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ORIGINAL RESEARCH

Genomic Epidemiology and Antimicrobial Resistance Profiles of *Clostridioides difficile* from Multi-Hospitals in a City in Eastern China

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Background: *Clostridioides difficile* is an important pathogen causing approximately 20–30% of the cases-with antibiotic-associated diarrhea and 90% of those with *Pseudomembranous enteritis*. However, limited surveillance of *C. difficile* infections (CDI) in China is done at present, especially in terms of multi-hospital epidemiological reports.

Methods: Between June 2020 and November 2020, we conducted a prospective study addressing antimicrobial susceptibility profiles and genomic epidemiology of *C. difficile* strains isolated from inpatients with diarrhea in seven tertiary hospitals in the same city.

Results: In total, 177 strains of toxin-producing *C. difficile* were isolated, and the dominant toxin gene profiles were tcdA+tcdB+ (84.2%, 149/177) and tcdA-tcdB+ (15.8%, 28/177). Furthermore, 130 isolates were successfully analyzed for antimicrobial susceptibility phenotype in which the rates of resistance to clindamycin, erythromycin, levofloxacin, and moxifloxacin were higher than to other antibiotics. All strains were susceptible to metronidazole and vancomycin. Fluoroquinolone-associated mutations (such as *gyrA*) were the most frequently found ones in the analyzed genomes. Moreover, 24 different sequence types (STs) were identified in the 130 isolates, and the most prevalent types were ST3 (26.2%, 34/130) followed by ST54 (16.9%, 22/130) and ST2 (10%, 13/130). The so-called highly virulent strain ribotyping 027 (B1/NAP1/ST1) was not identified. In addition, we also compared single nucleotide polymorphisms (SNPs) among the isolates and carried out genomic epidemiological studies on the isolates. We found that ST3 and ST54 could cause transmission in both intra- and inter-hospital settings.

Conclusion: Although it is the so-called hypervirulent epidemic strain, ribotyping 027 (ST1), was not detected. ST3 and ST54 can be transmitted through different hospitals. Therefore, it is necessary to conduct further molecular epidemiological monitoring of *C. difficile* and screening of patients admitted to key departments.

Keywords: *Clostridioides difficile* infection, genomic analysis, toxin gene, antibiotic resistance, transmission

Introduction

Clostridioides difficile is a leading pathogen that frequently causes hospital infections.[1] About 20–30% of the cases of antibiotic-associated diarrhea and 90% of Pseudomembranous enteritis cases are caused by *C. difficile. C. difficile* infection (CDI) is a toxin-mediated disease, and most *C. difficile* toxigenic strains produce two main toxins, TcdA and TcdB.[2] In addition, some strains additionally produce "binary toxins" (CDT), encoded by the *cdtA* and *cdtB* genes.[3] With the outbreak of hypervirulent ribotyping 027 (B1/NAP1/ST1) in Europe and North America, *C. difficile* has become a major challenge

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affecting public health and represents a significant burden for global health-care systems.[4] In 2011, *C. difficile* infected nearly 500,000 patients and caused around 29,000 deaths in the United States.[5] CDI is also the most frequent hospital-acquired infection in Europe,[6,7] and 150,000 CDI cases and 8000 deaths occur every year, resulting in medical expenses and direct economic losses of about 3 billion Euros.[8] A meta-analysis showed that the incidence rate of hospital infection with CDI in China was 14%.[9] In 2015, *C. difficile* ribotyping 027 was detected for the first time on mainland China after which sporadic occurrences of ribotyping 027 were reported.[10] However, outbreaks in hospitals were also reported.[11] Therefore, it is still important to increase focus on the genomic epidemiological surveillance of *C. difficile*.

With the development of sequencing technology, whole genome sequencing (WGS) has been established as the best method to analyze epidemiology and explores the transmission dynamics of bacterial pathogens.[12] In 2017, the United Kingdom (UK) conducted a multicenter study using WGS to monitor the differences in hospital transmission among medical institutions.[13] In a single center study over four consecutive years in China, WGS was used for genomic epidemiological surveillance and prevention of healthcare-associated infections.[14] Therefore, WGS can be used to compare single nucleotide variations (SNPs) among isolates in non-repetitive core genomes, and as a highly discriminatory power and standardized typing method, it provides value when analyzing *C. difficile* strains and evaluating their genetic diversity, and also helps to provide strain dissemination information.[15]

Researchers outside of North America and Europe emphasize the importance of CDI epidemiological monitoring. In China, the genomic epidemiological characteristics of *C. difficile* isolates are still not completely understood although some studies have focused on the molecular typing characteristics of *C. difficile* in a single medical institution.[14] However, minimal data concerning the transmission capacity of different *C. difficile* strains among hospitals and the correlation of strains among multiple medical institutions at the gene level are available. Based on these reasons, we conducted a prospective WGS study in the same city with multiple centers. By performing an antimicrobial susceptibility analysis and focusing on the genomic epidemiology of *C. difficile* and the phenomenon of transmission within and between hospitals, we tracked the epidemic trend of *C. difficile* and established a foundation for the prevention and monitoring CDI transmission.

Materials and Methods

Definitions

Diarrhea: Diarrhea is defined as three loose stools within at least one 24 h period.[16]

CDI: A case of CDI is defined as clinical findings compatible with CDI and microbiological evidence of *C. difficile* toxin genes (*tcdA* and/or *tcdB*) based on polymerase chain reaction (PCR) results without reasonable evidence of another cause of diarrhea.[16]

Community-associated CDI: Symptoms of CDI occur within 48 h of hospitalization, and the patient has no history of hospitalization in the past 12 weeks.[17]

Healthcare-associated CDI: Symptom of CDI occurs more than 48 h after admission or less than 4 weeks after discharge from a health-care facility or hospital.[17]

Cloning transmission: A threshold of ≤2 SNPs was established to identify strains possibly involved in transmission events.[18]

C. difficile Isolates

Only one sample from each inpatient in seven tertiary hospitals in Ningbo, Zhejiang Province, China, was collected between June 1, 2020 and November 30, 2020. During the cultivation process, anaerobic isolation of *C. difficile* was performed using the selective medium cycloserine–cefoxitin–taurocholate agar (CCFA-TA; Oxoid, UK), and the plates were incubated under anaerobic conditions for 48 h at 37°C. The suspected *C. difficile* colonies were identified using Brooke matrix-assisted laser desorption/ionization-time of flight mass spectrometry ([MALDI-TOF] Bruker Daltonik GmbH, Bremen, Germany).

Toxin Gene and Whole Genome Sequencing

The genome DNA of *C. difficile* was extracted using the Qiagen QiaAmp kit. With reference to the primer design in previous literature, *C. difficile* strain ATCC BAA-1870 (ribotyping 027) was used as the positive template control of toxin genes.[19,20]

Genomic DNA was sequenced using Illumina technology with 150 base pair terminal readings. The sequence data were processed and quality controlled according to a standard pipeline.[21] Multilocus sequence typing (MLST) with seven housekeeping genes (*adk, atpA, dxr, glyA, recA, oda*, and *tpi*) was performed on all isolates as described previously by Griffiths et al.[22] The distribution of sequence type (ST) and evolution branch of *C. difficile* was completed according to the PubMLST database using MLST v.2.10.7 Optimal k-mers fell between 47 and 93 bp according to the mean value for median contig size of genome assembly. STs were determined based on DNA sequencing data using the PubMLST sequence query page (https://pubmlst.org).

Antimicrobial Susceptibility Testing

Determination of the minimum inhibitory concentration (MIC) of nine antibiotics, including moxifloxacin, vancomycin, tetracycline, erythromycin, rifampicin, linezolid, metronidazole, clindamycin, and levofloxacin, was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.[23] Brucella agar (BBL BD, USA) with 5% fibrotic sheep blood, vitamin K (10 μg/mL), and hemin (5 μg/mL) was used for cultures. The resistance breakpoints determined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were used for vancomycin (>2 μg/mL), linezolid (>4 μg/mL), and rifampicin (>32 μg/mL) (http://www.eucast.org/clinical_ breakpoints/) as no CLSI recommendations for these antibiotics have been established. *C. difficile* ATCC 700057 was used as a quality control strain for susceptibility testing.

Core-Genome SNP Analysis

Variant calls for SNP analysis were performed using Snippy (http://github.com/tseemann/snippy) with default parameters. The chromosome of *C. difficile* M68 was set as the reference for all strains of *C. difficile* in this study. The alignment file was filtered from variants with elevated densities of base substitutions as putative repetitive regions, mobile genetic elements (MGEs) and recombination events by Gubbins v.2.4.1, and used to calculate the pairwise cgSNP.[24] The cgMLST analysis was performed using chewBBACA.[25] The maximum likelihood trees based on core genome were constructed using MEGA11 with 1000 bootstrap replicates and visualized using the Interactive Tree of Life (iTOL) web server.[26,27] The minimum spanning tree was constructed in PHYLOViZ 2.0 based on pairwise comparison of cgSNP and cgMLST.[28] Antimicrobial resistance genes were identified using the Comprehensive Antibiotic Resistance Database (CARD) Resistance Gene Identifier (RGI) software (https://card.mcmaster.ca/analyze/rgi).

Statistical Analysis

SPSS software version 21 for Windows (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

Results

Detection of Toxin Genes and Clinical Characteristics

A prospective study was conducted on the detection of continuous samples of *C. difficile* in seven hospitals for a period of 6 months. A total of 1954 non-repeated stool samples were collected, and 209 (10.7%, 209/1954) strains of *C. difficile* were identified by culture. Among the isolates, 177 (84.2%, 177/209) were toxigenic strains and the remaining 32 (15.8%, 32/209) were non-toxigenic. Over half (64.4%, 114/177) of patients contracted CDI 7 days after admission, and 65.5% (116/177) of patients were aged 65 or more years. Out of the 177 toxigenic strains, 149 (84.2%, 149/177) strains were positive for *tcdA* and *tcdB* genes (A+B+), and 28 (15.8%, 28/177) strains contained only *tcdB* genes (A-B+). A total of 16 (9.0%, 16/177) strains were found to carry the binary toxin genes (CDT+A+B+).

The demographics of CDI patients in different hospitals were compared in addition to the results of toxin gene detection. Among them, the highest isolation rate of *C. difficile* in Hospital 7 was 10.7% (31/290). The proportion of CDT+A+B+ strains was relatively high in Hospital 2, but no binary-toxin-producing strains were isolated from Hospitals l and 7. As Hospital 1 focuses on the collection of samples by departments (hematology department and gastroenterology department), it is different from the other hospitals in the proportion of men and women and age distribution. According to the definitions

Table 1 *C. difficile* Toxin Gene and Clinical Characteristics of Diarrhea Patients in Different Hospitals

Hospital	Separation Rate	Toxin Profile			Age		Gender		Length-of-Stay		
Number	CD/Number of Specimens	tcdA+tcdB+	tcdA-tcdB+	CDT+	≥65 Years Old	<65 Years Old	Man	Woman	<48 h	3–7 Days	≥7 Days
Hospital1	9.0% (42/467)	35 (83.3%)	7 (16.7%)	0	20	22	20	22	1	13	28
Hospital2	10.5% (45/429)	38 (84.4%)	7 (15.6%)	8	30	15	29	16	2	13	30
Hospital3	5.8% (6/104)	5 (83.3%)	1 (16.7%)	1	5	1	2	4	0	2	4
Hospital4	9.1% (15/165)	14 (93.3%)	1 (6.7%)	3	8	7	9	6	0	4	11
Hospital5	9.2% (23/249)	21 (91.3%)	2 (8.7%)	3	19	4	14	9	0	10	13
Hospital6	6.0% (15/250)	13 (86.7%)	2 (13.3%)	1	11	4	9	6	1	6	8
Hospital7	10.7% (31/290)	23 (74.2%)	8 (25.8%)	0	23	8	20	11	0	11	20
Total	9.1% (177/1954)	149 (84.2%)	28 (15.8%)	16	116	61	103	74	4	59	114

of CA-CDI and HA-CDI, we found that only 2.3% (4/177) of patients were CA-CDI, while the proportion of patients with HA-CDI (97.7%, 173/177) was significantly higher than CA-CDI ($P < 0.05$) as shown in Table 1.

Multi-Locus Sequence Typing

After recovery and culturing of the strains, 130 toxin-gene-positive isolates were successfully sequenced, including 123 A+B+ and 7 A-B+. Following comparison with the public database, a total of 24 STs types were detected. The most prevalent type was ST3 (26.2%, 34/130) followed by ST54 (16.9%, 22/130) and ST2 (10%, 13/130). Fortunately, none of the isolates was identified as ST-1 (BI/NAP1/RT027). The toxin-type of A+B+ is mainly consisted of ST3, ST54, and ST2. Seven strains typed as A-B+ were mainly distributed in ST37 (four strains) and ST81 (three strains) as shown in Table 2.

Table 2 The Results of Multi-Locus Sequence Typing (MLST) of *C. difficile* in Different Hospitals

Sequential Type	Distribution of Infection Isolates (Strains)							Total
	Hospital 1	Hospital 2	Hospital 3	Hospital 4	Hospital 5	Hospital 6	Hospital 7	(Strains)
ST2 (tcdA+tcdB+)	5	3				1	4	13
ST3 (tcdA+tcdB+)	5	15	2	2	3	2	5	34
ST5 (tcdA+tcdB+CDT+)	1					1		2
ST8 (tcdA+tcdB+)	5	2			3	1		11
ST11 (tcdA+tcdB+CDT+)				1				1
ST14 (tcdA+tcdB+)		1						1
ST17 (tcdA+tcdB+)		1						1
ST21 (tcdA+tcdB+)					2			2
ST35 (tcdA+tcdB+)	1	1			2	2		6
ST37 (tcdA-tcdB+)			1	1	1	1		4
ST42 (tcdA+tcdB+)	1	4	1				1	7
ST54 (tcdA+tcdB+)	3	1	1	6	5		6	22
ST55 (tcdA+tcdB+)	1					2	1	4
ST80 (tcdA+tcdB+)				2				2
ST81 (tcdA-tcdB+)	1						2	3
ST99 (tcdA+tcdB+)		1	1					2
ST102 (tcdA+tcdB+)	1				1			2
ST103 (tcdA+tcdB+)		1		1				2
ST110 (tcdA+tcdB+)							1	1
ST122 (tcdA+tcdB+CDT+)		1						1
ST129 (tcdA+tcdB+)	1						1	2
ST278 (tcdA+tcdB+)	1							1
ST415 (tcdA+tcdB+CDT+)		5						5
ST512 (tcdA+tcdB+)	1							1
Total	28	34	6	15	17	10	21	130

The distribution of STs in all hospitals was diverse. ST3 was distributed in all hospitals, while some STs were unique to special hospitals, such as ST415 to Hospital 2. Another phenomenon the presence of different ST diversity and prevalence of STs in each hospital. For example, ST3 was mostly concentrated in Hospital 2 and accounted for 44.1% (15/34), while ST54 was the most frequent ST in Hospital 4 (40.0%, 6/15). Some correlations were observed between the STs and the wards in some hospitals. ST3 cases were mainly distributed in the intensive care unit (ICU) in Hospital 2 and the rehabilitation department in Hospital 7.

Antimicrobial Susceptibility Testing

One-hundred and thirty strains from *C. difficile* isolates underwent agar dilution, and the results showed that erythromycin (61.5%, 80/130) and clindamycin (83.8%, 109/130) had high resistance rates followed by levofloxacin (40%, 52/130), moxifloxacin (32.3%, 42/130), and tetracycline (30.8%, 40/130). Low resistance rates were found for rifampicin (5.3%, 7/130). All isolates were susceptible to vancomycin, metronidazole, and linezolid. In this study, 59 strains (45.4%, 59/130) were confirmed to be multidrug resistant (Figure 1).

C. difficile presents resistance to different types of antibiotics due to the fact that its genome contains multidrug resistance genes. In this study, the majority of fluoroquinolone-resistant *C. difficile* isolates were found to have *gyrA* mutations (84.6%, 44/52). Fourteen of them had both *gyrA* and *gyrB* mutations, but five isolates did not have mutations in *gyrA* and/or *gyrB*, and may have other types of antimicrobial resistance mechanisms. The ST3 isolate is the predominant type of fluoroquinolone-resistant strain with an antimicrobial resistance rate of 79.4% (27/34), and it only mutates on *gyrA*, while five ST415 strains have *gyrA* and *gyrB* mutations. Among 109 strains of clindamycin-resistant *C. difficile*, 75 strains were found to carry the *ermB* gene alone (Figure 1). Almost all *ermB* positive isolates also showed resistance to erythromycin. It is worth noting that ST3 is also the predominant type of MLSB (CLI and ERY) resistant strains.

Detection of Virulence Genes

In this study, virulence genes were characterized by the diversity of *slpA, cwp84, cwp66, rmlB, rmlC, rfbA*-1, and others. We observed that sequencing of the *cwp66* gene in the isolates showed three different types of sequence levels. Its antigenicity and immunogenicity were higher in CDI patients[29] and mainly distributed in ST5, ST8, and ST35. However, *cwp84* showed relatively uniform sequence levels, and only one type with 100% identity was detected in sequence analysis. Its antigenicity and immunogenicity were lower. In addition, as a highly polymorphic protein, 109 (83.8%, 109/130) *C. difficile* isolates with targeted primers of the S layer protein A (SlpA) gene that were positive based on PCR amplification were found, and both ST8 and ST35 lacked SlpA. It is worth noting that the sequence-level results of *rmlB, rmlC* and *rfbA*-1 are consistent, and the main deletion types included ST54, ST35, and ST37 (Figure 1).

Transmission Within and Among Hospitals

We compared SNPs between isolates and researched the clonal transmission characteristics of the SNP phylogeny. In terms of the whole development cluster of this study, WGS indicated that substantial genetic diversity exists.[18] A total of 23 clone groups were identified from 130 isolates, and each group consisted of two or more strains. Up to 76 isolates (58.5%, 76/130) were identified to have genetic correlation (SNP $\leq$ 2). Among them, 41 isolates were scattered in the same ward or in the same hospital, and some were found even among different hospitals, suggesting that *C. difficile* transmission might have occurred. However, 31 isolates (23.8%) had more than 10 SNPs, indicating that the isolates were genetically distinct from each other, while 29 isolates (22.3%) had SNP differences <10 but >2. From the above data, the possible epidemiological links between genetic related cases were determined (Figure 2).

The largest transmission cluster was ST3 cluster formed by 26 isolates covering all seven hospitals while the highest number of strains came from Hospital 2. It is interesting to note that one isolate (ID 3062) from Hospital 5 was the center of the ST3 transmission net (Figure 2). ST54 was another ST with more transmission clusters, which were divided into five different subclusters. Except for two subclusters confined to the same hospital (Hospital 7), the other three subclusters showed transmission of ST54 strains between different hospitals. Clusters of ST415, ST80, ST21, and ST81 were found only in Hospitals 2, 4, 5, l 7, respectively. These data indicate that localized transmissions happened according to different hospitals. By reviewing clinical data, 34 patients (26.2%, 34/130) had been admitted to the same

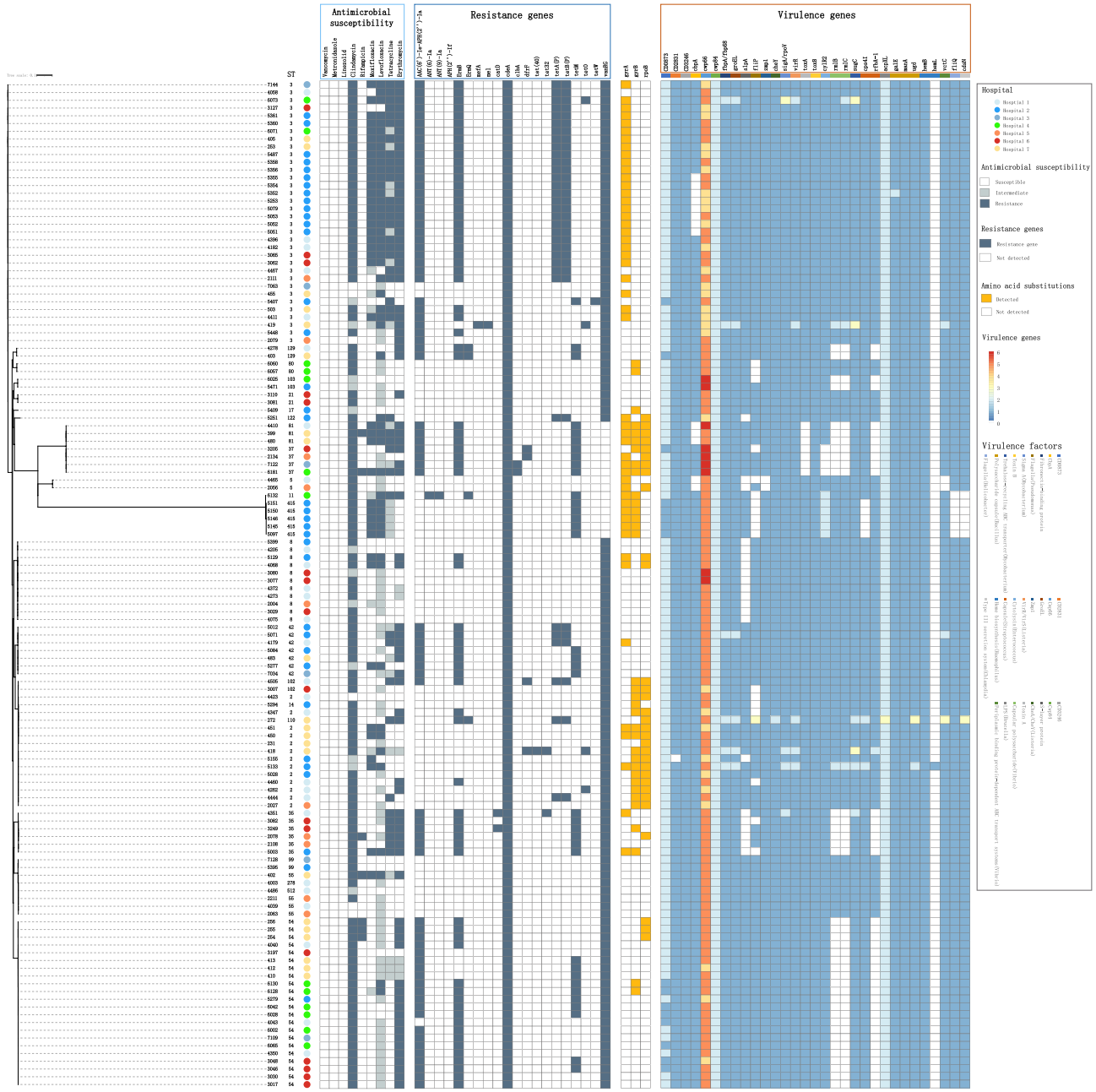

Figure 1 Multi-locus sequence typing (MLST) and antimicrobial resistance gene analysis results of 130 *C. difficile* strains. A phylogenetic tree was constructed based on the whole genome sequence, describing the strain number, MLST typing, antimicrobial resistance and virulence gene in each hospital. The sensitive, intermediate, and antimicrobial resistance phenotypes are represented by white, gray, and dark blue rectangles, respectively.

ward concurrently, while 41 patients (31.6%, 41/130) had been admitted to the same hospitals but not to the same wards and at different times. Other potential transmission routes, including asymptomatic colonized patients or sources in a wider environment, may have existed. For the remaining 28 patients (27.2%, 28/103) had no spatial-temporal overlap in health-care settings.

Discussion

To the best of our knowledge, this study is the first prospective multicenter study addressing the genomics of *C. difficile* in China. The study describes *C. difficile* toxin genes, antibiotic resistance, and transmission. Based on the relevant

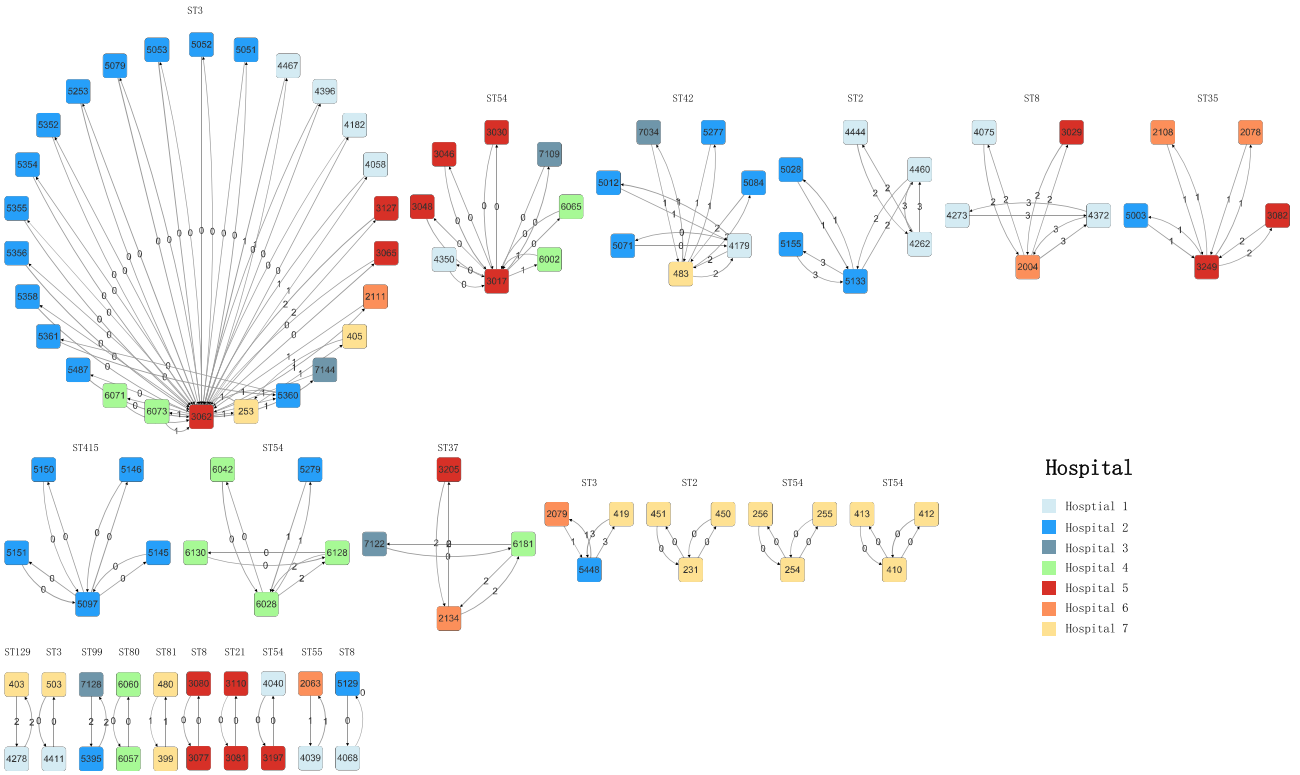

Figure 2 One-hundred and thirty *C. difficile* strains form developmental clusters of clonal transmission based on different and single nucleotide polymorphisms (STs and SNPs, respectively).

clinical information and WGS data, we found that *C. difficile* can be directly disseminated throughout different hospitals in the same city.

The results of our study show that nearly two-thirds of CDI cases occurred after 1 week of admission. Elderly people who had been hospitalized for a long time, suffered from complications, and received antimicrobial treatment were more vulnerable to infection.[30] Together with older patients, these results are consistent with other reports in which the CDI incidence is reported to be high in older adults.[31] Although 15.8% of CDI patients were infected with A-B+ strains, only seven isolates were successfully sequenced and identified as ST37 and ST81, a finding that was lower than other reports in China.[32] This inconsistency may be due to the geographical difference in some STs. The failure of most A-B+ strains in this study to resuscitate may also be related and further research is needed to explain such a failure. In previous epidemiological studies, ST3 and ST54 were the predominant strains of *C. difficile* in mainland China.[9] Our study shows similar results found in other studies, which means the common popular STs do not change by spatial–temporal scale.

At present, *C. difficile* is resistant to many antibiotics, especially erythromycin, clindamycin, and fluoroquinolones.[33] It was previously reported that fluoroquinolone resistance is widely believed to play an important role in the transmission, such as virulent strain ribotyping 027.[34] Resistance rates to fluoroquinolones in this study were similar to the reports in many European countries in 2014.[35] More studies have confirmed that moxifloxacin resistance of *C. difficile* is an important sign of CDI transmission in the medical environment.[36] Our data show that the resistance rate of ST3 to fluoroquinolones exceeded 70%, which may partly explain why ST3 was the most common ST. In addition, the incidence of multidrug resistant isolates in this study was significantly higher, which may have led to the prevalence of pathogens in hospital environments and the continued emergence of clones. On the other hand, we found that *rmlB, rmlC*, and *rfbA*-1 genes, which are reported to be associated with metabolism and biosynthesis,[37,38] existed in all ST3 isolates but were absent in ST54. Although more experiments are needed to confirm this, we inferred that these genes may make ST3 easier to spread than ST54.

As *C. difficile* is an important pathogen of hospital-acquired infections, which can be transmitted between wards and hospitals,[39] we detected genetically related pairs of isolates in patients with CDI. Clustering analysis suggests that the interspecies clonal transmission (≤2 SNP) of *C. difficile* strains between wards and hospital is possible, with several identified clusters harboring isolates from different wards and hospitals. ST3 is the largest cluster observed in this study, and it covers isolates from all seven participating hospitals, suggesting that the ST3 isolates may be well established in the city, which is consistent with Eyre et al reported that ST3 is more likely linked to healthcare-associated acquisition.[40] The fact that most ST3 strains presenting resistance to moxifloxacin may constitute a concern in terms of circulation of these strains as fluoroquinolone resistant strains were found to be associated with nosocomial outbreaks.[41] ST3 is usually identified in asymptomatic patients and can colonize for long periods without any clinical symptoms.[42] This asymptomatic spread may contribute to the spread of ST3 in hospitals because patients without diarrhea are not screened. ST54 is another popular ST in China.[43] However, unlike ST3 strains undergoing clonal transmission, ST54 is spread by sporadic transmission.

Our data show that 26.2% (34/130) of the cases have contact history in the same ward, and also have genetic correlation with previous cases, but only some of these cases share time and space in the same hospital ward, indicating that many genetically related isolates have no direct contact evidence, and there may be other transmission routes, such as environmental contact. Recent studies show that 24% of the cases occur due to the hospital environment.[6] In addition, 23.8% of HA-CDI cases from non-ward transmission show that *C. difficile* spores have unlimited viability and may become a continuous source of transmission, which can be widely spread within the spatial-temporal intersection of hospitals.[44] In 2017, the clinical guidelines for CDI in the United States recommended providing separate rooms for CDI patients as much as possible to reduce the probability of infection to other patients and contact isolation and hand hygiene are the key to preventing and controlling CDI.[45] Therefore, early identification of *C. difficile* cross-infection may help prevent hospital transmission and reduce the risk of contracting such infections for uninfected patients.

Our research also had some limitations. First, 6 months of samples were collected, the time span was not long enough, and the availability of *C. difficile* isolates to fully explain the direction of hospital transmission was limited. In addition, environmental samples were not collected at the same time during the study. Moreover, because *C. difficile* forms spores, which lead to repeated infection, and the persistence of spores in the environment promotes their transmission,[46] we cannot evaluate these potential transmission sources. Fidaxomicin is associated with improved sustained clinical cure and significantly reduces CDI recurrence compared with vancomycin and recommended by several guidelines.[16,45] However, at this time, fidaxomicin has not yet been used in China, and lack of this antibiotic susceptibility is one of the limitations of this study.

Conclusions

This study presents the first multicenter study investigating the genomic relatedness of *C. difficile* from hospitalized patients in China and offers meaningful insight into the transmission of *C. difficile* in the country. Based on the genomic epidemiology of *C. difficile*, we found ST3 and ST54 were the main prevalent STs and transmitted among different wards and hospitals. Different patterns in CDI epidemiology underscore the importance of local surveillance and infection control.

Ethics Approval

This study was approved by the Ethics Committee (No. 202101001) and reached a plan after discussion. The Ethics Committee believed that the protocol did not change the patient's treatment plan, did not disclose privacy, and did not cause adverse consequences; thus, consent was waived. This study complies with the Declaration of Helsinki.

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Disclosure

The authors report no conflicts of interest in this work.

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