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Case Report

Multiplex PCR testing for travelers' diarrhea—friend or foe?

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With the advent of high-throughput multiplex DNA extraction PCR diagnostic modalities for the diagnosis of infectious diseases, particularly gastrointestinal enteric infections, the increased sensitivity and specificity of this modality has been hailed by most as an advance in our ability to make specific etiological diagnoses in acute and chronic gastroenteritis and travelers' diarrhea (Platts-Mills et al., 2012; Raich and Powell, 2014). The potential advantages and drawbacks of this modality of testing are illustrated by the following case.

A 59-year-old Norwegian man, with residence in Scotland, traveled as part of a work assignment to several South American countries in early 2016. The patient is a member of the entourage of a prominent rock and roll band that traveled through South America on tour. Although the members of the entourage stay in 5 star hotels, there are multiple other exposures to local food and beverages and local populations. These include 'local catering' at the performing venue (usually a large stadium), which is set up to feed many people in a short period of time. In addition, there are exposures to thousands of local individuals, not just at the concert venue, but also at various points along the way.

His travels took him to Los Angeles in the USA, Santiago in Chile, Buenos Aires in Argentina, Montevideo in Uruguay, Rio de Janeiro, Sao Paolo, and Porto Alegre in Brazil, and Lima in Peru, before he arrived in Bogota in Colombia 58 days after the tour began. He was well until his arrival in Bogota, after spending 3 days in Lima. On the first night in Bogota he awakened in the middle of the night with nausea and vomiting and vomited for about 3 hours. He felt tired, but otherwise well the next day, and ate cautiously. He remained well until the fourth day in Bogota, when he developed the acute onset of watery diarrhea associated with lower abdominal cramping. Symptoms were present for approximately 4 hours when he decided to take one dose of ciprofloxacin 500 mg. Feeling no better 2 hours later, he switched to azithromycin 500 mg, one dose, and began to feel better over the course of the next 3–4 hours, although unformed stool remained for 24 hours. He then traveled to Mexico City and on the second day after arrival, he felt nauseated and had intermittent soft stool. By the next day his symptoms had resolved. Taking advantage of a break in his work schedule, he traveled to Guatemala for a few days. Upon arrival he began to have intermittent watery, semi-formed diarrhea with nausea, belching, and burping. Low-level and intermittent symptoms continued. Upon his return to Mexico City, a stool sample was obtained in Cary–Blair medium and was sent to New York for analysis. The BioFire FilmArray GI panel showed the presence of five pathogens: enteroaggregative *Escherichia coli* (EAEC), enteropathogenic *E. coli* (EPEC), Cryptosporidium, Giardia, and sapovirus (Figure 1).

This case highlights the advantages and potential disadvantages of high-throughput multiplex DNA extraction PCR stool analysis. Within 1 hour of its arrival in New York, the stool specimen results were available. Compared to historical methods of diagnosis, including stool culture and stool microscopy, this represents a distinct advantage (Jones, 2012; McAuliffe et al., 2013). In addition, in terms of completeness, three of this patient's pathogens would not have been discovered with conventional modalities of diagnosis. Diarrheagenic *E. coli* such as EPEC and EAEC are typically not tested for on bacterial stool culture (McAuliffe et al., 2013). In addition, viral agents of gastroenteritis such as sapovirus are not routinely tested for either (McAuliffe et al., 2013). In addition, Giardia and Cryptosporidium are notoriously difficult to diagnose with stool microscopy because of intermittent shedding of parasites and the need to evaluate multiple specimens (McHardy et al., 2014).

Therefore, in some respects, this diagnostic modality can make the clinician look like a diagnostic genius; however, on the other hand, it is still necessary for good clinical judgment to prevail to guide the management of such patients, especially when multiple

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FilmArray ®
GI Panel

BIOFIRE
www.BioFireDx.com

Run Summary			
Sample ID:	Patient's Name	Run Date:	21 Mar 2016 10:49AM
Detected:	Enteroaggregative *E. coli* (EAEC) Enteropathogenic *E. coli* (EPEC) *Cryptosporidium* *Giardia lamblia* Sapovirus	Controls:	Passed

Result Summary	
Bacteria	
Not Detected	*Campylobacter*
Not Detected	*Clostridium difficile* toxin A/B
Not Detected	*Plesiomonas shigelloides*
Not Detected	*Salmonella*
Not Detected	*Vibrio*
Not Detected	*Vibrio cholera*
Not Detected	*Yersinia enterocolitica*
Diarrheagenic E. coli/Shigella	
✓ Detected	Enteroaggregative *E. coli* (EAEC)
✓ Detected	Enteropathogenic E. coli (EPEC)
Not Detected	Enterotoxigenic *E. coli* (ETEC) lt/st
Not Detected	Shiga-like toxin-producing *E. coli* (STEC) stx1/stx2
Ø N/A	*E. coli* O157
Not Detected	Shigella/Enteroinvasive *E. coli* (EIEC)
Parasites	
✓ Detected	*Cryptosporidium*
Not Detected	*Cyclospora cayetanensis*
Not Detected	*Entamoeba histolytica*
✓ Detected	*Giardia lamblia*
Viruses	
Not Detected	Adenovirus F 40/41
Not Detected	Astrovirus
Not Detected	Norovirus GI/GII
Not Detected	Rotavirus A
✓ Detected	Sapovirus

Run Details			
Pouch:	GI Panel v2.1	**Protocol:**	Stool FA v2.3
Run Status:	Completed	**Operator:**	
Serial No.:	xxxx4437	**Instrument:**	ITI FA "FA2094"
Lot No.:	245015		

Figure 1. The patient's Film Array GI Panel results.

pathogens are present and the relative contribution of each to the patient's illness must be taken into account.

In this particular case, the author's approach to the five pathogens discovered would be first to look at the various elements of his clinical presentation, keeping in mind the fact that it was quite possible that these pathogens were all acquired during the same contaminated meal but had differential clinical and temporal manifestations due to the different incubation periods of bacterial, viral, and parasitic microbes.

The initial illness that occurred the first night in Bogota was a vomiting illness which lasted 3 hours. This most likely represented a viral gastroenteritis, in this case sapovirus. The onset a few days later of cramping and watery diarrhea is suggestive of diarrheagenic *E. coli*, in this case the EPEC and EAEC. The onset several days later of low-level loose stool and intermittent symptoms is suggestive of a protozoan pathogen, such as Cryptosporidium or Giardia.

In this case, it was suspected that the catering at the Stadium in Lima, Peru, the stop immediately prior to the stay in Bogota, Colombia, was the source of this patient's infections. Thirty-eight of the 75 people in the entourage developed a diarrheal illness upon arrival in Bogota and multiplex DNA extraction PCR was done on 10 of them, including this index patient. Although there were a number of different pathogens found in the other tested patients, there was no result as dramatic or extensive as this patient's.

Although the formulation of the clinical with the microbiological as outlined here is conjecture, it is believed that this type of analysis is important when confronted with this type of laboratory result. One of the challenges of molecular diagnostics is to be able to reconcile the clinical with the stool findings in order to know what to treat and what not to treat.

The advantages of DNA extraction PCR include the following: very rapid results, very sensitive and specific diagnostic capabilities, and the ability to diagnose pathogens that have been relatively undiagnosable until now. On the other hand, this technology will pick up pathogens that are non-viable and pathogens that may not be the active or most important cause of the patient's diarrhea, and good clinical judgment must be exercised in determining the management of and treatment approach to these patients (Platts-Mills et al., 2012).

In general, when diarrheagenic *E. coli* are found in mixed infections, if symptoms are no longer present or mild, or associated

with other more likely causes of diarrhea such as viruses or protozoan parasites, it is justifiable to withhold antibiotics and either not treat (if virus) or target the parasite if the clinical illness is consistent. In this case, the patient had already self-treated with one dose each of a fluoroquinolone (ciprofloxacin) and azithromycin, which may have mitigated the clinical illness from the diarrheagenic *E. coli*. Since this was the patient's only treatment, once these results became available he was treated with nitazoxanide, which theoretically should cover both Cryptosporidium and Giardia. In fact the patient did feel better within 5 days of starting this medication.

Unfortunately approximately 4 weeks later, the patient began to have constipation, abdominal bloating, and gas, and was diagnosed with small intestinal bacterial overgrowth/post-infectious irritable bowel syndrome (IBS), which persisted for another 6 weeks. He was treated with rifaximin 550 mg three times a day for 14 days before it resolved. He has not had a recurrence of the gastrointestinal symptoms since.

Interestingly this patient, on another multi-city trip in 2015, acquired a protracted gastrointestinal illness after visiting Dallas, Texas, and was found to have Cyclospora, also diagnosed by the FilmArray GI Panel. A local outbreak of Cyclospora was reported in Dallas at around the same time.

Traditionally enteric infection is conceptualized as a binary state. The pathogen is either present in the gastrointestinal tract or not. Molecular diagnostics may provide a more nuanced picture of infection and understanding of the concept of pathogenesis by requiring us to reconsider basic concepts of colonization, infection, and disease. As noted here, a potential drawback of culture-independent diagnostics is that, by identifying multiple pathogens in association with a patient's illness, it may be unclear which of these organisms is actually responsible for the clinical picture. This methodology of diagnosis does not discriminate between viable and non-viable organisms and may pick up microbes at non-pathogenic levels. Due to high rates of asymptomatic carriage in developing countries, this may be a problem and the relative importance of each pathogen may be unclear (Raich and Powell, 2014).

These limitations notwithstanding, this case illustrates both the utility and pitfalls of multiplex DNA extraction PCR. As we become more familiar with this technology and are able to accumulate more diagnostic as well as outcomes data, the interpretation of the data and their clinical utility will likely increase.

Conflict of interest

None.

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