Enteric Infections Are Common in Patients with Flares of Inflammatory Bowel Disease

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OBJECTIVES: **Few studies have examined the role of non-*Clostridium difficile* enteric infections in flares of inflammatory bowel disease (IBD). Our objective was to investigate enteric infection detected by multiplex PCR stool testing in patients with IBD.**

METHODS: **We performed a cross-sectional analysis of 9403 patients who underwent 13,231 stool tests with a gastrointestinal pathogen PCR panel during a diarrheal illness from March 2015 to May 2017. Our primary outcome was the presence of an infection. Secondary outcomes included endoscopic and histologic predictors of infection, and IBD outcomes following testing.**

RESULTS: **A total of 277 patients with Crohn's disease (CD), 300 patients with ulcerative colitis (UC), and 8826 patients without IBD underwent 454, 503, and 12,275 tests, respectively. Compared to patients without IBD, patients with IBD were less likely to test positive (CD 18.1%, UC 16.1%, no IBD 26.6%, $p<0.001$). Compared to patients without IBD, CD had a higher prevalence of norovirus ($p=0.05$) and *Campylobacter* ($p=0.043$), whereas UC had a lower prevalence of norovirus ($p=0.001$) and a higher prevalence of *Campylobacter* ($p=0.013$), *Plesiomonas* ($p=0.049$), and *Escherichia coli* species ($p<0.001$). Of 77 patients who underwent endoscopy, there were no major endoscopic or histologic predictors of a positive test. Patients who tested negative were more likely to have IBD therapy escalated ($p=0.004$). Enteric infection did not impact IBD outcomes following testing (log-rank 0.224).**

CONCLUSIONS: **Non-*Clostridium difficile* enteric infections were identified in 17% of symptomatic patients with IBD. Endoscopic and histologic findings may not differentiate flare from infection. Norovirus and *E.coli* may play an important role in flare of IBD.**

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INTRODUCTION

Inflammatory bowel disease (IBD), comprising Crohn's disease (CD) and ulcerative colitis (UC), develops from a combination of genetic susceptibility and environmental factors that elicit an deleterious inflammatory response [1]. The intestinal microbiota regulates mucosal immunity through a number of pathways and dysbiosis is thought to be a major environmental factor in the pathogenesis and maintenance of IBD [2–5]. Enteric infection is a common cause of dysbiosis and is frequently identified in patients with IBD [6].

Several observational studies have demonstrated a link between enteric infection, functionally altered commensal bacteria, and the subsequent development of IBD [7–10]. An increased risk of IBD was observed in patients with a previous episode of *Salmonella* or *Campylobacter jejuni* gastroenteritis [8, 11, 12]. IBD patients with *Clostridium difficile* (*C. difficile*) infection had more pronounced dysbiosis and significantly worse clinical outcomes compared to patients without IBD, including longer hospital stays, higher colectomy rates, higher recurrence rates, and increased mortality [7, 13–16]. In addition, several studies have implicated enteric infection in relapse of known IBD, including viral pathogens [7, 17–20].

In a recent cross-sectional study on stool polymerase chain reaction (PCR) testing during an exacerbation of symptoms in patients with known IBD, we identified enteric infection in 26.8% of tests with *C. difficile*, the most common (12.9%) followed by *Escherichia coli* (*E.coli*) species (8.1%) and viruses (5.1%) [7]. This study, however, was limited by lack of a control group consisting of patients without IBD, meaningful measures of clinical IBD

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outcomes, and endoscopic or histologic findings to help elucidate flare from enteric infection.

Despite growing research regarding the prevalence of enteric infection in patients with flare of IBD, little is known regarding the distribution of infections in patients with IBD compared to patients without IBD. In addition, the similar clinical presentations and laboratory findings in relapse IBD and enteric infection pose substantial barriers to diagnosis and treatment. Enteric infection may be the sole etiology for an exacerbation in symptoms, coexist as a complicating factor, or represent asymptomatic colonization. As such, the clinical importance and diagnostic approach to differentiating non-*C. difficile* enteric infection from flare remain unknown. In addition, highly sensitive and specific molecular multiplex assays have started to replace conventional microbiological tests as a rapid and accurate means of approaching diarrhea [21, 22]. These assays allow for the identification of specific organisms not previously and readily diagnosable by the clinician.

The objective of this study was to evaluate the distribution of non-*C. difficile* enteric infections detected by multiplex PCR stool testing in symptomatic patients with CD, UC, and without IBD. The secondary objective was to compare clinical, endoscopic, and histologic associations, and clinical outcomes, in symptomatic IBD patients with and without enteric infection.

METHODS

Study population and variables

We performed a cross-sectional study using the electronic medical records of patients at New York Presbyterian-Columbia University Medical Center, a quaternary care institution in New York City that serves patients from the surrounding urban area, the tristate region (NY, NJ, and CT), as well as people seeking care from more distant regions. We identified all outpatients and inpatients who underwent stool testing with a FilmArray gastrointestinal pathogen PCR panel (BioFire Diagnostics, Salt Lake City, UT) during an episode of diarrhea during the 26-month period spanning March 2015 through May 2017.

We recorded the following values from the medical record: Date of PCR test, PCR results, date of birth, zip code, place of PCR test (e.g., emergency department, outpatient visit, inpatient hospitalization, and endoscopy), sex, race, ethnicity, presence of IBD using International Classification of Diseases (ICD) coding, date of IBD diagnosis, and IBD subtype. Repeat gastrointestinal pathogen PCR tests within 6 months and any repeat positive tests on the same patient were excluded. Patients diagnosed with IBD within 7 days of a PCR test were classified as IBD patients. All 577 patients with IBD and a random sample of 25 patients without IBD were assessed to confirm that identified records had correct diagnoses codes, PCR test dates, and results. Of those sampled patients, all patients were correctly classified.

Enteric pathogen testing

The gastrointestinal pathogen panel PCR tests for 22 analytes in stool including 13 bacteria, 5 viruses, and 4 parasites including *Campylobacter* (*jejuni, coli*, and *upsaliensis*), *Clostridium difficile* (Toxin A/B), *Plesiomonas shigelloides*, Salmonella, *Yersinia enterocolitica*, *Vibrio* (*parahaemolyticus, vulnificus*, and *cholerae*), enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), Shiga-like toxin-producing *E. coli* (STEC), *E. coli* O157, Shigella/enteroinvasive *E. coli* (EIEC), *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Entamoeba histolytica, Giardia lamblia*, adenovirus (AdV) F40/41, astrovirus, norovirus GI/GII, rotavirus A, and sapovirus (I, II, IV, and V). In our institution, an alternative PCR test is utilized for *C. difficile* (Xpert *C. difficile*, Cephid, Sunnyvale, CA) and as such, these results are not reported with the gastrointestinal pathogen panel PCR and were not examined in this study. Patients with a positive *C. difficile* PCR within 7 days of a gastrointestinal pathogen panel PCR were excluded. The gastrointestinal pathogen panel PCR is capable of the simultaneous detection and identification of nucleic acids from multiple bacteria, viruses, and parasites directly from stool samples in Cary Blair transport media. The multiplex PCR process takes approximately 1 h. The clinical sensitivity and specificity is 94.5–100% for all targets [22, 23].

Endoscopic evaluation

In a subset of patients with IBD who underwent endoscopic evaluation within 30 days before or after submitting a gastrointestinal pathogen panel PCR test, we collected IBD phenotype, duration of IBD, medication exposures, including 5-Aminosalicylate (5-ASA) agents, corticosteroids, immunomodulators, biologics, and other medication exposures such as previous and current use of a proton pump inhibitor, antibiotic use within 90 days before and at PCR testing, inflammatory markers at PCR testing including erythrocyte sedimentation rate (ESR) and C-reactive protein, active inflammation noted on computed tomography (CT) or magnetic resonance (MR) imaging within 2 weeks of testing, endoscopic findings on esophagogastroduodenoscopy (EGD), ileoscopy, colonoscopy, or flexible sigmoidoscopy, histologic findings, requirement for hospitalization, and length of stay.

For endoscopic and findings on examination of the upper GI tract, we assessed endoscopy reports for the presence of esophagitis, gastropathy with gastric location, duodenopathy (as defined as erythema, erosions, or petechial hemorrhage), and whether a biopsy was obtained. We assessed pathology reports for the notation of esophagitis, chronic active gastritis with *Helicobacter pylori*, chronic active gastritis without *H. pylori*, and chronic inactive gastritis without *H. pylori*.

For endoscopic and findings on examination of the lower GI tract, we assessed endoscopy reports for the presence of ileitis, colitis with severity, ulcers, focality of endoscopic inflammation, either focal or diffuse, and whether a biopsy was obtained. We assessed pathology reports for the documentation of ileitis, colitis, chronicity of inflammation, including acute, chronic, and mixed, architectural distortion, and the presence of granulomas.

As there was no established protocol for the endoscopic approach to IBD patients who underwent stool testing, this was done at the discretion at the individual gastroenterologist. In addition, we did not differentiate findings that would suggest enteric infection

versus flare, but rather, recorded all data reported in endoscopy and pathology reports for analysis.

IBD outcomes following enteric pathogen testing

All patients with IBD were retrospectively followed after initial symptom resolution until the last date of outpatient or inpatient follow-up (through April 30, 2018) to assess their course of IBD. We collected data regarding treatment for positive PCR results and change in IBD management after the results of PCR testing. Follow-up time for all patients was accrued from the time of first stool PCR testing with patients censored at loss to follow-up evaluation, end of the study period, death, or the following IBD-related data including hospitalization, emergency department visit, steroids prescription, other IBD therapy escalation, complication (e.g., stricture, obstruction, fistula, and abscess), and surgical intervention.

Outcomes and statistical analyses

Our primary outcome was the presence of any positive gastrointestinal pathogen PCR panel according to the presence of IBD and IBD subtype. We measured for associations between variables, the presence of IBD, and IBD subtype with PCR stool test results via the Chi-square test for categorical variables and the *t*-test for continuous variables. We used Chi-square analysis to test for the association between endoscopic and histologic findings in patients with IBD and the presence of enteric infections. We evaluated time to IBD-related outcomes after a gastrointestinal pathogen PCR panel by constructing Kaplan–Meier curves and using the log-rank test. All tests were considered significant at a two-sided p value less than 0.05. SPSS software (IBM) was used to perform all statistical analyses. The study was approved by the Columbia University Medical Center Institutional Review Board.

RESULTS

Of 9403 inpatients and outpatients who underwent 13,231 stool tests with a gastrointestinal pathogen PCR panel during an episode of diarrhea, we identified 277 patients with CD, 300 patients with UC, and 8826 patients without IBD who underwent 454, 503, and 12,275 stool tests, respectively (Table 1). Forty-eight patients with IBD and 461 patients without IBD had a positive *C. difficile* PCR submitted at the same time as a gastrointestinal pathogen panel PCR and were excluded. Among patients with CD, 82 (18.1%) tests were positive for a total of 122 pathogens (Table 2). In patients with UC, 81 (16.1%) tests were positive for a total of 115 pathogens. In patients without IBD, 3263 (26.6%) tests were positive for 4431 pathogens. Compared to patients without IBD, patients with IBD were less likely to test positive for an enteric infection ($p < 0.001$). Among those with a positive test, patients with IBD were older than those without IBD (median 33.0 for CD vs 30.6 years for non-IBD, $p = 0.045$; 49.6 for UC, $p < 0.001$).

In terms of the distribution of infections, compared to patients without IBD, patients with CD had a higher prevalence of norovirus (17.4% vs 24.6%, $p = 0.05$) and *Campylobacter* (7.6% vs 13.1%, $p = 0.043$), and a lower prevalence of parasites (6.1% vs 0.8%, $p = 0.01$, Table 2). Among patients with a positive test result, repeat positive testing was more common in patients with CD (20.7% vs 12.4%, $p = 0.041$). Patients with UC had a lower prevalence of parasites compared to patients without IBD (0.9% vs 6.1%, $p = 0.014$) and viruses (16.5% vs 31.9%, $p = 0.007$), specifically norovirus (7.8% vs 17.4%, $p = 0.019$), and a higher prevalence of bacteria (82.6% vs 62.1%, $p < 0.001$), specifically *Campylobacter* (13.9% vs 7.6%, $p = 0.013$), *Plesiomonas* (2.6% vs 0.7%, $p = 0.049$), and *E. coli* species (64.3% vs 47.6%, $p < 0.001$), including enteroaggregative *E. coli* (EAEC; 20.9% vs 13.5%, $p = 0.028$), and enteropathogenic *E. coli* (EPEC; 33.9% vs 22.1%, $p = 0.004$). Patients with UC had a lower prevalence of viruses compared to patients with CD (16.5% vs 32.8%, $p = 0.004$), specifically norovirus (7.8% vs 24.6%, $p < 0.001$). After adjusting for age, sex, race, ethnicity, and season, patients with CD had a higher odds of norovirus and lower odds of parasites; patients with UC had a lower odds of viruses, specifically norovirus, and parasites, and a higher odds of bacteria, specifically *Campylobacter*, and *E. coli* species (Supplementary Table **1**).

Of 77 patients who underwent 22 upper endoscopies, 2 ileoscopies, 61 colonoscopies, and 13 flexible sigmoidoscopies within 30 days of a gastrointestinal pathogen PCR panel, 26 (33.8%) tested positive for 33 enteric pathogens. *E. coli* species (14, 42.4%) and Norovirus (5, 15.2%; Supplementary Table **2**) were the most common pathogens detected. Patients with and without an enteric infection who underwent endoscopic evaluation were similar in age ($p = 0.601$), sex ($p = 0.084$), and IBD subtype ($p = 0.806$; Table 3). Endoscopic findings of esophagitis were more common in patients with an infection (44.4% vs 7.7%, $p = 0.043$), but there were no other endoscopic predictors of a positive test including gastropathy ($p = 0.251$), duodenopathy ($p = 0.595$), ileitis ($p = 0.43$), colitis ($p = 0.58$), the distribution of colitis ($p = 0.585$), the severity of colitis ($p = 0.273$), the presence of colonic ulcers ($p = 0.693$), or the focality of colitis ($p = 0.693$; Table 4). In patients who underwent biopsy, no histologic finding, including esophagitis ($p = 0.795$), gastritis ($p = 0.106$), duodenitis ($p = 0.142$), ileitis ($p = 0.998$), colitis ($p = 0.082$), chronicity ($p = 0.21$), architectural distortion ($p = 0.65$), or granulomata ($p = 0.46$) were associated with a positive pathogen test result. These findings did not differ between viruses and bacteria.

Of 577 patients with IBD, 117 tested positive on the first stool test. Of 87 who tested positive for a bacteria, 46 (52.9%) received antimicrobial therapy. IBD patients with a negative gastrointestinal pathogen PCR panel were more likely to have IBD medications added or up-titrated (50.0% vs 18.6%), whereas patients with a positive gastrointestinal pathogen panel were more likely to have IBD medications held or no changes to their IBD management (81.3% vs 50.0%; $p = 0.001$).

During a median follow-up of 10.5 months (range, 0–36.3) after resolution of the initial flare, there were no differences in time to IBD-related hospitalization (log-rank, 0.268), emergency department visit (log-rank, 0.777), steroid prescription (log-rank, 0.968), other IBD therapy escalation (log-rank, 0.221), complication of IBD (log-rank, 0.765), surgery (log-rank, 0.575), or a composite outcome including the above variables between 117 IBD patients with and 460 IBD patients without an enteric infection (log-rank,

Table 1 Characteristics of 9403 inpatients and outpatients who underwent 13,231 stool tests with a gastrointestinal pathogen PCR panel during an episode of diarrhea

	No inflammatory bowel disease ($n=8826$)			Crohn's disease ($n=277$)				Ulcerative colitis ($n=300$)				
	Total	PCR negative	PCR positive	Total	PCR negative	PCR positive	*p* value[a]	Total	PCR negative	PCR positive	*p* value[b]	*p* value[c]
Number of stool tests	12,275	9011 (73.4%)	3263 (26.6%)	454	372 (81.9%)	82 (18.1%)	0.001	503	422 (83.9%)	81 (16.1%)	0.001	0.498
Mean age at test (years)	42.9	46.3	33.7	37.7	37.8	37		47.1	47.3	45.9		
Median age at test (range)	47.3 (0–102)	51.7 (0–102)	30.6 (0–100)	31.6 (3–92)	31.3 (3–92)	33 (3–81)	0.045	49.7 (2–93)	50.1 (2–93)	49.6 (8–90)	0.001	0.001
Age group												
<18	2983	1761 (59%)	1222 (41%)	97	79 (81.4%)	18 (18.6%)		57	49 (86%)	8 (14%)		
18–29	1319	925 (70.1%)	394 (29.9%)	110	96 (87.3%)	14 (12.7%)		87	72 (82.8%)	15 (17.2%)		
30–49	2180	1613 (74%)	567 (26%)	197	82 (76.6%)	25 (23.4%)		108	90 (83.3%)	18 (16.7%)		
50–69	3660	2958 (80.8%)	702 (19.2%)	98	78 (79.6%)	20 (20.4%)		165	135 (81.8%)	30 (18.2%)		
>70	2132	1754 (82.3%)	378 (17.7%)	42	37 (88.1%)	5 (11.9%)	0.002	86	76 (88.4%)	10 (11.6%)	0.001	0.251
Place of test												
Outpatient	3549	2591 (73%)	958 (27%)	148	118 (79.7%)	30 (20.3%)		174	145 (83.3%)	29 (15.7%)		
Inpatient	7242	5287 (73%)	1955 (27%)	271	229 (84.5%)	42 (15.5%)		274	231 (84.3%)	43 (15.7%)		
Emergency room	1336	1019 (76.3%)	317 (23.7%)	30	21 (70%)	9 (30%)		50	41 (82%)	9 (18%)		
Endoscopy unit	147	114 (77.6%)	33 (22.4%)	5	4 (80%)	1 (20%)	0.459	5	5 (100%)	0	0.439	0.359
Season												
Summer	2783	1963 (70.5%)	820 (29.5%)	104	87 (83.7%)	17 (16.3%)		146	119 (81.5%)	27 (18.5%)		
Spring	3545	2674 (75.4%)	817 (24.6%)	124	100 (80.6%)	24 (19.4%)		132	109 (82.6%)	23 (17.4%)		
Fall	2771	2093 (75.5%)	678 (24.5%)	122	95 (77.9%)	27 (22.1%)		115	96 (83.5%)	19 (16.5%)		
Winter	3175	2281 (71.8%)	894 (28.2%)	104	90 (86.5%)	14 (13.5%)	0.362	110	98 (89.1%)	12 (10.9%)	0.392	0.177
Sex												
Male	6008	4301 (71.6%)	1707 (28.4%)	232	188 (81%)	44 (19%)		214	173 (80.8%)	41 (19.2%)		
Female	6266	4710 (75.2%)	1556 (24.8%)	222	185 (82.9%)	38 (17.1%)	0.888	289	249 (86.2%)	40 (13.8%)	0.842	0.119
Race												
Asian/Pacific Islander	566	423 (74.7%)	143 (25.3%)	11	7 (63.6%)	4 (36.4%)		4	3 (75%)	1 (25%)		
Non-Hispanic Black	1327	1050 (79.1%)	277 (20.9%)	40	36 (90%)	4 (10%)		44	40 (90.9%)	4 (9.1%)		
American Indian/Alaskan	20	10 (50%)	10 (50%)	0	0	0		0	0	0		
Other/unknown	5884	4092 (69.5%)	1792 (30.5%)	173	135 (78%)	38 (22%)	0.185	223	190 (85.2%)	33 (14.8%)		
Non-Hispanic White	4477	3436 (76.7%)	1041 (23.3%)	230	194 (84.3%)	36 (15.7%)		232	189 (81.5%)	43 (18.5%)	0.002	0.094
Hispanic ethnicity												
Hispanic	2187	1539 (70.4%)	648 (29.6%)	116	88 (75.9%)	28 (24.1%)		85	64 (75.3%)	21 (24.7%)		
Non-Hispanic	10087	7472 (74.1%)	2615 (25.9%)	338	284 (84%)	54 (16%)	0.002	418	358 (85.6%)	60 (14.4%)	0.227	0.920
Residential zip code												
New York City	7580	5400 (71.2%)	2180 (28.8%)	262	214 (81.7%)	48 (18.3%)		276	229 (83%)	47 (17%)		
Surrounding Tri-State area	4444	3408 (76.7%)	1036 (23.3%)	180	147 (81.7%)	33 (18.3%)		219	186 (84.9%)	33 (15.1%)		
Other	250	203 (81.2%)	47 (18.8%)	12	11 (91.7%)	1 (8.3%)	0.266	8	7 (87.5%)	1 (12.5%)	0.229	0.632

[a]Crohn's disease compared to no inflammatory bowel disease
[b]Ulcerative colitis compared to no inflammatory bowel disease
[c]Crohn's disease compared to ulcerative colitis

Table 2 Distribution of pathogens in patients with Crohn's disease, ulcerative colitis, and without IBD

	No IBD	Crohn's disease	*p* value[a]	Ulcerative colitis	*p* value[b]	*p* value[c]
Total pathogens identified	4431	122		115		
Viruses	1412 (31.9%)	40 (32.8%)	0.844	19 (16.5%)	0.007	0.004
Adenovirus F40/41	106 (2.4%)	2 (1.6%)	0.999	0	0.115	0.499
Astrovirus	113 (2.6%)	1 (0.8%)	0.373	2 (1.7%)	0.999	0.614
Norovirus GI/GII	771 (17.4%)	30 (24.6%)	0.05	9 (7.8%)	0.019	0.001
Rotavirus A	204 (4.6%)	3 (2.5%)	0.279	4 (3.5%)	0.584	0.716
Sapovirus (I, II, IV, V)	218 (4.9%)	4 (3.3%)	0.427	4 (3.5%)	0.498	0.999
Bacteria	2750 (62.1%)	81 (66.4%)	0.639	95 (82.6%)	0.001	0.276
Campylobacter species	338 (7.6%)	16 (13.1%)	0.043	16 (13.9%)	0.013	0.888
Plesiomonas shigelloides	30 (0.7%)	0	0.999	3 (2.6%)	0.049	0.117
Salmonella species	166 (3.7%)	5 (4.1%)	0.805	0	0.022	0.061
Yersinia enterocolitica	93 (2.1%)	1 (0.8%)	0.520	2 (1.7%)	0.999	0.614
Vibrio species	12 (0.3%)	1 (0.8%)	0.298	0	0.999	0.999
Vibrio cholerae	4 (0.1%)	1 (0.8%)	0.127	0	0.999	0.999
Escherichia coli species	2107 (47.6%)	57 (46.7%)	0.920	74 (64.3%)	0.001	0.157
Enteroaggregative *E. coli* (EAEC)	598 (13.5%)	17 (13.9%)	0.920	24 (20.9%)	0.028	0.524
Enteropathogenic *E. coli* (EPEC)	981 (22.1%)	28 (23%)	0.862	39 (33.9%)	0.004	0.310
Enterotoxigenic *E. coli* (LT/ST)	186 (4.2%)	4 (3.3%)	0.632	4 (3.5%)	0.999	0.999
Shiga-like toxin-producing *E. coli* STX/ST2	147 (3.3%)	3 (2.5%)	0.799	4 (3.5%)	0.797	0.716
E. coli 0157	24 (0.5%)	1 (0.8%)	0.494	0	0.999	0.999
Shigella/enteroinvasive *E. coli* (EIEC)	171 (3.9%)	4 (3.3%)	0.999	3 (2.6%)	0.803	0.999
Parasites	269 (6.1%)	1 (0.8%)	0.010	1 (0.9%)	0.014	0.999
Cryptosporidium	107 (2.4%)	0	0.119	0	0.115	—
Cyclospora cayetanesis	18 (0.4%)	0	0.999	0	0.999	—
Entamoeba histolytica	2 (0.1%)	0	0.999	0	0.999	—
Giardia lamblia	142 (3.2%)	1 (0.8%)	0.594	1 (0.9%)	0.269	0.999
Multiple pathogens	871/3263 (26.7%)	29/82 (35.4%)	0.100	23/81 (28.4%)	0.791	0.527

[a]Crohn's disease compared to no inflammatory bowel disease
[b]Ulcerative colitis compared to no inflammatory bowel disease
[c]Crohn's disease compared to ulcerative colitis

0.224; Fig. 1). In exploratory analyses, of 87 patients who tested positive for a bacteria, those who received antimicrobial therapy (46, 52.9%) were equally as likely to remain in remission during the follow-up period compared to patients who did not receive antimicrobial therapy (41, 47.1%; log-rank, 0.442; Supplementary Figure **1**).

DISCUSSION

In this cross-sectional analysis of patients who underwent a gastrointestinal pathogen panel PCR test, non-*C. difficile* enteric infection was detected in 18.1%, 16.1%, and 26.8% of tests in patients with CD, UC, and without IBD, respectively. Endoscopic and histologic findings did not differentiate flare of IBD from enteric infection. This study confirms previously reported pilot data demonstrating that enteric infection was common in symptomatic patients with IBD, understandably lower than in symptomatic patients without IBD [7, 24]. Our findings, utilizing a multiplex PCR panel, differ significantly from recent data suggesting a much lower rate of non-*C. difficile* bacterial infection using limited and heterogeneous diagnostic methods, ranging from 0.88% in patients with CD to 2.5% in patients with UC [25].

In the present study, there were significant differences in the distribution of infections between symptomatic patients with CD, UC, and without IBD. In CD, *norovirus* and *Campylobacter*

Table 3 Characteristics of 77 patients with IBD who underwent GI PCR stool testing and endoscopy

	Negative gastrointestinal pathogen PCR test $n=51$	Positive gastrointestinal pathogen PCR test $n=26$	*p* value
IBD subtype			
Crohn's disease	25 (49%)	12 (46.2%)	
Ulcerative colitis	26 (51%)	14 (53.8%)	0.806
IBD phenotype			
Isolated ileal/upper GI only	3 (5.9%)	4 (15.4%)	
Any colonic involvement	48 (94.1%)	22 (84.6%)	0.170
Gender			
Male	29 (56.9%)	20 (76.9%)	
Female	22 (43.1%)	6 (23.1%)	0.084
Race/ethnicity			
Caucasian	29 (56.9%)	12 (46.2%)	
Hispanic	15 (29.4%)	10 (38.5%)	
Black	5 (9.%)	1 (3.8%)	
Asian	0	1 (3.8%)	
Other/unknown	2 (3.9%)	2 (7.7%)	0.403
Average duration of IBD at test (years)	6.8±8.7	6.7±8.2	0.960
Average age at test (years)	38.7±21.8	36.2±17.1	0.601
Previous *Clostridium difficile* infection	3 (5.9%)	4 (15.4%)	0.170
Previous partial or total colectomy	8 (15.7%)	3 (11.5%)	0.623
Antibiotic exposure within 90 days	7 (13.7%)	7 (26.9%)	0.156
Medications at testing			
5-ASA	16 (31.4%)	8 (30.8%)	0.957
Corticosteroids	9 (17.6%)	9 (34.6%)	0.096
Immunomodulators	5 (9.8%)	3 (11.5%)	0.814
Biologics	8 (15.7%)	3 (11.5%)	0.623
Immunomodulator with biologic	2 (3.9%)	1 (3.8%)	0.987
Proton pump inhibitor	6 (11.8%)	4 (15.4%)	0.655
Antibiotics	5 (9.8%)	3 (11.5%)	0.814
Inflammatory markers at testing			
Elevated ESR or C-RP	41 (80.4%)	18 (81.8%)	0.887
Median ESR	35	43	0.480
Median C-RP	25.9	29.2	0.223
CT or MR imaging at testing			
No active inflammation	4/28 (14.3%)	4/15 (26.7%)	
Active inflammation	24/28 (85.7%)	11/15 (73.3%)	0.320

were more common, whereas in UC, bacteria were more common overall, including *Campylobacter*, *Plesiomonas*, and *E. coli* species. These findings suggest that viruses, such as norovirus, may play an important role in modulating the mucosal immune response in patients with CD while bacterial pathogens, such as *E. coli* species, may play an important role in modulating the mucosal immune response in patients with UC.

There have been several studies examining the potential role of viruses in the pathogenesis of CD [26–30]. In particular, norovirus has been implicated not only in the pathogenesis of IBD, but also in exacerbations of IBD. These findings have been replicated in animal models where norovirus infection in the setting of a polymorphism in the CD susceptibility autophagy gene *ATG16L1* produces CD in mice [27, 31]. Our findings may yield further evidence for the role of the enteric virome in flares of CD, although the significance of the decreased prevalence of viruses in UC remains unclear. The ability of norovirus infection to induce TH1 immune responses provides a potential explanation for the selective association with CD, which typically displays a stronger TH1 signature compared with UC [31–34]. Norovirus tropism for particular regions of the gastrointestinal tract may also be a contributing factor [35]. In addition, data regarding *E. coli* infection in patients with UC is limited. We are the first to report an increased prevalence of *E. coli* species EPEC and EAEC in UC compared to patients without IBD.

As we and other groups have previously reported, enteric infection testing significantly impacts IBD management such that patients with an enteric infection are less likely to have IBD therapies added or escalated [7, 25]. However, few studies have examined the clinical significance of enteric infection in patients with IBD and none have evaluated the impact of targeted antimicrobial therapy. Previous studies have demonstrated worse outcomes in patients with IBD flare complicated by *Campylobacter* and *Salmonella* [36, 37]. Conversely, more recent data have suggested that patients with non-*C. difficile* infection are more likely to remain in remission within 1 year compared to those with *C. difficile* or non-infectious flare [25].

In an effort to evaluate the clinical significance of enteric infection in IBD, we examined data from a subset of patients who underwent endoscopy near PCR testing. Clinical, endoscopic, and histologic findings did not differentiate flare of IBD from enteric infection. Although our sample was small and non-random, these data may be consistent with existing endoscopic literature on *C. difficile* in patients with IBD, where studies have shown the rate of endoscopic pseudomembranes to be low and not accounted for by the use of immunosuppression [38]. Our results suggest that infection and non-infectious flare may elicit similar clinical, endoscopic, and histologic findings, and PCR stool testing may identify potential pathogens.

In addition to endoscopic and histologic findings, we found no difference in relevant long-term IBD outcomes after initial symptom resolution between patients with and without enteric infection, including hospitalizations, emergency department visits, steroids prescriptions, other IBD therapy escalations, complications, and surgical interventions. In exploratory analyses, our

Table 4 Endoscopic and histologic findings in 77 patients with IBD who underwent GI PCR stool testing and endoscopy

	Negative gastrointestinal pathogen PCR test $n=51$	Positive gastrointestinal pathogen PCR test $n=26$	*p* value
Endoscopic procedures			
Upper endoscopy	13 (25.5%)	9 (34.6%)	
Ileoscopy	1 (2%)	1 (3.8%)	
Colonoscopy	44 (86.3%)	17 (65.4%)	
Flexible sigmoidoscopy	6 (11.8%)	7 (26.9%)	0.153
Median number of days from GI PCR to endoscopy (range)	2.5 (0–23)	5.9 (0–29)	0.745
Upper GI tract			
Endoscopic findings	13	9	
Esophagitis	1/13 (7.7%)	4/9 (44.4%)	0.043
Gastropathy	7/13 (53.8%)	7/9 (77.8%)	0.251
Cardia/fundus	0	0	
Body	1/7 (14.3%)	0	
Antrum	2/7 (28.6%)	4/7 (57.1%)	
Entire stomach	4/7 (57.1%)	3/7 (42.9%)	0.364
Duodenopathy	3/13 (23.1%)	3/9 (33.3%)	0.595
Upper GI tract			
Histologic finding	11	9	
Esophagitis	3/11 (50.3%)	2/9 (22.2%)	0.795
Gastritis	9/11 (81.8%)	5/9 (55.6%)	0.106
Chronic active gastritis with HP	0	2/5 (40%)	
Chronic active gastritis	2/9 (22.2%)	0	
Chronic inactive gastritis	7/9 (77.8%)	3/5 (60%)	
Duodenitis	6/11 (54.5%)	2/9 (22.2%)	0.142
Lower GI tract			
Endoscopic findings	51	25	
Ileitis	11/51 (21.6%)	9/25 (36%)	0.431
Colitis	45/51 (88.2%)	24/25 (96%)	0.580
Location			0.585
Pancolitis	16/45 (35.6%)	9/24 (37.5%)	
Right-sided colitis	8/45 (17.8%)	7/24 (29.2%)	
Left-sided colitis	21/45 (46.7%)	8/24 (33.3%)	
Severity			0.273
Mild	9/45 (20%)	6/24 (25%)	
Moderate	18/45 (40%)	13/24 (54.2%)	
Severe	18/45 (40%)	5/24 (20.8%)	
Ulcer	21/45 (46.7%)	14/24 (58.3%)	0.508
Focality			0.693
Focal	17/45 (37.8%)	11/24 (45.8%)	
Diffuse	28/45 (62.2%)	13/24 (54.2%)	
Lower GI tract			
Histologic findings			
Ileitis	11/11 (100%)	9/9 (100%)	0.998
Colitis	46/47 (97.9%)	22/25 (88%)	0.082
Chronicity			0.211
Acute	6/46 (13%)	5/22 (22.7%)	
Chronic	29/46 (63%)	14/22 (63.6%)	
Acute and chronic	11/46 (23.9%)	3/22 (13.6%)	
Architecture distortion	21/46 (45.7%)	10/22 (45.5%)	0.646
Granulomas	4/46 (8.7%)	1/22 (4.5%)	0.460

finding of equivalent outcomes in patients with bacterial enteric infection who received antimicrobial therapy suggests that targeting a non-*C. difficile* infectious source may not offer any long-term therapeutic benefit. These data require further study.

There are several limitations to the current study inherent to a retrospective study design. Our analyses do not prove a cause-and-effect relationship between diarrhea, an exacerbation in symptoms of IBD, and enteric infections. Individual patient information concerning precise presenting symptoms, medication exposures, recent travel, sexual behavior, other comorbid conditions, and management after stool testing was not available for full analysis. There may be selection bias in patients who underwent endoscopic evaluation. In addition, thresholds for testing in specific populations may influence rates of overall infection; however, we do not believe this would particularly influence the distribution of particular infections detected, as described in this manuscript. The PCR test became available to clinicians in 2015 and as such, follow-up time was limited. Although the patient population was ethnically and geographically diverse, the majority of patients resided in the Northeast United States. In addition, PCR testing fails to discriminate between active infection and asymptomatic colonization, and there is considerable uncertainty regarding clinical interpretation and cost-effectiveness of such multiplex assays [39]. However, as this is a symptomatic cohort, the probability of asymptomatic carriage is likely low. The FilmArray gastrointestinal pathogen PCR panel does not assess for the presence of Cytomegalovirus (CMV), a pathogen of increasing importance in IBD, and given testing constraints in our institution, we were unable to analyze *C. difficile* infection. Moreover, endoscopy and biopsy were performed in a non-random manner, and other factors may have influenced decision-making in these procedures.

In summary, enteric infection was common, identified in 17% of symptomatic patients with IBD, and the distribution of pathogens

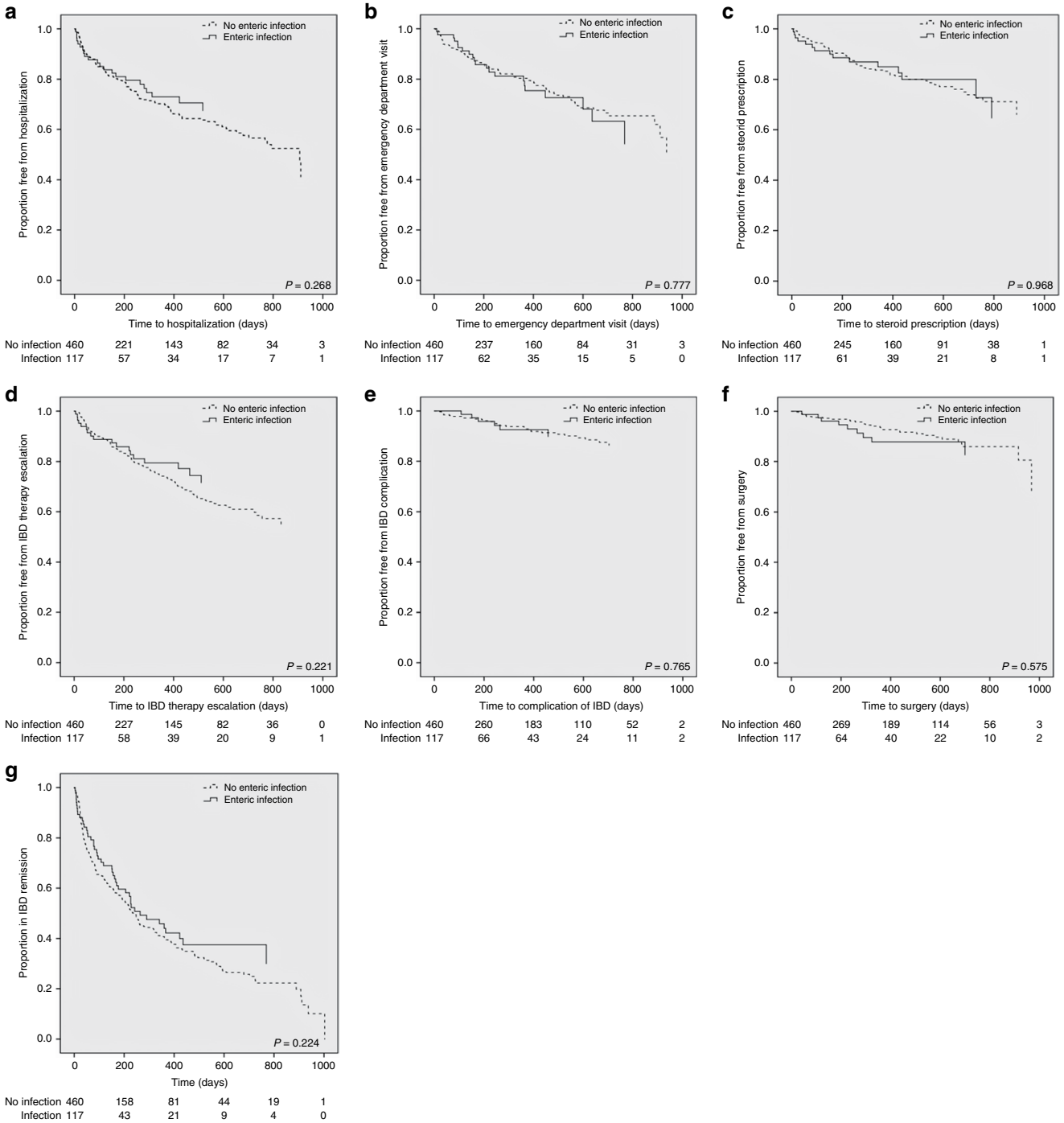

Fig. 1 Time to IBD-related outcomes after resolution of the initial flare between patients with and without enteric infection including time to hospitalization (**a** log-rank 0.268), emergency department visit (**b** log-rank 0.777), steroid prescription (**c** log-rank 0.968), other IBD therapy escalation (**d** log-rank 0.221), complication of IBD (**e** log-rank 0.765), surgery (**f** log-rank 0.575), or a composite outcome of all end points (**g** log-rank 0.224)

differed significantly from patients without IBD. Although broad PCR testing impacted IBD management, endoscopy and histology did not differentiate flare from infection. As such, PCR testing should be considered as a diagnostic step in patients with an apparent relapse of IBD. The identification and treatment of an enteric infection did not appear to impact long-term IBD outcomes, however, more robust data is lacking regarding the clinical relevance and outcomes of specific non-*C. difficile* enteric infection in patients with IBD. Further study is required to investigate the interaction between specific enteric infections, mucosal immunity, and the phenotypic presentations of IBD. In addition, further study is required to evaluate the impact of various enteric infections on the course of IBD, and the impact GI PCR testing on treatment strategies and outcomes.

CONFLICT OF INTEREST

Guarantor of the article: Benjamin Lebwohl.

Specific author contributions: JEA: Study concept and design; acquisition of data; analysis and interpretation of data; drafting of the manuscript; and statistical analysis. AJ: Study concept and design; and acquisition of data. PHRG: Study concept and design; drafting of the manuscript; and critical revision of the manuscript for important intellectual content. GL: Critical revision of the manuscript for important intellectual content. SL: Critical revision of the manuscript for important intellectual content. KC: Critical revision of the manuscript for important intellectual content. BL: Study concept and design; drafting of the manuscript; and critical revision of the manuscript for important intellectual content.

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Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Enteric infection is frequently identified in patients with IBD.
- ✓ Patients with IBD and an enteric infection are less likely to have IBD therapies added or escalated.
- ✓ Little is known regarding the distribution and clinical sequela of non-*C. difficile* enteric infections in IBD.

WHAT IS NEW HERE

- ✓ Non-*C. difficile* enteric infection was detected in 18.1%, 16.1%, and 26.8% of tests in patients with CD, UC, and without IBD, respectively.
- ✓ Endoscopic and histologic findings did not differentiate flare of IBD from enteric infection.
- ✓ In CD, *norovirus* and *Campylobacter* were more common, whereas in UC, bacteria were more common overall.
- ✓ The identification and treatment of an enteric infection did not impact long-term IBD outcomes.

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