

Emergence of *Clostridium difficile* infection in tuberculosis patients due to a highly rifampicin-resistant PCR ribotype 046 clone in Poland

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Abstract *Clostridium difficile* infection (CDI) is a major cause of nosocomial diarrhea. CDI is known to develop after antibiotic administration, but anti-tuberculosis agents have rarely been implicated. We documented an outbreak caused by a highly rifampicin-resistant *C. difficile* strain of polymerase chain reaction (PCR) ribotype 046 in patients with active tuberculosis.

Introduction

Clostridium difficile infection (CDI), the most common infectious cause of hospital-acquired diarrhea, is usually preceded by the use of antibiotics such as fluoroquinolones (FQ), cephalosporins, broad-spectrum penicillins, and clindamycin [1]. Anti-tuberculosis drugs are not commonly associated with the development of CDI [2–5]. Recently, FQ have been linked to CDI, particularly those cases caused by

polymerase chain reaction (PCR) ribotype 027 [6, 7]. The objective of our study was to determine the characteristics of *C. difficile* isolates associated with an outbreak of infection in a tuberculosis unit. Patients were hospitalized in the Specialized Hospital of Lung Diseases and Tuberculosis (SHLDT) in Bystra, South Poland. The clinical findings were also assessed.

Materials and methods

Setting and study design

This study was conducted in the 250-bed SHLDT harboring a 46-bed tuberculosis unit, located in South Poland. During the study period, a total of 8,158 patients were hospitalized in the SHLDT. From September 2009 through December 2010, CDI was suspected in 23 symptomatic patients. CDI was diagnosed in ten patients, corresponding to an incidence of 12.3/10,000 patients admitted to the SHLDT. CDI was diagnosed by an immunoenzymatic assay (*C. difficile* TOX A/B II, TechLab, Blacksburg, VA, USA) and *C. difficile* culture for patients who developed diarrhea. The clinical characteristics of the ten patients were observed prospectively and *C. difficile* isolates from the patients were collected for further study.

Definition of CDI

Diarrhea was defined as the passage of three or more unformed stools in 24 h or less. CDI was diagnosed when patients had diarrhea and *C. difficile* toxin(s) was/were detected in fecal samples and/or cultures revealed the presence of *C. difficile*. We defined hospital-acquired CDI

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(HA-CDI) when diarrhea developed at least 48 h after hospitalization or within 2 months of hospital discharge if a patient had a complicated clinical status involving admission to the Intensive Care Unit or surgical intervention [8].

Data collection

The clinical data collected in this study were age, length of hospitalization, medication history, underlying conditions, and the use of antibiotics or chemotherapeutics, especially anti-tuberculosis medication. All adults (mean age 64.3 years) who were suspected of CDI hospitalized at the SHLDT in the period between September 09, 2009 and December 31, 2010 were included in the study. Stool characteristics, frequency of defecation, and abdominal pain were monitored.

Microbiological studies

All fecal samples from ten *C. difficile* toxin-positive diarrheic patients were inoculated anaerobically on *C. difficile*-selective cycloserine-cefoxitin agar plates (CLO, bioMérieux SA, Marcy l'Etoile, France). Colonies were identified as *C. difficile* on the basis of characteristic growth on selective medium, yellow-green fluorescence under UV light (365 nm), the specific odor, and API 20A (bioMérieux SA, Marcy l'Etoile, France). PCR assays to detect *tcdA* and *tcdB* genes and the specific deletion in the *tcdA* gene were conducted as previously described [9]. Primers described by Stubbs et al. were used for amplification of the binary toxin genes *cdtA* and *cdtB* [10]. Three strains were included in this study as controls: toxigenic *C. difficile* VPI 10463 (A+B+), non-toxigenic *C. difficile* NIHBRIGS 8050 (A-B-), and *C. difficile* GAI 95601 (A-B+) (from H. Kato, Institute of Anaerobic Bacteriology, Gifu University School of Medicine, Gifu, Japan). PCR ribotyping was performed as described by Stubbs et al. [11]. Banding patterns were compared with those of the library of PCR ribotypes at the Anaerobe Reference Laboratory (ARU), Cardiff, Wales, UK. Strains belonging to different PCR ribotypes were used as internal controls.

Antimicrobial drug susceptibility testing

Cultures of *C. difficile* strains on Columbia agar plates (bioMérieux SA, Marcy l'Etoile, France) were suspended and adjusted to an OD₉₅₀ of 1 on the McFarland scale (using a bioMérieux ATB1550 densitometer) and grown as lawns on Brucella agar plates. Plastic strips (E-test, bioMérieux SA, Marcy l'Etoile, France) with gradients of antibiotics, clindamycin (CL), erythromycin (EM), ciprofloxacin (CI), moxifloxacin (MX), metronidazole (MTZ), vancomycin (VA), and rifampicin (RI), were placed on each plate and

incubated anaerobically at 37 °C for 48 h, according to the manufacturer's instructions. According to the Clinical and Laboratory Standards Institute (CLSI) recommendations [12], antibiotic resistance was defined as follows: minimum inhibitory concentration (MIC) ≥ 8.0 mg/L for clindamycin and erythromycin, MIC ≥ 4.0 mg/L for ciprofloxacin and moxifloxacin. Resistance to metronidazole and vancomycin was defined according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST): for metronidazole MIC ≥ 1 mg/L and for vancomycin MIC > 2 mg/L [13]. No CLSI guidelines exist for determining the susceptibility of *C. difficile* to the rifamycins, so these ranges were determined based on the natural breakpoints observed previously, and rifampin susceptibility was categorized according to the MICs determined (≤ 0.002 $\mu\text{g/mL}$, 0.003–32 $\mu\text{g/mL}$, and > 32 mg/L) [14]. Quality control strains (*Bacteroides fragilis* NCTC 11295, *B. thetaotaomicron* ATCC 29741, *Escherichia coli* ATCC 25922, and *Staphylococcus aureus* ATCC 25923) were always included.

Results

Clinical findings

The demographic data, medication history, and clinical and laboratory characteristics of CDI are presented in Table 1. Ten patients with an age range from 48 to 86 years (median age 64.3 years) were diagnosed with a CDI between September 2009 and December 2010. There were four women and six men: six patients were hospitalized in the tuberculosis unit and four patients in other units: pulmonary ($n=2$), internal medicine ($n=1$), and thoracic surgery ($n=1$). Of the ten patients, five had active pulmonary tuberculosis as diagnosed by sputum smear and culture. These patients were treated with anti-tuberculosis agents, including isoniazid, rifampicin, and pyrazinamide. Watery diarrhea was observed in all patients, and provided the major clinical manifestation. Three patients developed CDI recurrence. Five patients died because of CDI and the attributed mortality was 50 % (5/10).

Microbiological analysis

Ten *C. difficile* strains were available for investigation. All ten isolates contained *tcdA* and *tcdB* genes. The PCR detecting the repeated sequences in the toxin A gene was positive for a single isolate and generated a 700-bp product similar to that obtained for the Japanese control GAI 95601 *C. difficile* strain. PCR ribotyping classified the ten isolates according to their ribotypes: 046 ($n=7$), 001 ($n=1$), 002 ($n=1$), and 017 ($n=1$). All strains were resistant to

Table 1 Clinical summary for ten cases of *Clostridium difficile* infection (CDI) in the Specialized Hospital of Lung Diseases and Tuberculosis (SHLDT) in Bystra, South Poland

Date of hospitalization	Age (years)/sex/number of strain	Unit	Underlying diseases	Anti-TB drugs	Other AB	Date of diagnostic CDI	Symptoms of CDI	Fatality associated with CDI	PCR ribotype/rifampicin RI (R) or RI (S)
27.11.09–17.12.09	82/F/CD14	TBU	Non-active TB, atherosclerosis, hypertension	–	AMOX	09.12.09	Diarrhea, hypovolemic shock, extreme dehydration	Yes	046/RI (R)
13.10.09–04.01.10	86/F/CD12	TBU	AP TB, chronic congestive heart diseases	RI, I	CI	09.12.09	Recurrent diarrhea, heart failure (associated with CDI)	Yes	046/RI (R)
03.11.09–08.01.10	80/F/CD15	TBU	AP TB, failure, chronic obstructive pulmonary	RI, I, P	–	11.12.09	Diarrhea	–	046/RI (R)
19.11.09–02.02.10	48/M/CD13	TBU	AP TB, alcoholism, cirrhosis of the liver	RI, I, P	–	09.12.09	Recurrent diarrhea, neurological symptoms (associated with CDI)	Yes	046/RI (R)
17.02.09–09.04.10	67/F/CD18	TBU	AP TB, lung empyema, cirrhosis of the liver	RI, I, P	–	30.12.09	Recurrent severe diarrhea	Yes	046/RI (R)
19.11.09–12.02.10	51/M/CD4	TBU	AP TB, pulmonary mycetoma	RI, I, P	CI	12.01.10	Diarrhea	–	046/RI (R)
04.02.10–03.03.10	86/M/CD22	INT	Respiratory systems diseases	–	AMOX, CI	25.02.10	Diarrhea	–	046/RI (R)
17.03.10–25.03.10	72/M/CD30	PUL	Pneumonia, lung empyema	–	AMOX, CI, CM, MZN	22.03.10	Severe diarrhea	Yes	017/RI (R)
29.11.10–05.02.11	88/M/CD61	PUL	Pneumonia, chronic obstructive pulmonary renal failure	–	AMOX, TX	30.12.10	Diarrhea	–	001/RI (S)
14.09.10–23.09.10	69/M/CD50	TS	Lung tumor	–	CEF	23.09.10	Diarrhea	–	002/RI (S)

AB antibiotics, CDI *C. difficile* infection, TBU tuberculosis unit, INT internal unit, PUL pulmonology, TS thoracic surgery, TB tuberculosis, AP TB active pulmonary tuberculosis, RI rifampicin, I isoniazid, P pyrazinamide, AMOX amoxicillin, CI ciprofloxacin, CM clindamycin, MZN metronidazole, TX trimethoprim/sulfamethoxazole, CEF cefazolin (perioperative prophylaxis), RI (R) rifampicin resistant, RI (S) rifampicin sensitive

ciprofloxacin (CI; MIC \geq 32 mg/L), eight strains to moxifloxacin (MX; MIC \geq 32 mg/L), seven strains to clindamycin (CM; MIC \geq 256 mg/L) and erythromycin (EM; MIC \geq 256 mg/L), and seven to rifampicin (RI; MIC \geq 32 mg/L). All strains were susceptible to metronidazole (MZN; MIC range 0.047–0.38 mg/L). The MIC range for vancomycin (VA) was 0.75–1 mg/L. Three strains had reduced susceptibility to vancomycin (MIC=1 mg/L) (according to the EUCAST breakpoints). All strains belonging to PCR ribotype 046 were highly resistant to moxifloxacin, clindamycin, erythromycin, and rifampicin.

Discussion

CDI caused by *C. difficile* is a major hospital problem. During the past decade, the hypervirulent ribotype 027 strain, expressing the binary toxin, caused outbreaks in North America and Europe [6, 7]. PCR ribotype 046 was less commonly detected in a European survey performed in 2008; it was found in 2 % of all the tested isolates, whereas types 014/020, 001, and 078 belonged to the most frequently encountered types [8]. Only five strains belonging to PCR ribotype 046 were found in the University Hospital in Warsaw, Poland, between 2004 and 2006 [9]. All historic strains

were highly resistant to clindamycin and erythromycin (MICs ≥ 256 mg/L), but fully susceptible to moxifloxacin and rifampicin (unpublished data). In our present study, resistance to moxifloxacin was found in all strains belonging to PCR ribotype 046. Resistance to newer fluoroquinolones has been described not only in the hypervirulent strain 027, but also in other emerging PCR ribotypes circulating in hospital settings [15, 16].

Anti-tuberculosis agents are very rarely associated with CDI [2–4]. Agents such as isoniazid and pyrazinamide have little or no effect on the intestinal flora, but rifampicin has an antibiotic effect on a wide range of bacteria [17]. *C. difficile* is usually highly resistant to rifampicin, and rifampicin derivatives have been used for CDI treatment [18]. The emergence of rifampicin-resistant *C. difficile* has been reported in patients with prolonged rifampicin use [19]. In our study, all PCR ribotype 046 strains isolated from patients with prolonged rifampicin therapy were highly resistant to this drug. Multidrug resistance (i.e., resistance to clindamycin, moxifloxacin, and rifampicin) was limited primarily to ribotype 027 isolates [16].

Our findings suggested that: (1) patients who are treated with anti-tuberculosis agents, especially rifampicin, who developed acute diarrhea during or after therapy should be evaluated for CDI, (2) treatment with rifampicin can lead to high-level resistance to rifampicin in *C. difficile* strains, (3) the emergence of multidrug-resistant *C. difficile* PCR ribotype 046 may be detrimental to anti-tuberculosis chemotherapy.

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Conflict of interest The authors declare that they have no conflicts of interest.

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