CONCISE COMMUNICATION

The Perils of Multiplex Gastrointestinal Pathogen Panels: Pseudo-outbreaks of Salmonellae and *Entamoeba histolytica* in Immunocompromised Hosts

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Two distinct clusters of gastroenteritis due to Salmonellae and *Entamoeba histolytica* (EH) were identified using a multiplex gastrointestinal pathogen panel (GPP) at a tertiary-care cancer center. Despite temporo-spatial overlap, our investigation did not corroborate transmission or true infection. In clinical practice, GPPs may render false-positive results.

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Acute diarrheal illness, common among hospitalized immunocompromised patients has many etiologies, including infection, medications (eg, laxatives), and chemotherapy side effects.[1] Rapid assessment of infection is essential to ensure timely therapeutic intervention and implementation of isolation precautions in vulnerable patient populations. Multiplex PCR-based panels for gastrointestinal pathogens offer rapid and simultaneous analysis of multiple bacterial, viral, and parasitic pathogens, with highly convenient same-day turnaround of results. Additionally, these assays are less dependent on the technical expertise of laboratory personnel than on traditional methods. For the 2 predominant multitarget gastrointestinal pathogen panels (GPPs) cleared by the US Food and Drug Administration (FDA), sensitivity and specificity are high. However, clinical experience with routine implementation, particularly in specialized populations and for pathogens uncommon in high-income countries, is insufficient.[2–4] This report describes the investigation of 2 clusters of uncommon infections, one *Entamoeba histolytica* (EH) and the other Salmonellae, in hospitalized patients. Upon in-depth investigation, both clusters were determined to be pseudo-outbreaks related to GPP testing.

METHODS

The study was conducted at the Memorial Sloan Kettering Cancer Center, a 471-bed tertiary-care cancer hospital in New York, New York, where Luminex xTAG GPP (Luminex Diagnostics, Toronto, Canada) had been implemented in 2014 for the evaluation of stool from patients with diarrheal illness. The infection control department performs routine surveillance for all pathogens included on the panel. The Luminex GPP has been cleared by the FDA for 14 targets: 8 bacteria, 3 viruses, and 3 parasites. Among them, 5 are reportable to the New York State Department of Health, including Salmonellae and EH. Additionally, under New York State (NYS) and New York City (NYC) Health Code laws, laboratories are required to submit certain isolates for confirmatory testing. According to the protocol, isolate recovery for all samples positive by GPP for Salmonellae were attempted using Hektoen Enteric agar plates (Hardy Diagnostics, Santa Maria, CA). All positive raw stool samples on GPP are routinely saved and frozen at −80°C.

Cluster 1

From April 19, 2017, to May 4, 2017, 5 cases of EH were identified via routine laboratory evaluation of clinical cases of non–travel-related diarrhea using the Luminex xTAG GPP. Among them, 3 were adult hematopoietic stem cell transplant (HSCT) recipients; 2 cases were diagnosed 2 days apart on the same HSCT unit, while the third occurred in an HSCT recipient residing on another unit. The 2 additional cases occurred children with recent inpatient admission or exposure to our facility's pediatric day hospital.

Cluster 2

Between July 1 and July 5, 2017, 3 cases of infection with *Salmonella* spp were identified by GPP in hospitalized patients admitted to the same oncology unit. The first 2 cases occurred within 1 day in patients residing in adjoining rooms. The third case was identified 3 days later on the same unit. The 3 patients were cared for by different clinical teams with very limited overlap of support staff.

No clinical or ancillary staff reported contemporaneous diarrheal illness at the time of either cluster, and no common diet- or procedure-related links could be established among the cases. All patients received targeted antibacterial or antiparasitic therapy for the identified pathogen. Clinical and demographic characteristics of all cases are described below (Table 1).

RESULTS

Testing

The mean overall inpatient and outpatient GPP testing volume per month for 2017 was 317, compared to 311 for 2016 (Figure 1). This difference was not significant ($P = .60$; 95% CI, −33.8 to 21). No selective increase in testing was detected among the units with cases. Notably, the 3 cases of *Salmonella* identified on GPP in October 2017 were identified in outpatients without known prior temporo-spatial overlap. One of

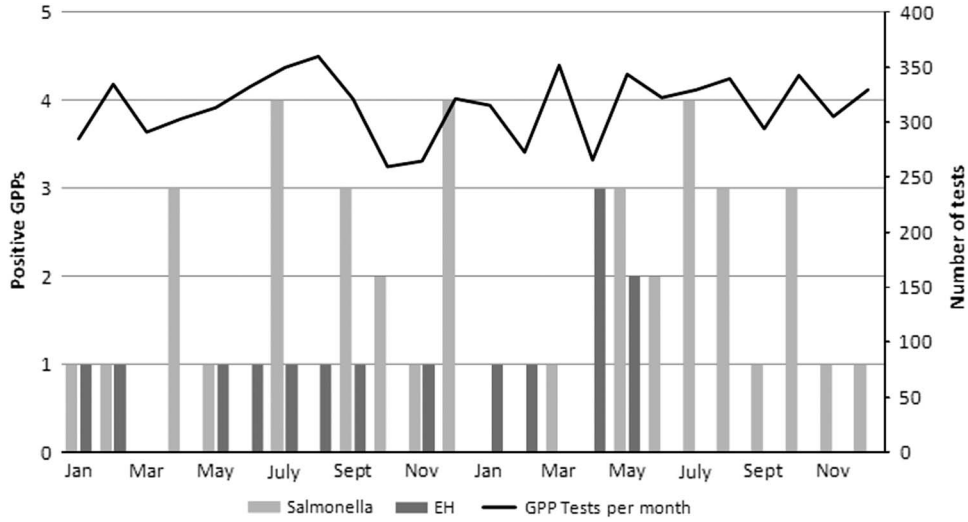

FIGURE 1. Salmonellae- and *Entamoeba histolytica*–positive GPPs and overall tests per month January 2016 through December 2017.

these patients remained GPP positive in December. No copathogens were identified on GPP or by other routine clinical diagnostic testing (Table 1).

Retesting

A second analysis of the positive specimens was performed with another FDA-approved multiplex assay, FilmArray (BioFire Diagnostics, Salt Lake City, UT). Residual samples, when available, were also sent to the New York City and/or New York State Department of Health for additional confirmatory testing, which included EH and *E. dispar* polymerase chain reaction (PCR; suspected EH cases) and stool culture (suspected Salmonellae cases). These alternative methods did not confirm EH or *Salmonella* spp in any of the respective specimens. Confirmatory testing results are summarized in Table 1.

DISCUSSION

The clusters described here were initially compelling because possible outbreaks because of corroborating gastrointestinal symptoms and close temporo-spatial proximity among cases. Clinical symptoms of both *Salmonella* and EH are nonspecific and can mimic other types of colitis common among oncology patients such as *Clostridium difficile* colitis, cytomegalovirus colitis, and gastrointestinal graft-versus-host disease. Further investigation revealed no epidemiologic exposure for the identified pathogen among all patients. While confirmatory results were in progress, clinicians elected to treat patients due to their immunocompromised state and lack of alternate microbiologic explanation before ultimately attributing symptoms to medication side effects. While no adverse events related to treatment of suspected EH or *Salmonella* were identified, these patients did endure unnecessary exposure to antibacterials and amebicides. Additionally, substantial personnel time and resources at the local and public health levels were required for the investigation of these pseudo-outbreaks. The associated costs may be an unanticipated expense for health systems that transition to multiplex testing from traditional methodologies.

Entamoeba histolytica, the causative agent of amebiasis, is a common parasitic infection transmitted to humans through contaminated food and water. While it is ubiquitous in many parts of the world, it remains an uncommon cause of diarrheal illness among hospitalized patients in developed countries. The clinical manifestations of intestinal EH infection can range from asymptomatic colonization to severe colitis. For the xTAG GPP, poor specificity for EH was recognized in an early evaluation of the assay for multipathogen testing.[5]

Salmonellae, gram-negative bacteria, frequently cause foodborne gastrointestinal illness. Disease manifestations range from self-limited diarrheal illness to bacteremia and invasive infections especially among immunocompromised hosts. Hospital-based outbreaks due to Salmonellae have been reported, and the occurrence of any cluster should be promptly investigated.[6,7]

Multiplex panels have several distinct advantages, especially in terms of turnaround time from sample collection to actionable results and the limited need for specialized laboratory training. However, these advantages may be undercut by real-world performance in certain patient populations. Currently, data are limited in the reported literature on the real-world performance characteristics of GPP and their associated clinical implications in the evaluation of diarrheal illness in developed nations or among immunocompromised hosts. The specificity issue with Salmonellae and EH detection

TABLE 1. Patient Demographics and Gastrointestinal Pathogen Confirmatory Testing

Case	Age/ Gender	Underlying Disease (+ t-HSCT)	Signs/ Symptoms	Chemotherapy	Treatment	Luminex Result[a]	BioFire GPP Result	Acid Fast Stain	*Giardia* FA	*Cryptosporidium* FA	Trichorme Stain	*E. dispar* PCR	EH PCR
Entamoeba histolytica													
1	42/M	AML (Y)	Diarrhea	Y	Metronidazole, parmomycin	+	−	−	−	−	−	−	Cluster 1
2	66/M	AML (Y)	Diarrhea, colitis on imaging	Y	Metronidazole, parmomycin	+	−	−	−	−	−	−	
3	65/M	DLBCL (Y)	Diarrhea	Y	Metronidazole, parmomycin	+	−	−	−	−	−	−	
4	8/M	ALL (N)	Diarrhea, abdominal pain, fever	Y	Metronidazole, parmomycin	+	−	ND	ND	ND	ND	ND	ND
5	5/F	ALL (Y)	Diarrhea, abdominal pain	Y	Metronidazole, parmomycin	+	−	ND	ND	ND	ND	ND	ND
						Initial Luminex GPP[a]	Luminex GPP (repeat)[a]	BioFire GPP	MSK culture[b]		NYC & NYS DOH culture		
Salmonella													
1	25/M	GCT (Y)	Diarrhea, abdominal pain, fever	Y	β-lactam	+	−	−	No growth		No growth		Cluster 2
2	68/F	Lung (N)	Diarrhea	Y	β-lactam	+	−	−	No growth		No growth		
3	41/F	ATLL (Y)	No symptoms	N	β-lactam	+	−	+	No growth		ND		

NOTE. HSCT, hematopeotic stem cell transplant; GPP, gastrointestinal pathogen panel; FA, fluorescent antibody; PCR, polymerase chain reaction; EH, *Entamoeba histolytica*; AML, acute myeloid leukemia; DLBCL, diffuse large B-cell lymphoma; GCT, germ cell tumor; ATLL, adult T-cell leukemia/lymphoma; MSK, Memorial Sloan Kettering; NYC & NYS DOH, New York City and New York State departments of health; ND, not done.

[a]All Luminex median fluorescence intensity results exceeded the manufacturer's thresholds for a positive result.

[b]Overall Salmonellae recovery in culture from January 2016 to December 2017 was 27% (10 of 37 isolates).

was highlighted in a small report from Netherlands, in which 11 of 43 additional positives using xTAG GPP could not be confirmed, including 5 EH and 4 *Salmonella* cases.[8] These results, coupled with those of our study, support the need for confirmatory testing in epidemiologically implausible circumstances. A large health-system analysis of GPP implementation noted the large number of positive results of uncertain clinical significance as the main detraction to wide-scale implementation of these testing strategies, while others have identified GPP use in hospitalized patients as a potential diagnostic stewardship target.[9,10]

In summary, clusters of a single pathogen identified by multiplex GPP diagnostic assay require investigation to confirm the likelihood of a true outbreak, especially in the absence of convincing epidemiologic data for exposure to the identified pathogen. Further evaluation of GPP diagnostic assays is necessary to document the true sensitivity and specificity of these multiplex assays in real-world clinical settings, especially among specialized patient populations.

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