EDUCATIONAL COMMENTARY – TESTING ENTERIC PATHOGENS

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LEARNING OBJECTIVES

On completion of this exercise, the participant should be able to

- describe the etiology and impact of gastrointestinal infections in the United States and worldwide;
- summarize the current methods for detection of enteric pathogens;
- identify the limitations of the current methods for detection of enteric pathogens;
- recognize the emerging role of molecular diagnostics in the diagnosis of infectious disease;
- explain the advantages of molecular testing for the detection of enteric pathogens; and
- identify the limitations and potential consequences of transitioning from conventional laboratory testing for enteric pathogens to molecular techniques.

Introduction

Infections of the intestinal tract can be caused by a variety of bacterial, viral, and parasitic agents. These infections are transmitted by contaminated food or water and typically result in diarrhea. Most healthy individuals will experience a self-limited illness that lasts a few days, yet in others, severe dehydration, bacteremia, chronic symptoms, and serious complications can develop, including malnutrition and death. Worldwide, there are approximately 1.7 billion cases of diarrheal disease each year, responsible for the death of 760,000 children.\(^1\) Although mortality from diarrheal disease is low in industrialized nations, these illnesses remain a major public health burden. In the United States, the cost of hospitalization due to gastrointestinal infections exceeds $6 billion dollars annually.\(^2\) Owing to similar symptoms, it is challenging to determine the etiologic agent, and in 80% of diarrhea cases the cause is not identified, often resulting in inappropriate treatment.\(^2\)

Current Diagnostic Methods

The differential diagnosis of diarrheal illness is extremely broad: a multitude of infectious agents or preformed toxins are potentially responsible. Evaluating patients for all possible causes would be extremely costly, so the physician must rely on clinical clues to determine the most appropriate workup. Clinical history including travel, dietary, and immune status along with a physical examination will guide the physician to appropriate laboratory testing.\(^3\) The Infectious Diseases Society of America (IDSA) recognized the complexity of this situation, and in 2001 released guidelines to assist in the workup of diarrheal illnesses. The guidelines provide an algorithm to determine the most appropriate laboratory testing in the workup and treatment of these patients.\(^4\)
Currently, the diagnostic process is complex: several methods are involved and they are usually spread across multiple departments or even laboratories. The physician must order several tests, and each test may have specific collection/transport requirements. The stool specimen that is received in the microbiology laboratory may then be split for testing in other departments or even packaged for shipment to a reference laboratory. Conventional testing utilizes culture methods to isolate bacterial pathogens and microscopy to detect parasites. Antigen-detection tests are also available for certain agents and toxins. Limited testing using traditional methodologies is available for viral infections.

**Limitations of Current Methods**

Traditional testing methods for detecting enteric pathogens have several limitations. Most bacterial pathogens are detected through culture techniques. Routine stool cultures are performed to detect the presence of *Salmonella* sp., *Shigella* sp., *Campylobacter* sp., and *E coli* O157. Most laboratories will also test for the presence of *Yersinia enterocolitica* or *Vibrio* sp. upon special request. Cultures take 3 to 4 days to complete, delaying diagnosis and leaving the patient at risk for untreated infections and for transmission of the disease to others. Normal fecal flora and pathogens can appear similar on culture media, requiring technical expertise and subjective interpretation. This results in a great deal of technical time spent working up negative stool samples. Although 95% of samples are negative, they still require as much time as a positive culture. In addition, *Campylobacter* species requires specialized conditions for growth in culture and is known to revert to a viable but nonculturable form. *Shigella* has reduced viability outside the body, further diminishing the sensitivity of culture methods.

Conventional testing for gastrointestinal parasites includes microscopy and stool antigen tests. Microscopy is technically demanding: it is time-consuming and relies on the experience and skill of the technologist. Sensitivity can also be low owing to intermittent shedding of protozoa; thus, it may be necessary to examine multiple samples.

The introduction of antigen testing to the battery of tests for enteric pathogens has been beneficial. This technique has been especially helpful in improving the detection of viral agents (*Norovirus*, *Rotavirus*), Shiga toxin–producing *Escherichia coli* (STEC), and protozoan agents (*Giardia*, *Cryptosporidium*, *Entamoeba histolytica*). Antigen testing is a fast and effective alternative but it is not available for all pathogens. In addition, sensitivity is limited and most assays detect a single pathogen.

**Molecular Methods**

Molecular diagnostics is significantly affecting the diagnosis of infectious diseases. There are now several FDA-approved nucleic acid amplification tests for bacterial, viral, and mycobacterial infections. Molecular assays consistently demonstrate superior sensitivity when compared with traditional diagnostic methods. For this technology to be clinically useful for the detection of enteric pathogens, the application will need to detect multiple pathogens in a single platform. This syndrome-based approach to molecular
testing is already available for the diagnosis of respiratory infections. The ability to detect as many as 20 bacteria and viruses in a nasopharyngeal specimen has revolutionized respiratory testing by providing physicians with a more comprehensive picture from a single specimen in about an hour. Rapid molecular multiplex testing is on the forefront of a revolution in enteric diagnostics. The FDA has recently approved molecular multiplex tests that detect and identify multiple gastrointestinal bacterial, viral, and parasitic pathogens simultaneously from a single patient sample.

The road to this accomplishment was paved by molecular studies conducted by various groups. A study by de Boer et al concluded that prescreening stool specimens with 2 real-time multiplex polymerase chain reaction (PCR) assays, followed by guided culture/microscopy of positive samples, increased the detection rate of enteric pathogens three-fold compared with that of conventional methods alone. Their findings also revealed that molecular screening detected mixed infections in a single sample more often than conventional methods. During the study, all samples were screened for multiple pathogens, even pathogens that were not requested by the physician. The parasite Giardia was discovered in patient specimens from which the physician had requested only bacterial cultures. Clearly, pathogens can be missed if physicians do not order appropriate testing, and the authors concluded that it is worthwhile to screen specimens for multiple pathogens. During an outbreak of diarrhea in Germany in 2011, Malecki and colleagues detected a novel strain of enterohemorrhagic E. coli using a PCR-based multiplex panel for gastroenteritis. The laboratory methods in use at the time did not allow recognition of this E. coli strain. These studies provide evidence that the use of multiplex molecular assays will improve diagnosis of enteric infections, increasing sensitivity and speed of detection.

The commercially-available molecular multiplex systems vary in the spectrum of targets available. Some manufacturers have designed a single panel with as many as 23 targets (bacteria including Clostridium difficile, viruses, and parasites) and others are designing more focused panels. There is controversy over which of these is the better approach. Those that include all potential targets (“shotgun approach”) eliminate the need for algorithms and decision making when ordering tests. Some believe that this approach is necessary because it will pick up all potential pathogens and unexpected agents will not be missed in the case that the physician does not request appropriate testing. Other manufacturers are using a more cost-effective approach and are designing focused panels that utilize clinical algorithms: enteric bacteria panel vs parasite panel vs hospital-acquired C difficile panel. Each laboratory will need to evaluate the needs of its own institution in choosing a system that provides the best approach for testing.
Advantages and Limitations of Molecular Methods

There are significant advantages to implementing multiplex molecular technology for detection of enteric pathogens. Most importantly, this will result in increased detection of pathogens in a faster timeframe, allowing physicians to institute directed therapy earlier. *Campylobacter* species are a leading cause of bacterial enteritis worldwide, and culture methods often have a low yield. Molecular methods result in greater detection of these bacteria. One study tested 127 stool specimens from patients with gastroenteritis and found that 18 specimens were culture-positive for *Campylobacter*, while PCR detected the pathogen in 58 specimens. Another advantage is the increased ability to detect multiple pathogens, which will allow physicians to recognize and treat patients with mixed infections. Recognition of unsuspected causes of gastroenteritis will potentially lead to a reduction in outbreaks.

A change from the traditional laboratory testing methods to molecular techniques will have a substantial impact on the laboratory as well. The workload in traditional testing is laborious and time-consuming. Molecular methods will reduce labor and material requirements and eliminate the subjective interpretation associated with culture techniques. Although molecular techniques are costly, their ability to detect multiple pathogens with one test panel is more cost-effective than performing multiple tests using conventional methods. This technology will create a single-stool workflow.

Although the use of molecular methods will positively affect the diagnosis of enteric infections, there are some limitations. One disadvantage of any nonculture method is that there is no isolate available for antibiotic susceptibility testing or for additional testing by public health laboratories. This will hinder the public health laboratories’ ability to provide subtyping for epidemiology purposes, in turn limiting their ability to study and characterize outbreaks. Another limitation of this single-stool workflow involves the detection of certain ova or parasites that are not available on molecular testing menus. Although these parasites are rare, they will require microscopy for detection. One last consideration in using molecular techniques for enteric pathogens is the significance of the findings when the method is unable to distinguish between viable and nonviable pathogens. The isolation of an enteric pathogen from stool culture is a clear indicator of viability. The significance of the nucleic acid of an enteric pathogen detected in a stool sample and its association with disease is not yet understood. Further studies are needed to address the relationship of these findings.

Molecular techniques can also detect additional bacterial species that are not found with traditional culture methods. Molecular assays have the ability to detect certain *Campylobacter* species not associated with human infections. The increased sensitivity of this methodology leaves open questions as to the significance of the findings. Current knowledge about acute gastroenteritis including diagnosis and epidemiology is based on traditional cultures and microscopic examination. The pattern may be changing as we move away from culture toward rapid non-culture methods.
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Conclusion
Molecular technology is making great strides in diagnosis of infectious diseases. Traditional laboratory testing methods relying on microbial morphology and growth variables are transitioning to a workflow structure where molecular testing is capable of identifying and characterizing pathogens. The molecular testing industry is marketing expanded menus and instruments that enable laboratories of any size to consolidate testing on a single platform, allowing more laboratories to institute this type of testing in-house. The ability to automate sample preparation, extraction, and amplification on a single system saves time and improves laboratory efficiency while improving standardization of test results.

Gastrointestinal infections will continue to inflict a significant toll on the health care system. The use of rapid molecular diagnostic tests for stool pathogens may provide a means to reduce the impact of this burden. Detection of multiple pathogens in a single test panel along with a rapid turnaround time have the potential to transform diagnosis and treatment of enteric infections.

REFERENCES AND SUGGESTED READING