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

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Abstract

Background : Clostridium difficile is considered to be the main pathogen responsible for hospital-acquired infections in western countries, but few studies on C. difficile have been carried out in China. This study performed a prospective study to describe the prevalence, molecular epidemiological characteristics and risk factors of Clostridium difficile infection (CDI) and Clostridium difficile colonization (CDC) among patients in intensive care units (ICUs), with the aim of providing strategies for efficient CD prevention and control.

Methods: Stool samples were collected from adult patients on admission to an 18-bed ICU department, and were anaerobically cultured for C. difficile . The identified isolates were tested for toxin genes, followed by multilocus sequence typing to analyze the genotypes. Patients were divided into CDI, CDC and control groups according to clinical features. The medical records of these groups were collected and further analyzed using logistic regression 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). However, 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 ST-2 was the epidemic clone. In the CDC group, the dominant strains were A+B+ and ST-81 was the epidemic clone.

Conclusions: The prevalence of C. difficile colonization and infection in our ICU patients 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 take into consideration the duration of hospital stays, enteral nutrition, underlying comorbidities, as well as the use of combined antibiotics. Moreover, metronidazole could be a protective factor for both CDI and CDC.

Background

Clostridium 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][(1)]. 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]. A similar situation occurred in some Asian countries [6, 7], bringing economic challenges.

Clostridium difficile is a Gram-positive spore-forming anaerobic bacterium, which 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 hypervirulent 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 wasestimated 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 ofCDI 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 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. 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.

Clostridium 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 37C 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 thegenotyping 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.

Figure 1 Study flowchart of CDI and CDC among ICU patients

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) (P value <0.001) and metabolic disorders (OR = 3.28) (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), antiviral drugs (15.2% versus 1.52%) (OR = 11.607) and fluoroquinolone (21.2% versus 3%) (OR = 8.615) during their hospitalization (P <0.05). Additionally, a larger proportion of CDI patients were administered more than one type of antibiotic (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, P = 0.001).

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

Clinical features and risk factors for ICU patients with CDC

For CDC patients, the median hospital stay was 62, 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, 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 vacomycin was regarded as an independent risk factor (OR = 18.168, P = 0.047), whereas metronidazole was as a protection factor (OR = 0.013, 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

Molecular characteristics of *C. difficile*

The toxin type was detected among the 58 positive isolated strains 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 the positive strains. In total, 18 serotypes (STs) were identified. In the CDI group, ST–2, ST–81, ST–54 and ST–3 were the major STs constituting 19%, 15%, 12% and 12% of strains, respectively. In the CDC group, ST–81, ST–35, ST–37 and ST–54 were the dominant types accounting for 20%, 12%, 12% and 12% of strains, respectively, as shown in Figure 2.

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.

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.

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.

Discussion

Over recent decades, there has been a continuous increase in cases of CDI and CDC among hospitalized patients in almost all medical settings. Patients in ICUs often suffer from various comorbidities and many are immunocompromised, greatly increasing the potential risk of developing a CDI and leading to difficulties in treatment [26]. A systematic 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 the present study, the prevalence of CDI among ICU patients was 4.12%, much higher than most studies reported in European countries [27]. Of the patients with CDI in this study, 28.7% had diarrhea. This was much higher than the 8% reported in another Chinese study [28]. As for CDC, few studies have systematically assessed

CDC in ICUs to date. 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 varies geographically. The high detection rate of *C. difficile* in China may be due to specific characteristics of the Chinese population and the highly sensitive detection methodology used in this study. The high acquisition of toxigenic *C. difficile* may be related to the increasing awareness of disease prevention and effective disinfection procedures.

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], and many of the risk factors are found in patients in ICU settings. In the present study, we found that medication with multiple antibiotics significantly increased the risk of CDI. Specifically, the increased use of fluoroquinolones [33] in hospitalized patients has contributed to the incidence of CDI, as previous suggested [32, 33]. Regarding underlying conditions, our study found a significant association between the occurrence of CDI and metabolic diseases. However, the mechanisms involved remain unclear, and further studies are needed among this population.

Routine interventions such as PPI medication, surgery and enteral feeding, are especially relevant for patients in ICUs because of the severity of the patients' condition. Several retrospective studies have demonstrated that patients are more than twice as likely to contract CDI if they have received treatment with PPIs [30, 34]. PPIs also cause a change in the gastrointestinal flora, which may create a niche for CDC [35]. In addition, many prospective cohort studies [16, 36, 37] have shown that enteral feeding at least doubled the risk of CDI. However, these factors were not reflected in our research, which may be because of the different geographical regions and populations used in the studies; however, further studies are needed to confirm this.

For patients with CDC in our study, large differences in the number of comorbidities and in the duration of hospital stays were detected among CDC groups. The data on the duration of hospital stays were consistent with a previous study [38]. Patients with multiple diseases usually have poor underlying immune function and are therefore predisposed to acquiring CDC. 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 oral vancomycin have been the main antimicrobial agents for the treatment of CDI [39]. Oral metronidazole has been shown to be an effective inducer of clinical responses, with the advantages of low cost and the reported association with reducing the selection risk of vancomycin-resistant *Enterococcus* [20]. In the treatment analysis of three randomized controlled trials comparing symptomatic treatment of metronidazole and vancomycin [40–42], no significant difference was found between them [43, 44]. In our study, we found that using metronidazole was a protective factor

against CDI, which was consistent with a previous study [30]. Rodriguez *et al.* [45] also suggested that using metronidazole before an operation might lower the incidence of CDI. These results suggested that the preventive use of metronidazole might contribute to the prevention of CDI and CDC. Conversely, oral vancomycin has been shown to be a risk factor for CDC, which is consistent with the findings of Johnson *et al* [46].

Most of the CD isolates identified among the two groups in this study had a toxin A+B+ phenotype. ST2 was the most common epidemic strain type in the CDI group and ST81 was the most common strain type among cases with CDC. This finding differed from that of another recent study that reported ST54 as the most common genotype [47]. In addition, neither ST1 nor ST11, which were epidemic in western countries [48], were detected in China. Our map showing the temporospatial relationships between strains indicated that *C. difficile* dispersed among normal colonized patients is likely to be a potential source of infection and transmission to clinical patients leading to CDC and CDI [31, 49].

There are several limitations in our study. First, the samples were collected from a single center and may therefore not be representative of all healthcare institutions because of patient heterogeneity in China. 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, environmental samples from the patients' wards were not obtained as part of this study, so 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 [50–52]. 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 provides prospectively independent comparisons in ICUs and demonstrates the results of molecular epidemiology. The overall prevalence of CDI and CDC was 4.12% and 3.12%, respectively. A fever, metabolic disorders, the use of fluoroquinolone and exposure to multiple antibiotics were significantly associated with CDI. Longer hospital stays, the number of comorbidities and a history of using vancomycin were found to be associated with acquiring CDC. As for metronidazole, protective effects were seen for the two groups. The most common epidemic strains were ST2 and ST81 in the two groups, respectively. It is essential for medical staff to recognize the importance of *C. difficile*-related diseases in patients in ICUs because of their high risk of CDI/CDC. Patients in ICU are often immunocompromised and their treatments are complicated by comorbidities. Therefore, these results highlight the importance of antibiotic management and appropriate isolation in the prevention and control of *C. difficile*-related diseases. The role of asymptomatic carriers in the transmission of *C. difficile* deserves further study because there are identifiable risk factors and strain types that can be used for detection, which may provide a basis for screening and isolation.

Declarations

List of abbreviations

ICUs: Intensive care units

CDI: *Clostridium difficile* infection

CDC: *Clostridium difficile* colonization

MLST: Multilocus sequence typing

ST: Sequence type

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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Tables

Due to technical limitations, Tables 1 & 2 are only available for download from the Supplementary files section.

Figures

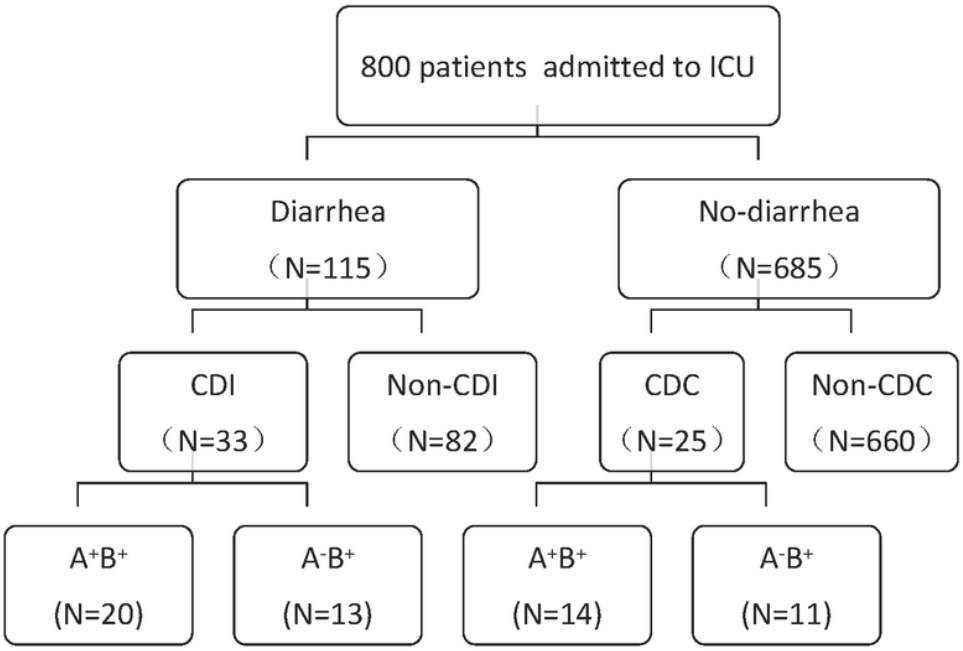

Figure 1

Study flowchart of CDI and CDC among ICU patients

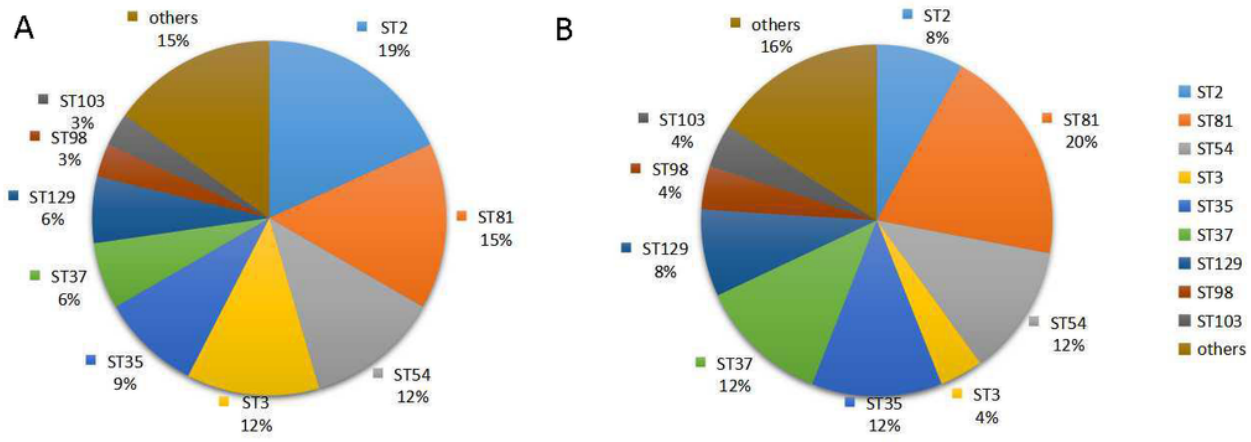

Figure 2

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.

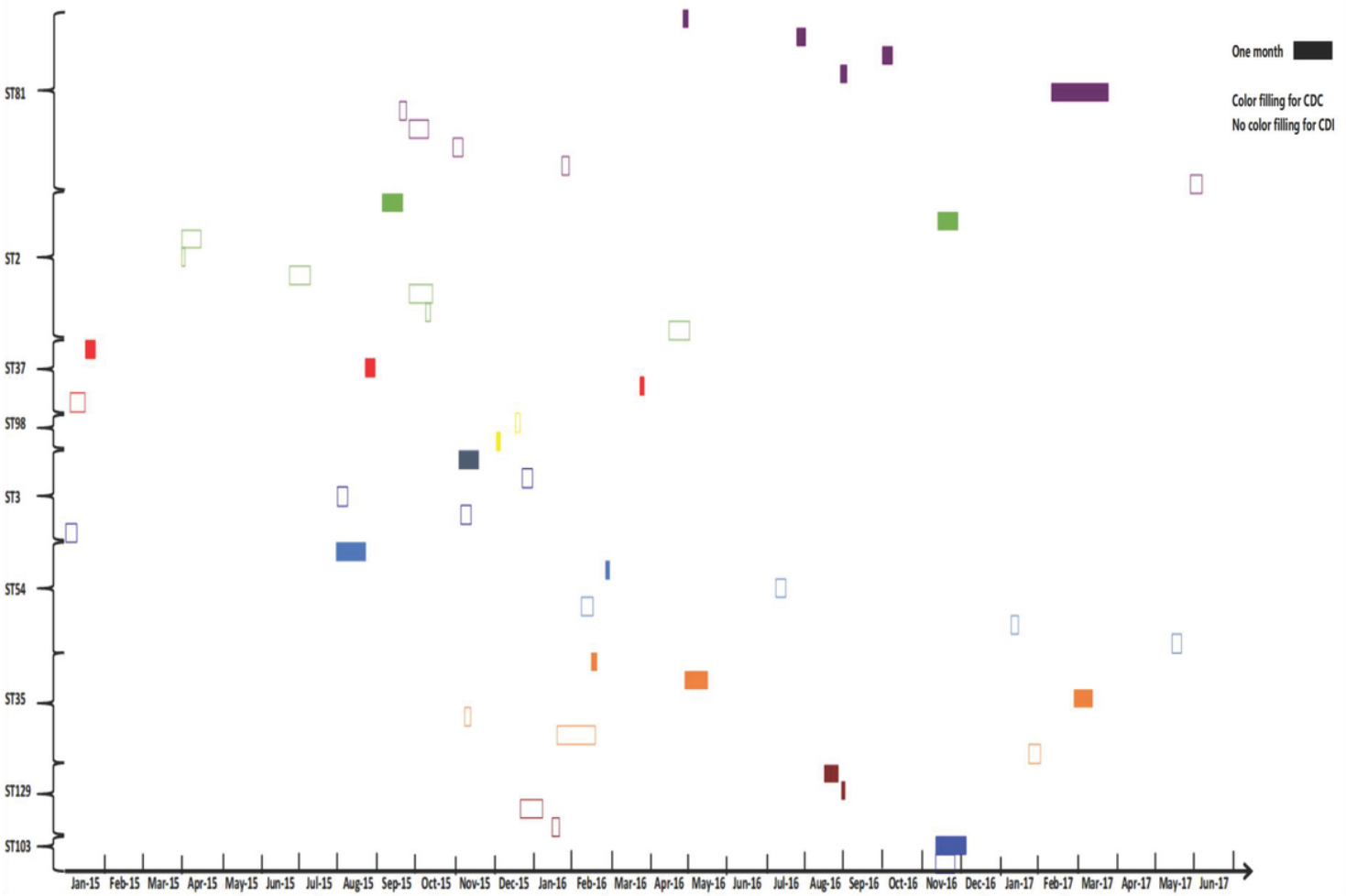

Figure 3

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.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplement1.pdf
- supplement2.pdf