SYSTEMATIC REVIEW

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Efficacy of Molecular Methods for the Diagnosis of Enteropathogenic Microorganisms Associated with Diarrhoea: A Systematic Review

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ABSTRACT

Background & Aims: Diarrhoea has a significant health impact and requires accurate diagnostic approaches, despite the limitations of many existing methods. This review examines various molecular techniques aimed at facilitating the rapid diagnosis of diarrhoeal diseases caused by bacterial, viral and parasitic pathogens.
Methods: A comprehensive systematic literature review was conducted using six prestigious databases, including WOS, Scopus, Science Direct, Embase, PubMed and LILACS Plus. This rigorous approach allowed the synthesis of reviews on molecular diagnostic techniques for infectious diarrhoeal diseases.
Results: This research began with a careful systematic literature review of 2,760 scientific papers published in the last ten years (2014-2024), culminating in the inclusion of 18 studies following rigorous screening and eligibility criteria. In particular, multiplex polymerase chain reaction (PCR), isolation of genomic DNA from stool samples and the Luminex xTAG Gastrointestinal Pathogen Assay emerged as the predominant molecular methods. These techniques demonstrated remarkable consistency in sensitivity, specificity and rapid diagnostic capability, particularly in the context of acute infectious diarrhoeal diseases. They also demonstrated the ability to simultaneously detect and identify multiple pathogens, including bacteria, viruses and parasites.
Conclusions: The use of multiplex real-time PCR assays has not only improved the detection rates of enteropathogens, but has also revealed previously unrecognised gaps in the diagnosis of infectious diarrhoea. Our study highlights the importance of using molecular methods for comprehensive diagnosis in terms of sensitivity, specificity and rapid diagnostic capability. This will provide healthcare professionals with timely and accurate diagnostic data, enabling more effective treatment strategies for this public health problem.

Key words: diarrhoeal pathogens – dysbiosis; enteropathogens – gut microbes – infectious diarrhoea – molecular diagnosis – sequencing.

Abbreviations: PCR: polymerase chain reaction; TTR: time-to-result.

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INTRODUCTION

Diarrhoea is a pervasive global health challenge that affects people from all walks of life [1]. Despite significant advances in medical science, conventional diagnostic methods often suffer from limitations such as long turnaround times, high costs and intermittent inaccuracies [2]. This condition places a significant burden on the global public health infrastructure, particularly in low- and middle-income countries and across diverse populations [3, 4]. Despite concerted efforts in health services and interventions, diarrhoeal diseases remain a major cause of morbidity and mortality, particularly among children under five. Shockingly, this population bears the brunt, with diarrhoea being the second leading cause of death, responsible for 15% of deaths in this age group [1, 5, 6].

The staggering annual toll of 1.6 million deaths is overwhelmingly in the developing world, underscoring the need for improved diagnostic and therapeutic paradigms. The impact goes beyond individual health outcomes, exerting a profound economic toll, straining already scarce resources and hindering the achievement of universal access to healthcare [1, 5]. Moreover, the financial burden associated with treatment exacerbates the socio-economic hardship of affected households, reinforcing the cycle of poverty [3].

Diarrhoea manifests in a variety of presentations influenced by various factors such as pathophysiology (osmotic, secretory,

inflammatory, motility), duration (acute/chronic) and underlying causes (pathogens, treatments, diseases). Rapid and accurate diagnosis of the underlying aetiology is essential for effective intervention and treatment [7, 8]. However, conventional diagnostic methods, such as stool culture and histological examination of biopsy samples, are expensive, dependent on operator expertise and time-consuming, with a long time-to-result (TTR). Meanwhile, traditional approaches such as patient history, physical examination and basic laboratory tests have limitations in terms of accuracy, speed, and cost-effectiveness [9, 10].

In the field of diarrhoea investigation, techniques that facilitate rapid and accurate diagnosis play a key role in effective management and prevention of complications. A key advantage is the ability to provide timely results without exposing patients to invasive risks or discomfort [11]. Traditional methods such as stool culture, while reliable, are inherently time-consuming, often requiring several days for microbial growth, thereby delaying diagnosis. The advent of innovative approaches capable of providing accurate and rapid results holds great promise for improving diagnostic efficacy and subsequent management [12, 13].

Emerging non-invasive techniques, such as stool and blood biomarker assessment, and various imaging modalities offer viable alternatives to invasive procedures such as colonoscopy [11, 13]. These modalities have the potential to expedite diagnosis at a more affordable cost and with lower associated risks, thereby reducing hospitalisation rates and minimising complications. Ongoing research is investigating whether these non-invasive approaches can match or exceed the diagnostic accuracy of conventional invasive methods. Successful implementation of such non-invasive techniques could effectively avoid the delays, costs and inherent risks associated with invasive testing [14].

To date, there is a noticeable lack of systematic reviews in the literature that comprehensively evaluate the sensitivity, specificity and rapid diagnostic capabilities of molecular methods. This gap highlights the need to compare conventional diagnostic techniques with advanced molecular methods, with the overall objective of refining diagnostic accuracy and efficiency. Thus, the aim of this review is to fill this gap by critically evaluating and comparing the efficacy, accuracy and speed of diagnostic approaches for the identification of enteropathogenic microorganisms associated with infectious diarrhoea. In doing so, we aim to delineate the most optimal diagnostic strategy from the spectrum of available methods, thereby contributing to the advancement of diagnostic practice in the field of infectious diarrhoeal diseases.

METHODS

Design and Protocol

We incorporated the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement to increase the precision of our search results (Fig 1). Our systematic review follows the strict research protocol introduced by Donthu et al. [15]. This meticulous adherence ensures a clear demarcation between the deliberate exclusion of meta-analyses from our study.

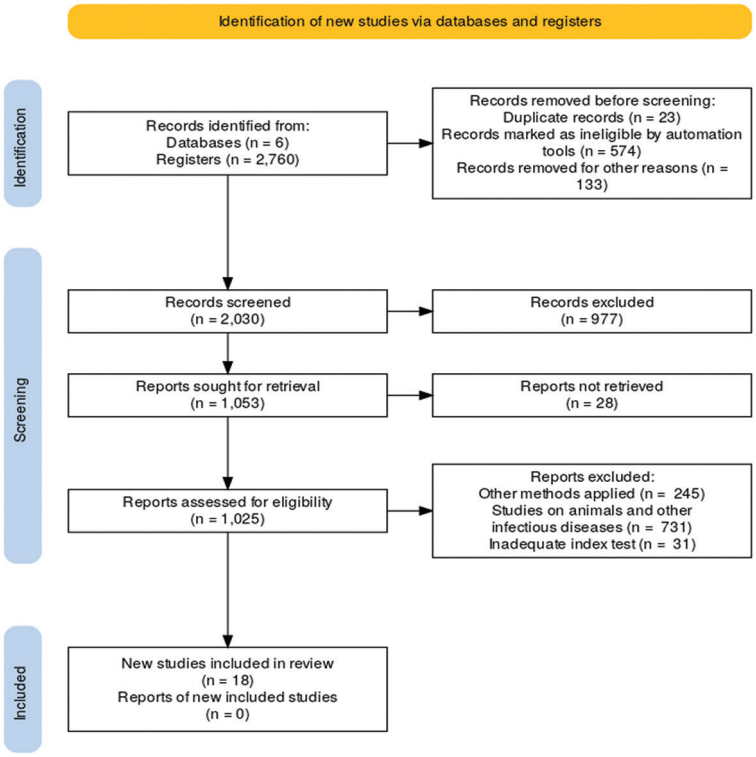

Fig. 1. Diagram of the PRISMA statement and the steps involved in identifying bibliographic data and refining searches. Source: modified from Haddaway et al. [17].

The specific criteria that guided our selection process are shown in Table I. We selected Rayyan to complement the PRISMA flowchart and enhance the systematic review process by introducing efficiency, automation and collaboration capabilities. While the PRISMA flowchart remains essential for transparent reporting of the review methodology and results (Fig. 1), Rayyan's digital platform optimises the execution of the review. It accelerates the screening phase, reduces the likelihood of bias through customisable screening criteria, and promotes collaborative research through real-time tracking and seamless integration with reference management tools such as Mendeley, which was used in this study.

The question to be answered was: What are the molecular methods that are associated with enteropathogenic microorganisms in infectious diarrhoea due to their sensitivity, specificity and ability to rapidly diagnose?

Literature Search Strategy

The search was conducted using six prestigious databases, including Web of Science (WOS), Scopus, Science Direct, Embase, PubMed and LILACS Plus. Only scientific papers published in the last ten years (2014-2024) were included. The search language was English. MeSH terms were used: "dysbiosis", "enteropathogens", "sequencing", "infectious diarrhoea" and "molecular diagnostics". For each term, the corresponding entry terms were added and the Boolean operators "OR", "AND" and "NOT" were used to form the final search strategy (Table I) [16].

Eligibility Criteria and Study Selection

To ensure the highest level of rigour and quality, we further refined our search by imposing specific publication date restrictions. Only original research articles published in rigorously indexed scientific journals using a rigorous double peer review system, were included in our review, thereby reducing the inclusion of lower quality, non-refereed studies.

Exclusion criteria included a range of study types, including non-observational studies (such as systematic reviews, clinical trials, case reports and conference proceedings), case-control studies, conference abstracts and letters to the editor. In addition, participants with specific conditions identified in the literature review, such as pregnant women, children, adolescents or adults with chronic diseases such as chronic kidney disease, HIV/AIDS, SARS-CoV-2 and COVID-19, were excluded. Studies involving non-human (animal) subjects and those using diagnostic methods other than molecular or molecular diagnostic approaches were also excluded from the analysis.

Ethical Considerations

Given the nature of this project as a systematic review of literature retrieved from scientific databases, it did not require review by an ethics committee. No instruments were used on individuals and there was no direct contact with patients or carers. In addition, the research was not conducted in a healthcare setting. As such, the project falls into the "no risk" category of Resolution 8430 of 1993.

RESULTS

Article Selection Process

Initially, the search of the specified databases yielded 2,760 publications, as detailed in Table I. These records were consolidated and processed using the RAYYAN web application, resulting in the elimination of 23 duplicate entries. Subsequently, 574 records were excluded based on pre-defined automation criteria, with a further 133 records excluded for various reasons that did not meet the pre-defined standards of the study. Following this screening, the remaining 2,030 studies were further assessed using the title and abstract.

Of these, 977 were deemed irrelevant to the research question and were therefore excluded. Finally, 1,053 articles

Table I. Criteria for the retrieval of cited documents in our data set

Items	Criteria
Time horizon:	2014-2024
Database:	Clarivate Analytics' Web of Science Core Collection™; Scopus; Science Direct; Embase; PubMed and LILACS Plus.
The keywords combination and Booleans/ Search Equation†:	"Enteropathogens" [MeSH Terms] OR "Intestinal pathogens" [Text Word] OR "Enteric pathogens" [Text Word] OR "Enteric parasites" [Text Word] AND "Dysbiosis" [MeSH Terms] OR "Gastrointestinal Dysbiosis" [Text Word] OR "Microbial dysbiosis" [Text Word] OR "Gut Microbes" [Text Word] OR "Gut dysbiosis" [Text Word] AND "Infectious Diarrhoea" [MeSH Terms] OR "Enteric infection" [Text Word] OR "Diarrhea Outbreaks" [Text Word] OR "Diarrheal Pathogens" [Text Word] OR "Etiology-Specific Diarrhea" [Text Word] OR "Infectious Gastroenteritis" [Text Word] AND NOT "SARS-CoV-2" [MeSH Terms] OR "Diarrheic COVID-19" [Text Word] OR "No Human" [Text Word] OR "HIV/AIDS" [MeSH Terms] AND "Molecular methods" [MeSH Terms] OR "Molecular diagnosis" [Text Word] OR "Nucleic Acid Extraction" [Text Word] OR "Polymerase Chain Reaction" [Text Word] OR "Genetic detection" [Text Word] OR "Metagenomics" [Text Word]
Seriation by type of document:	Only original research articles
Software used††:	VosViewer®; Posit PBC™ formerly known as RStudio. It is a rebranding that reflects the expansion into Python and VS Code and its web interface Biblioshiny: the shiny app for bibliometrics.
Application of artificial intelligence:	Rayyan, a web-based and mobile application designed to streamline and facilitate the process of conducting systematic reviews and syntheses of the literature.

†: The research equation shown in Table I does not follow any of those outlined by other authors. Therefore, the study is a robust template for possible replication, for which it is sufficient to follow the research and eligibility criteria used here; ††: adapted from Aria and Cuccurullo [16].

were considered eligible for final assessment. However, during the subsequent full-text review, 28 studies were excluded due to inaccessibility, either because they were not retrievable in full text or because of restrictions imposed by open access availability. After careful evaluation, 1,025 studies met the eligibility criteria. Reasons for exclusion identified using RAYYAN included the use of alternative methodologies (n=245), studies focusing on animals or other infectious diseases (n=731), and insufficient adequacy of the index test (n=31) in the full-text review. Finally, 18 articles that met the inclusion criteria were selected for inclusion in this systematic review. For a comprehensive overview, see the full PRISMA flowchart in Fig. 1 [17].

Characteristics of Included Studies

The 18 articles selected for inclusion in the study were carefully characterised using several key parameters. These parameters included year of study, journal of publication, geographical location of study, study design, specific enteropathogenic microorganisms associated with infectious diarrhoea and the molecular diagnostic method used, ensuring a comprehensive review of consistency. This comprehensive characterisation provided a nuanced understanding of the landscape surrounding the molecular diagnosis of infectious diarrheal diseases associated with enteropathogenic microorganisms (Table II). By delving into these details, the study aimed to elucidate patterns, trends and potential variations across different contexts, thereby enriching the depth of insight gained from the systematic review.

Of the 18 studies included in our analysis, 12 were cross-sectional studies and the remaining 6 were cohort studies. Our review revealed a predominant focus on shigellosis or *Shigella* infection caused by *Shigella spp*. as the most extensively studied infectious disease associated with enteropathogenic microorganisms. This was closely followed by enteroaggregative *Escherichia coli, Campylobacter, Giardia, Salmonella* and Psoriasis, all of which featured prominently in the literature reviewed.

Notably, the study with the largest sample size included a staggering 11,400 samples from 5,700 cases and matched controls, yielding valid results from 5,304 matched case-control pairs. This robust dataset provided a comprehensive understanding of the diagnostic landscape surrounding infectious diarrhoeal diseases. In terms of the diagnostic methods used, multiplex polymerase chain reaction (PCR) was the predominant technique, used in 12 of the 18 studies (66.6%). This was followed by the isolation of genomic DNA from stool samples, which was used in 2 studies (13.3%). Other molecular diagnostic techniques, including the BioFire FilmArray Gastrointestinal (GI) Panel, metagenomic sequencing and the Luminex xTAG Gastrointestinal Pathogen Panel (GPP), were also used, although to a lesser extent.

Interestingly, while all studies showed a preponderance of cases in children under 5 years of age, it is worth noting that the analyses included the general population affected by acute gastroenteritis without age discrimination, providing a comprehensive overview of the epidemiological landscape of infectious diarrhoeal diseases (Table II).

DISCUSSION

In our exploration of diagnostic modalities for diarrhoea, we encountered a rich diversity of biomarkers and molecular techniques in 18 selected studies in this review. In particular, twelve studies highlighted the importance of pathogen cultivation as a cornerstone biomarker, often complemented by the use of multiplex polymerase chain reaction (PCR) as the primary point-of-care diagnostic tool for pathogen detection [18, 19, 23-25, 27-30, 32]. In contrast, two separate studies focused on the detection of glutamate dehydrogenase (GDH) as a diagnostic biomarker [20, 31], while a further two studies focused on the identification of bacterial lipopolysaccharide (LPS) antigens, using immunochromatography as the preferred detection method [24, 36].

In addition, six studies looked at the analysis of serum and faecal proteins as diagnostic indicators, covering a wide range of proteins including calprotectin, lactoferrin, C-reactive protein (CRP), fibroblast growth factor 19 (FGF19), total free faecal bile acids (TFFBA), soluble triggering receptor expressed on myeloid cells (sTREM), procalcitonin (PCT) and serum C4 concentration [18, 21-24, 32]. In addition, two studies investigated target genes as potential biomarkers for the detection of diarrhoea, each focusing on specific genes such as the *C. difficile* toxin A and B gene, stx-1, stx-2, eae and ipaH (invasion plasmid antigen H) gene [26, 31]. This multi-faceted approach highlights the complexity and breadth of diagnostic strategies being used in diarrhoeal disease research.

PCR methods showed a remarkable increase in the overall detection rate of bacteria-positive stool samples, outperforming culture methods by 60% and identifying 39.4% more bacterial pathogens [37, 38]. In addition, Luminex® GPP served as a valuable screening tool, complementing other diagnostic techniques to detect a broad spectrum of pathogens in patients with persistent diarrhoea [39]. However, it is important to note that the use of Luminex® GPP remains significantly under-reported [40], with a smaller number of studies from Latin America and Colombia [35].

When compared to conventional testing methods, this diagnostic assay, multiplex PCR-based real-time PCR, has demonstrated remarkable sensitivity and specificity of over 94% and 98%, respectively [41]. It is worth noting, however, that no study to date has established an appropriate reference standard to comprehensively evaluate the accuracy of xTAG GPP against conventional testing protocols [42].

Due to the scarcity of evidence from Latin America and Colombia, only three studies were included in this review [33-35]. The selection of studies (n=18) focused predominantly on developed countries, where molecular diagnostic methods are commonly used, facilitating rapid and accurate diagnosis of disease. For example, Hayman et al. [20] included two observational studies conducted during a *Campylobacter* outbreak and routine surveillance initiatives. In addition, the study included populations from the UK and New Zealand, specifically looking at cases of *Giardia* infection and acute gastrointestinal illness. This deliberate focus on developed countries provides valuable insights into the use and effectiveness of molecular diagnostic approaches in settings with well-established healthcare infrastructures.

Table II. Molecular tests for the rapid detection of enteric pathogens associated with infectious diarrhoea

Study	Assay/reference test	Index test/PCR target	Enteropathogens identified	Molecular methods
Mohtar et al. [18]	Allplex™ full gastrointestinal assay	Allplex™ full gastrointestinal assay	Enteroaggregative *E. coli,* Enterotoxigenic *E. coli*,, Enteropathogenic *E. coli*,, *Blastocystis hominis*, Rotavirus, *Salmonella, Shigella*, and Adenovirus	Allplex gastrointestinal assay, which is a multiplex one-step real-time RT-PCR assay. This assay can detect and identify 25 gastrointestinal pathogens simultaneously, including bacteria, viruses, and parasites
Kanwar et al. [19]	BioFire FilmArray Gastrointestinal (GI) panel	*Shigella*/EIEC	The enteropathogens identified in this study included Shigella, enteroinvasive *Escherichia coli* (EIEC), enteropathogenic *Escherichia coli* (EPEC), and enteroaggregative *Escherichia coli* (EAEC).	BioFire FilmArray Gastrointestinal (GI) panel, a PCR-based assay, for detecting enteric pathogens like *Shigella*. The study did not conduct further confirmation of samples positive for *Shigella*/EIEC by the GI panel through independent PCR assay and sequencing.
Hayman et al. [20]	For *Cryptosporidium*, the diagnosis can be confirmed by detecting *Cryptosporidium* spp., oocysts using methods like antigen detection, rapid antigen test, enzyme immunoassay, or nucleic acid detection. For *Giardia*, similar methods are used, including antigen/DNA detection and visualisation of *Giardia* cysts or trophozoites.	Inhouse - endpoint PCR test targeting the gp60 gene for *Cryptosporidium* and gdh gene for *Giardia*	*Cryptosporidium* and *Giardia*	In-house endpoint PCR test, as well as comparisons with commercial real-time quantitative PCR (qPCR) for detection of protozoa in *Campylobacter* patients. These molecular methods were used to investigate the level of protozoa detection in cases missed through antigen-based assays, with a focus on improving sensitivity and accuracy of diagnosis
Shah et al. [21]	Culture of jejunal fluid aspirate for establishing the diagnosis of small intestinal bacterial overgrowth (SIBO)	16s rRNA gene from domain bacteria	Not identified a specific enteropathogens, focusing instead on microbial dysbiosis and the use of culture-independent techniques to study the gut microbiota in the context of functional dyspepsia and small intestinal bacterial overgrowth	Next generation sequencing technologies, shotgun metagenomic sequencing, metatranscriptomics, metaproteomics and metabolomics for studying the microbial diversity and function in the gastrointestinal tract. These methods provide a culture-independent and comprehensive assessment of the gut microbiome
Xiao et al. [22]	Metagenomics sequencing and bioinformatic analyses	Metagenomics sequencing of DNA extracted from stool samples	The enteropathogens identified in the study is not explicitly mentioned. The study focuses on alterations in gut microbiota composition, gene functions, and metabolites in psoriasis patients, rather than the identification of a specific enteropathogens	The molecular methods in the study include DNA extraction, metagenomics sequencing, quality control of sequencing data, taxonomic assignment prediction, diversity analysis, LEfSe analysis, COG and KEGG pathway annotations, and metabolite potential estimation
Tilmanne et al. [23]	The Luminex Gastrointestinal Pathogen Panel (Luminex)	The Luminex Gastrointestinal Pathogen Panel (GPP) assay targets 15 enteropathogens including bacteria, viruses, and parasites.	Rotavirus A, adenoviruses 40 and 41, noroviruses GI and GII, *Salmonella* spp., *Shigella* spp., *Campylobacter* spp. (jejuni, lari, and coli), *Clostridium difficile* toxin A/toxin B, Shiga toxin-producing *Escherichia coli*, enterotoxigenic *Escherichia coli*, enterohaemorrhagic *Escherichia coli* O157, *Yersinia enterocolitica, Vibrio cholerae, Giardia lamblia, Cryptosporidium* spp., and *Entamoeba histolytica*	Luminex xTAG Gastrointestinal Pathogen Panel for identifying enteropathogens in pediatric gastroenteritis, highlighting its advantages over routine diagnostic methods but also raising concerns about result interpretation and clinical significance
Kellner et al. [24]	Luminex xTAG gastrointestinal pathogen panel (GPP)	Luminex xTAG GPP	Salmonella spp., *Shigella* spp., *Escherichia coli* O157, and *Campylobacter* spp.	Nucleic acid amplification techniques (NAAT), specifically the Luminex xTAG gastrointestinal pathogen panel (GPP), for detecting multiple bacterial pathogens in children with acute gastroenteritis
Leli et al [25]	FilmArray GI panel (BioFire Diagnostics, Salt Lake City, UT) with that of standard culture for aetiological diagnosis of suspected infectious diarrhoea	Bacteria (such as *Campylobacter* spp., *Salmonella* spp., *E. coli* strains), viruses (such as norovirus, rotavirus, adenovirus), and protozoa (such as *Cryptosporidium, Giardia*)	*Salmonella* spp., *Campylobacter* spp., *Yersinia enterocolitica*, and Shiga-like toxin producing E. coli O157	The FilmArray gastrointestinal panel. This panel is a multiplex polymerase chain reaction (PCR) assay that simultaneously detects and identifies various pathogens, including bacteria, viruses, and parasites, in stool samples

Table II (continued)

Huang et al. [26]	xTAG GPP	The „Index test/PCR target" in this study is the xTAG Gastrointestinal Pathogen Panel (xTAG GPP) which detects 15 types of diarrheal pathogens through multiplex PCR	*Escherichia* coli O157, enterotoxigenic *E. coli,* Shiga-like toxin-producing *E. coli*, *Cryptosporidium* spp., *Giardia*, *Salmonella* spp, *C. difficile* toxin A/B, norovirus GI/GII	Multiplex polymerase chain reaction (PCR) for the simultaneous detection of multiple pathogens causing infectious gastroenteritis, including bacterial, viral, and parasitic pathogens
Platts-Mills et al. [27]	custom-designed TaqMan Array Cards for probe-based quantitative PCR assays for 29 enteropathogens	ipaH target for detecting *Shigella* infection using quantitative PCR.	*Shigella*; Sapovirus; Rotavirus Adenovirus 40/41; Enterotoxigenic *Escherichia coli* (ETEC); Norovirus; Astrovirus *Campylobacter jejuni* or C. coli; *Cryptosporidium* spp.; Typical enteropathogenic *E. coli*	Quantitative PCR and custom-designed TaqMan Array Cards for assessing the aetiology of diarrhoea in children in low-resource settings. These methods helped refine estimates of pathogen quantity and associations with diarrhoea
Rogawski et al. [28]	TaqMan Array Cards for quantitative PCR assays targeting 29 enteropathogens, with the presence of ipaH considered diagnostic of *Shigella* spp	ipaH target, which was considered diagnostic of *Shigella* spp.	*Shigella*, enteroaggregative *Escherichia coli, Campylobacter, Giardia,* and *E. bieneusi.*	Quantitative PCR to detect 29 enteropathogens in stool samples collected from children in the MAL-ED study using custom-designed TaqMan Array Cards.
Liu et al. [29]	Quantitative real-time PCR (qPCR) with a custom TaqMan Array Card for 32 enteropathogens in stool samples from cases and controls in the GEMS study	ipaH target for detection of *Shigella* spp.	*Shigella* spp; Rotavirus Adenovirus 40/41; ST-ETEC (heat-stable enterotoxin-producing *E. coli*); *Cryptosporidium* spp. *Campylobacter* spp	Quantitative real-time PCR (qPCR) to test for 32 enteropathogens in stool samples from cases and controls, allowing for a more accurate and detailed assessment of the causes of diarrhoea
Fiedoruk et al. [30]	PCR	Isolating genomic DNA from Stool samples using Polymerase Chain Reaction (PCR)	Toxigenic *C. difficile*, *Campylobacter jejuni*, *Salmonella* spp, and atypical enteropathogenic *E. coli* (aEPEC)	Isolating genomic DNA from stool samples, performing duplex and triplex PCRs, visualizing PCR products using agarose gel electrophoresis, conducting PCRs in duplicate, and retesting samples to exclude false-positive results
Becker et at. [31]	The Luminex® Gastrointestinal Pathogen Panel (GPP), parasitological microscopic examinations, and rapid antigen detection tests for the diagnosis of diarrhoeagenic pathogens in patients with persistent diarrhoea	Luminex ® xTAG Gastrointestinal Pathogen Panel (GPP)	Enterotoxigenic *Escherichia coli* (ETEC), *Giardia intestinalis*, *Shigella* species, and *Strongyloides stercoralis*	Luminex ® xTAG Gastrointestinal Pathogen Panel (GPP), a multiplex PCR assay that allows for the concurrent detection of 15 pathogens, including bacteria, viruses, and intestinal protozoa
Zhang et al. [32]	BioFire FilmArray GI test	5-gene panel targeting specific genes in common bacterial pathogens	Common bacterial pathogens such as *Campylobacter*, *Salmonella*, *Shigella*, *Clostridium difficile*, and pathogenic *Escherichia coli*.	Multiplex PCR-based tests, real-time PCR-based multiplex GI assays, 2-tube TaqMan-based real-time PCR assays, multiplex tandem PCR technology, low-density microarray detection, and dual priming oligonucleotide technology for detecting various enteric bacterial pathogens and other pathogens causing infectious gastroenteritis
Kann et al. [33]	PCR	Isolating genomic DNA from Stool samples using Polymerase Chain Reaction (PCR)	Protozoan parasites such as *Giardia intestinalis*, *Entamoeba histolytica, Cryptosporidium* spp., and *Cyclospora cayetanensis*, bacteria including *Campylobacter jejuni, Salmonella* spp., *Shigella* spp./enteroinvasive *E. coli* (EIEC), *Yersinia* spp., enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enterotoxin-producing *E. coli* (ETEC), and enteroaggregative E. coli (EAEC), as well as helminths like *Necator americanus*	Real-time Polymerase Chain Reaction (RT-PCR). This method was used to target and detect the various pathogens including protozoan parasites, bacteria, and helminths in the stool samples collected from the study participants

Table II (continued)

Fandino et al. [34]	The Luminex® Gastrointestinal Pathogen Panel (GPP), parasitological microscopic examinations, and rapid antigen detection tests for the diagnosis of diarrhoeagenic pathogens in patients with persistent diarrhoea	Luminex ® xTAG Gastrointestinal Pathogen Panel (GPP)	*Giardia intestinalis* *Entamoeba histolytica* *Cryptosporidium* spp. *Cyclospora cayetanensis* *Campylobacter jejuni* *Salmonella* spp. *Shigella* spp. /Enteroinvasive *E. coli* (EIEC) *Yersinia* spp. Enterohemorrhagic *E. coli* (EHEC) Enteropathogenic *E. coli* (EPEC) Enterotoxin-producing *E. coli* (ETEC) Enteroaggregative *E. coli* (EAEC) *Necator americanus* *Strongyloides stercoralis* *Ascaris lumbricoides*	Multiplex PCR-based tests, real-time PCR-based multiplex GI assays, 2-tube TaqMan-based real-time PCR assays
Lopez-Medina et al. [35]	Multiplex nucleic acid assay (xTAG GPP; Luminex Corporation)	Luminex ® xTAG Gastrointestinal Pathogen Panel (GPP)	Nine bacteria (*Campylobacter*, *Clostridium* difficile toxin A/B, *E. coli* (O157, enterotoxigenic, and Shiga-like toxin-producing), *Yersinia enterocolitica, Vibrio cholerae, Salmonella, Shigella*), three viruses (norovirus GI/GII, rotavirus A, adenovirus 40/41), and three parasites (*Giardia, E. histolytica, Cryptosporidium*)	Real-time Polymerase Chain Reaction (RT-PCR). This method was used to target and detect the various pathogens including protozoan parasites, bacteria, and helminths in the stool samples collected from the study participants.

Therefore, there is an urgent need for further applied research to elucidate the cost-effectiveness, sensitivity, specificity and rapidity of using these molecular diagnostic methods in the context of Colombian and/or Latin American settings [43-45]. Within this review, PCR emerged as the outstanding molecular technique, demonstrating exceptional efficacy in the diagnosis and identification of a wide range of enteric pathogens associated with infectious diarrhoea. Its superior sensitivity, specificity and speed made it the dominant method among those evaluated (their sensitivity and specificity of this test have been reported to be above 94% and 98%, respectively [41]). This underscores the importance of prioritising the research and validation of molecular diagnostic approaches tailored to the unique healthcare landscape of the Latin American and Colombian contexts.

Limitations of the Reviewed Studies

The limitations identified in the reviewed studies cover several key aspects. In particular, there is a notable lack of evaluation of patient outcomes and health economic impact, which could provide valuable insights into the wider impact of molecular diagnostic methods [18, 22, 32]. In addition, a recurring limitation is the omission of testing for pathogens not included in the pathogen panel, suggesting a potential gap in comprehensive pathogen detection.

Further investigation is warranted to assess the clinical utility of including additional pathogens in diagnostic panels and to address the inherent limitations of multiplex PCR, in particular its inability to provide information on antibiotic susceptibility or to detect novel genes or gene variants. Some of the studies included [23, 27, 28], have methodological limitations, such as the exclusive use of stool samples preserved in Cary-Blair medium for molecular testing, which may limit the scope of pathogen detection. In addition, the selective performance of reflex culture in some studies may introduce bias and affect the overall accuracy of molecular diagnostic results.

Although most of the research in this review mainly focuses on the use of multiplex PCR to identify pathogens, it is important to recognise that this method does not automatically provide information on antibiotic resistance. For example, the study by Rogawski et al. [28] used multiplex PCR-based methods to detect specific pathogens such as *Shigella* and *E. coli*, which are associated with significant health outcomes such as inflammatory diarrhoea and intestinal malabsorption. However, this approach did not extend to the identification of antibiotic resistance genes, which are essential to guide effective treatment strategies.

The identification of resistance genes, as highlighted in the study by Xiao et al. [22] , involved the prediction of significantly different functional categories and enriched pathways related to bacterial resistance mechanisms. This study highlighted the importance of understanding genetic pathways, functions and metabolites that are dysregulated in diseases such as psoriasis to gain insight into possible mechanisms of resistance. It is imperative that molecular diagnostic protocols incorporate resistance gene assessment. Enhancing our knowledge of pathogen resistance profiles, facilitating targeted therapy and preventing the spread of antibiotic resistance all depend on this integration. Molecular diagnostics can provide a more comprehensive understanding of infectious diseases by combining in-depth genetic analyses, such as resistance gene detection. This can ultimately improve patient outcomes and inform public health strategies.

Limitations of Molecular Techniques: the Role of Culture Examination as the Gold Standard

While culture examination remains the traditional gold standard, it has inherent limitations, including long turnaround times of several days to weeks. In contrast, molecular techniques such as PCR offer a distinct advantage over traditional culture methods by providing rapid results, often within hours. This rapid turnaround time allows for the prompt initiation of targeted therapy and the implementation of effective infection control strategies [46, 47].

Despite the rapid turnaround time of molecular methods, culture testing remains essential to validate the presence of pathogens detected by molecular assays. This validation process helps reduce the risk of false positives and ensures the accuracy of diagnostic results, ultimately improving patient care and infection control measures [48, 49]. For instance, a study by Beal et al. [50] compared the turnaround times of PCR-based methods with culture for several infectious diseases and highlighted the significant reduction in time-to-result achieved by the use of molecular techniques. In addition, the findings of a systematic review by Kaur et al. [51], aimed to elucidate the diagnostic accuracy and potential clinical applications of these tests, particularly in the context of diarrhoeal diseases. The results highlighted the importance of rapid diagnostic tests in improving patient outcomes and reducing the burden on healthcare systems, and identified opportunities and challenges in diarrhoeal diagnostics and the need for continued research and development in this area.

In one study in particular, limitations include the statistical power of the study to determine the aetiology of diarrhoea, the focus on long-term outcomes rather than specific aetiological factors, assumptions about appropriate antibiotic use, and the limitations of standardised definitions of diarrhoea [29]. In addition, the need to validate the *Shigella* score for clinical applicability and the observed high rates of inappropriate antibiotic use underscore the complexity and multifaceted nature of the challenges inherent in diarrheal disease research [19].

Among the notable shortcomings identified, a significant proportion of studies suffered from inadequate data availability, with two studies in particular lacking comprehensive data sets [30, 31]. In addition, a subset of studies failed to perform quantitative analyses or strain typing, limiting the depth of insight into microbial strains and their epidemiological implications [26, 36].

In addition, two studies raised concerns about bias due to the omission of cost estimates, potentially biasing the economic evaluation of molecular diagnostic methods [23, 29]. Furthermore, the reliance on retrospective designs in two studies introduces inherent limitations in data interpretation and inference. These multiple limitations highlight the need for more methodologically rigorous investigations using standardised protocols. Such efforts are essential to validate the true clinical utility of emerging diagnostic tools in the context of diarrhoeal diagnosis.

The heterogeneous nature of these limitations underscores the critical need for expanded, high-quality trials. These studies play a key role in informing clinical guidelines and facilitating the widespread adoption of new non-invasive diagnostic techniques, thereby improving the effectiveness of diarrhoeal disease management strategies.

The future research agenda includes a multi-faceted approach aimed at improving the effectiveness and scope of molecular diagnostics in the management of diarrhoeal diseases. Key priorities include evaluating the impact of multiplex PCR on patient outcomes and cost-effectiveness, and exploring the potential expansion of pathogen panels to include additional diarrhoeal pathogens. There is also a critical need to further investigate the benefits of using multiplex PCR for the diagnosis of gastroenteritis, with a particular focus on improving assay specificity to differentiate between closely related pathogens such as *Shigella* and Enteroinvasive *Escherichia coli* (EIEC). In addition, efforts should be directed towards validating the superior detection capabilities of molecular PCR-based assays compared to traditional culture methods [52-54].

Expanding the applications of sensitive molecular tests in surveillance and diagnostic accuracy, and conducting comprehensive cost-benefit analyses in different healthcare settings are essential components of the future research agenda. In addition, improving test sensitivity and understanding the impact of false-negative results on disease transmission are paramount. Innovations in diagnostic methods to improve the detection of protozoal diarrhoea should be pursued vigorously [55]. Clinicians are advised to be cautious about relying on test results alone and to consider the need for multiple testing, especially during the transition to molecular testing.

There is also an urgent need for more targeted approaches to treatment initiation, using the increased availability of sensitive PCR diagnostic tests to optimise patient care and effectively reduce the risk of disease transmission [56]. This comprehensive research agenda underscores the need for continued advances in molecular diagnostics to address the global burden of diarrhoeal diseases.

In a comprehensive analysis, this systematic review critically evaluates the efficacy of molecular methods in detecting enteropathogenic microorganisms associated with diarrhoeal diseases. It highlights the transformative potential of these techniques in reshaping diagnostic paradigms, paving the way for improved individual health outcomes and strengthened epidemiological control strategies [57]. Echoing the concerns of Domanovich et al. [58], the escalating threat of antimicrobial resistance (AMR) underscores the imperative for robust and cost-effective diagnostic solutions. Molecular techniques are emerging as a beacon of hope in this endeavour, offering rapid identification of pathogens such as *Helicobacter pylori*, along with genetic markers indicative of antibiotic resistance, thus providing a formidable arsenal in the battle against AMR.

The implementation of these methods presents formidable challenges. The complex biochemical and metabolic characteristics of *Helicobacter pylori* pose significant hurdles to its isolation in culture, necessitating phenotypic validation of molecular findings [59]. Although molecular techniques offer accelerated detection, their reliance on phenotypic validation underscores the need for rigorous validation protocols. Furthermore, our review highlights the evolving diagnostic landscape, particularly the prevalent emphasis on stool samples as the gold standard in the current literature. While molecular

analysis of stool samples shows promising results, the paucity of studies on culture isolation from stool samples highlights a critical gap in our understanding. The lack of follow-up makes it difficult to fully evaluate this approach and thus determine its diagnostic accuracy.

CONCLUSIONS

The introduction of molecular methods for rapid diagnosis of enteropathogens represents a transformative leap forward in the management of diarrheal diseases. These methods offer numerous advantages, including the rapid delivery of actionable results, which are critical for initiating timely treatment interventions to prevent serious complications resulting from delayed diagnosis. In addition, their ability to accurately differentiate between bacterial and non-bacterial causes of diarrhoea plays a key role in curbing antibiotic misuse, thereby addressing the growing threat of antimicrobial resistance. Notably, these molecular tests have demonstrated sensitivity and specificity greater than 94% and 98%, respectively, compared to conventional tests, further underscoring their diagnostic power and potential impact on improving patient outcomes and public health.

Conflicts of interest: None to declare.

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REFERENCES

1. World Health Organization. Diarrhoeal disease. Fact Sheets Diarrhoeal Disease 2024. Available from: https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease Accessed: April 10, 2024.
2. Babady E, Mead P. Molecular Diagnosis of Gastrointestinal Infections. In: Eslick GD. Gastrointestinal Diseases and their Associated Infections. Elsevier. 2019:167–185. doi:10.1016/B978-0-323-54843-4.00013-1
3. Zhou HL, Bessey T, Wang SM, Mo ZJ, et al. Burden and etiology of moderate and severe diarrhea in children less than 5 years of age living in north and south of China: Prospective, population-based surveillance. Gut Pathog 2021;13:33. doi:10.1186/s13099-021-00428-2
4. Keddy KH, Saha S, Kariuki S, et al. Using big data and mobile health to manage diarrhoeal disease in children in low-income and middle-income countries: societal barriers and ethical implications. Lancet Infect Dis 2022;22:e130–e142. doi:10.1016/S1473-3099(21)00585-5
5. Shine S, Muhamud S, Adanew S, Demelash A, Abate M. Prevalence and associated factors of diarrhea among under-five children in Debre Berhan town, Ethiopia 2018: a cross sectional study. BMC Infect Dis 2020;20:174.doi:10.1186/s12879-020-4905-3
6. Mokomane M, Kasvosve I, de Melo E, Pernica JM, Goldfarb DM. The global problem of childhood diarrhoeal diseases: emerging strategies in prevention and management. Ther Adv Infect Dis 2018;5:29–43. doi:10.1177/2049936117744429
7. Arasaradnam RP, Brown S, Forbes A, et al. Guidelines for the investigation of chronic diarrhoea in adults: British Society of Gastroenterology, 3rd edition. Gut 2018;67:1380–1399. doi:10.1136/gutjnl-2017-315909
8. Camilleri M, Sellin JH, Barrett KE. Pathophysiology, Evaluation, and Management of Chronic Watery Diarrhea. Gastroenterology 2017;152:515-532.e2. doi:10.1053/j.gastro.2016.10.014
9. Erhun K. The importance of stool tests in diagnosis and follow-up of gastrointestinal disorders in children. Turk Arch Pediatr 2019;54:141-148. doi:10.14744/TurkPediatriArs.2018.00483
10. Mărginean CO, Meliț LE, Săsăran MO. Traditional and Modern Diagnostic Approaches in Diagnosing Pediatric Helicobacter pylori Infection. Children (Basel) 2022;9:994. doi:10.3390/children9070994
11. Kaur MR, Patel H, Haris Ali M, et al. Evaluation of non-invasive diagnostic tools for diarrhea: A systematic review of point-of-care tests and biomarkers. Ann Med Surg (Lond) 2024;86:2951-2962. doi:10.1097/MS9.0000000000001946
12. Lee JY, Cho SY, Hwang HSH, et al. Diagnostic yield of stool culture and predictive factors for positive culture in patients with diarrheal illness. Medicine 2017;96:e7641. doi:10.1097/MD.0000000000007641
13. Mawazo A, Bwire GM, Matee MIN. Performance of Widal test and stool culture in the diagnosis of typhoid fever among suspected patients in Dar es Salaam, Tanzania. BMC Res Notes 2019;12:316. doi:10.1186/s13104-019-4340-y
14. Rout D, Sachdev S, Marwaha N. Evaluation of new non-invasive & conventional invasive methods of haemoglobin estimation in blood donors. Indian J Med Res 2019;149:755. doi:10.4103/ijmr.IJMR_301_17
15. Donthu N, Kumar S, Mukherjee D, Pandey N, Lim WM. How to conduct a bibliometric analysis: An overview and guidelines. J Bus Res 2021;133:285–296. doi:10.1016/j.jbusres.2021.04.070
16. Aria M, Cuccurullo C. Bibliometrix: An R-tool for comprehensive science mapping analysis. J Informetr 2017;11:959–975. doi:10.1016/j.joi.2017.08.007
17. Haddaway NR, Page MJ, Pritchard CC, McGuinness LA. PRISMA 2020: An R package and Shiny app for producing PRISMA 2020-compliant flow diagrams, with interactivity for optimised digital transparency and Open Synthesis. Campbell Syst Rev 2022;18:e1230. doi:10.1002/cl2.1230
18. Mohtar J, Mallah H, Mardirossian JM, et al. Enhancing enteric pathogen detection: implementation and impact of multiplex PCR for improved diagnosis and surveillance. BMC Infect Dis 2024;24:171. doi:10.1186/s12879-024-09047-z
19. Kanwar N, Jackson J, Bardsley T, et al. Impact of Rapid Molecular Multiplex Gastrointestinal Pathogen Testing in Management of Children during a Shigella Outbreak. J Clin Microbiol 2023;61:e0165222. doi:10.1128/jcm.01652-22
20. Hayman DTS, Garcia-Ramirez JC, Pita A, et al. Diagnosis of protozoa diarrhoea in Campylobacter patients increases markedly with molecular techniques. PLOS Glob Public Health 2023;3:e0001527. doi:10.1371/journal.pgph.0001527
21. Shah A, Talley NJ, Holtmann G. Current and Future Approaches for Diagnosing Small Intestinal Dysbiosis in Patients With Symptoms of Functional Dyspepsia. Front Neurosci 2022;16:830356. doi:10.3389/fnins.2022.830356
22. Xiao S, Zhang G, Jiang C, et al. Deciphering Gut Microbiota Dysbiosis and Corresponding Genetic and Metabolic Dysregulation in Psoriasis Patients Using Metagenomics Sequencing. Front Cell Infect Microbiol 2021;11:605825. doi:10.3389/fcimb.2021.605825
23. Tilmanne A, Martiny D, Quach C, et al. Enteropathogens in paediatric gastroenteritis: comparison of routine diagnostic and molecular methods. Clin Microbiol Infect 2019;25:1519–1524. doi:10.1016/j.cmi.2019.07.021

24. Kellner T, Parsons B, Chui L, et al. Comparative Evaluation of Enteric Bacterial Culture and a Molecular Multiplex Syndromic Panel in Children with Acute Gastroenteritis. J Clin Microbiol 2019;57:e00205-19. doi:10.1128/JCM.00205-19
25. Leli C, Di Matteo L, Gotta F, et al. Evaluation of a multiplex gastrointestinal PCR panel for the aetiological diagnosis of infectious diarrhoea. Infect Dis 2020;52:114–120. doi:10.1080/23744235.2019.1688861
26. Huang SH, Lin YF, Tsai MH, et al. Detection of common diarrhea-causing pathogens in Northern Taiwan by multiplex polymerase chain reaction. Medicine (Baltimore) 2018;97:e11006. doi:10.1097/MD.0000000000011006
27. Platts-Mills JA, Liu J, Rogawski ET, et al. Use of quantitative molecular diagnostic methods to assess the aetiology, burden, and clinical characteristics of diarrhoea in children in low-resource settings: a reanalysis of the MAL-ED cohort study. Lancet Glob Health 2018;6:e1309–e1318. doi:10.1016/S2214-109X(18)30349-8
28. Rogawski ET, Liu J, Platts-Mills JA, et al. Use of quantitative molecular diagnostic methods to investigate the effect of enteropathogen infections on linear growth in children in low-resource settings: longitudinal analysis of results from the MAL-ED cohort study. Lancet Glob Health 2018;6:e1319–e1328. doi:10.1016/S2214-109X(18)30351-6
29. Liu J, Platts-Mills JA, Juma J, et al. Use of quantitative molecular diagnostic methods to identify causes of diarrhoea in children: a reanalysis of the GEMS case-control study. Lancet 2016;388:1291–1301. doi:10.1016/S0140-6736(16)31529-X
30. Fiedoruk K, Daniluk T, Rozkiewicz D, et al. Conventional and molecular methods in the diagnosis of community-acquired diarrhoea in children under 5 years of age from the north-eastern region of Poland. Int J Infect Dis 2015;37:145–151. doi:10.1016/j.ijid.2015.06.028
31. Becker SL, Chatigre JK, Gohou JP, et al. Combined stool-based multiplex PCR and microscopy for enhanced pathogen detection in patients with persistent diarrhoea and asymptomatic controls from Côte d'Ivoire. Clin Microbiol Infect 2015;21:591.e1-10. doi:10.1016/j.cmi.2015.02.016
32. Zhang H, Morrison S, Tang YW. Multiplex Polymerase Chain Reaction Tests for Detection of Pathogens Associated with Gastroenteritis. Clin Lab Med 2015;35:461–486. doi:10.1016/j.cll.2015.02.006
33. Kann S, Bruennert D, Hansen J, et al. High Prevalence of Intestinal Pathogens in Indigenous in Colombia. J Clin Med 2020;9:2786. doi:10.3390/jcm9092786
34. Fandiño LC, Verjan N. A common Salmonella Enteritidis sequence type from poultry and human gastroenteritis in Ibagué, Colombia. Biomedica 2019;39:50–62. doi:10.7705/biomedica.v39i1.4155
35. López-Medina E, Parra B, Dávalos DM, López P, Villamarín E, Pelaez M. Acute gastroenteritis in a pediatric population from Cali, Colombia in the post rotavirus vaccine era. Int J Infect Dis 2018;73:52–59. doi:10.1016/j.ijid.2018.06.006
36. Xie J, Tarr GAM, Ali S, et al. Pigment visibility on rectal swabs used to detect enteropathogens: A prospective cohort study. J Clin Microbiol 2019;57:e00213-19. doi:10.1128/JCM.00213-19
37. Amjad M. An Overview of the Molecular Methods in the Diagnosis of Gastrointestinal Infectious Diseases. Int J Microbiol 2020;2020:8135724. doi:10.1155/2020/8135724
38. Vicente B, De Freitas A, Freitas M, Midlej V. Systematic Review of Diagnostic Approaches for Human Giardiasis: Unveiling Optimal Strategies. Diagnostics 2024;14:364. doi:10.3390/diagnostics14040364
39. Duong VT, Phat VV, Tuyen HT, et al. Evaluation of Luminex xTAG Gastrointestinal Pathogen Panel Assay for Detection of Multiple Diarrheal Pathogens in Fecal Samples in Vietnam. J Clin Microbiol 2016;54:1094–1100. doi:10.1128/JCM.03321-15
40. Zhan Z, Guo J, Xiao Y, et al. Comparison of BioFire FilmArray gastrointestinal panel versus Luminex xTAG Gastrointestinal Pathogen Panel (xTAG GPP) for diarrheal pathogen detection in China. International Journal of Infectious Diseases 2020;99:414–420. doi:10.1016/j.ijid.2020.08.020
41. Claas EC. Performance of the xTAG® gastrointestinal pathogen panel, a multiplex molecular assay for simultaneous detection of bacterial, viral, and parasitic causes of infectious gastroenteritis. J Microbiol Biotechnol 2013;23:1041–1045. doi:10.4014/jmb.1212.12042
42. Freeman K, Mistry H, Tsertsvadze A, et al. Multiplex tests to identify gastrointestinal bacteria, viruses and parasites in people with suspected infectious gastroenteritis: a systematic review and economic analysis. Health Technol Assess 2017;21:1–188. doi:10.3310/hta21230
43. Yalley AK, Ahiatrogah S, Kafintu-Kwashie AA, et al. A Systematic Review on Suitability of Molecular Techniques for Diagnosis and Research into Infectious Diseases of Concern in Resource-Limited Settings. Curr Issues Mol Biol 2022;44:4367–4385. doi:10.3390/cimb44100300
44. Castillo-Rodríguez L, Ovalle-Bracho C, Díaz-Jiménez D, Sánchez-Vanegas G, Muvdi-Arenas S, Castañeda-Orjuela C. Cost-effectiveness analysis of Mucosal Leishmaniasis diagnosis with PCR-based vs parasitological tests in Colombia. PLoS One 2019;14:e0224351. doi:10.1371/journal.pone.0224351
45. Guzmán Ruiz Y, Vecino-Ortiz AI, Guzman-Tordecilla N, et al. Cost-effectiveness of the COVID-19 test, trace and isolate program in Colombia. Lancet Reg Health Am 2022;6:100109. doi:10.1016/j.lana.2021.100109
46. Kralik P, Ricchi M. A Basic Guide to Real Time PCR in Microbial Diagnostics: Definitions, Parameters, and Everything. Front Microbiol 2017;8:108. doi:10.3389/fmicb.2017.00108
47. Collins S, Jorgensen F, Willis C, Walker J. Real-time PCR to supplement gold-standard culture-based detection of *Legionella* in environmental samples. J Appl Microbiol 2015;119:1158–1169. doi:10.1111/jam.12911
48. Kaprou GD, Bergšpica I, Alexa EA, Alvarez-Ordóñez A, Prieto M. Rapid Methods for Antimicrobial Resistance Diagnostics. Antibiotics (Basel) 2021;10:209. doi:10.3390/antibiotics10020209
49. Sinha M, Jupe J, Mack H, Coleman TP, Lawrence SM, Fraley SI. Emerging Technologies for Molecular Diagnosis of Sepsis. Clin Microbiol Rev 2018;31:e00089-17. doi:10.1128/CMR.00089-17
50. Beal SG, Assarzadegan N, Rand KH. Sample-to-result molecular infectious disease assays: clinical implications, limitations and potential. Expert Rev Mol Diagn 2016;16:323–341. doi:10.1586/14737159.2016.1134325
51. Kaur MR, Patel H, Ali MH, et al. Evaluation of non-invasive diagnostic tools for diarrhea: A systematic review of point-of-care tests and biomarkers. Ann Med Surg 2024;86:2951-2962. doi:10.1097/MS9.0000000000001946
52. Dung TTN, Phat VV, Vinh C, et al. Development and validation of multiplex real-time PCR for simultaneous detection of six bacterial pathogens causing lower respiratory tract infections and antimicrobial resistance genes. BMC Infect Dis 2024;24:164. doi:10.1186/s12879-024-09028-2
53. Danielson PB, McKiernan HE, Legg KM. Integrated Polymerase Chain Reaction Technologies (Sample-to-Answer Technologies). In: Molecular Diagnostics. Third edition. Elsevier; 2017:59–78. doi:10.1016/B978-0-12-802971-8.00005-5
54. Chen JQ, Healey S, Regan P, Laksanalamai P, Hu Z. PCR-based methodologies for detection and characterization of Listeria

monocytogenes and Listeria ivanovii in foods and environmental sources. Food Science and Human Wellness 2017;6:39–59. doi:10.1016/j.fshw.2017.03.001

55. Fitri LE, Candradikusuma D, Setia YD, et al. Diagnostic Methods of Common Intestinal Protozoa: Current and Future Immunological and Molecular Methods. Trop Med Infect Dis 2022;7:253. doi:10.3390/tropicalmed7100253
56. Shang M, Guo J, Guo J. Point-of-care testing of infectious diseases: recent advances. Sensors & Diagnostics 2023;2:1123–1144. doi:10.1039/D3SD00092C
57. Zhang S, Li X, Wu J, et al. Molecular Methods for Pathogenic Bacteria Detection and Recent Advances in Wastewater Analysis. Water (Basel) 2021;13:3551. doi:10.3390/w13243551
58. Domanovich-Asor T, Craddock HA, Motro Y, Khalfin B, Peretz A, Moran-Gilad J. Unraveling antimicrobial resistance in Helicobacter pylori Global resistome meets global phylogeny. Helicobacter 2021;26:e12782. doi:10.1111/hel.12782
59. Spagnuolo R, Scarlata GGM, Paravati MR, Abenavoli L, Luzza F. Change in Diagnosis of Helicobacter pylori Infection in the Treatment-Failure Era. Antibiotics 2024;13:357. doi:10.3390/antibiotics13040357