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Three-Hour Molecular Detection of *Campylobacter*, *Salmonella*, *Yersinia*, and *Shigella* Species in Feces with Accuracy as High as That of Culture▽

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Campylobacter jejuni* and *Salmonella*, *Shigella*, and *Yersinia* species (along with Shiga toxin-producing *Escherichia coli*) are the most common causes of acute bacterial diarrheal disease in the United States. Current detection techniques are time-consuming, limiting usefulness for patient care. We developed and validated a panel of rapid PCR assays for the detection and identification of *C. jejuni*, *C. coli*, *Salmonella*, and *Yersinia* species and *Shigella* and enteroinvasive *E. coli* in stool samples. A total of 392 archived stool specimens, previously cultured for enteric pathogens, were evaluated by PCR. Overall, 104 stool specimens had been culture positive (*C. jejuni*/*coli* [$n = 51$], *Salmonella* species [$n = 42$], *Shigella* species [$n = 6$], and *Yersinia* species [$n = 5$]). Compared to culture, the overall sensitivity and specificity of PCR detection of these organisms were 92 and 98% (96/104 and 283/288), respectively, from fresh or Cary Blair stool ($P = 0.41$); 87 and 98% (41/47 and 242/246), respectively, from fresh stool ($P = 0.53$); and 96 and 98% (55/57 and 41/42), respectively, from Cary Blair stool ($P = 0.56$). For individual genera, PCR was as sensitive as the culture method, with the exception of *Salmonella* culture using selenite enrichment for which PCR was less sensitive than culture from fresh, but not Cary Blair ($P = 0.03$ and 1.00, respectively) stools. This PCR assay panel for the rapid diagnosis of acute infectious bacterial diarrheal pathogens has a sensitivity and specificity equivalent to that of culture for stools in Cary Blair transport medium. Paired with reflexive culture of stools testing positive, this should provide an improvement in care for patients with acute infectious diarrheal disease.

Despite advances in water treatment, food safety, and sanitary conditions, acute diarrheal disease remains a leading cause of morbidity and mortality worldwide. Most bacterial enteric infections in the United States originate within the food supply chain. According to the Centers for Disease Control and Prevention, 43% of laboratory-confirmed bacterial enteric infections in the United States are caused by *Salmonella* species, followed by *Campylobacter* species (33%), *Shigella* species (17%), Shiga toxin-producing *Escherichia coli* (4.1%), and *Yersinia* species (0.9%) (4).

Although most common agents of bacterial enteric infection are easily cultivated on standard selective and differential bacteriologic media, isolation and final identification are time-consuming, leaving patients without a diagnosis for several days, and putting them at risk for untreated infection and spread of infection to others. Alternatively, empirical antimicrobial therapy may have adverse consequences for some diarrheal pathogens, such as *E. coli* O157:H7 (16). At Mayo Clinic (Rochester, MN), the time to final identification for *Salmonella*, *Shigella*, and *Yersinia* species from stool culture ranges from 3 to 5 days and that for *Campylobacter* species ranges from 2 to 4 days.

We recently described a rapid real-time PCR assay for detecting Shiga toxin-producing *E. coli* in stool that showed performance equivalent to that of culture for detecting *E. coli* O157:H7 and which additionally detects non-O157 Shiga toxin-producing *E. coli* (6). We have also developed a stool PCR assay that is as accurate as culture for detecting toxigenic *Clostridium difficile* in stool samples (12). These assays are currently the only ones used for detection of the associated pathogens in our laboratory. Based upon the success of Shiga toxin and *C. difficile* stool PCR, we developed and validated assays to rapidly detect and differentiate *Campylobacter*, *Salmonella*, and *Yersinia* species, and *Shigella* species/enteroinvasive *E. coli* in stool and compared the results to those of routine stool cultures on specimens submitted for testing for enteric pathogens.

(This study was presented in part at the 110th General Meeting of the American Society for Microbiology, San Diego, CA, 23 to 27 May 2010.)

MATERIALS AND METHODS

Clinical specimens. A total of 392 stool specimens, submitted as fresh stools ($n = 293$) or in Cary Blair transport medium ($n = 99$) for routine culture of enteric pathogens, were cultured and stored at −70°C between October 2007 and February 2009. This study was reviewed and approved by the Mayo Clinic Institutional Review Board.

Stool culture. Stool culture for *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia* species was performed using BBL Hektoen enteric, BBL cefsulodin irgasan novobiocin, and BBL Campy CVA agars (BD Diagnostics, Sparks, MD) incubated at 35°C in room air, 30°C in room air, and 42°C in a microaerophilic

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TABLE 1. Primers and probes

Strain set[a]	Primer or probe	Sequence (5′–3′)
C. jejuni/*C. coli cadF* (set 602)		
Primers	CAMP 221F	CTGCTAAACCATAGAAATAAAATTTCTCAC
	CAMP 221R	CTTTGAAGGTAATTTAGATATGGATAATCG
Probes	CAMP 221fl	ACATCAGAATAATGCTCTAACCCAAATTCTAAT-FITC
	CAMP 221fl2	TCCGAGTAATGTTCTAAACCTAGTTCTAAT-FITC
	CAMP 221iLC610	Red610-CATCACCATCTTCATAGGCTACTTGACCTATAGT-PO4
Salmonella sp. *invA* (set 604)		
Primers	SALM 178F	TGCATAATGCCAGACGAAAGAG
	SALM 178R	ATCATTTCTATGTTCGTCATTCCA
Probes	SALM 178fl	GAGGATTCTGTCAATGTAGAACGACCC-FITC
	SALM 178iLC610	Red610-TAAACACCAATATCGCCAGTACGATATTCAGTGCG-PO4
Shigella sp./enteroinvasive *E. coli ipaH* (set 663)		
Primers	SHIG 172F	ATAGAAGTCTACCTGGCCT
	SHIG 172R	GGGAGAACCAGTCCGTAA
Probes	SHIG 172fl	CAAATGACCTCCGCACTGCC-FITC
	SHIG 172iLC670	Red610-AGCCACGGTCAGAAGCCG-PO4
Yersinia sp. *lysP* (set 664)		
Primers	lysP 156F	GGCATCATGAAAGGCGG
	lysP 156R	TGATTCACCAGCAGCAATAC
Probes	lysP 156fl	GGTTCTCGGCGATGATTGGTGTGG-FITC
	lysP 156iLC670	Red610-ATGATTGTCGGTTTCTCCTTCCAGGGAACTGAGC-PO4

[a] Sets: TIB MolBio, Adelphia, NJ.

environment, respectively. Stool was also inoculated into selenite broth and incubated at 35°C in room air for 8 to 16 h, followed by subculture to BBL Hektoen enteric agar. Stool was additionally cultured to Trypticase soy agar with 5% sheep blood and eosin methylene blue agar. Suspicious colonies were tested by using standard methods.

Primer and probe design. Primers and probes (Table 1) were designed by using the LightCycler Probe Design Software 2.0 (Roche Diagnostics, Indianapolis, IN) and Oligo 6.71 (Molecular Biology Insights, Cascade, CO).

Positive PCR controls. Positive control plasmids were constructed for the four target genes (Table 1) by using the pCR2.1 TOPO TA cloning kit (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's instructions. Sources for the inserted target sequences were *Campylobacter jejuni* ATCC 35919, *Campylobacter coli* ATCC 43472, *Salmonella enterica* ATCC 35987, *Shigella sonnei* ATCC 25931, and *Yersinia enterocolitica* ATCC 9610. Plasmids were purified by using a High Pure plasmid isolation kit (Roche Applied Science, Indianapolis, IN). The sizes of the cloned inserts were confirmed by restriction enzyme digestion (EcoRI; Invitrogen Corp). Plasmid inserts were sequenced by using the M13 forward and reverse primers included in the TOPO TA cloning kit to assure proper insert orientation. Plasmids were diluted in Tris-EDTA buffer (pH 8.0) and stored at 4°C.

Stool processing and extraction for PCR. Sterile cotton swabs were used to transfer a pea-sized amount of formed or semiformed fresh stool into 1 ml of 1:1 Stool Transport and Recovery medium (STAR; Roche Applied Science, Indianapolis, IN) buffer-sterile water. For liquid specimens and those transported in Cary Blair medium, a 100-μl aliquot was placed in 1 ml of 1:1 STAR buffer-sterile water. The resultant stool slurries were vortexed and centrifuged at 20,800 × *g* for 10 s. Then, 200 μl of the supernatant was transferred to a MagNA Pure sample cartridge (Roche Applied Science). DNA extraction was performed on a MagNA Pure LC 2.0 using the MagNA Pure LC total nucleic acid isolation kit (Roche Applied Science).

PCR. The four assays were independently optimized on the LightCycler 2.0 platform using LightCycler software version 4.1 (Roche Applied Science). Next, 15-μl portions of PCR master mix containing final concentrations of 1× Roche LC Fast Start DNA Master HybProbe (*Taq* DNA polymerase, PCR buffer, deoxyribonucleoside triphosphate with dUTP substituted for dTTP, 1 mM $MgCl_2$), 3 mM (additional) $MgCl_2$, and 1× concentrations of each of the LightCycler primer-probe sets (Table 1) were added to a 20-μl LightCycler cuvette. Extracted nucleic acid (5 μl) was then added to each cuvette containing the respective master mix. The cycling program was as follows: denaturation at 95°C for 10 min; amplification for 45 cycles of 10 s at 95°C, 15 s at 55°C (single acquisition), and 15 s at 72°C; melting-curve analysis/amplicon detection for 0 s at 95°C, 20 s at 59°C, 20 s at 40°C (ramp rate of 0.2°C/s), and 0 s at 85°C (ramp rate of 0.2°C/s and continuous acquisition); and finally cooling 30 s at 40°C. Positive and negative controls were included in each run. The positive control consisted of plasmids constructed for each of the aforementioned assays diluted in 1:1 STAR buffer-sterile water at a final concentration of 1,000 targets/μl. The negative control contained 1,000 CFU of *Escherichia coli* ATCC 25922/μl.

Analytical sensitivity and specificity, cross-reactivity, and inhibition. Analytical sensitivity was assessed by spiking a series of dilutions of clinical isolates of *C. jejuni*, *C. coli*, *Salmonella enterica* serovar Typhimurium, *Shigella boydii*, and *Yersinia enterocolitica* into fresh and Cary Blair transported stools. To determine the analytical specificity, predicted amplified product, primer, and probe sequences were subjected to BLAST searches using the National Center for Biotechnology Information (NCBI) genomic database (http://www.ncbi.nlm.nih.gov). Cross-reactivity studies were performed using a previously described panel of 66 organisms (6), *Helicobacter pylori* ($n = 3$) and *Helicobacter cinaedi*, and an enteric pathogens inclusivity panel (Table 2). Inhibition studies were performed by spiking 50 stool extracts negative for enteric pathogens with each plasmid control (final concentration, 100 targets/μl) and assaying the resultant mixtures by PCR.

Clinical sensitivity and specificity. The clinical sensitivity and specificity were assessed by assaying the aforementioned stools by PCR and comparing the results to those of culture. For *Salmonella* PCR, results were compared to culture with or without selenite enrichment. Discordant results were subjected to repeat extraction and PCR, attempted cultivation using enrichment methods, and isolate testing (when available) by PCR. For organisms cultured from fewer than 30 human stool specimens, additional spiking studies were performed by adding a known low quantity of organism to stool specimens from 30 uninfected (i.e., culture- and PCR-negative) subjects.

Statistical analysis. The clinical sensitivity and specificity of the PCR assays for detection of enteric pathogens were determined. Comparisons of culture and PCR for *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia* species were made by using McNemar's test, a test of paired proportions. P values of <0.05 were considered statistically significant. Statistical analysis was performed by using SAS software version 9.1 (SAS, Inc., Cary, NC).

RESULTS

Analytical sensitivity and specificity, cross-reactivity, and inhibition. The analytical sensitivity of the *Campylobacter* PCR assay for the detection of *C. jejuni* was 16 CFU/ml in fresh and

TABLE 2. Enteric pathogen inclusivity panel

Organism	Strain	Detected
Campylobacter jejuni	ATCC 29428	Yes
Campylobacter jejuni	ATCC 35919	Yes
Campylobacter jejuni	ATCC 33560	Yes
Campylobacter jejuni	ATCC 33291	Yes
Campylobacter coli	Patient isolate	Yes
Campylobacter coli	ATCC 33559	Yes
Campylobacter coli	ATCC 43472	Yes
Campylobacter upsaliensis	Patient isolate	
Campylobacter fetus	ATCC 33248	
Campylobacter sputorum	ATCC 33710	
Campylobacter hyointestinalis	ATCC 35217	
Campylobacter lari	ATCC 35221	
Escherichia coli[a]	ATCC 43893	Yes
E. coli[a]	CDC TD215	Yes
E. coli[a]	CDC EDL 1282	Yes
Salmonella arizonae	Patient isolate	Yes
Salmonella bongori	ATCC 43975	Yes
Salmonella choleraesuis	ATCC 23565	Yes
Salmonella enterica	ATCC 35987	Yes
Salmonella enterica serovar Paratyphi	CDC AB9-C12	Yes
Salmonella enterica serovar Typhi	CAP D-2-79	Yes
Salmonella enterica serovar Typhimurium	ATCC 14028	Yes
Shigella boydii	CAP D-01-96	Yes
Shigella dysenteriae	CDC 82-002-72	Yes
Shigella flexneri	ATCC 29903	Yes
Shigella sonnei	ATCC 25931	Yes
Yersinia enterocolitica	ATCC 9610	Yes
Yersinia kristensenii	NYS 3-85	
Yersinia pseudotuberculosis	CAP LPS A-01	Yes
Yersinia frederiksenii/*Yersinia intermedia*	Patient isolate	Yes
Yersinia frederiksenii	ATCC 29912	Yes
Yersinia intermedia	ATCC 29909	Yes
Yersinia pseudotuberculosis	ATCC 907	Yes
Yersinia aldovae	DSMZ 18303	Yes
Yersinia alecsiciae	DSMZ 14987	Yes
Yersinia bercovieri	DSMZ 18528	Yes
Yersinia massiliensis	DSMZ 21859	Yes
Yersinia mollaretii	DSMZ 18520	Yes
Yersinia rohdei	DSMZ 18270	Yes
Yersinia ruckeri	DSMZ 18506	Yes
Yersinia similis	DSMZ 18211	Yes

[a] That is, an enteroinvasive *E. coli* strain.

Cary Blair stools, and for the detection of *C. coli* it was 4,000 CFU/ml in fresh stools and 400 CFU/ml in Cary Blair stools. For the *Salmonella* PCR assay, the analytical sensitivity was 990 CFU/ml in fresh and 99 CFU/ml in Cary Blair stools. The *Shigella*/enteroinvasive *E. coli* PCR assay had an analytical sensitivities of 52 CFU/ml in both fresh and Cary Blair stools for *Shigella* spp. and 500 CFU/ml in both fresh and Cary Blair stools for enteroinvasive *E. coli*. The *Yersinia* PCR assay had analytical sensitivities of 700 CFU/ml in fresh stools and 70 CFU/ml in Cary Blair stools. Amplified product, primer, and probe sequences for each of the assays were subjected to NCBI database searches using BLAST software; no significant homology was noted outside of the genera targeted by these assays. Isolates of *Salmonella*, *Shigella*, *Campylobacter*, and *Yersinia* species ($n = 12$) included in the cross-reactivity panel were detected with the respective assays. The remaining isolates in the cross-reactivity panel and the *Helicobacter* species were not detected with the four assays. The *C. jejuni*/*C. coli* PCR assay detected all isolates of both species within the inclusivity panel; however, other members of the genus *Campylobacter* were not detected (Table 2). The *Salmonella* and *Shigella*/enteroinvasive *E. coli* assays detected all *Salmonella* and *Shigella* species tested, respectively; the *Shigella*/enteroinvasive *E. coli* assay detected enteroinvasive *E. coli*. Thirteen species of *Yersinia* were detected; however, the assay did not detect *Yersinia kristensenii*. PCR inhibition was not detected in any of the 50 spiked extracts evaluated by all four assays.

TABLE 3. Clinical sensitivity and specificity of the PCR assays for the detection of enteric pathogens

Organism	Stool type	No. of strains/total no. of strains (%)		P[f]
		Sensitivity	Specificity	
C. jejuni/*C. coli*[a]	Any stool type	49/51 (96)	336/341 (99)	0.26
	Fresh stool	23/23 (100)	266/270 (99)	0.05
	Cary Blair stool	26/28 (93)	70/71 (99)	0.56
Salmonella spp. (HE)[b]	Any stool type	34/34 (100)	355/358 (99)	0.08
	Fresh stool	12/12 (100)	281/281 (100)	1.00
	Cary Blair stool	22/22 (100)	74/77 (96)	0.08
Salmonella spp. (SE+HE)[c]	Any stool type	37/42 (88)	350/350 (100)	0.03
	Fresh stool	12/17 (71)	276/276 (100)	0.03
	Cary Blair stool	25/25 (100)	74/74 (100)	1.00
Shigella spp.[d]	Any stool type	6/6 (100)	386/386 (100)	1.00
	Fresh stool	5/5 (100)	288/288 (100)	1.00
	Cary Blair stool	1/1 (100)	98/98 (100)	1.00
Yersinia spp.[e]	Any stool type	4/5 (80)	387/387 (100)	0.32
	Fresh stool	1/2 (50)	291/291 (100)	0.32
	Cary Blair stool	3/3 (100)	96/96 (100)	1.00

[a] *C. jejuni* ($n = 49$) and *C. coli* ($n = 2$).
[b] HE, direct culture to Hektoen enteric agar.
[c] *Salmonella enterica* serovars Typhimurium ($n = 11$), II 2:b:enxz15 ($n = 1$), I 6,8:NM ($n = 1$), I 45:b:minus ($n = 1$), Hadar ($n = 1$), Montevideo ($n = 2$), Enteritidis ($n = 5$), II 58:Iz13z28:26 ($n = 1$), Litchfield ($n = 1$), Hartford ($n = 1$), Nigeria ($n = 2$), Muenchen ($n = 1$), Agona ($n = 1$), I 4,5,12:i: ($n = 1$), Saintpaul ($n = 1$), Anatum ($n = 2$), Reading ($n = 1$), and unknown ($n = 8$). SE+HE, selenite enrichment and subculture to HE agar.
[d] *S. flexneri* ($n = 1$), *S. boydii* ($n = 1$) and *S. sonnei* ($n = 4$).
[e] *Y. enterocolitica* ($n = 3$), *Y. fredricksenii* ($n = 1$), and *Y. intermedia* ($n = 1$).
[f] That is, the *P* value determined using McNemar's test comparing the culture and PCR results.

Clinical sensitivity and specificity. Overall, 104 stool specimens were culture-positive for *C. jejuni*/*coli* ($n = 51$), *Salmonella* species ($n = 42$), *Shigella* species ($n = 6$), and *Yersinia* species ($n = 5$). Compared to culture, the overall clinical sensitivity and specificity of PCR detection of these organisms were 92 and 98% (96/104 and 283/288), respectively, from fresh or Cary Blair stool (p, 0.41); 87 and 98% (41/47 and 242/246), respectively, from fresh stool (p, 0.53); and 96 and 98% (55/57 and 41/42), respectively, from Cary Blair stool (p, 0.56). The clinical sensitivities of the PCR assays performed on fresh (87%; 95% confidence interval [CI], 74 to 95%] and Cary Blair (96%; 95% CI, 88 to 100%) stools were not significantly different from one another ($P = 0.14$).

The *Campylobacter* PCR assay had a clinical sensitivity and specificity of 96 and 99%, respectively (Table 3). Repeat PCR testing on the two samples missed by PCR was negative; isolates from these samples were unavailable to test by PCR. Five specimens tested PCR positive and culture negative. Repeat

PCR testing was negative in all five. The associated stools were placed into *Campylobacter* enrichment broth (Neogen Corp., Lansing, MI) and incubated for 48 h, followed by subculture to Campy CVA agar; no growth was observed.

The *Salmonella* PCR assay had a clinical sensitivity and specificity of 100 and 99%, respectively, compared to direct culture to Hektoen enteric agar, and 88 and 100%, respectively, compared to selenite enrichment with subculture to Hektoen enteric agar (Table 3). There were three PCR-positive and five PCR negative stools that were *Salmonella* culture positive only following selenite broth enrichment culture with subculture to Hektoen enteric agar. For stool transported in Cary Blair medium, the *Salmonella* PCR assay was 100% sensitive and 96% specific compared to direct culture to Hektoen enteric agar. The assay had a sensitivity and specificity of 100% compared to culture with preparatory selenite enrichment for stool in Cary Blair. Overall, for *Salmonella* culture using selenite enrichment, PCR was less sensitive than culture from fresh, but not Cary Blair transport medium-preserved stools ($P = 0.03$ and 1.00, respectively).

The clinical sensitivity and specificity for the *Shigella*/enteroinvasive *E. coli* assay were both 100% for detection of *Shigella* species, but the number of positive specimens with culture-confirmed shigellosis was small (Table 3). A total of 30 uninfected stools were spiked with ~4,000 CFU of *S. boydii*/ml and ~5,000 CFU of enteroinvasive *E. coli*/ml to add supportive data. The spiking studies were concordant with the expected results. The *Yersinia* assay was 80% sensitive and 100% specific (Table 3). The PCR assay missed a single culture positive specimen, which had grown *Yersinia enterocolitica*; the isolate yielded a positive result when tested by PCR. Repeat PCR testing of the stool sample was negative. Although there was an apparent difference in the sensitivity between fresh stool (50%) and Cary-Blair stool (100%), the overall number of culture positive stools was low. In order to supplement the clinical data, 30 uninfected stools were spiked with ~7,000 CFU of *Y. enterocolitica*/ml and tested by PCR. Spiking studies were concordant with the expected results.

DISCUSSION

We validated a panel of PCR assays for the detection of *Salmonella*, *Campylobacter*, and *Yersinia* species and of *Shigella* species/enteroinvasive *E. coli*. This panel performed comparably to culture for the detection of these organisms in stool but yields results in 3 h (or less) versus 2 to 5 days with conventional culture. Overall, the PCR assay panel performed well on fresh stool or stool in Cary Blair medium. The sensitivity of the *Salmonella* PCR assay was not as good as culture when using selenite enrichment for fresh stool but was as good as culture when using selenite enrichment for stool in Cary Blair medium (or culture without selenite enrichment for either fresh stool or stool in Cary Blair medium). Cary Blair medium provides stability, through pH buffering activity, to fecal specimens submitted for routine culture (3). With the described PCR assay, processing, extraction, and amplification/detection take 2.5 to 3 h per run of up to 30 patient specimens. Paired with reflexive culture of stools testing positive (e.g., for antimicrobial susceptibility testing as needed), this assay panel should (i) improve the care of patients with acute bacterial gastroenteritis through improved turnaround time, "leading to more timely and directed therapeutic intervention" (15), (ii) mitigate the inappropriate use of antibiotics, and (iii) aid epidemiologic investigations (14).

Although there have been publications describing conventional and real-time PCR assays for the detection of *Salmonella* species, *Shigella* species, enteroinvasive *E. coli*, and/or *Campylobacter* species in stool, we believe this to be the most comprehensive real-time PCR assay panel described for the detection of enteric pathogens and that this panel was evaluated against the largest number of human stool samples to date. Iijima et al. described a real-time PCR assay for detection of *S. enterica* and *C. jejuni* in stool samples; however, of the human stool specimens tested, only nine were culture positive for *S. enterica*, and 16 were culture positive for *C. jejuni* (8). Our PCR panel also includes *Shigella* and *Yersinia* species and enteroinvasive *E. coli*, and our clinical evaluation included a substantially larger number of culture-positive samples.

Conventional PCR assays require gel electrophoresis of amplified PCR product, which, when applied in clinical laboratories, increases the risk of amplified product contamination and turnaround time (i.e., compared to closed-system real-time PCR assays, as described here). Abu Elamreen et al. described a conventional PCR panel that included detection of *Shigella* and *Salmonella* species and *C. jejuni*/*coli*; however, only nine of the specimens studied were culture positive for any of these organisms (1). Other investigators have described conventional PCR assays for the detection of *Campylobacter* species in stool (2, 11). Huong et al. described a conventional PCR assay for the detection of *C. jejuni* and *coli* in stool samples (7). Although that assay yielded more positive results than did culture (275/358 [77%] versus 202/358 [56%]), culture was performed on frozen stools, which likely decreased its sensitivity (7). In addition, 16/202 (8%) of the culture-positive stools were PCR negative, apparently as a result of inhibitors, which were shown to be effectively removed by our specimen-processing approach (7). Takeshi et al. described conventional PCR assays for detection of *C. jejuni* and *Salmonella* species in patients with bloody diarrhea but studied only 24 patients (13). Finally, Dutta et al. described a conventional PCR assay for enteroinvasive *E. coli* and *Shigella* detection in stool (5).

Logan et al. described a nested PCR assay for the detection of *Campylobacter* species which increases the possibility of contamination versus a non-nested assay (10). Further, these researchers only evaluated stools from 38 subjects (of whom half were culture positive) (10).

Although *Y. enterocolitica* is the most frequently isolated *Yersinia* species in diarrheal stools, other species of *Yersinia*, including *Y. intermedia* and *Y. fredericksenii*, may be encountered from clinical specimens (9) and are detected by our *Yersinia* assay. A limitation of the assay is that it does not detect *Y. kristensenii*, a species that accounted for 3/194 (1.5%) *Yersinia* stool isolates at our institution from 1985 to 1999 (9).

A limitation of our study is that culture for enteroinvasive *E. coli* was not routinely performed; however, this type of *E. coli* is rare in the United States. In addition, the ideal enteric-pathogen PCR study would be prospectively performed; however, such as single-institution study would require several years to yield the number of culture-positive specimens studied here.

Clinical application of the PCR assay panel described will offer a substantially shorter turnaround time compared to conventional culture, although the cost benefit of this assay panel versus conventional culture deserves further study. Real-time, closed-system PCR is arguably easier to perform than is conventional culture and identification. Accordingly, clinical application of these assays may circumvent the need for skilled microbiology technologists to interpret stool cultures, which is important since many laboratories are struggling to find experienced technologists. Rapid testing for enteric bacterial disease is a new tool for clinicians in their care of patients and public health personnel in their investigation and control of the spread of enteric bacterial diseases. It may provide cost savings in that PCR positive specimens can be selected for focused culture for antimicrobial susceptibility testing.

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