Molecular diagnostic practices for infectious gastroenteritis

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To the Editor: Diarrheal disease is characterized by loose, watery stools or the frequent need for a bowel movement, and infectious gastroenteritis is a major cause of diarrheal illness worldwide. Despite improvements in public health and the economy, diarrheal disease remains a major public health problem with an associated burden.[1] Notably, most cases of infectious gastroenteritis are associated with contaminated food and water sources containing infectious agents. Many kinds of infectious pathogens, including bacteria (*Escherichia coli*, *Salmonella,* and *Shigella*), viruses (*Rotavirus, Adenovirus,* and *Norwalk*), and protozoa (*Giardia*, *Cyclospora,* and *Isospora*), cause infectious gastroenteritis.[2] A comprehensive clinical assessment of patients with infectious gastroenteritis is critical to establish an effective approach to the initial diagnostic tests and treatment. In addition to the clinical and exposure history, the stool characteristics of patients with infectious gastroenteritis can provide important clues to possible causes, but rapid and specific testing of the pathogen also contributes to the diagnosis of infectious gastroenteritis. Current pathogen testing methods, including stool culture, antigen detection, and ova and parasite microscopy, involve a number of procedures, and findings may take up to 2 to 3 days to become available. These methods also have limitations such as being labor-intensive, requiring certain laboratory staffing, training, and resource. What's more, positive yield of bacterial pathogens is known to be very low. Previous studies have shown 2.4% to 32% positive rates in stool cultures and even lower rates in cultures from intensive care unit patients (0.1%).[3]

With the emergence of new molecular detection technologies, diagnostic tools for infectious gastroenteritis have undergone several innovations from scatological cultivation to next-generation sequencing (NGS), leading to improvements in the efficiency of infectious gastroenteritis pathogen diagnosis. Several molecular diagnostic methods relying on nucleic acid amplification and detection have been developed. Currently, there are several commercially available multiplex polymerase chain reaction (PCR)-based testing platforms. Each platform simultaneously evaluates stool specimens for the presence of multiple pathogens, including bacteria, viruses, and parasites.[4] Detailed information on these four panels is listed in Table 1. Recent studies have shown that these tests could generally correctly identify pathogens detected by conventional testing; moreover, they also generate a considerable number of additional positive results of unclear clinical importance.

In addition to multiplex platforms, there are also single PCR-based platforms that target specific pathogens. Considering the clinical and epidemiological relevance, we focus only on the detection of toxigenic *Clostridium difficile* (*C. difficile*) here, as the leading cause of hospital-acquired infection. There are currently several molecular *C. difficile tes*ts, including Xpert *C. difficile*/Epi (Cepheid), illumigene *C. difficile* (Meridian Bioscience, Cincinnati, OH, USA), GeneOhm Cdiff PCR (BD Diagnostics, San Diego, CA, USA), and Simplexa *C. difficile* Direct (Focus Diagnostics, San Diego, CA, USA) and so on. Paitan *et al*[5] compared six commercial molecular tests for rapid detection of toxigenic *C. difficile*. All six assays resulted in high diagnostic values with >90% sensitivity and close to 100% specificity. However, the high sensitivity of molecular tests also raises concerns about overdiagnosis and overtreatment. Some patients with a positive molecular test result and a negative toxin immunoassay test result had outcomes comparable to those of patients without *C. difficile* by either method. Therefore, the exclusive reliance on molecular tests for *C. difficile* diagnosis without tests for toxins or host response is likely to result in overdiagnosis, overtreatment, and increased health care costs. We also implemented the FilmArray GI panel and Xpert *C. difficile/Epi* in our center. The positive rates of these two platforms in 2019 were 25.0% and 26.7%, respectively. The most detected pathogens in the FilmArray

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Table 1: Characteristics of different platforms for detection of common enteric pathogens.

Features	xTAG (Luminex)	FilmArray (BioFire Diagnostics)	Fecal Pathogens B (AusDiagnostics)	QIAstat-Dx (QIAGEN)
Number of pathogens	15	22	15	24
Pre-treatment time (min)	30–45	2	45–60	2
Turnaround time (h)	5	1	3–4	1
Throughput (samples/run, including controls), *n*	96	1	24	1
Starting material	100 μL of fresh or newly frozen stool (raw or in transport medium)	200 μL of Cary-Blair stool	Fresh stool	200 μL of Cary-Blair stool
Detection method	Bead hybridization following PCR	Melting curve following nested PCR	Melting curve following nested PCR	Melting curve following real-time PCR
System	Open (not an integrated system, requiring DNA extraction before loading for analysis and post PCR handling)	Closed (fully integrated system including DNA extraction, PCRs, and detection)	Open (DNA extraction required before loading for analysis)	Closed (fully integrated system including DNA extraction, PCRs, and detection)

PCR: Polymerase chain reaction; DNA: Deoxyribonucleic acid.

GI panel were *C. difficile, Norovirus*, and *Salmonella*, which is consistent with the epidemiological data.

NGS is characterized by the ability to rapidly and deeply sequence mixed DNA or RNA genomes and has already had a substantial impact on our understanding of the epidemiology of many diarrhea-associated pathogens. With NGS becoming cheaper, it has huge potential in routine diagnostics.[6] Recently, whole-genome sequencing was used to examine the origin of the *Salmonella* outbreak in China.[7] In addition to providing pathogen identification, direct sequencing can also offer extensive additional information on, for instance, virulence and resistance genes, or the presence of other pathogens, for example, fungi and DNA viruses. As prices and turnaround times for NGS are declining, this type of analysis may become available in first-line diagnostic setups offering rapid diagnostics and providing valuable information to help direct patient treatment.

While infectious gastroenteritis is self-limiting for the most part, the identification of a pathogen by culture is required for the management of patients with severe or prolonged diarrhea, symptoms consistent with invasive disease, or a history that may predict a complicated disease course. Furthermore, non-culture-based diagnostic methods, particularly nucleic acid amplification tests, often as multiple PCR tests in a syndromic panel, are advancing the modern medical microbiology laboratory. Despite the evolution of diagnostics, the optimal use of these diagnostic tests requires the recognition of their limitations and judicious use of supporting clinical and laboratory evidence.

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Conflicts of interest

None.

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