

Epidemiology and Antibiotic Resistance of Foodborne Diseases Among Children in Shanghai: A Four-Year Surveillance Study, 2021–2024

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Objective: The primary objective of this study was to examine the epidemiology and antibiotic resistance of food-borne diseases in pediatric populations in Shanghai from 2021 to 2024.

Methods: Data were collected from patients presenting with suspected food-borne illnesses at the intestinal clinic of the Children's Hospital of Fudan University during the period from July 1, 2021 to June 30, 2024. Fresh stool samples from all enrolled patients were analyzed for six pathogens: *Salmonella*, *Vibrio parahaemolyticus*, *Diarrheagenic Escherichia coli*, *Campylobacter*, *Shigella*, and *Norovirus*. Detection of *Salmonella*, *Shigella*, *Diarrheagenic Escherichia coli* and *Norovirus* was performed using polymerase chain reaction (PCR) methods, while culture assays were utilized for *Salmonella*, *Shigella*, *Vibrio parahaemolyticus*, and *Campylobacter*. Antimicrobial susceptibility testing of the isolated strains (102 *Salmonella* and 90 *Campylobacter* isolates) was performed in accordance with CLSI M100 and M45 guidelines, 2024.

Results: A total number of 795 cases were included in the study, revealing an overall positivity rate of 68.3% (543/795). This positivity rate indicates that at least one of the six pathogens tested was positive. The predominant pathogen identified was *Diarrheagenic Escherichia coli*, which accounted for 49.2% (391/795) of cases. Co-infections were identified in 20.0% (159/795) of the samples analyzed. *Salmonella* resistance rates were high, at 74.5% for ampicillin and 24.5% for multi-drug resistance. *Campylobacter* exhibited over 80% resistance to ciprofloxacin and tetracycline; 18.9% of isolates were resistant to all three tested antibiotics.

Conclusion: Food-borne pathogens are widely prevalent among children with suspected food-borne illnesses in Shanghai, and the high levels of antibiotic resistance observed in *Salmonella* and *Campylobacter* highlight a critical public health concern. These findings underscore the necessity for strengthened surveillance of pediatric food-borne diseases, rational clinical use of antibiotics, and implementation of targeted preventive strategies to reduce the burden of drug-resistant infections in this vulnerable population.

Keywords: food-borne diseases, *Salmonella*, *Diarrheagenic Escherichia coli*, antimicrobial susceptibility testing

Introduction

Foodborne diseases represent a severe global public health threat, with hundreds of millions of cases reported annually worldwide and a considerable number of disability-adjusted life year lost as a result.¹ To address this pressing challenge and supplement Shanghai's existing surveillance system, the present study focuses on developing predictive models for childhood foodborne diseases, alongside a systematic analysis of pediatric surveillance data in Shanghai from 2021 to 2024.

In line with sentinel surveillance protocols, the study targets six key foodborne pathogens: *Salmonella*, *Vibrio parahaemolyticus*, *Diarrheagenic Escherichia coli*, *Campylobacter*, *Shigella*, and *Norovirus*. The number of clinically

diagnosed cases in this study may differ from globally estimated case numbers. For example, while norovirus is the foodborne pathogen with the highest globally estimated case numbers, its clinical case statistics typically show lower infection rates than most bacterial infections because the majority of infected individuals do not seek medical care. Currently, surveillance of foodborne diseases among children in Shanghai remains inadequate. Many medical institutions lack a comprehensive understanding of foodborne illnesses, are deficient in corresponding diagnostic testing capabilities, and implement non-standardized detection methods. Given the heterogeneous institutional capabilities, the findings of this study are expected to underpin the development of standardized guidelines, guiding foodborne disease surveillance efforts across the city and optimizing the overall monitoring framework.

Specifically, this study aims to clarify the epidemiological characteristics, pathogen distribution profiles, and antimicrobial resistance patterns of childhood foodborne diseases, thereby providing evidence-based support for formulating targeted prevention and control strategies and refining clinical management protocols.

Materials and Methods

Patient Enrollment and Specimen Collection

This study is part of the foodborne disease surveillance program conducted by the Shanghai Center for Disease Control and Prevention (CDC). Throughout the research process and after data collection, the authors had no access to detailed information that could identify individual participants. Participants could no longer be located, and this research project did not involve personal privacy or commercial interests. Therefore, the study was approved by the Ethics Committee of the Children's Hospital of Fudan University and consent was exempted by the ethics committee. Patients presenting with diarrhea at the outpatient clinic of the Children's Hospital of Fudan University between July 1, 2021, and June 30, 2024, were included in the surveillance cohort.

Participants were deemed eligible if they satisfied three specific criteria: ① they exhibited acute diarrhea, defined as experiencing more than three episodes per day, with or without additional symptoms such as fever or vomiting; ② they had a history of consuming unclean food; and ③ they were able to provide fresh fecal specimens at the time of their visit. An unclean dietary history was defined as the presence of any one of the following exposure conditions in cases within 72 hours prior to the onset of symptoms: Consumption of undercooked poultry, eggs, or unpasteurized dairy products; Ingestion of unboiled drinking water or unbranded drinking water of unknown origin; Consumption of food items from unlicensed street vendors, or expired/spoiled food products. Conversely, individuals suspected of having chronic diarrhea, antibiotic-associated diarrhea, food poisoning, or diarrhea without a history of unclean food consumption were excluded from the study.

All newly collected fecal specimens from enrolled patients were utilized for the detection of *Salmonella*, *Vibrio parahaemolyticus*, *Diarrheagenic Escherichia coli*, *Campylobacter*, *Shigella*, and *Norovirus*. In instances of delayed transportation or analysis, the specimens were preserved in Cary-Blair transport medium and maintained at a temperature of 4°C. Clinical information and dietary histories were obtained from the electronic medical records (EMR). Co-infection was defined as positivity for two or more pathogens.

PCR Assay

PCR is used to detect *Salmonella* and *Shigella*. RT-PCR is used to detect *Diarrheagenic Escherichia coli* and *Norovirus*. The PCR kits for *Salmonella* and *Shigella* were purchased from Jiangsu Biopertectus Technologies Co. Ltd. The primer sets for detecting *Salmonella* and *Shigella* target the *ssaR* gene and *ipaH* gene, respectively. The multiplex real-time fluorescent PCR kits targeting various strains of pathogenic *Escherichia coli*, including *enteropathogenic Escherichia coli* (EPEC), *enterohemorrhagic Escherichia coli* (EHEC)/*Shiga toxin-producing Escherichia coli* (STEC), *enterotoxigenic Escherichia coli* (ETEC), *enteroinvasive Escherichia coli* (EIEC), and *enteroaggregative Escherichia coli* (EAEC) were purchased from Beijing Zhuocheng Huisheng Biotechnology Co. Ltd.² Primer targets and gene markers for five pathogenic *Escherichia coli* strains include *Stx1* gene, *eae* gene, *bfpB* gene, *Stx2* gene, *ipaH* gene, *sth* gene, *stp* gene, *lt* gene, *aggR* gene, *pic* gene, *astA* gene, and *uidA* gene. Typing criteria are detailed in [Table S1](#). Furthermore, the RT-PCR assay kits for *Norovirus* were obtained from Hubei Langde Medical Technology Co., Ltd. The gene target and gene

marker used for detecting *Norovirus* is *RdRp* gene. The PCR instrument for *Salmonella* and *Shigella* detection is the Roche 480, with analysis software version 1.5.1.62. The instrument for RT-PCR detection of *Diarrheagenic Escherichia coli* and *Norovirus* is the ABI 7500, with analysis software version v2.0.6. For internal quality control (IQC), blank controls, negative controls, and positive controls are included in each batch of tests. For external quality control (EQC), interlaboratory comparisons are conducted with other laboratories once or twice a year.

Culture Assay

Culture assay^{3,4} was employed for *Salmonella*, *Shigella*, *Vibrio parahaemolyticus*, and *Campylobacter*. Specimens, including pus, blood, mucus-like feces, or rectal swabs, were inoculated into xylose lysine desoxycholate (XLD) agar (Komaja) and SBG bacteriostatic (Komaja) enrichment solution for the isolation of *Salmonella* and *Shigella*. Both the XLD agar and SBG liquid medium were incubated at 35 °C for a duration of 18 to 24 hours. Colonies suspected to be *Salmonella* or *Shigella* manifested as small black colonies on the XLD agar. The SBG liquid medium, which exhibited a yellow and turbid appearance after overnight incubation, was subsequently recultured on XLD agar for further isolation of *Salmonella* and *Shigella*. For the isolation of *Vibrio parahaemolyticus*, TCBS agar (Komaja) was employed as the culture medium, following the same incubation protocol as previously described. *Vibrio parahaemolyticus* colonies appeared as green or blue on the TCBS agar.

The filtration methods employed serve to eliminate non-Campylobacter elements, thereby enhancing the efficacy of direct culture techniques.³ This process involves the application of sterile cellulose acetate filters with a pore size of 0.45 µm onto the surface of CAMALI agar plates, which is a commercially available selective medium for *Campylobacter* (Komaja). Following the dilution of approximately 1 gram of stool in 10 milliliters of sterile saline, 10 to 15 drops of the resulting fecal suspension are deposited onto the filters. The plates are then incubated at 37°C for a duration of 20 to 30 minutes. Subsequently, the filters are removed, and the plates are incubated at 42°C for 48 hours under microaerobic conditions, specifically 5% O₂, 10% CO₂, and 85% N₂. The presumptive *Campylobacter* appear in grey small colonies on the CAMALI agar.

All strains of *Salmonella*, *Shigella*, *Vibrio parahaemolyticus*, and *Campylobacter* were identified by Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF, MALDI Biotyper, Bruker Daltronics). The IQC strains consist of *Salmonella typhimurium* ATCC 14028, external quality assessment (EQA) strains provided by the National Center for Clinical Laboratories (NCCL) of the National Health Commission, and clinically isolated strains verified by species identification. For EQC, the laboratory participates in the EQA program organized by the NCCL of the National Health Commission three times annually.

Antimicrobial Susceptibility Testing

Antibiotic susceptibility was tested using the E-test (Taikang Biologicals, China) and disk diffusion methods (Oxoid, UK), in accordance with the reference standards established by the Institute for Clinical and Laboratory Standards M100 and M45, 2024 (Wayne, Pennsylvania, USA). The antimicrobial agents utilized in this study were procured from Oxoid, UK, and included ampicillin, ampicillin/sulbactam, ceftriaxone, azithromycin, methotrexate/sulfamethoxazole, and levofloxacin for susceptibility testing of *Salmonella*, *Shigella* and *Vibrio parahaemolyticus* isolates. For the assessment of *Campylobacter* susceptibility, erythromycin, azithromycin, tetracycline, doxycycline, and ciprofloxacin were employed. Multidrug resistance was operationally defined as resistance to three or more classes of antibiotics.

Statistical Analysis

Statistical analysis was performed using SPSS software version 24.0 (IBM, USA), employing the appropriate chi-square test or Fisher's exact test. A *P*-value of < 0.05 was considered statistically significant, and the Bonferroni correction was applied for multiple comparisons. Power values were calculated based on *P*=0.05 (corrected *P*=0.0083), sample size, and effect size, which were used to evaluate test power (Power ≥ 0.8 indicates sufficient power). Power calculation is performed using G*Power 3.1.

Results

The Characteristics of the Enrolled Patient

A total number of 795 cases were included in the study. The clinical characteristics of these participants are presented in Table 1. Notably, 63.3% of the patients were enrolled during the third (Q3) and fourth (Q4) quarters of the study period. The median age of the patients was 4.1 years, with 13.8% of the cohort being under 1 year of age, 28.1% aged between 1 and 3 years, 21.5% aged between 3 and 6 years, and 36.6% aged 6 years or older. A slightly majority of the patients were male, comprising 60.9% (484/795). Diarrhea characterized by loose stools and fever was the most prevalent clinical

Table 1 The Clinical Characteristics of the Enrolled Patients

Groups	Number (%)
Patient enrolled time (quarter)	Number (%)
Q1	88 (11.1)
Q2	204 (25.6)
Q3	387 (48.7)
Q4	116 (14.6)
Gender	Number (%)
Male	484 (60.9)
Female	311 (39.1)
Age, year (median, range)	(4.1y, 0–17y)
Age group	Number (%)
<1y	110 (13.8)
≥1-3y	223 (28.1)
≥3-6y	171 (21.5)
≥6y	291 (36.6)
Clinical manifestations	Number (%)
Fever	451 (56.7)
Abdominal cramps	194 (24.4)
Vomiting	104 (13.1)
Diarrhea faecal specimen characteristics	724 (91.1), Number (%)
Loose faecal specimen	361 (45.4)
Watery faecal specimen	227 (28.6)
With bloody faecal specimen	93 (11.7)
History of unclean diet	585 (73.6), Number (%)
Fruit, vegetable	139 (17.5)
Milk and dairy products	124 (15.6)

(Continued)

Table 1 (Continued).

Groups	Number (%)
Drink	91 (11.4)
Meat	88 (11.1)
Seafood	75 (9.4)

symptom observed. Additionally, 73.6% of the patients reported having consumed an unclean diet prior to admission. A subset of parents claimed a history of suspicious diet yet failed to identify the specific category of the suspected food.

The Infectious Rates of the Targeted Pathogens

All participants underwent testing for the presence of *Salmonella*, *Vibrio parahaemolyticus*, *Diarrheagenic Escherichia coli*, *Campylobacter*, *Shigella*, and *Norovirus* in their fecal specimens. In this study, a total of 543 participants tested positive for at least one of these target pathogens, resulting in an overall infection rate of 68.3% among enrolled foodborne disease patients. This figure includes co-infections, defined as simultaneous infection by two or more pathogens.

The infectious rate for *Diarrheagenic Escherichia coli* was found to be 49.2% (391/795), which was significantly higher than that of the other pathogens. This was followed by *Salmonella* at 18.1% (144/795), *Campylobacter* at 15.1% (120/795), and *Norovirus* at 6.7% (54/795). Statistically significant differences were observed in the infectious rates of the various pathogens ($P < 0.001$), as shown in Figure 1.

In a cohort of patients diagnosed with *Diarrheagenic Escherichia coli* infections, *enteroadherent Escherichia coli* (EAEC) emerged as the most prevalent strain, accounting for 64.5% (253/391). This was followed by *enteropathogenic Escherichia coli* (EPEC), which represented 34.1% (143/391). Among patients with *Salmonella* infections, *Salmonella*

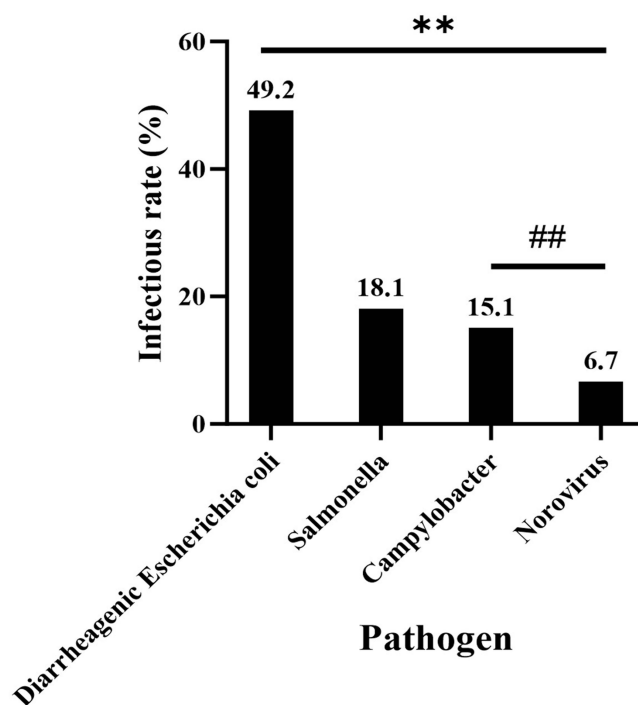


Figure 1 Infectious rate of four pathogens.

Notes: **: The infection rate of *Diarrheagenic Escherichia coli* was significantly higher than that of the other three pathogens, with the difference being statistically significant ($P < 0.001$). #: The infection rate of *Campylobacter* was significantly higher than that of *Norovirus*, with the difference being statistically significant ($P < 0.001$).

species were the most frequently identified pathogens, comprising 53.5% (77/144), with *Salmonella typhimurium* and *Salmonella enteritidis* being the next most common strains, at 20.1% (29/144) and 13.2% (19/144), respectively. Other *Salmonella species* also accounted for 13.2% (19/144). In the context of *Campylobacter* infections, *Campylobacter jejuni* was the predominant species, representing 85.0% (102/120), while *Campylobacter coli* constituted 15.0% (18/120).

Co-infections were identified in 20.0% (159/795) of the samples analyzed. The predominant co-infections involved *Salmonella* and *Diarrheogenic Escherichia coli*, accounting for 42.1% (67/159) of the cases. This was followed by co-infections of *Campylobacter* and *Diarrheogenic Escherichia coli*, which constituted 37.1% (59/159) of the instances. Additionally, co-infections involving more than two pathogens are also present in rare case.

The Clinical Manifestation of the Patients Infected by Targeted Pathogens

The seasonal distribution of infections was observed to be 51.1% in the first quarter (Q1), 57.8% in the second quarter (Q2), 76.0% in the third quarter (Q3), and 70.7% in the fourth quarter (Q4). January to March constitutes the first quarter

Table 2 Symptom, Food, Age and Seasonal Distribution of Patients with Different Pathogens

Variables	<i>Salmonella</i> (n=144)	<i>Campylobacter</i> (n=120)	<i>Diarrheogenic Escherichia coli</i> (n=391)	<i>Norovirus</i> (n=54)	P-value*	P-value**	Power
Clinical Symptom							
Fever	103(71.5)	78(65.0)	256(65.5)	27(50.0)	0.045	0.360	0.62
Vomiting	17(11.8)	24(20.0)	47(12.0)	17(31.5)	<0.001	<0.001	0.98
Abdominal cramps	29(20.1)	44(36.7)	78(19.9)	4(7.4)	<0.001***	<0.001	0.99
Diarrhea							
Loose stool	63(43.8)	59(49.2)	190(48.6)	33(61.1)	0.191	1.528	0.35
Water stool	53(36.8)	34(28.3)	131(33.5)	19(35.2)	0.529	4.232	0.20
With bloody stool	21(14.6)	20(16.7)	47(12.0)	2(3.7)	0.0123***	0.792	0.47
Age					<0.001	<0.001	1.00
<1	19(13.2)	3(2.5)	36(9.2)	7(13.0)			
≥1-3	69(47.9)	20(16.7)	139(35.5)	16(29.6)	–	–	–
≥3-6	35(24.3)	36(30.0)	59(15.1)	16(29.6)	–	–	–
≥6	21(14.6)	61(50.8)	157(40.2)	15(27.8)	–	–	–
Quarter					<0.001	<0.001	0.99
Q1	3(2.1)	14(11.7)	21(5.4)	9(16.7)	–	–	–
Q2	16(11.1)	12(10.0)	106(27.1)	9(16.7)	–	–	–
Q3	107(74.3)	64(53.3)	216(55.2)	26(48.1)	–	–	–
Q4	18(12.5)	30(25.0)	48(12.3)	10(18.5)	–	–	–
Unclean food					<0.001	<0.001	0.95
Total	144 (100)	120 (100)	391 (100)	54 (100)	–	–	–
Fruit, vegetable	26(18.1)	18(15.0)	65(16.6)	7(13.0)	–	–	–
Meat	13(9.0)	21(17.5)	46(11.8)	7(13.0)	–	–	–
Drink	20(13.9)	18(15.0)	49(12.5)	10(18.5)	–	–	–
Seafood	9(6.2)	18(15.0)	41(10.5)	2(3.7)	–	–	–
Milk and dairy products	18(12.5)	7(5.8)	47(12.0)	5(9.2)	–	–	–

Notes: 1. Data are presented as n (%). 2. The P-value is derived through the analysis of case numbers. *Chi-squared test; **after Bonferroni correction; ***Fisher's exact test was used when the expected frequency of any cell was less than five. 3. Categorical variables were analyzed using the chi-square test. After multiple group comparisons, the Bonferroni method was applied for multiple comparison correction; Power values were calculated based on $P=0.05$ (corrected $P=0.0083$), sample size, and effect size, which were used to evaluate test power (Power ≥ 0.8 indicates sufficient power). 4. The symbol "–" indicates that the subcategory P-value was not calculated separately, as the overall group comparison already reached statistical significance. 5. The positive rate of vomiting induced by the four pathogens was statistically significantly different ($P<0.001$). The positive rate of abdominal cramps caused by the four pathogens showed a statistically significant difference ($P<0.001$). The positive rate of the four pathogens varied statistically significantly across different age groups ($P<0.001$). The positive rate of the four pathogens differed statistically significantly among different quarters ($P<0.001$). The positive rate of the four pathogens was statistically significantly different in relation to various histories of contaminated food consumption ($P<0.001$).

(Q1), April to June the second quarter (Q2), July to September the third quarter (Q3), and October to December the fourth quarter (Q4).

The age distribution of affected individuals was as follows: 50.0% were under one year of age, 79.4% were between one and three years, 66.1% were between three and six years, and 68.0% were over six years. A significant peak in infections was identified during Q3 ($P<0.001$), with the age group of one to three years being particularly susceptible ($P<0.001$).

An analysis of the clinical characteristics associated with *Salmonella* infection revealed that 71.5% of patients presented with fever, 11.8% with vomiting, and 20.1% with abdominal cramps ($P<0.001$). Among patients experiencing diarrhea, the predominant fecal characteristic was loose stools, reported by 43.8% of individuals ($P<0.001$). The age group of one to three years was identified as particularly vulnerable ($P<0.001$), coinciding with the peak incidence of infections in Q3 ($P<0.001$). The most frequently reported dietary history among patients included the consumption of fruits and vegetables (18.1%), followed by drink (13.9%) and milk and dairy products (12.5%) ($P<0.001$) (Table 2).

The examination of the clinical features associated with *Campylobacter* infection revealed that 65.0% of patients presented with fever, 20.0% with vomiting, and 36.7% with abdominal cramps ($P<0.001$). Among patients experiencing diarrhea, the predominant fecal characteristic was loose stools, observed in 49.2% of cases ($P<0.001$). The demographic most affected was the cohort aged three years and older ($P<0.001$), with an observed peak in infections occurring in the fourth quarter of the year ($P<0.001$). Additionally, no significant differences were noted in the dietary histories of the patients.

The examination of the clinical characteristics associated with *Diarrheagenic Escherichia coli* infections revealed that 65.5% of patients experienced fever, 12.0% exhibited vomiting, and 19.9% reported abdominal cramps ($P<0.001$). The predominant fecal characteristic among patients with diarrhea was identified as loose stools, occurring in 48.6% of cases ($P<0.001$). The demographic most affected was the 1–3 year age group ($P<0.001$), with an observed peak in infections during the third quarter of the year ($P<0.001$). No significant differences were noted in dietary history.

In contrast, the analysis of the clinical characteristics related to *Norovirus* infections indicated that 50.0% of patients presented with fever, 31.5% with vomiting, and 7.4% with abdominal cramps ($P<0.001$). The most frequently observed fecal characteristic in patients with diarrhea was also loose stools, reported in 61.1% of cases ($P<0.001$). Furthermore, the investigation into *Norovirus* infection rates across various demographic factors, including age, seasonal quarter, and dietary history, did not reveal any statistically significant differences.

A stratified analysis of pathogenic infection incidence across various demographic groups, incorporating clinical manifestations, age categories, and dietary history, revealed notable disparities. After Bonferroni correction, five key variables remained statistically significant across the four pathogen groups (all $P < 0.001$), with corresponding statistical power values consistently ≥ 0.8 ; this confirmed sufficient test validity and effectively ruled out the risk of type II errors, thereby ensuring high reliability of the findings. Specifically, vomiting exhibited the highest incidence in the *Norovirus* group (31.5%) and the lowest in the *Salmonella* group (11.8%), with a statistical power of 0.98, indicating that the intergroup difference is real and easily detectable. For abdominal cramps, the *Campylobacter* group presented the maximum incidence (36.7%) versus the minimum in the *Norovirus* group (7.4%), and a statistical power of 0.99 reflected exceptional stability in capturing this variation. Age distribution analysis revealed a predominant composition of children aged ≥ 6 years in the *Campylobacter* group (50.8%), in contrast to the *Salmonella* group where children aged 1–3 years constituted the majority (47.9%), with a statistical power of 1.00, confirming clear differences in age distribution among groups. In term of quarter distribution, all four pathogens were mainly prevalent in Q3, but the *Salmonella* group had the highest proportion in Q3 (74.3%), signifying notable seasonal variation across groups with the stability of this result corroborated by a statistical power of 0.99. For unclean food exposure, although the exposure rate was 100% in all groups, there were significant differences in the distribution of different types of unclean food, for example, the *Campylobacter* group exhibited a higher seafood exposure rate (15.0%) than other cohorts, and a statistical power of 0.95 confirmed the authenticity and credibility of this intergroup difference (Table 2).

Chi-Square Test for Trend Analysis of Seasonal Pathogen Prevalence

Seasonal prevalence varied significantly across the four pathogens ($P < 0.001$, power = 0.99; Table 2). A chi-square test for trend was conducted with quarters treated as ordinal variables (Q1 = 1, Q2 = 2, Q3 = 3, Q4 = 4), revealing a statistically significant linear seasonal trend for all pathogens ($\chi^2_{trend} > 3.84$, $P < 0.001$). This trend was marked by increasing infection prevalence from Q1 to Q3, followed by a decline in Q4.

Notably, *Salmonella* exhibited the most prominent trend ($\chi^2_{trend} \approx 838,657.4$, $P < 0.001$), with 74.3% of cases concentrated in Q3 versus $\leq 12.5\%$ in Q1–Q2 and Q4. *Campylobacter* showed a distinct pattern ($\chi^2_{trend} \approx 462500$, $P < 0.001$), peaking in Q3 (53.3%) and with a secondary peak in Q4 (25.0%). Diarrheagenic *Escherichia coli* displayed a highly significant trend ($\chi^2_{trend} \approx 14,731,457.8$, $P < 0.001$), with a Q3 peak (55.2%) and a relatively high Q2 prevalence (27.1%), suggesting a longer transmission window than the other two bacteria. Norovirus had the lowest χ^2_{trend} value among the four pathogens (32799.4, $P < 0.001$), consistent with its relatively uniform prevalence across Q1, Q2, and Q4 (16.7%–18.5%) and a moderate Q3 peak (48.1%), indicating weaker seasonal aggregation.

Pooled analysis of all four pathogens further confirmed a strong linear seasonal trend ($\chi^2_{trend} = 79,994,526.5$, $P < 0.001$), verifying that summer and early autumn (Q3) constitute the core high-incidence period for pediatric foodborne pathogen infections in the study population.

Antibiotic Resistance of the *Salmonella* and *Campylobacter*

In the current investigation, a total of 102 *Salmonella* isolates were evaluated for their susceptibility to various antibiotics. The resistance rates of these isolates to ampicillin, ampicillin-sulbactam, ceftriaxone, azithromycin, methotrexate-sulfamethoxazole, and levofloxacin were 74.5% (76/102), 27.5% (28/102), 20.6% (21/102), 10.8% (11/102), 34.3% (35/102) and 7.8% (8/102) respectively (Table 3). Notably, 11.8% (12/102) of the isolates exhibited susceptibility to all six antibiotics, indicating that 88.2% (90/102) were resistant to at least one antibiotic. Furthermore, 24.5% (25/102) of the isolates demonstrated multidrug resistance, with 2.0% (2/102) being resistant to all six antibiotics tested. The two predominant antibiotic resistance patterns identified in this study included ampicillin combined with methotrexate-sulfamethoxazole, which was present in 13.7% (14/102) of the isolates. Specifically, the resistance rates for *Salmonella typhimurium* were 70.8% (17/24) for ampicillin, 20.8% (5/24) for ampicillin-sulbactam, 16.7% (4/24) for ceftriaxone, 4.2% (1/24) for azithromycin, 50.0% (12/24) for methotrexate-sulfamethoxazole, and 0% for levofloxacin. In contrast, *Salmonella enterica* exhibited resistance rates of 75.0% (12/16) to ampicillin, 37.5% (6/16) to ampicillin-sulbactam and 6.3% (1/16) to methotrexate-sulfamethoxazole, with no resistant strains detected for ceftriaxone, azithromycin, or levofloxacin.

In the present study, a total of 90 *Campylobacter* isolates were tested for antibiotic susceptibility. The observed resistance rates for erythromycin, azithromycin, tetracycline, doxycycline and ciprofloxacin were 23.3% (21/90), 23.3%

Table 3 Analysis of Antibiotic Resistance Rates in *Salmonella* and *Campylobacter*

Antibiotic	<i>Salmonella</i> (n=102)	<i>Campylobacter</i> (n=90)
Ampicillin	76 (74.5)	NA
Ampicillin-sulbactam	28 (27.5)	NA
Ceftriaxone	21 (20.6)	NA
Azithromycin	11 (10.8)	21 (23.3)
Methotrexate-sulfamethoxazole	35 (34.3)	NA
Levofloxacin	8 (7.8)	NA
Erythromycin	NA	21 (23.3)
Tetracycline	NA	79 (87.8)
Doxycycline	NA	79 (87.8)
Ciprofloxacin	NA	80 (88.9)

Note: Data are presented as n (%).

Abbreviation: NA, not available.

(21/90), 87.8% (79/90), 87.8% (79/90), and 88.9% (80/90), respectively (Table 3). Notably, 3.3% (3/90) of the isolates exhibited susceptibility to all five antibiotics, while 18.9% (17/90) demonstrated resistance to all five. Furthermore, the predominant antibiotic resistance patterns identified in this study included a combination of tetracyclines and ciprofloxacin, which was present in 63.3% (57/90) of the isolates. Specifically, the resistance rates for *Campylobacter jejuni* to erythromycin, azithromycin, tetracycline, doxycycline and ciprofloxacin were recorded at 17.7% (14/79), 17.7% (14/79), 86.1% (68/79), 86.1% (68/79), and 87.3% (69/79), respectively. In contrast, the resistance rates for *Campylobacter coli* to the same antibiotics were significantly higher, with rates of 63.6% (7/11) for both erythromycin and azithromycin, and 100% (11/11) for tetracycline, doxycycline, and ciprofloxacin.

Discussion

The World Health Organization (WHO) defines food-borne diseases as infectious or toxic conditions that arise from the ingestion of contaminated food or water.⁵ These diseases can be classified into three categories: poisoning, which results from toxins produced by pathogens; infections, which occur due to the consumption of food containing pathogens; and toxin-mediated infections, where toxins are produced as pathogens multiply within the human gastrointestinal system.^{6,7} Food-borne illnesses constitute a significant public health issue in both developed and developing countries; however, the impact is notably more severe in developing nations.^{8,9} The WHO estimates that approximately 30% of individuals in developed countries experience food-borne illnesses annually, while developing countries may experience up to 2 million deaths each year attributable to these diseases.

Food-borne pathogens include a variety of microorganisms, such as bacteria, viruses, fungi, and various parasites,¹⁰ which are primary contributors to food spoilage and food-borne illnesses.¹¹ These microorganisms present a considerable risk to food safety, resulting in human infections following the consumption of animal products that have been contaminated with pathogens or their toxins.

The current surveillance system for food-borne diseases in Shanghai reveals several significant shortcomings, particularly in the following domains: (1) There exists a notable deficiency in awareness of food-borne diseases among certain medical institutions, which may lead to the oversight or misdiagnosis of these conditions during clinical evaluations and treatments; (2) Some healthcare facilities lack the necessary capabilities to identify pathogens linked to food-borne diseases, highlighting the need for improved standardization of detection methods and enhanced promptness in diagnostic processes; (3) The diagnostic and treatment capacities of medical institutions addressing food-borne diseases within the city are inconsistent. As a result, there is an urgent requirement to establish comprehensive and stable normative guidelines to promote the advancement of food-borne disease surveillance in Shanghai. In response to these challenges, this study was conducted to improve the monitoring of food-borne diseases in the city and to acquire insights into the fundamental characteristics and prevalence trends of such diseases among the pediatric population.

The findings of this study indicate that males exhibit a higher susceptibility to food-borne illnesses, a result that aligns with the conclusions of prior research.^{12,13} The predominant clinical manifestations observed were diarrhea characterized by loose stools and fever. Notably, 73.6% of the patients reported consuming contaminated food prior to admission, with fruits and vegetables identified as the most frequently implicated sources of contamination.

In our investigation, we identified the presence of *Salmonella*, *Campylobacter*, *Diarrheagenic Escherichia coli*, and *Norovirus*, with *Diarrheagenic Escherichia coli* exhibiting the highest positive rate, followed by *Salmonella*, *Campylobacter*, and *Norovirus*. This pattern diverges from the findings of earlier studies concerning food-borne pathogens in adult populations. Among adults, *Norovirus* is the most prevalent intestinal pathogen, followed by *Vibrio parahaemolyticus*, *Diarrheagenic Escherichia coli*, and *Salmonella*.^{14,15} Among the various types of *Diarrheagenic Escherichia coli*, EAEC was the most prevalent, corroborating the results of a previous study conducted in Shanghai.¹⁶

The finding from the clinical symptom study indicated that *Norovirus* was associated with the highest incidence of vomiting, corroborating the results of previous research.^{14,17} Conversely, fever was predominantly linked to *Salmonella* infections, while *Campylobacter* was responsible for the highest occurrence of abdominal cramps. Given that a majority of foodborne pathogens can induce acute gastroenteritis characterized by gastrointestinal symptoms, distinguishing between infections caused by different pathogens based solely on symptoms presents a significant challenge.

Our research revealed that children aged 1 to 3 years exhibited the highest rates of detection for total foodborne pathogens, including *Salmonella* and *Diarrheagenic Escherichia coli*. This trend may be attributed to the relatively low levels of hand hygiene practices among infants and toddlers within this age group.¹⁸ Furthermore, the underdeveloped gut microbiota in infants and toddlers may contribute to the elevated infection rates observed in this demographic.¹⁹

Salmonella is recognized as a significant global pathogen associated with outbreaks of both disseminated gastroenteritis and foodborne gastroenteritis, particularly affecting children under the age of five, with a heightened incidence observed in developed nations.^{20,21} These findings align with the outcomes of the current study, which indicated that *Salmonella* infections were most prevalent during the third quarter of the year, with fruits and vegetables identified as the primary sources of infection, corroborating previous research.^{22,23}

Similarly, *Campylobacter* is a prominent pathogen implicated in foodborne illness, accounting for an estimated 800,000 cases annually in the United States alone.^{24–26} The present study revealed a higher positive rate of *Campylobacter* infections in children over three years of age, which may be attributed to the primary sources of human campylobacteriosis, including the consumption of poultry meat, contaminated water, unpasteurized dairy products, and direct contact with farm animals.^{27,28} Consequently, there is an imperative to enhance food safety management and environmental monitoring practices.

Diarrhea resulting from infections with *Diarrheagenic Escherichia coli* represents a significant public health concern for both children and adults in developing nations, particularly due to its association with morbidity and mortality among children under the age of five.²⁹ The findings of the current study indicate that *Diarrheagenic Escherichia coli* was most frequently identified in children aged 1 to 3 years, with the highest detection rates occurring in the third quarter, corroborating the results of previous research.³⁰ The majority of foodborne outbreaks linked to *Escherichia coli* are attributed to the consumption of undercooked food that is contaminated with *Diarrheagenic Escherichia coli* at both the source and during food preparation. Enhancements in sanitation and hygiene practices may represent the most effective strategy for preventing infections caused by *Diarrheagenic Escherichia coli* and associated foodborne outbreaks.³¹

In contrast to earlier studies conducted on adults,^{14,15} *Vibrio parahaemolyticus* was not detected in the present investigation. Additionally, the prevalence of shigellosis among children in Shanghai was found to be exceedingly low,¹⁶ a finding that aligns with the results of this study, which also revealed no detection of *Shigella*.

In the third quarter, *Salmonella* and *Diarrheagenic Escherichia coli* exhibited the highest incidence rates, a finding that aligns with the observations made by Enserink³² and Gong.¹⁵ This seasonal pattern is likely influenced by environmental factors,^{33,34} including ambient temperature, precipitation, and humidity, all of which may affect exposure frequency, host immunity, and pathogenicity. Conversely, *Norovirus* was detected consistently throughout the year, showing no seasonal variation or differences in detection across age groups or tribes, which consistent with Lindsay³⁵ findings.

The results of this study indicate that the extensive use of antimicrobial agents has led to significant resistance in *Salmonella* among food-borne pediatric patients in Shanghai from 2021 to 2024. Currently, third-generation cephalosporins are the preferred treatment for *Salmonella* enterica infections,^{36,37} with a recorded resistance rate of 20.6%. Furthermore, the multi-resistance rate reached 24.5%, with the two predominant resistance patterns being ampicillin combined with methotrexate-sulfamethoxazole, observed at a rate of 13.7%. The findings of this study may contribute valuable insights for future clinical management of *Salmonella* infections.

Antibiotic resistance presents a significant global challenge in the management of *Campylobacter* infections.³⁸ Recent reports from various regions indicate a notable increase in resistance to macrolide, quinolone and tetracycline antibiotics, which are commonly employed in the treatment of *Campylobacter spp* infections. Despite this rising resistance, these antibiotics continue to be the preferred therapeutic options for patients diagnosed with campylobacteriosis.^{39,40} In the current investigation, the resistance rates of *Campylobacter* to ciprofloxacin, tetracyclines and macrolides were found to be 88.9%, 87.8% and 23.3%, respectively. Notably, the resistance rates for tetracycline and erythromycin exceeded those documented in prior studies (67.4% and 14%, respectively), while the resistance rate for ciprofloxacin was marginally lower than previously reported (90.1%).⁴¹ Furthermore, the overall resistance rate to all three antibiotic classes in this study was 18.9%. The predominant patterns of antibiotic resistance identified were tetracyclines combined with

ciprofloxacin, observed at a rate of 63.3%, corroborating the findings of Tryjanowski,⁴² which indicated that *Campylobacter* exhibits multi-drug resistance.

In conclusion, this research offers comprehensive insights into the epidemiology, pathogen diversity, and antibiotic resistance associated with food-borne diseases among children in Shanghai. The study identified *Diarrheagenic Escherichia coli* as the predominant pathogen. Notably, *Salmonella* and *Diarrheagenic Escherichia coli* were more frequently observed in children aged 1 to 2 years, while *Campylobacter* was more prevalent in children over the age of three. A seasonal pattern was noted, with food-borne illnesses occurring more frequently during the third and fourth quarters of the year. Further seasonal trend analysis confirms that the third quarter (Q3) is the core high-incidence period for studying foodborne pathogen infections in children. The current landscape of antibiotic resistance in *Salmonella* and *Campylobacter* is concerning, with evidence of multidrug resistance.

However, this study is not without limitations: it only focuses on 6 pathogens and does not detect other foodborne pathogens such as *Staphylococcus aureus* and *Listeria monocytogenes*. Future research can expand the scope based on laboratory capabilities. In addition, medical-seeking behavior largely affects the distribution results of foodborne pathogens, leading to differences between reported clinical cases and population-estimated cases.

Nonetheless, the findings hold significant potential for informing the enhancement of public health policies aimed at preventing food-borne illnesses in children and guiding the judicious use of antibiotics in clinical settings to mitigate the ongoing spread of drug resistance in *Salmonella* and *Campylobacter*. Our results underscore the necessity for proactive and thorough surveillance of food-borne illnesses in children, which will facilitate a deeper understanding of these conditions and aid in the formulation of targeted prevention strategies.

Data Sharing Statement

Data can be available from the corresponding author and first author.

Ethics Statement

This study has been approved by the Research Ethics Committee of the Children's Hospital of Fudan University, with the affiliated institution being the Children's Hospital of Fudan University. The approval number is Fudan Children's Ethics Review (2025) 192. This study adheres to the Declaration of Helsinki. Informed consent was not needed was not required to participate in this study in accordance with the national legislation and the institutional requirements, the applicable regulations or guidelines are cited as follows: https://www.gov.cn/zhengce/zhengceku/2023-02/28/content_5743658.htm. Medical records and patients' information were retrospectively reviewed and collected. All authors guarantee the completeness and accuracy of the data provided.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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