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MAJOR ARTICLE

Syndromic Panel Testing Among Patients With Infectious Diarrhea: The Challenge of Interpreting *Clostridioides difficile* Positivity on a Multiplex Molecular Panel

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Background. Including *Clostridioides difficile* (CD) in gastrointestinal multiplex molecular panels (GIPCR) presents a diagnostic challenge. Incidental detection by polymerase chain reaction (PCR) without consideration of pretest probability (PTP) may inadvertently delay diagnoses of other treatable causes of diarrhea and lead to prescription of unnecessary antibiotics.

Methods. We conducted a retrospective study to determine the frequency at which clinicians characterize PTP and disease severity in adult patients who test positive for CD by GIPCR. We organized subjects into cohorts based on the status of their CD PCR, glutamate dehydrogenase enzyme immunoassay (GDH), and toxin A/B detection, as well as by high, moderate, or low CD PTP. We used multivariable regression models to describe predictors of toxin positivity.

Results. We identified 483 patients with positive CD PCR targets. Only 22% were positive for both GDH and CD toxin. Among patients with a low PTP for CDI, 11% demonstrated a positive CD toxin result compared to 63% of patients with a high PTP. A low clinician PTP for CD infection (CDI) correlated with a negative CD toxin result compared to cases of moderate-to-high PTP for CDI (odds ratio, 0.19 [95% confidence interval, .10–.36]). Up to 64% of patients with negative GDH and CD toxin received CD treatment. Only receipt of prior antibiotics, fever, and a moderate-to-high clinician PTP were statistically significant predictors of toxin positivity.

Conclusions. Patients with a positive CD PCR were likely to receive treatment regardless of PTP or CD toxin results. We recommend that CD positivity on GIPCR be interpreted with caution, particularly in the setting of a low PTP.

Keywords. *Clostridioides difficile* infection; molecular panel; pretest probability.

Advances in molecular diagnostics have improved our ability to detect gastrointestinal (GI) pathogens such as *Clostridioides difficile* (CD) rapidly in a culture-independent manner by nucleic acid amplification testing (NAAT), such as polymerase chain reaction (PCR). Test configurations range from detection of single pathogens to large, multiplex panels that detect >20 pathogens. Such large panels are designed to detect numerous GI pathogens, which include common community-acquired bacteria and viruses, travel-associated bacteria, nosocomial targets such as CD, and uncommonly identified protozoal organisms. All panel pathogens could potentially trigger GI symptoms; however, disease-specific risk factors vary, making certain pathogens less likely based on the patient's clinical syndrome. While multiplex gastrointestinal PCR (GIPCR) panels allow for rapid, simultaneous identification of multiple GI organisms, clinicians are unable to tailor the organism selection based on their clinical suspicion. Panel use leads to routine testing of both common and uncommon organisms irrespective of patient-specific exposures. Additionally, the need for treatment varies as the majority of diarrheal illnesses resolve with supportive care alone, whereas some cases may benefit from antimicrobial treatment.

Clostridioides difficile infections (CDIs) are among the most common nosocomial infections identified within the United States [1–4]. After CD acquisition, patients may develop CD colonization with manifestations ranging from no symptoms to diarrhea from another cause [3, 5, 6]. Subsequent exposure to antibiotics can lead to disruption of the GI microbiome, leading to loss of colonization tolerance and symptomatic disease with manifestations ranging from mild diarrhea to fulminant colitis [7].

The optimal diagnostic test for CD remains uncertain and each available test method has advantages and disadvantages

Received 23 August 2022; editorial decision 31 April 2023; accepted 06 April 2023; published online 10 April 2023

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https://doi.org/10.1093/ofid/ofad184

[1, 3, 4]. Cell culture cytotoxicity neutralization assay and toxigenic culture are sensitive tests considered to be the gold standard tests but are labor intensive and time consuming, limiting clinical utility. Toxin A&B enzyme immunoassay is rapid and specific but has poor sensitivity, whereas glutamate dehydrogenase enzyme immunoassay (GDH) is rapid and sensitive but is not specific for toxin-producing CD infections.

To tackle the challenge of CD diagnosis, healthcare systems commonly implement a multistep algorithm, which includes CD toxin testing plus stool tests for GDH and/or NAAT. NAAT for CD toxin genes is highly sensitive for organism detection but cannot differentiate colonization from CD disease [1, 4], yet it has been adopted as a standalone strategy in many clinical laboratories. The Infectious Diseases Society of America (IDSA) guidelines for CD diagnosis among patients with consistent symptoms state that either NAAT alone or a multistep algorithm may be used to diagnose CDI [8]. NAAT alone is only admissible in the setting of a preagreed institutional criteria which specifies that patients have unexplained new onset frequency of ≥3 unformed stools within 24 hours and without known laxative use [8].

Given that GI molecular panels bypass CD toxin testing and report CD results of unclear clinical significance, we wish to determine how clinicians use GIPCR panels in the diagnosis of CDI, to what degree CDI pretest probability (PTP) is documented prior to GIPCR interpretation, and what additional clinical variables predict CD toxin positivity among patients with CD positivity by NAAT. We hypothesize that the use of GIPCR without adequate consideration of CDI PTP leads to overdiagnosis and overtreatment of CDI.

MATERIALS AND METHODS

We conducted a retrospective study of adult patients who tested positive for CD on a multiplex molecular panel (GIPCR, BioFire) at the central laboratory of an integrated healthcare network from 1 June 2017 to 15 December 2018. Inclusion criteria consisted of patients ≥18 years of age with a positive GIPCR target for CD. We excluded patients <18 years of age, patients without clinical documentation in our electronic medical records system, and patients who were immunocompromised as defined by active chemotherapy, solid organ or stem cell transplant, or diagnosis of inflammatory bowel disease. We also excluded subsequent GIPCR tests occurring within a single encounter.

Patient Consent Statement

This project was reviewed and approved by the Intermountain institutional review board. A waiver of informed consent was granted as our retrospective study did not include factors necessitating patient consent.

Setting

Intermountain Healthcare is an integrated health system with 24 acute care hospitals, 36 urgent care clinics during the study period, and a network of primary care clinics. Standard testing for CD is with GDH and toxin A/B by enzyme immunoassay (Quik Chek Complete; TechLab) performed at hospital-based laboratories. Discordant results are reflexed to toxin B detection by reverse-transcription PCR (Xpert *C. difficile*, Cepheid), which is performed at the Intermountain central laboratory. Standalone testing for CD by PCR is not permitted, and formed stools (Bristol stool types 1–5) are rejected. GIPCR is a standalone order, with use for hospital-onset diarrhea discouraged. Both repeat testing for CD by standard method and repeat GIPCR within 7 days of an initial test are prohibited. Since the inception of GIPCR use in the central laboratory, samples positive for CD are backwards reflexed to GDH/toxin A/B testing with results of the GIPCR CD target delayed until reflex testing has been completed. Specific comments are reported with all CD test results to assist clinicians with test interpretation.

Outcomes

Regarding laboratory test results, we identified the number (%) of GIPCR tests positive for CD targets and the number (%) of CD-positive PCR tests confirmed by GDH and toxin testing. Our primary clinical outcome was CD toxin positivity. We collected data regarding the following variables: gastroenteritis severity and characteristics, antibiotic exposure within 3 months prior to GIPCR testing, whether an alternate explanation for diarrhea was present, whether the positive CD test was an index or recurrent episode, treatment based on positive CD result, hospital admission, and identification of additional GI pathogens.

Data Collection

We collected cohort data by querying our electronic data warehouse for laboratory, administrative, and demographic details. Additional information was collected through manual chart review. We used RedCap to record data for each chart. We arranged patients into the following cohorts: (1) CD target positive on GIPCR, GDH positive, CD toxin positive; (2) CD target positive on GIPCR, GDH positive, CD toxin negative; (3) CD target positive on GIPCR, GDH negative, CD toxin negative; and (4) CD target positive on GIPCR, no reflex run.

We defined patients with CDI as those who had both positive PCR and toxin tests. Patients with a positive CD PCR, negative GDH, and negative toxin were considered to represent CD colonization or a PCR false positive. Cases of positive CD PCR,

positive GDH, and negative toxin were listed as unclear regarding interpretation.

We subsequently reviewed patient records to describe CD PTP at the time GIPCR was ordered. We classified PTP as follows:

1. Not done: clinician did not document clinical decision making regarding CDI.
2. Low: Clinician mentioned CD in assessment and documented low clinical suspicion.
3. Moderate: Clinician discussed risk for CD in the assessment along with suspicion of disease but did not initiate empiric antibiotic therapy.
4. High: Clinician described antibiotic exposure in the admission documentation, documented suspicion for CDI, and initiated empiric antibiotic therapy.

We classified gastroenteritis severity based on the American College of Gastroenterology 2016 gastroenteritis guidelines, which define mild disease as disease without impact on patient daily activities, moderate disease as requiring adjustment in patient daily activities, and severe disease as leading to complete disability [9]. We also documented stool frequency if recorded and exposure to systemic antibiotics within 90 days prior to testing.

Statistical Analysis

Using our clinical and laboratory variables, we constructed univariate and multivariable regression models to determine which variables would predict CD toxin positivity. We completed statistical analysis using Stata. Variables included white blood cell count >15 x 10^9/L, antibiotics in the 3 months prior to evaluation, fever, abdominal pain, tachycardia, hypotension, nausea/vomiting, >1 GIPCR target positivity, clinician PTP, and GIPCR testing location. Only univariate variables with $P = \leq.2$ were included in multivariable analysis.

RESULTS

Between the testing period of June 2017 to December 2018, we identified 1326 patients with a positive bacterial or protozoal GIPCR target that included the following pathogens: CD, *Campylobacter* species, *Salmonella* species, *Shigella*, *Escherichia coli* O157, *Plesiomonas shigelloides*, *Yersinia enterocolitica*, *Vibrio* species, *Cryptosporidium* species, *Cyclospora cayetanensis*, *Entamoeba histolytica*, or *Giardia lamblia*. We excluded 547 GIPCR tests due to lack of patient documentation, immunocompromised status, or repeat GIPCR tests within a single encounter. Among the 779 remaining patients (Supplementary Table 1), 483 patients had a positive CD target; the 296 patients with negative CD targets were excluded (Figure 1).

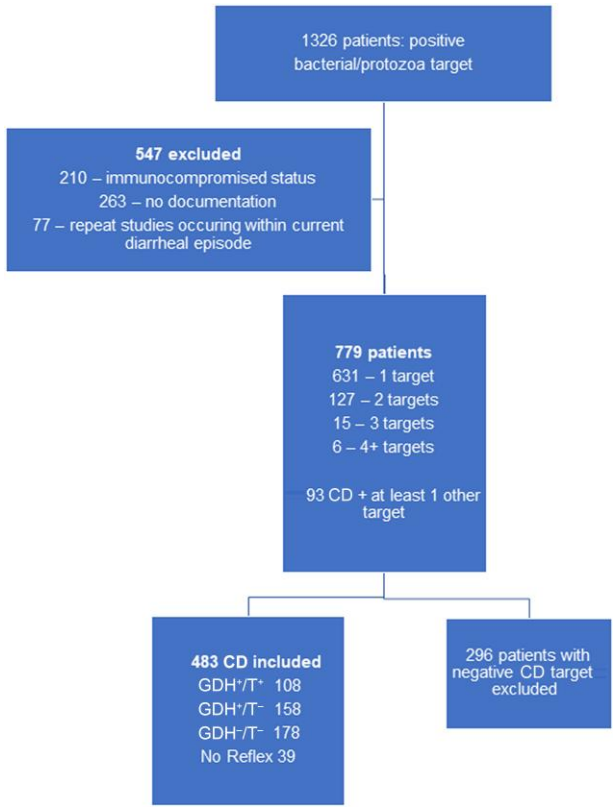

Figure 1. Flowchart outlining creation of patient cohorts. Excluded cases may fall under more than one category for exclusion. Abbreviations: CD, *Clostridioides difficile*; GDH^+/T^+, glutamate dehydrogenase positive/toxin positive; GDH^+/T^-, glutamate dehydrogenase positive/toxin negative; GDH^-/T^-, glutamate dehydrogenase negative/toxin negative.

Collection of GIPCR samples occurred within a variety of clinical settings (Supplementary Table 2). More than half of CD PCR-positive patients (51%) were evaluated in the emergency room or urgent care. Another 32% of patients were seen in the inpatient setting. Only 17% of patients were tested in clinic or evaluated over the phone.

We grouped patients into cohorts based on results of subsequent CD testing (Figure 1). Among the 483 patients with positive CD PCR targets, 444 cases were reflexed to GDH/toxin testing. Cohort 1 included 108 cases (22%) with both GDH and toxin positivity (G^+/T^+). Cohort 2 included 158 cases (33%) with a negative toxin and positive GDH test (G^+/T^-). Cohort 3 included 178 cases (37%) with both negative GDH and toxin results (G^-/T^-). Of the 444 cases with reflex CD testing, 336 cases (76%) were CD toxin negative.

Documentation of disease-specific details varied among all included patients. In >95% of cases, disease severity was not documented. Clinicians did not document frequency of diarrhea in 279 (58%) cases. When documented, 11% of tested patients with CD PCR positivity had <3 diarrheal episodes per day (Table 1). We found that 218 cases had antibiotic exposures

Table 1. Clinical and Demographic Variables

Characteristic	GDH^+/T^+ (n = 108)	GDH^+/T^- (n = 158)	GDH^-/T^- (n = 178)
Age, y, mean (SD)	60.9 (19.0)	56.1 (18.9)	58.6 (21.4)
Alternative explanation for diarrhea present	3 (2.8)	16 (10.1)	20 (11.3)
Index positive test	90 (84)	94 (81.7)	63 (70.8)
WBC count, x 10^9/L, mean (SD)	13.7 (7.5)	10.8 (6.7)	10.1 (4.2)
Temperature, °C, mean (range)	37.1 (35.4–39.7)	36.9 (35.1–39.7)	36.9 (34.9–39.7)
Fever	26 (24)	15 (9.5)	18/167 (11)
Hypotension	5 (5)	5 (3.2)	5 (3)
Abdominal pain	65 (61)	85 (54)	106 (60)
Nausea/vomiting	27 (25)	49 (31)	67 (38)
Tachycardia	57 (54)	66 (42)	70 (41)
Test indication documented	71 (67)	79 (51)	83 (47)
No. of stools			
Not characterized	64 (59)	88 (56)	105 (59)
<3	5 (5)	20 (13)	22 (12)
3–5	13 (12)	25 (16)	26 (15)
6–10	12 (11)	14 (9)	12 (7)
>10	14 (13)	11 (7)	12 (7)
Antibiotic within 90 d	67 (62)	73 (46)	58 (33)
>1 positive target	13 (12)	30 (19)	42 (24)
Disease severity documented	8 (7)	9 (6)	7 (4)
Admission			
No	65 (69)	96 (84)	117 (80)
Yes—non-ICU	29 (31)	16 (14)	27 (18)
Yes—ICU	0	2 (2)	2 (1)

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: GDH^+/T^+, glutamate dehydrogenase positive/toxin positive; GDH^+/T^-, glutamate dehydrogenase positive/toxin negative; GDH^-/T^-, glutamate dehydrogenase negative/toxin negative; ICU, intensive care unit; SD, standard deviation; WBC, white blood cell.

Table 2. *Clostridioides difficile* Infection Treatment Patterns Among Patients Who Tested Positive for Both *C difficile* and Another Bacterial or Viral Organism on Gastrointestinal Multiplex Molecular Panel

Interventions	G^+/T^+ and Second Positive Target	G^+/T^- and Second Positive Target	G^-/T^- and Second Positive Target
Additional bacterial target(s) positive	n = 7	n = 12	n = 20
No treatment	0	1 (8)	2 (10)
Treat CDI and additional bacteria	1 (14)	5 (42)	6 (30)
Treat CDI only	6 (86)	5 (42)	8 (40)
Treat bacteria only	0	1 (8)	4 (20)
Additional viral target(s) positive	n = 5	n = 16	n = 23
No treatment	0	2 (12.5)	6 (26)
Treat CDI	5 (100)	14 (87.5)	17 (74)

Data are presented as No. (%).

Abbreviations: GDH^+/T^+, glutamate dehydrogenase positive/toxin positive; GDH^+/T^-, glutamate dehydrogenase positive/toxin negative; GDH^-/T^-, glutamate dehydrogenase negative/toxin negative.

in the 90 days prior to GIPCR testing. The majority of G^+/T^+ cases received antibiotics before testing (62%) compared to only a third (33%) of G^-/T^- cases (Table 1). An alternative explanation for diarrhea at the time of testing was more common in patients who were toxin negative (10.7%) compared to those who were toxin positive (2.8%) (Table 1) with receipt of laxatives within 48 hours of testing as the most common alternative cause (28.2%).

CDI treatment was likely for all cohorts. The G^+/T^+ cohort had a 99% treatment rate and the G^-/T^- cohort had a 64% treatment rate (Supplementary Table 3). Approximately 16% of the G^-/T^- cohort (29/178) had significant diarrhea with >3 episodes prior to GIPCR testing. While toxin positivity is not 100% sensitive for CDI, a negative toxin should prompt consideration of alternative etiology for diarrheal symptoms. To evaluate the likelihood of an alternative diagnosis, we also reviewed the number of cases with additional GIPCR target positivity. Among cases with >1 positive PCR target, 80% (72) were toxin negative, 14% (13) were toxin positive, and 6% (5) had no additional toxin testing. Seventy percent of toxin-negative cases with a second positive bacterial target and 74% of toxin-negative cases with a positive viral target (Table 2) still received treatment for CD.

While the clinical thought process may not always be fully reflected in clinical documentation, we attempted to classify clinician PTP by the reasoning documented during the encounter combined with empiric treatment prior to receiving the test result. Among patients with a low PTP for CDI, 11% demonstrated a positive CD toxin result. In comparison, 63% of patients with a high PTP and 36% of patients with a moderate PTP tested positive for CD toxin (Figure 2). Patients with a low PTP for CD infection had lower odds of toxin positivity compared to patients for whom the CDI PTP was moderate to high (odds ratio [OR], 0.19 [95% confidence interval [CI], .10–.36]).

We used univariate and multivariate regression models to describe predictors of toxin positivity among patients with CD-positive targets on GIPCR. We identified the following statistically significant clinical predictors of toxin positivity: prior antibiotics (OR 2.9 [95% CI, 1.0–4.0]), fever (OR, 3.4 [95% CI, 1.6–7.4]), and moderate clinician PTP (OR, 3.0 [95% CI, 1.4–6.4]) or high clinician PTP (OR, 8.5 [95% CI, 2.3–32.1]) (Table 3).

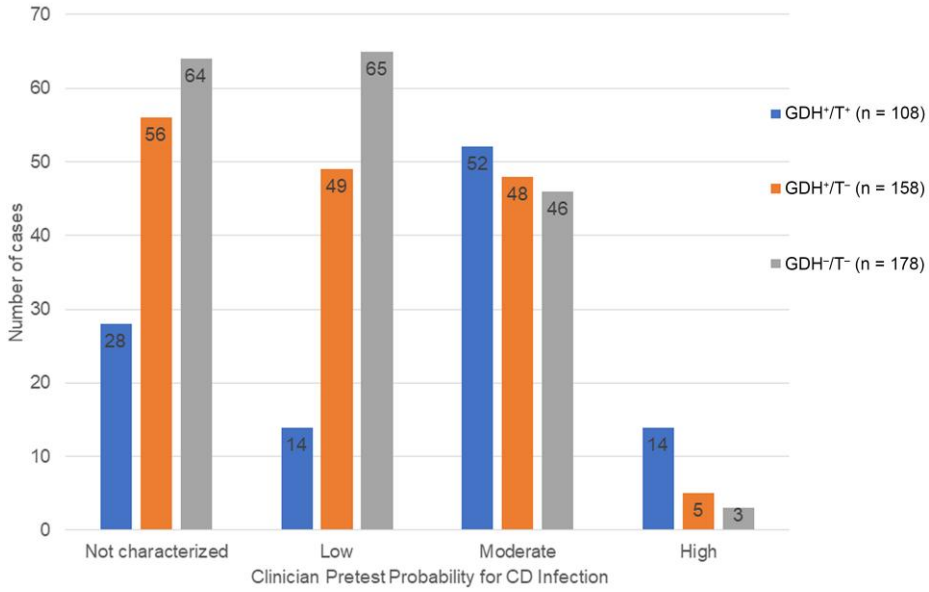

Figure 2. *Clostridioides difficile* cohorts based on glutamate dehydrogenase enzyme immunoassay/toxin results grouped by clinician pretest probability. Abbreviations: CD, *Clostridioides difficile*; CDI, *Clostridioides difficile* infection; GDH^+/T^+, glutamate dehydrogenase positive/toxin positive; GDH^+/T^-, glutamate dehydrogenase positive/toxin negative; GDH^-/T^-, glutamate dehydrogenase negative/toxin negative.

Table 3. Univariate and Multivariate Regression Model Describing Predictors of Toxin Positivity

Predictors for Toxin Positivity	Univariable, OR (95% CI)	*P* Value	Multivariable, OR (95% CI)	*P* Value
WBC count >15 x 10^9/L	2.9 (1.6–5.3)	.001	1.6 (.7–3.4)	.2
Prior antibiotics	2.5 (1.6–3.9)	<.0001	2.0 (1.0–4.0)	**.04**
Fever	2.9 (1.6–5.1)	<.0001	3.4 (1.6–7.4)	**.002**
Abdominal pain	1.2 (.8–1.9)	.4	NA	
Tachycardia	1.6 (1.1–2.6)	.03	1.3 (.7–2.6)	.4
Hypotension	1.6 (.5–1.7)	.4	NA	
Nausea/vomiting	0.7 (.4–1.1)	.09	1.0 (.5–2.0)	.9
>1 PCR target positive	0.5 (.3–1.0)	.04	0.4 (.1–1.0)	**.05**
Provider PTP				
Low vs not done	0.5 (.3–1.0)	.05	0.8 (.3–2.2)	.7
Moderate vs not done	2.3 (1.3–3.9)	.002	3.0 (1.4–6.4)	**.004**
High vs not done	7.2 (2.8–18.7)	<.0001	8.5 (2.3–32.1)	**.002**
Test location				
Inpatient vs clinic	0.8 (.4–1.6)	.2	0.4 (.07–2.0)	.2
ED vs clinic	1.5 (.8–2.8)	.2	1.1 (.2–5.3)	.9

Only prior antibiotics, fever, and moderate or high provider PTP were statistically significant as predictors. More than 1 target positive demonstrated trend ($P = .05$) toward inverse relationship. Only univariate variables with $P \leq .2$ were placed into multivariate analysis.

Abbreviations: CI, confidence interval; ED, emergency department; NA, not applicable; OR, odds ratio; PCR, polymerase chain reaction; PTP, pretest probability; WBC, white blood cell.

DISCUSSION

We show in a multicenter analysis that CD positivity is a common finding among patients tested with a multiplex panel; however, when tested using an alternative method, only 24% of subsequent tests conclusively diagnosed a CD infection. Given that clinical PTP varies between panel pathogens, positive results should be correlated with the clinical syndrome and ideally be confirmed with additional testing. As illustrated by our CD PCR-positive cohorts, 76% of patients with a positive CD PCR tested negative for CD toxin, and 37% tested negative for both toxin and GDH. The majority of toxin-negative cases still received CD treatment even though many were positive for other organisms and thus may have had an alternative diagnosis. Treatment of false positives or CD colonization leads to unnecessary healthcare expense and antibiotic exposure.

Current CD guidelines still include an option for NAAT testing alone provided patients meet preagreed institutional criteria; however, it remains unclear if this recommendation only applies to specific CD NAAT or also includes multiplex PCR panels with CD targets [8]. The inclusion of CD targets on multiplex molecular panels risks incidental CD identification among patients who may have low pretest probabilities. Some microbiology laboratories blind CD results to limit overdiagnosis with the caveat that the diagnosis of community-acquired CDI may be missed in the absence of dedicated CD testing [10]. Interestingly, inpatient samples, which did not undergo reflex CD testing, made up the highest proportion of studies. Appropriate CD testing may pose a challenge even when reflex protocols exist.

Established data suggest that NAAT testing alone may overdiagnose CDI among patients with low PTP [2–4, 11–13] and that treatment of PCR-positive/toxin-negative cases may not improve outcomes [14]. Polage et al found that nearly half of patients with positive CD PCR studies remain asymptomatic

and do not require treatment [12]. Toxin positivity is associated with increased mortality and disease severity [12, 13, 15, 16], but the requirement for reflex toxin testing after NAAT is often determined by individual institutions guided by IDSA recommendations [8]. For patients with high PTP, selection of CD-specific testing is warranted over broad nonspecific molecular panels. Patients who are CD PCR positive and toxin negative may still represent CDI rather than colonization in the appropriate clinical setting with a high PTP [7]. Similarly, for patients with low CD PTP, CD positivity on GIPCR should be interpreted with caution.

In the characterization of diarrheal illness, we found that clinicians frequently fail to document CD PTP, diarrheal symptoms, and known predictors of the CDI syndrome. Known predictors of CD toxin positivity include fever and recent exposure to antibiotic therapy [1]. Our analysis demonstrates that a high clinician PTP also strongly predicts CD toxin positivity. Conversely, a low PTP is associated with toxin negativity. When clinicians consider known CDI features to determine likelihood of disease etiology, clinical judgment correlates with diagnosis. However, when presented with a positive CD PCR, clinicians are likely to treat regardless of PTP, an alternative diagnosis, or negative toxin results.

Our study has several limitations. Many host factors that impact CDI risk, such as proton pump inhibitor use, were not included in collected data. We recognize that our patient population is skewed toward patients presenting with higher gastroenteritis severity and is not generalizable to the immunosuppressed or populations with inflammatory bowel diseases. Perhaps the most significant limitation is the fact that our retrospective design is impacted by the quality of clinical documentation, and we cannot correlate the completeness or incompleteness of documentation to disease severity or PTP. Clinician notes may only provide a limited view of clinician assessment. For example, only 296 of the 444 cases included enough information to infer PTP based on our nonstandardized criteria. However, even in the absence of clinical documentation, we did identify concern for overdiagnosis and overtreatment of positive CD identified by GIPCR. Future work includes the creation of a clinical prediction tool to aid in CD diagnosis.

GI multiplex molecular panels are convenient, sensitive tests with a rapid turnaround time and a large variety of tested pathogens. While such panels have improved laboratory efficiency, an "all-or-none" approach for the diagnosis of infectious gastroenteritis/colitis can lead to confusion and overtreatment, especially when results are unexpected and PTP is not factored into the treatment decision. Including CD, the most common cause of healthcare-associated colitis, in a large panel of community-acquired GI pathogens increases the likelihood of overdiagnosing and overtreating CDI. Characterizing PTP is essential for interpreting GI panel test results.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. S. K. T. and B. K. L. designed the study; S. K. T. and N. G. collected the data; M. P. and S. K. T. analyzed the data with input from D. L. and B. K. L.; and M. P. was the major contributor in writing this manuscript. All authors assisted in manuscript revision and approved the final manuscript.

Financial support. This work was supported by the Intermountain Healthcare Division of Infectious Diseases and Clinical Epidemiology.

Potential conflicts of interest. B. K. L. served as a medical consultant to Luminex, is a member of the scientific advisory board for Seegene, and received research support from OpGen and Immunexpress. All other authors report no potential conflicts.

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