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Assessing Antimicrobial Stewardship in Paediatric *Clostridioides difficile* Positivity: To Treat or Not to Treat?

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Abstract

Background: Molecular syndromic stool panels are increasingly used in paediatric diarrheal syndromes; however, interpretation of *Clostridioides difficile* (*C. difficile*) detection remains challenging because colonisation is common in younger children. We aimed to assess the frequency of *C. difficile* detection using a syndromic gastrointestinal panel in a paediatric tertiary-care centre and to describe the subsequent microbiological work-up and CDI-directed treatment. **Methods**: We conducted a retrospective single-centre study of all BioFire FilmArray Gastrointestinal (GI) panels performed at San Marco Hospital (University Hospital "G. Rodolico-San Marco", Catania, Italy) from 1 January 2023 to 31 December 2025. Only the first *C. difficile*-positive result per patient was included; repeat positives within 30 days were excluded. Index-positive episodes were stratified by age (<1 year, 1 to <2 years, and ≥2 years). Data collected included co-detected pathogens, toxin A/B enzyme immunoassay (EIA) results, GeneXpert PCR findings, and CDI-directed therapy. **Results**: Among the 714 GI panels performed during the study period, 112 (15.7%) were positive for *C. difficile*. After exclusion of repeat positives, 91 index-positive episodes were analysed. Median age was 1.0 years (IQR 0.75–4.0), and 48/91 cases (52.7%) occurred in children younger than two years. Toxin A/B EIA was positive in 11/82 tested episodes (13.4%), whereas GeneXpert tcdB was positive in 75/84 episodes (89.3%). Co-detection of at least one additional enteric pathogen occurred in 40/91 cases (44.0%). CDI-directed therapy was administered in 9/91 episodes (9.9%), mainly in children aged ≥2 years. **Conclusions**: Detection of *C. difficile* by syndromic molecular panels was relatively frequent in our paediatric cohort but rarely associated with toxin positivity or the need for specific treatment. These findings suggest that many positive Nucleic Acid Amplification Test (NAAT) results may represent colonisation rather than true infection, particularly in younger children. Careful clinical interpretation of syndromic panel results is therefore essential to avoid overdiagnosis and unnecessary antimicrobial therapy.

Keywords: *Clostridioides difficile*; paediatric; multiplex PCR; gastrointestinal panel; diagnostic stewardship

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1. Introduction

Clostridioides difficile (*C. difficile*) is a Gram-positive, anaerobic, spore-forming bacterium and a leading cause of healthcare-associated diarrhoea and colitis. It was first described

in 1935 by Hall et al. as a component of the neonatal intestinal microbiota [1]. In 1978, George et al. isolated *C. difficile* and identified a preformed faecal toxin in a patient with pseudomembranous colitis; contemporaneous studies established toxin-producing *C. difficile* as the etiologic agent of antibiotic-associated pseudomembranous colitis [2,3].

Colonisation with *C. difficile* is common in early childhood, with rates exceeding 30% in infants and progressively declining to levels comparable to those observed in adults by school age [4]. A higher colonisation rate has been reported in specific paediatric populations, including children with inflammatory bowel disease, cystic fibrosis, malignancy, solid organ or hematopoietic stem cell transplantation, and preterm infants [5].

Exposure to *C. difficile* occurs primarily via the faecal–oral route, from environmental, animal, or healthcare-associated reservoirs. After ingestion, spores resist gastric acidity and transit to the intestine, where germination into metabolically active vegetative forms is regulated by the intestinal microenvironment, particularly the balance of bile acids within the gut lumen [6]. Once spores have germinated, disease is primarily mediated by toxin production, most notably toxin A (TcdA) and toxin B (TcdB), with a subset of strains also producing binary toxin (CDT). These toxins are internalized by colonic epithelial cells via endocytosis, disrupt cytoskeletal integrity, induce epithelial damage and apoptosis, and trigger a pro-inflammatory cascade characterized by neutrophil recruitment and formation of the characteristic pseudomembranes [7].

Clinically, *C. difficile* infection (CDI) is heterogeneous and often non-specific. Symptoms may include watery or bloody diarrhoea, mucus in the stool, abdominal pain, nausea, vomiting, and/or fever. However, clinical features alone lack sufficient specificity to reliably distinguish CDI from other causes of diarrhoea. A systematic review by Manzoor et al. demonstrated the limited diagnostic accuracy of symptoms in CDI, emphasizing the need for an integrated diagnostic approach incorporating risk factors and microbiological testing [8].

Over the past decade, rapid multiplex syndromic molecular assays for gastrointestinal pathogens have been increasingly adopted in routine practice. These nucleic-acid amplification platforms enable simultaneous detection of multiple bacterial, viral, and parasitic pathogens directly from stool specimens, potentially improving diagnostic turnaround time, infection control, and antimicrobial stewardship [9].

In paediatrics, however, interpretation of *C. difficile* detections by molecular assays is particularly challenging. Colonisation is common in children under two years of age, and co-detection of alternative enteric pathogens is frequent, complicating attribution of symptoms to *C. difficile* [10]. Accordingly, contemporary diagnostic stewardship frameworks stress that CDI diagnosis should be based on a combination of compatible clinical features, risk-factor assessment, and judicious interpretation of laboratory results [11].

In this context, we aimed to quantify the frequency of *C. difficile* detection on syndromic GI panel testing in a paediatric tertiary-care hospital over a three-year period and to characterise the subsequent microbiological evaluation and CDI-directed therapy following molecular detection.

2. Results

During the study period, 714 GI panels were performed. *C. difficile* was detected in 112 panels (15.7%). After exclusion of 21 repeat positive results occurring within 30 days, 91 index *C. difficile*-positive episodes were included in the final analysis. The median age at index positivity was 1.0 year (IQR 0.75–4.0; range 28 days to 14 years). Of the 91 index episodes, 26 (28.6%) occurred in infants (<1 year), 22 (24.2%) in toddlers (1 to <2 years), and 43 (47.3%) in children ≥2 years (Table 1). Most GI panels yielding index positivity

were ordered in inpatient settings (Paediatrics, No. 65; Paediatric pulmonology, No. 15), whereas 11 tests were requested directly from the Paediatric emergency unit.

Table 1. Study population and testing overview.

Variable	No.	%
GI panels performed	714	—
Panels positive for *C. difficile*	112	15.7
Repeat positives excluded	21	18.75 of positives
Index positive episodes analysed	91	12.7 of all panels
Age, median (IQR), years	1.0 (0.75–4.0)	—
Age group: <1 year	26	28.6
Age group: 1 to <2 years	22	24.2
Age group: ≥2 years	43	47.3

Toxin A/B EIA results were available for 82 episodes and were positive in 11 (13.4%) and negative in 71 cases (86.6%). GeneXpert tcdB results were available for 84 episodes and were positive in 75 (89.3%) and negative in 9 cases (10.7%). Binary toxin genes were detected in 3 episodes (3.3% of all index cases). No tcdC deletion at nucleotide 117 and no NAP1/hypervirulent strain profile were identified among the available GeneXpert results (Table 2).

Table 2. Supplementary testing among index *C. difficile*-positive episodes.

Assay/Result	No.	%
Toxin A/B EIA performed	82	90.1
–Positive	11	13.4 of tested
Negative	71	86.6 of tested
GeneXpert tcdB performed	84	92.3
tcdB positive	75	89.3 of tested
tcdB negative	9	10.7 of tested
Binary toxin genes detected (cdtA/cdtB)	3	3.3
TcdC deletion at nucleotide 117 (GeneXpert)	0	0.0
NAP1/hypervirulent profile (GeneXpert)	0	0.0

At least one additional enteric pathogen was co-detected in 40 episodes (44.0%). A single co-detected pathogen was identified in 30 episodes, whereas 10 episodes had two or more additional pathogens. The most frequently co-detected organisms were diarrheagenic *Escherichia coli* (any pathotype; No. 16), norovirus (No. 13), rotavirus (No. 10), and *Salmonella* spp. (No. 6) (Table 3).

Table 3. Co-detections on the syndromic panel.

Finding	No.	%
Any additional pathogen co-detected	40	44.0
Single co-detection	30	33.0
≥2 co-detections	10	11.0

Table 3. *Cont.*

Finding	No.	%
Most frequent co-detections (presence):		
E. coli	16	17.6
Norovirus	13	14.3
Rotavirus	10	11.0
Salmonella	6	6.6
Campylobacter	4	4.4
Sapovirus	1	1.1

CDI-directed therapy was administered in 9 episodes (9.9%). Treatment was uncommon in children <2 years (2/48, 4.2%) and occurred predominantly in children aged ≥2 years (7/43, 16.3%). Among treated patients, 8/9 (88.9%) received metronidazole monotherapy, and 1/9 (11.1%) received combined metronidazole and vancomycin (Table 4).

Table 4. CDI-directed therapy.

Therapy	No.	%
Any CDI-directed therapy	9	9.9
Metronidazole monotherapy	8	88.9 of treated
Metronidazole + vancomycin	1	11.1 of treated
Treated by age group:		
<1 year	1	3.8
>1 to <2 years	1	4.5
≥2 years	7	16.3

3. Discussion

Our three-year retrospective analysis provides a real-world overview of how syndromic stool panel testing identifies *C. difficile* in paediatric clinical practice and how these findings translate into further diagnostic evaluation and therapeutic decisions.

In our cohort, *C. difficile* was detected in approximately one in six syndromic stool panels. Notably, nearly half of index-positive cases occurred in children younger than two years of age. Previous studies demonstrated colonisation rates exceeding 30–40% during early infancy, with a gradual decline toward adult levels later in childhood. Consequently, molecular detection of *C. difficile* in this population does not necessarily indicate clinically significant infection. Current guidelines recommend against routine testing in infants and advise cautious testing in toddlers 1–2 years old unless alternative causes of diarrhoea have been excluded [12]. In this context, a positive molecular result, particularly in the absence of toxin positivity, may represent colonisation rather than active disease.

Consistent with this concept, toxin A/B positivity in our cohort was relatively uncommon and was observed in only approximately 13% of tested episodes. Although our study design does not allow definitive diagnosis of CDI, the discordance between molecular detection and toxin assays aligns with the growing recognition that molecular detection alone may overestimate clinically relevant infection. This issue is particularly relevant in paediatric settings, where colonisation rates are higher and the clinical presentation of diarrheal illness is often nonspecific.

An additional finding of our study was the high frequency of pathogen co-detection. Nearly half of the index-positive episodes were associated with at least one additional

enteric pathogen identified by the syndromic panel. Viral pathogens such as norovirus and rotavirus, as well as diarrheagenic *Escherichia coli*, were among the most frequently detected organisms. These findings further complicate the attribution of gastrointestinal symptoms to *C. difficile* alone and underscore the importance of interpreting molecular results within the broader microbiological and clinical context. In many cases, the presence of an alternative pathogen may represent the primary cause of symptoms, while *C. difficile* detection reflects incidental colonisation.

Despite relatively frequent molecular detection, CDI-directed therapy was administered in only a small proportion of cases. Less than 10% of index-positive episodes resulted in treatment, and therapy was predominantly initiated in children ≥2 years. Notably, treatment consisted mainly of metronidazole monotherapy, with combined metronidazole and vancomycin used in a single case. This pattern likely reflects clinicians' awareness of age-related colonisation and current guideline recommendations, which discourage routine testing for *C. difficile* in infants and recommend cautious interpretation of positive results in toddlers. The limited use of CDI-directed therapy observed in our cohort, therefore, suggests that, in routine clinical practice, clinicians often interpret positive molecular results within an appropriate clinical framework.

Our findings support the concept that diagnostic stewardship plays a crucial role in the appropriate use of syndromic molecular panels. While multiplex gastrointestinal assays provide rapid and comprehensive pathogen detection, their broad analytical sensitivity can also increase the likelihood of identifying organisms that may not be clinically relevant. In the case of *C. difficile*, particularly in younger children, reliance on molecular detection alone may lead to overestimation of clinically significant infection and potential overtreatment. Current diagnostic guidelines generally recommend a multistep approach to *C. difficile* testing, combining sensitive screening assays such as NAAT or GDH detection with confirmatory toxin testing [13]. In our study, toxin testing was available for most episodes and remained positive in only a minority of cases, further emphasizing the importance of confirmatory assays in distinguishing colonisation from active disease.

Interestingly, markers associated with hypervirulent strains were rarely detected in our cohort. No NAP1/027 profiles or tcdC deletion at nucleotide 117 were identified among the available GeneXpert results. Although the clinical significance of these markers remains debated, their absence may partly reflect the epidemiology of *C. difficile* in paediatric populations, where hypervirulent strains appear to be less prevalent than in adult cohorts. Therapeutic decisions in paediatric CDI are severity-based. Non-severe cases are typically treated with oral metronidazole or vancomycin, whereas severe or fulminant disease warrants vancomycin alone or in combination with metronidazole [14]. Fidaxomicin has emerged as an effective alternative to vancomycin and has been approved for paediatric use in some settings; this recommendation is supported by background paediatric literature and by the phase 3 SUNSHINE trial, which demonstrated higher global cure rates and favourable tolerability compared with vancomycin in children and adolescents [15,16].

Our study has several limitations that should be acknowledged. First, its retrospective single-centre design limits generalisability to other healthcare settings. Second, detailed clinical information, including symptom severity, antibiotic exposure, underlying comorbidities, and stool frequency, was not systematically available for all patients. The absence of these variables precludes more precise clinical classification of CDI and limits the ability to identify predictors of treatment decisions. Finally, the study focused exclusively on index-positive episodes and did not assess outcomes such as recurrence or clinical response to therapy.

Despite these limitations, our findings provide valuable insight into the real-world interpretation of *C. difficile* detections in paediatric syndromic testing. The high rate of

molecular detection, combined with low toxin positivity and infrequent treatment, suggests that many NAAT-positive results likely represent colonisation rather than true infection. Overall, these observations reinforce the importance of age-aware diagnostic strategies and careful clinical interpretation of syndromic panel results. Integrating molecular findings with toxin assays, clinical presentation, and patient age is essential to avoid overdiagnosis of CDI and to prevent unnecessary antimicrobial therapy in paediatric patients.

4. Materials and Methods

4.1. Study Design and Setting

We conducted a retrospective, single-centre observational study at San Marco Hospital (University Hospital "G. Rodolico-San Marco", Catania, Italy), a tertiary-care paediatric referral centre serving a large regional population. The study was designed and reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [17] (Table S1).

4.2. Population and Data Sources

We reviewed all BioFire FilmArray Gastrointestinal (GI) panels (BioMérieux, Marcy-l'Étoile, France) performed between 1 January 2023 and 31 December 2025. The study population included paediatric patients aged 28 days to 14 years at the time of testing. Data were extracted from the laboratory information system and, when necessary, complemented by review of electronic medical records. For patients with multiple *C. difficile*-positive panels, only the first (index) positive result was included. Subsequent positive results within 30 days of the index test were excluded to minimise double-counting of the same episode and reduce potential misclassification related to persistent colonisation, prolonged shedding, or early recurrence.

4.3. Variables and Definitions

Index *C. difficile*-positive episodes were stratified into three predefined age groups: infants (<1 year), toddlers (1 to <2 years), and children (≥2 years). For each index episode, we collected the following variables:

(i) Ordering location/service;
(ii) Co-detected enteropathogens identified by the syndromic GI panel;
(iii) Results of supplementary diagnostic tests;
(iv) Administration of CDI-directed therapy.

According to a local diagnostic protocol developed in collaboration with the Department of Laboratory Medicine, detection of *C. difficile* by the syndromic GI panel prompted reflex supplementary testing. This included toxin A/B detection by enzyme immunoassay (EIA) and molecular testing using the GeneXpert PCR (Cepheid, Sunnyvale, CA, USA) assay targeting tcdB, binary toxin genes (cdtA/cdtB), and the tcdC deletion at nucleotide 117. CDI-directed therapy was defined as administration of antimicrobial agents primarily indicated for treatment of CDI (e.g., metronidazole, oral vancomycin, and/or fidaxomicin) initiated during the index episode, as documented in the medical record.

4.4. Bias

To reduce information bias, all eligible GI panels during the predefined study period were included consecutively. Restricting analyses to index positive episodes minimised overrepresentation of patients with repeated testing. However, given the retrospective design, variability in clinical documentation and testing indications may have introduced residual misclassification.

4.5. Statistical Analysis

Statistical analyses were performed using Microsoft Excel (Microsoft® Excel® Microsoft 365 MSO-2602 Build 16.0.19725.20126-64 bit). Given the descriptive nature of the study, categorical variables were presented as absolute counts and percentages. Continuous variables, specifically age, were reported as median and interquartile range (IQR) due to their non-normal distribution. Data was compiled from laboratory records and checked for accuracy before analysis.

5. Conclusions

In this three-year retrospective study, detection of *C. difficile* through syndromic gastrointestinal panels was relatively common in paediatric patients, particularly in children under two years of age and often in the presence of alternative enteric pathogens. However, toxin positivity was infrequent and CDI-directed therapy was initiated in only a minority of cases, indicating that a substantial proportion of molecular detections likely reflect colonisation rather than clinically significant infection.

The frequent co-detection of other enteric pathogens further complicates the attribution of symptoms to *C. difficile* alone and highlights the challenges in distinguishing colonisation from true infection when highly sensitive molecular assays are used.

These findings emphasize the importance of integrating laboratory results with clinical evaluation and patient age when interpreting syndromic panel findings. Careful diagnostic stewardship is essential to avoid overdiagnosis of CDI and unnecessary antimicrobial treatment in paediatric populations.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/gidisord8020019/s1. Table S1: STROBE checklist.

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Informed Consent Statement: Patient consent was waived due to the retrospective design of the study and the use of fully anonymized data, which posed minimal risk to participants and made obtaining consent impracticable.

Data Availability Statement: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

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