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Characteristics of cases with foodborne diarrheagenic *Escherichia coli* infection in Huzhou, China

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Abstract: Diarrheagenic *Escherichia coli* (DEC) is a common pathogen around the world that can cause disease with symptoms of diarrhoea. We collected all clinical DEC isolates from diarrhoea samples in three sentinel hospitals for active surveillance of foodborne diseases in Huzhou, China, between 2020 and 2022. The isolates were characterised according to demographic characteristics, time distribution, distribution of suspected culprit foods, antimicrobial results of susceptibility testing, analysis of virulence genes, and pulsed-field gel electrophoresis (PFGE) typing. The positive detection rate was 7.28%, the highest for enteroaggregative *E. coli* (EAEC) strains, followed by enterotoxic *E. coli* (ETEC) strains. The predominant virulence genes were *astA*/*pic* in EAEC (57.25%) and *estIa*/*estIb* in ETEC (17.94%) strains. The proportion of cases was relatively high in children aged 6 years old (15.27%), and the peak of incidence was between June and September. The rates of drug resistance in DEC were high in Huzhou, and the spectrum of drug resistance was wide. The highest rate of drug resistance was for AMP (63.51%), and multiple drug resistance was common. The household was the leading site of DEC infection, and meat and meat products were the main suspected culprit foods (18.15%). EAEC strains showed 52.4–100.00% sequence identity, and ETEC strains showed 52.2–100.00% sequence identity. All cases of DEC infection in Huzhou were sporadic between 2020 and 2022. Strengthening continuous surveillance will be useful in the risk assessment of foodborne diseases caused by DEC.

Keywords: foodborne pathogens; foodborne disease; drug sensitivity; etiology; pulsed-field gel electrophoresis

Foodborne diseases are those with infectious and toxic clinical characteristics caused by pathogenic factors in food entering the human body through ingestion (Yuan et al. 2018). These diseases have serious impacts on both public health and national economies (Kumagai et al. 2015; Sundstrom et al. 2018; Wang et al. 2022b). Bacterial foodborne diseases remain the primary problems related to food safety, and outbreaks of these diseases frequently occur in China (Chen et al. 2019). The numbers of events and cases of bacterial food poisoning account for 71.03% and 76.78% of the total, respectively (Liu et al. 2022). Diarrheagenic *Escherichia coli* (DEC) is the most important pathogen responsible for foodborne diseases and one of the main pathogens causing human diarrhoea (Desroches et al. 2018; Duan et al. 2018). Surveillance of infectious diarrhoea in China showed that DEC is also an important pathogen in outpatients with diarrheal disease (Zhou et al. 2021).

DEC can cause human morbidity through contaminated food, and infectious diarrhoea has the highest incidence among all infectious diseases in China (Wen et al. 2021). DEC are divided into five categories according to the presence of different virulence genes: enterotoxic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), and enteroaggregative *E. coli* (EAEC) (Wang et al. 2013). The detection rate of DEC in stool samples from patients with acute diarrhoea has increased recently (Nyholm et al. 2015). The reported DEC detection rates of 6.30–37.5% in patients with diarrhoea (He et al. 2018; Natarajan et al. 2018) seriously impact public health and economic development worldwide. For foodborne disease monitoring and food safety, it is important to determine the prevalence of foodborne diseases and the distribution of pathogenic types of the responsible organisms. Here, we examined basic information and laboratory test results of cases of DEC in Huzhou, China, between 2020 to 2022 to gain insight into infection and epidemic trends of foodborne diseases caused by DEC and improve the prevention and treatment of these diseases.

MATERIAL AND METHODS

Data. Foodborne disease surveillance data from three sentinel hospitals in Huzhou, China, during 2020–2022, including demographic information of patients, symptoms and signs, and laboratory test results, were collected. The definition of surveillance cases was based on the National Foodborne Disease Surveillance Work Manual. The chief complaint was suspected food-induced diarrhoea as the main symptom, with stool number ≥ 3 times a day and abnormal stool characteristics (loose stool, watery stool, mucous, pus, or blood in stool, etc.).

Isolation and identification of bacteria. Faecal samples were directly inoculated onto MacConkey medium (Hope Bio-Technology, China) for culture. More than five suspected colonies were analysed by matrix-assisted laser desorption and ionisation time-of-flight mass spectrometry to identify bacteria, and the characteristic genes of the identified strains were detected using a nucleic acid testing kit (Zhoucheng Biological Co., Ltd., China).

Antimicrobial susceptibility testing. The drug susceptibility of the DEC strains was examined using the micro broth dilution method with commercial drug sensitivity plates. The minimum inhibitory concentration (MIC) was determined by naked-eye interpretation of the drug-sensitivity plates. The sensitivity (S), moderate sensitivity (I), and resistance (R) were defined according to the criteria of the National Committee for Clinical Laboratory Standardization (CLSI 2020). In drug sensitivity testing, *E. coli* ATCC 25922 was used as the quality control strain.

Analysis of virulence genes. Nucleic acid was extracted using the bacterial DNA extraction reagent from the kit mentioned above. The supernatant containing DNA was collected and stored at −80 °C until use. Virulence genes of the DEC isolates were analysed by fluorescence polymerase chain reaction (PCR) following the manufacturer's instructions.

Pulsed-field gel electrophoresis. Chromosomal DNA was digested with *Xba* I in a water bath at 37 °C for 4 h, after which pulsed-field gel electrophoresis (PFGE) for DEC was performed following the standard procedure according to the Chinese Pathogen Identification Net. After electrophoresis, the bands were observed after staining with GelRed. The PFGE patterns were analysed using BioNumerics 7.1 (AppliedMaths, Belgium), and the band pattern similarity was measured according to the Dice coefficient. The unweighted pair group method with arithmetic mean was used for clustering, with tolerance of strip location difference set to 1.50% and the optimisation value set to 1.00%.

Statistical analysis. Statistical analyses were performed with Microsoft Excel (version 2010) and SPSS (version 22.0). Comparisons between groups were performed using the chi-squared test. In all analyses, $P < 0.05$ was taken to indicate statistical significance.

RESULTS

Positive detection of diarrheagenic *Escherichia coli*. In total, 6 813 faecal samples (anal swabs) from foodborne diarrhoea cases were collected, and 496 DEC-positive samples were found, indicating a positive detection rate of 7.28%. The positive rates of diarrhoea-causing DEC significantly differed between different years ($\chi^2 = 7.400$, $P = 0.025$) (Table 1).

Table 1. Detection of different types of DEC in Huzhou, 2020–2022

Year	Number of cases	Positive number	EAEC	ETEC	EPEC	EHEC	EIEC
		(*n*; %)					
2020	2 115	127 (6.01)	74 (3.50)	44 (2.08)	6 (0.28)	1 (0.05)	2 (0.09)
2021	2 154	170 (7.89)	117 (5.43)	41 (1.90)	11 (0.51)	0 (0.00)	1 (0.05)
2022	2 544	199 (7.82)	122 (4.80)	45 (1.77)	27 (1.06)	2 (0.08)	3 (0.12)
Total	6 813	496 (7.28)	313 (4.59)	130 (1.91)	44 (0.65)	3 (0.04)	6 (0.09)

DEC – diarrheagenic *Escherichia coli*; EAEC – enteroaggregative *E. coli*; ETEC – enterotoxic *E. coli*; EPEC – enteropathogenic *E. coli*; EHEC – enterohemorrhagic *E. coli*; EIEC – enteroinvasive *E. coli*

The predominant virulence genes were *astA/pic* in EAEC (57.25%) and *estIa/estIb* in ETEC (17.94%) strains. The *eae* gene was detected in 8.87% of EPEC strains (Table 2).

Population distribution of infected cases. Among the 6 813 specimens collected, 3 771 were from male patients, among which 289 were positive (positive detection rate 7.66%), and 3 042 were from female patients, among which 207 were positive (positive detection rate 6.81%); this difference was not significant ($P > 0.05$). The patients ranged in age from 2 to 81 years, with the highest positive rate of 15.27% in children 6 years old. The lowest positive rate of 1.17% was seen in infants under 1 year old. The difference in the positive detection rate of DEC among different age groups was significant ($\chi^2 = 344.24$, $P < 0.05$) (Table 3).

Antimicrobial resistance profiles. Table 4 shows the drug sensitivity testing results of the 496 DEC strains to 14 antibiotics. The sensitivity of the strains ranged from 0.00% to 100.00%. The greatest sensitivity was to Imipenem (IPM) (100%) followed by Ceftazidime (CAZ) (91.93%), and Meropenem (MEM) (84.68%). The rates of resistance to the 14 antibiotics ranged from 0.00 to 63.51%, with the highest rate to Ampicillin (AMP) (63.51%), followed by Streptomycin (STR) (62.70%) and Nalidixic (NAL) (57.86%).

Among the drug-resistant strains, 47 (9.48%) were resistant to a single drug, 42 (8.67%) were resistant to two drugs, and 341 (68.75%) were multidrug-resistant to 3–10 antibiotics. There were 92 kinds of drug resistance spectrum: NAL, 49 strains (9.88%); AMP-NAL-SXT, 34 strains (6.85%); STR-SXT, 27 strains (5.44%); AMP-STR-SXT, AMP-AMS-STR-SXT, and

Table 2. Genotype distribution of DEC stains in Huzhou, 2020–2022

DEC type	Virulence	Isolates	Prevalence (%)
EAEC	*astA/pic*	284	57.25
	aggR	9	1.81
	astA/pic,aggR	20	4.03
EPEC	*eae*	44	8.87
EHEC	*eae,stx1/stx2*	3	0.60
EIEC	*ipaH*	6	1.21
ETEC	*estIa/estIb*	89	17.94
	elt	20	4.03
	estIa/estIb,elt	21	4.23
Total	–	496	100.00

DEC – diarrheagenic *Escherichia coli*; EAEC – enteroaggregative *E. coli*; EPEC – enteropathogenic *E. coli*; EHEC – enterohemorrhagic *E. coli*; EIEC – enteroinvasive *E. coli;* ETEC – enterotoxic *E. coli*

Table 3. Detection of DEC in different populations in Huzhou, 2020–2022

Demographic characteristics	Number of cases	EAEC	ETEC	EPEC	EHEC	EIEC	Total	χ^2	P
		(n; %)							
Genders								1.841	0.175
Male	3 771	196 (5.20)	61 (1.62)	28 (0.74)	2 (0.05)	2 (0.05)	289 (7.66)		
Female	3 042	117 (3.85)	69 (2.27)	16 (0.53)	1 (0.03)	4 (0.13)	207 (6.81)		
Age (years)								344.240	0.000
0–5	3 078	29 (9.42)	1 (0.03)	2 (0.06)	0 (0.00)	4 (0.13)	36 (1.17)		
6–19	452	54 (11.95)	9 (1.99)	4 (0.88)	0 (0.00)	2 (0.44)	69 (15.27)		
20–39	1 766	136 (7.70)	76 (4.30)	18 (1.02)	2 (0.11)	0 (0.00)	232 (13.14)		
40–59	1 027	80 (7.79)	35 (0.10)	12 (1.17)	1 (0.10)	0 (0.00)	128 (12.46)		
≥ 60	490	14 (2.86)	9 (1.84)	8 (1.63)	0 (0.00)	0 (0.00)	31 (6.33)		
Total	6813	313 (4.59)	130 (1.91)	44 (0.65)	3 (0.04)	6 (0.08)	496 (7.28)		

DEC – diarrheagenic *Escherichia coli*; EAEC – enteroaggregative *E. coli*; ETEC – enterotoxic *E. coli*; EPEC – enteropathogenic *E. coli*; EHEC – enterohemorrhagic *E. coli*; EIEC – enteroinvasive *E. coli*

AMP-STR-GEN-SXT, 17 strains each (4.43%). AMP, STR, AMS, and other antibiotics appeared most frequently in multidrug resistance spectra to three or more drugs.

Time distribution of positive cases. Cases of DEC infection and positive detection occurred in every month throughout the year. However, there was a distinct peak in the number of visits to a medical practitioner in the summer (from June to September). The greatest number of visits to a medical practitioner occurred in August, followed by July. The number of visits to a medical practitioner was relatively small in winter and spring, with the lowest rate in January, followed by February. The positive detection rate of DEC also showed a significant temporal trend, essentially the same as in the number of medical visits. From 2020 to 2021, the highest positive detection rate was in August, followed by July. In 2022, the month

Table 4. Antimicrobial susceptibility testing of DEC strains Huzhou, 2020–2022

Antibiotics	Sensitive	Intermediate	Resistant
	(n; %)		
Ampicillin	181 (36.49)	0 (0.00)	315 (63.51)
Ceftazidime	453 (91.33)	29 (5.85)	14 (2.82)
Ampicillin/sulbactam	243 (48.99)	181 (36.49)	72 (14.52)
Imipenem	496 (100.00)	0 (0.00)	0 (0.00)
Streptomycin	185 (37.30)	0 (0.00)	311 (62.70)
Nalidixic	209 (42.14)	0 (0.00)	287 (57.86)
Colistin	0 (0.00)	457 (92.14)	39 (7.86)
Tetracycline	419 (84.48)	14 (2.82)	63 (12.70)
Chloramphenicol	343 (69.15)	0 (0.00)	153 (30.85)
Cefotaxime	382 (77.02)	5 (1.01)	109 (21.98)
Amikacin	262 (52.82)	72 (14.52)	162 (32.66)
Meropenem	420 (84.68)	14 (2.82)	62 (12.50)
Trimethoprim-sulfamethoxazole	281 (56.65)	0 (0.00)	215 (43.35)
Ciprofloxacin	324 (65.32)	49 (9.88)	123 (24.80)

DEC – diarrheagenic *Escherichia coli*

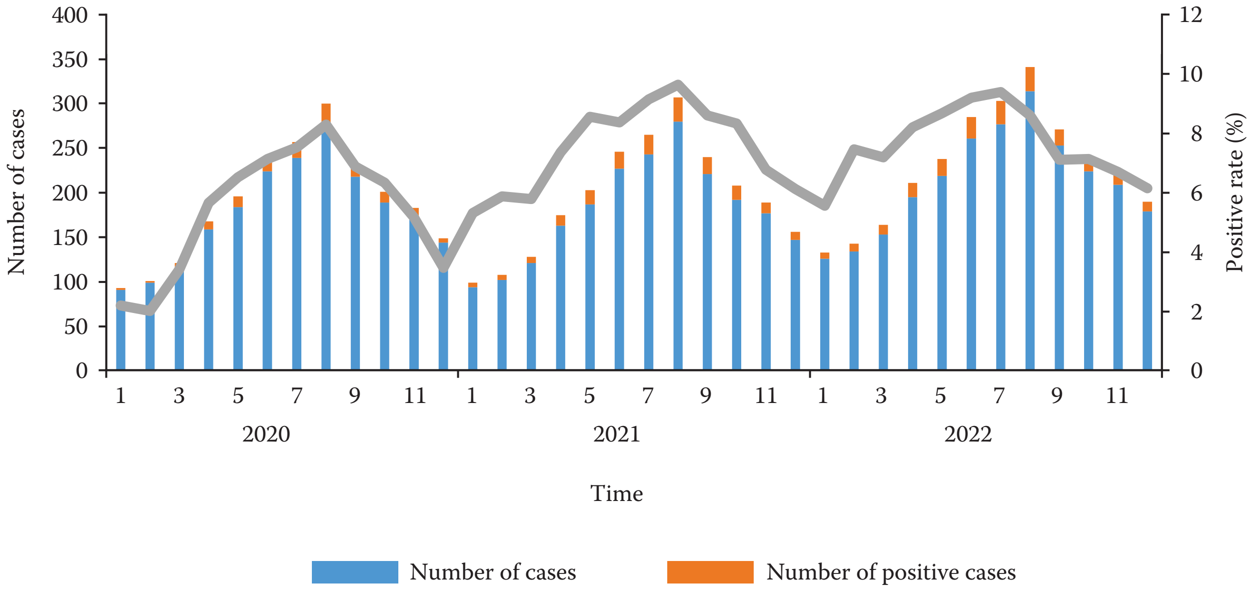

Figure 1. Detection rate and temporal distribution of DEC in patients with foodborne disease in Huzhou, 2020 to 2022

DEC – diarrheagenic *Escherichia coli*

with the highest positive detection rate was July, followed by June and August (Figure 1).

Distribution of suspected culprit foods in positive cases. Beverages and frozen beverages were the main suspected culprit foods for DEC infection (25.20%) followed by meat and meat products (18.15%) and fruit and fruit products (16.33%) (Table 5). Households were the main sites of exposure to suspected culprit foods for DEC infections (67.77%) followed by restaurants and street food vendors (5.98%).

Pulsed-field gel electrophoresis (PFGE) and clustering analysis. A subset of 120 DEC strains was randomly selected for analysis by PFGE. DNA was isolated and digested with *Xba* I, and 117 bands were obtained on PGFE (70 EAEC strains, 36 ETEC strains, 11 EPEC strains). The results showed that the similarity between different DEC strains was low. Therefore, this study divided the dominant EAEC and ETEC strains into two clusters. Based on the numbers and locations of bands, there were 69 different PFGE types with 70 strains of EAEC, and the band pattern similarity was 52.40–100.00%. There were 35 different PFGE types among 36 ETEC strains, and the band pattern similarity was 52.20–100.00% (Figures 2 and 3).

Table 5. Distribution of suspected culprit foods in cases of DEC infection in Huzhou, 2020–2022

Suspiciously exposed food	2020	2021	2022	Total
	(*n*; %)			
Meat and meat products	26 (20.47)	30 (17.64)	34 (17.09)	90 (18.15)
Milk and dairy products	10 (7.87)	11 (6.47)	11 (5.53)	32 (6.45)
Eggs and egg products	0 (0.00)	1 (5.88)	4 (2.01)	5 (1.01)
Fruits and fruit products	22 (17.32)	28 (16.47)	31 (15.58)	81 (16.33)
Grains and grain products	17 (13.39)	25 (14.70)	21 (10.55)	63 (12.70)
Aquatic animals and aquatic animal products	8 (6.30)	9 (5.29)	23 (11.56)	40 (8.06)
Vegetables and their products	3 (2.36)	7 (4.12)	11 (5.53)	21 (4.23)
Drinks and frozen drinks	32 (25.20)	46 (27.06)	47 (23.62)	125 (25.20)
Other food (or unknown)	9 (7.09)	13 (7.65)	17 (8.54)	39 (7.86)
Total	127 (100.00)	170 (100.00)	199 (100.00)	496 (100.00)

DEC – diarrheagenic *Escherichia coli*

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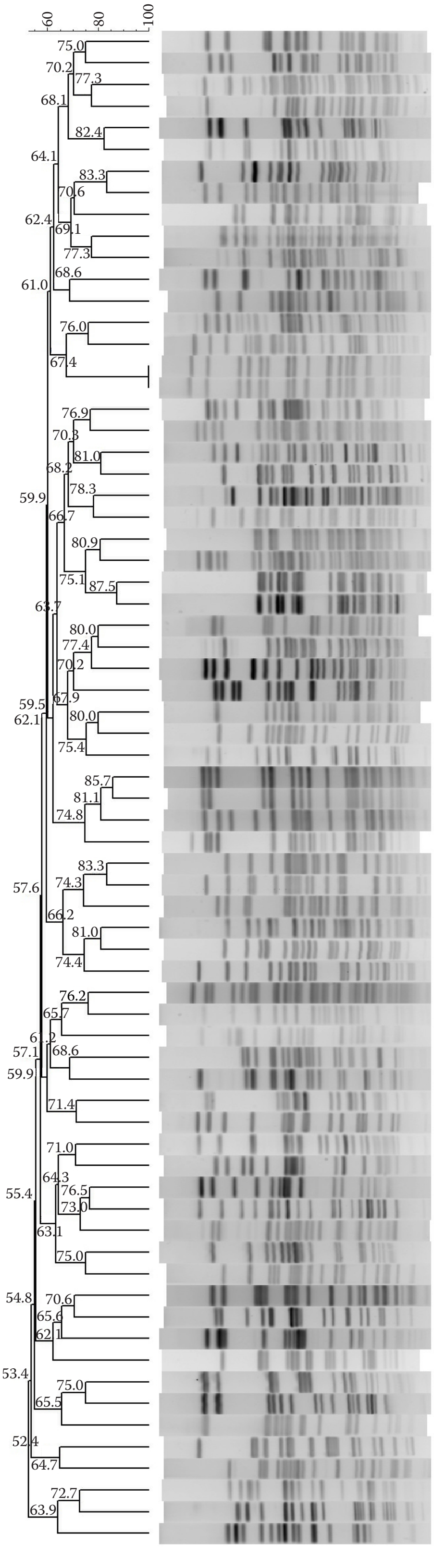

Key	District	Year	Drug resistance
ZJHH21_126	Changxing	2021	CHL-SXT-TET-CIP-NAL-STR
ZJHH22_076	Changxing	2022	SXT-TET-CIP-NAL-STR-AMP-AMS
ZJHH22_352	Changxing	2022	CHL-SXT-TET-CIP-NAL-AMP
ZJHH22_372	Deqing	2022	CHL-TET-STR-AMP
ZJHH20_067	Deqing	2020	CTX-CIP-NAL-STR-AMP
ZJHH20_095	Changxing	2020	SXT-TET-STR
ZJHH20_073	Changxing	2020	SXT-TET-STR
ZJHH21_135	Deqing	2021	CHL-SXT-CTX-TET-CIP-NAL-STR-AMP
ZJHH21_128	Changxing	2021	CHL-SXT-CTX-TET-CIP-NAL-STR-AMP
ZJHH20_074	Changxing	2020	CHL-SXT-TET-NAL-AMP
ZJHH22_367	Wuxing	2022	CHL-SXT-TET-CIP-NAL-STR-AMP-AMS
ZJHH20_099	Wuxing	2020	All sensitive
ZJHH21_254	Wuxing	2021	CHL-SXT-CTX-TET-CIP-NAL-STR-AMP-AMS
ZJHH22_365	Deqing	2022	SXT-NAL-AMP
ZJHH22_374	Wuxing	2022	CHL-SXT-TET-STR-AMP
ZJHH21_264	Changxing	2021	All sensitive
ZJHH21_265	Deqing	2021	CHL-SXT-TET-STR-AMP
ZJHH21_121	Changxing	2021	TET
ZJHH21_259	Wuxing	2021	CHL-SXT-TET-CIP-STR-AMP
ZJHH22_220	Changxing	2022	SXT-CTX-TET-NAL-AMP-AMS
ZJHH22_355	Changxing	2022	All sensitive
ZJHH22_099	Changxing	2022	All sensitive
ZJHH22_377	Wuxing	2022	SXT-CTX-CAZ-TET-STR-AMP-AMS
ZJHH21_245	Changxing	2021	SXT-TET-STR
ZJHH22_366	Deqing	2022	CHL-SXT-CTX-TET-CIP-NAL-STR-AMP
ZJHH21_125	Deqing	2021	NAL
ZJHH22_077	Changxing	2022	SXT-TET-STR-AMP
ZJHH21_137	Wuxing	2021	CHL-TET-STR-AMP
ZJHH22_357	Wuxing	2022	CHL-SXT-CTX-TET-NAL-STR-AMP
ZJHH20_071	Deqing	2020	CHL-SXT-CT-TET-CIP-NAL-STR-AMP-AMS
ZJHH20_072	Deqing	2020	TET-NAL-STR-AMP
ZJHH21_138	Wuxing	2021	SXT-TET-STR-AMP
ZJHH22_378	Changxing	2022	TET-AMP
ZJHH22_213	Wuxing	2022	CHL-SXT-TET-CIP-NAL-STR-AMP
ZJHH21_248	Deqing	2021	TET-NAL-AMP-AMS
ZJHH21_255	Wuxing	2021	TET-NAL
ZJHH21_246	Changxing	2021	SXT-CTX-TET-NAL-STR-AMP
ZJHH21_119	Wuxing	2021	TET-NAL-AMP
ZJHH21_258	Wuxing	2021	CTX-CIP-NAL-AMP
ZJHH22_376	Wuxing	2022	CTX-CIP-NAL-AMP
ZJHH21_261	Deqing	2021	All sensitive
ZJHH22_075	Changxing	2022	TET-STR-AMP
ZJHH22_362	Changxing	2022	CTX-TET-AMP
ZJHH22_358	Wuxing	2022	CTX-CAZ-AMP-AMS
ZJHH21_252	Wuxing	2021	SXT-TET-AMP
ZJHH22_073	Changxing	2022	TET-NAL-STR-AMP
ZJHH22_081	Deqing	2022	SXT-CTX-CIP-NAL-AMP
ZJHH20_097	Changxing	2020	NAL-AMP-AMS
ZJHH20_098	Changxing	2020	SXT-CTX-TET-NAL-NAL-AMP
ZJHH22_072	Deqing	2022	All sensitive
ZJHH22_360	Changxing	2022	SXT
ZJHH22_071	Deqing	2022	CIP-NAL
ZJHH22_078	Changxing	2022	All sensitive
ZJHH20_068	Changxing	2020	NAL-STR
ZJHH22_218	Changxing	2022	SXT-CTX-CAZ-TET-CIP-NAL-AMP-AMS
ZJHH21_263	Changxing	2021	CHL-SXT-CTX-TET-CIP-NAL-STR-AMP
ZJHH20_065	Changxing	2020	CHL-SXT-TET-NAL-STR
ZJHH22_371	Wuxing	2022	SXT-NAL-STR-AMP
ZJHH21_249	Wuxing	2021	CHL-SXT-TET-AMP
ZJHH22_219	Changxing	2022	SXT-TET-AMP
ZJHH20_070	Deqing	2020	CHL-SXT-CTX-CAZ-TET-CIP-NAL-STR-AMP-AMS
ZJHH21_118	Wuxing	2021	All sensitive
ZJHH20_064	Changxing	2020	TET-NAL-AMP
ZJHH20_066	Deqing	2020	SXT-CTX-TET-NAL-AMP
ZJHH21_266	Deqing	2021	All sensitive
ZJHH22_353	Wuxing	2022	TET-AMP
ZJHH22_370	Wuxing	2022	NAL
ZJHH20_096	Changxing	2020	SXT-TET-NAL-STR-AMP
ZJHH22_217	Deqing	2022	NAL-AMP-AMS
ZJHH22_074	Changxing	2022	CTX-CIP-NAL-AMP

Figure 2. Pulsed-field gel electrophoresis clustering analysis of 70 enteroaggregative *Escherichia coli* strains

AMI – Amikacin; AMP – Ampicillin; AMS – Ampicillin/sulbactam; CAZ – Ceftazidime; CHL – Chloramphenicol; CIP – Ciprofloxacin; CT – Colistin; CTX – Cefotaxime; IPM – Imipenem; MEM – Meropenem; NAL – Nalidixic; STR – Streptomycin; SXT – Trimethoprim-sulfamethoxazole; TET – Tetracycline

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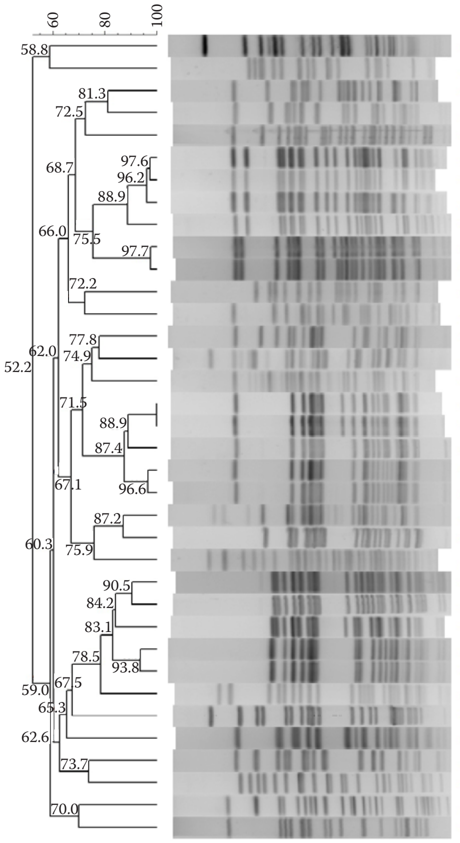

Key	District	Year	Drug resistance
ZJHH20_069	Changxing	2020	CHL-SXT-STR-AMP-AMS
ZJHH21_131	Deqing	2021	CIP-NAL-AMP
ZJHH21_134	Wuxing	2021	All sensitive
ZJHH21_257	Wuxing	2021	SXT-CT-MEM-CTX-CAZ-CIP-NAL-AMI-STR-AMP
ZJHH22_369	Wuxing	2022	SXT-TET-NAL-AMP
ZJHH21_124	Deqing	2021	NAL-AMP
ZJHH21_129	Deqing	2021	NAL-AMP
ZJHH21_127	Changxing	2021	CIP-NAL-AMP
ZJHH22_363	Changxing	2022	CTX-TET-NAL-AMP
ZJHH21_247	Wuxing	2021	SXT-TET-NAL
ZJHH21_250	Wuxing	2021	NAL
ZJHH21_136	Wuxing	2021	All sensitive
ZJHH21_139	Wuxing	2021	NAL
ZJHH20_103	Changxing	2020	TET-CIP-NAL-AMP
ZJHH22_080	Changxing	2022	TET-NAL-AMP
ZJHH21_130	Deqing	2021	TET-AMP
ZJHH21_120	Changxing	2021	TET-CIP-NAL-AMP
ZJHH21_123	Changxing	2021	TET-CIP-NAL-AMP
ZJHH22_375	Wuxing	2022	CTX-NAL-AMP
ZJHH20_101	Changxing	2020	CIP-NAL-AMP
ZJHH21_262	Wuxing	2021	NAL
ZJHH20_100	Wuxing	2020	NAL
ZJHH22_361	Changxing	2022	SXT-TET-CIP-NAL-STR
ZJHH21_132	Changxing	2021	TET-CIP-NAL-AMP
ZJHH21_251	Wuxing	2021	SXT-TET-NAL-STR
ZJHH22_354	Wuxing	2022	SXT-TET-NAL
ZJHH21_122	Changxing	2021	NAL
ZJHH20_102	Changxing	2020	SXT-TET-STR-AMP
ZJHH20_104	Changxing	2020	SXT-AMP
ZJHH21_256	Wuxing	2021	All sensitive
ZJHH22_356	Changxing	2022	NAL
ZJHH21_253	Deqing	2021	All sensitive
ZJHH21_260	Deqing	2021	All sensitive
ZJHH22_364	Changxing	2022	SXT-TET-CIP-NAL-STR
ZJHH22_216	Deqing	2022	CTX-NAL-AMP
ZJHH22_368	Wuxing	2022	SXT-TET-STR-AMP

Figure 3. Pulsed-field gel electrophoresis clustering analysis of 36 enterotoxin *Escherichia coli* strains

AMI – Amikacin; AMP – Ampicillin; AMS – Ampicillin/sulbactam; CAZ – Ceftazidime; CHL – Chloramphenicol; CIP – Ciprofloxacin; CT – Colistin; CTX – Cefotaxime; IPM – Imipenem; MEM – Meropenem; NAL – Nalidixic; STR – Streptomycin; SXT – Trimethoprim-sulfamethoxazole; TET – Tetracycline

DISCUSSION

The burden of diarrheal diseases caused by DEC is heavy in China (Zhang et al. 2017b). Previous reports have indicated that the detection rates and dominant bacterial communities of DEC are also different between different regions of the country (Zhang et al. 2017b; Wang et al. 2022a). Data analysis in this study revealed positive rates of DEC in active surveillance cases of foodborne diseases of 6.01, 7.89, and 7.82% in Huzhou in 2020, 2021, and 2022, lower than the total detection rate of DEC in diarrheal outpatients (28.55%) reported by Li et al. (Yang et al. 2020) and in Haidian District of Beijing (11.8%) (Liu et al. 2021). However, it was higher than in Shanghai (5.71%) (Wang et al. 2022a) and Jiangsu (2.48%) (Qin et al. 2020), further indicating that the detection rates may be significantly different between regions, possibly due to climate and dietary habits. The dominant types in Huzhou were EAEC and ETEC, consistent with the prevalent types detected in Jinan (Yang et al. 2020) and some districts of Beijing (Wen et al. 2021). The positive detection rate was slightly higher in males (5.79%) than females (5.16%), which may have been because males have more opportunities to eat out, more frequent social activities, less awareness of personal hygiene, and more exposure opportunities. The most significant number of DEC infection cases was seen in children aged 6 years, followed by adults 20 years old and 40 years old. There were significant differences in positive rates among different age groups ($P < 0.05$), consistent with the literature (Zhang et al. 2017b; Wen et al. 2021; Yu et al. 2022). There were cases of DEC infection every month, and the peak infection period was between April and November. This is consistent with previous reports (Tang et al. 2018; Duan et al. 2022; Yu et al. 2022) suggesting that foodborne DEC infection has a seasonal distribution and is more prevalent in summer. Therefore, efforts to prevent and control foodborne DEC infection should be strengthened in summer, particularly for those caused by EAEC.

In this study, *astA*/*pic* was the predominant EAEC virulence gene and *estIa*/*estIb* was the predominant ETEC virulence gene. However, other virulence genes were also detected, indicating the diversity of virulence genes in the bacterial populations. These results are consistent with those reported for the Haidian District (Bai et al. 2022).

Foodborne diseases are often related to exposures within the family (Zhang et al. 2017a). The highest positive detection rates were reported in beverages, frozen beverages, and meat products (Chen et al. 2016). In the present study, beverages and frozen beverages were the main suspected culprit foods responsible for DEC infection in Huzhou, accounting for 23.81% of cases, followed by meat and meat products (17.14%) and fruit and fruit products (15.24%), similar to some other provinces and cities in China (Chen et al. 2016; Wang et al. 2022a). The suspected place of exposure was mainly in the family home, which is consistent with previous reports in Jiangsu Province (Zhang et al. 2017a; Liu et al. 2021).

In this study, the incidence of DEC drug resistance was very high in the Huzhou area. Among the 14 antibiotics examined, all strains were sensitive only to IMP, and there were different numbers of resistant strains to the others. The antibiotics with the highest resistance rates were AMP (63.51%), STR (62.70%), and NAL (57.86%), which was different from the profiles in other regions (Wang et al. 2021). This discrepancy may be related to the different kinds of drugs used and different sources of strains between areas in China. 341 strains were resistant to three or more antibiotics; AMP, STR, and AMS appeared the most frequently in multidrug resistant (MDS) strains. Our results suggest that drug-resistant DEC is a problem in Huzhou, and the spectrum of multidrug resistance is broad, which challenges the rational selection of antibiotics and drug combinations.

Analysis of strains from different sources can reveal correlations between other cases and whether the infections are sporadic or clustered. Such studies can also trace the source of pathogens, especially for the analysis and treatment of clustered outbreaks, which is of practical significance. Here, PFGE molecular typing was used to analyse the relations of EAEC and ETEC strains, which were detected at high rates. The PFGE band types of DEC in the Huzhou area were abundant and showed polymorphism at the gene level. Three EAEC clones had similarity exceeding 85.00%, and each clone contained two strains without apparent homology. There were six clones with ETEC similarity above 85.00%, and each clone contained two to five strains, among which two strains had 100% type similarity; one of the clones contained five strains, and the other contained four strains. Data analysis showed that these isolates came from different regions or times and represented non-clustered outbreaks.

CONCLUSION

We analysed the characteristics of foodborne DEC infections in Huzhou, China. A variety of virulence types were detected. The household was the main place of DEC infection, and meat and meat products were the main suspected culprit foods. The rates of drug resistance in DEC were high in Huzhou, and multidrug-resistant strains were common. It is necessary to pay close attention to the emergence of drug-resistant strains and enhance antimicrobial management. Our results will be useful for controlling and treating foodborne illnesses caused by DEC in Huzhou, China.

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