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An epidemiological surveillance study (2021–2022): detection of a high diversity of *Clostridioides difficile* isolates in one tertiary hospital in Chongqing, Southwest China

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Abstract

Background *Clostridioides difficile* is a bacterium that causes antibiotic-associated infectious diarrhea and pseudomembranous enterocolitis. The impact of *C. difficile* infection (CDI) in China has gained significant attention in recent years. However, little epidemiological data are available from Chongqing, a city located in Southwest China. This study aimed to investigate the epidemiological pattern of CDI and explore the drug resistance of *C. difficile* isolates in Chongqing.

Methods A case-control study was conducted to investigate the clinical infection characteristics and susceptibility factors of *C. difficile*. The features of the *C. difficile* isolates were evaluated by testing for toxin genes and using multi-locus sequence typing (MLST). The susceptibility of strains to nine antibiotics was determined using agar dilution technique.

Results Out of 2084 diarrhea patients, 90 were tested positive for the isolation of toxigenic *C. difficile* strains, resulting in a CDI prevalence rate of 4.32%. Tetracycline, cephalosporins, hepatobiliary disease, and gastrointestinal disorders were identified as independent risk factors for CDI incidence. The 90 strains were classified into 21 sequence types (ST), with ST3 being the most frequent (n = 25, 27.78%), followed by ST2 (n = 10, 11.11%) and ST37 (n = 9, 10%). Three different toxin types were identified: 69 (76.67%) were $A^+B^+CDT^-$, 12 (13.33%) were $A^-B^+CDT^-$, and 9 (10%) were $A^+B^+CDT^+$. Although substantial resistance to erythromycin (73.33%), moxifloxacin (62.22%), and clindamycin (82.22%), none of the isolates exhibited resistance to vancomycin, tigecycline, or metronidazole. Furthermore, different toxin types displayed varying anti-microbial characteristics.

Conclusions The strains identified in Chongqing, Southwest China, exhibited high genetic diversity. Enhance full awareness of high-risk patients with HA-CDI infection, particularly those with gastrointestinal and hepatocellular

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diseases, and emphasize caution in the use of tetracycline and capecitabine. These findings suggest that a potential epidemic of CDI may occur in the future, emphasizing the need for timely monitoring.

Keywords *C. difficile*, Molecular epidemiology, Risk factors, Antibiotics resistance

Background

Clostridioides difficile infection (CDI) is a highly prevalent diseases in healthcare settings and is the leading cause of antibiotic-related diarrhea worldwide [1]. The incidence, severity, and mortality rates of CDI have significantly increased since the emergence of hypervirulent strains of toxigenic *C. difficile* in the 21st century [2]. In the United States, CDI is responsible for an estimated 13, 000 deaths and incurs approximately $1 billion in medical expenses [3]. In Asia, CDI has been reported in 14.8% of individuals with diarrhea, with East Asia having the highest rate (19.5%) among Asian populations [4, 5]. Recently data from a meta-analysis of 14 regions of China revealed an incidence rate of 11.4% for CDI [6]. However, the epidemiology of CDI in Chongqing remains poorly understood due to limited epidemiological data.

The characteristics and genetic diversity of *C. difficile* exhibit regional variations. In Europe and North America, the most prevalent strain is ST1, whereas in Asia, ST37 is more commonly detected [7–9]. In China specifically, the predominant STs of *C. difficile* vary between northern (ST2 and ST81) and southern regions (ST54, ST3, and ST37) [6]. These regional differences highlight the importance of studying the prevalence of *C. difficile* across different geographical areas.

Antibiotic exposure is the primary risk factor for the development of CDI [10]. Moreover, the widespread use of antibiotics leads to an upsurge in drug resistance, the emergence of multi-drug-resistant strains, and a decline in the cure rate of CDI [11]. Studies have shown that *C. difficile* in China displays distinct patterns of antibiotic resistance and genotype characteristics [11–15]. While the frequency of *C. difficile* infection has been investigated in certain regions of Southwest China, such as Kunming [12, 16, 17], limited data is available regarding the epidemiological features and drug sensitivity of CDI in Chongqing. Therefore, this study aimed to investigate the prevalence, identify its risk factors, and evaluate the antibiotic sensitivity among patients in a tertiary hospital in Chongqing, China, from January 2021 to September 2022. The findings of this study have implications for prevention and management of CDI in Chongqing.

Methods

Study design and definitions

This case-control study was conducted from January 2021 to September 2022 at the First Affiliated Hospital of Chongqing Medical University, which is a tertiary teaching hospital located in Chongqing, Southwest China. The hospital has a total of 3200 beds and serves as the Chongqing Antibacterial Drug Resistance Monitoring Center. During the survey, diarrhea patients' unformed stool was collected for results of toxin isolation and culture. The detection of toxin A and toxin B antigens in collected feces was carried out using the enzyme linked fluorescence assay (ELFA) (Vidas mini, Bio Merieux, France). After examining medical histories, patients who were in the hospital for more than or equal to two days and who received antibiotics prior to diarrhea were included in this case-control research. Patients who were diagnosed with HA-CDI were enrolled in the case group, whereas patients who were diagnosed with non-CDI were enrolled in the control group, based on results from toxin detection and separation cultivation Results. Except for the aforementioned patients, all other patients are classified as Community-related diarrhea. Community-related diarrhea was excluded from the case-control study due to the lack of patient information. Prior approval was obtained from the institutional review boards of Chongqing Medical University, and the requirement for informed consent was waived.

The criteria used to identify cases of diarrhea was the occurrence of three or more unformed stools within a 24-hour period. Diarrhea cases were confirmed based on positive stool test for *C. difficile* toxins, detection of toxigenic *C. difficile*, or colonoscopy/histopathologic findings indicating pseudomembranous colitis [18]. Healthcare-associated *C. difficile* infection (HA-CDI) was defined as symptoms occurring more than 48 h after admission or within 12 weeks following discharge. Cases not meeting this definition were classified as community-associated CDI (CA-CDI), which also included outpatients [19]. Any second CDI episode within 14 days of a previous positive incident was considered as a duplicate case and was excluded from the study [20]. Recurrent CDI (rCDI) referred to CDI events that occurred eight weeks after a previous incident [21–23]. Severe diarrhea was determined by the presence of bloody diarrhea, hypovolemia, leukocytosis (white blood cells $>12\times10^9$ cells/L), hypoalbuminemia (albumin level <20 g/L), fever (above 38 °C), or pseudomembranous colitis [24].

The following data was collected during the study: Demographics, Prior hospitalization, Disease type, Use of antibiotics, Clinical symptoms, CDI history, White blood cell count level. CDI history to determine the percentage of recurring infections. The severity of CDI was assessed by the level of the white blood cell. This is important because many patients with diarrhea had kidney damage

prior to infection, making it difficult to accurately measure the severity of CDI by the creatinine level.

C. difficile isolation and toxin gene detection by PCR

The analysis of stool samples was conducted using the *C. difficile* toxin A/B test kit. The positive samples were subjected to the following procedures: *(1)* Alcohol pretreatment: in short, take 1 g of the fecal sample mixed evenly with 1mL75% of alcohol and stand still for 30 min; *(2) Culturing the pretreated samples on* cefoxitin cyclomerize fructose agar (CCFA, Oxoid, UK) for three days; (3) Incubating the plates at 37 °C under anaerobic jar containing 90% N_2 and 10% CO_2;4). As previously noted, the strain was determined by colony and Gram staining, together with polymerase chain reaction PCR) technique to find the housekeeping gene tpi of *C. difficile* [20]; 5) Extracting DNA from *C. difficile* strain using the TIAN amp Bacteria DNA Kit, and PCR was performed to detect specific *C. difficile* toxin genes, including *tcdA*, *tcdB*, binary toxin CDT (*cdtA* and *cdtB*), and toxin regulatory genes such as *cdu2*, *cdd3*, *tcdC*, *tcdD*, and *tcdE* [25–27].

Multi-locus sequence typing (MLST)

Multi-locus sequence typing (MLST) was carried out and evaluated as reported previously, and seven housekeeping genes (*adk*, *atpA*, *dxr*, *glyA*, *recA*, *sodA*, and *tpi*) were amplified and sequenced [28]. The result was uploaded to the *C. difficile* MLST database (https://pubmlst.org/organisms/clostridioides-difficile) and acquired the allele profile and ST. The MLST data is displayed by using the minimum spanning tree produced by BioNumerics version 7.6. In brief, the smallest generating tree represents the distribution and relationship of the MLST sequence type. The number of isolates of each related type is represented by the size of a circle. The illustration on the straight lines connecting the two circles shows different positions between them. The gray region covers the type that is less than or equal to two different spots. The colored region represents an evolutionary branch.

Antimicrobial susceptibility testing

C. difficile isolates were tested for susceptibility to multiple antibiotics using agar dilution technique following the guideline set by the Clinical and Laboratory Standards Institute (CLSI) [29]. The antibiotics tested included moxifloxacin, erythromycin, rifampicin, vancomycin, tetracycline, metronidazole, clindamycin, nitazoxanide, and tigecycline. To this end, a suspension equivalent to a 0.5 McFarland standard was prepared for each isolate using nutritional broth. This suspension was then swabbed onto brucella agar supplemented with heme and vitamin K1 and 5% sheep blood. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) [30] or CLSI [29] recommendations were used to evaluate the results of antibiotic susceptibility tests. Additionally, the breakpoints for rifampicin, and vancomycin were determined based on previous studies [31, 32]. Isolates that demonstrated resistance to at least three different antibiotics were classified as multi-drug resistant (MDR) [33].

Statistical analyses

SPSS version 27 was used for data entry and analysis. The quantitative variables will be presented with mean and standard deviation if the frequency of the observations has a normal distribution; otherwise, they will be presented with median and interquartile range, while categorical variables are expressed as frequency and percentage. For the analysis of CDI-related risk factors, binary logistic regression analysis was conducted first, and then multivariate logistic analysis was conducted for the factors with $p<0.01$. Odds ratios (*OR*), 95% confidence intervals (*CI*), and P-values were calculated to evaluate the variations across groups. The significance level was established at $p<0.05$.

Result

Characteristics and incidence rate of CDI in hospital

During the study period, 2084 patients with diarrhea were admitted. Among them, 85 patients had community-related diarrhea, and 239 patients with hospital-related diarrhea were identified as AAD, accounting for 11.96% (239/1999). Ninety of the overall diarrhea patients were diagnosed with CDI, with a prevalence rate of 4.32% (90/2084). Of those CDI patients, 83 were classified as HA-CDI cases (34.73%, 83/239) while seven were CA-CDI patients (8.24%, 7/85). As shown in Table 1, there was a slightly higher number of male patients (n=51, 56.67%) compared to female patients (n=39, 43.33%). The average age of CDI patients was 63±13 years. The majority of CDI cases occurred in patients over 65 years old (n=54, 60%), followed by those between 30 and 65 years old (n=30, 33.33%). CDI cases were observed across different hospital departments, mainly from the gastroenterology department (16.67%), department of hematology (12.22%), and rehabilitation medicine department (13.33%). Most CDI patients were admitted during the spring months (March-May) (42.22%, 38/83), followed by the winter (December-February) (23.33%, 21/83). Among the CDI patients, seven individuals (7.78%) have severe symptoms. Additionally, three patients experienced recurrent CDI, although no hospital outbreaks were reported. Vancomycin was the most commonly prescribed antibiotic (62.65%; 52 of 83), with a 100% cure rate. Metronidazole was used in 8.34% (8/83) of cases, and 87.5% of those patients were cured. One case did not react to metronidazole treatment, requiring

Table 1 The 90 CDI patients' basic information and clinical symptoms

variable	CDI (n = 90)	Percent (%)
Gender:		
Male	51	56.67
female	39	43.33
Age:		
Age (Median, IQS)	59(23)	
0–30	6	6.67
30–65	30	33.33
> 65	54	60
Season:		
Spring (March-May)	38	42.22
Summer (June–August)	18	20
Autumn (September-November)	13	14.44
Winter (December-February)	21	23.33
Ward of hospitalization:		
Gastroenterology department	15	16.67
Department of Hematology	11	12.22
Respiratory department	5	5.56
Neurology Department	4	4.44
Rehabilitation Medicine Department	12	13.33
ICU	10	11.11
Outpatient Department	7	7.78
Other departments	26	28.89
Type of infection:		
CA-CDI	7	7.78
HA-CDI	83	92.22
Severity	7	7.78
rCDI	3	3.6
Therapeutic drugs:		
Metronidazole	8	8.89
Vancomycin	52	57.78
Tegacyclin	3	3.33
Teicoplanin	3	3.33
others	17	18.89

IQS: interquartile spacing; HA-CDI: healthcare-associated *C. difficile* infection; CA-CDI: community-acquired *C. difficile* infection; ICU: intensive care unit; Severity: A leukocyte count of greater than 15×10^9/L is indicative of a serious illness; rCDI: recurrent *C. difficile* infection

a 14-day of vancomycin, which eventually led to a successful outcome.

Clinical characteristics and risk factors of CDI

In this study, 156 HA-AAD patients who were not infected were included in the case-control study as a control group, while a total of 83 patients with HA-CDI were included in the case group. However, 17 HA-AAD patients were left out of the case-control study due to incomplete case data. In actuality, 139 patients with HA-AAD were included in the control group. The clinical characteristics and risk factors associated with CDI were examined by conducting a cohort study involving 83 HA-CDI patients, employing a multivariate logistic regression model. As demonstrated in Table 2, gastrointestinal disease, hepatobiliary disease, cephalosporin, tetracyclines, and WBC count ($>9.5\times10^9$/L) are highly associated with the CDI occurrence ($p<0.01$). However, this study found that chronic kidney disease, quinolones, hypoproteinemia, radiotherapy, chemotherapy, and surgery, which have been reported as risk factors for CDI, were not closely related to the CDI ($p>0.05$).

Molecular epidemiology of *C. difficile*

Toxins produced by *C. difficile* are strongly related to the intestinal disease. Therefore, toxin and its regulatory gene were further investigated in this study. Table 3 shows that out of a total of 90 isolates, the detected toxin gene divided them into three groups: $A^+B^+CDT^-$ (n = 69, 76.67%), $A^-B^+CDT^-$ (n = 12, 13.33%), and $A^+B^+CDT^+$ (n = 9, 10%). Among 90isolated strains, 21 ST types were detected using MLST. The relationship between ST kinds was depicted in Fig. 1 by the minimum spanning tree. The most common ST type was ST3 (n = 25, 27.78%), followed by ST2 (n = 10, 11.11%), ST37 (n = 9, 10%), and ST42 (n = 8, 8.89%). These STs belong to three clade groups: clade 1 (n = 69, 76.67%), clade 3 (n = 9, 10%), and clade 4 (n = 13, 14.44%). No strains belonging to clade 2 had been found. Almost all $A^-B^+CDT^-$ strains, except for one (ST82), belonged to clade 4 (ST37, ST81). It is worth noting that three STs, namely ST5, ST221, and ST201, were found to belong to clade 3. In terms of source of *C. difficile* in the hospital, ST3 was mainly from the hematology department, and neurology department, while ST2 was more common in the hematology department (as Fig. 2 shows). Additionally, Fig. 3 shows that CDI patients infected with $A^-B^+CDT^-$*C. difficile* strain had higher levels of A/B toxin compared to those infected with the other two toxin strains ($A^+B^+CDT^-$, $A^+B^+CDT^+$).

Detection of antimicrobial susceptibility

As shown in Table 4, the study determined the minimum inhibitory concentration (MIC) of nine antimicrobial agents for 90 isolated strains. It was found that the majority of isolates exhibited resistance to erythromycin (73.33%), moxifloxacin (62.22%), and clindamycin (82.22%). However, none of the isolates exhibited resistance to vancomycin, tigecycline, or metronidazole. Only a small percentage of isolates were resistant to rifampin (8.89%). The drug sensitivity of nitazoxanide varied between 0.125 and 4 μg/ml. Among the 90 *C. difficile* isolates, 41 (45.56%) showed multiple drug resistance.

Different antimicrobial phenotypes were observed based on toxin types. Isolates with the $A^-B^+CDT^-$ phenotype demonstrated higher frequencies of resistance to rifampin (25%), erythromycin (83.33%), and clindamycin (91.67%), compared to those with the

Table 2 Susceptibility factors and distribution characteristics of patients with CDI

variable	HA-CDI (n = 83)	Control (n = 139)	Univariate analysis		Multivariate analysis	
			P-value	*OR*	*P*-value	*OR* (95%*CI*)
Demographic data:						
Age ≥ 65 years	49	66	0.361	1.446		
Male	49	83	0.824	1.092		
History of disease:						
Gastrointestinal disease	52	30	< 0.001***	4.356	< 0.001***	4.838 (2.475, 9.458)
Hepatobiliary disease	49	26	0.04*	3.359	0.009**	2.492 (1.258, 4.939)
Cardiovascular disease	30	33	0.160	1.791		
Kidney disease	20	24	0.263	0.556		
Autoimmune disease	16	22	0.142	2.274		
Diabetes mellitus	18	14	0.182	2.088		
Surgical History	23	22	0.193	1.885		
Chemotherapy	24	8	0.508	1.465		
Antibiotic use history:						
No. of antibiotics (≥ 3)	25	28	0.491	1.778		
β-Lactams	27	22	0.239	1.795		
Quinolone	16	28	0.346	0.623		
Cephalosporin	54	40	0.007**	3.392	0.009**	2.451 (1.251,4.803)
Penicillins	21	34	0.559	0.733		
Aminoglycosides	8	14	0.213	0.438		
Neoglycopeptides	28	27	0.450	1.514		
Tetracyclines	8	1	0.024*	15.214	0.006**	22.459 (2.447,206.121)
Meropenem	25	26	0.144	1.894		
Biological parameters						
WBC count > 9.5×10^9/L	24	12	0.005*	4.526	0.009*	0.010 (0.165, 0.808)
Hypoalbuminemia	31	35	0.132	1.861		

WBC: white blood cell; *OR*: odds ratio; *CI*: confidence interval, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. +: positive; -: negative

Table 3 Molecular typing and toxin types of 90 *C. difficile* isolates

Clade	MLST	PaLoc							CDT		No.of isolates
		tcdA	tcdB	tcdC	tcdD	tcdE	Cdu2	Cdd3	cdtA	cdtB	
1	ST2	+	+	+	+	+	+	+			10
	ST3	+	+	+	+	+	+	+			25
	ST8	+	+	+	+	+	+	+			2
	ST33	+	+	+	+	+	+	+			1
	ST35	+	+	+	+	+	+	+			3
	ST149	+	+	+	+	+	+	+			1
	ST42	+	+	+	+	+	+	+			8
	ST48	+	+	+	+	+	+	+			1
	ST54	+	+	+	+	+	+	+			6
	ST63	+	+	+	+	+	+	+			2
	ST82	+	+	+	+	+	+	+			2
	ST102	+	+	+	+	+	+	+			3
	ST129	+	+	+	+	+	+	+			2
	ST111	+	+	+	+	+	+	+			1
	ST278	+	+	+	+	+	+	+			1
	ST696	+	+	+	+	+	+	+			1
3	ST5	+	+	+	+	+	+	+	+	+	6
	ST221	+	+	+	+	+	+	+	+	+	1
	ST201	+	+	+	+	+	+	+	+	+	2
4	ST37		+	+	+	+	+	+			9
	ST81		+	+	+	+	+	+			3

MLST: multi-locus sequence typing; ST: sequence type; *PaloC*: Pathogenicity determining region of *C. difficile*; CDT: binary toxin; +: positive; -: negative

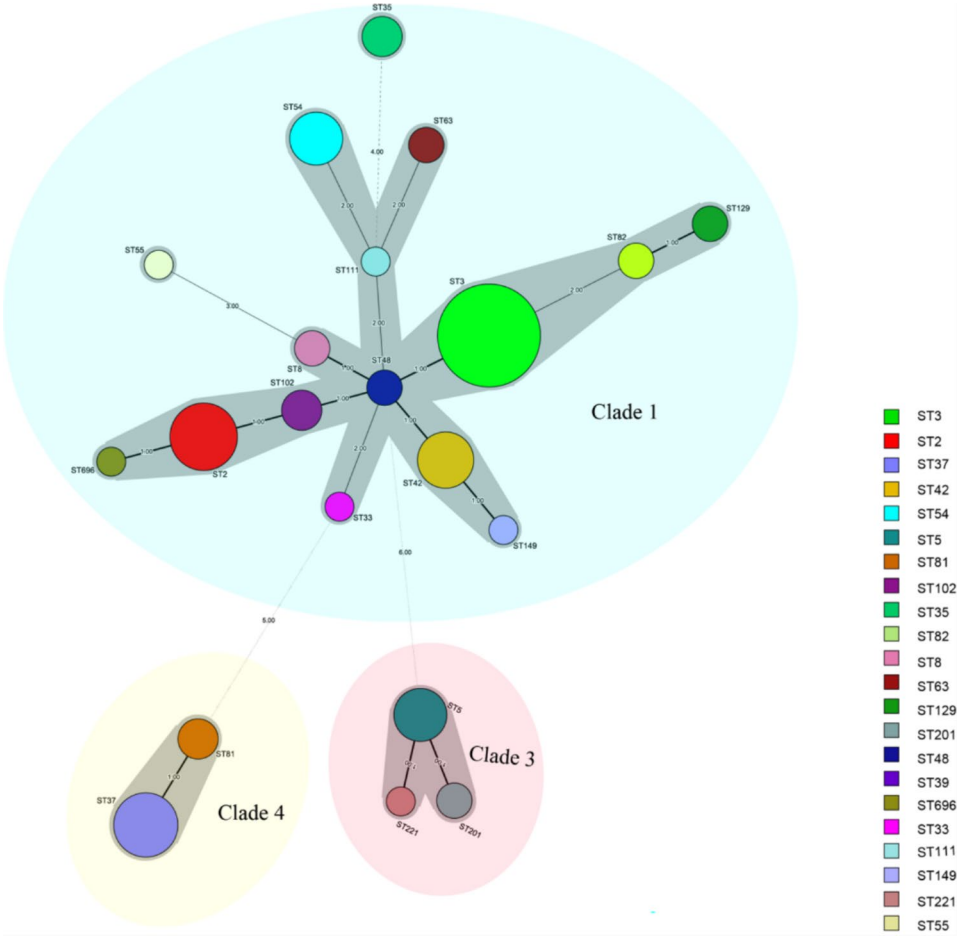

Fig. 1 Sequence types (STs) by MLST method are displayed in the minimal spanning tree. The circle's size represents each ST type's separations. The number of sites between two circles appears on the straight line. Gray zones encompass all types with less than or equal to two distinct states

$A^+B^+CDT^-$ and $A^+B^+CDT^+$ phenotypes. Notably, isolates with $A^+B^+CDT^+$ showed relatively high resistance rates to moxifloxacin (88.89%) compared to other drugs, although the resistance rates were still lower overall.

Discussion

C. difficile is the leading cause of healthcare-associated infectious diarrhea in Europe and North America [1, 34]. But in China, particularly in the southern metropolis of Chongqing, epidemiological data about *C. difficile* are rather few [6]. The objective of the study was to evaluate the molecular epidemiology, antibiotic susceptibility, and demographic characteristics of CDI patients in Chongqing, China, between 2021 and 2022. In patients with diarrhea, the overall CDI rate in Chongqing was discovered to be 4.37% (90/2084), which was lower than that in eastern and central China [4, 29]. The study's AAD prevalence was 11.95% (239/1999), and it was shown that HA-CDI accounted for 34.72% of AAD, consistent with other reports, demonstrating the high incidence rate of CDI in AAD patients [35].

Previous investigations have identified advanced age (≥65 years old), antibiotic abuse, exposure to health care environment, and various complications or diseases are the risk factors for CDI [36, 37]. Our findings, consistent with previous reports, demonstrated that advanced age (mean age: 63 years old) and antibiotics abuse were among the high-risk factors for CDI. In our study, cephalosporins ($p=0.009$, $OR=2.451$) and tetracycline ($p=0.006$, $OR=22.459$) emerged as independent significant risks for CDI. Additionally, gastrointestinal disease ($p<0.001$, $OR=0.240$) and hepatobiliary ($p=0.009$, $OR=2.492$) disease were identified as independent risk factors for CDI, highlighting the importance of intestinal barrier damage and flora disorder in CDI development [38]. Contrary to what has been shown in prior studies, our study did not find chronic renal disease to be a specific risk factor for HA-CDI. And it might be attributable to variations in patient populations and the CDI epidemic's dynamic character [39]. Furthermore, our findings indicated that CDI could spread outside of hospitals and among younger patients, similar to some studies [40–42].

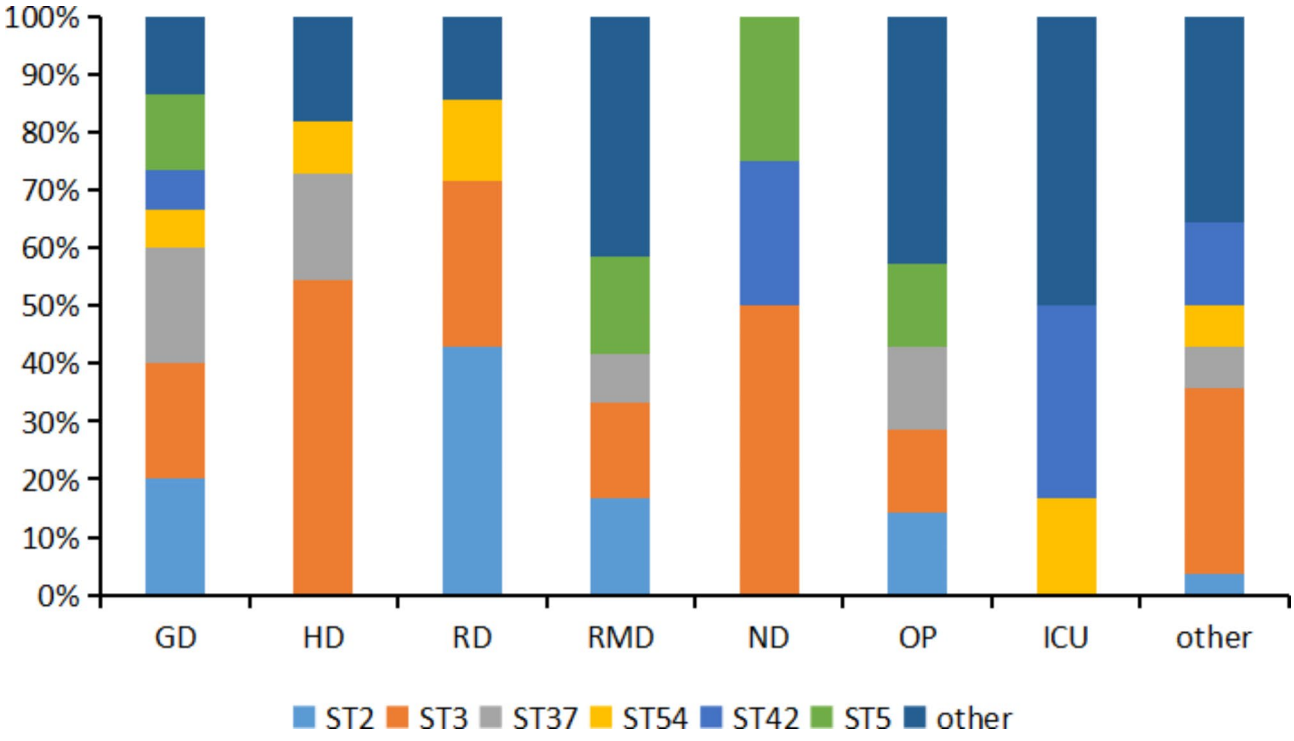

Fig. 2 Genotype distribution by hospital departments. GD: Gastroenterology department; OP: Outpatient; HD: Hematology department; ND: Neurology department; RMD: Rehabilitation medicine department; RD: Respiratory department; ICU: Intensive care unit

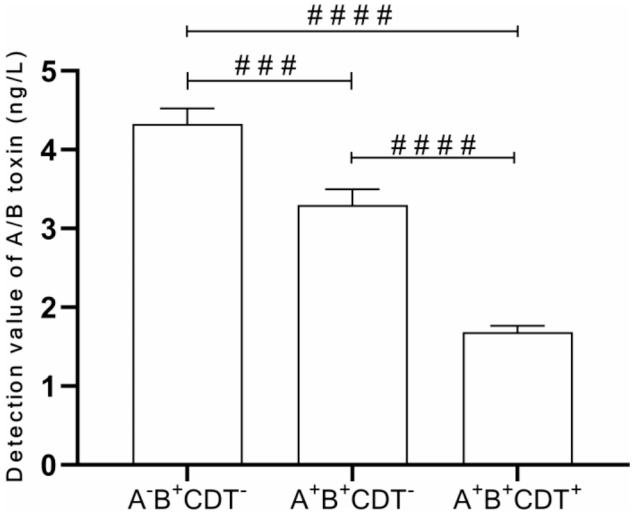

Fig. 3 Detection value of *C. difficile* A/B toxin in stool samples from patients with three types of toxin strains. **# # #**: $p < 0.001$, **# # # #**: $p < 0.0001$

Our research hint that the risk variables for CDI change with demographics, location, and sampling period. Additionally, there is a tendency for infected populations to spread to youth and local communities. These emphasize the importance and urgency of ongoing epidemiological monitoring of CDI.

As a result of our study, we discovered a large variety of *C. difficile* isolates in Chongqing, South-west China. Based on MLST analysis, 90 separated strains were divided into 21 ST types and assigned to three evolutionary branches. The primary ST type is ST3, followed by ST2, ST37, and ST42.They differ slightly from the distribution and other parts of China, such as Beijing and Shanghai [43, 44]. This implies a regional variation in the primary types of *C. difficile* strains. However, the prevalence of certain ST types differs significantly from that observed in 2014–2016, indicating a dynamic microevolution and heterogeneity within clade 1 [39, 45], which highlights the potential evolution of *C. difficile* in Chongqing. It is worth noting that nine CDT-positive *C. difficile* belonged to clade 3, including the common ST5 seen in China and two uncommon varieties (ST201, ST221). The number and variety of CDT-positive strains have increased in 2021–2022 compared to previous studies on *C. difficile* in Chongqing from 2014 to 2016 [34]. CDT-positive strains are known to cause more severe infections, and higher mortality rates. As a result, careful observation and assessment of this strain are required [46].

Antibiotics play a crucial role in the initiation and treatment of CDI [47]. The observed trend of antimicrobial resistance in this study was consistent with previous reports [17, 37, 47], where all strains remained susceptible to first-line drugs such as metronidazole, vancomycin, and tigecycline, as indicated in Table 4. Likewise, the cure rates for metronidazole, vancomycin, and tigecycline were found to be 87.5%, 100%, and 100%, respectively,

Table 4 Drug resistance characteristics of 90 *C. difficile* Isolates

Antibiotics	Break-point (μg/mL)	All samples (n = 90)				$A^+B^+CDT^-$ (n = 69)				$A^-B^+CDT^+$ (n = 12)				$A^+B^+CDT^+$ (n = 9)			
		MIC50	MIC90	Range	Resistant rate (%)	MIC50	MIC90	Range	Resistant rate (%)	MIC50	MIC90	Range	Resistant rate (%)	MIC50	MIC90	Rang	Resistant rate (%)
Metronidazole[a]	>=32	0.25	0.5	0.125-4	0	0.125	1	0.125-4	0	0.25	0.25	0.125-1	0	0.5	0.5	0.125-2	0
Vancomycin[a]	> 2	0.031	0.125	0.031-1	0	0.063	0.125	0.031-1	0	0.063	0.125	0.031–0.125	0	0.031	0.063	0.031-1	0
Erythromycin[c]	>=8	8	32	0.25–512	73.33	4	16	0.25–256	78.26	8	32	0.25–512	83.33	4	16	1–64	33.33
Tetracycline[a]	>=16	0.5	2	0.125-8	0	0.5	4	0.125-8	10.2	0.5	0.5	0.125-4	0	2	4	0.25-4	0
Tigecycline[a]	> 0.25	0.031	0.063	0.016–0.125	0	0.031	0.063	0.031–0.125	0	0.031	0.031	0.031–0.063	0	0.016	0.031	0.016–0.063	0
Nitazoxanide[d]	——	0.5	2	0.125-4	——	0.5	1	0.125-4	——	0.5	2	0.125-4	——	1	2	0.125-4	——
Clindamycin[c]	>=8	16	64	1-512	82.22	16	16	1-128	84.05	8	8	2-512	91.67	4	8	1-128	55.56
Moxifloxacin[c]	>=8	8	16	0.25–128	62.22	8	16	0.25–128	60.87	4	8	0.5–32	50	4	8	2–32	88.89
Rifampicin[c]	>=8	0.031	0.031	0.016-64	8.89	0.063	0.063	0.031-16	5.8	0.125	16	0.031-64	25	0.016	0.063	0.016-16	11.11

[a] MIC breakpoint is advised by the EUCAST or CLSI; [b] Breakpoints were proposed by a previous study; [c] No drug sensitivity breakpoint, report with specifc values; MIC: minimum inhibitory concentration

demonstrating their efficacy for treating CDI in Chongqing. However, some studies have suggested that certain *C. difficile* strains exhibit reduced susceptibility to these drugs, highlighting the need for continuous monitoring [12, 48]. Moreover, a high rate of drug resistance of the isolates against moxifloxacin, erythromycin, and clindamycin was observed, consistent with previous reports [15]. Subsequently, the MICs of nitazoxanide against *C. difficile* were investigated to assess its suitability as an alternative therapy for CDI [49]. As expected, the majority of *C. difficile* strains exhibited low MICs for nitazoxanide, suggesting its potential as a new therapeutic option for CDI. Additionally, the strain's toxin type was found to be linked to drug resistance, with varying antibacterial characteristics among different toxin types. $A^-B^+CDT^-$isolates demonstrated higher resistance to rifampicin (25%), erythromycin (83.33%), and clindamycin (91.67%) compared to $A^-B^+CDT^-$ and $A^-B^+CDT^+$ isolates. Although $A^+B^+CDT^+$ strains had the highest resistance to moxifloxacin (88.89%), resistance was lower to other drugs. To treat and manage CDI, it may be helpful to comprehend antibiotic resistance in relation to toxin type. Furthermore, differences in drug resistance have been identified among various ST strains, highlighting the significance of genetic epidemiology research and ongoing monitoring for CDI.

Our research was limited to a single hospital in Chongqing, China, which might not accurately reflect the prevalence and variety of CDI in the general population. A multi-center investigation would enable a more thorough comprehension of CDI in Chongqing. Additionally, our study was unable to fully explore CA-CDI due to a paucity of participants and clinical data. Exploring the prevalence pattern and risk factors related to CA-CDI requires more study.

Conclusions

In conclusion, this study presented a comprehensive survey of CDI in Chongqing, Southwest China. The overall incidence of CDI in diarrhea patients was 4.32%. Inpatients undergoing tetracycline and cephalosporin therapy and inpatients suffering from gastrointestinal disorders and hepatobiliary disease are thus at high risk for HA-CDI. Increased number and genetic diversity of *C. difficile* strains indicate the possibility of a future outbreak of hardships, and the importance of CDI continuity testing and epidemiological studies of strain molecules in Chongqing.

Abbreviations

C. difficile	*Clostridioides difficile*
ST	Sequence types
CDI	*Clostridioides difficile* infection
HA-AAD	Healthcare-associated antibiotic-associated diarrhea
HA-CDI	Healthcare-associated *Clostridioides difficile* infection
CA-CDI	Community-associate *Clostridioides difficile* infection

rCDI	Recurrent *Clostridioides difficile* infection
WBC	White blood cell
CCFA	Cefoxitin cycloserine fructose agar
PCR	Polymerase chain reaction
MLST	Multi-locus sequence typing
CLSI	Clinical and Laboratory Standards Institute
EUCAST	European Committee on Antimicrobial Susceptibility Testing
MIC	Minimum inhibitory concentration
MDR	Multi-drug resistant
OR	*O*dds ratios
CI	*C*onfidence intervals
IQR	Interquartile range
ICU	Intensive care unit

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Not applicable.

Authors' contributions
ZT designed the study. YHC completed drug sensitivity testing and was a major contributor in writing the manuscript. CMZ, and Q Y J complete clinical data and sample collection and toxin testing. YZ and XP Hua completed statistical analysis. Y W, K H, and R K R I review and edit the manuscript; Y T, and X G carried out the routine sample tests including anaerobic culture and toxin genetic detection. WW J and SIY completed molecular typing. All authors reviewed, revised, and approved the final manuscript.

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Data Availability
The published paper and its supplemental information files contain the datasets created and analyzed during the current investigation. Due to the need for more research, many datasets are not currently accessible to the public but are available upon reasonable request from corresponding author.

Declarations

Competing interests
The authors have no conflicts of interest to declare.

Ethics approval and consent to participate
All methods of our research were carried out in accordance with relevant guidelines and regulations, and were in accordance to guidelines of Declaration of Helsinki. This was a laboratory-based, single-hospital retrospective study of *C. difficile* permitted by the ethics committee of Chongqing Medical University [Refference Number: 2021105]; because of the retrospective nature of the study, the requirement for informed consent was waived by the ethics committee of Chongqing Medical University.

Consent for publication
Not applicable.

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