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Isolation of *Campylobacter* Species from Stool Samples by Use of a Filtration Method: Assessment from a United States-Based Population

Irving Nachamkin,[a,b] Phi Nguyen[b]

Department of Pathology and Laboratory Medicine, University of Pennsylvania Perelman School of Medicine,[a] and Clinical Microbiology Laboratory, Hospital of the University of Pennsylvania,[b] Philadelphia, Pennsylvania, USA

ABSTRACT Fecal samples submitted to our clinical microbiology laboratory from patients in the Philadelphia region were prospectively analyzed for *Campylobacter* species other than *C. jejuni* and *C. coli* using a filtration method and microaerobic conditions with increased H_2 concentrations. Of 225 samples tested, 13 (5.8%) yielded *Campylobacter* species, with frequent isolation of *C. concisus*. The majority of *Campylobacter* species were not clinically significant. Additional studies in U.S. populations are warranted.

KEYWORDS *Campylobacter jejuni*, campylobacter, campylobacteriosis, culture methods, gastrointestinal infection

Campylobacter species other than *C. jejuni* and *C. coli*, such as *C. concisus*, *C. upsaliensis*, *C. ureolyticus*, *C. sputorum*, and others, are not isolated commonly from routine stool cultures due to the nonthermophilic nature of the species and/or inhibition by antimicrobial agents in commonly used selective medium (1). Except for *C. upsaliensis*, these species may not be detected in recently developed molecular multiplex stool pathogen test kits. Little is known about the occurrence of these species in U.S. patients. While species such as *C. upsaliensis* are known pathogens (2), the pathogenicity of many other species, such as *C. concisus*, is controversial (3). Recovery of *Campylobacter* spp. from stool cultures requires the addition of a filtration method and sufficient H_2 in the microaerobic environment (1). The purpose of this study was to determine the frequency and clinical relevance of *Campylobacter* species from stool cultures in a United States-based clinical laboratory.

RESULTS AND DISCUSSION

We processed 225 fecal samples submitted to the Hospital of the University of Pennsylvania (HUP) Clinical Microbiology Laboratory for routine testing for gastrointestinal pathogens from September to December 2016 for the presence of *Campylobacter* spp. using the filtration method. *Campylobacter* spp., including *C. jejuni*, *C. coli*, *C. lari* ($n = 10$), and other species ($n = 13$), were recovered from 23 samples processed by this method. All of the samples from which *C. jejuni*, *C. coli*, or *C. lari* isolates were recovered using the filtration method were also positive in the Verigene enteric panel multiplex molecular assay (Table 1). Of the 13 isolates other than *C. jejuni*, *C. coli*, and *C. lari*, 4 were recovered using 0.45-μm nitrocellulose filters (30.7%), 8 (61.5%) were recovered on 0.65-μm nitrocellulose filters, and 13 (100%) were recovered from 0.6-μm polycarbonate filters, suggesting the superiority of polycarbonate filters for recovery of *Campylobacter* spp. (Fig. 1). These results are consistent with those reported by Nielsen et al. (4), who also found that polycarbonate filters were superior to cellulose acetate filters for recovery of *C. concisus* from stool samples.

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Address correspondence to Irving Nachamkin, irving.nachamkin@uphs.upenn.edu.

TABLE 1 *Campylobacter* species isolated by the filtration method

Organism	No. positive by filtration	No. recovered on 0.45-μm nc/0.65-μm nc/0.6-μm pc[a]	No. positive for *Campylobacter* spp. by Verigene multiplex testing	Hospital status (no. inpatient/ no. outpatient)	Other pathogens detected[b]
C. jejuni, *C. coli*, *C. lari*	10	9/10/10	10	2/8	None
C. concisus	8	4/5/8	0	4/4	*Salmonella* (V), Norovirus (V), *Cryptosporidium* (I)
C. ureolyticus	3	0/3/3	0	2/1	None
C. sputorum	1	0/0/1	0	0/1	None
C. showae	1	0/0/1	0	1/0	None

[a]nc, nitrocellulose; pc, polycarbonate.
[b]Detection was by Verigene system (V) or immunoassay (I).

Of the 13 non-*C. jejuni*, -*C. coli*, -*C. lari* isolates, 4 (30.8%) were categorized as being clinically significant. *C. concisus* was recovered from stool samples of 8 of the 13 patients, equally distributed between male (age range, 37 to 57 years) and female (age range, 27 to 77 years) patients. Six of the 8 isolates were categorized as not clinically significant. In 3 of the 8 patients with *C. concisus*, well-recognized conventional pathogens were detected (*Salmonella* in 1, *Cryptosporidium* in 1, and *Norovirus* in 1), and 3 patients had other, noninfectious etiologies (1 patient had an inflammatory bowel disease [IBD] flare, 1 had a stroke [unclear why a sample was submitted], and 1 had bile salt-induced diarrhea). Two isolates were considered to be clinically significant based on available information in the medical record. One isolate was from a patient who had traveler's diarrhea and in whom no other routine enteric pathogens were detected. The patient had not been treated with antimicrobial agents, and the illness was self-limited. The other patient had HIV infection and 2 weeks of diarrhea that resolved without specific antimicrobial therapy.

There were 3 patients who had *C. ureolyticus* isolated from stool samples, and all three were female patients (aged 53, 82, and 86 years). Isolates from 2 of the 3 patients with *C. ureolyticus* were categorized as not clinically significant. Two isolates were from patients with gastrointestinal (GI) malignancies and were thought to be the cause of GI symptoms. One isolate was categorized as clinically significant and was from a patient with traveler's diarrhea; no other conventional pathogens were detected and the diarrhea was self-limited, resolving without specific antimicrobial treatment. There was one isolate of *C. sputorum* categorized as clinically significant from a male patient aged 36 years who had traveled to Mexico several months prior (>30 days), had no other enteric pathogens detected during that time, and had slowly resolving intermittent diarrhea that did not require antimicrobial therapy. *C. showae* isolated from one patient

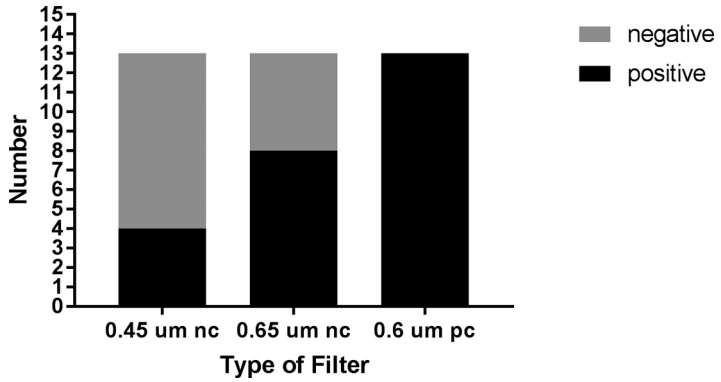

FIG 1 Recovery of *Campylobacter* species other than *C. jejuni*, *C. coli*, or *C. lari* using different types of filters. nc, nitrocellulose; pc, polycarbonate.

(a female aged 52 years) was categorized as not clinically significant (the patient had familial polyposis).

The HUP Clinical Microbiology Laboratory uses a reflex culture method for *Campylobacter* spp. on any stool sample positive by the Verigene Enteric Panel multiplex assay. There were 12 samples positive by Verigene that were processed for filtration where *C. jejuni*, *C. coli*, or *C. lari* was recovered from routine reflex culture in the clinical microbiology laboratory. Ten were positive by filtration for 83.3% sensitivity for filtration for these organisms. There was no instance where the Verigene multiplex assay was positive for *Campylobacter* spp., the culture was negative by reflex culture, and *Campylobacter* species isolates were recovered by filtration.

The filtration method was first described as a method for isolating *C. jejuni* from stool samples (5). Subsequently, a number of studies performed outside the United States recognized the importance of a filtration method for isolating non-*C. jejuni*, non-*C. coli Campylobacter* species from stool samples (6–8). *C. concisus* was the most frequently isolated *Campylobacter* species in our survey. In a recent study by Nielsen et al. (9), *C. concisus* was the *Campylobacter* species most frequently isolated from fecal samples using filtration, and *C. ureolyticus* was also detected, but they did not report on the clinical significance of these isolates. Similarly, Vandenberg et al. (7) showed that *C. concisus* and other species were frequently isolated from fecal samples using the filtration method in a Belgium microbiology laboratory; however, clinical details were not reported. We are not aware of any study from a U.S. laboratory on the use of the filtration method for isolating *Campylobacter* species from fecal samples.

The role of *C. concisus* as a cause of gastrointestinal infection has been the subject of debate for many years. There are no case-control studies to help delineate whether *C. concisus* is a significant enteropathogen; however, some studies suggest an etiologic role in certain patient populations (10). A recent study by Nielsen et al. (11) did not show, however, a difference in azithromycin therapy versus placebo in a small group of patients with *C. concisus*-associated diarrhea. In a questionnaire survey of patients with *C. concisus* isolated from fecal samples, Nielsen et al. concluded that the patients had a milder course of infection compared with patients who had *C. jejuni/C. coli* isolated from stool samples but were more likely to have prolonged symptoms (12). The role of other species in gastrointestinal infection such as *C. ureolyticus* isolated from 3 patients in our survey is less certain (13).

Campylobacter species other than *C. jejuni*, *C. coli*, or *C. lari* were isolated in 5.7% of fecal samples in a survey of patients from the Philadelphia region. Our study suggests that *Campylobacter* species other than *C. jejuni*, *C. coli*, and *C. lari* can be isolated frequently from U.S. patients with a filtration system and increased H_2 microaerobic conditions. In most circumstances, we did not find that the isolates were clinically significant; however, several patients did not have other reasons for their diarrheal illness, which suggested that these species may be clinically relevant in certain patients. Further studies in U.S. populations are warranted.

MATERIALS AND METHODS

We prospectively cultured fecal samples submitted to the Hospital of the University of Pennsylvania (HUP) Clinical Microbiology Laboratory from September to December 2016 (~10 weeks) using a filtration method (1). Stool samples, primarily from outpatients, were submitted in Cary-Blair transport medium and refrigerated if not processed the day of collection. The filtration method used was as follows. Three brucella blood agar plates (Becton Dickinson BBL brucella agar with 5% sheep blood, hemin, and vitamin K; Becton, Dickinson, Sparks, MD) were used as the nonselective medium. For comparison, three different filter types were used, 47-mm cellulose acetate filters, (0.45 μm and 0.65 μm; Sartorius, Goettingen, Germany) and polycarbonate (0.6 μm; EMD Millipore Corp., Billerica, MA) filters. A single filter was placed onto the surface of the plate, 10 drops of fecal material from the Cary-Blair transport vial, gently mixed prior to dispensing of the drops, were placed on each filter, each drop in a separate location on the filter, and plates were incubated for 1 h at 37°C in ambient air. Filters were then removed and plates placed into anaerobic jars, processed to create microaerobic conditions (6% O_2, 7% CO_2, 7% H_2, 80% N_2) using an evacuation-replacement protocol, (Anoxomat System, Advanced Instruments, Inc., Norwood, MA) and incubated at 37°C. Plates were examined on day 2 and day 3 for colonies resembling *Campylobacter* spp., Gram stained, and subjected to matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) identification (Vitek MS, bioMérieux, Durham, NC) and 16S rRNA gene sequence analysis. All stool samples were tested routinely with a multiplex GI panel that included *Campylobacter*

spp. (*C. jejuni*, *C. coli*, *C. lari*), *Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Yersinia* spp., Stx1, Stx2, rotavirus, and norovirus (Verigene enteric pathogens test, Luminex Corp., Austin, TX). Samples positive for one of the bacterial panel targets were routinely cultured (reflex culture) to provide an isolate for antimicrobial susceptibility testing and to submit to the Pennsylvania State Bureau of Laboratories. For isolation of *Campylobacter* spp., fecal material from the original sample in Cary-Blair transport medium was plated onto Campy CVA agar medium (BBL Campy CVA Agar, Becton, Dickinson, Sparks, MD) and incubated at 42°C in microaerobic conditions for 72 h. Some samples, if ordered by the medical provider, were tested for intestinal parasites by antigen immunoassay for detection of *Giardia* and *Cryptosporidium* spp. (*Giardia*/*Cryptosporidium* Quik Chek, Alere, Waltham, MA) and *C. difficile* glutamate dehydrogenase (GDH) antigen and toxin A/B (Cdiff Quik Chek Complete, Techlab, Blacksburg, VA) with indeterminate samples (antigen positive/toxin negative) tested for *tcdB* (BD Max Cdiff, BD Diagnostics, Sparks, MD).

We categorized isolates as clinically significant, not significant, or of unclear significance. To determine the clinical significance of isolates, patient medical charts were retrospectively reviewed for relevant clinical data, including the date of culture, patient age and gender, hospital status (inpatient/outpatient), primary diagnosis at the time of culture, onset of gastrointestinal symptoms, indications for submitting the culture, presence of fever, chills, nausea, vomiting, and/or abdominal pain, description of diarrhea (i.e., watery, bloody, other), other underlying conditions (e.g., IBD, gastrointestinal malignancy), travel history (within the past 30 days or previously), treatment with antimicrobial agents for GI illness or other illnesses in the past 30 days, and resolution of symptoms. A *Campylobacter* isolate was considered clinically significant if (i) the clinical presentation described in the chart was noted by the provider as consistent with a gastrointestinal infection or (ii) charted notes recorded a strong suspicion or high likelihood of infectious gastrointestinal infection and (iii) no other recognized caused of infectious gastroenteritis was detected or documented in the medical record. Isolates were considered not significant if (i) documented reasons for the current gastrointestinal findings were attributed to noninfectious causes such as postsurgical complications or gastrointestinal malignancy or (ii) other recognized causes of infectious gastrointestinal infection were detected in the sample by other laboratory tests. All other cases were categorized as being of unclear significance.

This study was reviewed and approved by the institutional review board of the University of Pennsylvania.

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