Risk factors for Clostridioides difficile infection and colonization among patients admitted to intensive care units in Shanghai, China

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Abstract

Background : Clostridioides difficile is considered to be the main pathogen responsible for hospital-acquired infections. This study performed a prospective study to describe the prevalence, molecular epidemiological characteristics and risk factors of Clostridioides difficile infection (CDI) and Clostridioides difficile colonization (CDC) among patients in intensive care units (ICUs), a tertiary hospital in China, with the aim of providing strategies for efficient CD prevention and control. Methods: Stool samples were collected and anaerobically cultured for C. difficile . The identified isolates were tested for toxin genes and multi-locus sequence typing. The medical records of patients who were divided into CDI, CDC and control groups were collected and analyzed to investigate the risk factors. Results: Of the 800 patients included in the study, 33 (4.12%) and 25 (3.12%) patients were identified with CDI and CDC, respectively. An association was found between CDI patients and having a fever (OR=13.993) or metabolic disorder (OR=7.972), and treatment with fluoroquinolone (OR=42.696) or a combination of antibiotics (OR=2.856). CDC patients were characterized by longer hospital stays (OR=1.137), an increased number of comorbidities (OR=36.509), respiratory diseases (OR=0.043) and treatment with vancomycin (OR=18.168). Significantly, treatment with metronidazole was simultaneously found to be a protective factor in the two groups (OR=0.042; OR=0.013). Eighteen sequence types (STs) were identified. Among the CDI group, the isolates were predominantly toxin A and toxin B positive (A+B+) strains and genotype ST2 was the epidemic clone. In the CDC group, the dominant strains were A+B+ and ST81 was the epidemic clone. Conclusions: The prevalence of CDC and CDI in our ICUs was relatively high, suggesting the importance of routine screening to detect the acquisition of this pathogen. Future prevention and treatment strategies for C. difficile -related disease should consider hospital stays, enteral nutrition, underlying comorbidities, and the use of combined antibiotics. Moreover, metronidazole could be a protective factor for both CDI and CDC, which could be used empirically .

Background

Clostridioides difficile is a Gram-positive spore-forming anaerobic bacterium, which has been listed as the leading cause of hospital-acquired diarrhea in many developed countries[1]. This pathogen secretes two main toxins, toxin A and toxin B, which mediate *C. difficile*-associated colitis and diarrhea [2]. The incidence of *C. difficile* infection (CDI) is steadily rising worldwide and the mortality rate has concordantly increased [3, 4]. One report stated that the number of patients in hospital with CDI more than doubled in the last decade in the USA [5]. In some Asian countries, the similar situation occurred [6, 7], leading to prolonged stays and higher costs in ICUs and bringing significant economic burdens.

Clostridioides difficile can colonize individuals without causing any detectable symptoms of infection. Such asymptomatic *C. difficile*-colonized patients may present a potential risk to other susceptible individuals as infection reservoirs [8, 9]. It is thought that asymptomatic *C. difficile*-colonized patients may serve as potential vehicles for transmission of *C. difficile* in medical settings[10], where there is a significantly higher risk of CDI[11]. The global spread of emerging hyper-virulent toxigenic strains is of particular concern [12].

As for the patients in intensive care units (ICUs), mainly were receiving antimicrobial therapy and had comorbidities [13]. CDI patients in ICUs were reported to have prolonged hospital stays [14, 15], higher hospital costs [16], as well as higher mortality rates [17]. The current prevalence of CDI among ICU patients was estimated to be 0.4%–4% [18]. In one study, about 10%–20% of ICU patients were colonized with *C. difficile* without any symptoms of infection [18]. Therefore, the presence of *C. difficile* may have a particular impact on the morbidity and mortality of patients in ICUs.

However, the incidence of toxigenic *C. difficile* infection or colonization among ICU patients in China remains largely uninvestigated. In addition, little is known about the epidemiology of strains in terms of typing, or about the in-depth risk factors. We therefore aimed to perform a prospective study to provide a better understanding of the prevalence, molecular epidemiological characteristics and risk factors of CDI and *C. difficile* colonization (CDC) among patients in the ICUs of a large-scale teaching hospital in China.

Methods

Study design, case definitions and data collection

We conducted a prospective study on adult patients admitted to our ICUs, an 18-bed department in Shanghai Ruijin Hospital, from January 2015 to June 2017. Patients were screened for the presence of *C. difficile* within 48 hours of admission [19], and were then tested every week or at the onset of symptoms of diarrhea. The surveillance continued until patients died or were discharged from hospital. This study was approved by the ethics committee of Ruijin Hospital in Shanghai, China.

According to European guidelines [20], the diagnosis of CDI was defined based on the symptom of diarrhea and laboratory findings of toxigenic *C. difficile*, while CDC was defined [21] as a patient positive for toxigenic *C. difficile* but without diarrhea. To reduce the influence of confounding factors, we chose *C. difficile*-negative patients with diarrhea as controls for CDI and those without diarrhea as controls for CDC. The control groups were randomly selected from ICU patients who had been admitted to the hospital during the same time period and who had no history of CDI/CDC in the previous eight weeks.

For all patients involved in this study, demographic data as well as clinical features were recorded, including the duration of hospital stays, mortality, surgery (in the previous six months), as well as the history of antibiotic use, gastric acid suppressants (The history of antibiotic use and gastric acid suppressants indicated the previous one month before the onset of diarrhea for CDI and its control, and the previous one month before developing CDC and the index hospital stays for its controls.)and enteral nutrition. Primary diagnosis diseases were divided into six major categories: gastrointestinal disease, respiratory disease, cardiovascular disease, renal disease, neurological disease and metabolic disorders including diabetes, hypertension or hyperlipidemia. As for the laboratory test indices, body temperature, leukocyte count, serum albumin levels and serum creatinine levels were measured. All laboratory indicators were recorded when patients were diagnosed with CDI/CDC. Meanwhile, related laboratory indicators were tested on admission for patients in two control groups.

Clostridioides difficile strain isolation and collection

Stool samples were collected from ICU patients at a set time period and were plated onto *C. difficile* agar base supplemented with norfloxacin and moxalactam (Oxoid Ltd., Basingstoke, UK) and cultured anaerobically at 37°C for 48–72 hours. Colonies were identified according to morphological features, a latex agglutination test (*C. difficile* Agglutination Test Kit; Oxoid Ltd.) and *gluD* gene detection. Feces and *C. difficile* isolates were also subjected to toxin A&B detection using an enzyme-linked fluorescence assay with a VIDAS automatic analyzer (Biomerieux, Marcyl'Etoile, France)[22-24].

Multilocus sequence typing (MLST)

MLST was performed for the genotyping of *C. difficile* strains. Briefly, DNA extraction was performed using a DNA extraction kit (Sangon Biotech, Shanghai, China). Seven housekeeping genes (*adk*, *atpA*, *dxr*, *glyA*, *recA*, *sodA* and *tpi*) were amplified from all strains and sequenced based on the method established by Griffths *et al*[25]. The obtained sequences were aligned with sequences in the MLST database (http://pubmlst.org/*clostridium difficile*).

Data analysis

Continuous variables were expressed as medians and standard deviations, and were compared using Student's t test. As for categorical data, variables were presented as frequencies or percentages, and were tested using the Chi-square test or Fisher's exact test. Univariate analysis was performed to evaluate the potential risk factors relevant to cases. Only those statistically significant variables from the univariate analysis were included in the multivariate logistic regression model. The results of logistic regression analysis were presented as odds ratios (ORs) with 95% confidence intervals (95% CIs).

All analyses were performed with the Statistical Program for Social Sciences version 22.0 for Windows (SPSS version 22.0), and a P value less than 0.05 was considered statistically significant.

Results

Patient population

As shown in Figure 1, of 800 adult patients admitted to ICU during the study period, 115 patients developed diarrhea and 33 (28.70%) were identified as having a CDI. Twenty-five toxigenic *C. difficile* strains were also isolated from non-diarrhea patients, which were defined as CDC cases. The overall prevalence of CDIs and CDCs was 4.12% and 3.12%, respectively, all of which were healthcare facility-associated. Only one patient showed recurrence of infection, one patient transitioned from colonization to infection and two patients had infections of two different types. To assess the potential risk factors and clinical features, 66 non-CDI and 50 non-CDC patients were included as control groups. CDI and CDC patients had a median age of 54.15 and 62 years old, the proportion of men was 66.7% and 68%, and the number of days after admission when patients tested positive was 17.06±12.97 and 31.16±33.85 days, respectively. Neither age nor sex showed any significant difference between groups.

Clinical features and risk factors for ICU patients with CDI

As illustrated in Table 1, univariate analysis was conducted to show the differences between the CDI group and the controls with diarrhea in terms of clinical characteristics, diagnosis and treatment. The CDI group were more likely to suffer from fever (OR=6.786, 95% CI 2.634-17.483) (P value <0.001) and metabolic disorders (OR=3.28, 95% CI 1.363-7.893) (P <0.05) compared with the non-CDI group. CDI patients also displayed a larger number of comorbidities (P <0.05). Compared with the control group, patients with CDI more frequently received enteral feeding (78.8% versus 50%) (OR=3.714, 95% CI 1.416-9.74), antiviral drugs (15.2% versus 1.52%) (OR=11.607, 95% CI 1.296-103.948) and fluoroquinolone (21.2% versus 3%) (OR=8.615, 95% CI 1.678-44.247) during their hospitalization (P <0.05). Additionally, a larger proportion of CDI patients were administered more than one type of antibiotic drugs(P <0.05). To further assess the potential risk of CDI, multivariable logistic regression analysis was performed. The results showed that having a fever or metabolic disorder, or treatment with fluoroquinolone or combined antibiotics, were risk factors associated with the development of CDI among ICU patients. However, treatment with metronidazole was found to be a protective factor (OR=0.042, 95% CI 0.006-0.288, P=0.001).

Table 1 Univariate and multivariate analysis of the demographic, clinical characteristics, and risk factors in CDI groups

Characteristics	CDI group (n=33)	non-CDI group (n=66)	Univariate Analysis		Multivariable logistic regression Analysis	
	n(%)/mean±SD	n(%)/mean±SD	OR (95% CI)	P value	OR (95% CI)	P value
Male	22（66.7）	39(60)	0.722 (0.301-1.732)	0.465		
Age(mean±SD)	54.15±20.89	58.97±14.87	-	0.242		
Clinical features						
Hospital duration (days) (mean±SD)	35.39±27.61	30.08±33.11	-	0.429		
Fever (≥ 38°C)	19（57.6）	11(16.7)	6.786 (2.634-17.483)	<0.001*	13.993 (3.292-59.472)	<0.001*
Leukocyte count (10^9 /L) (mean±SD)	9.79±5.35	10.61±6.24	-	0.992		
Serum albumin (g/L) (mean±SD)	30.30±6.02	29.97±7.01	-	0.816		
Serum creatinine rise>50% (μmol/L)	2（6.06）	12（18.18）	-	0.103		
Mortality	6(18.2)	11(16.7)	1.111 (0.371-3.325)	0.851		
Classification of primary diagnosis						
Gastrointestinal	29(87.9)	55(83.8)	1.45 (0.424-4.959)	0.552		
Respiratory	12(36.4)	26(39.4)	0.879 (0.37-2.086)	0.770		
Cardiovascular	6(18.2)	14(21.2)	0.825 (0.285-2.39)	0.723		
Renal	5(15.2)	17(25.8)	0.515 (0.171-1.546)	0.231		
Neurologic	8(24.2)	9(13.6)	2.027 (0.701-5.862)	0.187		
Metabolic disorders	22(66.7)	25(37.9)	3.28 (1.363-7.893)	0.007*	7.972 (1.767-35.971)	0.007*
NO. of comorbidities[a] 1-2	15(45.5)	43(65.2)	-	0.037*		

3-4	16(48.5)	21(31.8)				
≥5	2(6.1)	1(1.5)				
Treatments and procedures						
Surgical intervention	7(21.2)	15(22.7)	0.915 (0.332-2.523)	0.864		
Enteral feeding	26(78.8)	33(50)	3.714 (1.416-9.74)	0.006*		
PPI use	17(51.5)	43（65.2）	0.568 (0.243-1.330)	0.191		
Antibiotics use	31(93.9)	57（86.4）	2.447 (0.497-12.042)	0.258		
Antiviral drugs	5(15.2)	1（1.52）	11.607 (1.296-103.948)	0.007*		
Antifungal agents	6(18.2)	6（9.1）	2.222 (0.657-7.522)	0.191		
Cephalosporin	9(27.3)	26（29.4）	0.577 (0.232-1.435)	0.234		
Fluoroquinolone	7(21.2)	2（3.0）	8.615 (1.678-44.247)	0.003*	42.696 (3.895-468.058)	0.002*
Carbapenem	24(72.7)	35（53.0）	2.362 (0.955-5.843)	0.060		
Vancomycin	10(20.3)	13（19.7）	1.773 (0.68-4.624)	0.239		
Metronidazole	5(15.2)	22（33.3）	0.357 (0.121-1.052)	0.056	0.042 (0.006-0.288)	0.001*
NO. of antibiotics received[a]						
0	2(6.1)	9（13.6）	-	0.024*	2.856 (1.362-5.99)	0.005*
1-2	20(6.1)	48（72.7）				
≥3	11(33.3)	9（13.6）				

Numerical data are given as the mean ± SD, and categorical data are described as frequencies (percentages).

* P < 0.05; [a] The variables "No. of comorbidities" and “No. of antibiotics received” were made categorical, and the Cochran–Armitage trend test was used to analyze the differences in these variables between the two groups.

Clinical features and risk factors for ICU patients with CDC

For CDC patients, the median hospital stay was 62 days, significantly longer than that for non-CDC patients (P<0.05), which was further verified in the multivariable logistic regression model. The colonization of *C. difficile* did not cause a significant difference in the laboratory test indices including the laboratory leukocyte count, or serum albumin or creatinine levels. However, patients with respiratory or neurological disease were more likely to acquire *C. difficile* asymptomatically. The number of comorbidities was a potential risk factor for CDC patients (OR=36.509, 95% CI 2.602-512.183, P=0.08). As for the treatment procedure, surgical intervention, enteral feeding, antifungal agent usage, as well as carbapenem medication, were found more frequently in CDC patients (P<0.05). The multivariable model analysis showed that vancomycin was regarded as an independent risk factor (OR=18.168, 95% CI 1.036-318.503, P=0.047), whereas metronidazole use was a protective factor(OR=0.013, 95% CI 0-0.512, P=0.021) for *C. difficile* carriage (Table 2).

Table 2 Univariate and multivariate analysis of the demographic, clinical characteristics, and risk factors in CDC groups

Characteristics	CDI group (n=33)	non-CDI group (n=66)	Univariate Analysis		Multivariable logistic regression Analysis	
	n(%)/mean±SD	n(%)/mean±SD	OR (95% CI)	P value	OR (95% CI)	P value
Male	17(68.0)	31(62.0)	0.768 (0.278-2.121)	0.610		
Age(mean±SD)	62±18.93	59.06±10.54	-	0.660		
Clinical features						
Hospital duration (days) (mean±SD)	61.28±66.12	16.98±11.48	-	0.003*	1.137(1.05-1.23)	0.002*
Fever (≥ 38°C)	9(36.0)	7(14.0)				
Leukocyte count (10^9 /L) (mean±SD)	9.84±5.32	8.75±5.07	-	0.389		
Serum albumin (g/L) (mean±SD)	31.12±5.55	33.80±10.53	-	0.238		
Serum creatinine rise>50% (μmol/L)	2(8.0)	5(10)	-	0.779		
Mortality	3(12.0)	2(4.0)	3.273 (0.51-21.002)	0.190		
Classification of primary diagnosis						
Gastrointestinal	18(72.0)	30(60.0)	1.714 (0.606-4.852)	0.307		
Respiratory	15(60.0)	19(38.0)	2.447 (0.916-6.541)	0.071	0.043（0.002-0.969）	0.048*
Cardiovascular	7(28.0)	18(36.0)	0.691 (0.243-1.969)	0.488		
Renal	9(36.0)	15(30.0)	1.313 (0.475-3.626)	0.600		
Neurologic	6(24.0)	3(6.0)	4.947 (1.121-21.838)	0.024*		
Metabolic disorders	12(48.0)	26(52.0)	0.852 (0.326-2.227)	0.744		
NO. of comorbidities						
1-2	13(52.0)	33(66.0)	-	0.139	36.509 (2.602-512.183)	0.008*
3-4	9(36.0)	17(34.0)				
≥5	3(12.0)	0(0)				

Treatments and procedures						
Surgical intervention	10(40.0)	4(8.0)	7.667 (2.094-28.068)	0.001*		
Enteral feeding	16(64.0)	16□32.0□	3.778 (1.376-10.372)	0.008*		
PPI use	8(32.0)	25□50.0□	0.471 (0.172-1.288)	0.139		
Antibiotics use	24(96.0)	41□82.0□	5.268 (0.628-44.178)	0.093		
Antiviral drugs	2(8.0)	3□6.0□	1.362 (0.213-8.729)	0.743		
Antifungal agents	7(28.0)	4□8.0□	4.472 (1.166-17.146)	0.021*		
Cephalosporin	9(36.0)	24□48.0□	0.609 (0.227-1.636)	0.324		
Fluoroquinolone	5(20.0)	8□16.0	1.313 (0.381-4.525)	0.666		
Carbapenem	18(70.0)	21□42.0□	3.551 (1.258-10.027)	0.014*		
Vancomycin	12(48.0)	7□14.0□	5.67 (1.851-17.374)	0.001*	18.168□1.036-318.503□	0.047*
Metronidazole	2(8.0)	12(24.0)	0.275 (0.056-1.342)	0.094	0.013 □0-0.512□	0.021*
NO. of antibiotics received						
0	1(4.0)	9(18.0)	-	0.076		
1-2	16(64.0)	33(66.0)				
≥3	8(32.0)	8(16.0)				

Numerical data are given as the mean ± SD, and categorical data are described as frequencies (percentages).

* P < 0.05; [a] The variables "No. of comorbidities" and “No. of antibiotics received” were made categorical, and the Cochran–Armitage trend test was used to analyze the differences in these variables between the

two groups.

Molecular characteristics of *C. difficile*

The toxin type was detected among 58 *Clostridioides difficile* strains isolated from CDI and CDC patients, and 34 (58.6%) were A+B+ (positive for both *tcdA* and *tcdB*) and 24 (41.3%) were A−B+ (negative for *tcdA* and positive for *tcdB*). As for the two defined groups, 20 (60.6%) strains were A+B+ and 13 (39.4%) strains were A−B+ in the CDI group, and 14 (56%) strains were A+B+ and 11 (44%) strains were A−B+ in the CDC group.

Then, MLST was performed on these strains. In total, 18 sequence types (STs) were identified. In the CDI group, ST2, ST81, ST54 and ST3 were the major STs constituting 19%, 15%, 12% and 12% of strains, respectively. In the CDC group, ST81, ST35, ST37 and ST54 were the dominant types accounting for 20%, 12%, 12% and 12% of strains, respectively, as shown in Figure 2.

Based on the STs of strains, a map was constructed to compare the temporospatial relationship for the same STs from two groups during the study period, as shown in Figure 3. Two overlaps were detected within the CDI group in ST2 and one overlap was detected between the CDI and CDC groups in ST103. No overlaps were detected among other STs.

Discussion

Over recent decades, there has been a continuous increase in cases of CDI and CDC among hospitalized patients in many medical settings [1, 6, 7, 21]. Patients in ICUs often suffer from various comorbidities, greatly increasing the potential risk of developing CDI and leading to difficulties in treatment of underlying medical conditions [26]. A review reported that about 2% of ICU patients suffered from CDI, which was significantly higher than 0.9% among patients on general wards [27]. In our study, we found that the prevalence of CDI was 4.12%, much higher than most studies reported in European countries [27]. And 28.7% of the patients with diarrhea in ICU developed CDI which was much higher than the 8% reported in another Chinese study [28]. As for CDC, the detection rate of CDC in our study was 3.12%, relatively lower than the 7% reported in a retrospective study in Kuwait [29]. Above all, the prevalence of CDI and CDC varied geographically. The high acquisition of toxigenic *C.difficile* may result from the increased screening for the disease and highly sensitive detection methodology used, due to the enhanced awareness of the disease prevention. Moreover, it could be the true fact that the incidence of *C.difficile* changed distinctly, especially in ICU patients.

The main risk factors for CDI include antibiotic exposure, age > 60, longer hospital stays, severe dyspepsia, a history of gastric acid inhibitor use [30], enteral feeding and proton pump inhibitor (PPI) medication [31]. ICU admission is also a common pathogenic factor [32]. In the present study, we found that medication with multiple antibiotics significantly increased the risk of developing CDI. Specifically, the increased use of fluoroquinolones [33] contributed to the incidence of CDI, as previously suggested [32, 33]. Routine interventions especially relevant for patients in ICUs, such as surgery, enteral feeding and PPI medication, doubled the risk of CDI infection [16, 30, 34-36]. PPIs caused a change in the gastrointestinal flora, which may create a niche for CDC [37]. Presently, neither surgery history nor PPI medication were found different in CDI patients. Whereas CDI patients were more likely to receive enteral feeding in our univariable analysis, but not in the multivariable logistic regression analysis. Regarding underlying conditions, our study found a significant association between the occurrence of CDI and metabolic diseases. However, the mechanisms involved remain unclear and need further studies.

For patients with CDC in our study, large differences in the number of comorbidities and in the duration of hospital stays were detected which was consistent with a previous study [38]. However, CDC occurred rarely in patients with respiratory diseases and the reason for this remains to be clarified. Exposure to a variety of antibiotics is a risk factor for CDI, but not for CDC. The significant discrepancy between the results may indicate that the destruction of intestinal microbiota caused by antibiotic exposure is not a key feature of CDC.

For decades, metronidazole and vancomycin are main antimicrobial agents for the treatment of CDI [39]. In the treatment analysis of metronidazole and vancomycin [40-42], no significant difference was found[43, 44]. In our study, metronidazole usage was found to be a protective factor against CDI, which was consistent with a previous study [30] [45].However, oral vancomycin could be a risk factor for CDC, in accordance with the findings of Johnson et al [46]. These results suggested that the preventive use of metronidazole may contribute to the prevention of CDI and CDC, while it would be cautious for the medication of vancomycin in clinic.

ST2 and ST81 were the most common strain types in CDI and CDC group respectively. This finding differed from that in another study reporting ST54 as the most common genotype [47]. In addition, neither ST1 nor ST11, which were epidemic in western countries [48], were detected presently. Our map showed the temporospatial relationships between strains, indicating that *C. difficile* dispersed among normal colonized patients could be a potential source of infection, but there is still no definite evidence demonstrating the transmission from colonized subjects to others.

There are several limitations in our study. First, the samples were collected from a single center and may not be representative of all healthcare institutions. However, to our knowledge, our research is one of the limited studies to report the clinical features and molecular characteristics of *C. difficile* among patients in ICUs in China. To overcome this limitation, long-term multi-center studies should be carried out in the future. Second, ICU wards were always rooms isolated and strictly disinfected, so the environmental samples were not obtained and we could not fully assess *C. difficile* transmission. To identify the risk

factors for developing CDI and CDC, most previous studies compared positive cases with *C. difficile*-negative cases [49-51]. However, most negative cases had no diarrhea, so the risk factors identified for CDI in these cases are unlikely to be specific. To overcome this shortcoming, we used two sets of patients with or without diarrhea as negative controls.

Conclusions

Our study provided prospectively independent comparisons in ICUs and characterized the molecular epidemiology. The overall prevalence of CDI and CDC was 4.12% and 3.12%, respectively. Fever, metabolic disorders, the use of fluoroquinolone and multiple antibiotics exposure were significantly associated with CDI. Longer hospital stays, comorbidities and the use of vancomycin were found to be associated with acquiring CDC. As for metronidazole, protective effects were detected for both groups. The most common epidemic strains were ST2 and ST81 in the two groups, respectively. Therefore, these results highlight the importance of antimicrobial stewardship and isolation of the pathogen in the prevention and treatment of *C. difficile*-related diseases. The role of asymptomatic carriers in the transmission of *C. difficile* needs further investigation. In conclusion, it is essential for medical staff to emphasize the importance of *C. difficile*-related diseases, especially for ICU patients.

List Of Abbreviations

ICUs: Intensive care units

CDI: *Clostridioides difficile* infection

CDC: *Clostridioides difficile* colonization

MLST: Multilocus sequence typing

ST: Sequence type

Declarations

Ethics approval and consent to participate

The Ruijin Hospital Ethics Committee approved the study protocol and obtained informed consent verbally because this study only involved patients stool samples and all data collected were anonymized. All participants provided verbal consent prior to participation.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

CYC, ZLH, DDF and PYB contributed to the study design. CYC, ZLH, MEQ, JC, NQ, WC, WDS contributed to the collection of clinical samples, related experiments and case records. CYC, ZLH and DDF contributed to the data analysis. CYC, ZLH, DDF and PYB drafted the manuscript. All authors read and approved the final manuscript.

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Figures

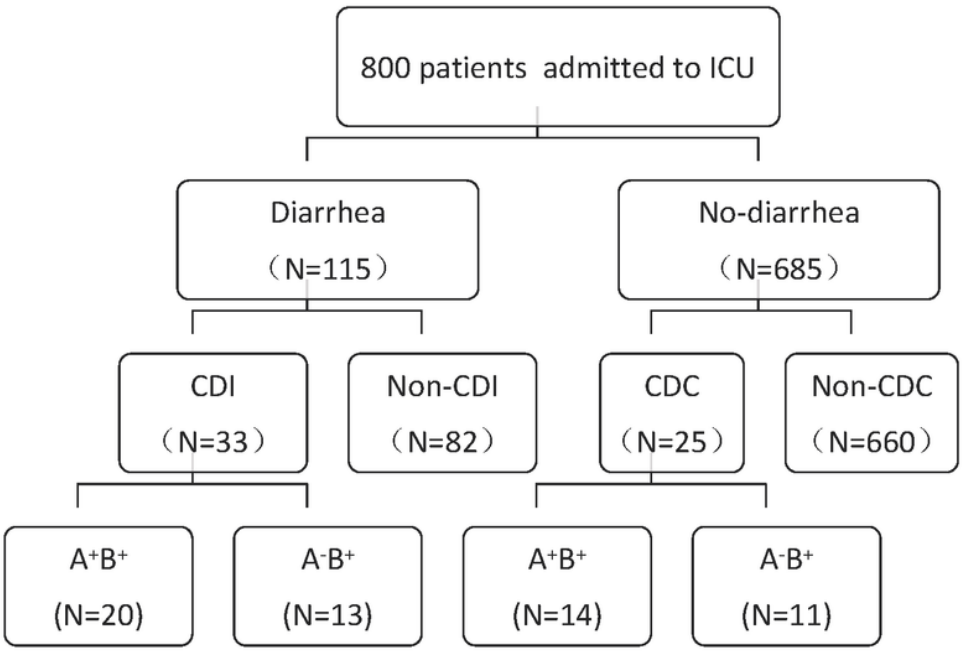

Figure 1

Study flowchart of CDI and CDC among ICU patients

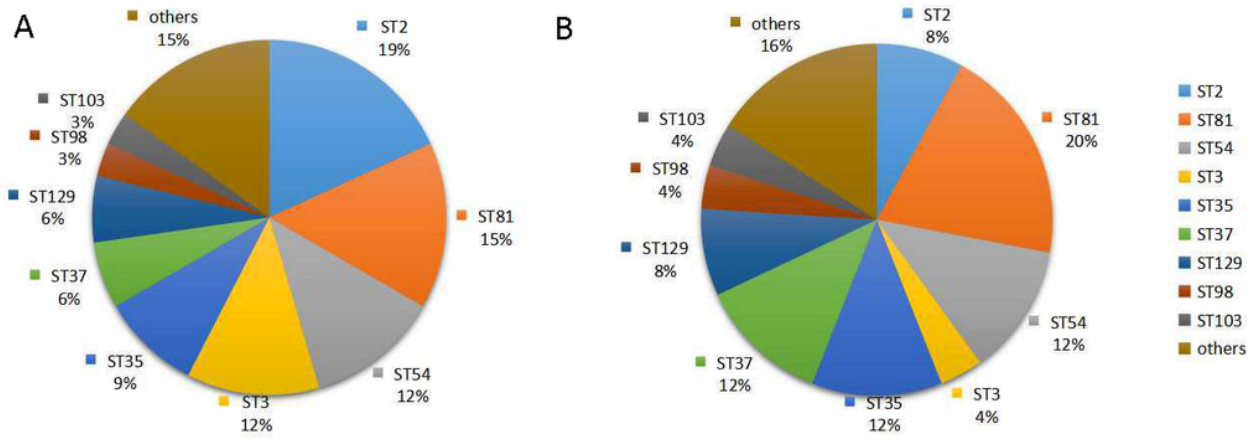

Figure 2

Proportion of the sequence types of CD strains isolated from patients in ICUs: A Proportion of the sequence types in CDI group. B Proportion of the sequence types in CDC group.

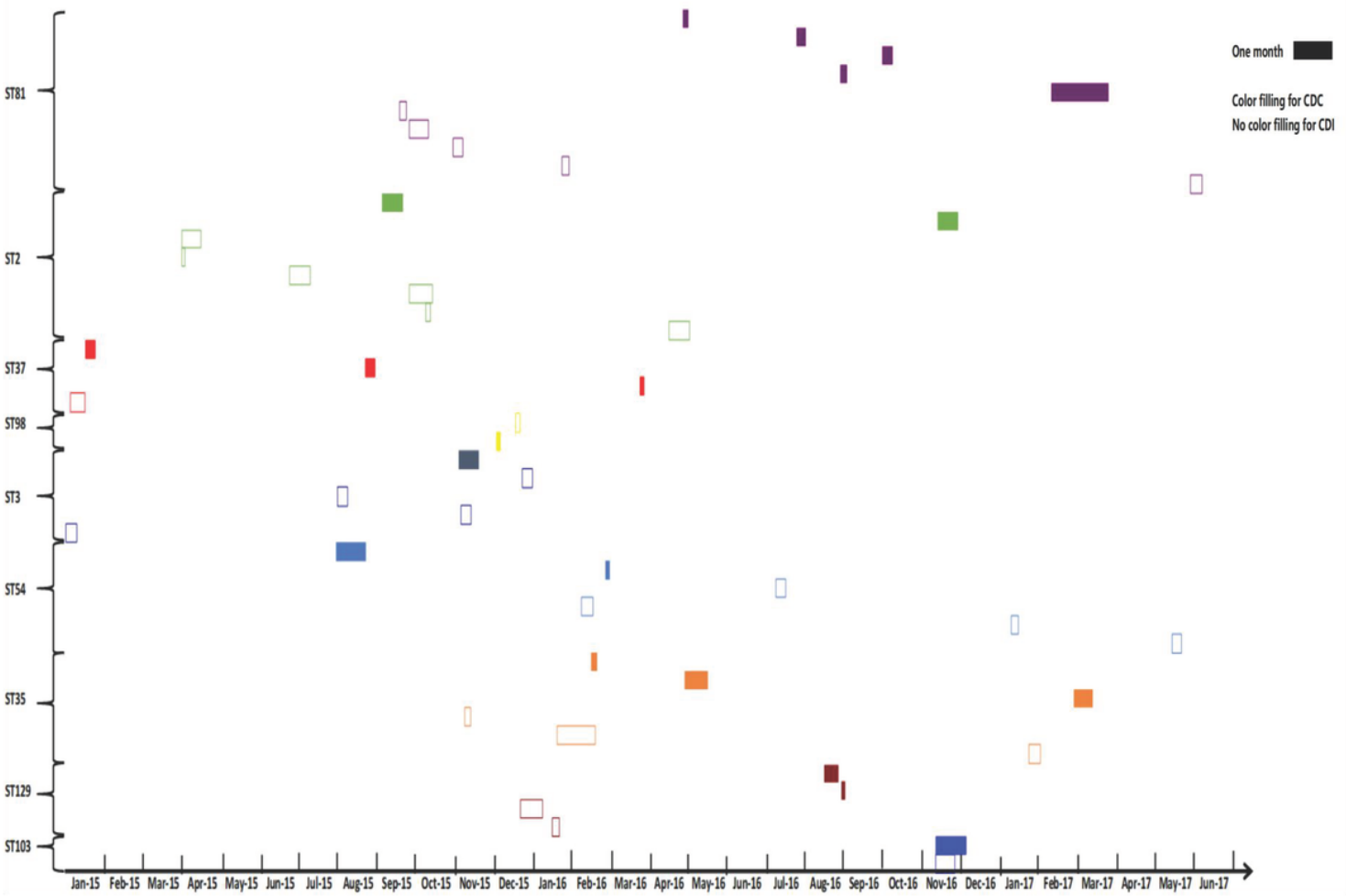

Figure 3

Time-space cluster map of different STs from CDI and CDC patients in ICUs Y-axis shows multilocus STs. X-axis shows the duration of the study period. Each small box represents the duration from the date of detection of C. difficile in the stool of a hospitalized ICU patient to the date at which the organism was no longer detectable.