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Seven-year overview of antimicrobial resistance in diarrheagenic *Escherichia coli* from sporadic human diarrhea cases in 20 Chinese provinces

Haihong Han[1], Weiwei Li[1], Jikai Liu[1], Xiaoai Zhang[2], Xiang Huo[3], Yong Sun[4], Jiancai Chen[5], Ruyue Fan[6], Jing Zhang[7], Yong Chen[8], Hongxia Yang[9], Shuai Chen[10], Yanfen Li[11], Dongmei Tan[12], Xuemin Ma[13], Penghui Fan[1], Shaofei Yan[1] and Yunchang Guo[1*]

Abstract

Diarrheagenic *Escherichia coli* is one of the most prevalent foodborne pathogens and poses significant clinical challenges due to increasing rates of antibiotic resistance. In this study, 9,068 diarrheagenic *E. coli* isolates from sporadic diarrhea cases across 20 provinces of China from 2016 to 2022 were systematically analyzed to determine the prevalence and distribution of resistance. A total of 14 antibiotics were included in the antimicrobial resistance surveillance. Antimicrobial susceptibility testing indicated that the majority of the isolates (89.3%) were resistant to at least one antimicrobial agent, with a significant proportion (60.3%) demonstrating multidrug resistance. Isolates showed high levels of resistance to ampicillin (68.2%) and high sensitivity to imipenem (97.0%). Over the 7 years, an increasing trend in resistance to cefoxitin and chloramphenicol among the isolates was found. Enteroaggregative *E. coli* was identified as the predominant pathotype. Furthermore, antimicrobial resistance rates were higher among the isolates from infants under 1 year of age compared to other populations and resistance levels differed among pathotypes. In summary, our findings revealed the detailed resistance characteristics of diarrheagenic *E. coli* strains isolated from human sporadic diarrhea cases in China, offering valuable insights that can inform clinical antibiotic treatment strategies and priorities.

Keywords Foodborne disease, Diarrheagenic *Escherichia coli*, Antimicrobial resistance

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*Correspondence:
Yunchang Guo
gych@cfsa.net.cn
[1] NHC Key Laboratory of Food Safety Risk Assessment, Food Safety Research Unit (2019RU014) of Chinese Academy of Medical Science, China National Center for Food Safety Risk Assessment, Beijing 100022, China
[2] Beijing Center for Disease Control and Prevention, Beijing 100013, China
[3] Jiangsu Provincial Center for Disease Control and Prevention, Nanjing 210009, China
[4] Anhui Provincial Center for Disease Control and Prevention, Hefei 230601, China
[5] Zhejiang Provincial Center for Disease Prevention and Control, Hangzhou 310051, China
[6] Shandong Provincial Center for Disease Prevention and Control, Jinan 250014, China
[7] Gansu Provincial Center for Disease Prevention and Control, Lanzhou 730000, China
[8] Shanghai Center for Disease Prevention and Control, Shanghai 200336, China
[9] Shanxi Provincial Center for Disease Prevention and Control, Taiyuan 030012, China
[10] Hunan Provincial Center for Disease Control and Prevention, Changsha 410005, China
[11] Henan Provincial Center for Disease Control and Prevention, Zhengzhou 450016, China
[12] Guangxi Center for Disease Prevention and Control, Nanning 530028, China
[13] Ningxia Center for Disease Prevention and Control, Yinchuan 750004, China

Introduction

Escherichia coli is a key facultative anaerobic species that predominantly resides in the large intestine of humans and warm-blooded animals, typically without causing illness. However, certain strains of *E. coli*, known as diarrheagenic *E. coli* (DEC), are significant contributors to diarrheal diseases [1]. DEC strains are classified into five distinct pathotypes based on their preferred sites of colonization, mechanisms of pathogenesis, and subsequent clinical symptoms and consequences: enteroaggregative *E. coli* (EAEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), and enteroinvasive *E. coli* (EIEC). These DEC strains represent a well-known group of foodborne pathogens, found in a variety of foods, including raw meats, pasteurized milk, vegetables, water, and have been implicated in human infections [2]. ETEC, EPEC, and EHEC are particularly notable among the high-burden pathogens of 22 known foodborne bacterial, protozoal, and viral diseases globally, with EPEC ranking as the second leading cause of foodborne-related mortality, after *Salmonella Typhimurium* [3].

Antimicrobial therapy is crucial for treating severe and prolonged diarrhea caused by DEC. However, antimicrobial resistance (AMR) in these strains, driven by the overuse of antibiotics, presents a formidable challenge, rendering many first-line treatments ineffective. Commonly prescribed medications, such as ampicillin, ciprofloxacin, and the combination of sulfamethoxazole and trimethoprim, are gradually losing their efficacy due to resistance [4]. Moreover, the emergence of *E. coli* strains that produce extended-spectrum beta-lactamase (ESBL) and exhibit multidrug-resistant (MDR) complicates treatment options, as these strains are increasingly resistant to a wider array of antibiotics. The ongoing global trend of increasing antimicrobial resistance in *E. coli* underscores the importance of continuous surveillance and proactive efforts to combat the spread of resistance.

In China, a proactive, laboratory-based national surveillance system, the National Foodborne Disease Surveillance System, is in operation. Under this system, each provincial Center for Disease Control and Prevention (CDC) is tasked with collecting at least ten stool specimens monthly from patients with sporadic diarrhea at designated sentinel hospitals. The surveillance is designed to detect five targeted foodborne pathogens, including *Salmonella* species, *Shigella* species, *Vibrio parahaemolyticus*, DEC, and Norovirus. The findings indicate that DEC is the second most prevalent bacterial cause of sporadic diarrhea in China [5]. Antimicrobial resistance surveillance has also been implemented to monitor trends in antibiotic resistance among foodborne pathogens, including DEC.

In this study, antimicrobial resistance surveillance data were compiled from 9,068 DEC isolates between 2016 and 2022 across 20 provinces in China. The prevalence and distribution of resistant pathogenic *E. coli* strains isolated from sporadic diarrhea cases were analyzed. These data can assist policymakers in formulating and adjusting antibiotic use policies and help reduce the spread of resistant strains through targeted preventive measures.

Results

Epidemiological distribution

The minimum inhibitory concentrations (MICs) of 9,068 DEC isolates were reported by 20 Chinese provincial CDCs from samples collected between 2016 and 2022. These isolates originated from human patients with sporadic diarrhea, ranging in age from 0 to 98 years. The number of isolates reported varied significantly by province, with the fewest from Guizhou (44) and the most from Beijing (2,399). Over the 7-year study period, the annual number of isolates reported ranged from 688 in 2016 to 1,813 in 2021 (Table 1).

Antimicrobial resistance

In total, 89.3% of the isolates (8,098 out of 9,068) were resistant to at least one antibiotic. Additionally, 60.3% (5,464 out of 9,068) were identified as MDR strains. Among these MDR strains, nearly half (49.0%, 2,677 out of 5,464) exhibited resistance to more than five classes of antimicrobial agents. The identification of 901 distinct antimicrobial resistance profiles illustrated a wide range of resistance mechanisms. Two predominant MDR profiles were highlighted: ACSuT, characterized by resistance to ampicillin, chloramphenicol, sulfonamide, tetracycline, and ACSuTAs, which adds resistance to ampicillin/sulbactam. These profiles were found in 11.6% (1,051 out of 9,068) and 5.7% (520 out of 9,068) of all DEC isolates, respectively.

Ampicillin resistance was most common, observed in 68.2% (6,162 out of 9,034) of the isolates, while imipenem resistance was least common, observed in only 1.6% (140 out of 8,789) of the isolates. Additionally, 21.2% were resistant to the antibiotic ciprofloxacin. A high percentage of isolates, 41.9%, showed reduced sensitivity to ciprofloxacin, which includes both intermediate and resistant isolates [6]. Rates of resistance to the third-generation cephalosporins ceftazidime and cefotaxime were 6.3% and 27.4%, respectively. Among human DEC isolates in China, 17.1% showed resistance to chloramphenicol (Fig. 1). A trend analysis spanning 7 years, evaluated using a linear regression model, revealed an increase in resistance to chloramphenicol, from 10.8% in 2016 to 19.2% in 2022. Similarly, resistance to cefoxitin

Table 1 Annual and geographical distribution of pathotypes of 9,068 diarrheagenic *Escherichia coli* isolates from sporadic diarrhea cases in China, 2016–2022

Groups	EAEC	EPEC	ETEC	EHEC	EIEC	Unknown[a]	Total
Year							
2022	659	263	252	17	7	62	1260
2021	814	339	466	28	10	156	1813
2020	589	286	198	39	15	7	1134
2019	771	395	474	12	22	4	1678
2018	663	359	352	12	19	12	1417
2017	468	273	312	9	14	2	1078
2016	322	184	167	5	10	-	688
Province							
Anhui	420	128	187	2	10	8	755
Beijing	897	676	780	20	24	2	2399
Gansu	382	80	47	30	12	-	551
Guangxi	184	38	45	4	3	-	274
Guizhou	34	3	6	-	1	-	44
Hebei	66	49	17	2	-	8	142
Henan	239	45	57	-	10	-	351
Heilongjiang	48	8	10	1	-	1	68
Hubei	30	25	32	-	-	2	89
Hunan	149	135	111	1	2	5	403
Jilin	35	20	4	1	-	-	60
Jiangsu	550	337	221	16	18	140	1282
Neimenggu	95	25	4	3	1	-	128
Ningxia	85	78	26	16	4	28	237
Shandong	194	141	217	8	2	6	568
Shanxi	199	131	75	1	4	-	410
Shanghai	274	53	214	2	-	1	544
Tianjin	52	43	34	3	-	2	134
Xingjiang	43	5	4	2	3	-	57
Zhejiang	310	79	130	10	3	40	572

[a] "Unknown" indicates strains identified as diarrheagenic *Escherichia coli*, but the specific pathotypes are not available

EAEC Enteroaggregative *E. coli*, *EPEC* Enteropathogenic *E. coli*, *EHEC* Enterohemorrhagic *E. coli*, ETEC Enterotoxigenic *E. coli*, *EIEC* Enteroinvasive *E. coli*

also showed an upward trend, rising from 3.4% in 2016 to 11.2% in 2022 (Fig. 2).

Pathotype and age distributions

A high percentage of the isolates, 97.3% (8,825 out of 9,068), were successfully identified at the pathotype level. EAEC emerged as the predominant pathotype in sporadic clinical isolates in China, representing 47.3% (4,286 out of 9,068) of the total. ETEC and EPEC were the second and third most common pathotypes, accounting for 24.5% and 23.1% of the isolates, respectively. EIEC and EHEC were identified in only approximately 1% of cases.

There was considerable variation in resistance rates and age distributions across different pathotypes. Notably, EAEC strains had significantly higher rates of resistance to ampicillin compared to other pathotypes, whereas EPEC strains showed low resistance to nalidixic acid. ETEC strains exhibited low resistance to a range of antibiotics, including ampicillin/sulbactam, chloramphenicol, gentamicin, ciprofloxacin, tetracycline, and trimethoprim-sulfamethoxazole. Conversely, EIEC strains had higher rates of resistantance to cefazolin and cefotaxime than those of other strains, while EHEC strains were particularly resistant to ciprofloxacin (Fig. 3).

Among isolates obtained from infants under 1 year of age, EAEC was the predominant pathotype, accounting for 70.1% of infections in males and 61.5% in females. The prevalence of EAEC infections declined to approximately 50% in children aged 1–4 years. In the pediatric population under 1 year of age, the proportion of EPEC infections was 20%, and this increased to 35% in

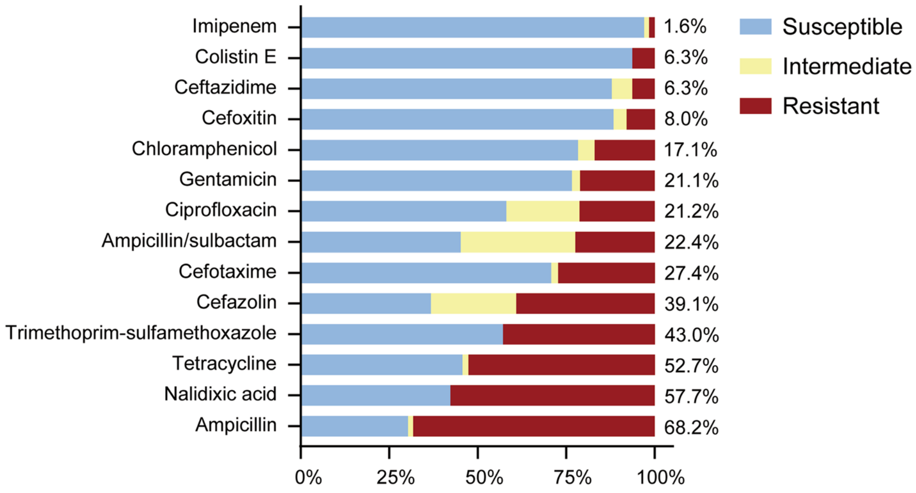

Fig. 1 Antibiotic resistance profile of diarrheagenic *Escherichia coli* isolated from sporadic cases, 2016–2022 China. The percentages indicated on the right side represent the proportion of resistant bacteria

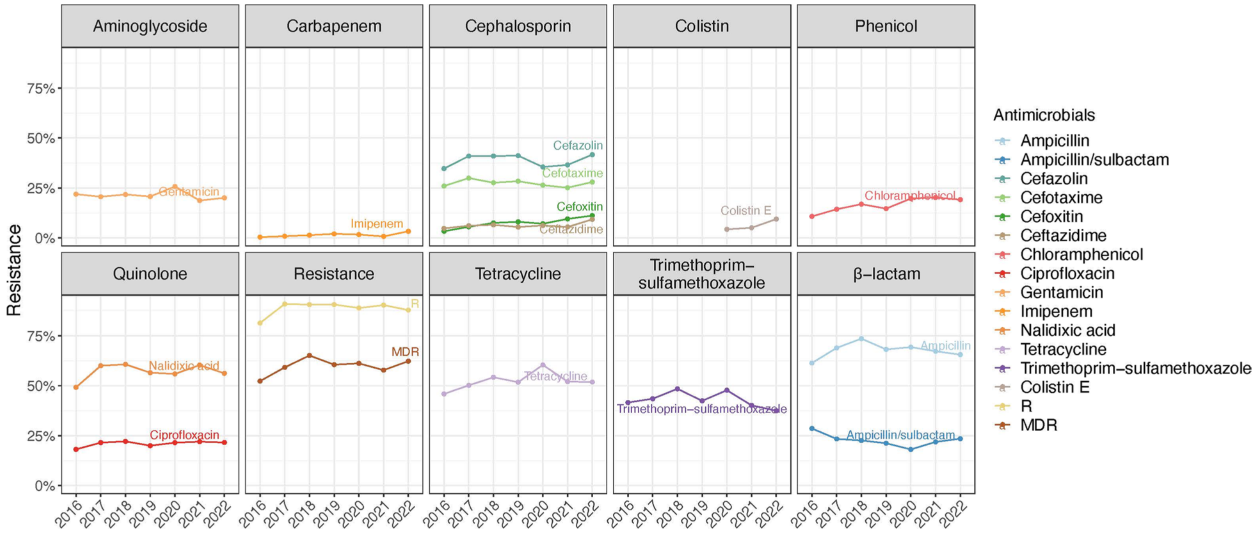

Fig. 2 Pooled resistance rates of diarrheagenic *Escherichia coli* isolated over the years. "R" in the legend represents pooled resistance, meaning resistantance to at least one of the 14 antimicrobial agents. MDR: multidrug resistance

children aged 1–4 years. ETEC infections were rare in children under 5 years, representing only 5% of cases; however, their prevalence increased with age, peaking at 33% among adults aged 25–64 years. For EIEC and EHEC strains, no clearly susceptible populations were detected due to the small number of strains tested (Fig. 4).

Table 2 illustrates the impact of age and sex on the prevalence of antimicrobial-resistant *E. coli*. The isolates from infants under 1 year of age had higher rates of resistance to several antibiotics, including ampicillin, cefazolin, cefotaxime, and cefoxitin, compared to isolates from older age groups. Additionally, the isolates from younger children were more likely to exhibit resistance to ampicillin/sulbactam, ceftazidime, tetracycline, chloramphenicol, gentamicin, and trimethoprim-sulfamethoxazole. However, resistance to ciprofloxacin, nalidixic acid, imipenem, and colistin did not show a clear age-related pattern. No significant sex-related differences in the prevalence of antimicrobial-resistant DEC were found.

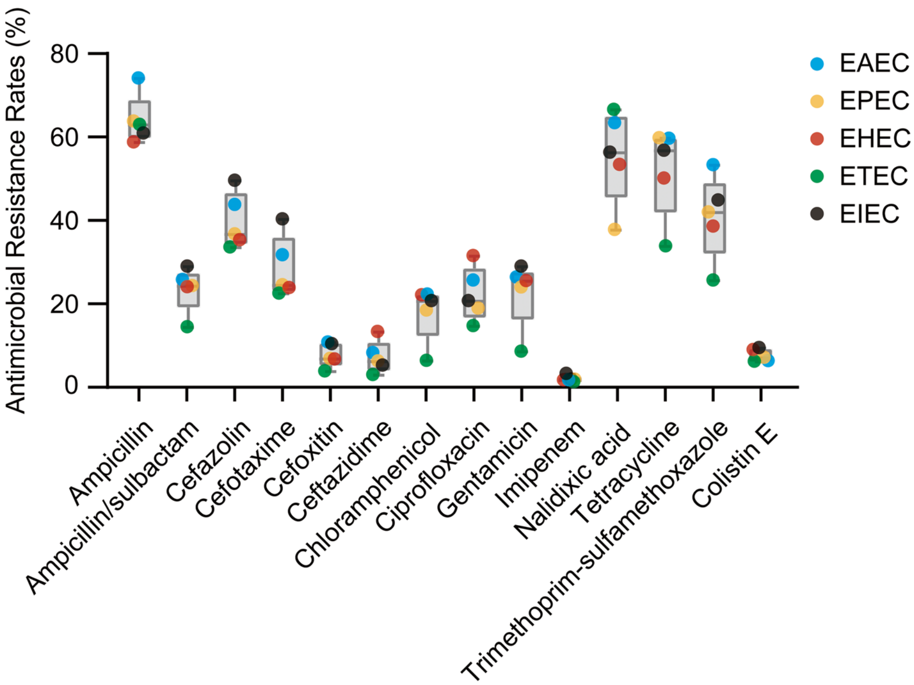

Fig. 3 Resistance rates of different pathotypes of *Escherichia coli* to various antimicrobial agents. EAEC: Enteroaggregative *E. coli*, EPEC: Enteropathogenic *E. coli*, EHEC: Enterohemorrhagic *E. coli*, ETEC: Enterotoxigenic *E. coli*, EIEC: Enteroinvasive *E. coli*

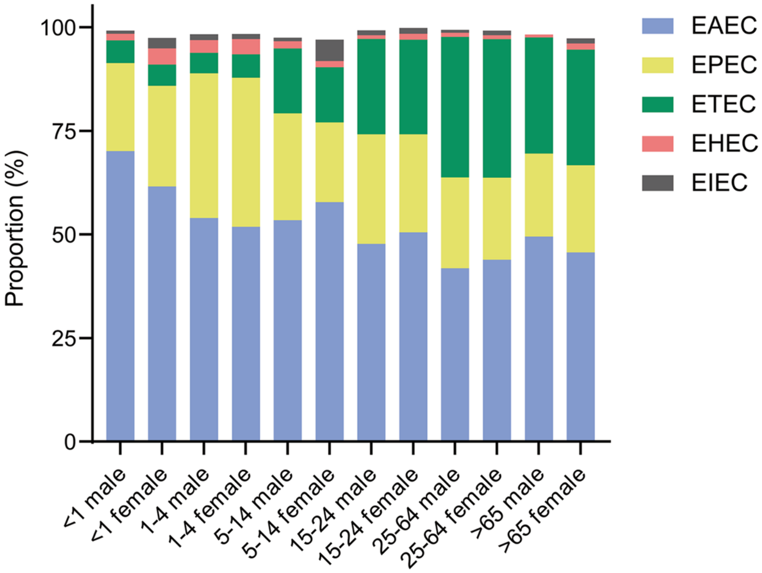

Fig. 4 Composition of diarrheagenic *Escherichia coli* pathotypes in different populations

Discussion

DEC strains, particularly EAEC, EPEC, and ETEC, are major causes of diarrheal diseases, with a vast network of transmission routes that encompass human populations, animal reservoirs, food supplies, and environmental sources. These strains contribute to an estimated 30%–40% of all diarrhea episodes among children under the age of 5 years and are among the leading causes of mortality among young children [7]. The alarming rise of antibiotic-resistant strains at the global level has presented a challenge for the management of DEC infections, severely narrowing the range of effective antimicrobial therapies.

In a proactive and strategic response to the growing rates of antimicrobial resistance, China initiated a series of well-coordinated national action plans in 2014.

Table 2 Resistance profile of diarrheagenic *Escherichia coli* isolates grouped by different populations

Antimicrobial Agent	**Population groups (Resistance %)**											
	< 1 year		**1–4 years**		**5–14 years**		**15–24 years**		**25–64 years**		**> 65 years**	
	male	**female**	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**female**	**male**	**female**
Ampicillin	73.2	74.4	71.6	69.7	69.1	66.4	67.6	64.6	67.1	67.1	68.1	69.4
Ampicillin/sulbactam	29.4	25.3	24.7	24.4	25.6	24.4	22.8	22.4	22.1	20.9	25.3	19.3
Cefazolin	51.6	48.7	43.1	43.9	42.1	35.6	38.9	39.2	38.2	37.2	37.3	36.0
Cefotaxime	34.7	33.3	32.8	31.2	26.8	28.2	29.5	29.4	25.6	24.4	26.3	22.8
Cefoxitin	11.8	15.4	10.3	8.7	8.1	8.9	6.8	9.7	7.1	6.8	8.5	7.2
Ceftazidime	9.5	10.3	9.2	10.8	6.8	8.2	6.2	6.7	4.5	5.5	5.3	4.9
Chloramphenicol	19.7	19.2	21.5	22.6	20.8	25.2	16.8	18.1	14.4	13.6	16.5	15.4
Ciprofloxacin	16.7	26.9	24.4	18.7	22.5	17.0	20.5	21.8	20.9	19.4	27.5	23.3
Gentamicin	31.2	25.6	30.2	28.3	22.0	24.4	21.6	19.7	18.1	18.6	19.0	18.1
Imipenem	1.8	2.9	2.0	0.2	1.3	0.0	3.0	2.3	1.5	1.3	2.8	1.3
Nalidixic acid	57.5	55.8	55.5	53.6	59.2	52.6	57.8	61.5	58.1	57.9	66.4	52.4
Tetracycline	59.8	55.8	64.4	60.2	57.2	62.2	54.4	54.0	49.2	47.6	49.3	46.6
Trimethoprim-sulfamethoxazole	47.2	55.4	48.1	51.2	46.1	51.1	43.2	45.2	41.2	37.4	42.7	42.1
Colistin E	7.4	13.3	6.3	5.1	5.0	3.2	9.8	5.8	7.6	7.9	8.7	6.0

These plans focus on foodborne pathogens, including DEC, with the aim of meticulously monitoring the emergence and spread of antibiotic resistance. They are also designed to identify critical resistance patterns and to thoroughly assess the effectiveness of implemented intervention strategies, thereby strengthening the country's defenses against this global health challenge.

First-line antimicrobials, such as ampicillin, nalidixic acid, ciprofloxacin, tetracycline, and sulfamethoxazole/trimethoprim, are foundational in treating human *E. coli* infections. However, we detected widespread resistance among DEC isolates, particularly against penicillin-class antibiotics, with 68.2% of isolates exhibiting ampicillin resistance. Despite this high resistance to penicillin antibiotics, pairing it with sulbactam increased the susceptibility of DEC to ampicillin, reducing the resistance rate to 22.4%. A significant proportion of DEC isolates demonstrated low susceptibility to ciprofloxacin (41.9%), and resistance to the synthetic quinolone nalidixic acid was alarmingly high at 57.7%. Furthermore, 31.8% of DEC isolates with decreased susceptibility to ciprofloxacin were still susceptible to nalidixic acid, suggesting that the continued use of fluoroquinolones may inadvertently promote the spread of plasmid-mediated quinolone resistance determinants. High resistance rates were also observed for tetracycline (52.7%) and the combination of sulfamethoxazole/trimethoprim (43.0%), highlighting the limitations of our antimicrobial arsenal. The resistance rates for these antimicrobial agents in China exceed those reported for *E. coli* O157 isolates by the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) in the United States, with reported resistance rates of 6.6% for ampicillin, 0.0% for ciprofloxacin, 5.0% for nalidixic acid, 9.9% for tetracycline, and 0.6% for sulfamethoxazole/trimethoprim [8].

ESBL-producing *E. coli* strains, known as superbugs for their ability to hydrolyze third-generation cephalosporins [9], accounted for over 20% of human DEC isolates from patients with diarrhea in China, markedly higher than the estimate of 1.7% in the United States [8]. These critical priority pathogens listed by the World Health Organization (WHO) [10] show encouraging susceptibility to carbapenems, especially imipenem (98.4%). Notably, beta-lactamase genes in ESBL-producing DEC isolates can be readily transferred among species via mobile elements, raising concerns about AMR proliferation. Resistance to first-line drugs has also led to a 60.3% MDR rate in DEC isolates, significantly higher than the estimate of 8.3% reported by NARMS [8].

The growing resistance to chloramphenicol and cefoxitin in DEC isolates, as well as to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, and azithromycin in *Salmonella* isolates from China [10] is indicative of the widespread and often unnecessary use of antibiotics in clinical and agricultural settings. Echoing the emphasis of the One Health initiative on the interconnectedness of health across human, animal, and environmental domains, measures to curb the use of antimicrobials in food animals have been adopted in several countries [11]. The escalating resistance over the past 7 years observed in this study indicates the urgent need for China to investigate the root causes of AMR spread and to enforce

Table 3 Antimicrobial agents used for susceptibility testing of diarrheagenic *Escherichia coli* isolates, 2016–2022

WHO category level	Importance	Antimicrobial Class	Antimicrobial Agent	Years Tested	Antimicrobial Agent Concentration Range (µg/mL)	MIC Interpretive Standard (µg/mL)		
						Susceptible (S)	Intermediate (I)	Resistant (R)
I	Critically important[a]	**Cephalosporins (3rd, 4th and 5th generation)**	Cefotaxime (CTX)	2016–2022	0.25 ~ 8	1	2	4
			Ceftazidime (CAZ)	2016–2022	1 ~ 32	4	8	16
		Polymyxins	Colistin E (CT)	2019–2022	0.12 ~ 4	2	-	4
		Quinolones	Nalidixic acid (NAL)	2016–2022	2 ~ 64	16	-	32
			Ciprofloxacin (CIP)	2016–2022	0.03 ~ 32	0.25	0.5	1
		Aminoglycosides	Gentamicin (GEN)	2016–2022	1 ~ 32	4	8	16
		Carbapenems	Imipenem (IPM)	2016–2022	0.25 ~ 8	1	2	4
		Penicillins	Ampicillin (AMP)	2016–2022	2 ~ 64	8	16	32
		Penicillins (with β-lactamase inhibitors)	Ampicillin/sulbactam (AMS)	2016–2022	2/1 ~ 64/32	8/4	16/8	32/16
II	Highly important	Cephalosporins (1st and 2nd generation) and Cephamycins	Cefazolin (CFZ)	2016–2022	0.5 ~ 16	2	4	8
			Cefoxitin (CFX)	2016–2022	2 ~ 64	8	16	32
		Phenicols	Chloramphenicol (CHL)	2016–2022	2 ~ 64	8	16	32
		Tetracyclines	Tetracycline (TET)	2016–2022	1 ~ 32	4	8	16
		Folate pathway inhibitors	Trimethoprim-sulfamethoxazole (SXT)	2016–2022	0.25/4.75 ~ 8/152	2/38	-	4/76

[a] The antimicrobials shown in bold are Highest Priority Critically Important Antimicrobials, and others are High Priority Critically Important Antimicrobials

stricter regulations on antibiotic use in both humans and livestock.

In this study, analyses of relationships between both pathotype and age distributions, and the prevalence of antimicrobial-resistant DEC, revealed varying levels of resistance across different pathotypes and a significant trend toward increased resistance in EAEC strains from infants under 1 year of age. EAEC was the most prevalent pathotype in sporadic clinical isolates in China, followed by ETEC and EPEC. This distribution mirrors patterns in Mongolia and South Korea, with the exception of a lower incidence of ETEC [12, 13]. These findings highlight the importance of regional pathotype comparisons and variation in *E. coli* pathotypes in clinical environments. Our results confirm higher rates of resistance in younger individuals, likely due to their exposure to specific infections and treatments, a trend supported by previous findings [14], and reveal no effect of sex on resistance. Consistent with our previous research on *Vibrio parahaemolyticus* [15], our results emphasize the significant roles of pathotype, serotype, and demographic factors in shaping resistance patterns. Notably, there are geographical differences in the *E. coli* pathotype distribution; for example, EAEC is predominant in Mexico and Paraguay, consistent with our results, ETEC is predominant in Colombia, and EPEC is most frequent in Chile [16, 17]. In our research, we observed that ETEC strains demonstrated low rates of resistance to ciprofloxacin and tetracycline. This finding aligns with the work of Fatema-Tuz, who investigated the antimicrobial resistance patterns of ETEC isolated from both patients with diarrhea and environmental sources in Bangladesh [18].

This study provides a thorough overview of antimicrobial resistance in specific DEC strains, alongside an epidemiological examination of effects of age and sex. Furthermore, it provides crucial data to guide clinicians and policymakers in developing effective treatments and strategies against DEC infections. The findings underscore the necessity of sustained antimicrobial resistance surveillance in China, which is essential for early detection, tracking resistance trends, and evaluating interventions. This study advances the collective goal of improving protection against antimicrobial resistance and encourages interdisciplinary efforts to reduce the spread of resistant strains, especially in vulnerable groups like infants.

Limitations

The study had four key limitations. First, AMR data were not comprehensively collected across all Chinese provinces, potentially skewing the true prevalence of antimicrobial-resistant DEC. Second, the scope of the study was limited to a subset of diarrheal cases from selected hospitals, with variable diagnostic capabilities, which may not fully capture the national DEC resistance profile. Third, some isolates were not accurately typed owing to testing limitations, potentially affecting the pathotype-specific resistance data. Fourth, while standard methods were used, differences in commercial antimicrobial panels and concentrations along with the failure to obtain MICs of certain agents for some isolates could introduce inconsistencies. Furthermore, the study focused solely on the phenotypic resistance of *E. coli*, suggesting a need for further studies of genetic resistance factors.

Conclusion

Our 7-year study of the AMR profiles of DEC isolates from patients with active foodborne disease in China unveiled a high rate of resistance, with a significant majority of isolates exhibiting MDR. We identified variation in resistance profiles among different *E. coli* pathotypes and a discernible age-specific pattern in AMR, with a particularly concerning upward trend in resistance to chloramphenicol and cefoxitin. The resistance profiles of human DEC isolates in China highlighted the need to generate updated and comprehensive epidemiological data with a “One Health” approach to provide more information and minimize the impacts of foodborne infections caused by resistant DEC.

Materials and methods

Isolation of diarrheagenic *E. coli* strains

In accordance with the protocols of the National Laboratory Manual for the Foodborne Disease Surveillance System, fecal samples or rectal swabs from patients with diarrhea who sought care at designated sentinel hospitals were screened for *E. coli* using traditional culture methods across 20 provinces in China from 2016 to 2022. A refined multiplex PCR approach, described by Müller et al. [19], was then employed to classify the isolates based on the presence of 12 specific genes: *uidA*, *escV*, *bfpB*, *stx1*, *stx2*, *elt*, *estIa*, *estIb*, *invE*, *astA*, *aggR*, and *pic*. The *E. coli*-specific *uidA* gene was expected to be detected in all DEC strains. The pathotyping criteria were as follows. EAEC was identified by at least one positive gene among *aggR*, *astA*, and *pic*. EPEC was characterized by the presence of *bfpB* (either positive or negative), positive *escV*, and the absence of both *stx1* and *stx2*. ETEC was recognized by positivity for at least one of *elt*, *estIa*, or *estIb*. EHEC was defined by the presence of either a positive or negative *escV*, negative *bfpB*, and positive *stx1* and/or *stx2*. EIEC was confirmed by a positive *invE* gene. This study spanned a diverse range of provinces, including Anhui, Beijing, Gansu, Guangxi, Guizhou, Hebei, Henan, Heilongjiang, Hubei, Hunan, Jilin, Jiangsu, Inner Mongolia, Ningxia, Shandong, Shanxi, Shanghai, Tianjin, Xinjiang, and Zhejiang.

To guarantee the integrity and uniformity of the surveillance data, each isolate was rigorously identified and tested for AMR at the respective provincial CDCs. The experimental outcomes along with the epidemiological data for the isolates were reported in detail through the National Molecular Tracing Network for Foodborne Disease Surveillance, known as Tranet, which is a standardized and reliable dataset for national monitoring and analysis.

Antimicrobial susceptibility testing

A commercial antimicrobial susceptibility testing panel (Fosun Diagnostics, Shanghai, China) based on the broth microdilution technique was used, consisting of 14 antibiotics spanning 10 classes (i.e., ampicillin, ampicillin/sulbactam, cefazolin, cefotaxime, ceftazidime, cefoxitin, tetracycline, chloramphenicol, nalidixic acid, ciprofloxacin, gentamicin, trimethoprim-sulfamethoxazole, imipenem, and colistin E). The isolates were categorized as susceptible, intermediate, or resistant based on the MIC values determined against the Clinical and Laboratory Standards Institute M100-S32 clinical breakpoints. *E. coli* ATCC 25922 was used as a standard strain to ensure reliability and reproducibility. All of the antibiotics tested were recognized as critically or highly important according to the WHO Medically Important Antimicrobials list [20] (Table 3).

Data analysis

Isolates that were resistant to at least one of the 14 agents were considered AMR strains, and MDR strains were identified as those showing resistance to three or more classes of agents. The data are presented in the form of counts or percentages (%). Descriptive statistical methods were employed to assess the prevalence of resistance across various antimicrobial agents and to calculate the AMR and MDR rates. To compare resistance levels, Pearson’s chi-square tests and, where appropriate, chi-square tests with continuity correction were utilized (with a significance threshold of $P \leq 0.05$). Additionally, a linear regression model was applied to analyze trends in resistance over time using R software, version 4.1.2.

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Authors' contributions
H.H. took the lead in analyzing the data and drafting the manuscript. W.L. provided a thorough review of the data. J.L., along with X.Z., X.H., Y.S., J.C., R.F., J.Z., Y.C., H.Y., S.C., Y.L., D.T., and X.M., contributed significantly to conducting the experiments and submitting the data for analysis. P.F. and S.Y. were responsible for the meticulous statistical analyses. Y.G. was the driving force behind the study, providing the vision and overseeing its management. All authors have carefully reviewed and approved the final version of the manuscript.

Declarations

Ethics approval and consent to participate
The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of the China National Center for Food Safety Risk Assessment (protocol code 2017015; date of approval: 2017–11-14).

Competing interests
The authors have declared that no conflicts of interest exist.

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