Etiological Agents of Infectious Diarrhea: Implications for Requests for Microbial Culture

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Gastrointestinal infections remain a frequent disease worldwide. In order to increase our knowledge of the epidemiology for our patient population, we retrospectively analyzed the results obtained for stool samples received at the clinical microbiology laboratory of the University Hospital of Geneva during a 4-year period. A total of 13,965 specimens from 7,124 patients (1.96 specimens per patient) were cultured, yielding 369 (2.6%) Salmonella spp., 408 (2.9%) Campylobacter spp., and 79 (0.6%) Shigella spp. The cumulative positivity rate of 6.1% decreased to 2.7% when patients received antimicrobial agents (P < 0.001). The positivity rate for 5,912 specimens obtained from patients hospitalized for ≤3 days was 12.6%, whereas it dropped to 1.4% for patients hospitalized for >3 days (P < 0.001). Of 3,837 stool samples originating from pediatric patients, 8.8% were positive, and 5.1% of 10,128 samples from adults were positive (P < 0.001). The cytotoxin of Clostridium difficile was detected in 379 of 3,723 samples analyzed (10.2%), and rotaviruses were detected in 190 of 1,601 samples (11.9%). We recommend that the use of cultures for enteric bacterial pathogens be restricted to patients hospitalized for ≤3 days, with the exceptions of follow-up samples, specimens from immunocompromised patients, and patients whose first sample was culture negative or in the rare event of nosocomial food-borne outbreaks. For patients under antimicrobial therapy, testing for cytotoxin of C. difficile should primarily be requested; this analysis should also be accepted for samples from patients not receiving antimicrobial agents at the time of specimen collection. By applying these restrictions, we could have saved at least $5,000 annually.

Materials and Methods

Fecal specimens were obtained from patients seeking medical care at the University Hospital of Geneva, a 1,500-bed health care center providing primary and tertiary care for Geneva and the surrounding area (population, ~700,000). This is the main hospital in this urban area, with approximately 40,000 admissions and about 400,000 ambulatory visits yearly. The stool samples were collected in plastic containers containing 8 ml of Cary-Blair transport medium. In the laboratory, these samples were plated onto MacConkey agar, Hektoen enteric agar (both from BioMérieux, Lyon, France), and Campylobacter selective agar (Karmali; Oxoid, Basingstoke, United Kingdom). Approximately 1 g of the sample was inoculated into 10 ml of selenite F broth (Becton Dickinson microbiology Systems, Cockeysville, Md.). The MacConkey and Hektoen agar plates were incubated for 18 to 24 h at 35°C. The selenite F broth was subcultured onto Hektoen enteric agar and MacConkey agar after 18 to 24 h of incubation. The Campylobacter plates were incubated in a microaerophilic atmosphere at 42°C for 48 h. Suspect colonies on the primary or subculture plates were identified by standard laboratory procedures (21). Cultures for other potential pathogens were performed on specific request.

Ward personnel were instructed to discard the Cary-Blair transport medium in the plastic containers before collecting specimens when cytotoxigenic C. difficile was suspected to be a possible cause of diarrhea. The TechLab C. difficile toxin/antitoxin kit was used in conjunction with McCoy tissue cultures to detect cytotoxicity in fecal samples (VPI Research Park, Blacksburg, Va.) (10, 21).

Stool samples were analyzed for rotaviruses upon special request. The SlideX Rotakit 2, a simple latex agglutination technique, was performed according to the instructions of the manufacturer (BioMérieux).

To evaluate the influence of antimicrobial agents administered to patients on the stool culture results, we considered the information transmitted by our computerized requests or on the request forms. The computerized requests obliged the requesting physician or nurse to introduce a reply in the field “antimicrobial therapy.” Tests with controls to verify the real presence or absence of antimicrobial agents were not performed.

Factors associated with positive stool culture results were initially analyzed by univariate analysis. The strength of the association between these factors and outcome (stool culture positive for Salmonella spp., Shigella spp., and Campylobacter spp.), odds ratios, and their corresponding 95% confidence intervals were calculated and interpreted as described by Cornfield (9). Differences in proportions were compared by the chi-square test, using Yates’ correction when necessary. Independent variables were defined by multivariate logistic regression analysis. The five variables chosen at the baseline for each stool sample used in the analysis were as follows: age, gender, hospital department, length of hospital stay (≤3 days, 4 to 5 days, and >5 days), and the presence of antimicrobial treatment at the time of sampling. The model was constructed by using an entrance P value of ≤0.15. The relative importance of the independent variables selected by the model for predicting stool culture positivity was assessed by using an increase in the percentage of chi-square values uniquely associated with each variable in the

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During the 4-year period from August 1991 to July 1995, 14,878 stool samples were received in the bacteriology laboratory. Cultures for *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. were performed with 13,965 specimens from 7,124 patients, resulting in an average of 1.96 cultures per patient. These cultures yielded 369 (2.6%) *Salmonella* spp., 14,878 stool samples were received in the bacteriology laboratory. Cultures for *Salmonella* spp., and 408 (2.9%) *Shigella* spp., were performed with 13,965 specimens from 7,124 patients. Cultures for *Salmonella* spp., and 408 (2.9%) *Shigella* spp., were performed with 13,965 specimens from 7,124 patients, resulting in an average of 1.96 cultures per patient. These cultures yielded 369 (2.6%) *Salmonella* spp., 79 (0.6%) *Shigella* spp., and 408 (2.9%) *Campylobacter* spp., which comprised co-infections with *Salmonella* spp. and *Shigella* spp. of which 3 were co-infections with *Salmonella* spp. and *Shigella* spp. and 6 were co-infections with *Salmonella* spp. and *Campylobacter* spp. The presence of *C. difficile* toxin was detected in 379 (10.2%) of 3,723 samples analyzed, and rotaviruses were detected in 190 (11.9%) of 1,601 samples analyzed.

The combined positivity rate for *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. in 5,912 specimens obtained from patients hospitalized for ≤3 days was 12.6%. It dropped to 1.4% (P < 0.001) for samples from patients hospitalized for >3 days (n = 8,053; Table 1). For pediatric patients (≤16 years old) the positivity rate was significantly higher (P < 0.001) than that for adult patients (338 of 3,837 samples [8.8%] versus 518 of 10,128 samples [5.1%], respectively). Of the 369 *Salmonella* spp. cultured, 316 (85.6%) were isolated from samples collected from patients who had been hospitalized for ≤3 days. Similarly, 359 of 408 (87.7%) *Campylobacter* spp. and 71 of 79 (89.9%) *Shigella* spp. were isolated from samples collected from patients who had been hospitalized for ≤3 days. For the remaining 110 samples culture positive for enteropathogenic bacteria (53 *Salmonella* spp., 49 *Campylobacter* spp., and 8 *Shigella* spp.), 37 (20 *Salmonella* spp., 13 *Campylobacter* spp., and 4 *Shigella* spp.) were identified from patients who had been hospitalized for >3 days and whose stool sample collected at ≤3 days of hospitalization had already been positive, and 22 (11 *Salmonella* spp., 9 *Campylobacter* spp. and 2 *Shigella* spp.) were collected from patients whose previous stool specimen obtained >3 days after admission had been positive. Furthermore, for the 110 positive samples collected after 3 days of admission, cultures of another specimen collected during the first 3 days of hospitalization were requested for 45 patients. Of the specimens from these 45 patients, 37 were positive. Of the 51 patients hospitalized for >3 days with one or more positive stool cultures, medical records were available for review for 48 (94%) patients (the medical records for 22 patients with two or more positive stool samples collected ≥3 days after admission are included in this sample). For four patients, a specimen other than a stool specimen (blood, pleural fluid, synovial fluid, and urine) positive for *Salmonella* spp. was collected during the first day of hospitalization. For 13 patients (3 infected with *Salmonella* spp., 9 infected with *Campylobacter* spp., and 1 infected with a *Shigella* sp.), the stool samples were collected on the 4th day of hospitalization. For eight patients (five *Salmonella* spp., two *Campylobacter* spp., and one *Shigella* sp.) a stool sample obtained during the first 3 days of hospitalization had been culture negative; the culture of a second specimen collected >3 days after admission revealed the enteric pathogen for these patients. For eight immunocompromised patients (five cancer patients and three positive human immunodeficiency virus-patients), enteropathogenic

<table>
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<th>Variable</th>
<th>No. of cultures with the following result for Campylobacter spp., or Shigella spp.:</th>
<th>% Positive</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>P value</th>
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<td>Negative</td>
<td>Positive</td>
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* CI, confidence interval.
* Refers to length of hospital stay at the time of sample collection.
* NS, not significant (P > 0.05).

RESULTS

During the 4-year period from August 1991 to July 1995, 14,878 stool samples were received in the bacteriology laboratory. Cultures for *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. were performed with 13,965 specimens from 7,124 patients, resulting in an average of 1.96 cultures per patient. These cultures yielded 369 (2.6%) *Salmonella* spp., 79 (0.6%) *Shigella* spp., and 408 (2.9%) *Campylobacter* spp., which comprised co-infections with *Salmonella* spp. and *Shigella* spp. of which 3 were co-infections with *Salmonella* spp. and *Shigella* spp. and 6 were co-infections with *Salmonella* spp. and *Campylobacter* spp. The presence of *C. difficile* toxin was detected in 379 (10.2%) of 3,723 samples analyzed, and rotaviruses were detected in 190 (11.9%) of 1,601 samples analyzed.

The combined positivity rate for *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp. in 5,912 specimens obtained from patients hospitalized for ≤3 days was 12.6%. It dropped to 1.4% (P < 0.001) for samples from patients hospitalized for >3 days (n = 8,053; Table 1). For pediatric patients (≤16 years old) the positivity rate was significantly higher (P < 0.001) than that for adult patients (338 of 3,837 samples [8.8%] versus 518 of 10,128 samples [5.1%], respectively). Of the 369 *Salmonella* spp. cultured, 316 (85.6%) were isolated from samples collected from patients who had been hospitalized for ≤3 days. Similarly, 359 of 408 (87.7%) *Campylobacter* spp. and 71 of 79 (89.9%) *Shigella* spp. were isolated from samples collected from patients who had been hospitalized for ≤3 days. For the remaining 110 samples culture positive for enteropathogenic bacteria (53 *Salmonella* spp., 49 *Campylobacter* spp., and 8 *Shigella* spp.), 37 (20 *Salmonella* spp., 13 *Campylobacter* spp., and 4 *Shigella* spp.) were identified from patients who had been hospitalized for >3 days and whose stool sample collected at ≤3 days of hospitalization had already been positive, and 22 (11 *Salmonella* spp., 9 *Campylobacter* spp. and 2 *Shigella* spp.) were collected from patients whose previous stool specimen obtained >3 days after admission had been positive. Furthermore, for the 110 positive samples collected after 3 days of admission, cultures of another specimen collected during the first 3 days of hospitalization were requested for 45 patients. Of the specimens from these 45 patients, 37 were positive. Of the 51 patients hospitalized for >3 days with one or more positive stool cultures, medical records were available for review for 48 (94%) patients (the medical records for 22 patients with two or more positive stool samples collected ≥3 days after admission are included in this sample). For four patients, a specimen other than a stool specimen (blood, pleural fluid, synovial fluid, and urine) positive for *Salmonella* spp. was collected during the first day of hospitalization. For 13 patients (3 infected with *Salmonella* spp., 9 infected with *Campylobacter* spp., and 1 infected with a *Shigella* sp.), the stool samples were collected on the 4th day of hospitalization. For eight patients (five *Salmonella* spp., two *Campylobacter* spp., and one *Shigella* sp.) a stool sample obtained during the first 3 days of hospitalization had been culture negative; the culture of a second specimen collected >3 days after admission revealed the enteric pathogen for these patients. For eight immunocompromised patients (five cancer patients and three positive human immunodeficiency virus-patients), enteropathogenic
bacteria (three Salmonella spp. and five Campylobacter spp.) could be isolated in a specimen obtained ≥3 days after admission. Of these eight immunocompromised patients, seven showed symptoms of gastrointestinal disorder at the time of stool collection. Seven patients were considered to be healthy carriers (four patients carried Salmonella spp. and three patients carried Campylobacter spp.). Two patients were constipated on the day of admission and developed diarrhea ≥3 days later, when Campylobacter spp. could be identified in their stool specimens. Intensive care was required for two comatose polytrauma patients with Salmonella spp. or Campylobacter spp. in their stool samples collected ≥3 days after admission. In four patients (two infected with Salmonella spp. and 2 infected with Campylobacter spp.), no particular reason for requesting cultures of stool specimens collected ≥3 days after admission could be determined.

In only two patients (including a patient with cancer) could nosocomial infection be suspected retrospectively. Both patients were hospitalized on the same ward and suffered from diarrhea, and the rare species Salmonella indiana could be isolated from their stool samples, which were collected within a 10-day interval.

Salmonella spp., Shigella spp., and Campylobacter spp. were detected more often in the feces of patients who did not receive antimicrobial therapy at the time of specimen collection (Table 1). For these patients, 700 of 8,154 (8.6%) samples were positive for at least one of the three enteric pathogens, whereas 156 of 5,811 (2.7%) samples were positive among patients receiving antimicrobial treatment (P < 0.001).

Among the subgroup of 5,912 patients hospitalized for ≤3 days, we observed a significant difference (P < 0.001) in the yield of enteropathogenic bacteria between patients (n = 4,599) without antimicrobial treatment (13.7% positive) and the patients (n = 1,313) receiving antibiotics at the time of stool collection for culture (8.8% positive). On the other hand, the positivity rate was 1.6% for patients hospitalized for >3 days with antimicrobial treatment (P < 0.001) and 12.8% (n = 4,498) and 12.8% (n = 3,555). This difference did not reach statistical significance (P = 0.15).

Factors independently associated with stool cultures positive for Salmonella spp., Shigella spp., or Campylobacter spp. were age less than 16 years (P < 0.014), the absence of antimicrobial treatment (P < 0.001), and cultures performed during the first 3 days of hospitalization (P < 0.001). The last factor constituted the strongest predictor of stool culture positivity; in terms of relative influencing importance, stool cultures requested for patients hospitalized for ≤3 days explained 73% of the results, the absence of antimicrobial treatment explained 17% of the results, and age under 16 years explained 7.5% of the results.

To determine the value of repeat specimens, we separately analyzed samples collected within 3 days and multiple samples collected within an interval of >3 days. This period was chosen since laboratory results were rarely available when a specimen was collected ≤3 days of the time of collection of a first sample. For 182 patients, two or more samples were collected within ≤3 days, at least one specimen of which was positive for Salmonella spp., Shigella spp., or Campylobacter spp. (105 patients with two specimens, 55 patients with three specimens, 19 patients with four specimens, and three patients with five specimens). For 77 patients (42.3%), at least one specimen was culture negative. Considering the sequence of sample collection, the first sample revealed that 158 of 182 (86.8%) patients were positive for one or more species on culture; a second specimen improved the yield to 97.8% (Fig. 2). In one patient, only the fourth specimen was positive, and that was for Shigella flexneri. Similar results were achieved when restricting the analysis to the 55 patients for whom three stool samples were cultured. The first sample was positive for 43 (78.2%) patients, and the first and second samples were positive for 52 (94.5%) of the 55 patients who had at least one sample positive. For the subgroup of 105 patients for whom two specimens were cultured and of which at least one was positive, for 71 (67.6%) patients both cultures were positive, for 26 (24.8%) patients only the first one was positive, and for 8 (7.6%) patients only the second one was positive (P = 0.004). Of the 34 patients for whom either only the first or only the second sample was positive on culture, 6 (17.6%) received antibiotics when the first sample was collected, while 21 (61.8%) were treated with antibiotics when the second sample was collected (P = 0.007). Among these, 4 of 6 and 4 of 21, respectively, were positive.

Analyzing repeat specimens collected during an interval of >3 and ≤30 days (i.e., a period of diarrheal illness when culture results should have been available at the time when a further sample was collected), culture-positive samples were followed by a negative culture for a sample collected, on average, 13 days later. For 13 patients, a negative culture was followed by a positive culture of a sample collected, on average, within 9 days, and for 27 patients, two positive samples were collected, on average, 11 days apart.

The positivity rate for cytotoxin of C. difficile in 149 fecal specimens from pediatric patients hospitalized for ≤3 days was 8.7%, whereas it was 7.8% for 306 samples from patients hospitalized for >3 days (P = 0.9; not significant). For adult patients hospitalized for ≤3 days, 94 of 737 samples (12.8%) tested contained detectable cytotoxin of C. difficile, whereas for those patients hospitalized for >3 days 248 of 2,531 samples (9.8%) were positive (P = 0.025) (Fig. 3). Rotaviruses were detected in 107 of 858 samples (12.5%) from pediatric patients hospitalized for ≤3 days and in 83 of 701 samples (11.8%) from patients hospitalized longer than 3 days (P = 0.7; not significant) (Fig. 3). In none of 42 stool samples obtained from adults were rotaviruses detected.

Cytotoxin of C. difficile was detectable in 180 of 1,433 (12.6%) samples collected from patients not receiving antimicrobial therapy at the time of sample collection, which was higher than the overall positivity rate (10.2%). Also, more positive samples were noted among the patients taking the following antimicrobial agents: amoxicillin-clavulanic acid, 31 of 259 (12.0%); imipenem, 28 of 197 (14.2%); and clindamycin, 4 of 27 (14.8%). Less than 10% positive samples were observed among patients taking fluocoxacin (5.8%; n = 52), ceftriax-
one (5.8%; n = 52), glycopeptides (5.9%; n = 101), combinations of two or more agents (6.8%; n = 1,120), amoxicillin (7.4%; n = 54), macrolides (7.5%; n = 40), quinolones (8.4%; n = 166), and trimethoprim-sulfamethoxazole (8.8%; n = 57). In a different context, cytotoxin of C. difficile was detectable in 10 of 55 samples (18.2%) when the patients received metronidazole. Of these 10 samples (from nine patients), 7 were from patients with a previous specimen positive for cytotoxin of C. difficile at most 14 days previously.

Two or more specimens were collected from 68 patients within 3 days for analysis of C. difficile cytotoxin and at least one sample was positive (46 patients with two samples, 20 patients with three samples, and two patients with four samples). The first specimen revealed that 58 patients (85.3%) were positive for cytotoxin, the second specimen revealed that 66 (97.1%) patients were positive for cytotoxin, and the third specimen revealed that all patients had one or more positive specimens. For specimens collected >3 days apart, 29 patients provided samples that were positive both times, 83 provided negative samples followed by a positive sample, and 39 provided positive samples followed by a negative sample; all samples were collected within an average interval of 13 days.

The recovery of enteric pathogens from our patient population varied by age group. One-third of all Salmonella spp. (112 of 369) were isolated from samples from children under 5 years; another 31% (126 of 369) were isolated from patients younger than 5 years old. Of the 408 Campylobacter spp. identified, 126 (31%) originated from patients younger than 5 years. Most Shigella spp. (28%) could be identified from patients 25 to 29 years old. The majority (97%) of rotavirus were from children younger than the age of 5 years, and 30% (113 of 379) of patients with a sample positive for C. difficile cytotoxin were older than 75 years.

**DISCUSSION**

Infectious diarrhea is a major public health concern (7, 12). Fortunately, most of these diseases are self-limiting, but the diagnosis of the infecting microbe is important for the prevention and control of food-borne or C. difficile-associated epiemics (3, 12, 14), as well as for severely ill and immunocompromised patients. With the discovery of an increasing number of potential enteric pathogens and as new rapid techniques for the detection of these pathogens become available (5, 17, 20), microbiology laboratories and physicians ordering tests need to adapt their attitude in order to improve the efficiency of the diagnosis of infectious diarrhea (11, 18, 21, 22).

It has previously been observed that Salmonella spp., Shigella spp., and Campylobacter spp. rarely cause diarrhea in patients hospitalized for >3 days (6, 11, 18, 22). In these studies the results were evaluated during a shorter time period, and consequently, in those studies fewer samples were analyzed than in the present study. We analyzed our results over a recent 4-year period because seasonal and epidemiological variations influence the outcome of the analysis. In our institution, only 12.9% of all Salmonella spp., Shigella spp., and Campylobacter spp. were isolated from stool samples collected from patients after their third day of hospitalization (n = 8,056). By logistic regression, a request for stool culture during the first 3 days after admission was identified as the strongest predictor of positivity for enteropathogens, independent of patient age and the presence of antimicrobial treatment. Similar findings were reported in another study over a 6-month period, with a positivity rate of 6.4% for Salmonella spp., Shigella spp., and Campylobacter spp. (6). The recovery rates of Salmonella spp., Shigella spp., and Campylobacter spp. were lower in two other series (11, 22) (2.0 and 2.6%, respectively). The relatively high positivity rate for stool cultures for enteropathogens in our study may be related to the severity of diarrhea and the rapid access to hospitalization in our institution. It is the main primary health care center and is the only tertiary health care center in the area. Ambulatory patients had a lower positivity rate (Fig. 1), probably due to milder gastrointestinal disorders and delays until medical consultation was required.

Restricting the bacterial stool cultures to specimens collected ≤3 days after admission (18) would have revealed 87.1% of all Salmonella spp., Shigella spp., and Campylobacter spp. in the current study. Follow-up specimens collected from patients who have been in the hospital for >3 days with a documented infection due to Salmonella spp., Shigella spp., or Campylobacter spp. represented 7.4% of the culture-positive samples. The inclusion of specimens collected on day 4 of hospitalization would provide an increased positivity rate of 1.5%. In this study we considered the day of admission as the first day of hospitalization (Fig. 1 and 3). It is unclear whether other investigators considered the day of admission as day 0 or day 1 (6, 11, 22). Finally, a further 1% increased yield would have been achieved if all stools from immunosuppressed patients were cultured, as proposed by others (6, 18).

It is commonly recognized that a single negative stool culture result may not be sufficient to completely rule out bacterial infection (21). Indeed, in the present study, for the group of 55 patients from whom three samples were collected within 3 days for culture, one or more of which was positive, the first specimen was positive for only 78.2% of these patients. A second sample increased the detection rate to 94.5% for these patients. Of the patients from whom multiple stool samples were collected, a first sample was collected from eight patients during the first 3 days of hospitalization and was culture negative, but a second sample collected >3 days later was positive for Salmonella spp. (n = 5), Shigella spp. (n = 1), or Campylobacter spp. (n = 2).

Antimicrobial therapy at the time of stool collection had a considerable influence on the culture results. As for bacteriological cultures in general, we expected the higher positivity rate for enteropathogenic bacteria from patients who did not receive antimicrobial agents compared to those under antimicrobial treatment (8.6 versus 2.7%, respectively). In the present study we have demonstrated that antimicrobial treatment affects culture results independently of other variables, in particular, age, hospital department, and length of hospital stay at the time of sampling. The presence of antimicrobial agents in specimens causes false-negative culture results since the growth of susceptible bacteria is inhibited. Many anti-
crobial agents are still excreted in feces for >2 days after administration of the final dose of medication (4, 28). Aminopenicillins, trimethoprim-sulfamethoxazole, and quinolones can prolong the excretion period of Salmonella spp. (4, 28). This may explain our observation of Salmonella sp. excretion during aminopenicillin or trimethoprim-sulfamethoxazole treatment. It is not surprising that for many patients treated with metronidazole Salmonella spp. were still detectable in their feces, since these organisms are naturally resistant to metronidazole. Sample collection during the early phase of treatment may be a reason why 3 of 253 patients (1.2%) receiving trimethoprim-sulfamethoxazole excreted Shigella spp. that were susceptible to this agent. This situation may also explain the high rate (6 of 147 samples) of Campylobacter spp. in specimens from patients receiving quinolones. Indeed, it has been reported that 50% of patients with enteritis due to Campylobacter spp. and receiving quinolones still excreted Campylobacter spp. 12 to 17 days after treatment initiation (28). The appearance of quinolone-resistant strains after quinolone treatment has been described and could explain the persistent excretion of Campylobacter spp. (1).

The surprisingly high rate of C. difficile cytotoxin in the stools of patients who had not being receiving antimicrobial agents when samples were collected (12.1% in the present study) has also been reported by others (27). It has been observed that C. difficile-associated diarrhea and colitis may occur up to 10 weeks after the offending antimicrobial agent has been discontinued (16). We noticed a low rate of cytotoxicogenic C. difficile in patients receiving fluocoxacin, ceftriaxone, glycopeptides, amoxicillin, macrolides, quinolones, or trimethoprim-sulfamethoxazole. On the other hand, higher incidences were noted in patients who received amoxicillin-clavulanic acid, imipenem, or clindamycin, confirming other reports (8, 14, 27). The relatively high rate of positive samples with detectable C. difficile cytotoxin (10 of 55; 18.2%) among patients receiving metronidazole was associated with follow-up specimens ordered within 14 days of a previously positive sample for seven patients. So, the samples were collected and analyzed as follow-up for a previously positive result; metronidazole was not the offending agent but was the agent administered to treat C. difficile colitis, as recommended in our institution (27). Symptoms of C. difficile enterocolitis may persist for up to 16 days during metronidazole treatment (27). In only two patients, a treatment failure or relapse with metronidazole may have occurred, and the treatment was changed to vancomycin, as recommended by others (19, 24). Considering the importance of unrecognized C. difficile enterocolitis in hospitals and the high incidence of transmission, we recommend that at least two stool samples be analyzed, since in our study analysis of a second specimen increased the sensitivity from 85.3% to 97.1%.

On the basis of these observations we recommend our ordering physicians to request the cultures for Salmonella spp., Shigella spp., and Campylobacter spp. mainly for patients hospitalized for ≥3 days. For patients who have been in the hospital for >3 days, cultures may be acceptable if (i) follow-up samples are required for epidemiological reasons, (ii) the patients are immunocompromised, or (iii) a first fecal specimen obtained ≤3 days of hospitalization has been culture negative for a patient highly suspected of having infectious diarrhea or (iv) in the rare event of nosocomial food-borne outbreaks (25). Applying these restrictions and considering reagent costs of $3 and 4 min of technologist time for a negative stool culture (18), we could have saved at least $20,000 in reagent costs and ≥12 weeks of technologist time during the study period. On the other hand, rejecting stool cultures as mentioned, we would have missed 13 (1.5%) stool cultures positive for Salmonella spp. or Campylobacter spp., and 7 of these were from subjects considered to be healthy carriers.

For patients receiving antimicrobial agents, Salmonella spp., Shigella spp., and Campylobacter spp. may still be recovered, but the yield is significantly lower. Therefore, specimens should be collected before antimicrobial treatment whenever possible. During antimicrobial therapy, tests for C. difficile cytotoxin should mainly be requested; however, it may also be detected in feces from patients not receiving antimicrobial agents at the time of stool collection for testing. For adult human immunodeficiency virus-positive or geriatric patients, rotaviruses may have to be excluded for epidemiological reasons, especially during the winter season (2, 13, 23).

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REFERENCES