Molecular epidemiology of community-onset *Clostridioides difficile* infections at a tertiary hospital in mainland China: A ten-year (2010-2019) retrospective study

Yunbo Chen
Zhejiang University School of Medicine First Affiliated Hospital
Lihong Bu
Jinhua Municipal Central Hospital
Tao Lv
Zhejiang University School of Medicine First Affiliated Hospital
Lisi Zheng
Zhejiang University School of Medicine First Affiliated Hospital
Silan Gu
Zhejiang University School of Medicine First Affiliated Hospital
Jianqin He (1198015@zju.edu.cn)
The First Affiliated Hospital of Zhejiang University

Research Article

Keywords: Clostridioides difficile infection, community, molecular epidemiology

Posted Date: September 16th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-877500/v1

Abstract

Background

Clostridioides difficile infection (CDI) is an increasingly common disease in healthcare facilities and community settings. However, there are limited reports of community-onset CDI (CO-CDI) in China. We retrospectively analyzed the molecular epidemiology of CO-CDI at a tertiary hospital over a period of 10 years.

Methods

A total of 1307 stool samples from 1213 outpatients were tested by culturing. The presence of toxin genes (*tcd A*, *tcd B*, *cdtA* and *cdtB*) were confirmed by PCR. Toxigenic strains were typed using multilocus sequence typing (MLST). Susceptibility to 9 antimicrobials was evaluated using the E-test.

Results

Eighty-nine of 1213 outpatients (7.3%) had CO-CDI, 4 of these patients (4.5%) had one or more recurrence, and there were 95 strains of toxigenic *C. difficile*. Among these strains, 82 (86.3%) had the *tcdA* and *tcdB* genes (A + B+) and 5 of these 82 strains were positive for the binary toxin genes (*cdtA* and *cdtB*); the other 13 strains (13.7%) had the *tcdB* gene only (A-B+). There were 15 different STs and the most prevalent were ST-54 (23.2%), ST-35 (16.8%), and ST-2 (13.7%). All strains were susceptible to metronidazole and vancomycin, and had low resistance to moxifloxacin and tetracycline, but had high resistance to ciprofloxacin, clindamycin, and erythromycin. Twenty-three isolates (24.2%) were multidrug-resistant.

Conclusions

Outpatients with CDI were common during this period in our hospital. The *C. difficile* isolates had high genetic diversity. All isolates were susceptible to metronidazole and vancomycin, and nearly one quarter of all isolates had multidrug resistance.

Introduction

Clostridioides difficile is an anaerobic Gram-positive, spore-forming, toxin-producing bacillus that is able to colonize and proliferate in the human gut following changes in the indigenous colonic microbiota. The numbers of *C. difficile* may increase greatly following antibiotic administration, and this can lead to diarrhea, colitis, and pseudomembranous colitis, a condition known as a *C. difficile* infection (CDI)(1). Two structurally similar toxins, toxin A (TcdA, an enterotoxin) and toxin B (TcdB, a cytotoxin), are the major virulence factors associated with CDI(2). However, some toxigenic strains, such as *C. difficile* BI/NAP1/RT027 clones causing large epidemics across the developed world, can also produce a third toxin, *C. difficile* binary toxin (CDT), which is associated with increased morbidity and mortality (3).

Most studies of the trends and molecular epidemiology of CDI focused on hospitalized patients. The major and well-established risk factors for hospital-onset CDI (HO-CDI) are excessive or non-standard use of antibiotics, older age, prior and prolonged hospitalization, and multiple comorbidities(4). However, CDI is no longer an exclusively nosocomial infection, and recent studies have reported the onset of symptoms in the community(5). In fact, community-onset CDI (CO-CDI) represents nearly half of incident cases and it is now considered a major cause of community diarrhea(6). Moreover, the epidemiology of HO-CDI and CO-CDI are related, given the frequent movement of patients between hospitals and the community(7). Studies in general populations have compared the characteristics of patients with CO-CDI and HO-CDI and reported that those with CO-CDI were younger and had milder disease. However, some CO-CDI cases still require hospitalization and these individuals may lack the classic health care facility (HCF) risk factors for CDI(8). Therefore CO-CDI may account for a considerable reservoir of CDI, so prevention efforts should also include interventions that reduce CO-CDI(9).

Studies of the molecular epidemiology of CDI in mainland China have mostly examined hospitalized patients, although a few studies have described CO-CDI. We initiated this study to retrospectively analyze the molecular epidemiology of CO-CDI at a tertiary hospital over a period of about 10 years to identify the epidemic strains of *C. difficile*, compare the results with other epidemiological data, and provide an overview of CDI in a single region of China.

Methods

Collection of C. difficile isolates

This retrospective study was performed at The First Affiliated Hospital, School of Medicine, Zhejiang University, a 2500-bed tertiary teaching hospital in Hangzhou, Zhejiang, China. Stool samples were collected from outpatients with diarrhea and submitted to the clinical microbiology laboratory between 1 January 2010 and 31 August 2019. Only samples requested for *C. difficile* assay by clinicians were included in the analysis. Patient demographic characteristics, including age, sex, and clinical department of admission, were recorded.

Stool specimens were treated with alcohol to a final concentration of 75% for spore selection before anaerobic isolation of *C. difficile* using the selective medium cycloserine-cefoxitin-taurocholate agar (CCFA-TA; Oxoid) supplemented with 7% sheep blood at 35 °C for 48 h. Then, the *C. difficile* isolates were confirmed by matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) analysis using the Bruker Daltonics Microflex LT system (Bruker Daltonik GmbH, Bremen, Germany).

Definitions

A case of CDI was defined as the presence of diarrhea (≥ 3 loose stools/day) with a positive toxin-producing *C. difficile* in the stool. CO-CDI was defined as CDI with onset of symptoms in the community, and did not include patients who first developed symptoms after admission(9). Recurrent CDI was defined as a CDI that was diagnosed within 8 weeks of a prior episode; relapse with the same strain or another strain of *C. difficile* were both designated as recurrent CDI(10).

Detection of toxin genes by PCR

Bacteria obtained after growth for 48 h in anaerobic blood agar culture were suspended in 1 mL of distilled water in a microcentrifuge tube. Bacterial genomic DNA was then extracted using the simplified alkaline lysis method. All isolated strains were tested for *tcdA*, *tcdB*, and the binary toxin genes (*cdtA* and *cdtB*) using the polymerase chain reaction (PCR), as previously described(11, 12).

Multilocus sequence typing (MLST) and analysis

MLST was used to genotype all toxigenic isolates based on seven housekeeping genes (*adk*, *atpA*, *dxr*, *glyA*, *recA*, *sodA*, and *tpi*), as previously described(13). Allele designations were obtained from the *C. difficile* PubMLST batch profile query page (http://pubmlst.org/cdifficile/) to obtain the sequence types (STs).

Antibiotic susceptibility testing

The antibiotic susceptibility of each toxigenic isolate was analyzed using E-test strips (bioMérieux, Marcy-l'Étoile, France), as previously described(11). Each minimum inhibitory concentration (MIC) was read at the point at which the zone of complete inhibition intersected with the MIC scale, using Clinical and Laboratory Standards Institute (CLSI) guidelines. The selected resistance breakpoints included 8 mg/L for erythromycin, clindamycin, tetracycline, and the fluoroquinolones, and 32 mg/L for metronidazole, according to the CLSI interpretative categories approved for anaerobic bacteria. The resistance breakpoints determined by The European Committee on Antimicrobial Susceptibility Testing (EUCAST) were used for vancomycin (> 2 mg/L), linezolid (> 4 mg/L), and rifampicin (> 32 mg/L) (http://www.eucast.org/clinical_breakpoints/). The control isolate was *C. difficile* ATCC 700057.

Data Analysis

SPSS version 23.0 for Windows (SPSS) was used for statistical analysis.

Ethical approval

Participants or their legally authorized representatives gave informed consent to be included in the study. Ethical approval for the study was granted by the First Affiliated Hospital, School of Medicine, Zhejiang University (Approval number1554-1).

Results

Patient characteristics

We analyzed 1307 samples from 1213 patients at our institution (median age: 48 years, 47.4% males) and diagnosed CDI in 89 patients (7.3%, median age: 56 years, 60.1% males) from 1 January 2010 and 31 August 2019 (Table 1). The number of CDI episodes per total tests performed was 49/507 for the clinical gastroenterology department, 7/232 for the emergency department, 8/117 for the clinical infectious disease department, 10/71 for the clinical hematology department, and 15/286 for other departments. Thus, the highest positive rates of CDI were in the clinical hematology department (13.2%) and the clinical gastroenterology department (9.4%). Among the 89 positive patients, 3 (3.4%) had a single recurrence and 1 (1.1%) had multiple recurrences (total recurrence incidence: 4.5%). The median age of the 4 patients with recurrences was 53 years, and 3 of them were males.

Analysis of all outpatients with diarrhea indicated the median age was significantly greater in those with than without CDI (56 *vs.* 47 years, $p = 0.004$). Moreover, stratifying the data by age classes indicated an increased percentage of patients with diarrhea due to *C. difficile* as age increased (Fig. 1). However, there were no differences according to gender (data not shown).

Molecular epidemiology of the isolates

We isolated 126 (9.6%) strains from 1307 stool samples, and identified 75.4% (95/126) toxigenic strains and 24.6% (31/126) non-toxigenic strains. Among the 95 toxigenic strains, 82 (86.3%) were positive for *tcdA* and *tcdB* genes (A+B+) and 13 (13.7%) contained only the *tcdB* gene (A−B+). Five of the A+B+ isolates (5.3%, 5/95) were positive for *cdtA* and *cdtB*.

We used MLST to analyze the toxigenic strains, and divided them into 15 different STs. The most prevalent STs were ST-54 (23.2%, 22/95), ST-35 (16.8%, 16/95), ST-2 (13.7%, 13/95), ST-3 (9.5%, 9/95), and ST-37 (9/95). We also identified 4 ST-5 isolates that were positive for *tcdA*, *tcdB*, and the binary toxin genes, two from the clinical gastroenterology department, one from the clinical hematology department, and one from another clinical department.

In the clinical gastroenterology department, ST-54 (22.2%, 12/54), ST-2 (16.7%, 9/54), ST-3 (11.1%, 6/54) were the most common, whereas ST-54 (45.5%, 5/11) and ST-37 (27.3%, 3/11) were the most common in the clinical hematology department. Among the 4 patients with recurrence, 2 had relapses with the same STs (ST-54 and ST-285), 1 was re-infected another ST (ST-5 then ST-35), 1 was infected 4 times with different STs (ST-2, ST-54, ST-102, and then ST-54).

Antimicrobial resistance

Our analysis of the distribution of the MICs of the different antimicrobial agents (Table 2) indicated that metronidazole and vancomycin had *in vitro* activity against all of the toxigenic isolates within a narrow range. Two ST-37 isolates had resistance to linezolid, with MICs above 4 μg/mL. Most isolates were resistant to clindamycin (69.4%) and erythromycin (62.1%). Analysis of the quinolones indicated 100% resistance to ciprofloxacin and 22.1% resistance to moxifloxacin. The resistance to tetracycline was 14.7% and the resistance to rifampicin was 7.4%. Four of the 7 strains resistant to rifampicin were ST-37. The isolates with binary toxin genes had lower MICs to all tested antibiotics and were all susceptible to erythromycin and moxifloxacin.

Analysis of multidrug-resistant strains, defined as those with resistance to at least 3 antibiotics, indicated that 24.2% (23/95) of the toxigenic isolates were multidrug-resistant. These multidrug-resistant strains were ST-35 (7/23), ST-37 (6/23), ST-81 (3/23), ST-3 (3/23), ST-2 (2/23), and ST-54 (2/23).

Table 1
Demographics of outpatient study participants.

Characteristic	All outpatients (%)	Patients without CDI (%)	Patients with CDI (%)
Total (n)	1213	1124	89
Gender (n)			
Male	575 (47.4)	521 (46.4)	54 (60.7)
Female	638 (52.6)	603 (53.6)	35 (39.3)
Clinical Department (n)			
CIDD	117 (9.6)	109 (9.7)	8 (9.0)
ED	232 (19.1)	225 (20.0)	7 (7.9)
CGD	507 (41.8)	458 (40.7)	49 (55.1)
CHD	71 (5.9)	61 (5.4)	10 (11.2)
ODs	286 (23.6)	271 (24.1)	15 (16.9)
Median age, years (range) (year)	48(2–93)	47(2–93)	56(2–93)
Male	49 (2–93)	47(2–93)	56 (19–93)
Female	5548(2–92)	47(2–91)	55 (2–92)
Visiting CIDD	51(11–93)	50(11–93)	62(32–92)
Visiting ED	55(14–92)	54(14–92)	65(53–86)
Visiting CGD	54(2–93)	53(2–87)	62(2–93)
Visiting CHD	36(2–84)	36(2–84)	38(16–51)

Abbreviations: CIDD, Clinical Infectious Disease Department; ED, Emergency Department; CGD, Clinical Gastroenterology Department; CHD, Clinical Hematology Department

Table 2
Resistance of the 95 toxigenic *C. diffiicle* isolates to different antibiotics.

STs (No.of isolates)	Percentage of isolates(%)	Vancomycin	Metronidazole	Linezolid	Clindamycin	Rifampicin	Ciprofloxacin	Moxifloxacin	Erythromycin	Tet
ST-2(13)	13.68	0(0)	0(0)	0(0)	7(53.8)	1(7.7)	7(100)	2(15.4)	3(23.1)	1(7.
ST-3(9)	9.47	0(0)	0(0)	0(0)	8(88.9)	0(0)	9(100)	4(44.4)	4(44.4)	1(1
ST-5(4)	4.21	0(0)	0(0)	0(0)	1(25)	0(0)	4(100)	0(0)	0(0)	0(0
ST-8(4)	4.21	0(0)	0(0)	0(0)	0(0)	0(0)	4(100)	0(0)	0(0)	0(0
ST-11(1)	1.05	0(0)	0(0)	0(0)	1(100)	0(0)	1(100)	0(0)	0(0)	1(1
ST-33(3)	3.16	0(0)	0(0)	0(0)	1(33.3)	0(0)	3(100)	0(0)	0(0)	0(0
ST-35(16)	16.84	0(0)	0(0)	0(0)	11(68.8)	1(0.6)	16(100)	3(18.8)	14(87.5)	8(5
ST-37(9)	9.47	0(0)	0(0)	2(22.2)	8(88.9)	4(44.4)	9(100)	5(55.6)	9(100)	2(2
ST-53(1)	1.05	0(0)	0(0)	0(0)	1(100)	0(0)	1(100)	0(0)	0(0)	0(0
ST-54(22)	23.16	0(0)	0(0)	0(0)	18(81.8)	1(4.5)	22(100)	3(13.6)	21(95.5)	1(4.
ST-81(4)	4.21	0(0)	0(0)	0(0)	4(100)	0(0)	4(100)	3(75)	4(100)	0(0
ST-102(2)	2.11	0(0)	0(0)	0(0)	1(50)	0(0)	22(100)	0(0)	1(50)	0(0
ST-129(3)	3.16	0(0)	0(0)	0(0)	3(100)	0(0)	3(100)	0(0)	3(100)	0(0
ST-139(2)	2.11	0(0)	0(0)	0(0)	0(0)	0(0)	2(100)	0(0)	0(0)	0(0
ST-285(2)	2.11	0(0)	0(0)	0(0)	0(0)	0(0)	2(100)	0(0)	0(0)	0(0
Total number (95)	100	0(0)	0(0)	2(2.2)	66(69.4)	7(7.4)	95(100)	21(22.1)	59(62.1)	14(

Discussion

Our 10-year surveillance of community-onset CDI at a tertiary hospital of mainland China indicated that 89 patients (7.3%) had CDI and 4.5% of these patients experienced at least one recurrence. This observation is line with our earlier study, which identified 8.9% of toxigenic *C. difficile* isolates from inpatients(11). There were two reasons for the small number of CO-CDI patients in this study. Unlike other studies of CO-CDI, which recruited patients admitted within 48 h, we only analyzed individuals who visited outpatient clinics, so it is likely that most patients with severe or difficult-to-treat CO-CDI were admitted to a hospital. Another reason for our smaller number of CO-CDI cases may be the poor awareness of CDI among general healthcare workers, which could have led to missed cases. In the study by Bauer et al., there was no specific request to test for *C. difficile*, which could have caused six of ten cases to be missed(14).

Our outpatients with CDI were older than those without CDI (56 *vs.* 47 years). However, the median age of our CDI patients was similar to that of inpatients with CDI in our previous study (56 years)(15). A previous study reported that patients with CO-CDI were younger than those with HO-CDI(5). However, the present study clearly showed that CDI patients tended to be older, in that nearly half of the cases (43/89) were 60 or more years-old. Moreover, the median age of the 10 patients from the clinical hematology department was 38 years, and this biased the total median age. Epidemiological studies in Western countries showed that CDI were more common in females than males(16), and a large study of 113 laboratories across England also reported that 67% of CA-CDI cases were in females(16, 17). However, males accounted for more than half of the outpatients in the present study, similar to the proportion of inpatients in our previous study(15). This difference may be due to gender differences in care-seeking behaviors among different countries.

More than half of our cases were from the clinical gastroenterology department, indicating that diarrhea or gastrointestinal discomfort were the most common reasons for visiting the clinic. Patients visiting this department were older than those who visited other outpatient departments. This is consistent with the interpretation that older individuals are more susceptible to CDI(18). Another interesting founding in this study was that the positive rate for CDI was highest in our clinical hematology department, and the median age was younger in this department. Our review of the records of these patients indicated that most of them were follow-up patients who received chemotherapy or haematopoietic stem cell transplants (HSCTs) and these cases of *C. difficile* were healthcare-facility acquired community onset *C. difficile* infection. There is evidence that patients receiving chemotherapy for haematological malignancies or HSCTs have an increased risk for CDI(19). Thus, it is important for clinicians to consider testing for *C. difficile* in outpatients developing diarrhea who are older (≥60 years) or immunocompromised. Recurrences of CDI are serious, and the management of these patients is challenging. A meta-analysis found that the recurrence rate of CDI was 13 to 50% among all patients after an initial episode(20). The recurrence in the present study was 4.5%, similar to that reported by Tsai et al. (4.7%)(21), but lower than reported in another study (15.9%)(22). We speculate this may be because our CO-CDI patients were not as severely ill as hospitalized patients and because some of our patients were lost to follow-up due to visiting other hospitals.

A+B+ strains were the most common toxin types in the present study, similar to our previous study(11). The epidemiology of *C. difficile* is region-specific. Thus, CDI cases in Europe are mostly from RT002 and RT056(16). In the present study, ST-54, ST-35, and ST-2 were the most common STs, similar to our previous study which identified ST-54, ST-35, and ST-37 as the three most common STs among patients hospitalized with CDI(12). Although ST-2 was not among the major STs isolated from inpatients in our previous studies(12), ST-2 is a major ST of *C. difficile* isolated from patients with community associated CDI in many European countries(23). Future studies are needed to examine the differences of ST diversity of *C. difficile* isolates in those with community onset and hospital onset CDI. Interestingly, the distributions of STs differed among our different departments. For example, ST-54 was the most common in the clinical gastrointestinal department and clinical hematology department, but ST-37 was the most common in the clinical infectious disease department. In general, the STs of inpatients and outpatients are similar, and a previous study showed 79% of the same RTs in healthcare facilities and community settings(24). This may be attributed to outpatients who were hospitalized and reviewed after discharge, especially in the hematology clinic and the infection clinic. Fortunately, none of the isolates was identified as the hypervirulent ST-1 (BI/NAP1/027), but there was one ST-11 (ribotype [RT] 078) isolate from the clinical hematology department.

Vancomycin and metronidazole are the only two antimicrobial agents used to treat CDI in mainland China. Fortunately, all toxigenic *C. difficile* isolates from our outpatients were sensitive to these two antibiotics and the MICs were low. Compared with our previous study, the isolates from outpatients and hospitalized patients had similar resistance profiles(11). However, we observed a low resistance to tetracycline, slightly lower than reported for hospital-acquired CDI but not significantly different from reports in the Asia-Pacific region(25). These differences in antimicrobial resistance may be attributed to differences in exposure to antibiotics in outpatients and individuals in the community, and to the use of different antibiotics in different regions. Another important finding of the present study is that nearly one-quarter of the isolates were multidrug-resistant, much lower than previously reported for isolates from inpatients(11). Based on this, we speculate that most of the strains we identified were from community, where antibiotic use is much less common.

There were some limitations in this study. Firstly, we only analyzed patients visiting clinics, and this may have reduced the number of CO-CDI for those who were admitted within 48 h. Secondly, we did not classify CDI as community-acquired, or healthcare-acquired. Thirdly, we were missing data regarding antibiotic exposure, comorbidities, and outcomes, and could therefore not identify risk factors associated with CDI. Therefore, further research is needed to address these limitations.

Conclusion

In conclusion, this retrospective study described community-onset CDI at a tertiary hospital over a period of about 10 years. There were many outpatients with CDI, the *C. difficile* isolates had high genetic diversity, and ST-54 was the most common ST. All isolates were susceptible to metronidazole and vancomycin, the resistance rates varied for the other tested antibiotics, and nearly one-quarter of the isolates were multidrug-resistant. Physicians should consider CDI during the differential diagnosis of a patient who presents with community-onset diarrhea or abdominal pain, either alone or in combination. Further studies that analyze whole-genome sequences and epidemiological data are required to investigate the genetic relationships of hospitalized patients and outpatients, especially follow-up patients.

Declarations

Ethics approval and consent to participate. Participants or their legally authorized representatives gave informed consent to be included in the study. Ethical approval for the study was granted by the First Affiliated Hospital, School of Medicine, Zhejiang University (Approval number1554-1).

Consent for publication. Not applicable.

Availability of data and materials. All data generated or analysed during this study are included in this published article

Competing interests. The authors declare that they have no competing interests.

Funding. This work was supported by a grant from National Nature Science Foundation of China (No. 82073609).

Authors' contributions. Yunbo Chen analyzed the data and drafted the paper. Lihong Bu revised the analyzed data. Tao Lv, Lisi Zheng and SilanGu performed the experiments. Jianqin He designed and revised the paper. Yunbo Chen and Lihong Bu contributed equally to this work. All authors contributed and approved the final article.

Acknowledgements. Not applicable.

Authors' information (optional). This section is optional.

References

1. Rupnik M, Wilcox MH, Gerding DN. Clostridium difficile infection: new developments in epidemiology and pathogenesis. Nat Rev Microbiol. 2009;7(7):526-36.

2. Pothoulakis C. Pathogenesis of Clostridium difficile-associated diarrhoea. Eur J Gastroenterol Hepatol. 1996;8(11):1041-7.

3. McEllistrem MC, Carman RJ, Gerding DN, Genheimer CW, Zheng L. A hospital outbreak of Clostridium difficile disease associated with isolates carrying binary toxin genes. Clin Infect Dis. 2005;40(2):265-72.

4. Bassetti M, Villa G, Pecori D, Arzese A, Wilcox M. Epidemiology, diagnosis and treatment of Clostridium difficile infection. Expert Rev Anti Infect Ther. 2012;10(12):1405-23.

5. Crobach MJT, Notermans DW, Harmanus C, Sanders I, De Greeff SC, Kuijper EJ. Community-Onset Clostridioides Difficile Infection in Hospitalized Patients in The Netherlands. Open Forum Infect Dis. 2019;6(12):ofz501.

6. Guh AY, Mu Y, Winston LG, Johnston H, Olson D, Farley MM, et al. Trends in U.S. Burden of Clostridioides difficile Infection and Outcomes. N Engl J Med. 2020;382(14):1320-30.

7. Tan XQ, Verrall AJ, Jureen R, Riley TV, Collins DA, Lin RT, et al. The emergence of community-onset Clostridium difficile infection in a tertiary hospital in Singapore: a cause for concern. Int J Antimicrob Agents. 2014;43(1):47-51.

8. Kwon SS, Gim JL, Kim MS, Kim H, Choi JY, Yong D, et al. Clinical and molecular characteristics of community-acquired Clostridium difficile infections in comparison with those of hospital-acquired C. difficile. Anaerobe. 2017;48:42-6.

9. Mora Pinzon MC, Buie R, Liou JI, Shirley DK, Evans CT, Ramanathan S, et al. Outcomes of Community and Healthcare-onset Clostridium difficile Infections. Clin Infect Dis. 2019;68(8):1343-50.

10. Bauer MP, Kuijper EJ, van Dissel JT. European Society of Clinical Microbiology and Infectious Diseases (ESCMID): treatment guidance document for Clostridium difficile infection (CDI). Clin Microbiol Infect. 2009;15(12):1067-79.

11. Chen YB, Gu SL, Shen P, Lv T, Fang YH, Tang LL, et al. Molecular epidemiology and antimicrobial susceptibility of Clostridium difficile isolated from hospitals during a 4-year period in China. J Med Microbiol. 2018;67(1):52-9.

12. Chen YB, Gu SL, Wei ZQ, Shen P, Kong HS, Yang Q, et al. Molecular epidemiology of Clostridium difficile in a tertiary hospital of China. J Med Microbiol. 2014;63(Pt 4):562-9.

13. Griffiths D, Fawley W, Kachrimanidou M, Bowden R, Crook DW, Fung R, et al. Multilocus sequence typing of Clostridium difficile. J Clin Microbiol. 2010;48(3):770-8.

14. Bauer MP, Veenendaal D, Verhoef L, Bloembergen P, van Dissel JT, Kuijper EJ. Clinical and microbiological characteristics of community-onset Clostridium difficile infection in The Netherlands. Clin Microbiol Infect. 2009;15(12):1087-92.

15. Xu Q, Chen Y, Gu S, Lv T, Zheng B, Shen P, et al. Hospital-acquired Clostridium difficile infection in Mainland China: A seven-year (2009-2016) retrospective study in a large university hospital. Sci Rep. 2017;7(1):9645.

16. Fawley WN, Davies KA, Morris T, Parnell P, Howe R, Wilcox MH. Enhanced surveillance of Clostridium difficile infection occurring outside hospital, England, 2011 to 2013. Euro Surveill. 2016;21(29).

17. Ofori E, Ramai D, Dhawan M, Mustafa F, Gasperino J, Reddy M. Community-acquired Clostridium difficile: epidemiology, ribotype, risk factors, hospital and intensive care unit outcomes, and current and emerging therapies. J Hosp Infect. 2018;99(4):436-42.

18. Donskey CJ. Clostridium difficile in Older Adults. Infect Dis Clin North Am. 2017;31(4):743-56.

19. Gu SL, Chen YB, Lv T, Zhang XW, Wei ZQ, Shen P, et al. Risk factors, outcomes and epidemiology associated with Clostridium difficile infection in patients with haematological malignancies in a tertiary care hospital in China. J Med Microbiol. 2015;64(Pt 3):209-16.

20. Garey KW, Sethi S, Yadav Y, DuPont HL. Meta-analysis to assess risk factors for recurrent Clostridium difficile infection. J Hosp Infect. 2008;70(4):298-304.

21. Tsai CS, Hung YP, Lee JC, Lee NY, Chen PL, Syue LS, et al. Community-onset Clostridium difficile infection at a tertiary medical center in southern Taiwan, 2007-2015. J Microbiol Immunol Infect. 2018;51(2):243-50.

22. Visconti V, Brunetti G, Cuomo MR, Giordano A, Raponi G. Nosocomial-acquired and community-onset Clostridium difficile infection at an academic hospital in Italy: Epidemiology, recurrences and toxin genes distribution. J Infect Chemother. 2017;23(11):763-8.

23. Bauer MP, Notermans DW, van Benthem BH, Brazier JS, Wilcox MH, Rupnik M, et al. Clostridium difficile infection in Europe: a hospital-based survey. Lancet. 2011;377(9759):63-73.

24. Furuya-Kanamori L, Riley TV, Paterson DL, Foster NF, Huber CA, Hong S, et al. Comparison of Clostridium difficile Ribotypes Circulating in Australian Hospitals and Communities. J Clin Microbiol. 2017;55(1):216-25.

25. Luo Y, Cheong E, Bian Q, Collins DA, Ye J, Shin JH, et al. Different molecular characteristics and antimicrobial resistance profiles of Clostridium difficile in the Asia-Pacific region. Emerg Microbes Infect. 2019;8(1):1553-62.

Figures

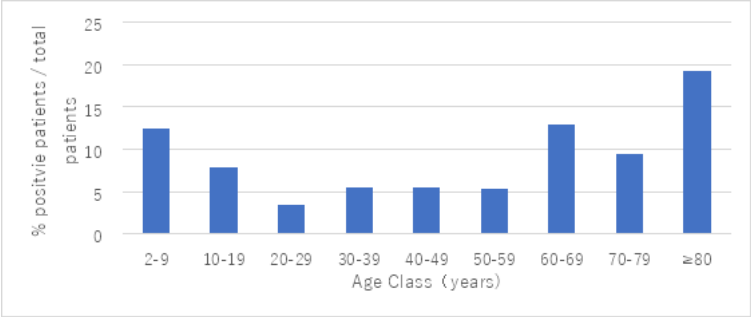

Figure 1

Percentages of outpatients with CDI in different age classes.