Article:
Macfarlane-Smith, LR, Ahmed, S and Wilcox, MH orcid.org/0000-0002-4565-2868 (2018) Molecular versus culture-based testing for gastrointestinal infection. Current Opinion in Gastroenterology, 34 (1). pp. 19-24. ISSN 0267-1379

https://doi.org/10.1097/MOG.0000000000000405

Molecular versus Culture-Based Testing for Gastrointestinal Infection

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Abstract

Purpose of review

Molecular-based diagnostic methods for the detection of gastrointestinal pathogens are becoming increasingly commonplace in microbiology laboratories. This review aims to summarise recent developments in this field and discuss the clinical application and limitations of implementing these techniques.

Recent Findings

Recent evaluations of multiplex PCR assays show increased sensitivity when compared to standard microbiological culture-based methods. In addition to shorter turnaround times, assays can detect an increased repertoire of pathogens from a single specimen and provide useful information for infection prevention and control practices. However; there are many limitations associated with their use, including clinical interpretation of results and lack of concordance between different test panels. Newer technologies, such as metagenomic analysis, can provide comprehensive information useful to both patient management and public health surveillance.

Summary

Molecular techniques are capable of replacing culture in the diagnosis of gastrointestinal infections. However, whether all positive results represent true infection is still debateable, as is the clinical significance of identifying more than one pathogen. As it currently stands, microbiological culture remains vital for public health surveillance, monitoring antibiotic resistance and managing outbreaks**.**

Keywords

Gastrointestinal infection; molecular-based diagnosis; multiplex PCR; enteric pathogens

Introduction

Gastrointestinal infections are associated with a high incidence of morbidity (and mortality in developing world settings), particularly amongst children, and have important health, economic and social consequences for both communities and healthcare systems worldwide [1]. The majority of these infections are self-limiting and, as such, investigation and treatment are not required. However, in severe or protracted cases or in outbreak situations, identification of the aetiological agent can aid patient treatment, guide infection prevention and control practices and facilitate further epidemiological investigations.

Traditional diagnostic techniques for the routine detection of enteric pathogens, such as microscopy, culture and enzyme immunoassays (EIA), are both time-consuming and laborious. The lengthy turnaround times for standard microbiological culture of samples is not useful for clinicians and can have a significant impact on institutions, such as hospitals or nursing homes, where early detection and prevention of disease spread is crucial. Prolonged turnaround times may also limit the opportunity to intervene either at patient or cluster / investigation levels.

Current guidelines are not consistent on recommended practice for the laboratory diagnosis of gastrointestinal infections. For example, Public Health England's (PHE) syndromic algorithm for Gastroenteritis and Diarrhoea describes which infections should be considered according to the different clinical presentations; nucleic acid amplification tests (NAAT) are only recommended for the diagnosis of viral infections (norovirus, rotavirus, astrovirus and sapovirus) [2]. In contrast, the American College of Gastroenterologists recommend, where possible, the use of culture-independent methods in place of traditional culture based methods [3].

The introduction of molecular technologies into a clinical microbiology laboratory, such as multiplex polymerase chain reaction (PCR) assays, can benefit the diagnosis of gastrointestinal infection by facilitating

simultaneous detection of pathogens (bacterial, parasitic and viral) directly from faeces. Molecular-based diagnosis gives a laboratory the potential to increase sample throughput, increase the amount of information obtained from a single test and decrease sample turnaround times [4-10]. Additionally, simple workflows and a reduction in the need for technical expertise are appealing attributes of these diagnostic methods.

Evidence to Support Molecular-Based Diagnostic Testing

There are several commercially-available multiplex PCR assays, including EntericBio Real-Time Gastro Panel 2 (Serosep), FTD Gastroenteritis panels (Fast Track Diagnostics) and xTAG® Gastrointestinal Pathogen Panel (GPP; Luminex). Other options include complete testing platforms, for example, the BD MAX™ Enteric Bacterial Panel (BD), FilmArray® Gastrointestinal Panel (bioMerieux), and microarray systems, such as the NanoCHIP® Gastrointestinal Panel (Savyon Diagnostics).

As these tests become more prevalent in clinical microbiology laboratories, it is essential that adequate evaluation of molecular tests for the diagnosis of gastrointestinal infections is undertaken. Recent evaluation studies demonstrate that the use of molecular-based diagnostics increases the sensitivity of testing for some enteric pathogens, with overall high negative predictive values [4-10]. However, the UK National Institute for Health and Care Excellence (NICE) published their diagnostics guidance in January 2017 [11], based on a Diagnostic Assessment Report commissioned by the National Institute of Health Research (NIHR) Health Technology Assessment (HTA) Programme [12]. A meta-analysis was undertaken of the clinical and cost-effectiveness of three integrated multiplex PCR assays: xTAG® GPP, FilmArray™ Gastrointestinal Panel and the Faecal Pathogens B assay (AusDiagnostics). The comparator was the PHE syndromic algorithm for Gastroenteritis and Diarrhoea [2]. The final recommendations of the guidance state that there is presently insufficient evidence to recommend the routine adoption of such assays into diagnostic laboratories. It was commented that these tests show potential; however, further research is needed into their effect on health outcomes and resource use in clinical practice.

Whole-genome sequencing (WGS) has been shown to be useful in the evaluation of molecular diagnostic assays, in particular, to investigate the validity of positive test results that are not confirmed by culture [13]. WGS data from 1724 *Campylobacter* isolates was used to perform *in-silico* analysis of primers and probe from an existing 'in-house' PCR assay. The assay specificity was found to be high, with 99.7% of isolates correctly identified.

Conventional culture-based methods typically underestimate the presence of gastrointestinal pathogens. Molecular techniques can detect nucleic acid from organisms at very low levels within samples, leading to an overall increased positivity rate [4-10, 14]. GEMS (Global Enteric Multicentre Study) was a case-control study of diarrhoea in children <five years old in Africa and Asia, published in 2013 [15]. Three years later, a reanalysis of the samples was undertaken using quantitative real-time PCR (qPCR) and compared with the original results obtained using standard microbiological methods [14]. An underestimation of the incidence of certain pathogens, including *Shigella* spp., adenovirus 40/41, Shiga toxin-producing *Escherichia coli* (STEC) and *Campylobacter jejuni* / *C. coli* was evident. Using model-derived quantitative cut-offs, the majority of samples (>80%) showed pathogens at diarrhoea-associated quantities.

An important advantage of employing molecular diagnostics is their ability to enhance infection prevention and control interventions. In order to limit transmission, patients with suspected gastrointestinal infection should be managed in isolation, ideally in a side room with contact precautions (gloves and apron). The rapid turnaround time of molecular multiplex panels allows correct application of source isolation, thus, freeing up side rooms once the causative agent is found or, conversely, an infective cause has been ruled out [11, 16]. Prompt identification of the offending pathogen also ensures that appropriate epidemiological investigations can be initiated quicker, thus, preventing further cases. Furthermore, if required, early targeted treatment can be commenced.

Very few data exist on the use of molecular diagnostics for follow-up testing of patients with gastrointestinal infection. Park *et al* (2017) found that almost half of patients with initial positive results obtained with the FilmArray™ Gastrointestinal Panel remained positive for up to four weeks. The authors suggest that the use of molecular tests as a test of cure is not helpful to clinicians. It was also observed that follow up testing after an initial negative result is unlikely to offer new information and, therefore, is not recommended unless there is concern regarding the validity of the initial negative result [17].

Limitations Associated with the Use of Molecular-Based Diagnostic Testing

Although multiplex PCR technologies allow an increased number of pathogens to be detected using a single test, the assay must hold the capability to detect the organism in the first place. With commercially-available gastrointestinal multiplex PCR panels, the range of target pathogens is fixed and cannot be adjusted based upon the clinical picture of the patient. Novel 'in-house' assays are regularly described in the literature; these allow expansion and variation of the pathogen panels offered by commercial companies. Developers have the freedom to tailor the repertoire of pathogens targeted by the assay to suit their needs, often based upon local epidemiology [18, 19]. In some regions of the world, intestinal parasites are important pathogens, and are underestimated due to the lack of sensitive and accurate diagnostic methods. The majority of gastrointestinal panels only include a limited number of parasites, namely *Cryptosporidium*, *Entamoeba* and *Giardia*. A multiplex PCR assay targeting 20 intestinal parasites was developed and evaluated in Senegal [18]. The target pathogens were chosen based upon results from local epidemiological data. Mass deworming is frequently carried out in order to control infection with soil transmitted helminths (STH). The inclusion of STH in an 'in-house' multiplex PCR panel was shown to assist in the monitoring of the prevalence of STH post-treatment [19].

There is also a potential to overestimate disease burden, as molecular assays will also detect non-viable organisms, and asymptomatic carriage of enteric pathogens has been widely recognised in adult and paediatric populations [14, 15, 17, 20, 21]. In routine use, such overestimation of pathogenic versus

colonising microorganisms cannot, of course, be distinguished from false-positive results. Furthermore, as the repertoire of target pathogens detected by molecular assays increases, situations arise where no independent reference standard exists for certain organisms. As such, the accuracy (sensitivity and specificity) of an assay cannot be calculated [6]. This leads to uncertainty about the interpretation and clinical significance of certain results.

Freeman et al., (2017) performed a meta-analysis of 10 evaluation studies which each compared molecular gastrointestinal pathogen panel assays with standard microbiology testing [6]. It was concluded that, whilst high positive agreement with traditional diagnostic methods could be shown by certain studies, this was not the overall case. The authors suggest that the performance of these assays varies for different enteric pathogens. Concordance between different multiplex PCR panels was also found to be variable in a further study by Huang et al. (2016) [22]. Three multiplex PCR panels were evaluated: Verigene Enteric Pathogens Test (Verigene), FilmArrayTM Gastrointestinal Panel and xTAG® GPP. For rotavirus and Shigella spp., 100% concordance was seen between the FilmArrayTM and xTAG® GPP. Only 71% agreement was seen for rotavirus when the Verigene Enteric Pathogens Test and xTAG® GPP were compared. Much lower concordances were reported when the FilmArrayTM and Verigene Enteric Pathogens Test were compared (rotavirus: 71.4%, Shigella spp.: 95.3%). Sub-optimal performance issues in the molecular detection of Salmonella spp. have also been documented [5, 16]. In a large evaluation of the EntericBio Real-Time Gastro Panel 2, 19% of Salmonella spp. culture-positive samples were missed [5].

There will always be a necessity to perform other tests in conjunction with molecular assays. There are two prime examples. Firstly, the optimal diagnosis of *Clostridium difficile* infection (CDI) requires the detection of free faecal toxin, rather than the presence of (toxigenic) bacteria alone. The move away from toxin-detection to NAAT testing has resulted in increased CDI reporting, which has been confused in some reports claiming higher CDI incidence [23]. A recent European observational, systematic, prospective study highlighted the lack of standardised testing for CDI as is a potential confounder when comparing infection rates. It was observed that, although testing rates were similar between the centres included in the study,

significantly higher rates of CDI were seen in centres using standalone toxin tests or non-toxin detecting methods when compared to those adopting the recommended two-stage algorithm (NAAT/GDH EIA followed by cytotoxin testing) [24]. Thus, NAAT positive samples should be tested using a toxin assay to confirm CDI. Secondly, current molecular assays for enteric bacterial pathogens do not provide antibiotic susceptibility data, thus, samples testing positive, for example, for *Salmonella* and *Campylobacter,* will require additional culture and susceptibility testing.

It has been suggested that commercial molecular tests may have limitations in certain patient populations, highlighting the care that must be taken by a diagnostic laboratory when assigning tests. McMillen *et al*. (2017) looked at the diagnostic value of a multiplex PCR assay in an oncology patient population, who are at risk for invasive adenovirus disease [25]. The most common type detected in all samples, including stool, was human adenovirus (HAdV) C/2. Whilst multiplex respiratory panels are designed to target a wide range of HAdV types, the gastrointestinal panels primarily focus on HAdV F40/4. The authors conclude that gastrointestinal panel assays cannot be reliably used for diagnosis of adenovirus gastroenteritis in high risk patients.

Mixed gastrointestinal infections are typically not recognised with standard microbiological culture. By contrast, studies suggest approximately 20-50% of positive samples detected using molecular techniques have more than pathogen identified [4, 9, 14, 20, 26]. The combination of pathogens involved in these co-infections varies between studies. Murphy *et al*. (2016) reported Enteroaggregative *E. coli* (EAEC) and Enteropathogenic *E. coli* (EPEC), *C. difficile* and norovirus, EPEC and norovirus, *C. difficile* and EPEC, and EPEC and Enterotoxigenic *E. coli* (ETEC) as the most frequently seen pathogen combinations [26]. Whereas, in a prospective population-based surveillance study of American children aged <six years, *C. difficile* and *norovirus* most commonly occurred together [20]. In reanalysing results from the GEMS study, 38.9% of cases were found to have mixed infections with ≥2 pathogens, but no specific co-occurrences were observed [14]. This variability is likely to be influenced by the target pathogens included in the molecular panels, specificities of the assay targets, and local epidemiology and disease burden. A

case-control study of children under six year olds in Ghana found that 27.8% of the symptomatic cases and 31.4% of the asymptomatic controls had two pathogens detected by the xTAG® GPP assay. This suggests that these pathogens are more likely to be colonising the gastrointestinal tract rather than causing infection [27]. Currently, the clinical significance of these mixed infections, as well as how best to manage them, remains largely unknown.

Disease Surveillance and Public Health Implications

Knowledge about gastrointestinal infections and the epidemiology of bacterial enteric pathogens is derived predominantly from investigations using culture-derived isolates. A consequence of the increased use of molecular-based diagnostic tests is the absence of a cultured pathogen. This restricts the further work that can be performed by a public health laboratory, including antimicrobial sensitivities, isolate characterisation and epidemiological investigations. It has been suggested that culture as a reflex to a positive molecular result needs to be adopted [28, 29]. Marder *et al*., (2017) found that of the infections diagnosed in the United States (U.S.) using molecular methods, a reflex culture was performed on only 60% of samples [29]. Attempted culture in a reflex manner will invariably lead to a delay in time to inoculation and could affect the success of isolating the microorganism. Van Lint *et al.* (2016) found that a delay of three days increased the likelihood of culture failure, which could potentially have a detrimental effect on outbreak control [4].

As previously mentioned, the positivity detection rate is higher with molecular diagnosis, which can influence reported epidemiological trends [30, 31]. In the U.S., the increased use of culture-independent detection tests (CIDTs) in laboratories, such as EIA and multiplex PCR panels, has been paralleled by significant increases in the reported incidence of *Cryptosporidium* (+45%), STEC (+43%) and *Yersinia* (+91%) [29, 32]. Overall, the number of *Campylobacter, Salmonella, Shigella* and STEC infections diagnosed by CIDTs only (without confirmation) increased by 122% [29].

The Future of Molecular Diagnostics

The field of clinical microbiological diagnostics is rapidly evolving. Currently, the Centers for Disease Control and Prevention (CDC) is coordinating the introduction of WGS and advanced bioinformatics into U.S. public health laboratories/systems for the enhancement of infectious disease prevention and control [33].

In the future, metagenomic techniques may be routinely used for the diagnosis of infections. Metagenomic analysis has been used to provide an insight into the presence and diversity of pathogenic organisms in a human faecal specimen [34]. Culture-independent shotgun metagenomics were applied to two foodborne outbreaks in the U.S. [35]. The analysis demonstrated that the outbreaks were caused by different *Salmonella* Heidelberg genotypes. The results were consistent with culture-based typing methods. In a further study, stool specimens obtained from patients with persistent diarrhoea were subjected to microscopy, multiplex PCR, and metagenomic analysis [36]. Across the samples, metagenomic analysis identified between 8-11 potential enteric pathogens and also showed the presence of antibiotic resistance genes. Metagenomics provides a promising alternative to current methods and accumulates all clinically relevant information.

Conclusion

There is no doubt that the introduction of molecular-based assays into the routine diagnostic laboratory will continue, and will enable more rapid and sensitive detection of gastrointestinal pathogens. The decision of whether to adopt such an assay and which one to select will depend on a number of factors, including assay performance, repertoire of target organisms, cost, workflow, throughput and patient population to be tested and will, ultimately, depend on laboratory and service priorities. Thorough evaluation of these methods must be undertaken, with consideration given to the unexpected

consequences on organism detection, surveillance and the ability to perform further pathogen characterisation.

Key points

- The introduction of molecular-based assays into the routine diagnostic laboratory will continue, and will enable more rapid and sensitive detection of gastrointestinal pathogens.
- There are advantages and disadvantages to their use in clinical laboratories
- Thorough evaluation of these methods must be undertaken, with consideration given to the unexpected consequences on organism detection, surveillance and the ability to perform further pathogen characterisation.

Acknowledgements

None

Financial Support and Sponsorship

None

Conflicts of Interest

Professor Mark Wilcox has previously received honoraria from bioMerieux. The remaining authors have no conflicts of interest.

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