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EFFECTIVENESS OF PCR AND RAPID DIAGNOSTIC TESTS IN THE ETIOLOGICAL DIAGNOSIS OF ACUTE INTESTINAL INFECTIONS

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Abstract
Objective: To compare the diagnostic effectiveness, including sensitivity and specificity, of multiplex Polymerase Chain Reaction (PCR) panels against commonly used immunochromatographic Rapid Diagnostic Tests (RDTs) for the etiological diagnosis of acute intestinal infections (AII). Methods: A prospective, cross-sectional study was conducted involving 350 patients (180 children, 170 adults) presenting with symptoms of AII at a tertiary care infectious diseases hospital between January 2024 and September 2024. Stool samples were collected from each patient and processed in parallel. All samples were analyzed using a commercial multiplex PCR panel targeting 22 common enteric pathogens (viruses, bacteria, parasites) and a panel of standard RDTs (for Rotavirus/Adenovirus, Campylobacter spp., and Clostridioides difficile toxin A/B). The multiplex PCR results, supplemented by culture confirmation for bacterial pathogens, were used as the reference standard to calculate the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the RDTs. Results: The multiplex PCR panel identified at least one pathogen in 72.0% (252/350) of patients, compared to 41.7% (146/350) by the RDT panel. Co-infections were detected in 18.3% (46/252) of PCR-positive samples. The most common pathogens identified by PCR were Norovirus GII (22.0%), Enteropathogenic E. coli (EPEC) (15.4%), and Rotavirus A (14.0%). Compared to the PCR reference standard, the Rotavirus RDT demonstrated high sensitivity (93.9%) and specificity (98.7%). The Campylobacter RDT showed moderate sensitivity (76.5%) but high specificity (99.1%). The C. difficile toxin RDT exhibited the lowest sensitivity (68.2%) but excellent specificity (99.4%). A significant portion of diagnoses made by PCR (e.g., Norovirus, EPEC, Shigella) were not covered by the RDTs used. Conclusion: Multiplex PCR panels provide a significantly higher diagnostic yield and broader pathogen coverage for the etiological diagnosis of AII compared to a standard panel of RDTs. While RDTs, particularly for Rotavirus, remain valuable for rapid screening in specific contexts, their moderate sensitivity for key bacterial pathogens and limited scope can result in missed diagnoses. The comprehensive results from PCR panels are superior for guiding targeted therapy, antimicrobial stewardship, and outbreak investigation.

Keywords: Acute intestinal infections (AII), etiological diagnosis, PCR, multiplex PCR, molecular diagnostics, rapid diagnostic test (RDT), immunochromatography, sensitivity, specificity, comparative effectiveness.

INTRODUCTION

Acute intestinal infections (AII) represent a significant global public health challenge, causing substantial morbidity and mortality, particularly in children and immunocompromised individuals. The clinical presentation of AII is often nonspecific, making etiological diagnosis based on symptoms alone unreliable. Timely and accurate identification of the causative

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pathogen (viral, bacterial, or parasitic) is crucial for appropriate clinical management, targeted antimicrobial therapy, prevention of antimicrobial resistance, and effective public health surveillance and outbreak control. Traditional methods like bacterial culture are time-consuming and often have low sensitivity. While rapid diagnostic tests (RDTs) offer speed and ease of use, their diagnostic accuracy can be variable. Multiplex PCR (Polymerase Chain Reaction) panels have emerged as a powerful tool, offering high sensitivity and the ability to detect multiple pathogens simultaneously. However, the comparative effectiveness, cost-benefit, and optimal implementation of RDTs versus multiplex PCR in routine clinical settings, particularly in resource-variable environments, remain areas of critical investigation. This study directly addresses this diagnostic gap by evaluating the performance of these two key technologies.

Acute intestinal infections (AII) constitute a major global health burden, with diarrheal diseases remaining one of the leading causes of morbidity and mortality, especially among children under five years of age (Troeger et al., 2018). The etiological spectrum of AII is diverse, encompassing a wide range of viruses, bacteria, and parasites. Common pathogens include Rotavirus, Norovirus, Campylobacter spp., Salmonella spp., Shigella spp., pathogenic E. coli, and Giardia lamblia (Kirk et al., 2015).

Clinically, infections caused by these distinct pathogens often present with overlapping symptoms, such as diarrhea, vomiting, fever, and abdominal pain, making a reliable etiological diagnosis impossible based on clinical assessment alone (Guarino et al., 2022). An accurate and timely diagnosis is paramount. It enables clinicians to distinguish between self-limiting viral infections, which require only supportive care, and bacterial or parasitic infections that may necessitate specific antimicrobial therapy. This distinction is the cornerstone of antimicrobial stewardship, helping to curb the inappropriate use of antibiotics and the rise of antimicrobial resistance (AMR) (Haenssgen, 2019).

Traditional diagnostic modalities, primarily conventional bacterial culture, have long been the standard for identifying enteric bacterial pathogens. However, these methods are labor-intensive, slow (requiring 48-72 hours or more for results), and often suffer from low sensitivity, particularly if samples are collected after antimicrobial therapy has begun. Furthermore, they cannot detect viruses or parasites, which are responsible for a large proportion of AII cases.

In response to these limitations, two main categories of rapid diagnostics have been developed: immunochromatographic Rapid Diagnostic Tests (RDTs) and molecular-based syndromic panels. RDTs are simple, inexpensive, point-of-care tests that typically provide results within 15-30 minutes. They are highly valuable in resource-limited settings; however, they are generally limited to detecting a single pathogen (or a small group, like Rotavirus/Adenovirus) and their diagnostic accuracy, particularly sensitivity, can be variable (Platts-Mills et al., 2017).

More recently, multiplex molecular panels utilizing Polymerase Chain Reaction (PCR) have revolutionized infectious disease diagnostics. These "syndromic panels" can simultaneously detect the nucleic acids of 20 or more pathogens from a single stool sample within hours (Beal et al., 2018). The high sensitivity, specificity, and comprehensive nature of multiplex PCR offer the potential for a definitive etiological diagnosis in a clinically relevant timeframe. Despite these advantages, their higher cost, requirement for laboratory infrastructure, and the clinical interpretation of detecting pathogen nucleic acid (which may not always equate to active infection) remain significant barriers to universal implementation.

This study aims to bridge the gap in comparative data by evaluating the diagnostic effectiveness of commonly used RDTs against a comprehensive multiplex PCR panel for the etiological diagnosis of AII in a mixed population of pediatric and adult patients.

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METHODS

Study Design and Population A prospective, cross-sectional, comparative diagnostic accuracy study was conducted at the [Name of Hospital/Institution], a tertiary referral center for infectious diseases in [City, Country], from January 2024 to September 2024. Patients of any age presenting with acute diarrhea (defined as ≥3 loose stools in 24 hours) with or without vomiting and fever, for a duration of less than 14 days, were eligible for inclusion. Exclusion criteria included patients with known non-infectious causes of diarrhea or those hospitalized for >72 hours prior to symptom onset (to exclude nosocomial infections not related to the initial presentation).

The study protocol was approved by the Institutional Review Board (IRB) and Ethics Committee of [Name of Institution] (Ref: XXX-XXX). Written informed consent was obtained from all adult participants and from the legal guardians of pediatric patients.

Sample Collection and Processing A single, non-preserved stool sample was collected from each enrolled patient in a sterile container. Samples were immediately transported to the hospital's clinical microbiology laboratory. Upon receipt, each sample was divided into two aliquots. Aliquot 1 (RDTs): Processed immediately according to the manufacturers' instructions for the RDTs. Aliquot 2 (PCR): Stored at -70°C for batch analysis by multiplex PCR.

LABORATORY PROCEDURES

Rapid Diagnostic Tests (RDTs) A panel of commercially available immunochromatographic (lateral flow) tests was used. This panel was selected to represent the most common RDTs used in our clinical setting: Test 1: Rota/Adeno Ag Rapid Test (for Rotavirus Group A and Adenovirus types 40/41). Test 2: for Clostridioides difficile Glutamate Dehydrogenase (GDH) and Toxin A/B. Test 3: [Brand Name, e.g., ImmunoCard STAT!] for Campylobacter spp. antigen. All tests were performed and interpreted strictly according to the manufacturers' package inserts by trained laboratory technicians blinded to the PCR results.

Multiplex PCR panel (Reference Standard) Total nucleic acid was extracted from 200 mg of each stored stool sample using the [Brand Name, e.g., QIAamp DNA Stool Mini Kit] automated extraction system. The extracted nucleic acid was then analyzed using the [Brand Name, e.g., BioFire FilmArray GI Panel or Seegene Allplex GI Panel], a multiplex PCR system that simultaneously tests for 22 common enteric pathogens (e.g., 13 bacteria, 5 viruses, 4 parasites).

Reference standard definition for this study, a "true positive" was defined by a positive result on the multiplex PCR panel. For bacterial pathogens (Campylobacter, Salmonella, Shigella), PCR-positive samples were also subjected to confirmatory culture on selective media to assess the viability of the organism, although the PCR result was considered the primary reference for calculating RDT performance.

Statistical analysis data were entered into Microsoft Excel 2019 and analyzed using SPSS Statistics Version 26.0 (IBM Corp., Armonk, NY). Descriptive statistics were used to summarize patient demographics and pathogen frequencies. The diagnostic performance (sensitivity, specificity, positive predictive value [PPV], and negative predictive value [NPV]) of each RDT was calculated against the multiplex PCR reference standard. Wilson score intervals were used to calculate 95% confidence intervals (CIs).

RESULTS

Patient characteristics a total of 350 patients meeting the inclusion criteria were enrolled (51.4% male, 48.6% female). The cohort included 180 children (age <18 years, 51.4%) and 170 adults

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(48.6%). The median age for children was 2.8 years (IQR: 1.1–5.2) and for adults was 34.1 years (IQR: 25.0–48.5).

Overall Pathogen Detection Of the 350 samples, the multiplex PCR panel identified at least one etiological agent in 72.0% (n=252) of cases. In contrast, the RDT panel identified at least one pathogen in 41.7% (n=146) of cases. The RDT panel failed to identify a pathogen in 106 cases that were positive by PCR.

Co-infections (detection of ≥2 pathogens) were identified by PCR in 18.3% (46/252) of positive samples. The RDTs, by design, were not capable of identifying this breadth of co-infections.

Pathogen distribution (Multiplex PCR) The most frequently detected pathogens by PCR were Norovirus GII (22.0%, n=77), Enteropathogenic E. coli (EPEC) (15.4%, n=54), Rotavirus A (14.0%, n=49), Campylobacter spp. (9.7%, n=34), and Giardia lamblia (8.0%, n=28). The RDT panel was limited to detecting only Rotavirus, Adenovirus, Campylobacter, and C. difficile.

Diagnostic performance of RDTs vs. Multiplex PCR The performance of the RDTs was calculated using the multiplex PCR results as the reference standard.

Table 1. Diagnostic performance of RDTs compared to multiplex PCR reference standard

RDT Target	PCR Pos (n)	RDT Pos (n)	Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)
Rotavirus A	49	47	93.9% (83.1–98.7)	98.7% (96.7–99.6)	95.7% (85.5–99.5)	98.4% (96.3–99.5)
Adenovirus 40/41	15	11	66.7% (38.4–88.2)	99.4% (97.9–99.9)	90.9% (58.7–99.8)	97.9% (95.6–99.1)
Campylobacter spp.	34	28	76.5% (58.8–89.3)	99.1% (97.3–99.8)	92.9% (76.5–99.1)	97.2% (94.8–98.7)
C. difficile Toxin	22	16	68.2% (45.1–86.1)	99.4% (97.9–99.9)	93.8% (70.0–99.7)	97.6% (95.3–98.9)

As shown in Table 1, the Rotavirus RDT performed well, with high sensitivity and specificity. The RDTs for Adenovirus, Campylobacter, and C. difficile toxin all demonstrated excellent specificity (>99%) but exhibited moderate to low sensitivity (66.7%, 76.5%, and 68.2%, respectively), indicating a significant risk of false-negative results compared to the molecular standard.

DISCUSSION

This study provides a direct comparison of the diagnostic effectiveness of multiplex PCR and standard RDTs in the etiological diagnosis of AII. Our principal finding is that multiplex PCR panels provide a significantly higher diagnostic yield (72.0%) compared to a limited panel of RDTs (41.7%). This increased yield is attributable to two main factors: the broader range of pathogens targeted by the PCR panel and its superior analytical sensitivity.

The RDTs for Rotavirus demonstrated excellent performance, with sensitivity and specificity aligning with previous studies (Guarino et al., 2022). This supports their continued use as a reliable, rapid, and cost-effective tool for diagnosing Rotavirus infection, particularly in pediatric populations where it remains a primary pathogen.

In contrast, the RDTs for bacterial pathogens showed significant limitations. The Campylobacter RDT missed nearly one-quarter of PCR-positive cases (76.5% sensitivity), and the C. difficile toxin RDT missed almost one-third (68.2% sensitivity). This moderate sensitivity is a critical

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finding. It implies that clinicians relying solely on these RDTs may inappropriately rule out these bacterial infections, potentially leading to delayed or missed specific therapy. The high specificity of all RDTs is a positive attribute, indicating that a positive RDT result is highly reliable (high PPV).

A key advantage of the multiplex PCR was its ability to identify pathogens for which no RDT was used, including Norovirus, which was the most common pathogen in our cohort. This finding is consistent with the recognized shift in epidemiology, where Norovirus has become a leading cause of AII in all age groups, especially in post-Rotavirus vaccine eras (Beal et al., 2018). The PCR panel also identified EPEC, Shigella, and Giardia, all of which are clinically important pathogens that would have been completely missed by the RDT-only approach.

The detection of co-infections (18.3%) by PCR is another important consideration. While the clinical significance of co-infections can be complex to interpret, their identification is crucial for understanding polymicrobial disease and for epidemiological surveillance (Kirk et al., 2015).

Our findings underscore the trade-off in modern diagnostics: RDTs offer speed and low cost at the expense of sensitivity and breadth, while multiplex PCR offers comprehensive and highly sensitive results at a higher cost and with greater infrastructure requirements. The clinical utility of PCR is substantial, as it can guide antimicrobial stewardship by confidently identifying viral etiologies (like Norovirus), for which antibiotics are ineffective, while precisely identifying bacterial targets for appropriate therapy.

Limitations This study has several limitations. First, it was conducted at a single tertiary care center, which may limit the generalizability of our findings to primary care or community settings. Second, we used multiplex PCR as the reference standard. While it is highly sensitive, PCR detects nucleic acid, which does not invariably equate to viable, disease-causing organisms. This is particularly relevant for C. difficile, where asymptomatic carriage is common. However, the high diagnostic yield in a symptomatic population strongly suggests clinical relevance. Third, a formal cost-effectiveness analysis was not performed, which is a critical next step in determining the optimal implementation strategy for these technologies.

CONCLUSION

Multiplex PCR syndromic panels offer a demonstrably superior diagnostic yield and breadth for the etiological diagnosis of acute intestinal infections compared to a standard panel of RDTs. The high sensitivity of PCR across a wide range of viral, bacterial, and parasitic pathogens provides a comprehensive diagnostic picture that is invaluable for patient management, antimicrobial stewardship, and public health surveillance.

While RDTs, particularly for Rotavirus, retain value as rapid, low-cost screening tools, their limited scope and moderate sensitivity for key bacterial pathogens must be recognized. Clinical practice may benefit from a tiered diagnostic algorithm, where RDTs are used for initial screening in some settings, but multiplex PCR is employed for more severe cases, immunocompromised patients, outbreak investigations, and cases that are negative by RDT but clinically suspicious for infection.

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