Gut Pathogens

REVIEW

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Prevalence of pathogens associated with neonatal gastrointestinal infections: a systematic review and meta-analysis

Xinyu Liu[1], Rui Zhang[1], Mengdie Wang[1], Chuncai Tang[2], Feifei Yang[1], Qingjuan Yang[2], Changyong Huang[2], Ying Zhang[2], Zhengmin Ren[2], Liqiao Liu[2], Guozhong Zhou[3*] and Jia Li[2*]

Abstract

Gastrointestinal infections represent a significant global health burden, ranking as the second leading cause of mortality among infants and children. Identifying of pathogens causing neonatal gastrointestinal infections has presented tough challenges. This study aimed to summarize the prevalence of common pathogens associated with neonatal gastrointestinal infections through a comprehensive systematic review and meta-analysis of published literature. The last search was performed on January 08, 2025, from databases including EMBASE, PubMed, Cochrane Libary, and Web of Science. The outcome variable was infection rate, and the detection methods used were blood culture, tissue culture, or molecular biology methods. Two researchers independently extracted the research data and evaluated its quality using the JBI Critical Appraisal Tools. Twenty-three studies met the inclusion criteria. The pooled prevalence rates of common pathogens were as follows: Bacteria, including *Escherichia* (22.2%; 95% CI 8.3–40.4%, $I^2 = 98\%$), *Clostridium* (21.8%; 95% CI 2.2–53.8%, $I^2 = 96\%$), *Klebsiella* (19.2%; 95% CI 8.3–33.4%, $I^2 = 97\%$), *Staphylococcus* (13.6%; 95% CI 6.0–23.7%, $I^2 = 91\%$), *Enterococcus* (12.4%; 95% CI 1.8–30.3%, $I^2 = 96\%$), and *Streptococcus* (6.8%; 95% CI 2.5–12.9%, $I^2 = 43\%$). Fungi, including *Candida* (3.8%; 95% CI 0.6–9.6%, $I^2 = 84\%$). Viruses, including Rotavirus (11.6%; 95% CI 1.0–31.5%, $I^2 = 94\%$) and Adenovirus (4.1%; 95% CI 0.5–11.0%, $I^2 = 58\%$). Peritoneal culture methods demonstrated significantly higher positivity rates compared to other detection methods. *Escherichia coli* exhibited consistently high positivity rates across the three main detection methods. *Klebsiella* showed the highest positivity rates among bacterial isolates in both blood and peritoneal cultures. Pathogen detection and prevalence in necrotizing enterocolitis (NEC) cases were markedly higher compared to other conditions. This meta-analysis identifies key pathogens in gastrointestinal infections, including *Klebsiella pneumoniae*, *Escherichia coli*, *Candida*, Rotavirus, Adenovirus, and others that are suspected before clinical sample results are available. It also highlights that intestinal pathogen infections are linked to an increased risk of neonatal necrotizing enterocolitis (NEC) and emphasizes the advantages of peritoneal culture in detecting these infections.

Keywords Neonatal, Gastrointestinal infection, Pathogens, Necrotizing enterocolitis, Meta-analysis

*Correspondence:
Guozhong Zhou
20220247@kust.edu.cn
Jia Li
l.jjjj@163.com
Full list of author information is available at the end of the article

Background

Gastrointestinal infections represent a significant global health burden and are the second leading cause of mortality among infants and children [1, 2]. While these infections can occur at any age, neonates are especially susceptible due to their immature intestinal immune response, intestinal dysbiosis, physiological fragility, and increased exposure to pathogens [3, 4]. The literature consistently documents outbreaks of gastrointestinal infections within neonatal wards [5, 6]. The World Health Organization reports that 9.1% of the 5.3 million annual deaths among children under five years old are attributable to gastrointestinal infections [7]. It is crucial to enhance early diagnosis and treatment of neonatal gastrointestinal infection.

Traditional methods for detecting intestinal pathogens primarily rely on microbiological and immunological techniques, such as blood and stool cultures [8]. While these conventional methods are generally reliable, they can be time-consuming and labor-intensive, and may sometimes fail to identify certain pathogenic microorganisms, potentially delaying optimal treatment interventions [9]. Recently, molecular biology methods, such as polymerase chain reaction (PCR) and metagenomic next-generation sequencing (mNGS) have been increasingly uesd in conjunction with traditional techniques to enhance pathogen detection [10]. Although broad-spectrum molecular diagnostic methods like mNGS provide comprehensive information [11, 12], they are also costly [13]. In contrast, PCR or multiplex PCR (MPCR) methods, while cost-effective, have a limited capacity for diagnosing a wide range of microorganisms.

Understanding the infection spectrum is crucial for designing targeted primers, thereby enhancing the efficiency and accuracy of PCR and MPCR [14]. Additionally, by gaining insights into the prevalence of various pathogens, clinicians can predict potential pathogenic microorganisms during the diagnostic process, enabling informed decisions regarding antibiotic selection [15]. Clarifying the infection spectrum of neonatal gastrointestinal infections is vital for optimizing the application of molecular diagnostic methods and guiding empirical antibiotic use. Therefore, this study aims to conduct a meta-analysis to comprehensively determine the microbial infection spectrum of neonatal gastrointestinal infections, providing valuable evidence for clinical diagnosis and treatment strategies.

Method

Literature search strategy

This systematic review was registered with PROSPERO (Identifier CRD42023478114). We attempted to include all the recently published trials. The search strategy is detailed in Fig. 1. To identify these studies we searched PubMed, Web of Science, Cochrane Library, and EMBASE databases for published trails to evaluate neonatal gastrointestinal infections. The following combinations of terms or keywords were used: (Infant OR Newborn OR Neonate) AND (Intestinal Disease) AND (Culture OR Multiplex PCR OR 16S rRNA OR mNGS). The detailed retrieval strategy is shown in Appendix Table S1. We applied no date or language restrictions. When we encounter non-English articles, we use translation tools, consult experts, and contact authors to ensure full understanding. The last search was performed on January 08, 2025.

Eligibility criteria

Titles and abstracts were initially screened to exclude studies that did not meet the inclusion criteria. Subsequently, full-text analysis was performed to ensure adherence to all inclusion criteria, with studies failing to meet any criteria being excluded. Two investigators (XYL and RZ) independently screened the titles and abstracts, and discrepancies were resolved by a third investigator (GZZ).

The inclusion criteria were as follows: (1) Clinical studies, trials, and observational research focusing on the prevalence, outcomes, diagnosis, or treatment of gastrointestinal disorders in neonates; (2) Participants including male and female newborns; (3) All newborns regardless of gestational age; (4) Retrospective or prospective studies with or without control groups; (5) Studies employing detection methods that successfully identified pathogens.

The exclusion criteria were as follows: (1) Non-neonatal studies; (2) Studies unrelated to gastrointestinal infections; (3) Case reports with fewer than five participants; (4) Research focused solely on detecting a specific pathogen; (5) Research published in the form of abstracts or reviews; Additionally, if multiple articles were published for the same trial, only one paper was included.

Data extraction and quality assessment of the included studies

Data were extracted using a custom-designed Microsoft Excel spreadsheet. Extracted information included author names, article titles, publication years, study locations, study types, designs, participant numbers, test subjects, patient symptoms, gender ratios, antibiotic usage, pathogen detection methodologies, number of positive cases, positivity rates, pathogens, and associated data. Two investigators (XYL and RZ) independently conducted data extraction, cross-checked the extracted data, and discrepancies were resolved by a third investigator (GZZ).

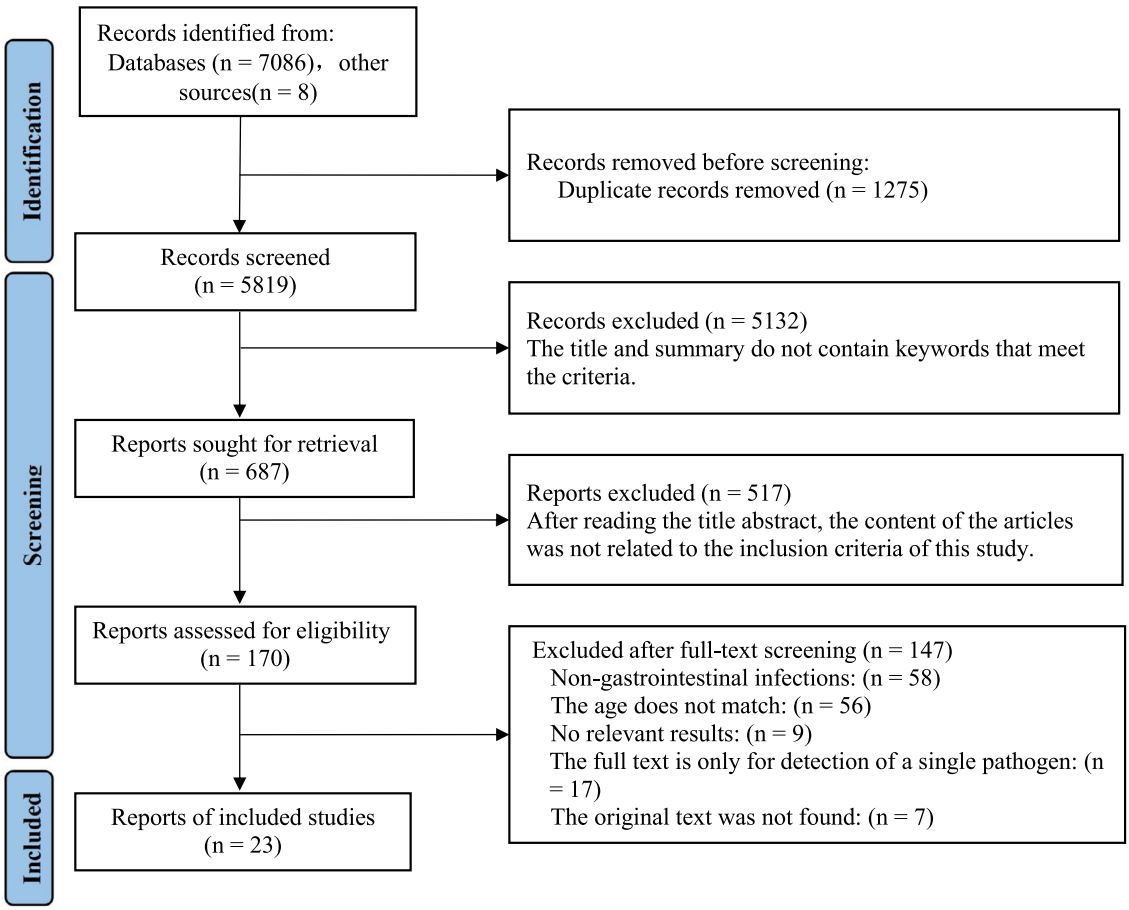

Fig. 1 Flowchart of the literature selection process

The quality of the included articles was assessed using the Joanna Briggs Institute (JBI) critical appraisal tool [16]. Each study was evaluated for its description of sample collection, recruitment methods, subjects and environment, subject numbers and characteristics, results, result reliability, statistical analysis methods, and response rate ("yes", "no", "unclear", or "not applicable"). For each study, the count of "yes" responses across these nine criteria was calculated; a higher number of "yes" responses indicates a lower risk of bias in the study. Studies were categorized as having a high risk of bias if the percentage of "yes" responses was less than 50%, moderate risk if between 50 and 69%, and low risk if 70% or more of responses were "yes" [17, 18]. Two investigators (XYL and RZ) independently conducted the quality assessment, and discrepancies were resolved by a third investigator (GZZ).

Statistical analysis

A meta-analysis was conducted to investigate the detection positivity rates of neonatal gastrointestinal infections and pathogen positivity rates. The primary objective was to evaluate event rates across multiple studies and derive pooled estimates, with a focus on the positivity rates of identified pathogens and the various detection methods employed. Heterogeneity between studies was assessed using the Cochrane Q test, in conjunction with the I^2 statistic. If $P > 0.1$ and $I^2 < 50\%$, a fixed-effect model was selected; if $P \leq 0.1$ or $I^2 \geq 50\%$, significant heterogeneity was indicated, and further analysis of its sources was performed. Meta-analysis requires a sufficient number of studies to accurately estimate the pooled effect, typically at least three independent studies. In cases where certain species were represented by fewer than three studies, these species were grouped under their respective bacterial genera for further examination. Subgroup analysis and meta-regression were conducted to explore the sources of heterogeneity, and sensitivity analysis was used to assess the robustness of the results. Funnel plots were employed to visually examine potential biases, and Egger's test was applied to evaluate publication bias. All

analyses were performed using R (version 4.2.3) with the "meta" package.

Results

As shown in Fig. 1, 7,094 articles were identified from the electronic search. After removing duplicates, 5,819 articles remained. Of these, 5,132 articles were excluded due to the absence of all specified keywords simultaneously. Following a thorough review of titles and abstracts, an additional 517 articles were excluded. Subsequent intensive full-text scrutiny led to the exclusion of 147 articles that did not meet the selection criteria. Ultimately, we included 23 articles, conducted between 1975 and 2022, including four case–control studies [19–22], and nineteen case series [23–41].

In total, 5,475 newborn patients with gastrointestinal infections were recruited in the twenty-three studies. Among these patients, 791 experienced intestinal perforation [25, 26, 31], 3,140 presented with diarrhea [20, 24, 30, 36], 174 exhibited gastroenteritis [29, 33, 39], 1127 were diagnosed with necrotizing enterocolitis (NEC) [19, 21–23, 27, 28, 32, 34, 35, 38, 40, 41], 18 suffered from rectal bleeding [27], and 225 were asymptomatic [37]. More characteristics of the included studies are presented in Table 1.

Quality assessment of the included studies

The quality assessment of the included studies, conducted using the JBI critical appraisal tool, is presented in Appendix Table S3. Among the 23 studies included,

Table 1 Study characteristics

Author	Country	Publication year	Type of study	Patients (n)	Subjects (n)	Major symptoms	Detection method
Holland et al. [31]	Australia	2003	Case series	23	20	Intestinal perforation	Blood culture, Peritoneal culture
Crawley et al. [29]	Australia	1993	Case series	79	79	Gastroenteritis	Stool culture, EIA
Canioni et al. [27]	France	1997	Case series	18	18	Rectal bleeding	Intestinal biopsy
Bell et al. [25]	America	1980	Case series	31	28	Intestinal perforation	Blood culture
Appleton et al. [24]	Britain	1978	Case series	84	84	Diarrhea	Virus isolation and culture
Alfa et al. [23]	Canada	2002	Case series	32	32	NEC	Blood culture, Stool culture
Duangmani et al. [30]	Thailand	1985	Case series	93	93	Diarrhea	Stool culture
Corebima et al. [19]	India	2023	Case control	32	15	NEC	Blood culture
Chan et al. [28]	China	1994	Case series	125	125	NEC	Blood culture, Peritoneal culture, Others
Kosloske et al. [32]	Mexico	1980	Case series	25	23	NEC	Blood culture, Peritoneal culture
Khan et al. [20]	Bangladesh	2009	Case series	2933	2511	Diarrhea	Stool culture
Mohammad et al. [33]	Kuwait	2020	Case series	4	4	Gastroenteritis	Multiplex RT-PCR
Liu et al. [21]	China	2019	Case control	65	9	NEC	Blood culture
Yelak et al. [39]	Israel	2019	Case series	91	91	Gastroenteritis	Stool culture
Senerwa et al. [36]	Kenya	1989	Case control	30	30	Diarrhea	-
Saenz de Pipaon Marcos et al. [35]	Spain	2008	Case series	44	44	NEC	Blood culture
Zhong et al. [41]	China	2013	Case series	277	79	NEC	Blood culture, Stool culture, Peritoneal culture
Yu et al. [22]	China	2023	Case control	194	83	NEC	Blood culture, Peritoneal culture
Yu et al. [40]	Australia	1984	Case series	47	47	NEC	Blood culture, Peritoneal culture, Stool culture
Spencer et al. [37]	Chile	1988	Case series	225	57	Asymptomatic	Stool culture
Rowe et al. [34]	America	1994	Case series	116	99	NEC	Peritoneal culture
Stone et al. [38]	America	1979	Case series	170	64	NEC	Blood culture, Peritoneal culture
Butzer et al. [26]	Germany	2024	Case series	737	719	Intestinal perforation	Blood culture, Peritoneal culture

NEC necrotizing enterocolitis, *EIA* Enzyme immunoassay, *RT-PCR* Real-time Polymerase Chain Reaction

7 were classified as having a low risk of bias, 15 exhibited a moderate risk of bias, and 1 showed a high risk of bias.

Diagnostic methods for neonatal gastrointestinal infections

In one study, the detection of clear pathogenic microorganisms in sepsis patients led to a 100% pathogen detection rate, which helped identify NEC patients. Excluding this study, we analyzed the diagnostic methods for neonatal intestinal infections across the remaining 22 studies. Of these, 10 employed multiple testing methods, while 12 used a single method. Blood culture was the most common diagnostic method, with a combined positive rate of 34.7% (95% CI 24.3–46.0%, $I^2 = 87\%$) among the patients tested in 9 studies. Peritoneal culture, used in 10 studies, showed a combined positivity rate of 55.8% (95% CI 33.5–77.0%, $I^2 = 96\%$) for the tested patients. Stool culture, used in 8 studies, showed a combined positivity rate of 21.3% (95% CI 10.7–34.2%, $I^2 = 92\%$) for the tested patients. Additional methods, including intestinal biopsy tissue culture, umbilical swab culture, umbilical duct culture, gastric aspirate culture, pharyngeal swab culture, enzyme immunoassay (EIA), virus isolation culture, multiplex Real-time Polymerase Chain Reaction (RT-PCR), and electron microscopy, were each used in only one study and thus were not included in the combined analysis.

Subgroup analysis and meta regression

To explore the sources of heterogeneity, a subgroup analysis and Meta-regression of neonatal intestinal infection positivity rates were conducted based on detection methods, economic development levels, regions, and sample sizes. After stratification, the Q value corresponding to the p-value was still significantly less than 0.05, indicating high heterogeneity; thus the random-effects model was applied. The results showed that the positivity rate of peritoneal culture (55.8%) was significantly higher than other detection methods. The positivity rate in high-income countries (56.5%) was the highest. In terms of regions, the positivity rate in the Americas (74.3%) was higher than in Asia (33.9%) and Europe (40.6%). The positivity rate in small sample studies (53.5%) was higher than in large sample studies (41.2%), with a statistically significant difference ($P < 0.05$) (Table 2). Meta-regression analysis revealed that economic development level, sample size, region, and detection methods all had significant effects on the dependent variable. However, no specific sources of heterogeneity were identified.

Pathogen detection rates in neonatal gastrointestinal infections

In some studies, when a pathogen is detected in a patient using multiple testing methods, and others pathogens test positive with individual methods, the case is counted as a single positive instance to avoid inflating the positive rate. After analyzing the data, we summarized the microbial

Table 2 Subgroup analysis of positive rate of gastrointestinal infection detection in newborns

	Studies (n)	Participants (n)	Rate (%)	I^2(%)	*P*-value	*Q*	*Q-P*-value
All studies	22	4216	46.9 (36.8–57.0)	94	$p < 0.01$		
Test method						574.9365	$p < 0.01$
Blood culture	12	1065	34.7 (24.3–46.0)	87	$p < 0.01$		
Peritoneal culture	9	450	55.8 (33.5–77.0)	96	$p < 0.01$		
Stool culture	8	2917	21.3 (10.7–34.2)	92	$p < 0.01$		
Molecular methods	2	251	55.9 (10.0–95.9)	76	$p = 0.04$		
Others	4	34	31.0 (16.5–47.7)	87	$p < 0.01$		
Economic development level						337.4119	$p < 0.01$
High income countries	10	1061	56.5 (42.1–70.4)	95	$p < 0.01$		
Middle income countries	8	553	39.7 (26.7–53.5)	88	$p < 0.01$		
Low income countries	4	2602	35.5 (10.3–66.2)	92	$p < 0.01$		
Area						337.4119	$p < 0.01$
Asia	10	3143	33.9 (26.2–42.0)	82	$p < 0.01$		
Europe	5	791	40.6 (20.6–62.3)	90	$p < 0.01$		
America	5	248	74.3 (64.3–83.2)	59	$p = 0.04$		
Africa	2	34	55.9 (10.0–95.9)	76	$p = 0.04$		
Sample size						337.4119	$p < 0.01$
Small	11	320	53.5 (38.0–68.7)	89	$p < 0.01$		
Large	11	3896	41.2 (28.9–54.1)	95	$p < 0.01$		

spectrum of neonatal gastrointestinal infections. *Escherichia* showed the highest pooled positive rate (22.2%; 95% CI 8.3–40.4%, $I^2=98\%$), followed by *Clostridium* (21.8%; 95% CI 2.2–53.8%, $I^2=96\%$), *Klebsiella* (19.2%; 95% CI 8.3–33.4%, $I^2=97\%$), and *Staphylococcus* (13.6%; 95% CI 6.0–23.7%, $I^2=91\%$). Among fungi, *Candida* had the highest rate (3.8%; 95% CI 0.6–9.6%, $I^2=84\%$). For bacteria, *Klebsiella pneumoniae* had the highest rate (30.2%; 95% CI 11.3–53.5%, $I^2=95\%$), followed by *Escherichia coli* (22.7%; 95% CI 9.5–39.4%, $I^2=98\%$), *Staphylococcus epidermidis* (14.8%; 95% CI 1.3–39.2%, $I^2=88\%$), and *Clostridium perfringens* (13.6%; 95% CI 7.6–20.9%, $I^2=0\%$). Among viruses, Rotavirus had the highest rate (11.6%; 95% CI 1.0%–31.5%, $I^2=94\%$), followed by Adenovirus (4.1%; 95% CI 0.5–11.0%, $I^2=58\%$). Detailed data are available in Table 3.

In blood culture assays, *Escherichia* demonstrated the highest positive rate (10.0%; 95% CI 3.1–20.4%, $I^2=92\%$), followed by *Staphylococcus* (9.4%; 95% CI 4.3–16.2%, $I^2=88\%$). Peritoneal fluid culture assays revealed *Klebsiella* as having the highest positive rate (24.7%; 95% CI 6.6–49.5%, $I^2=95\%$), followed by *Enterococcus* (11.6%; 95% CI 0.6–33.5%, $I^2=96\%$). In stool culture assays, *Escherichia* exhibited the highest positive rate (11.0%; 95% CI 3.8–21.3%, $I^2=75\%$), followed by *Campylobacter* (3.9%; 95% CI 1.6%–7.2%, $I^2=15\%$). Detailed information can be found in Appendix Table S2.

Pathogen detection varied across patients with different symptoms, with NEC patients constituting the largest group. In NEC patients, *Escherichia* (24.3%; 95% CI 4.0–54.5%, $I^2=98\%$) and *Klebsiella* (22.0%; 95% CI 7.6–41.1%, $I^2=96\%$) were most frequently identified, with *Klebsiella pneumoniae* showing the highest infection rate (39.1%; 95% CI 9.3–74.4%, $I^2=97\%$).

Detailed NEC pathogen detection is presented in Table 4. In diarrhea cases, *Vibrio* and *Aeromonas* were the primary pathogens. For gastroenteritis, Rotavirus and *Campylobacter* were the most common. In gastrointestinal perforation, *Staphylococcus* and *Escherichia* predominated, while in lower gastrointestinal bleeding, *Escherichia* and *Klebsiella* were most frequently found.

Table 3 The positivity rate of the main pathogens causing gastrointestinal infections in newborns

Pathogen	Combine infection rate*			
	Studies (n)	Participants (n)	Rate (%)	I^2 (%)
Bacteria				
Escherichia	12	1366	22.2 (95% CI 8.3–40.4)#	98
Escherichia coli	13	1465	22.7 (95% CI 9.5–39.4)	98
Clostridium	5	265	21.8 (95% CI 2.2–53.8)#	96
Clostridium perfringens	3	102	13.6 (95% CI 7.6–20.9)	0
Klebsiella	13	1374	19.2 (95% CI 8.3–33.4)#	97
Klebsiella pneumoniae	7	317	30.2 (95% CI 11.3–53.5)	95
Staphylococcus	9	1204	13.6 (95% CI 6.0–23.7)#	91
Staphylococcus epidermidis	3	209	14.8 (95% CI 1.3–39.2)	88
Staphylococcus aureus	5	978	7.6 (95% CI 2.2–15.7)	82
Enterococcus	6	1059	12.4 (95% CI 1.8–30.3)	96
Streptococcus	7	1015	6.8 (95% CI 2.5–12.9)#	43
β-haemolytic streptococcus	3	212	4.2 (95% CI 0.3–12.7)	81
Enterobacter	7	1107	4.8 (95% CI 2.2–8.4)	74
Pseudomonas	6	1069	2.9 (95% CI 0.2–8.5)#	88
Pseudomonas aeruginosa	4	251	5.3 (95% CI 0.4–15.3)	87
Campylobacter	4	366	2.8 (95% CI 0.8–5.8)	53
Salmonella	5	3457	1.3 (95% CI 0.5–2.5)	72
Fungus				
Candida	6	996	3.8 (95% CI 0.6–9.6)	84
Viruses				
Rotavirus	3	256	11.6 (95% CI 1.0–31.5)	94
Adenovirus	3	167	4.1 (95% CI 0.5–11.0)	58

* If a pathogen is detected in the same patient using various methods, the case is counted as a single positive instance to prevent inflating positivity rates. Even with multiple positive tests, the case is only counted once; #Due to fewer than three studies for certain unlisted species, they were grouped by bacterial genera for analysis

Table 4 Pathogen detection in necrotizing enterocolitis

Pathogen	Studies (n)	Participants (n)	Infection rate (%)	I^2 (%)
Bacteria				
Escherichia	7	520	24.3 (95% CI 4.0–54.5)*	98
Escherichia coli	7	520	24.3 (95% CI 4.0–54.5)	98
Klebsiella	9	579	22.0 (95% CI 7.6–41.1)*	96
Klebsiella pneumoniae	4	241	39.1 (95% CI 9.3–74.4)	97
Clostridium	5	265	21.8 (95% CI 2.2–53.8)*	96
Clostridium perfringens	3	102	13.6 (95% CI 7.6–20.9)	0
Enterococcus	5	340	15.2 (95% CI 2.0–37.5)	95
Streptococcus	4	143	12.0 (95% CI 5.5–20.5)*	39
β-haemolytic strept	3	212	4.2 (95% CI 0.3–12.7)	81
Staphylococcus	7	520	10.9 (95% CI 3.2–22.4)*	92
Staphylococcus aureus	4	259	10.0 (95% CI 3.3–19.7)	82
Enterobacter	5	370	5.3 (95% CI 2.1–9.9)	65
Pseudomonas	5	350	4.0 (95% CI 0.4–11.3)*	84
Pseudomonas aeruginosa	4	251	5.3 (95% CI 0.4–15.3)	87
Fungus				
Candida	3	213	4.0 (95% CI 0.8–9.6)	51

* Due to fewer than three studies for certain unlisted species, they were grouped by bacterial genera for analysis

Sensitivity analysis

Sensitivity analysis (using the leave-one-out approach) was performed to assess the stability of the included study results. The analysis revealed that the neonatal gastrointestinal infection positivity rate ranged from 46.9 to 51.9%, indicating that the combined results were robust.

Publication bias

Funnel plots were generated to visually assess the potential for publication bias. The results indicated relatively low symmetry in the funnel plot (see Fig. 2). In conjunction with the results of Egger's test ($p=0.0022$), which showed a bias estimate of 2.8641 and standard error (*SE*) of 0.8196, the findings suggest the possibility of publication bias.

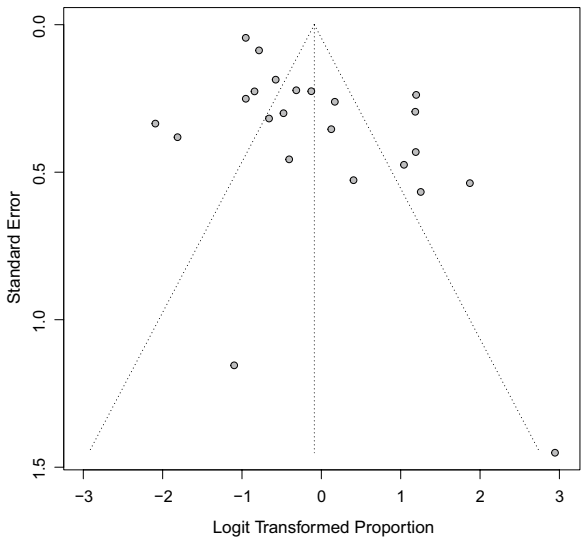

Fig. 2 Funnel plot of the detection positivity rates in neonates with gastrointestinal infections

Discussion

This meta-analysis provides the first comprehensive summary of the infection spectrum in neonatal gastrointestinal infections. By integrating three detection methods and analyzing co-infection rates, we identified key pathogens, including *Klebsiella pneumoniae, Escherichia coli, Staphylococcus epidermidis, Clostridium perfringens, Staphylococcus aureus, β-hemolytic streptococci, Pseudomonas aeruginosa, Campylobacter, Salmonella, Candida,* Rotavirus, and Adenovirus. Peritoneal culture showed a notably higher positive rate than other diagnostic methods. Our study found that NEC patients had a significantly higher proportion of detected pathogens, suggesting intestinal pathogen infection may trigger NEC. Understanding pathogen prevalence helps clinicians predict potential pathogens and make informed decisions on antibiotic selection. Clarifying the infection spectrum is crucial for optimizing molecular diagnostics and guiding empirical antibiotic use.

Previous studies consistently show a high positivity rate for peritoneal culture [42–44]. Our research also found significantly higher positivity rates in peritoneal culture

compared to other methods. Peritoneal cultures were obtained from abdominal drainage catheter placement or during surgery. These patients had suffered from severe peritonitis or intestinal perforation, which required laparoscopy or laparotomy. Although the positive rate of peritoneal cultures was high, it cannot be routinely used for diagnosing gastrointestinal infections, especially in non-surgical patients. Research on stool culture is less common, likely due to concerns that intestinal colonization may affect results. Additionally, there are challenges in using stool cultures to guide initial treatment decisions [45]. Other studies have reported similarly low positivity rates for stool cultures [46], and detecting genera such as Campylobacter is challenging using routine methods [47]. Consequently, stool cultures are rarely performed alone, and some patients may test positive in stool cultures but negative in blood cultures [48]. Molecular assays are less frequently used because they are complex, costly, and impractical for routine clinical use [49]. Only one study has utilized Multiplex RT-PCR for detection. Moreover, in several related studies, mNGS has demonstrated superior sensitivity and specificity compared to conventional diagnostic methods, enabling the detection of pathogens not detectable by traditional techniques. Furthermore, mNGS allows for the comprehensive identification of all potential pathogens in a single test, with a significantly higher positive rate than conventional methods [50]. For patients with unclear initial diagnoses, timely adjustment of targeted antibiotic therapy based on positive molecular diagnostic results has been shown to significantly improve clinical outcomes. We recommend using cost-effective conventional diagnostic methods in low-income regions, incorporating stool and blood cultures into routine testing to enhance diagnostic accuracy. Additionally, we suggest collecting peritoneal samples during necessary surgical interventions to support diagnosis. In middle- to high-income regions, molecular diagnostic methods can be added to conventional testing to further support accurate diagnosis.

Given the mortality rate of neonatal infections, clinicians must provide the most effective empirical treatment before microbiological diagnosis is confirmed. Our research identified *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus epidermidis*, *Clostridium perfringens*, *Staphylococcus aureus*, *β-hemolytic streptococci*, *Pseudomonas aeruginosa*, *Campylobacter*, *Salmonella*, *Candida*, Rotavirus, and Adenovirus as highly suspected pathogens based on laboratory findings. The bacteria most commonly detected in blood cultures are *Klebsiella pneumoniae* and *Escherichia coli*. Peritoneal cultures yielded the highest rates of *Klebsiella*, followed by *Enterococcus* and *Escherichia*. In stool cultures, the most commonly detected pathogens were *Escherichia*, *Campylobacter,* and *Salmonella*. *Escherichia coli*, a well-known opportunistic pathogen, is recognized as one of the primary causative agents of infant diarrhea globally [51]. In 2021, an estimated 44.4 million cases of diarrheal disease caused by Enterotoxigenic *Escherichia coli* (ETEC) resulted in approximately 28,230 deaths [52]. Our research confirms this observation by showing that *Escherichia coli* exhibits a higher detection rate across the three methods employed. *Escherichia coli* exhibited a notable positivity rate of 22.7% (95% CI 9.5−39.4%, $I^2 = 98\%$) among afflicted newborns using the three methods employed in this study. The high prevalence of Escherichia coli in the gastrointestinal tract is due to several factors. First, it is the most common commensal in both humans and animals [53]. Escherichia coli can induce attaching and effacing (A/E) lesions on intestinal epithelial cells [54, 55], facilitating the penetration of E. coli and other pathogens through altered epithelial cells. Additionally, rising antibiotic resistance poses a major challenge to effective treatment [56]. *Klebsiella pneumoniae* showed the highest positivity rates in both blood and peritoneal cultures, with a positivity rate of 30.2% (95% CI 11.3−53.5%, $I^2 = 95\%$) among sick newborns. These findings are consistent with previously reported outcomes of neonatal sepsis pathogen detection studies [51].

Bacterial colonization of the gut shortly after birth triggers the growth of bacteriophages. Current research suggests that intestinal infection and inflammation are primary causes of NEC [57–60]. Before NEC onset, changes in the intestinal flora occur, including reduced diversity, flora imbalance, and a decrease in probiotics. Enteric pathogens such as *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus epidermidis* were relatively dominant [59, 61], which is consistent with the findings of our study. Bacterial invasion of the gastrointestinal tract and the release of toxins or antigens damage intestinal epithelial cells, leading to NEC, which can enter the bloodstream and trigger systemic inflammation. Our study shows that NEC patients have a significantly higher incidence and proportion of detected pathogens compared to other conditions, highlighting the potential role of enteric infections in triggering NEC.

Limitations

This study has several limitations. Firstly, most studies are from developing countries, potentially introducing geographical bias, and limiting global applicability. Findings may not extend to high-resource healthcare environments, but they retain considerable significance. Therefore, further research needs to be conducted in high-resource countries to obtain more information.

Secondly, although the study includes 23 articles, the lack of sufficient case–control studies (only 4 out of 23) limits the ability to establish comparative outcomes rigorously. The larger proportion of case series reduces the strength of evidence for drawing conclusions about causal relationships, which may limit the generalizability of the findings. Lastly, some studies focus on a single pathogen, which may overlook co-infections or distort pathogen probabilities and rankings. To avoid bias in the infection spectrum, we excluded such studies, ensuring more accurate estimates of the overall infection profile, though this may limit the inclusiveness of our findings.

Conclusion

This meta-analysis described in detail the spectrum of infections in gastrointestinal Infections. Our research identified *Klebsiella pneumoniae*, *Escherichia coli*, *Candida*, Rotavirus, Adenovirus, and other pathogens as highly suspected based on laboratory testing. Additionally, intestinal pathogen infection is associated with an increased risk of neonatal NEC. Peritoneal culture has significant advantages in detecting gastrointestinal infections. Prospective studies are needed to address underresearched regions and incorporate emerging diagnostic methods for more targeted approaches to neonatal gastrointestinal infections. Future development of multiplex enteric panels, qPCR assays, or mNGS for blood or stool testing could greatly improve diagnostic accuracy and treatment strategies.

Abbreviations

PCR	Polymerase chain reaction
mNGS	Metagenomic next-generation sequencing
NICUs	Neonatal intensive care units
CI	Confidence interval
JBI	Joanna Briggs Institute
NEC	Necrotizing enterocolitis
EIA	Enzyme immunoassay
RT-PCR	Real-time polymerase chain reaction

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13099-025-00693-5.

Additional file 1

Author contributions

GZZ, JL, and XYL initiated the project and were responsible for the protocol design. XYL and RZ conducted a comprehensive literature review, gathered data, assessed study quality, and performed data analysis. MDW, CCT, FFY, QJY, CYH, YZ, ZMR, and LQL interpreted the data. XYL drafted the initial manuscript. All the authors were responsible for the critical revision of the manuscript and provided important intellectual input. All authors made substantial contributions to this article and approved the final submitted version.

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Availability of data and materials

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

All included studies were conducted in accordance with ethical standards and received approval from their respective ethics committees or institutional review boards. Furthermore, all data were anonymized to ensure patient confidentiality, and the studies adhered to relevant data protection regulations to safeguard the privacy and security of patient information.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Faculty of Life Science and Technology and the Affiliated Anning First People'S Hospital, Kunming University of Science and Technology, Kunming 650302, China. [2]Department of Neonatology, The Affiliated Anning First People'S Hospital of Kunming University of Science and Technology, Kunming 650302, Yunnan, China. [3]Department of Pain Medicine, The Affiliated Anning First People'S Hospital of Kunming University of Science and Technology, Kunming 650302, Yunnan, China.

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