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ORIGINAL RESEARCH

Detection of *Salmonella* spp. Related Co-Infections Among Children with Diarrheal Diseases in Guangzhou, China

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Background: Diarrheal diseases caused by gastrointestinal pathogens contribute to the high morbidity and mortality in children worldwide. *Salmonella* infection is one of the leading causes of diarrhea, especially in children under 5 years of age. This study aimed to assess the prevalence of *Salmonella* infection and its co-infection patterns in relation to clinical symptoms.

Methods: A total of 430 stool samples of children with diarrheal diseases were collected from Guangdong Women and Children's Hospital during January 2022 to December 2023 and used for detection. BioFire FilmArray Gastrointestinal (GI) Panel is an efficient and sensitive method used to assess infections caused by enteric pathogens simultaneously based on multiplex polymerase chain reaction (PCR) technologies.

Results: *Salmonella* spp. was classified as the predominant pathogen in all stool specimens, with an overall positivity rate of 36.74% (158/430), of which 35.44% (56/158) were identified as *Salmonella* single infection. For *Salmonella*-related bacterial co-infection pattern, *Salmonella* and enteropathogenic *Escherichia coli* combinations accounted for 12.66% (20/158) of *Salmonella*-related bacterial co-infection patterns, and *Salmonella* plus *Clostridium difficile* was found in 8.86% (14/158). For *Salmonella*-viral co-infection pattern, the most prevalent combination was *Salmonella* and adenovirus (6.33%, 10/158). Notably, the proportion of mucus stools recorded in *Salmonella* plus *C. difficile* infections was statistically higher than that in single *Salmonella* infections ($P < 0.05$).

Conclusion: This study provides a comprehensive understanding of the nature of *Salmonella* spp. co-infections in diarrheal diseases, and the possibility of clinical symptoms and enhanced treatments.

Keywords: enteric pathogens, gastrointestinal infection, diarrhea, FilmArray

Introduction

Diarrheal disease is the second leading cause of death in children under 5 years of age and was responsible for the deaths of 370,000 children in 2019.[1–3] In China, according to some regional studies, the overall incidence of diarrhea among children under 5 years old is 2.50–3.38 episodes per year for individuals.[4] Diarrhea is the passage of three or more loose or liquid stools per day, or more frequently than normal for the individual,[1] and is a common symptom of gastrointestinal infection (GI) that is mainly caused by single or multiple bacterial, viral, and parasitic organisms.[3,5–7] The most prevalent bacterial pathogens are *Escherichia coli, Salmonella* enterica, *Campylobacter* spp., and *Shigella* spp, while adenovirus, rotavirus, and norovirus were mainly responsible for viral infections. Infectious diarrhea demonstrates different seasonal distributions.[4]

Salmonella is a rod-shaped, gram-negative bacteria with no capsule and spore, and can be classified into typhoidal *Salmonella* and non-typhoidal *Salmonella* (NTS) serovars.[8] NTS infection is a leading cause of acute gastroenteritis in pediatric patients, especially children aged under 5 years.[9,10] *Salmonella* infection results in over 30,000 deaths annually, which was estimated by the Chinese Center for Disease Control (CDC) to be the cause of 70–80% of bacterial foodborne

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illnesses, ranking as one of the two diarrheal agents.[11,12] Zoonotic infection caused by NTS post a significant burden for public health.[13] A rising trend of Salmonellosis outbreak has been observed since the 1970s with high resistance to quinolone and beta-lactam, such as carbapenem.[14,15] From the nationwide surveillance study, NTS, *Shigella, C. jejuni* and *Y. enterocolitica* displayed a "Child-pattern", with a detection turn-point at 2–5 years.[11,16] However, it remains unclear whether *Salmonella*-related co-infection patterns influence diarrheal symptoms among children.

The comprehensive detection of gastrointestinal pathogenic microorganisms is challenging because conventional culture methods are time-consuming and serology tests show limited sensitivity.[17,18] The rapid and specific validation of gastrointestinal infectious agents is of great significance for clinical management. To overcome these difficulties, FilmArray™ technology (BioFire Diagnostics, Salt Lake City, Utah, USA) has produced rapid polymerase chain reaction (PCR) multiplexing, which is designed to simultaneously detect the most common gastrointestinal pathogens.[19,20] The FilmArray GI panel (FA) not only offers high sensitivity and specificity for identifying infectious pathogens in different physiological tracts,[21–23] but also provides a comprehensive way to reveal the nature of co-infection patterns among various pathogens.

The distribution of gastrointestinal pathogens, including *Salmonella* spp., shows significant regional characteristics. However, the distribution and clinical features of *Salmonella* related diarrhea in Guangzhou remain largely unknown. It is of crucial necessity to analyze the distribution and associated infection patterns of *Salmonella* among children, which would provide supporting information for promoting the understanding of Salmonellosis and improving bacterial gastroenteritis individualized treatment. The objectives of this study were as follows: 1) to test *Salmonella* in pediatric patients with diarrheal diseases using the FilmArray GI Panel to assess the overall positive rate of *Salmonella*; 2) to analyze the prevalence of *Salmonella* spp. via Film Array GI Panel and retrospective cultural results; and 3) to investigate the co-infection patterns of *Salmonella* with other enteric pathogens among clinical characteristics to assess the differences in symptoms.

Materials and Methods

Sample Enrollment

All stool samples in this study were obtained from patients as residual specimens of routine hospital testing procedure during January 2022 to December 2023 in Guangzhou, China. A total of 430 diarrheal stool samples from children under five years old were enrolled for FilmArray GI Panel (BioFire Diagnostics, Salt Lake City, UT, USA), and all samples were anonymized (Table S1). Second or subsequent samples from the identical patients or sample without clinical histories implicating infectious causes of diarrhea were excluded. The clinical results of fecal cultures for *Salmonella* spp. from 1580 patients were collected from the Laboratory Information System (LIS). These data were retrospectively categorized and analyzed to reveal the prevalence and distribution of *Salmonella* infection. Clinical information exported from LIS only retain gender, age, testing date, laboratory testing results and clinical syndromes. This study was approved by the Medical Ethics Committee of Guangdong Women and Children Hospital.

FilmArray GI Panel

All stool samples were tested using a commercially available multiplex PCR system, FilmArray Gastrointestinal (GI) Panel (BioFire Diagnostics, Salt Lake City, UT, USA). The FilmArray GI Panel was capable of simultaneously detecting and identifying nucleic acids from multiple bacteria, viruses, and parasites in stool samples, including *Campylobacter* (*C. jejuni*/*C. coli*/*C. upsaliensis), Clostridium difficile* (*C. difficile*) toxin A/B, *Plesiomonas shigelloides, Salmonella, Vibrio* (*V. parahaemolyticus*/*V. vulnificus*/*V. cholerae), Yersinia enterocolitica*, enteroaggregative *Escherichia coli* (EAEC), enteropathogenic *Escherichia coli* (EPEC), enterotoxigenic *Escherichia coli* (ETEC), Shiga-like toxin-producing *Escherichia coli* (STEC) stx1/stx2, *Shigella*/Enteroinvasive *Escherichia coli* (EIEC), *Cryptosporidium, Cyclospora cayetanensis, Entamoeba histolytica, Giardia lamblia*, Adenovirus F 40/41, Astrovirus, Norovirus GI/GII, Rotavirus A, Sapovirus (Genogroups I, II, IV, and V). According to FilmArray GI Panel Instruction, two process controls (RNA Process Control and PCR2 Control) were used in each essay and positive results indicated successful assay.

Salmonella Identification

Isolation and identification of *Salmonella* spp. was conducted according to previous research. Stool samples were plated and incubated onto XLD (Xylose Lysine Desoxycholate) agars at 36°C for 18–48h. The suspicious colonies (transparent, round, with or without black centers that reflect H_2S production) were further identified by MALDI-TOF-MS (Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrum).

Statistical Analysis

All data on clinical characteristics were imported into Microsoft Excel. GraphPad Prism 8 was used to display the figures. Statistical analyses, including *Chi-square* test or Fisher's exact test, were performed using the SPSS, version 26.0.

Results

Gender and Age Demographics of Patients

For the FilmArray GI Panel, 430 children aged <5 years with diarrheal diseases were enrolled in this study (Table 1), including 184 (42.79%) females and 246 (57.21%) males. Among them, 72 (39.13%) and 86 (34.96%) were positive for *Salmonella* spp. No statistically significant difference was observed between the sexes in the detection of *Salmonella* spp. ($\chi^2 = 0.788$, $P = 0.375$). The majority of stool samples were collected from children under 12 months of age (235/430), followed by 1–3 years old (161/430), and over 3 years old (34/430). The positivity rates of *Salmonella* spp. among the three age groups were 37.87% in children aged <12 months, 38.51% in children aged 1–3 years, and 20.59% in children aged >3 years. Moreover, 1580 patients who underwent fecal culture for *Salmonella* spp. were categorized and analyzed. Statistical differences were observed between children aged <12 months and those aged >3 years using both the FA ($\chi^2 = 3.866$, $P = 0.049$) and culture methods ($\chi^2 = 6.481$, $P = 0.011$). Among all stool specimens, 178 were subjected to both *Salmonella* detection methods.

Prevalence of Salmonella spp. in Monthly and Age Distribution

Either FA or culture detection of *Salmonella* spp. was defined as a positive result for *Salmonella* spp. prevalence. In the monthly distribution of *Salmonella* spp., the highest positive rate was observed in September (42.51%, 71/167), and the lowest rate was 8.49% (9/106) in February (Figure 1A). The positive rate increased from April to July, whereas a remarkable decline was observed from September to December, followed by a peak (Figure 1A). The positive detection rate of *Salmonella* spp. was similar among the three age groups. In accordance with the results in Table 1, the positivity rate in children aged >3 years was likely to be lower than in the other two age groups (Figure 1B).

Table 1 Gender and Age Distribution of Patients with Salmonella Spp. Detection

Methods	Groups	n	Positive, NO. (%)	Odd Ratio	χ^2	*P*
FA	**Gender**					
	Female	184	72 (39.13)	–	–	–
	Male	246	86 (34.96)	0.805–1.776	0.788	0.375
	Age					
	<12 months	235	89 (37.87)	–	–	–
	1–3 years	161	62 (38.51)	0.644–1.471	0.016	0.898
	3–5 years	34	7 (20.59)	0.983–5.624	3.866	0.049
Culture	**Gender**					
	Female	626	154 (24.60)	–	–	–
	Male	954	244 (25.58)	0.752–1.198	0.191	0.662
	Age					
	<12 months	955	240 (25.13)	–	–	–
	1–3 years	534	146 (27.34)	0.702–1.134	0.871	0.351
	3–5 years	91	12 (13.19)	1.183–4.127	6.481	0.011

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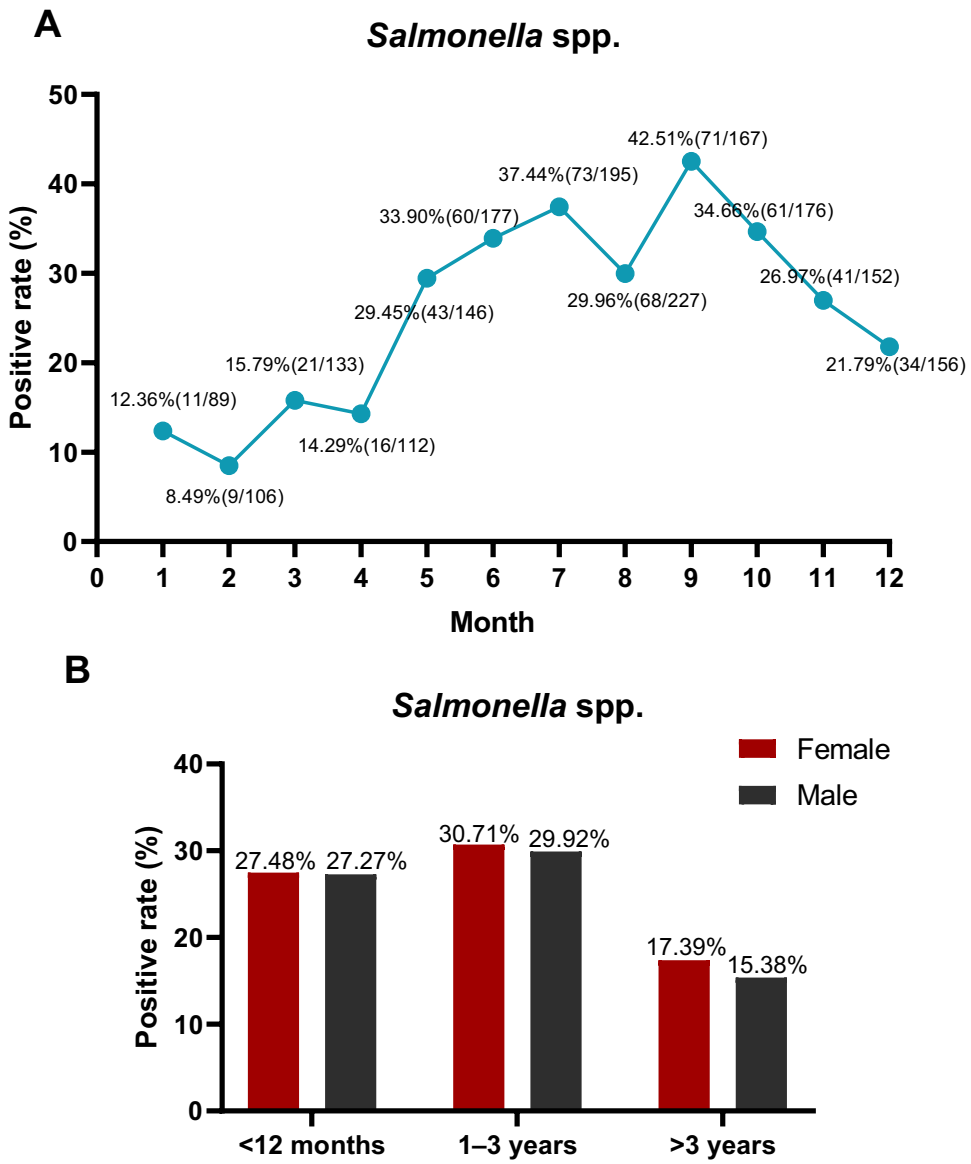

Figure 1 Prevalence of *Salmonella* spp. detected by FA and culture method. (**A**) Monthly distribution of *Salmonella* spp. positive rate from January 2023 to December 2024. (**B**) Distribution of *Salmonella* spp. in children (female vs male) aged under 12 months, 1–3 years and over 3 years respectively.

Prevalence of All Enteric Pathogens Detected by FilmArray

Of all the stool samples, 79.3% had at least one type of enteric pathogen (Table 2). The overall positive rate of *Salmonella* spp. was the highest among all the bacterial pathogens, which was detected in 36.74% (158/430), followed by EPEC (19.77%, 85/430), *C. difficile* (19.53%, 84/430), and *Campylobacter* (12.56%, 54/430). Among enteric viral pathogens, adenovirus contributed to the majority of infections (20.23%, 87/430). The proportions of bacterial single infection (Table 3), viral single infection, bacterial–bacterial infection, viral–viral infection and bacterial–viral infections were 24.19% (104/430), 10.00% (43/430), 18.60% (80/430), 3.02% (13/430), and 23.49% (101/430), respectively.

Co-Infection Patterns of Salmonella spp

Among the stool samples positive for *Salmonella* spp., 35.44% (56/158) were positive for *Salmonella* spp., whereas 37.34% (59/158) of stool samples were positive for both *Salmonella* spp. and other enteropathogenic bacteria. Moreover, 30 stool samples (18.99%) tested positive for *Salmonella* spp. and enteric viruses. A total of 13 (8.23%) stool samples were positive for *Salmonella* spp. and bacterial-viral co-infection. In the *Salmonella* mixed infection, *Salmonella* plus

Table 2 Frequency of Enteric Pathogens Detected by FA

Enteric Pathogens	Frequency	%
Salmonella spp.	158	36.74
EPEC	85	19.77
C. difficile	84	19.53
Campylobacter	54	12.56
ETEC	26	6.05
EAEC	23	5.35
Shigella/EIEC	10	2.33
STEC	1	0.23
Adenovirus	87	20.23
Norovirus	40	9.30
Rotavirus	30	6.98
Sapovirus	22	5.12
Astrovirus	12	2.79
No pathogen	89	20.70

Abbreviation: FA, FilmArray GI Panel.

Table 3 Infection Patterns of Enteric Pathogens

Infection Patterns	Frequency	%
Bacterial single infection	104	24.19
Salmonella spp.	56	13.02
Campylobacter	21	4.88
C. difficile	17	3.95
EPEC	8	1.86
EAEC	1	0.23
Shigella/EIEC	1	0.23
Viral single infection	43	10.00
Adenovirus	20	4.65
Rotavirus	11	2.56
Norovirus	6	1.40
Astrovirus	4	0.93
Sapovirus	2	0.47
Bacterial-bacterial co-infection	80	18.60
Viral-viral co-infection	13	3.02
Bacterial-viral co-infection	101	23.49

Abbreviation: FA, FilmArray GI Panel.

EPEC (12.66%, 20/158), *Salmonella* plus *C. difficile* (8.86%, 14/158) and *Salmonella* plus adenovirus (6.33%, 10/158) were the first three highest compositions. *Salmonella, C. difficile* and EPEC combination was detected in 4.43% (7/158) of all stool samples. *Salmonella* plus Norovirus, *Salmonella* plus EAEC were detected in 4.43% (7/158) and 2.53% (4/158) of the stool specimens, respectively (Table 4).

Clinical Symptoms in Different Salmonella Co-Infection Patterns

The percentage of female patients with *Salmonella* single infection, *Salmonella* plus *C. difficile, Salmonella* plus EPEC, and *Salmonella* plus adenovirus were 42.86%, 35.71%, 40.00%, and 50.00%, respectively (Table 5). Similarly, most of the patients in the four groups were under 1 year of age, and 80.00% of the children in *Salmonella* plus EPEC group were under 12 months of age. Clinical symptoms of fever were observed in 67.86% (38/56) of *Salmonella* single infections, 71.43% (10/14) of *Salmonella* infections plus *C. difficile*, 60.00% (12/20) of *Salmonella* infections plus EPEC, and 50%

Table 4 Infection Patterns of Salmonella Spp. Detected by FA

Infection Patterns	Frequency	%	Infection Patterns	Frequency	%
***Salmonella* single infection**	**56**	35.44	***Salmonella*-viral co-infection**	**30**	18.99
			Salmonella+Rotavirus	3	1.90
***Salmonella*-related bacterial co-infection**	**59**	37.34	*Salmonella*+Norovirus	7	4.43
Salmonella+*C. difficile*	14	8.86	*Salmonella*+Adenovirus	10	6.33
Salmonella+*C. difficile*+EPEC	7	4.43	*Salmonella*+Astrovirus	3	1.90
Salmonella+EAEC	4	2.53	*Salmonella*+Sapovirus	1	0.63
Salmonella+EAEC+EPEC+ETEC	1	0.63	*Salmonella*+Norovirus+Sapovirus	1	0.63
Salmonella+EPEC	20	12.66	*Salmonella*+Adenovirus+Norovirus	1	0.63
Salmonella+EPEC+ETEC	2	1.27	*Salmonella*+Adenovirus+Sapovirus	4	2.53
Salmonella+EPEC+*Shigella*	1	0.63			
Salmonella+ETEC	3	1.90	***Salmonella* multiple co-infection**	**13**	8.23
Salmonella+ETEC+STEC	1	0.63	*Salmonella*+*C. difficile*+Adenovirus	1	0.63
Salmonella+*Campylobacter*+*C. difficile*	2	1.27	*Salmonella*+*C. difficile*+EPEC+ETEC+Astrovirus	1	0.63
Salmonella+*Campylobacter*+EPEC	1	0.63	*Salmonella*+*C. difficile*+EPEC+Rotavirus	2	1.27
Salmonella+*Shigella*+EPEC	2	1.27	*Salmonella*+*C. difficile*+EPEC+Rotavirus+Sapovirus	1	0.63
Salmonella+*Shigella*+ETEC	1	0.63	*Salmonella*+*C. difficile*+*Shigella*+EPEC+Adenovirus	1	0.63
			Salmonella+*C. difficile*+*Shigella*+Norovirus +Sapovirus	1	0.63
			Salmonella+EAEC+ETEC+Adenovirus	1	0.63
			Salmonella+EAEC+ETEC+Adenovirus+Rotavirus	1	0.63
			Salmonella+EPEC+Adenovirus	2	1.27
			Salmonella+ETEC+Adenovirus	1	0.63
			Salmonella+*Shigella*+EPEC+Adenovirus	1	0.63

Table 5 Symptom Distribution of Different Salmonella Infection Patterns

Clinical Information	*Salmonella* Single Infection (n = 56)	*Salmonella*+*C. d* (n = 14)	*Salmonella*+EPEC (n = 20)	*Salmonella*+ADV (n = 10)
Gender				
Female	24 (42.86%)	5 (35.71%)	8 (40.00%)	5 (50.00%)
Male	32 (57.14%)	9 (64.29%)	12 (60.00%)	5 (50.00%)
Age				
<12months	34 (60.71%)	8 (57.14%)	16 (80.00%)	5 (50.00%)
1–3 years	19 (33.93%)	6 (42.86%)	4 (20.00%)	5 (50.00%)
>3 years	3 (5.36%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
Clinical characteristics				
Fever, n(%)	38 (67.86%)	10 (71.43)	12 (60.00%)	5 (50.00%)
Diarrhea, n(%)	56 (100.00%)	14 (100.00%)	20 (100.00%)	10 (100.00%)
Vomiting, n(%)	8 (14.29%)	1 (7.14%)	1 (5.00%)	0 (0.00%)
Diarrheal characteristics				
Bloody stool, n(%)	24 (42.86%)	6 (42.86%)	8 (40.00%)	1 (10.00%)
Watery stool, n(%)	10 (17.86%)	1 (7.14%)	0 (0.00%)	1 (10.00%)
Mucusy stool, n(%)	35 (62.50%)	13 (92.86%)[a]	9 (45.00%)	7 (70.00%)

Notes: [a]$P < 0.05$, compared with *Salmonella* single infection.
Abbreviations: *C. d, C. difficile*; EPEC, Enteropathogenic *Escherichia coli*; ADV, adenovirus.

of *Salmonella* infections plus adenovirus. Vomiting symptoms were recorded in 14.29% of patients with *Salmonella* single infections. All patients in the four groups had diarrhea, whereas the blood stools were recorded in 42.86% of *Salmonella* single infections and in 42.86% of *Salmonella* plus *C. difficile*. Notably, mucus stools were recorded in *Salmonella* plus *C. difficile* at a high rate of 92.86% (13/14), which was significantly higher than that in *Salmonella* infections alone ($P < 0.05$).

Discussion

Salmonellosis is responsible for one of the major causes of diarrhea, and salmonellosis outbreaks have post a great threat to worldwide developing regions, especially China.[14] *Salmonella* spp. is primarily transmitted through contaminated foods such as eggs, milk, meat, and drinking water.[12,24] Although children's consumption of nutritious foods has increased, their immature immune systems heighten susceptibility to *Salmonella* infections. This study found the high *Salmonella* positive rate among children with gastroenteritis, which was lower than the finding of Truong J et al and Spina A et al,[25,26] for the *Salmonella* infection is likely to be associated with climate, regions and living habit. Notably, co-infection with *Clostridium difficile* correlated with exacerbated diarrhea severity, highlighting the necessity to enhance pediatric dietary hygiene management and reinforce preventive strategies against gastrointestinal infections. Furthermore, healthcare institutions should optimize rapid pathogen detection technologies to facilitate comprehensive enteric pathogen screening in pediatric populations.

Given that the gastrointestinal tract is home to a large number of microorganisms, co-infection is a common enteric pathogen in diarrheal infections. The high burden of coinfection with multiple enteric pathogens in children with diarrhea should be given more attention.[27] The FilmArray GI Panel is an efficient and sensitive method for detecting enteric pathogens including the most prevalent bacteria, viruses, and parasites, which have a significant potential for improving the detection of important enteric microorganisms. From our data, the overall positivity rates of enteric pathogens and co-infections were higher than those reported elsewhere.[28,29] A key possible factor is that children under five years of age are more vulnerable to infectious diarrhea than children over five years of age, especially in developing countries.

In this study, children co-infected with *Salmonella* spp. and *C. difficile* displayed a high frequency of fever and mucus stools compared with *Salmonella* single infection. It has been reported that children coinfected with rotavirus and toxin-producing *C. difficile* have more severe clinical characteristics and are more vulnerable to severely dehydrated.[30] However, Shafiq M et al found that co-infection with other pathogens did not affect the severity of *C. difficile* infection or cause treatment failures.[31] Moreover, although co-infection with *Campylobacter* and *Salmonella* was observed at low levels, it was estimated that co-culture with *Salmonella* spp. significantly promoted the survival of *Campylobacter jejuni* under aerobic conditions.[32] Based on various gastrointestinal infection patterns and distinguished clinical symptoms, it would be reasonable to apply individualized treatments to the pediatric patients. Assessment of multiple possible infectious etiologies may contribute to better pharmacological management of acute gastroenteritis among children, whereas the strategies of the pathogenesis of concurrent infections require further investigation. Interactions between different enteropathogenic microorganisms are poorly understood. Therefore, it is reasonable to reveal and interpret the underlying mechanisms involved in pathogenic co-infections using multi-pathogen detection technology.

It was widely reported that age is a risk factor associated with *Salmonella* incidence, for the younger children were more vulnerable to infections. As estimated in a nationwide study, 34% of diarrheal patients infected with non-typhoid *Salmonella* are under five years of age.[33] In this study, the overall positive rate of *Salmonella* spp. among children under 5 years of age was 36.74% by the FilmArray GI Panel and 25.19% by the culture method, which may be attributed to the difference in sample size and patient source. Based on the surveillance network, the most prevalent pathogen in Beijing is *Shigella* spp., followed by *Vibrio parahaemolyticus, Salmonella* spp. and EPEC.[34] In another study of pathogens in children under five years of age with acute gastroenteritis in Guangzhou, China, the most prevalent pathogen was *Salmonella* spp.,[35] which is consistent with the results of this study. Taken together, the prevalence of *Salmonella* spp. may vary across regions, detection methods, and patient sources.

There are some limitations to this study, including: 1) The sample size in this study was relatively small and the samples were not evenly distributed in each month, so the prevalence of *Salmonella* spp. detected by the FilmArray GI Panel was not representative enough. 2) Not all stool samples for the FilmArray GI Panel were simultaneously assessed

using the conventional culture method, and the comparison of the two assays was limited. 3) The FilmArray GI Panel could not distinguish between *Salmonella* active infections and dead bacteria. 4) The FilmArray GI Panel could not determine whether the detected pathogens were pathogenic or enteric-colonized. 5) The strains and serotypes of *Salmonella* spp. could not be classified using the current FilmArray GI Panel.

In conclusion, this study showed that *Salmonella* is the predominant pathogen detected in diarrheal diseases in children under five years of age in Guangzhou, China. Furthermore, these results showed a high frequency of co-infection with *Salmonella* and other enteric pathogens, mainly *Clostridium difficile*, Enteropathogenic *Escherichia coli*, and adenoviruses. This study provides a comprehensive understanding of the clinical characteristics and outcomes of the potential interactions between *Salmonella* and other enteric microorganisms.

Data Sharing Statement

The data that support the findings are available from the corresponding author upon non-commercial request.

Ethics Approval

This study was approved by the Medical Ethics Committee of Guangdong Women and Children Hospital, China, and it was conducted according to the ethical guidelines of the Declaration of Helsinki.

Consent to Participants

The informed consent to participants was waived by the Medical Ethics Committee (NO. 202401096) of Guangdong Women and Children Hospital and the use of residual specimens was strictly anonymized in this study.

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Disclosure

The authors declare that they have no competing interests in publishing this paper.

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