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Research Article

JOURNAL OF PEDIATRICS, PERINATOLOGY AND CHILD HEALTH

ISSN: 2641-7405

Gastrointestinal Pathogens in Hospitalized Pediatric Patients in Northern Morocco: Insights from the BioFire® FilmArray® Gastrointestinal Panel

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Abstract

Background: Gastrointestinal infections are one of the leading causes of hospitalization in the pediatric population, yet its etiological diagnosis remains a persistent challenge in resource-limited settings where conventional microbiological methods lack both sensitivity and pathogen coverage. This study aimed to assess the diagnostic utility of the BioFire® FilmArray® Gastrointestinal Panel (GIP) and age-stratified distribution of enteric pathogens in hospitalized children.

Methods: A retrospective study was conducted among 101 pediatric patients who were admitted to the General University Hospital Mohammed VI of Tangier, Morocco, from June 2023 to January 2026. Participants were enrolled and stratified into three age groups: Group A (<6 months), Group B (6 months- 2 years), and Group C (>2 years). Stool samples were analyzed using the BioFire® FilmArray® GIP, a platform targeting simultaneously 22 enteric pathogens of bacterial, viral, and parasitic origin. Statistical analysis was performed to assess the association between infectious origins and age groups of our sample.

Results: Rate of positive tests was 73.3%, with a statistically significant age-dependent increase across groups. Bacterial pathogens were the leading agents, with enteroaggregative *Escherichia coli* (EAEC) and enteropathogenic *Escherichia coli* (EPEC) being the most frequently detected organisms. Co-detections (two or more agents) were identified in 43.6% of samples; 21.2% in Group A (<6 months) to 56.3% in Group B (6 months- 2 years) and 52.8% in Group C (>2 years).

Conclusion: This study highlights the importance of BioFire® FilmArray® Gastrointestinal Panel (GIP) in hospitalized children with suspected gastroenteritis, offering rapid detection of enteric pathogens. This technic improves diagnostic yield compared with conventional methods and supports timely, evidence-based clinical management of pediatric gastroenteritis.

Keywords: Bacteria; Gastroenteritis; Morocco; Multiplex Polymerase Chain Reaction; Parasites; Pediatrics; Viruses

Introduction

Gastrointestinal infections (GIs) are a global pediatric health concern. They are among the leading causes of morbidity and mortality worldwide. disproportionately affecting children under five years of age [1,2]. Their clinical presentation (diarrhea, vomiting, abdominal pain, and fever) is largely nonspecific, making it difficult to identify the causative pathogen based on clinical findings alone [3]. Although stool culture remains a reference method, its turnaround time of 2 to 5 days, limited sensitivity, and inability to detect

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Citation: Hajar Sabri, Reda Amrani Souhli, Youssra Amekran, Yousra El boussadni, Karima Rissoul. Gastrointestinal Pathogens in Hospitalized Pediatric Patients in Northern Morocco: Insights from the BioFire® FilmArray® Gastrointestinal Panel. Journal of Pediatrics, Perinatology and Child Health. 10 (2026): 51-59.

Received: March 28, 2026
Accepted: April 02, 2026
Published: April 13, 2026

Sabri H, et al., J Pediatr Perinatol Child Health 2026
DOI:10.26502/jppch.74050233

viral or parasitic agents restrict its clinical utility [4,5]. In the absence of an identified causative pathogen, empirical antibiotic therapy is commonly initiated, carrying risks of promoting antimicrobial resistance and potential harm in viral or self-limited infections [6-8].

In the last few years, there has been a significant progress in infections disease diagnostics, with the transition from traditional methods to syndromic-based diagnosis [9]. These methods use molecular assays that simultaneously detect several pathogens including bacteria, viruses, fungi and parasites within a few hours [10]. The BioFire® FilmArray® Gastrointestinal Panel (GIP) simultaneously targets 22 enteric pathogens and has shown high sensitivity and specificity in multiple clinical settings [4]. It requires minimal technical expertise, works directly from stool samples without prior culture, and has demonstrated high sensitivity and specificity across multiple clinical settings [4]. These features make it particularly useful in pediatric care, where a rapid and broad diagnostic workup is often needed [11]. Despite growing data on GIP performance in adult and mixed populations, few studies have looked at how detected pathogens differ by age group in hospitalized children [12].

This study was conducted at the General University Hospital Mohammed VI of Tangier, Morocco. It aimed to evaluate the distribution of gastrointestinal pathogens detected by the BioFire® FilmArray® GIP in hospitalized pediatric patients, to characterize age-specific differences in detection rates, and to describe co-infection patterns along with their clinical implications.

Materials and Methods

Study design and patients

This retrospective descriptive study analyzed 101 samples of pediatric patients who were admitted to the General University Hospital Mohammed VI of Tangier, Morocco, from June 2023 to January 2026, and underwent multiplex gastrointestinal panel (GIP) testing during the study period. We evaluated the results of samples of patients under 18 years old and both sexes were eligible for inclusion. Records with incomplete demographic or microbiological data were excluded.

Stool samples collected within routine clinical practice were examined using the BioFire® FilmArray® GI Panel at the request of an infectious disease specialist.

Data collection

For each pediatric patient, demographic and clinical data were extracted. Collected information included age, sex, clinical service of origin, symptoms, and GIP test results.

We categorized the samples into three groups: Group A, young infants (<6 months), Group B, toddlers (6 months- 2 years), and Group C, children (>2 years). Clinical services were classified into general pediatrics, pediatric emergency, neonatal intensive care unit (ICU), pediatric ICU and Pediatric surgery.

Gastrointestinal panel testing

Stool samples were analyzed using the BioFire® FilmArray® Gastrointestinal (GI) Panel, a fully automated multiplex PCR system. The panel simultaneously detects 22 enteric pathogens, including 13 bacteria, 5 viruses, and 4 parasites, directly from stool specimens.

The bacterial pathogens included *Campylobacter spp*, (*C. jejuni*, *C. coli*, *C. upsaliensis*), *Clostridioides difficile* (toxin A/B), Plesiomonas shigelloides, *Yersinia enterocolitica*, *Vibrio spp*. (*V. parahaemolyticus*, *V. vulnificus*) and *Vibrio Cholerae*, and diarrheagenic *Escherichia coli* pathotypes (enteropathogenic E. coli (EPEC), enteroaggregative E. coli (EAEC), enterotoxigenic E. coli (ETEC), Shiga toxin producing E. coli (STEC), and E. coli O157).

Viral targets included *Norovirus GI/GII*, *Rotavirus A*, *Adenovirus F (40/41)*, *Astrovirus*, and *Sapovirus* (*I*, *II*, *IV*, and *V*).

Parasitic targets included *Giardia lamblia*, *Cryptosporidium spp*., *Entamoeba histolytica*, and *Cyclospora cayetanensis*.

Statistical analysis

Data analysis was performed using SPSS version 26.0. Categorical variables were presented as frequencies and percentages. Comparisons between groups were performed using the chi-square test to assess associations between infection types and age groups. A p-value < 0.05 was considered statistically significant.

Results

A total of 101 pediatric patients were included: 61 males (60.4%) and 40 females (39.6%). They were distributed across three age groups: Group A (<6 months, n = 33), Group B (6 months–2 years, n = 32), and Group C (>2 years, n = 36). The most frequently reported symptom was diarrhea (73.3%), followed by vomiting (41.6%), fever (32.7%), and abdominal pain (15.8%). Most patients were admitted to the general pediatric ward (73.3%), with smaller proportions from the pediatric emergency unit (12.9%), neonatal Intensive Care Unit (ICU) (5.9%), and pediatric ICU (5.0%) (Table 1).

Among the 74 positive samples, single pathogen detection was observed in 30 samples (29.7%), whereas co-detections involving two or more pathogens were recorded in 44 samples (43.6%). Co-detection rates was strongly age-dependent, being the lowest in Group A (7/33,21.2%) and essentially higher in Group B (18/32, 56.3%) and Group C (19/36, 52.8%). Double detections cere the predominant

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co-detection pattern, representing 21 samples (20.8%), followed by triple detections in 15 samples (14.9%) and detections of four or more pathogens in 8 samples (7.9%) (Table 2).

Table 3 illustrates the distribution of all detected pathogens across the three age groups. The overall positivity rate was 73.3% (74/101), with a statistically significant increase across age groups: 51.5% in Group A (<6 months), 81.2% in Group B (6 months- 2 years), and 86.1% in Group C (>2 years) (p = 0.002).

Bacterial pathogens accounted for the majority of detections, with EAEC and EPEC being the most prevalent (20.8% each), followed by *Campylobacter* (17.8%), *Clostridium difficile toxin A/B* (11.9%), and *Salmonella* (7.6%). STEC showed a statistically significant age-dependent increase, with no detections recorded in Group A and B compared to 13.9% in Group C (p=0.037). Although a comparable age-dependent gradient was noted for *Shigella/EIEC*, the observed difference did fell short of statistical significance (p=0.065). Among viral pathogens *Norovirus GI/GII* (12.9%) and *Rotavirus A* (11.9%) were identified across all age groups, while *Adenovirus F 40/41* was detected exclusively in Group B (12.5%, p = 0.011). Parasitic pathogens followed a clear maturational gradient, with *Giardia lamblia* appearing in Group B (3.1%) and peaking in Group C (13.9%, p = 0.037), and *Cryptosporidium* exclusively identified to Group C (5.6%).

Table 4 summarizes the distribution of co-detected pathogen pairs across age groups, revealing both consistent and age-specific patterns. EAEC+ EPEC was the most frequent combination overall (n = 9), identified in all three groups, followed by EPEC + *Campylobacter* (n = 7), with a predominance Group A (n = 4). Age-specific co-detection patterns were observed in both Group B and Group C. In Group B, *Adenovirus F 40/41*, containing pairs and *Norovirus GI/GII*, *Salmonella* combination (n = 3) were identified. Whereas in Group C, ETEC-associated combinations were characterized including *Giardia lamblia*, *Shigella*, EIEC (n = 2). The absence of parasitic co-detections in Group A further support the age-dependent pattern of parasitic pathogen acquisition documented in this study.

Table 1: Demographic and clinical characteristics of patients included in the study.

Characteristics	Patients (N=101)		Percentage %
Gender	Male	61	**60.4%**
	Female	40	**39.6%**
Age group	< 6 months	33	**32.7%**
	6 months–2 years	32	**31.7%**
	> 2 years	36	**35.6%**
Symptoms	Diarrhea	74	**73.3 %**
	Vomiting	42	**41.6 %**
	Fever	33	**32.7%**
	Abdominal pain	16	**15.8 %**
Departments	Pediatric	74	**73.3%**
	Pediatric emergency	13	**12.9%**
	Neonatal ICU	6	**5.9%**
	Pediatric ICU	5	**5.0%**
	Pediatric surgery	3	**3.0%**

Table 2: Positivity rates of BioFire® FilmArray® Gastrointestinal Panel pathogens.

Parameter	< 6 months (n = 33)	6 months–2 years (n = 32)	> 2 years (n = 36)	Total (N = 101)
Negative samples	16 (48.5%)	6 (18.8%)	5 (13.9%)	27 (26.7%)
Positive samples	17 (51.5%)	26 (81.2%)	31 (86.1%)	74 (73.3%)
Single detections	10 (30.3%)	8 (25.0%)	12 (33.3%)	30 (29.7%)
Co-detections (≥2)	7 (21.2%)	18 (56.3%)	19 (52.8%)	44 (43.6%)
Co-detections (n)	7	18	19	44
Double detections	3/7 (42.9%)	9/18 (50.0%)	9/19 (47.4%)	21 (20.8%)
Triple detections	2/7 (28.6%)	7/18 (38.9%)	6/19 (31.6%)	15 (14.9%)
≥4 detections	2/7 (28.6%)	2/18 (11.1%)	4/19 (21.1%)	8 (7.9%)

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Table 3: Distribution of pathogens according to age groups.

Pathogens	Age groups			p-value	TOTAL (N=101)
	A: < 6 months (N=33)	B: 6 months – 2 years (N=32)	C: > 2 years (N=36)		
BACTERIA					
Campylobacter	7 (21.2%)	8 (25.0%)	3 (8.3%)	0.165	18 (17.8%)
Clostridium difficile toxin A/B	1 (3.0%)	5 (15.6%)	6 (16.7%)	0.158	12 (11.9%)
Plesiomonas shigelloides	1 (3.0%)	0 (0.0%)	2 (5.6%)	0.404	3 (3.0%)
Salmonella	1 (3.0%)	5 (15.6%)	1 (2.8%)	0.064	7 (6.9%)
Vibrio cholerae	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA	0 (0.0%)
Yersinia enterocolitica	1 (3.0%)	0 (0.0%)	1 (2.8%)	0.615	2 (2.0%)
EAEC (Enteroaggregative E. coli)	3 (9.1%)	9 (28.1%)	9 (25.0%)	0.124	21 (20.8%)
EPEC (Enteropathogenic E. coli)	8 (24.2%)	7 (21.9%)	6 (16.7%)	0.67	21 (20.8%)
ETEC (Enterotoxigenic E. coli)	1 (3.0%)	3 (9.4%)	6 (16.7%)	0.165	10 (9.9%)
STEC (Shiga-like toxin E. coli)	0 (0.0%)	1 (3.1%)	5 (13.9%)	0.037*	6 (5.9%)
E. coli O157	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA	0 (0.0%)
Shigella / EIEC	1 (3.0%)	2 (6.3%)	7 (19.4%)	0.065	10 (9.9%)
VIRUSES					
Adenovirus F 40/41	0 (0.0%)	4 (12.5%)	0 (0.0%)	0.011*	4 (4.0%)
Astrovirus	0 (0.0%)	0 (0.0%)	2 (5.6%)	0.158	2 (2.0%)
Norovirus GI/GII	3 (9.1%)	5 (15.6%)	5 (13.9%)	0.715	13 (12.9%)
Rotavirus A	3 (9.1%)	4 (12.5%)	5 (13.9%)	0.82	12 (11.9%)
Sapovirus	1 (3.0%)	1 (3.1%)	4 (11.1%)	0.262	6 (5.9%)
PARASITES					
Cryptosporidium	0 (0.0%)	0 (0.0%)	2 (5.6%)	0.163	2 (2.0%)
Cyclospora cayetanensis	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA	0 (0.0%)
Entamoeba histolytica	0 (0.0%)	0 (0.0%)	0 (0.0%)	NA	0 (0.0%)
Giardia lamblia	0 (0.0%)	1 (3.1%)	5 (13.9%)	0.037*	6 (5.9%)
Pathogens	**Age group**			**p-value**	**Total (N=101)**
	A: <6months (N=33)	**B: 6 months — 2 years (N=32)**	**C: >2 years (N=36)**		
No. of detected pathogens	31	55	69	—	**155**
No. positive samples / tested (%)	17/33 (51.5%)	26/32 (81.3%)	31/36 (86.1%)	**0.002***	**74/101 (73.3%)**

*EPEC, enteropathogenic Escherichia coli; EAEC, enteroaggregative Escherichia coli; ETEC, enterotoxigenic Escherichia coli; STEC, Shiga toxin-producing Escherichia coli; EIEC, enteroinvasive Escherichia coli. * p < 0.05.*

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Sabri H, et al., J Pediatr Perinatol Child Health 2026
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Table 4: Distribution of major co-detected pathogens by age group.

Pathogens	A: < 6 months (N=33)	B: 6m – 2 years (N=32)	C: > 2 years (N=36)
BACTERIA			
Campylobacter, EAEC	2	2	1
C. difficile toxin A/B, EAEC	0	4	1
C. difficile toxin A/B, EPEC	0	2	0
C. difficile toxin A/B, Plesiomonas shigelloides	0	0	2
C. difficile toxin A/B, Rotavirus A	0	2	0
EAEC, EPEC	3	2	4
EAEC, ETEC lt/st	1	1	2
EAEC, *Giardia lamblia*	0	0	2
EAEC, *Norovirus GI/GII*	0	0	2
EAEC, *Rotavirus A*	0	2	1
EAEC, STEC stx1/stx2	0	0	2
EPEC, *Campylobacter*	4	2	1
EPEC, ETEC lt/st	1	1	2
EPEC, *Norovirus GI/GII*	1	0	2
EPEC, *Salmonella*	1	2	0
ETEC lt/st, *Giardia lamblia*	0	0	2
ETEC lt/st, *Norovirus GI/GII*	0	0	2
ETEC lt/st, *Rotavirus A*	0	0	2
ETEC lt/st, *Sapovirus*	0	0	2
ETEC lt/st, STEC stx1/stx2	0	0	3
VIRUSES			
Adenovirus F 40/41, EAEC	0	2	0
Adenovirus F 40/41, EPEC	0	2	0
Norovirus GI/GII, Salmonella	0	3	0
PARASITES			
Giardia lamblia, Rotavirus A	0	1	3
Giardia lamblia, Shigella/EIEC	0	1	2
Co-detection rate	**8/33**	**18/32**	**19/36**

EPEC, enteropathogenic Escherichia coli; EAEC, enteroaggregative Escherichia coli; ETEC, enterotoxigenic Escherichia coli; STEC, Shiga toxin-producing Escherichia coli; EIEC, enteroinvasive Escherichia coli; C. difficile, Clostridium difficile.

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Sabri H, et al., J Pediatr Perinatol Child Health 2026
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Discussion

The overall positivity rate of 73.3% observed in our study is consistent with previously published data using the BioFire® FilmArray® GIP. Zhan et al. [13] reported a comparable rate of 65% in a Chinese multicenter study and Chung et al. [12] reported an overall positivity rate of 50.9% in a pediatric cohort, which is lower than the rate observed in our study. Multiplex gastrointestinal PCR panels results have described an age-related increase in enteropathogenic positivity; In a multicenter study using the same platform, children aged between one and five years receives significantly higher rates of positivity compared with infants younger than one year [4]. Likewise, Ho et al. [14] showed that age ≥1 year was independently associated with a greater likelihood of actionable gastrointestinal panel results, particularly in children aged 2–5 years. Consistent with these findings, our results showed increasing positivity with age, with the highest rate observed in children older than 2 years (86.1%).

Bacterial agents were predominant (71%), with EAEC and EPEC being the most frequently detected (20.8% each). Some recent molecular epidemiological investigations have shown that Enteropathogenic E. coli (EPEC) and Enteroaggregative E. coli (EAEC) are two of the most commonly reported diarrhoeagenic pathogens of acute diarrhea in children. In studies employing multiplex PCR panels, EPEC often ranks as the most prevalent pathotype detected, with EAEC also commonly identified at high rates, highlighting the contribution of these E. coli pathotypes towards the global pediatric diarrheal burden [15,16]. The high prevalence of diarrheagenic E. coli pathotypes in our study likely reflects local environmental and epidemiological conditions, as similarly reported in other developing-country settings [17]. STEC and *Shigella/EIEC* were both detected at significantly higher rates in older age groups ($p = 0.037$ and $p = 0.065$, respectively), consistent with the foodborne and environmental transmission dynamics of these pathogens, whose clinical relevance increase as children transition from exclusive breastfeeding to diversified dietary practices [17,18].

Among viral pathogens, *Norovirus GI/GII* (12.9%) and *Rotavirus A* (11.9%) were identified at similar rates across all age groups, reflecting their broad endemic transmission in pediatric populations with incomplete immunization coverage [19-21]. The persistent detection of *Rotavirus A* regardless of age may further sustained by the residual disruption of routine childhood vaccination programs in the post-COVID-19 era, with global routine vaccine uptake yet to fully recover to pre-pandemic levels as of 2023 [22,23].

The exclusive detection of *Adenovirus F 40/41* in Group B (12.5%, $p = 0.011$) is consistent with the established age-specific distribution of enteric Adenovirus infection, selectively affecting children under 24 months, as documented in a North African pediatric study [24]. Parasitic pathogens demonstrated a well-defined age-dependent distribution, being entirely absent from Group A and progressively more frequent in older children. *Giardia lamblia* was detected in Group B (3.1%) and Group C (13.9%, $p = 0.037$), while *Cryptosporidium* was identified exclusively in two Group C patients only. A review on global prevalence confirms that *Cryptosporidium* and *Giardia* are major causes of GIs in children and are widely reports across different geographic regions [15]. This age-dependent pattern reflects the progressive increase in environmental pathogen exposure as children become more mobile and transition to shared food and school settings [25].

The clinical presentation in our study (diarrhea (73.3%), vomiting (41.6%), fever (32.7%), and abdominal pain (15.8%) fit the typical picture of acute gastroenteritis. Symptoms, however, do not in themselves prove cause, as organisms, including EPEC, EAEC, or *Clostridium difficile*, are often noted [26]. Every one of these is a known colonizer in the infant gut and has the potential to remain in stool without causing disease [27-29]. C. difficile is a well-documented example: asymptomatic colonization reaches as high as 50% in neonates and young infants, and a positive result in this age group requires a cautious clinical approach and not automatic therapy [15,30].

The profile of EPEC and EAEC is similar, and they have been widely shed by healthy children in resource-limited settings; environmental contaminants keep their fecal-oral circulation intact irrespective of clinical condition [17,31]. Further complicating the situation, the FilmArray® GIP is unable to distinguish typical forms from atypical EPEC strains [4]. Clinical studies show that typical strains harbor both the Locus of Enterocyte Effacement (LEE) and the adherence factor plasmid and are related to frank diarrheal disease, while atypical strains are devoid of this plasmid and act as low-virulence commensals in children exposed to polluted contexts [16,32]. Given that the assay targets the eae gene shared by the two variants, a positive EPEC signal might reflect colonization by a clinically silent strain as well as actual infection [33,34]. The detection of EPEC and EAEC should be interpreted with caution [34]. Both pathotypes are also commonly co-detected with other enteropathogens, which raise the debate about their actual mode of pathogenesis versus asymptomatic carriage [34,35]. In a large pediatric study E. coli pathotypes are frequently detected in healthy children without symptoms, indicating asymptomatic carriage where detection alone does not imply a clinical disease requiring [36]. These findings underscore the interpretative difficulties of syndromic molecular testing and the necessity of clinical correlation when making a causal association between detection and disease [37,38].

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Co-detection between two or more pathogens was detected in 43.6% of samples compared to 31.5% for multicenter study (4), 30.2%, as reported by Spina et al. [27], and the 37.7% in Carmon et al. [39] in a hospitalized Israeli population [39]. Co-detections increased significantly with age: 21.2% in Group A compared with 56.3% in Group B and 52.8% in Group C. The interpretation of co-detections continues to be difficult since there are multiple pathogens, and different pathogens could present at once, hindering the detection of the primary causative agent [40]. This is complicated by the extended shedding of pathogens including Norovirus and Salmonella, where a positive PCR result may reflect a prior resolved infection rather than active disease [41].

In summary, these results reaffirm that the BioFire® FilmArray® GIP fills an important diagnostic gap in pediatric infectious gastroenteritis that has not been fully addressed by single-target assays or stool culture. Through rapid detection of 22 enteric pathogens within less than an hour, it enhances etiological assessment and supports timely bedside clinical decision-making in hospitalized children.

Limitations

The study was conducted in a single center with a relatively small simple size. Additionally, while the GIP provides a large and rapid detection of many gastrointestinal pathogens, it does not include certain viruses relevant in young infants, such as *Enterovirus* and *Parechovirus*.

Conclusion

This study highlights the diagnostic value of BioFire® FilmArray® GIP in hospitalized children with suspected gastroenteritis, particularly through the identification of age-related pathogen distribution patterns and co-detections not readily detected by conventional methods. Further studies integrating quantitative PCR are required to establish age-specific pathogen-load thresholds and strengthen antimicrobial stewardship in resource-limited pediatric settings.

Acknowledgments

The authors would like to thank the staff of the participating laboratory and hospital for their technical assistance and support during sample collection and analysis.

Funding Source

No external funding for this manuscript.

Financial Disclosure

All authors have indicated they have no financial relationships relevant to this article to disclose.

Conflict of Interest

The authors have no potential conflicts to disclose.

Authors' contributions

H.S and K.R, study conception and design. KR, supervision and critical evaluation. H.S and Y.A, writing and interpretation, R.A.S, data analysis. Y.E, reviewing.

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Sabri H, et al., J Pediatr Perinatol Child Health 2026
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Citation: Hajar Sabri, Reda Amrani Souhli, Youssra Amekran, Yousra El boussadni, Karima Rissoul. Gastrointestinal Pathogens in Hospitalized Pediatric Patients in Northern Morocco: Insights from the BioFire® FilmArray® Gastrointestinal Panel. Journal of Pediatrics, Perinatology and Child Health. 10 (2026): 51-59.