is typically only found in O1 El Tor or the epidemic O139 Bengal strains, although other serogroups (O75 and O141) occasionally harbor these elements as well and produce cholera-like disease.

Gastroenteritis caused by non-O1, non-O139 serogroups of *V. cholerae* is typically milder and self-limiting, since they normally lack the cholera toxin gene. These non-O1, non-O139 isolates nevertheless cause the vast majority of *V. cholerae* gastrointestinal infections in the United States. While disease caused by these isolates is typically mild, fatal cases of non-O1, non-O139 *V. cholerae* can occur (192).

V. parahaemolyticus is the most common cause of *Vibrio*-associated diarrhea in the United States. The most frequent symptoms linked to *V. parahaemolyticus* enteritis include diarrhea with abdominal cramps, with approximately half of all infected individuals having a febrile illness (193). Two prominent symptoms, nausea (76%) and vomiting (55%), help to distinguish diarrhea caused by this species from other vibriosis or other enteritides associated with bacteria.

Unlike with many other enteric pathogens, secondary complications due to *Vibrio* gastroenteritis are rare. The principle complication that can arise from enteric infection is secondary spread to the bloodstream, producing septicemia. In the case of *V. cholerae*, virtually all such bacteremias are caused by non-O1, non-O139 isolates (194). Other, infrequently encountered *Vibrio* species that have been demonstrated to cause septicemia subsequent to primary gastrointestinal infections include *V. fluvialis* and *G. hollisae* (195, 196).

In the case of cholera, most infections arise in areas of endemicity through contaminated water and nonhygienic conditions which perpetuate persistence of O1. However, persons can also develop cholera through ingestion of contaminated shellfish or seafood products containing high concentrations of *V. cholerae*. For other *Vibrio* and *Vibrio*-like infections, the two major risk factors for acquiring disease are consumption of contaminated seafood and foreign travel. *Vibrio* spp. have naturally been recovered from many different types of seafood, including oysters, mussels, clams, shrimp, and tilapia (197). A large number of seafood vehicles have been implicated in vibriosis outbreaks associated with non-*V. cholerae* vibrios (186, 193).

Yersinia enterocolitica and *Yersinia pseudotuberculosis*

There are currently 18 species within the genus *Yersinia*, nine of which are isolated from humans. *Yersinia enterocolitica*, the most well-established enteropathogen of the genera, has two subspecies described, *Y. enterocolitica* subsp. *enterocolitica* and *Y. enterocolitica* subsp. *paleartica*, which can be distinguished by sequencing

of the 16S rRNA gene (198). *Y. enterocolitica* subsp. *paleartica* O:3/4 is the dominant serotype worldwide (199). *Yersinia pseudotuberculosis* is also enteropathogenic but is more commonly associated with sepsis. *Y. frederiksenii*, *Y. kristensenii*, *Y. intermedia*, *Y. mollarettii*, *Y. bercovieri*, and *Y. rohdei* can be isolated from humans (including patients with diarrhea), but they are not believed to be pathogenic except in rare cases in individuals with underlying disorders (161, 200). Pathogenic strains of *Y. enterocolitica* are determined by the biotype and serotype.

Y. enterocolitica and *Y. pseudotuberculosis* can be isolated from a host of animals, birds, foods, and environmental sources (201). Animal sources of human infections include hares, rodents, cats (*Y. pseudotuberculosis*), and dogs (*Y. enterocolitica*). Environmental sources include soil, water, and sewage (161). Pigs are a major reservoir for both *Y. enterocolitica* and *Y. pseudotuberculosis* infections worldwide (201–203).

Between 1996 and 1999, FoodNet determined an annual incidence of *Y. enterocolitica* in the United States of 1.0/100,000 persons, with the greatest rates of infection in blacks and Asians (203). Between 1996 and 2009, FoodNet active surveillance noted a decline in the overall annual incidence (0.5/100,000 persons) of *Y. enterocolitica*, with rates in blacks also declining from 3.9 to 0.4 per 100,000 by 2009 (203). The overall rate of *Y. enterocolitica* reported by FoodNet in 2011 was 0.34 per 100,000 (143). The high infection rate in blacks has been associated with homemade chitterlings (pork intestines), and educational efforts have been cited as a possible explanation for the decrease in infections in this ethnic group. Infection rates are highest in children (201). In the United States, 32% of cases occurred in children <1 year old and 47% in children <5 years old (203). Similar epidemiology is seen outside the United States; in China, 44% of cases are reported in children <3 years of age (202). *Y. enterocolitica* infections are classically documented to occur in the autumn and winter; however, a study of yersiniosis in Europe conducted over a 3-year period found no clear seasonal pattern (201, 202), and winter trends in yersiniosis in high-risk populations have also diminished in the United States (203).

Y. pseudotuberculosis most commonly causes mesenteric adenitis, which manifests as an appendicitis-like syndrome with fever and right lower quadrant abdominal pain. *Y. pseudotuberculosis* can also cause severe septicemia (161). Symptoms associated with sepsis include fever, diarrhea, abdominal pain or tenderness, anorexia, nausea, vomiting, and malaise. Mortality rates range from 28% to 100% in treated and untreated cases, respectively (161).

Y. enterocolitica gastrointestinal disease ranges from self-limiting enteritis with diarrhea, low-grade fever, and abdominal pain to severe disease such as terminal ileitis and mesenteric lymphadenitis which also mimics appendicitis (203–205). Onset is generally 24 to 48 h following ingestion, with illness lasting between 7 and 14 days, but symptoms may persist for up to 2 to 12 months (201, 205). Bloody stools occur in 20 to 46% of cases, and host susceptibility, number of ingested organisms, and serotype are determining factors for severity of disease (201). Severe cases may require hospitalization due to dehydration; in one study, 27% of 571 patients were hospitalized (205).

Sepsis is uncommon and is often associated with cardiovascular, dermal, or pulmonary conditions and abscesses. Pharyngitis, with sore throat and fever as the predominant symptoms, is not unusual in yersiniosis; in one multistate outbreak, 14 of 172 (8%) patients reported pharyngitis. Fulminant symptoms, including difficulty swallowing and breathing, may occur and require immediate medical attention (161). In these cases, *Y. enterocolitica* can be isolated from throat cultures.

The two most common sequelae of *Y. enterocolitica* infection are reactive arthritis and erythema nodosum, an immunologically mediated disease resulting in inflammation of subcutaneous adipose tissue with eruption of painful nodular lesions (205). In one large study of 571 patients, 7% and 3% of 571 patients reported reactive arthritis or erythema nodosum, respectively (205). The onset of reactive arthritis generally occurs <3 weeks after enteritis, and the longer the duration of gastrointestinal symptoms, the greater the likelihood that reactive arthritis will develop (161). Joint inflammation generally subsides spontaneously after 1 to 12 months, but 10% of patients will develop chronic arthritis (206). Approximately 80% of patients developing reactive arthritis carry the HLA-B27 allele (206). Septic arthritis is less commonly encountered and is not associated with HLA-B27 (161).

Because some *Y. enterocolitica* serotypes are unable to synthesize siderophores (compounds that sequester iron from the host), patients with iron overload disease are more susceptible to infection (161, 201). *Y. enterocolitica* can be acquired from blood transfusions, as the organism readily grows at lower temperatures used to store blood products. The development and severity of disease are dependent on the species of *Yersinia* (other than *Y. enterocolitica*) and the *Y. enterocolitica* bioserotype acquired (200, 204).

Emerging Enteropathogens

This guideline provides technical information on enteropathogens most commonly encountered in clinical practice; however, there are many additional bacteria that have been associated with gastroenteritis. Limited information is available for the majority of these, and they are reviewed elsewhere (207). Several of these agents have enough clinical importance and high enough frequency to mention here. It should be stressed, however, that testing for these organisms is not part of routine bacterial stool cultures in the clinical microbiology laboratory at this point, due to the difficulty in differentiating these organisms from resident flora in stool.

Bacteroides fragilis. Strains of *B. fragilis* carrying an ~6-kb pathogenicity island produce a zinc metalloprotease enterotoxin that has been known by several different names, including *B. fragilis* toxin and fragilysin (208, 209). These enterotoxigenic *B. fragilis* strains (ETBF) not only have been implicated as a cause of diarrheal disease in children under 5 years of age but more recently have been associated with inflammatory diarrhea in children and adults (210). A meta-analysis of 17 studies that evaluated the association of ETBF with diarrheal disease found that 12 (71%) of the studies demonstrated a higher frequency of ETBF in patients with diarrhea than in controls (211). In contrast, a recent Indian study found no difference in the rate of isolation of ETBF as a sole pathogen from children with and without diarrhea (212). This suggests that other, mitigating factors may play a role in the infective process for ETBF.

Currently, there is no easy method to detect ETBF. Potential *B. fragilis* isolates can be recovered from stool on *Bacteroides* bile esculin agar (Becton Dickinson, Sparks, MD) and then tested for enterotoxigenicity *in vitro* using PCR for the *Bacteroides fragilis* toxin gene (*bft*) (212). Alternatively, the cytopathic effect (CPE) produced by fragilysin on HT29/C1 (human colon) cell lines can

be evaluated (211). Both methods are employed only for research purposes at this time.

Edwardsiella tarda. *E. tarda* is one of four species currently residing in the genus *Edwardsiella* of the family *Enterobacteriaceae* and is the only species considered pathogenic for humans. A common inhabitant of fish, reptiles, marine animals, and aquatic birds (213, 214), *E. tarda* can also be recovered from water. Approximately 80% of reported human illnesses attributed to *E. tarda* involve infections of the gastrointestinal tract (213). Data from a number of studies suggest that *E. tarda* is associated with 0.3% to 1.0% cases of gastroenteritis (161, 213). Asymptomatic carriage of *E. tarda* has been reported (12).

E. tarda-associated diarrhea can present in one of several forms, the most common of which is watery diarrhea. Other diarrheal syndromes linked to *E. tarda* include dysentery, chronic diarrhea, and enteric fever (213, 215). Risk factors for acquiring *E. tarda* diarrhea include consumption of contaminated fish or seafood, accidental ingestion of contaminated water, exposure to water from ornamental aquariums, and handling pet turtles (216–221). Person-to-person transmission has also been postulated but currently remains unsubstantiated (222). Two populations thought to be particularly susceptible to *E. tarda* infection are persons >50 years of age and young children <5 years of age (213, 223).

Escherichia albertii. *E. albertii* was described as a new species in the genus *Escherichia* in 2003 (224). Most of the initial strains were misidentified as *Hafnia alvei* prior to the establishment of *E. albertii* as a species and were isolated from fecal samples from Bangladeshi children experiencing diarrheal illnesses. Subsequent evidence suggests that *E. albertii* is isolated fairly frequently from patients with diarrheal disease (225, 226). The organism harbors known enteropathogenic virulence factors (227, 228) and has been associated with a major outbreak of gastroenteritis involving 48 persons (229).

E. albertii grows well on routine enteric agars, is frequently misidentified biochemically as *Hafnia*, *Salmonella*, *Citrobacter*, or inactive *E. coli* strains (230), and may not be included in the databases of commercial identification systems. The important phenotypic features distinguishing *E. albertii* from *E. coli* include a negative indole reaction and inability to ferment lactose, D-sorbitol, and D-xylose (224). Phylogenetic studies indicate that *Shigella boydii* type 13, already known not to belong to the true shigellae, is, in fact, a member of the species *E. albertii* (227). In the 10th edition of the *Manual of Clinical Microbiology*, the species is broken down into two biogroups. Biogroup 1 represents the original *E. albertii* strains and biogroup 2 represents isolates formerly referred to as *S. boydii* 13. *E. albertii* can be identified by 16S rRNA gene sequencing and by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS).

Klebsiella oxytoca. Since the late 1970s and early 1980s, *K. oxytoca* has been sporadically linked to cases of antibiotic-associated hemorrhagic colitis in Japan and other locations around the world (231). In 2006, in an elegant series of clinical observations and histopathological studies on six patients with antibiotic-associated hemorrhagic colitis (AAHC) convincingly established *K. oxytoca* as the etiological agent in persons negative for *Clostridium difficile* (232). *C. difficile*-negative patients who are at higher risk of developing *K. oxytoca* colitis include those previously receiving penicillins or on nonsteroidal anti-inflammatory drugs (232). At present, confirmation of *K. oxytoca* colitis in *C. difficile*-negative patients requires detection of the species-specific *K. oxytoca* cytotoxin by detection of CPE on HEp-2, CHO, or HeLa cells (232–234). In a recent study of 5,581 stool specimens submitted for *C. difficile* testing at an acute-care health system in China, 2.1% of specimens harbored *K. oxytoca*, but only 29.1% of these strains were cytotoxin producing (235).

A second highly suggested, but unproven, syndrome attributed to *K. oxytoca* is diarrhea. Although one study found no correlation between the presence of *K. oxytoca* and diarrhea (236), a later study found a high percentage of cytotoxin-positive *K. oxytoca* isolated from patients with health care-associated diarrhea that did not develop into AAHC (235). In the latter study, a specific selective medium termed SCITB (Simmons citrate-inositol-tryptophan-bile salts) was developed to recover *K. oxytoca* from stools. This medium has been shown to improve the recovery of *K. oxytoca* over that with MacConkey (MAC) agar by 30% (235). This medium could greatly aid in determining the significance of *K. oxytoca* from mild to moderate cases of diarrhea.

Providencia alcalifaciens. A British survey of travelers to Mediterranean countries between 1987 and 1988 found a significant association between the recovery of *P. alcalifaciens* and diarrheal disease (237). These initial findings have been subsequently supported by other studies describing individual cases of *P. alcalifaciens*-associated diarrhea and at least three outbreaks of gastrointestinal disease, including one large outbreak involving >270 children in Japan (238–240). *P. alcalifaciens* strains implicated in diarrheal disease are invasive in HEp-2 cell monolayers, although the type of diarrhea that they produce is secretory (239, 241); some strains additionally produce a cytolethal distending toxin (242). Persons most at risk of developing *P. alcalifaciens* diarrhea are those who are involved in foreign travel or have consumed contaminated foods containing the organisms (237, 243).

Most isolates of *P. alcalifaciens* recovered from diarrheal stools have been isolated in pure culture, as predominant flora, or without any other recognizable enteropathogens being detected (237, 240, 243). A selective medium, termed PAM (*Providencia alcalifaciens* medium), has been described for the recovery of this species from feces (244). This medium has subsequently been modified as PMXMP (polymyxin-mannitol-xylitol medium for *Providencia*) and used with success (242, 243).

GENERAL LABORATORY TESTING CONSIDERATIONS

At this time, diagnosis of bacterial gastroenteritis is by the routine stool culture. Two key exceptions to this are the use of antigen and/or nucleic acid amplification tests for the detection of (i) *C. difficile* and, to a lesser extent, (ii) STEC and (iii) *Campylobacter*. In addition, food poisoning caused by *B. cereus*, *C. perfringens*, and *S. aureus* is infrequently diagnosed by clinical laboratory testing, as few patients seek medical intervention for their symptoms, which are short-lived. In cases of outbreak investigations for these organisms, feces, food, and/or vomitus is collected by, or sent to, local or state public health officials, and testing is performed at public health laboratories. Laboratory diagnosis of listeriosis associated with human diarrhea is also extremely difficult to make at this time. Diagnosis can be made either via epidemiological linkage to other cases of diarrhea in which *L. monocytogenes* has been isolated or by isolation and identification of *L. monocytogenes* from stool in persons with gastrointestinal symptoms (120). Currently there is no standard protocol for detection of listeriae from human stools, and it is unclear what methods or procedures are

likely to yield maximum recovery rates. Because of these limitations, recovery of *L. monocytogenes* from stool should not be attempted routinely in clinical microbiology laboratories. This testing is better suited to reference and/or public health laboratories.

Specimen Collection

Feces collected in the acute phase of a diarrheal disease is the specimen of choice when bacterial gastroenteritis is suspected. If liquid or soft, approximately 5 ml should be collected (245), and if formed, 0.5 to 2 g is adequate for culture (245, 246). Clear instructions should be given for proper specimen collection. Feces should be collected in a clean, dry container with a tight lid and should not be contaminated with urine, barium, or toilet paper (which may contain barium salts). Specimen containers or collection devices should be labeled with the patient's full name and two additional patient identifiers, such as medical record number and date of birth.

Rectal swabs are generally considered less sensitive than stool for culture, but there are certain patient populations for which a properly collected rectal swab may be acceptable. For instance, a rectal swab is a useful specimen to collect from infants and young children, or when trying to recover *Shigella* (247–249). Rectal swabs must be inserted deep enough into the rectum, approximately 1 in. beyond the anal sphincter, and carefully rotated, so that feces can be collected and visible on the swab. The swab should then be placed in all-purpose transport medium and sent to the laboratory. In addition to feces, blood, bone marrow, and/or urine samples may be collected for patients presenting with symptoms consistent with typhoid fever. Duodenal contents may also be acceptable for these cases.

In cases of suspected extraintestinal *Salmonella* infections, blood and urine specimens should be collected in addition to stool. In suspected cases of typhoid fever, blood and/or bone marrow specimens should be collected in the first week of fever and stool and urine in subsequent weeks. The yield of *Salmonella* Typhi is best from bone marrow, in particular after antimicrobials have been started (250).

While in up to 94% of cases, the etiological agent is recovered from the first specimen submitted, collection of a second fecal specimens may be needed to rule out a bacterial cause of infection, especially in instances where patient symptoms persist (246). Many have documented that the yield of fecal culture for patients hospitalized for more than 3 days is poor, excluding *C. difficile* (8, 251–257). For this reason, laboratories should restrict testing to outpatients and those hospitalized for less than 3 days. Exceptions to this rule may include patients with HIV infection, severe neutropenia, suspected nondiarrheal manifestations of enteric infections (such as erythema nodosum, polyarthritis, etc.), a suspected nosocomial outbreak, or in some instances pediatric patients, from whom collection of a specimen may be difficult in the first 3 days of hospitalization.

Similarly, laboratories should develop and enforce strict specimen rejection criteria for *C. difficile* testing. Testing should not be performed on asymptomatic patients (i.e., those with formed stools), given the high rates of colonization among some patient populations. The exception to this policy is in cases where ileus is suspected (89), although this occurrence is rare and should involve physician consultation with the laboratory prior to testing. Repeat testing of negative specimens should also be discouraged or altogether prohibited. Several studies have shown the lack of value for repeat testing, regardless of method, for CDI (258–260). In addition, many laboratories have developed rejection criteria for specimens submitted within a defined interval of time following a positive result, as many patients will remain colonized with *C. difficile* following successful treatment (70). Finally, *C. difficile* testing should not be performed on infants (i.e., patients <1 year of age) due to their high colonization rate.

Transport Media and Storage Conditions

Transport media used for fecal specimens include Stuart's, Aimes, or Cary-Blair (261, 262) medium. Fresh stool specimens should be transported to the laboratory and processed within 2 h of collection (263); this is in particular critical to the survival of *Shigella* and *Campylobacter* (264). If the specimen cannot be processed within 2 h, it should be placed in Cary-Blair transport medium; refrigeration of the specimen in Cary-Blair medium at 4°C prior to processing will best conserve bacterial enteropathogens, with the exception of *Shigella* (265, 266).

Specimen Processing

Fecal specimens, if submitted fresh and not in preservative, should be macroscopically observed for areas that contain blood and/or mucus, as these will contain the highest number of enteric pathogens and should be used for culture. Gram staining is not typically useful when performed on stool, with the key exception of campylobacteriosis. In these cases, again with fresh, unpreserved stool specimens, the characteristic seagull-shaped campylobacters can be visualized in stool when carbol-fuchsin is used as a counterstain, with a sensitivity ranging from 66 to 94% in patients with acute enteritis (48). However, Gram staining on stool specimens is not routinely performed as part of stool culture testing and should be performed only on special request.

The battery of primary plating media used for routine bacterial fecal cultures will vary from laboratory to laboratory, depending on the patient population and organisms routinely isolated. Medium selection is also driven by test requisition. At a minimum, routine fecal culture setup should be designed to optimize the recovery of *Salmonella*, *Shigella*, *Campylobacter*, and STEC. Thus, fecal specimens received for culture should be planted to 4 media: (i) MacConkey (MAC) agar, (ii) a selective/differential medium designed for the recovery of *Salmonella* and *Shigella*, (iii) a medium designed for the recovery of *Campylobacter*, and (iv) a medium designed for the recovery of STEC O157 and/or enrichment broth for testing for the presence of Shiga toxins. Many laboratories also include a blood agar plate (BAP), in order to aid with the recovery of *Aeromonas* spp., *Plesiomonas* spp., and *Vibrio* spp., whereas other add this on request only. Table 2 presents an overview of some of the more common media used for fecal cultures, their intended use, and growth characteristics of some of the more common enteropathogenic bacteria when isolated on these media.

Selective media appropriate for fecal cultures include xylose-lysine-deoxycholate (XLD), salmonella-shigella (SS), Hektoen enteric (HE), brilliant green (BG), or bismuth sulfite (BS) medium or a chromogenic medium designed for the recovery and detection of specific enteropathogens. The value of chromogenic media has been demonstrated in a number of studies that reported improved sensitivity and specificity over traditional selective/differential media for the recovery of *Salmonella*, *Shigella*, STEC, *Vibrio*, and *Yersinia* (267–271). However, these media are more

TABLE 2 Commonly used media for recovery of pathogenic bacteria from stool samples

Medium	Intended use and notes
All-purpose broths	
Gram-negative (GN) broth	Selective enrichment for Gram-negative rods, specifically *Salmonella* and *Shigella* (subculture after 6–8 h of incubation, not part of routine setup unless for STEC EIA), can be used for STEC EIA
Selenite F broth	Selective enrichment for Gram-negative rods, specifically *Salmonella* and *Shigella* (subculture after 18–24 h of incubation, may inhibit growth of some *Shigella* species) (not part of routine setup)
Organism-specific broths	
Alkaline peptone water	Selective enrichment broth for *Vibrio*, when requested (subculture to TCBS after 24 h of incubation)
MAC broth	Can be used for STEC EIA, enrichment for *Y. enterocolitica* if incubated at 25°C (not part of routine setup)
All-purpose agars	
Hektoen enteric (HE)	Selective medium for Gram-negative rods, differentiates lactose fermenters (yellow-orange) from nonfermenters (blue or green), H_2S production can be detected (black precipitate)
MacConkey (MAC)	Selective medium for Gram-negative rods, differentiates lactose fermenters (pink) from nonfermenters (colorless)
Salmonella-shigella (SS)	Selective medium for Gram-negative rods, differentiates lactose fermenters (pink/red) from nonfermenters (colorless), H_2S production can be detected (black precipitate)
Xylose-lysine-deoxycholate (XLD)	Selective medium for Gram-negative rods, differentiates lactose fermenters (yellow) from nonfermenters (red), H_2S production can be detected (black precipitate)
Highly selective/differential agars	
Bismuth sulfite	Isolation of *Salmonella*, including *Salmonella* Typhi (black on this medium)
Brilliant green	Isolation of *Salmonella* (red, pink, or white with red halo on this medium), inhibits *Salmonella* Typhi and *Salmonella* Paratyphi
Blood agar with ampicillin	Isolation of *Aeromonas* (not part of routine setup unless specifically requested)
Campy Blood	Isolation of *Campylobacter*
Campy CVA	Isolation of *Campylobacter*
Campylosel	Isolation of *Campylobacter*
Cefsulodin-Irgasan-novobiocin (CIN)	Isolation of *Yersinia enterocolitica* or *Aeromonas* (deep red center and transparent margin [bull's eye appearance] on this medium) (not part of routine setup)
Charcoal selective	Isolation of *Campylobacter*
Charcoal-cefoperazone-deoxycholate agar (CCDA)	Isolation of *Campylobacter*
CHROMagar *Salmonella*	Isolation of *Salmonella* (mauve-rose on this medium)
CHROMagar O157	Isolation of O157 STEC (mauve on this medium)
CHROMagar STEC	Isolation of 6 most common STEC serogroups (mauve on this medium)
Cycloserine-cefoxitin-egg yolk/cycloserine-cefositin-fructose	Isolation of *Clostridium difficile* (not part of routine setup unless requested)
HardyChrom SS	Isolation of *Salmonella* (black on this medium) and *Shigella* (teal on this medium)
Inositol-brilliant green-bile salt	Isolation of *P. shigelloides* (white to pink on this medium) (not part of routine setup unless requested)
MacConkey agar with sorbitol (SMAC) or cefixime-tellurite SMAC (CT-SMAC)	Isolation of *E. coli* O157 (colorless on this medium)
Thiosulfate-citrate-bile salts-sucrose (TCBS)	Isolation of *Vibrio* (not part of routine setup unless requested), *V. cholerae* is yellow on this medium, *V. parahaemolyticus* is green on this medium, some vibrios are inhibited

expensive than traditional media and are not available for *in vitro* diagnostic use for *Vibrio* and *Yersinia* in the United States at this time.

Campylobacter selective media include the blood-free charcoal-cefoperazone-deoxycholate agar (CCDA) and charcoal-based selective agar and the blood-based Campy-CVA (cefoperazone, vancomycin, and amphotericin) and Skirrow medium. While Campy-CVA is most widely used in the United States, the use of a combination of media, including one that is charcoal based, increases the yield of *Campylobacter* by 10 to 15% (49). *Campylobacter* cultures must be incubated in a microaerobic atmosphere, typically 5% O_2, 10% CO_2, and 85% N_2, and at 42°C (48) to optimize recovery and prevent overgrowth of enteric organisms. *Campylobacter* media should be incubated for a minimum of 48 h and examined at 24 and 48 h.

The isolation and identification of STEC by culture methods is problematic, with the exception of O157 strains, most of which express a delayed or negative D-sorbitol reaction. Media to detect sorbitol-negative *E. coli* O157 strains include sorbitol-MacConkey (SMAC) agar and a modification on SMAC agar which contains cefixime and tellurite (CT-SMAC agar). Sorbitol-negative *E. coli* appears as colorless colonies on both of these media, but CT-SMAC agar has the advantage of eliminating many enteric flora that grow on SMAC agar, including commensal *E. coli*. CHROMagar O157 detects O157 serogroups, and CHROMagar STEC detects both O157 and the six most common STEC serogroups other than O157 isolated in the United States (O26, O45, O103, O111, O121, and O145). This product is labeled for research use only in the United States, but a recent study demonstrated that CHROMagar STEC had a sensitivity of 89.1% and specificity of 86.7% (272). In

contrast, a second study found the specificity of CHROMagar STEC to be 98.9% (273). If a laboratory attempts to detect STEC, an enrichment broth, typically GN broth, should also be inoculated and incubated at 37°C overnight for detection of Stx1 and Stx2 by immunoassay.

Several enteric pathogens require highly selective media and will not be optimally recovered by the selection of the 4 or 5 media described above for routine fecal cultures. These include aeromonads, vibrios, yersiniae, and a number of the emerging enteropathogens. The cost-effectiveness of routinely culturing for these organisms will depend on the patient population served by an individual laboratory and potentially on the peak time of the year that these pathogens are active. For example, laboratories in the Gulf States may consider routine use of media to optimize the recovery of vibrios in addition to the battery of primary stool culture media. A variety of specialized media have been designed and are commercially available for the recovery of these pathogens, but clearly not all can be stocked by routine clinical laboratories. Two that might be considered for areas these pathogens are more endemic include cefsulodin-Irgasan-novobiocin (CIN) agar and thiosulfate-citrate-bile salts-sucrose (TCBS) agar. CIN agar is used to recover both *Aeromonas* and *Yersinia enterocolitica*, whereas TCBS is used for the recovery of *Vibrio* (http://www.cdc.gov/cholera/pdf/laboratory-methods-for-the-diagnosis-of-vibrio-cholerae-chapter-4.pdf). Of note, TCBS agar has a short shelf life and should be made only as needed (it requires no autoclaving). While it is excellent for the recovery of *V. cholerae* and *V. parahaemolyticus*, not all pathogenic vibrio species will grow on this medium. For instance, *Grimontia hollisae* grows poorly if at all on TCBS agar. These species may be detected on blood agar, which can be screened for oxidase-positive or hemolytic colonies for further evaluation.

An enrichment broth was traditionally inoculated as part of routine fecal cultures; this practice was originally designed to aid in the recovery of low levels of *Salmonella*, *Shigella*, and *Campylobacter*. However, many laboratories have discontinued this practice, as the yield does not justify the cost (274). Historic data from individual laboratories should be used to help determine if subcultures from enrichment broth should be used or discontinued. A key exception to this is the use of alkaline peptone water, pH 8.5 to 8.6, enrichment for a minimum of 6 to 8 h at 35°C to 37°C for the recovery of *V. cholerae* if necessary. Failure to use enrichment culture in addition to direct plating will result in a significant percentage of *V. cholerae*-containing stools being reported out as negative (275).

Stool Culture Workup

Bacterial fecal culture workup consists of examining culture media for colonies that display phenotypic properties consistent with those of enteric pathogens (e.g., lactose negative, H_2S producers, etc.). These colonies are then traditionally further screened using select biochemical tests and, if yielding reactions consistent with an enteropathogen, identified by further biochemical and/or antigenic testing by a variety of algorithms. Ultimately, these traditional methods yield a precise identification, but they are time-consuming and labor-intensive. Further, the biochemical properties of certain bacterial isolates may differ from the common phenotype of the species, hampering unambiguous identification. The classic example of this is an inert *E. coli* strain masquerading as *Shigella*. Resolving these anomalous bacteria can be difficult for even specialized reference laboratories. Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) is a newer proteomic technology that has the potential to revolutionize the traditional algorithms used for the identification of enteric pathogens (276), and it can be a more cost-effective alternative to colony screening and biochemical testing.

Traditional algorithms for the identification of enteric pathogens. The traditional approach to fecal stool culture workup (excluding *Campylobacter* and STEC, which are described further below) is shown in Fig. 1A. The basis for this process is to minimize the cost associated with differentiating the plethora of nonpathogenic *Enterobacteriaceae* in a fecal specimen from the rare *Salmonella* and *Shigella*, which are isolated in only 3 to 5% of fecal specimens submitted for culture. Typically this is accomplished by selecting lactose nonfermenters and/or H_2S producers, isolated on the primary plates, and submitting these to a secondary screening test. This second screen may not be required if laboratories are using a chromogenic medium as part of the primary setup (Fig. 1C) or if the volume of fecal cultures is low enough that going directly to an identification kit is cost-effective. Regardless, the second screening method used by most laboratories consists of subculturing suspicious colonies to one or more tubed/slanted media. The classic "three-tube" system consists of a triple sugar iron (TSI) agar, a lysine iron agar (LIA), and a Christensen urea agar. Alternative tubed media for secondary screening include motility-indole-ornithine (MIO), motility-indole-lysine (MIL), and motility-indole-lysine-sulfide (MILS) (277–280). The most common biochemical reactions for enteric pathogens on TSI agar, LIA, and urea agar (negative unless otherwise noted) are listed in Table 3.

If not specifically requested, identification of aeromonads, vibrios, and *P. shigelloides* may be overlooked by the above approach. In particular, some strains of *Aeromonas* and *Vibrio* are lactose positive on MAC or sucrose-positive on HE and XLD media. If necessary, these organisms can be identified by screening the BAP for oxidase-positive or beta-hemolytic colonies. Caution must be exercised, however, as not all aeromonads are hemolytic, in particular some isolates of *Aeromonas caviae*. Oxidase-positive isolates are then further screened by evaluating the reactions on TSI agar and LIA and through the use of O/129 disks and salt tolerance for vibrios.

If *Yersinia* culture is requested, it may be difficult to isolate from feces using routine stool culture protocols, as at 35°C the organism grows more slowly than other flora. It is occasionally recovered as pinpoint colonies on MAC or SS agar at 24 h. These colonies should be reincubated at 25°C if possible, and if the colonies have grown after 24 h (to 1 mm), they should be examined for *Yersinia*; colonies that remain pinpoint are most likely fecal streptococci. If CIN agar is used, yersiniae are identified as bull's-eye colonies with pink centers on this medium, due to fermentation of D-mannitol. *Aeromonas* and some *Citrobacter*, *Serratia*, *Proteus*, and *Morganella* strains may mimic *Y. enterocolitica* colony morphology on this medium, and so these isolates require final identification. One strategy is to perform Voges-Proskauer and motility tests at 25°C and 35°C; *Y. enterocolitica* and *Y. pseudotuberculosis* should be positive only for these at 25°C.

Isolates that yield reactions consistent with enteropathogens on these secondary screening media are further identified using either a manual identification system such as API 20E or an auto-

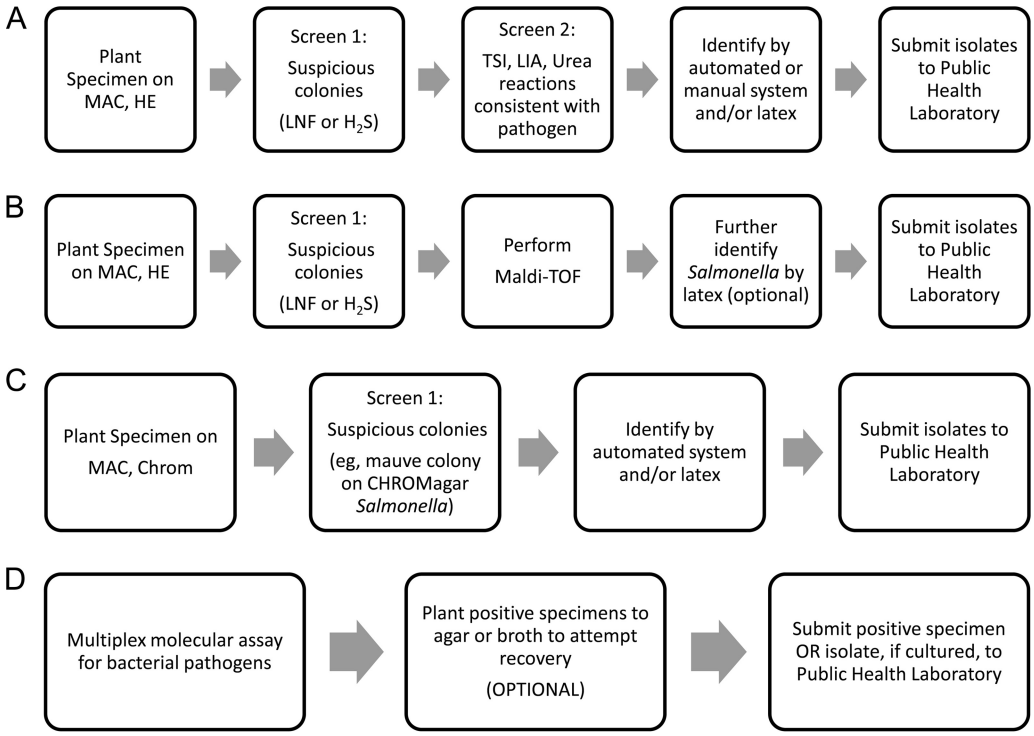

FIG 1 Strategies for the detection of *Salmonella* and *Shigella* on routine stool cultures. Where Hektoen enteric (HE) agar is specified, any agar listed in the text is appropriate. (A) Traditional approach to fecal stool culture workup for *Salmonella* and *Shigella*. See Table 3 for reactions of enteric pathogens. (B) Approach to identification of *Salmonella* and *Shigella* when using MALDI-TOF MS. See Table 4 for reactions of enteric pathogens. (C) Approach to identification of *Salmonella* and *Shigella* if chromogenic medium is used as part of the primary setup. (D) Approach to use of a multiplex molecular assay for the detection of *Salmonella* and *Shigella*.

mated identification system such as MicroScan, Vitek2, Phoenix, MALDI-TOF MS, etc. These systems perform well at identifying some enteropathogens but fall short for others. For instance, commercial systems are notoriously poor at the correct identification of *Vibrio* species to both the genus and species levels (281, 282). This is due in part to the salt requirement of halophilic species such as *V. parahaemolyticus* and *V. fluvialis* and to the fact that some species are phenotypically similar. A prime example of this situation is the phenotypic similarity between *V. fluvialis* and certain *Aeromonas* spp. For such situations, only a limited number of tests are available to separate these species, such as growth in 0% and 6% salt, O/129 susceptibility, and gas production from D-glucose, tests normally not available on commercial panels or in most diagnostic laboratories. Similarly, most systems are unable to identify *Aeromonas*, *Salmonella*, *Shigella*, or *Yersinia* beyond the genus level; however, in most instances, this level of identification is sufficient. Similarly, identification of *Salmonella* to the genus level when isolated from stool is generally sufficient, and clinical laboratories should forward isolates to regional or state public health laboratories for further characterization. However, laboratories may make a concerted effort to rule out typhoidal *Salmonella* serotypes, if possible, given the clinical significance of these organisms or if there will be a delay getting the isolate to the public health laboratory. *Salmonella* Typhi can be differentiated from other serotypes by the characteristic weak H_2S reaction on a TSI slant and a negative ornithine decarboxylase reaction. *Salmonella* Paratyphi A can be differentiated from other salmonellae by negative H_2S, lysine, and citrate reactions.

Antigenic testing, for example, by latex agglutination for somatic antigen, can be used to identify *Shigella* to the species level, as many commercial systems may misidentify these strains as *E. coli*. Antigen testing for the somatic, flagellar, and capsular antigens of *Salmonella* may also be used to supplement commercial test system identifications. Caution must be employed, as some *Citrobacter* and *E. coli* strains may posses the *Salmonella* Typhi Vi capsular antigen.

Campylobacter species can be identified based on characteristic Gram stain morphology, growth on selective media at 42°C, and positive catalase and oxidase reactions alone. *Campylobacter* appears as gray, flat colonies on plated media and often spreads along the streak lines. *Campylobacter* latex agglutination kits are available for culture confirmation to genus level, but sensitivity and specificity vary depending on the manufacturer (283). For the most part, routine reporting of species-level identification of *Campylobacter* when isolated from stool is unnecessary. *Campylobacter jejuni* can be readily differentiated from other thermotolerant *Campylobacter* spp. by the hydrolysis of sodium hippurate, but as some *C. jejuni* strains fail to hydrolyze hippurate, hippuricase-negative isolates should be reported as *Campylobacter* spp.

Sorbitol-nonfermenting *E. coli* isolated on SMAC or CT-SMAC agar should be tested in O157-specific antiserum or latex reagents in order to confirm O157 STEC. Caution must be used with these reagents, as other species of bacteria may cross-react with O157 antiserum, requiring biochemical confirmation of *E. coli* prior to reporting.

TABLE 3 Summary of identification characteristics and diagnostic test methods available in the United States[a]

Organism(s)	Growth characteristics	Key biochemical reactions	Clinical laboratory diagnostic test methods				Clinical symptoms used for diagnosis	Public health laboratory referral
			Culture	Chromogenic agar	Antigen detection	NAAT		
Aeromonas spp.[b]	BA, beta-hemolytic	Oxidase positive; TSI, K/A, gas; LIA, K/A or A/A	✓					
Bacillus cereus[b]	BA, beta-hemolytic						✓	Food and stool cultured for outbreak investigations
Campylobacter spp.	*Campylobacter* medium, gray-white colonies	Oxidase positive, catalase positive, sodium hippurate positive for *C. jejuni*	✓		✓	✓		
Clostridium difficile[b]	NA	NA			✓	✓		
Clostridium perfringens[b]	NA	NA					✓	Food and stool cultured for outbreak investigations
Edwardsiella tarda[b]	Lactose nonfermenter with H_2S production on HE, MAC, XLD, SS	TSI, K/A, H_2S, ± gas; LIA, K/K or K/NC, H_2S	✓					
Escherichia coli O157	SMAC, sorbitol nonfermenter	TSI, K/A or A/A, gas; LIA, K/A or A/A	✓	✓		✓		
Escherichia coli, STEC	SMAC, sorbitol fermenter (unless O157)	TSI, K/A or A/A, gas; LIA, K/A or A/A	✓		✓	✓		
Listeria monocytogenes[b]	BA, beta-hemolytic		✓					Food and stool cultured for outbreak investigations
Salmonella spp.	Lactose nonfermenter with H_2S production on HE, MAC, XLD, SS	TSI, K/A, H_2S, ± gas; LIA, K/K or A/A or K/NC (rare), H_2S	✓	✓	✓	✓		
Shigella spp.	Lactose nonfermenter on HE, MAC, XLD, SS	TSI, K/A; LIA, K/A or A/A	✓	✓		✓		
Staphylococcus aureus[b]	NA	NA					✓	Food and stool cultured for outbreak investigations
Yersinia enterocolitica[b]	CIN, pink bull's eye; lactose nonfermenter on MAC; ferments sucrose on HE	TSI, A/A or K/A; LIA, K/A or A/A; urea positive	✓					
Vibrio spp.[b]	TCBS, sucrose fermentation seen with *V. cholerae*; no sucrose fermentation for *V. parahaemolyticus*; *V. vulnificus* is sucrose fermentation variable	Oxidase positive; TSI, A/A or K/A; LIA, K/A or A/A	✓					

[a] Abbreviations: BA, blood agar; HE Hektoen enteric agar; MAC, MacConkey agar; XLD, xylose-lysine-deoxycholate agar; SS, salmonella-shigella agar; CIN, cefsulodin-Irgasan-novobiocin agar; TCBS, thiosulfate-citrate-bile salts-sucrose agar; SMAC, MAC with sorbitol; TSI, triple sugar iron agar; LIA, lysine iron agar; A, acid; K, alkaline; NC, no change; NA, not applicable.

[b] The organism is not part of the routine stool culture, and culture should be performed only on request.

MALDI-TOF MS. Because of the low cost associated with performing a MALDI-TOF identification, many laboratories have begun to identify bacterial isolates phenotypically consistent with enteric pathogens directly off the primary plates (Fig. 1B), rather than performing additional screening tests. MALDI-TOF MS can be performed within the FDA-approved package insert on colonies picked from the primary BAP and, in the case of the Vitek MS, from the MAC plate. Alternatively, testing can be run as a laboratory-developed test on colonies picked from other selective media routinely used for stool cultures (XLD, SS, HE, etc.) (284). Some have shown that SS and HE agars yield only genus-level identifications (284). For *Campylobacter*, colonies isolated on Campylosel and Columbia blood agar are FDA cleared on the Vitek MS and perform well for species-level identification (285–288). In contrast, spectra are not generated when isolates are taken directly from modified CCDA; colonies isolated on this medium require subculture on a less selective medium prior to MALDI-TOF analysis (286).

MALDI-TOF MS performs well for the identification of *Salmonella* (to the genus level), and *Campylobacter*. However, the current IVD and RUO systems and databases cannot differentiate *E. coli* from *Shigella* or *E. coli* from STEC or other *E. coli* pathotypes and cannot identify *Salmonella* to the serotype (276). The technology is rapidly approaching these targets, through special extraction protocols and selection of stable, pathogen-specific biomarkers (289–292), which can be internally verified by individual laboratories. In addition, one or both of the IVD MALDI-TOF systems can be used to identify the other enteric pathogens described in this article, with the exceptions of *B. cereus*, *P. shigelloides*, *E. albertii*, and *P. alcalifaciens*. The performance of MALDI-TOF MS is detailed in Table 4.

Susceptibility Testing

Antimicrobials are not routinely indicated for the treatment of otherwise healthy patients with bacterial gastroenteritis, as infections typically resolve spontaneously. In some cases, the use of antimicrobials can be detrimental to the host, such as the use of antimicrobials for STEC infection, which puts the patient at increased risk for HUS, or for *Salmonella*, which may increase the rate of clinical relapse and prolong carriage (3). Routine susceptibility testing is therefore also not indicated for bacteria isolated from stool cultures (293, 294). Exceptions to this generalization may be bacteria isolated from infants <6 months of age, the elderly or immunocompromised, or patients with prolonged disease. In these cases, communication with the physician will aid in determining the need for susceptibility testing and the antimicrobial agents to be tested. If the laboratory identifies *Salmonella* Typhi or *Salmonella* Paratyphi A from stool, consideration should be given to susceptibility testing. Additionally, the isolation of any of the organisms described here from an extraintestinal site warrants susceptibility testing.

Current standards for susceptibility testing conditions and interpretive criteria for members of the *Enterobacteriaceae* (*E. coli*, *E. tarda*, *Salmonella*, *Shigella*, and *Yersinia* spp.) are available from the Clinical and Laboratory Standards Institute (CLSI) M100 S24 standard (294). Recommendations for *Aeromonas*, *Campylobacter*, and *Vibrio* are provided by the CLSI in the M45 A2 document (293). In the United States, the CDC monitors antimicrobial resistance among *Campylobacter*, *Salmonella*, *Shigella*, *E. coli* O157, and *Vibrio* species other than *V. cholerae* as part of the National Antimicrobial Resistance Monitoring System (NARMS). Primary agents used to treat *Campylobacter* include ciprofloxacin and erythromycin. Fluoroquinolone resistance has been reported with increasing frequency in the United States among both human and animal isolates of *C. coli* and *C. jejuni*. In 1990, NARMS noted no resistance to ciprofloxacin, whereas in 2011, 24.2% of *Campylobacter* isolates tested were resistant to ciprofloxacin (295). This trend of increasing resistance may be related to the historical use of veterinary fluoroquinolones in animal feed as growth promoters (296). In contrast, resistance to erythromycin remains low, at 1.8% as reported in 2011. Because of increasing resistance, testing of *Campylobacter* isolates from individuals with severe illness or prolonged symptoms may be warranted. Susceptibility testing is performed by broth microdilution in cation-adjusted Muller-Hinton broth (CA-MHB) and incubation in a microaerobic atmosphere at either 35°C or 42°C. Disk diffusion for ciprofloxacin and erythromycin can be performed; no zone of inhibition indicates resistance, but the appearance of any zone of inhibition requires confirmation by an MIC method (293).

Resistance documented by NARMS for nontyphoidal *Salmonella* isolates to the antimicrobial agents commonly used to treat severe *Salmonella* infections was 2.5% to ceftriaxone, 1.2% to trimethoprim-sulfamethoxazole, and <1% to ciprofloxacin (295). In contrast, fluoroquinolone resistance was much higher in typhoidal isolates, with 7.3% of *Salmonella* Typhi and 2.1% of *Salmonella* Paratyphi A isolates documented as resistant to ciprofloxacin. An additional 64.2% and 95.1% of these isolates, respectively, had ciprofloxacin MICs in the intermediate range (0.12 to 0.5 μg/ml). Azithromycin is an alternative agent recommended for the treatment of invasive salmonellosis by the World Health Organization and the American Academy for Pediatrics. However, no CLSI breakpoints exist at present for *Salmonella* for azithromycin. An epidemiological cutoff (ECOFF) (i.e., MIC cutoff defining the upper end of the wild-type population) of ≥32 μg/ml has been used to indicate resistance, and based on this ECOFF, 0.2% of nontyphoidal *Salmonella* isolates were considered resistant to azithromycin by the CDC 2011 NARMS data (295).

Among *Shigella* isolates tested by NARMS in 2011, 2.4% were resistant to ciprofloxacin and 3.1% to azithromycin, again using an ECOFF breakpoint of ≥32 μg/ml. Notably, resistance to azithromycin was species dependent, with 10% of *Shigella flexneri* isolates ($n = 58$) testing resistant. Trimethoprim-sulfamethoxazole resistance among *Shigella* isolates in 2011 was high (66.9%), whereas only 1.7% resistance to ceftriaxone was documented. If laboratories perform susceptibility testing on *Salmonella* or *Shigella*, it is important to remember that first- and second-generation cephalosporins, cephamycins, and aminoglycosides may to be appear active *in vitro* but are not effective clinically, and the isolates should not be reported as susceptible (294).

In 2009, the CDC started annually tracking antimicrobial resistance in *Vibrio* spp other than *V. cholerae* as part of NARMS. In 2011, 95.1% of *V. alginolyticus* isolates were resistant to ampicillin, whereas 40.3% of *V. parahaemolyticus* and 4.8% of *V. vulnificus* isolates were resistant (295). No isolates in 2011 tested resistant to the fluoroquinolones or tetracycline, and 0.3% were resistant to trimethoprim-sulfamethoxazole. Antimicrobial therapy is not required to manage cholera but may shorten the duration and reduce severity of disease. Similarly, otherwise healthy individuals with diarrhea caused by non-*V. cholerae Vibrio* spp. usually re-

TABLE 4 MALDI-TOF for the identification of enteric pathogens

Organism	Performance of MALDI-TOF[a]	FDA-cleared organisms[b]	Notes	Reference(s)
Aeromonas	Present in IVD and commercial RUO databases; performance not well documented	Biotyper, genus only; Vitek MS, genus only	Preliminary data promising	322
Campylobacter	Excellent for genus and species identification; many species in RUO databases	Vitek MS, *C. jejuni*, *C. coli*	FDA cleared for colonies grown on Campylosel and brucella blood agar; cannot perform directly from mCCC	285–288
E. tarda	Present in IVD and commercial RUO databases; performance not well documented	Vitek MS only		
P. shigelloides	Present in commercial RUO databases; performance not well documented	No		
Salmonella	Good identification to genus	Biotyper, genus only; Vitek MS, genus only	Performance better with MAC vs SS or HE agar; serotype-specific biomarkers have been identified	291, 323
Shigella	Cannot distinguish from *E. coli* by use of IVD or commercial RUO databases	No	*Shigella*-specific biomarkers have been identified	292
STEC	Cannot distinguish STEC (or other pathotypes) from *E. coli* by use of IVD or commercial RUO databases	No	*E. coli* pathotype-specific biomarkers have been identified	290
Vibrio	Present in IVD and commercial RUO databases; performance not well documented	Vitek MS, *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*		
Yersinia	Present in IVD and commercial RUO databases; performance not well documented	Biotyper, *Y. enterocolitica*, *Y. pseudotuberculosis*; Vitek MS, *Y. enterocolitica*, *Y. pseudotuberculosis*, *Y. frederikenii*, *Y. intermedia*, *Y. kristensenii*		

[a] IVD, *in vitro* diagnostic device; RUO, research use only.
[b] FDA clearance as of 7 March 2014. Check with manufacturer or FDA.org for most up-to-date information.

TABLE 5 *C. difficile* diagnostic algorithms

Algorithm, test(s)[a]	Result	Action
GDH with reflex, GDH antigen	Negative	Report as "no *C. difficile* present"
	Positive	Reflex to NAAT; reporting based on NAAT results
GDH/toxin lateral-flow assay, GDH antigen and toxin A/B	Both negative	Report as "no *C. difficile* present"
	Both positive	Report as "*C. difficile* toxin present"
	Negative/positive or positive/negative	Reflex to NAAT
Toxin NAAT, NAAT	Negative	Report as "no *C. difficile* present"
	Positive	Report as "*C. difficile* toxin present"

[a] GDH, glutamate dehydrogenase; NAAT, nucleic acid amplification test.

cover spontaneously. When indicated, vibrios can be tested for susceptibility by a method similar to that used for the *Enterobacteriaceae*. For testing halophilic species, preparation of the inoculum in 0.85% NaCl solution for both disk diffusion and broth microdilution testing is recommended by the CLSI (293).

Susceptibility testing is not routinely indicated for *C. difficile*, as most isolates are susceptible to both metronidazole and vancomycin, agents commonly used to treat CDI. Almost all cases are currently identified by nonculture methods.

Nonculture Detection Methods

Recovery of *C. difficile* by anaerobic culture is possible but should not be used as a primary diagnostic test for *C. difficile* infection because of long turnaround times. Rather, three test methods are commonly used detect the presence of toxigenic *C. difficile* in stool specimens. These methods are glutamate dehydrogenase (GDH) enzyme immunoassays (EIAs), toxin A/B enzyme immunoassays, and toxin A/B gene nucleic acid amplification tests (NAATs). GDH is a cell wall enzyme that is abundant in *C. difficile*, and while EIAs for this antigen are a sensitive screen for the presence of *C. difficile*, they suffer from poor specificity, as they do not distinguish toxigenic from nontoxigenic strains (297). The presence of a toxigenic strain must therefore be confirmed on all GDH-positive specimens before a positive *C. difficile* result is reported. This can be accomplished in a number of ways, including cell culture cytotoxicity neutralization assay (CCCN), but most laboratories perform either a toxin A/B EIA or a toxin gene NAAT on GDH-positive specimens. Commercial toxin A/B EIAs suffer from poor sensitivity compared to both cytotoxigenic culture and CCCN. Sensitivity for the presence of toxigenic *C. difficile* is as low as 40 to 50% in some studies (297, 298). For this reason, detection of *C. difficile* by toxin EIA alone is no longer considered adequate to rule out CDI. Toxin gene NAATs offer the highest sensitivity and specificity among the different test options but are significantly more expensive than the EIA-based methods. Furthermore, because toxin A and/or B is required for CDI, the clinical significance of specimens that test negative for their presence by CCCN or EIA but are positive by NAAT (or culture) has been brought into question. Some have suggested that such cases represent colonization, rather than infection, by *C. difficile* (297, 299, 300). However, because of the poor sensitivity of the antigen tests for toxins A/B compared to cytotoxicity assays, performing toxin A/B detection tests alone will miss clinically significant cases (298).

Many have proposed testing algorithms, using one or more of the three test methods, aimed at optimizing *C. difficile* diagnostic performance and minimizing cost (301–306). Three of these testing algorithms are outlined in Table 5: (i) performing a GDH EIA with follow-up confirmatory toxin NAAT on GDH-positive specimens, (ii) performing a combination GDH and toxin A/B EIA and confirming specimens for which the two test results do not match by toxin NAAT, or (iii) performing NAAT alone on all specimens.

In addition to *C. difficile*, STEC infections are increasingly being diagnosed by culture-independent methods, as these methods allow for the detection of both O157 and non-O157 STEC infections (102). In 2009, the CDC published recommendations that stool samples submitted from patients with acute community-acquired diarrhea and patients with possible HUS be simultaneously cultured using selective and differential media for *E. coli* O157 and assayed for the detection of Stx1 and Stx2 (102). The Joint Commission issued a change to the hospital laboratory Element of Performance Standard for QSA.04.06.01 in 2013, which is consistent with the CDC recommendations. If laboratories are unable to perform a non-culture-based method for the detection of non-O157 STEC, this testing should be sent to a reference laboratory. Laboratory testing options are shown in Table 6. Stx immunoassays are performed on enrichment broths, typically GN broth, that have been incubated at 37°C overnight, and they detect (and in some cases differentiate) Stx1 and Stx2. Some assays are also FDA cleared for use directly on stool, with no broth enrichment, but performance varies among manufacturers. Several studies have demonstrated improved sensitivity for the detection of STEC over culture through the use of these immunoassays (307, 308), for both O157 and non-O157 STEC. Any broths that test positive for Stx should be forwarded to the local or state public health laboratory for isolate recovery and further characterization. The immunoassays can be performed off stool directly, but with much lower sensitivity (e.g., roughly 70%).

Finally, several antigen detection assays have been developed for the detection of *Campylobacter* from stool specimens. These enzyme immunoassays (EIAs) detect a surface antigen, called the *Campylobacter*-specific antigen, by use of either a microplate or lateral-flow immunochromatographic format. The antigen detection assay does not differentiate between *C. jejuni* and *C. coli*. Several studies have reported that while these antigen tests are more rapid and convenient than culture, up to 50% of positive results cannot be confirmed by other methods (309–313). Regardless, due to the low positive predictive value of these tests, laboratories may elect to confirm antigen-positive specimens by culture (310). Possible algorithms for the detection of *Campylobacter* are outlined in Table 7.

TABLE 6 Testing options for STEC

Option	Procedure[a]	Advantage(s)	Disadvantage(s)
Culture for O157	(i) Plant specimen to chromogenic or selective medium (SMAC, CT-SMAC), (ii) perform latex agglutination for O157 or MUG test to identify sorbitol-nonfermenting colonies of *E. coli*, (iii) refer isolate to public health laboratory	Generates isolate for further public health study	Does not detect non-O157 STEC, does not detect sorbitol-fermenting O157 STEC, misses non-O157 STEC
Toxin testing	(i) Inoculate specimen to GN broth and incubate overnight, (ii) perform antigen detection test for Shiga toxins I and II; (iii) refer positive broths to public health laboratory	Detects all STEC, may differentiate Stx1 and Stx2	Requires additional work on part of public health lab to find STEC from GN broth, antigen tests are expensive, may only be 50% as sensitive as NAAT
Culture and toxin testing	Perform both culture and toxin testing in parallel	Covers all STEC, rapid recovery of most frequent serotype (O157)	Cost and labor of additional testing to laboratory
Nucleic acid amplification test for stx_1 and stx_2	(i) Perform NAAT (laboratory developed or IVD) that detects the Shiga toxin genes directly from stool, (ii) attempt to recover positive isolates, inoculate to GN broth to forward to public health laboratory, or forward positive specimens to public health laboratory	Most sensitive method, multiplex assays allow detection of many enteric pathogens	Not well validated to date, does not yield isolate for public health laboratory

[a] SMAC, sorbitol MacConkey agar; CT-SMAC, cefixime-tellurite SMAC; MUG, 4-methylumbelliferyl-β-D-glucuronide.

Molecular Testing by Syndromic Panels

In recent years, syndromic panels for infectious diseases have gained popularity, in particular for screening patients who present with influenza-like illness for respiratory viruses. As of the writing of this article, five multiplex nucleic acid tests were FDA approved for the detection of organisms associated with gastroenteritis. The Prodesse ProGastro SSCS assay (Hologic Gen-Probe, San Diego, CA) detects *Salmonella*, *Shigella*, *Campylobacter*, and STEC. The Luminex xTAG GPP detects the organisms listed above as well as *C. difficile* toxin A/B, adenovirus 40/41, rotavirus A, norovirus, *Giardia lamblia*, *Cryptosporidium*, and *Entamoeba histolytica*. The BD MAX enteric bacterial panel (BD, Sparks, MD) detects *C. jejuni* and *C. coli*, *Salmonella* spp., *Shigella* spp./enteroinvasive *E. coli* (EIEC), and the stx_1 and stx_2 genes. The Nanosphere Verigene enteric pathogen test detects *Campylobacter* group, *Salmonella* spp., *Vibrio* group, *Y. enterocolitica*, and stx_1 and stx_2. The BioFire GI Panel detects *C. jejuni*, *C. coli*, *C. upsaliensis*, *C. difficile*, *P. shigelloides*, *Salmonella*, *Y. enterocolitica*, *V. parahaemolyticus*, *V. vulnificus*, *V. cholerae*, and the five diarrheagenic *E. coli* pathotypes (enteroaggregative, enteropathogenic, enterotoxigenic, Shiga toxin-producing, and enteroinvasive *E. coli*), along with *Shigella*. In addition to the bacterial targets, the Biofire GI Panel detects *Cryptosporidium*, *Entamoeba histolytica*, *Cyclospora cayetanensis*, *Giardia lamblia*, adenovirus 40/41, astrovirus, rotavirus A, sapovirus, and norovirus. While not FDA cleared, the Seeplex Diarrhea-V ACE (Seegene, Seoul, South Korea) has received CE marking for use in Europe. Numerous laboratory-developed multiplexed molecular assays that target enteric bacterial pathogens directly from fecal specimens have been described in the literature (314, 315).

Use of these tests to replace conventional cultures remains controversial. While it is clear that molecular assays offer improved sensitivity over culture (314, 316), there are concerns regarding

TABLE 7 Testing options for *Campylobacter*

Option (time for result)	Procedure[a]	Advantages	Disadvantages
Culture (2–4 days)	(i) Plant specimen to *Campylobacter* selective medium and incubate at 42°C under microaerobic conditions (report negative cultures after 48–72 h), (ii) *Campylobacter* identified by Gram stain and positive catalase and oxidase reactions (or MALDI-TOF), (iii) *Campylobacter* latex or sodium hippurate can be used to differentiate *C. jejuni*	Yields an isolate (for public health or susceptibility testing), most specific method	Requires special equipment (42°C incubator, ability to generate microaerobic atmosphere), *Campylobacter* is fastidious and many may not grow in culture unless conditions are ideal, takes 2–4 days for result
Antigen detection test (same day)	Perform antigen detection test following manufacturer's instructions	Rapid, easily batched	Concerns regarding specificity (may need to confirm positive results by culture), does not yield an isolate for further testing
NAAT	Perform NAAT (LDT or IVD) that detects *Campylobacter* directly from stool (may include extraction of nucleic acids)	Most sensitive method, multiplex assays allow detection of many enteric pathogens	Not well validated to date, does not yield isolate for further testing

[a] NAAT, nucleic acid amplification test; LDT, laboratory-developed test; IVD, *in vitro* diagnostic device.

specificity issues for the highly multiplexed panels (317). These molecular tests will detect organisms currently identified by routine stool cultures. However, it should be kept in mind that while routine fecal cultures may detect less frequent enteropathogens, they are designed to optimize recovery of *Salmonella*, *Shigella*, *Campylobacter*, and STEC. Perhaps the most pressing concern regarding the uptake of this new technology is that testing does not yield an isolate that could be used for surveillance for antimicrobial resistance or for subtyping to support the identification and investigation of outbreaks. Thus, laboratories that opt to use molecular assays should consult with their local or state public health laboratory to determine whether specimens positive for *Salmonella*, *Shigella*, *Campylobacter*, or STEC should be forwarded to the local or state public health laboratory for epidemiological studies (Fig. 1D).

Reporting Results

The laboratory report should reflect results for each organism routinely included in screening. For example, a negative routine fecal culture should be reported as "no *Salmonella*, *Shigella*, *Campylobacter*, or STEC isolated." If the laboratory routinely adds a TCBS for the detection of *Vibrio*, a negative result would be indicated by "no *Salmonella*, *Shigella*, *Campylobacter*, STEC, or *Vibrio* isolated." In contrast, recovery of any enteric pathogen, including those not specifically screened for, should be reported. The following organisms, for which the clinical significance of a stool isolate is less clear, should be reported with an indication of the number of colonies recovered on the primary plates: *Aeromonas*, *P. shigelloides*, *E. tarda*, and any of the emerging pathogens described in this article, as the clinical relevance of these may be less clear. For routine stool cultures, there is no need to report the presence of *B. cereus*, as this organism can be a transient member of the intestinal flora. However, heavy or pure growth of *B. cereus* may be reported as an unusual finding.

Antigen detection assays for an organism (e.g., *Campylobacter* spp.) or for toxins (e.g., Shiga toxin I and II or *C. difficile* toxin A/B) should be reported as positive or negative for the antigen/toxin tested. When molecular assays are used, the report should reflect the analyte(s) tested and a positive or negative result along with the test method used. For laboratory-developed tests (i.e., not FDA approved or cleared), a comment must be made regarding the laboratory's verification of the analytical performance of the assay. Additional comments about referral to a public health laboratory or reference laboratory for further identification may be used as warranted. However, testing by outside laboratories should not delay adding the result of a preliminary finding to the patient report.

Early parenteral volume expansion has been shown to significantly improve patient outcome for STEC infections (318). Further, because progression to HUS is associated with antimicrobial treatment in children (114), prompt reporting of results is critical for STEC infections. Therefore, laboratories should report probable and confirmed *E. coli* O157 isolates and Stx-positive specimens as soon as they are identified. Consideration should be given to a laboratory policy for calling positive results or the use of automated alerts via electronic medical records to notify the clinician, due to the critical nature of these infections.

CONCLUDING REMARKS

The potential number of bacteria associated with gastroenteritis is now estimated to approach or exceed 40 individual species (207). In contrast to this, both routine traditional and more contemporary molecular methods may screen for only 4 or 5 organisms (3, 246, 319). The number and type of agents routinely sought by the microbiology laboratory should be driven by geographic locale and patient history (foreign travel, disease symptomatology and underlying preexisting medical conditions). The importance of laboratory-clinician communication cannot be overemphasized for complicated cases, as patient-tailored testing is rarely feasible with the limited patient information that accompanies specimens received by the laboratory. Similarly, laboratories should ensure that physicians understand what organisms are screened for by the routine bacterial stool culture, as this is infrequently apparent to the general practitioner or even to many specialists. This being said, all clinical laboratories that perform stool cultures can provide useful information for patients with *Salmonella*, *Shigella*, STEC, and *Campylobacter*. The future role of bacterial stool cultures may be diminished by the emergence of nonculture diagnostic test methods for enteric pathogens. However, several pressing issues remain regarding these new technologies, including the performance of these tests and the issue of not providing an isolate to the public health laboratory as has been the practice in the past. These concerns are yet to be resolved (320). Nonetheless, these technologies have the potential for routine detection of enteropathogens that have eluded most clinical microbiology laboratories to date.

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