Gut Pathogens

RESEARCH

Open Access

Risk factors and clinical outcomes associated with multiple as opposed to single pathogens detected on the gastrointestinal disease polymerase chain reaction assay

Insa Mannstadt[1*], Alexa M. Choy[4], Jianhua Li[2], Daniel A. Green[3] and Daniel E. Freedberg[4]

Abstract

Background The use of gastrointestinal disease multiplex polymerase chain reaction (GI PCR) testing has become common for suspected gastrointestinal infection. Patients often test positive for multiple pathogens simultaneously through GI PCR, although the clinical significance of this is uncertain.

Methods This retrospective cohort study investigated risk factors and clinical outcomes associated with detection of multiple (as opposed to single) pathogens on GI PCR. We included adult patients who underwent GI PCR testing from 2020 to 2023 and had one or more pathogens detected. We compared patients with multiple versus those with single pathogens and hypothesized that immunosuppression would be a risk factor for detection of multiple pathogens. We further hypothesized that, during the 90 days after GI PCR testing, patients with multiple pathogens would have worse clinical outcomes such as increased rates of emergency department (ED) visits, death, hospitalization, or ambulatory care visits.

Results GI PCR was positive in 1341 (29%) of tested patients; 356 patients had multiple pathogens and 985 had one pathogen. The most common pathogens included Enteropathogenic *Escherichia coli* (EPEC, 27%), norovirus (17%), and Enteroaggregative *E. coli* (EAEC, 14%) in both multi- and singly positive patients. Immunosuppression was not associated with multiple pathogens (adjusted odds ratio [aOR] 1.35, 95% CI 0.96, 1.86). The factors most associated with multiple pathogens were Hispanic ethnicity (OR 1.86, 95% CI 1.42, 2.45) and chronic kidney disease (OR 1.69, 95% CI 1.13, 2.49). Patients with multiple pathogens were more likely to have ED visits during the 90 days after GI PCR testing (40% vs. 32%, $p < 0.01$), but they were not more likely to die, be hospitalized, or to have ambulatory medical visits.

Conclusions Immunosuppression was not associated with detection of multiple as opposed to single pathogens on GI PCR testing. There were worse clinical outcomes associated with detection of multiple pathogens, although these effects were modest.

Keywords Pathogens, Immunosuppression, Co-infection, Diagnosis

*Correspondence:
Insa Mannstadt
imm2147@cumc.columbia.edu
Full list of author information is available at the end of the article

Introduction

The gastrointestinal disease multiplex polymerase chain reaction (GI PCR) is common and growing in popularity as a tool to diagnose diarrheal illnesses with greater sensitivity compared to traditional culture. Traditional culture-based testing rarely proves positive for more than one pathogen in a given sample, but GI PCR often detects co-infections with multiple diarrhea-causing pathogens. While GI PCR can identify co-infections, it is not always clear whether all detected pathogens are clinically relevant or if some represent colonization, particularly in patients with altered immune function [1–4]. This distinction is crucial as it can significantly impact clinical management decisions [5].

Despite widespread use of GI PCR, few studies have characterized the prevalence and types of organisms present in samples with multiple positive results. Additionally, there is a lack of understanding of the clinical implications of detecting multiple pathogens as opposed to a single pathogen on patient outcomes. This study aims to fill this knowledge gap and provide valuable insights into the interpretation of GI PCR results, especially in immunocompromised patients.

We hypothesized that immunocompromised patients would be at increased risk for multiple as opposed to single pathogens on GI PCR testing. In individuals with weakened immune systems, such as those with HIV/AIDS or undergoing cancer treatment or organ transplantation, the body's normal defense mechanisms against colonization by gut pathogens are compromised [6–11]. Similarly, patients with comorbidities that disrupt the gut microbiome, such as cancer, diabetes, heart failure, chronic kidney disease, or inflammatory bowel disease (IBD), are more prone to enteric infections [9, 12–18].

We further hypothesized that the presence of multiple pathogens would be associated with measurably worse clinical outcomes even after adjusting for other factors—i.e., that these patients would have true co-infection which would lead to increased healthcare utilization compared to singly-infected patients. A null hypothesis is that the detection of multiple pathogens usually represents colonization, and that such patients fare similarly to those with just one pathogen present [1, 5, 19].

By examining outcomes in those with multiple as opposed to single pathogens on GI PCR, we aimed to inform the clinical question of infection versus colonization. The overarching goal of the study was to guide future GI PCR testing decisions and to better interpret results when patients test positive for multiple enteric pathogens.

Methods

Population

This was a single-center, retrospective cohort study conducted at Columbia University Irving Medical Center (CUIMC). Patients aged 18 years or older who had undergone a GI PCR test between February 2020 and March 2023 were included. Children were excluded because of the differences in gut pathogens affecting adults and children [20]. The primary analyses were focused on the subset of patients who tested positive for one or more pathogens (i.e., a positive GI PCR test result). In instances where multiple positive stool tests were recorded for a single patient, the first test result was selected to ensure that each included test represented a unique individual. To minimize loss to follow-up, only individuals who had received primary care or specialist outpatient care within the two-month period preceding the assay GI PCR test were included. The study protocol was approved by the institutional review board of CUIMC.

GI PCR testing

Patients were classified as testing positive for multiple pathogens if they had two or more organisms detected on GI PCR; they were classified positive for a single pathogen if only one organism was detected. The stool samples collected from the patients were processed using the FilmArray GI Panel (BioFire Diagnostics, Salt Lake City, UT) according to the manufacturer's instructions. Freshly excreted stool samples were collected by nurses and an aliquot of stool was placed directly into Cary Blair transport media at the bedside. These samples were mixed with the manufacturer's reagents, loaded onto a cartridge, and placed in the FilmArray instrument for automated analysis. The FilmArray GI Panel utilizes a closed-system disposable pouch to qualitatively detect DNA or RNA from 22 different gastrointestinal pathogens including bacteria, parasites, and viruses [21]. The treating physicians had access to the GI PCR results when formulating treatment plans for their patients.

Classification of immunosuppression

The main focus of interest was immunosuppression, which was classified categorically. Patients were classified as immunosuppressed if they had auto-immune diseases, history of solid organ transplant, or if they took an immunosuppressive medication in the 90 days before GI PCR testing (Supplemental Table 1) [22–26].

Co-variables

Using automated queries of the electronic medical record, we gathered demographic, clinical

Table 1 Patient characteristics at the time of GI PCR testing, stratified based on GI PCR result

Variable	Negative (N = 3363)	Positive PCR		P value
		Multiple (N = 356)	Single (N = 985)	
Female sex	2089 (62%)	183 (51%)	529 (54%)	0.49
Age (years)				
18–40	1013 (30%)	134 (38%)	367 (37%)	0.059
41–60	926 (28%)	117 (33%)	267 (27%)	
61 +	1399 (42%)	103 (29%)	342 (35%)	
Race				
Asian	67 (2%)	6 (2%)	21 (2%)	0.17
Black	353 (11%)	41 (12%)	119 (12%)	
Other	1139 (34%)	157 (44%)	369 (38%)	
White	1804 (54%)	152 (43%)	476 (48%)	
Ethnicity				
Hispanic	770 (23%)	134 (38%)	239 (24%)	< 0.01
Non-Hispanic	2019 (60%)	172 (48%)	581 (59%)	
Other	574 (17%)	50 (14%)	165 (17%)	
Insurance status				
Commercial	2267 (67%)	275 (77%)	779 (79%)	0.67
Medicaid no medicare	289 (9%)	47 (13%)	99 (10%)	
Medicaid with medicare	198 (6%)	24 (7%)	69 (7%)	
Medicare	145 (4%)	7 (2%)	21 (2%)	
Immunosuppression (overall)	726 (22%)	68 (19%)	147 (15%)	0.08
Immune-mediated disease[+]	210 (6%)	54 (15%)	112 (11%)	0.07
Solid organ transplant	296 (9%)	33 (9%)	79 (8%)	0.32
HIV/AIDS	75 (2%)	17 (5%)	38 (4%)	0.55
Immunosuppressive medication[+]	726 (22%)	68 (19%)	147 (15%)	0.08
Serum markers				
Albumin (< 3.4 g/dL)	54 (2%)	6 (2%)	15 (2%)	0.82
Creatinine (> 1.1 mg/dL)	359 (11%)	72 (20%)	148 (15%)	035
Comorbidities				
Cardiovascular disease	1143 (34%)	140 (39%)	338 (34%)	0.10
Diabetes	380 (11%)	51 (14%)	111 (11%)	0.16
COPD	114 (3%)	4 (1%)	19 (2%)	0.44
CKD or ESRD	187 (6%)	44 (12%)	76 (8%)	0.01
Obesity	866 (26%)	84 (24%)	258 (26%)	0.37
Inflammatory bowel disease	321 (10%)	31 (9%)	94 (10%)	0.72
Malignancy	440 (13%)	41 (12%)	116 (12%)	0.97
Smoker (ever)	110 (3%)	17 (5%)	35 (4%)	0.39
Autoimmune disease	530 (16%)	62 (17%)	144 (15%)	0.24
Medications received				
PPI in prior 90 days	647 (19%)	83 (23%)	204 (21%)	0.21
Antibiotics in prior 90 days	884 (26%)	112 (32%)	323 (33%)	0.40
Hospitalized in prior 30 days	253 (8%)	26 (7%)	88 (9%)	0.40

[*] Continuous variables reported as mean ± standard error or median (IQR). Categorical variables reported as N (%)

[+] As defined by Supplementary Table 1

characteristics, and comorbidities and classified them based on codes documented using the 10th revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10) coding system at the time of the GI PCR test (Supplemental Table 1). The ICD-10 system is used for billing in all U.S. healthcare settings and contains hierarchically structured medical diagnoses and reasons for healthcare visits

[22]. (Supplemental Table 1). As of May 2024, ICD-10 is a medical classification list that standardizes disease and health condition coding across the globe and is maintained by the World Health Organization [27]. Age and BMI were split into quartiles. Laboratory values were defined as normal or abnormal based on the institutional laboratory reference ranges as of May 1, 2024 [28]. Age and serum markers were categorized to aid in risk stratification and clinical decision-making, providing clearer ranges for interpretation.

Clinical outcomes

We compared clinical outcomes during the 90 days after GI PCR testing including mortality, hospitalization, ED visits, receipt of antibiotics, and ambulatory medicine visits, between groups testing positive for multiple versus single pathogens by gathering data from electronic medical records with automated queries and classifying outcomes as present or absent.

Statistical approach

Continuous data were expressed as means with standard deviations (SD) or as medians with interquartile ranges if the data were not normally distributed. Data were compared using t-tests for continuous data or chi-squared tests or Fisher's exact tests for categorical data. Two multivariable models were constructed. First, a model was constructed for the outcome of testing positive for multiple as opposed to single pathogens on GI PCR. This model included immunosuppression a priori, with additional variables added stepwise, retaining those in the final model that independently predicted multiple pathogens. Second, a model was constructed for each clinical outcome. The primary focus of interest in this model was testing positive for multiple as opposed to single pathogens and we additionally pre-specified that age, immunosuppression, and insurance status would be included because these factors are likely to associate with poor outcomes. Logistic regression modeling was used to investigate risk factors for detection of multiple as opposed to single pathogens on GI PCR. Crude (unadjusted) odds ratios were used for descriptive purposes and adjusted odds ratios were used to control for potential confounding variables and to estimate the independent effects of predictor variables. Two-tailed test with a p-value of ≤ 0.05 was considered statistically significant. All statistical analysis was performed using RStudio [44], using packages forestplot [45], lubridate [46], olsrr [47], vtable [48], checkmate [49], report [50], tibble [51], abind [52], R language and environment [53], Table 1 [54], reshape [55], ggplot2 [56], stringr [57], forcats [58], tidyverse [59], dplyr [60], purrr [61], readr [62], tidyr [63], and kableExtra [64].

Results

Patient characteristics

There were 4704 patients who underwent GI PCR testing during the study period. Of these, 29% tested positive (either singly or multiply) and were included in the main analyses. The majority of patients were female (60%), with a median age of 53 years (IQR 35–68). Over half were White (52%), and nearly a quarter were Hispanic (24%), (Table 1).

Multiple as opposed to single pathogens detected

Out of the total patients tested, 1341 (29%) had a positive result on the GI PCR test. Of those with a positive GI PCR test, 985 (73%) were positive for a single pathogen, while 356 (27%) had multiple pathogens detected. Among the identified pathogens, the most prevalent were Enteropathogenic *E. coli* (EPEC), norovirus, and Enteroaggregative *E. coli* (EAEC). These accounted for approximately 70% of GI PCR results in patients with a single pathogen detected and 60% of PCR results in patients with multiple pathogens detected (Fig. 1). Patients with multiple positive GI PCR were slightly more likely to be immunosuppressed without reaching statistical significance (19% vs 16%, $p=0.07$). They were more likely to be Hispanic (38% vs. 24%, $p<0.01$) and to have end-stage renal disease (12% vs. 8%, $p=0.01$) (Table 1). Among those with multiple pathogens detected on GI PCR, heat maps showed that the pathogen combinations most often co-present were EPEC and EAEC, EAEC and norovirus, and EPEC and norovirus. Higher rates of observed compared to expected combinations of co-positivity were seen for Enterotoxigenic *E. coli* (ETEC) and EAEC, Shiga toxin-producing *E. coli* (STEC) and ETEC, STEC and EAEC, and *Giardia* and *Campylobacter* (all $p<0.01$) (Fig. 2).

Logistic regression model for multiple vs. single pathogens

In the final model, immunosuppression was not significantly associated with multiple pathogens (aOR 1.35, 95% CI 0.96–1.86) (Table 2). Hispanic ethnicity was associated with increased risk for multiple pathogens (aOR 1.86, 95% CI 1.42–2.45).

Detection of multiple pathogens and clinical outcomes

Within 90 days of GI PCR testing, 24 (0.5%) patients died, 568 (12%) recorded ED or urgent care visits, 2673 (57%) recorded ambulatory medicine visits, and 161 (3.4%) were hospitalized. Patients with multiple positive pathogens were more likely to have ED/urgent care visits compared to those with single positive PCR results (40% vs. 32%, $p<0.01$) but were not more likely to experience any of the other outcomes (Fig. 3). Next, we used logistic regression modeling to investigate the independent

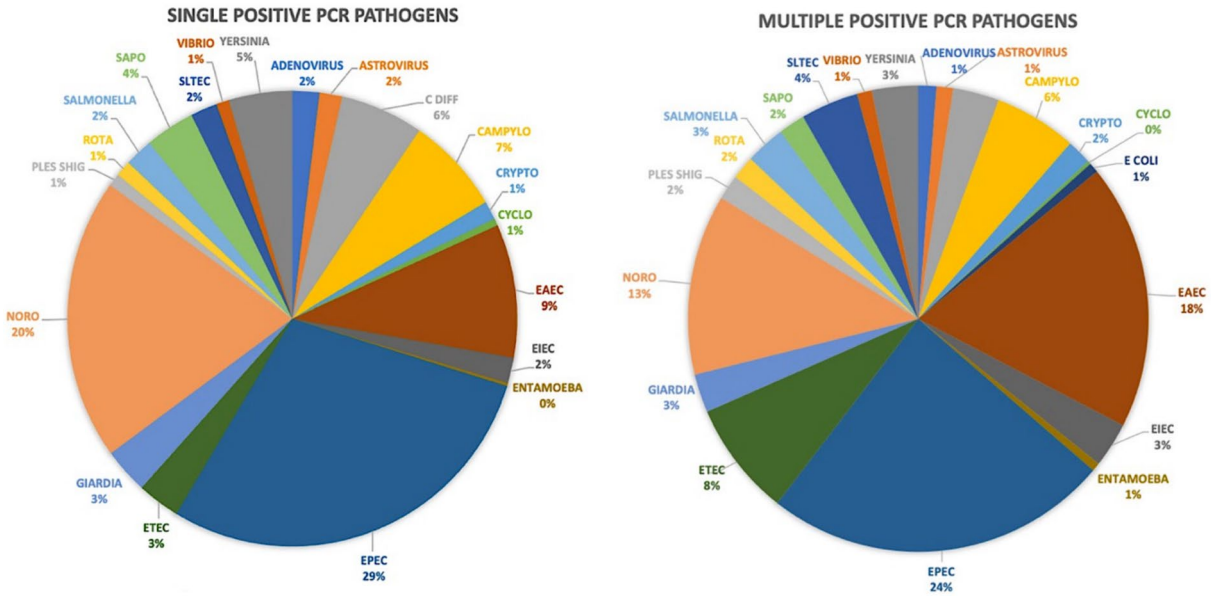

Fig. 1 Pie chart analyses of the study population's fecal samples. The left pie chart represents the distribution of pathogens in samples with only one detected pathogen, while the right pie chart shows the breakdown for samples containing multiple pathogens

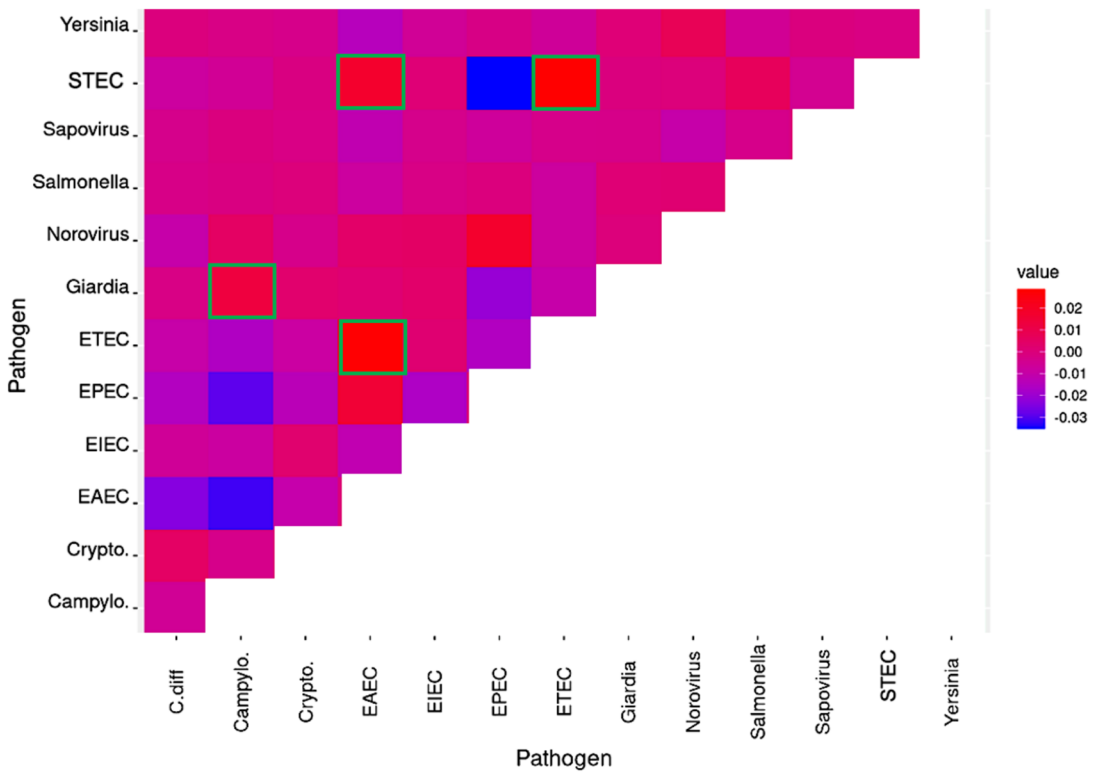

Fig. 2 Heat map illustrating the prevalence of co-infecting pathogens in patients with multi positive PCR results. Highlighted squares represent combinations of pathogens that occurred more frequently than expected, as determined by McNamar's test with a statistical significance threshold of $p < 0.05$

Table 2 Logistic regression model for risk factors for multiple as opposed to single pathogens on GI PCR

Patient variable	Unadjusted odds ratio (95% CI)	Adjusted odds ratio (95% CI)
Immunosuppression		
No immunosuppression	Reference	Reference
Immunosuppression	1.35 (0.97–1.84)	1.35 (0.96, 1.86)
Ethnicity		
Non-Hispanic	Reference	Reference
Hispanic	1.89 (1.44, 2.48)	1.86 (1.42, 2.45)
Age		
Age 18–41	Reference	Reference
Age 41–60	1.20 (0.89, 1.61)	1.08 (0.79, 1.45)
Age 65 +	0.83 (0.61, 1.11)	0.77 (0.57, 1.04)
Medication usage in prior 90 days		
No PPI	Reference	–
PPI	0.86 (0.65, 1.15)	–
No antibiotics	Reference	–
Antibiotics	0.94 (0.72, 1.22)	–
Comorbidities		
No CVD	Reference	–
CVD	1.24 (0.97, 1.59)	–
No CKD/ESRD	Reference	–
CKD/ESRD	1.69 (1.13, 2.49)	–
No diabetes	Reference	–
Diabetes	1.32 (0.92, 1.87)	–
Race		
White	Reference	–
Asian	0.90 (0.32, 2.13)	–
Black	1.08 (0.72, 1.60)	–
Other	1.33 (1.03, 1.73)	–

association between multiple pathogens and 90 day ED visits. After adjusting for other factors, detection of multiple (as opposed to single) pathogens was associated with increased risk for ED visits (aOR 1.44, 95% CI 1.11–1.87) (Table 3). Other factors that were independently associated with ED visits were immunosuppression (aOR 1.95, 95% CI 1.43–2.66), and Medicaid insurance (aOR 2.51, 95% CI 1.74, 3.62). The rates of receiving an antibiotic prescription were similar between patients with multiple vs. single positive GI PCR results (33% vs 32%). When looking specifically at the rates of receiving two or more antibiotic prescriptions, patients with multiple positive results had slightly higher rates compared to those with a single positive result (23% vs 19%, $p = 0.03$).

Discussion

This study assessed the clinical significance of detecting multiple as opposed to single pathogens on the GI PCR test, a common occurrence that was observed in 24% of all positive tests. We assessed risk factors for multiple pathogens, including immunosuppression. We also characterized the prevalence and types of enteric infections and the differences in clinical course and outcomes, comparing patients who tested positive for multiple gut pathogens versus those who tested positive for one pathogen alone. Overall, the baseline characteristics and outcomes of the two groups were more similar than we expected. A priori, we hypothesized that patients positive for multiple pathogens would be more likely to be immunosuppressed and would have increased medical comorbidities. We found that immunosuppression was not statistically associated with multiple pathogens. Downstream from this, we found only very modest differences in clinical outcomes when comparing those with multiple pathogens versus a single pathogen. Patients positive for multiple pathogens, including the more commonly detected but less clinically relevant EAEC, had a slightly higher rate of emergency room visits than those positive for a single pathogen, suggesting a potential additional health burden. However, the overall similarity between these two groups in terms of risk factors and the lack of thorough measures of clinical outcomes, such as severity of disease, duration of symptoms, or antibiotic requirement, makes it difficult to conclusively determine whether co-infection with multiple enteric pathogens represents a substantial health burden or is more likely an incidental finding. The higher prevalence of EAEC, an organism with less certain clinical relevance, among patients with multiple pathogens further supports the notion that these co-infections may not necessarily lead to worse clinical outcomes. While the increased emergency room visits among patients with multiple pathogens points to some additional health burden, the clinical course after GI PCR testing otherwise appeared largely similar between the two groups.

In contradiction to our results, prior studies have suggested that immunocompromise is associated with multiple gut pathogens, although many prior studies focus on enteric viruses and on children [3, 6, 29]. Specific immunocompromised subpopulations including children who are solid organ transplant recipients [29], those with HIV/AIDS [9, 12], and liver and stem cell transplant recipients [30, 31] are associated with higher rate of multiple pathogens on stool testing. The use of corticosteroids has also been associated with an increased likelihood of multiple pathogens on GI PCR among patients with IBD [32]. In a study of GI PCR testing in patients with HIV, Axelrad et al. found that 25% of men who have sex with men patients had multiple gut pathogens regardless of their degree of immunosuppression [11, 33]. Our study was not powered to look at specific categories of immunosuppression and it is likely that there

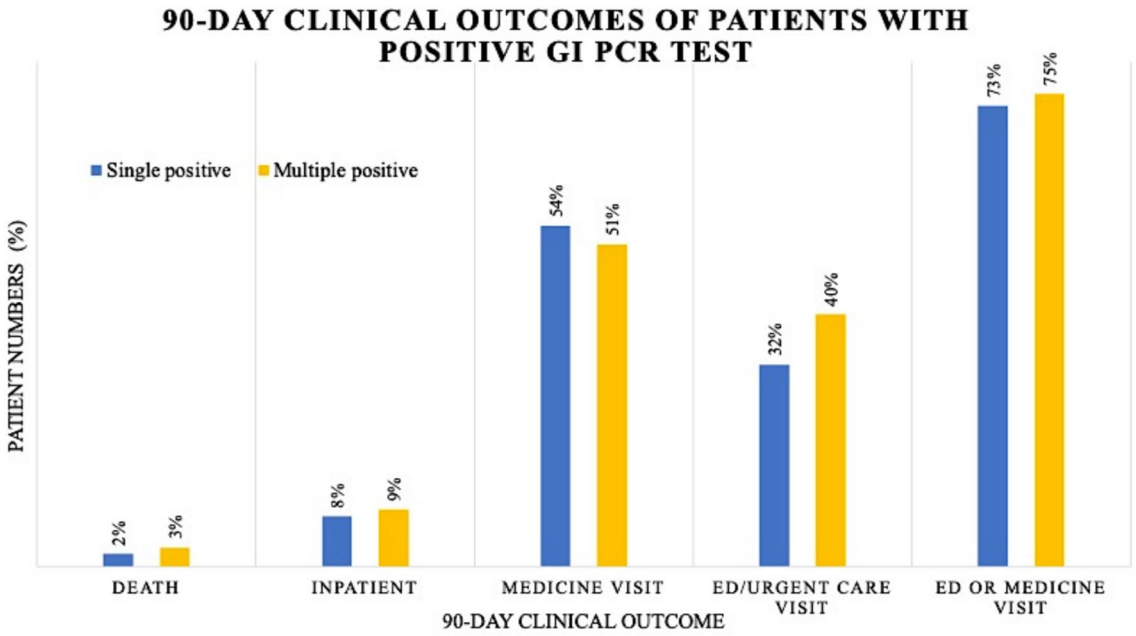

Fig. 3 Bar graph of the 90 day clinical outcomes or disease courses in patients with single (blue) or multiple (yellow) positive pathogen results on GI PCR assay. The graph compares the outcomes between patients with infections with a single organism versus patients with multiple concurrent infections

was heterogeneity in the degree of immunosuppression within the diverse group of immunosuppressed patients included in the study.

Interestingly, Hispanic ethnicity was the most important predictor of multiple pathogens on GI PCR. Prior research has documented differences in microbiome structure between racial and ethnic groups [34–37]. Hispanic ethnicity may be associated with the detection of multiple pathogens on GI PCR due to a combination of host genetics, geographic location, and socioeconomic factors such as diet, living environment, pathogen exposures, access to medical care, travel, and other social constructs that shape the gut microbiome and influence susceptibility to enteric pathogens [35–38]. This study could not determine the specific factors underlying the association between Hispanic ethnicity and higher incidence of multiple pathogens on GI PCR testing.

Prior studies of viral diarrhea in children have suggested that there is greater severity of diarrhea when multiple viruses are detected, but less is known in adults and with bacterial enteropathogens [39]. After adjusting for other factors including age, insurance status, and immunosuppression, detection of multiple pathogens was associated with a 44% increased risk for subsequent ED visits compared to detection of a single pathogen. Those with multiple pathogens were also more likely to receive more than one antibiotic, although overall rates of antibiotic use were similar comparing those with multiple vs. single pathogens. There was no association between multiple pathogens and other clinical outcomes (death, hospitalization, or increased likelihood of an ambulatory care visit). Future diagnostics—particularly those using sequencing technologies—may provide more granular clinical information by reporting on the relative abundance of a given organism which could influence the decision of whether and how to treat.

When we looked at patterns of co-positivity, we found several pathogen pairs which appeared at a rate greater than expected by pure chance: ETEC and EAEC, STEC and ETEC, STEC and EAEC, and *Giardia* and *Campylobacter*. Whether these represent synergistic relationships or rather shared environmental risk factors is unknown. In prior studies, ETEC has been found to co-occur more often with EPEC, and with *Campylobacter* [40]. Prior studies have also suggested that bacteria-bacteria pairs appear together more frequently than virus-bacteria pairs [40, 41]. It is plausible that viral-bacterial coinfection could augment the severity of diarrhea [42]. One study employed a cluster analysis and hierarchical clustering approach to PCR-based data and demonstrated that such co-infections were likely to be clinically relevant [43].

This study has strengths, and some limitations. This study builds on the limited body of existing research that investigates patient variables and clinical outcomes associated with multiple pathogens on GI PCR. It was relatively large and looked at the presence of multiple

Table 3 Logistic regression for patient variables associated with ED visits in 90 days following GI PCR

Patient variable	Unadjusted odds ratio (95% CI)	Adjusted odds ratio* (95% CI)
PCR pathogen result type		
Single positive pathogens	Reference	Reference
Multiple positive pathogens	1.47 (1.15, 1.89)	1.44 (1.11, 1.87)
Ethnicity		
Non-Hispanic	Reference	–
Hispanic	3.54 (2.73, 4.60)	–
Immunosuppression		
No immunosuppression	Reference	Reference
Immunosuppression	2.12 (1.57, 2.85)	1.95 (1.43, 2.66)
Age groups		
18–41 years	Reference	Reference
41–60 years	1.30 (0.98, 1.72)	1.05 (0.78, 1.42)
65 + years	1.39 (1.06, 1.83)	1.23 (0.91, 1.66)
Insurance type		
Private	Reference	Reference
Medicaid without medicare	2.29 (1.61, 3.26)	2.51 (1.74, 3.62)
Medicaid with medicare	4.49 (2.89, 7.10)	3.97 (2.52, 6.36)
Medicare only	0.94 (0.39, 2.09)	0.81 (0.33, 1.84)
Comorbidities		
No CKD/ESR	Reference	–
CKD/ESRD	2.61 (1.79, 3.83)	–
No CVD	Reference	–
CVD	2.59 (2.05, 3.28)	–
Race		
White	Reference	–
Asian	1.25 (0.51, 2.82)	–
Black	2.90 (2.03, 4.16)	–
Other	1.98 (1.54, 2.54)	–

pathogens from several angles. Limitations include a retrospective design, lack of granular data related to hygiene and lifestyle factors which may influence GI PCR positivity, and lack of detailed patient symptom and severity data. Future studies should investigate the impact of GI pathogens on patient outcomes and explore strategies to prevent and manage these infections.

In conclusion, we found that patients testing positive for multiple pathogens on GI PCR did not exhibit substantially different baseline characteristics or clinical outcomes compared to those testing positive for a single pathogen. The unexpected finding of Hispanic ethnicity as a predictor of multiple pathogens highlights the complex interplay between environmental, socioeconomic factors, and enteric infections. Patients who tested positive for multiple pathogens were more likely to have ER visits afterwards compared to those who tested positive for single pathogens, but no other harm was observed to be associated with multiple pathogens (no increased rate of death or hospitalization). On balance, these results argue that in many multi-positive GI PCR patients, one or more of the organisms is likely to be a colonizer.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13099-024-00638-4.

Supplementary Material 1. Table 1: Immunosuppression classification criteria including ICD-10 codes related to immune-mediated disease and immunosuppressants in 90 days prior to PCR

Acknowledgements
None.

Author contributions
All authors approved the final version of the manuscript. I.M. and D.E.F. wrote the main manuscript text and prepared tables 1–3 and Figs. 1–3. All authors reviewed the manuscript.

Funding
None to disclose.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
This study was approved by the Columbia University Institutional Review Board. Informed consent waived by the Columbia IRB under protocol number [Protocol #IRB-AAAU5707].

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Columbia University Vagelos College of Physicians and Surgeons, New York, NY, USA. [2] Department of Medicine, Columbia University Irving Medical Center, New York, NY, USA. [3]Clinical Microbiology, Department of Pathology and Cell Biology, Columbia University Irving Medical Center, New York, NY, USA. [4] Division of Digestive and Liver Diseases, Columbia University Irving Medical Center-New York Presbyterian Hospital, New York, NY, USA.

Received: 24 May 2024 Accepted: 22 August 2024
Published online: 30 August 2024

References

1. Hissong E, Mowers J, Zhao L, Greenson JK, Bachman M, Lamps LW. Histologic and clinical correlates of multiplex stool polymerase Chain reaction assay results. Arch Pathol Lab Med. 2022;146(12):1479–85. https://doi.org/10.5858/arpa.2021-0329-OA.
2. Stockmann C, Rogatcheva M, Harrel B, et al. How well does physician selection of microbiologic tests identify clostridium difficile and other pathogens in paediatric diarrhoea? Insights using multiplex PCR-based detection. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. 2015;21(2):179.e9-15. https://doi.org/10.1016/j.cmi.2014.07.011.
3. Huang SH, Lin YF, Tsai MH, et al. Detection of common diarrhea-causing pathogens in northern Taiwan by multiplex polymerase chain reaction.

Medicine. 2018;97(23): e11006. https://doi.org/10.1097/MD.0000000000011006.
4. Spina A, Kerr KG, Cormican M, et al. Spectrum of enteropathogens detected by the film array GI panel in a multicentre study of community-acquired gastroenteritis. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. 2015;21(8):719–28. https://doi.org/10.1016/j.cmi.2015.04.007.
5. Berdal JE, Follin-Arbelet B, Bjørnholt JV. Experiences from multiplex PCR diagnostics of faeces in hospitalised patients: clinical significance of Enteropathogenic *Escherichia coli* (EPEC) and culture negative campylobacter. BMC Infect Dis. 2019;19(1):630. https://doi.org/10.1186/s12879-019-4271-1.
6. Mengelle C, Mansuy JM, Prere MF, et al. Simultaneous detection of gastrointestinal pathogens with a multiplex luminex-based molecular assay in stool samples from diarrhoeic patients. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. 2013;19(10):E458-465. https://doi.org/10.1111/1469-0691.12255.
7. Liesman RM, Binnicker MJ. The role of multiplex molecular panels for the diagnosis of gastrointestinal infections in immunocompromised patients. Curr Opin Infect Dis. 2016;29(4):359–65. https://doi.org/10.1097/QCO.0000000000000276.
8. Forbes JD, Yu Chen C, Knox NC, et al. A comparative study of the gut microbiota in immune-mediated inflammatory diseases—does a common dysbiosis exist? Microbiome. 2018;6(1):221. https://doi.org/10.1186/s40168-018-0603-4.
9. Magahis PT, Satish D, Esther Babady N, et al. Prevalence of enteric infections in patients on immune checkpoint inhibitors and impact on management and outcomes. Oncologist. 2024;29(1):36–46. https://doi.org/10.1093/oncolo/oyad226.
10. Nagao-Kitamoto H, Kitamoto S, Kuffa P, Kamada N. Pathogenic role of the gut microbiota in gastrointestinal diseases. Intest Res. 2016;14(2):127–38. https://doi.org/10.5217/ir.2016.14.2.127.
11. Verma A, Hine AM, Joelson A, et al. The influence of hospitalization and HIV severity on gastrointestinal PCR panel evaluation of HIV-related acute diarrhea in New York city: a retrospective, cross-sectional study. Ther Adv Gastroenterol. 2022;15:17562848221092592. https://doi.org/10.1177/17562848221092593.
12. Pickard JM, Zeng MY, Caruso R, Núñez G. Gut microbiota: role in pathogen colonization, immune responses and inflammatory disease. Immunol Rev. 2017;279(1):70–89. https://doi.org/10.1111/imr.12567.
13. Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. Cell Res. 2020;30(6):492–506. https://doi.org/10.1038/s41422-020-0332-7.
14. Wilkins LJ, Monga M, Miller AW. Defining dysbiosis for a cluster of chronic diseases. Sci Rep. 2019;9(1):12918. https://doi.org/10.1038/s41598-019-49452-y.
15. Afzaal M, Saeed F, Shah YA, et al. Human gut microbiota in health and disease: unveiling the relationship. Front Microbiol. 2022. https://doi.org/10.3389/fmicb.2022.999001.
16. Cruz-López F, Martínez-Meléndez A, Garza-González E. How does hospital microbiota contribute to healthcare-associated infections? Microorganisms. 2023;11(1):192. https://doi.org/10.3390/microorganisms11010192.
17. Trøseid M, Holter JC, Holm K, et al. Gut microbiota composition during hospitalization is associated with 60 day mortality after severe COVID-19. Crit Care. 2023;27:69. https://doi.org/10.1186/s13054-023-04356-2.
18. Axelrad JE, Chen Z, Devlin J, Ruggles KV, Cadwell K. Pathogen-specific alterations in the gut microbiota predict outcomes in flare of inflammatory bowel disease complicated by gastrointestinal infection. Clin Transl Gastroenterol. 2023;14(2): e00550. https://doi.org/10.14309/ctg.0000000000000550.
19. Zautner AE, Groß U, Emele MF, Hagen RM, Frickmann H. More pathogenicity or just more pathogens?—on the interpretation problem of multiple pathogen detections with diagnostic multiplex assays. Front Microbiol. 2017;8:1210. https://doi.org/10.3389/fmicb.2017.01210.
20. Chen CH, Low YY, Liu YH, Lin HH, Ho MW, Hsueh PR. Rapid detection of gastrointestinal pathogens using a multiplex polymerase chain reaction gastrointestinal panel and its role in antimicrobial stewardship. J Microbiol Immunol Infect Wei Mian Yu Gan Ran Za Zhi. 2023;56(6):1273–83. https://doi.org/10.1016/j.jmii.2023.10.004.
21. FilmArray® Panels. BioFire diagnostics. https://www.biofiredx.com/products/the-filmarray-panels/. Accessed 30 July 2024
22. Elmahdi R, Ward D, Ernst MT, et al. Impact of immunosuppressive therapy on SARS-CoV-2 mRNA vaccine effectiveness in patients with immune-mediated inflammatory diseases: a Danish nationwide cohort study. BMJ Open. 2024;14(2): e077408. https://doi.org/10.1136/bmjopen-2023-077408.
23. Downey MR, Taskar V, Linder DF, et al. Incidence and risk factors for mucormycosis in renal transplant patients. J Investig Med Off Publ Am Fed Clin Res. 2022;70(2):396–401. https://doi.org/10.1136/jim-2021-001933.
24. Reichel F, Tesch F, Berger S, et al. Epidemiology and risk factors of community-acquired pneumonia in patients with different causes of immunosuppression. Infection. 2024. https://doi.org/10.1007/s15010-024-02314-w.
25. Patel NJ, Wang X, Lin M, et al. Factors associated with an electronic health record-based definition of postacute sequelae of COVID-19 in patients with systemic autoimmune rheumatic disease. J Rheumatol. 2024;51(5):529–37. https://doi.org/10.3899/jrheum.2023-1092.
26. Kolla E, Weill A, Zaidan M, et al. COVID-19 hospitalization in solid organ transplant recipients on immunosuppressive therapy. JAMA Netw Open. 2023;6(11): e2342006. https://doi.org/10.1001/jamanetworkopen.2023.42006.
27. International Classification of Diseases (ICD). https://www.who.int/standards/classifications/classification-of-diseases. Accessed 18 July 2024
28. NewYork-Presbyterian Columbia University Irving Medical Center Test Directory | Home. https://www.testmenu.com/nyphcolumbia. Accessed 12 July 2024
29. Stone JM, Savage A, Hudspeth M, et al. Multi-organism gastrointestinal polymerase chain reaction positivity among pediatric transplant vs non-transplant populations: a single-center experience. Pediatr Transplant. 2020;24(6): e13771. https://doi.org/10.1111/petr.13771.
30. Rogers WS, Westblade LF, Soave R, et al. Impact of a multiplexed polymerase chain reaction panel on identifying diarrheal pathogens in hematopoietic cell transplant recipients. Clin Infect Dis Off Publ Infect Dis Soc Am. 2020;71(7):1693–700. https://doi.org/10.1093/cid/ciz1068.
31. Ching CK, Nobel YR, Pereira MR, Verna EC. The role of gastrointestinal pathogen polymerase chain reaction testing in liver transplant recipients hospitalized with diarrhea. Transpl Infect Dis Off J Transpl Soc. 2022;24(4): e13873. https://doi.org/10.1111/tid.13873.
32. Varma S, Green PH, Krishnareddy S. Clinical factors associated with positive stool PCR for gastrointestinal pathogens in celiac and inflammatory bowel disease. J Clin Gastroenterol. 2022;56(3):e196–202. https://doi.org/10.1097/MCG.0000000000001657.
33. Hong S, Zaki TA, Main M, et al. Comparative evaluation of conventional stool testing and multiplex molecular panel in outpatients with relapse of inflammatory bowel disease. Inflamm Bowel Dis. 2021;27(10):1634–40. https://doi.org/10.1093/ibd/izaa336.
34. Byrd D, Wolf P. The microbiome as a determinant of racial and ethnic cancer disparities. Nat Rev Cancer. 2023. https://doi.org/10.1038/s41568-023-00638-7.
35. Deschasaux M, Bouter KE, Prodan A, et al. Depicting the composition of gut microbiota in a population with varied ethnic origins but shared geography. Nat Med. 2018;24(10):1526–31. https://doi.org/10.1038/s41591-018-0160-1.
36. Piawah S, Kyaw TS, Trepka K, et al. Associations between the gut microbiota, race, and ethnicity of patients with colorectal cancer: a pilot and feasibility study. Cancers. 2023;15(18):4546. https://doi.org/10.3390/cancers15184546.
37. Amato KR, Arrieta MC, Azad MB, et al. The human gut microbiome and health inequities. Proc Natl Acad Sci. 2021;118(25): e2017947118. https://doi.org/10.1073/pnas.2017947118.
38. Peters BA, Yi SS, Beasley JM, et al. US nativity and dietary acculturation impact the gut microbiome in a diverse US population. ISME J. 2020;14(7):1639–50. https://doi.org/10.1038/s41396-020-0630-6.
39. Hazan G, Goldstein Y, Greenberg D, et al. Comparing single versus multiple virus detection in pediatric acute gastroenteritis postimplementation of routine multiplex RT-PCR diagnostic testing. J Med Virol. 2024;96(1): e29344. https://doi.org/10.1002/jmv.29344.
40. Colgate ER, Klopfer C, Dickson DM, et al. Network analysis of patterns and relevance of enteric pathogen co-infections among infants in a diarrhea-endemic setting. PLoS Comput Biol. 2023;19(11): e1011624. https://doi.org/10.1371/journal.pcbi.1011624.

41. Pijnacker R, van Pelt W, Vennema H, et al. Clinical relevance of enteropathogen co-infections in preschool children-a population-based repeated cross-sectional study. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. 2019;25(8):1039.e7-1039.e13. https://doi.org/10.1016/j.cmi.2018.11.029.
42. Vergadi E, Maraki S, Dardamani E, Ladomenou F, Galanakis E. Polymicrobial gastroenteritis in children. Acta Paediatr Oslo Nor 1992. 2021;110(7):2240–5. https://doi.org/10.1111/apa.15854.
43. Backhaus J, Frickmann H, Hagen RM, et al. Gastrointestinal pathogens in multi-infected individuals: a cluster analysis of interaction. Microorganisms. 2023;11(11):2642. https://doi.org/10.3390/microorganisms11112642.
44. Posit team. RStudio: integrated development environment for R. Posit software, PBC, Boston, MA. 2024. http://www.posit.co/.
45. Gordon M, Lumley T. forestplot: Advanced Forest Plot Using 'grid' Graphics. R package version 3.1.3. 2023. https://gforge.se/packages/.
46. Grolemund G, Wickham H. Dates and times made easy with lubridate. J Statistical Softw. 2011;40(3):1–25.
47. Hebbali A. olsrr: Tools for Building OLS Regression Models. R package version 0.6.0. 2024. https://github.com/rsquaredacademy/olsrr, https://olsrr.rsquaredacademy.com/.
48. Huntington-Klein N. vtable: Variable Table for Variable Documentation. R package version 1.4.6. 2023. https://nickch-k.github.io/vtable/.
49. Lang M. checkmate: fast argument checks for defensive R programming. R J. 2017;9(1):437–45.
50. Makowski D, Lüdecke D, Patil I, Thériault R, Ben-Shachar M, Wiernik B. Automated results reporting as a practical tool to improve reproducibility and methodological best practices adoption. CRAN. 2023. https://easystats.github.io/report/.
51. Müller K, Wickham H. tibble: simple data frames. R package version 3.2.1. 2023. https://github.com/tidyverse/tibble, https://tibble.tidyverse.org/.
52. Plate T, Heiberger R. abind: combine multidimensional arrays. R package version 1.4–5. 2016.
53. R Core Team. R: A language and environment for statistical computing. R foundation for statistical computing, Vienna, Austria. 2024. https://www.R-project.org/.
54. Rich B. Table1: tables of descriptive statistics in HTML. R package version 1.4.3. 2023. https://github.com/benjaminrich/table1.
55. Wickham H. Reshaping data with the reshape package. J Statistical Softw. 2007;21(12):1–20.
56. Wickham H. ggplot2: elegant graphics for data analysis. Springer-Verlag New York. 2016. https://ggplot2.tidyverse.org.
57. Wickham H. Stringr: simple, consistent wrappers for common string operations. R package version 1.5.0. 2022. https://github.com/tidyverse/stringr, https://stringr.tidyverse.org.
58. Wickham H. Forcats: tools for working with categorical variables (factors). R package version 1.0.0. 2023. https://github.com/tidyverse/forcats, https://forcats.tidyverse.org/.
59. Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H. Welcome to the tidyverse. J Open Source Softw. 2019;4(43):1686.
60. Wickham H, François R, Henry L, Müller K, Vaughan D. dplyr: A grammar of data manipulation. R package version 1.1.3. 2023. https://github.com/tidyverse/dplyr, https://dplyr.tidyverse.org.
61. Wickham H, Henry L. purrr: functional programming tools. R package version 1.0.2. 2023. https://github.com/tidyverse/purrr, https://purrr.tidyverse.org/.
62. Wickham H, Hester J, Bryan J. readr: read rectangular text data. R package version 2.1.4. 2023. https://github.com/tidyverse/readr, https://readr.tidyverse.org.
63. Wickham H, Vaughan D, Girlich M. tidyr: tidy messy data. R package version 1.3.0. 2023. https://github.com/tidyverse/tidyr, https://tidyr.tidyverse.org.
64. Zhu H. kableExtra: construct complex table with 'kable' and pipe syntax. R package version 1.3.4. 2021. https://github.com/haozhu233/kableExtra, http://haozhu233.github.io/kableExtra/.

Publisher's Note