Eur J Clin Microbiol Infect Dis (2008) 27:1201–1207
DOI 10.1007/s10096-008-0564-9

ARTICLE

Epidemiology of *Clostridium difficile*-associated disease at University Hospital Basel including molecular characterisation of the isolates 2006–2007

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Received: 21 January 2008 / Accepted: 25 May 2008 / Published online: 17 June 2008

Abstract A prospective study was conducted during a one-year period between 2006 and 2007 to describe the epidemiology of *Clostridium difficile*-associated disease (CDAD) at University Hospital Basel, Switzerland (UHBS) and to determine phenotypic and genotypic features of *C. difficile* strains isolated at the Microbiology Laboratory UHBS including strains from regional non-university hospitals. We prospectively identified 78 CDAD cases at UHBS with an incidence of 2.65/1,000 hospitalised patients or 2.3/10,000 patient-days. Sixteen patients (20.5%) were infected with clindamycin-resistant strains of PCR-ribotype 027 during an outbreak at the geriatric hospital. Among 124 single-patient isolates, 28 (22.6%) were resistant to moxifloxacin and 34 (27.4%) were resistant to clindamycin, but all remained susceptible to metronidazole and vancomycin. Of 102 toxigenic isolates, 19 (18.7%) had an 18-bp deletion in the *tcdC* gene, eight (7.8%) a 39-bp deletion, and one (1.0%) a 54-bp deletion. Genes for binary toxin were present in 27 (21.8%). PCR-ribotype 027 was associated with older age (median age 83.5 vs. 65.5 years, $p<0.0001$) and longer duration of hospitalisation before onset of disease (median 15.5 vs. 9 days, $p=0.014$) with a trend towards higher crude mortality, more severe disease, and previous use of macrolides compared to ribotype non-027. Overall, severe disease correlated with use of a nasogastric tube and surprisingly shorter duration of hospitalisation before onset of disease. Today, laboratory-based and epidemiological surveillance systems are required to monitor CDAD cases and emergence of new epidemic strains.

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Introduction

Clostridium difficile is one of the most important nosocomial pathogens. It is the most frequent cause of antibiotic-associated diarrhoea [1, 2]. *C. difficile*-associated disease (CDAD) has become an increasing clinical problem in the hospital setting as well as in the community [3]. The spectrum of disease is wide—from asymptomatic colonization, mild or self-limiting diarrhoea to life-threatening pseudomembranous colitis [4]. Recently, a new virulent *C. difficile* strain characterised as toxinotype III, North American pulsed-field type 1, restriction-endonuclease analysis group type BI, and PCR-ribotype 027 has emerged worldwide causing outbreaks in North America and Europe [4, 5]. CDAD due to this hypervirulent strain is associated with increased morbidity and mortality [6–8]. It has been suggested that increased virulence results from hyperproduction of toxins A and B as a result of deletions in the putative negative regulator gene (*tcdC*) leading to truncated amino acid residues with insufficient function [9]. More severe disease and a higher case-fatality rate have also been associated with *C. difficile* strains producing actin-specific ADP-ribosyltransferase (binary toxin) [6, 10]. Other toxin-variant strains such as toxin A-negative/toxin B-positive and toxin A-positive/toxin B-negative strains are also associated with disease or outbreaks [11, 12].

In this study, we aimed to determine phenotypic and genotypic features of *C. difficile* strains isolated at the

Microbiology Laboratory of the University Hospital Basel, Switzerland during a one-year period between 2006 and 2007. Furthermore, we prospectively identified all CDAD cases including a first cluster of 16 CDAD patients with clindamycin-resistant *C. difficile* PCR-ribotype 027 at University Hospital Basel and the affiliated geriatric hospital in Basel (UHBS) [13] during the same study period. We analysed clinical and epidemiological patient data as well as the corresponding *C. difficile* isolates in order to compare strain characteristics with clinical presentation.

Material and methods

Microbiological analyses

All faecal specimens from patients suspected of having CDAD and submitted to the laboratory for *C. difficile* testing between June 2006 and July 2007 were included in the study. Specimens were sent by UHBS and surrounding hospitals. Samples were tested for toxin A/B using the enzyme-linked immunosorbent assay C.DIFF TOX A/B II (TechLab/Wampole, Blacksburg, VA, USA) according to manufacturer's instructions and simultaneously cultured as previously described [14]. DNA was extracted with LC MagnaPure systems (Roche Diagnostics, Rotkreuz, Switzerland). A single patient isolate was defined as an isolate per patient including repetitive isolates if at least four weeks apart. Genes for toxins A/B and binary toxin were detected by PCR [15]; the *tcdC* genotype was determined by sequencing [15, 16]. Ribotyping was performed by the methods of Stubbs et al. [17]. Antibiotic susceptibility to metronidazole, moxifloxacin, clindamycin, and vancomycin was determined by Etest (AB Biodisk, Solna, Sweden) and interpreted in accordance with the Clinical and Laboratory Standards Institute [18]. MICs ≥8 mg/l for moxifloxacin and MICs ≥8 mg/l for clindamycin were regarded as resistant; MICs ≤8 mg/l for metronidazole and MICs ≤2 mg/l for vancomycin were deemed susceptible.

Clinical data and definitions

The UHBS is a 680-bed tertiary care centre with 27,000 admissions and 167,000 outpatients per year. In 2001, the UHBS initiated a surveillance system for *C. difficile*. Cases are classified as asymptomatic carrier, mild or severe CDAD. For the purpose of this study, additional data from cases were prospectively completed by full-chart review using a standardized case report form over 12 months. Data cleaning was performed by manually reviewing the charts by a board-certified infectious diseases specialist and by checking for outliers in the analyses. The following date were collected: demographic data (age, sex), duration of hospitalisation, hospital ward, symptoms of CDAD (diarrhoea defined as more than three episodes per day), fever, relevant laboratory results (C-reactive protein, white blood cell count, albumin), antibiotic therapy, comorbidity using the Charlson index [19], McCabe classification [20], and

Table 1 Phenotypic and genotypic features of 124 *Clostridium difficile* single-patient strains isolated during a one-year period at the Microbiology Laboratory, University Hospital Basel

	All (n=124) n (%)	PCR-ribotype 027 (n=18) n (%)	PCR-ribotype non-027 (n=106) n (%)
Moxifloxacin resistant	28 (22.6)	18 (100)	10 (9.4)
Clindamycin resistant	34 (27.4)	18 (100)	16 (15.1)
Both resistant	21 (16.9)	18 (100)	3 (2.8)
Toxine A/B profile			
Tox A –/B –	21 (16.9)	0	21 (19.8)
Tox A +/B–	2 (1.6)	0	2 (1.9)
Tox A +/B +	101 (81.5)	18 (100)	83 (78.3)
Binary toxin +	27 (21.8)	18 (100)	9 (8.5)
tcdC genotype (n)		tcdC-sc1 (18)	tcdC-A (8), tcdC-UHBS2 (1)
tcdC genotype (n=102)			
Wildtype WT-1	7 (6.9)	0	7 (8.3)
Wildtype WT-2	3 (2.9)	0	3 (3.6)
Wildtype WT-3	4 (3.9)	0	4 (4.8)
Wildtype WT-4	60 (58.8)	0	60 (71.4)
tcdC-sc1 (18-bp deletion)	18 (17.7)	18 (100)	0
tcdC-A (39-bp deletion)	8 (7.8)	0	8 (9.5)
tcdC-B (18-bp deletion)	1 (1.0)	0	1 (1.2)
UHBS-2 (54-bp deletion)	1 (1.0)	0	1 (1.2)

tcdC putative negative regulator gene, + presence, – absence

underlying diseases according to the International Classification of Diseases (ICD, 10th edition). They were matched with the microbiological results obtained from *C. difficile* patient isolates during the same study period including cases with clindamycin-resistant *C. difficile* PCR-ribotype 027 from a cluster previously reported [13]. CDAD was defined by the presence of diarrhoea and a positive result for toxin A/B or a patient suffering from diarrhoea, a positive *C. difficile* stool culture, a clinical diagnosis in the chart, plus a therapy with vancomycin or metronidazole [21, 22]. A complicated clinical course was defined as admission to an intensive care unit, need for surgical intervention, or death. Recurrent CDAD was considered if a positive toxin or stool culture was detected within three months after first diagnosis; reinfection was defined when a new CDAD episode was diagnosed after three months during the same hospitalisation or during a new hospitalisation. A case of CDAD was classified as severe if one of the following complications occurred: paralytic ileus, toxic megacolon, severe dehydration, or severe sepsis. Cases were classified as moderate if at least three or as mild if less than three of the following symptoms were present: fever >38.5°C, severe diarrhoea, abdominal pain, and leukocytosis (>10^9/l).

Statistical analyses

SPSS statistical software version 14.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Odds ratios (OR) of binary, categorical, or continuous variables were determined by logistic regression (univariate). Statistical significance of dichotomous variables was achieved by using χ^2, Fisher's exact, or nonparametric tests when appropriate.

Results

Analysis of strains

The phenotypic and genotypic features of 124 single-patient isolates are given in Table 1. A total of 98 isolates (79%) were obtained from UHBS and 26 (21%) from surrounding hospitals. Overall, 103 strains (83.1%) were toxigenic (101 toxin A and B positive, two toxin A positive and B negative). PCR-ribotype 027 was detected in 18 isolates. The *tcdC* gene was not detected in 1/103 (1%) toxigenic isolates (strain was toxin A positive and B negative). Most frequent *tcdC* genotypes were wild-types, tcdC-sc1, and tcdC-A (Table 1). Genotypes tcdC-WT1 to WT4 showed no deletions in their sequences and were therefore considered as wild-type (GeneBank accession no. EU075382, EU075378, EU075379, and EU075380). tcdC-A

Table 2 Clinical features of 78 patients with *Clostridium difficile*-associated disease (CDAD) at the University Hospital Basel, Switzerland

Characteristic	*n* (%)
Sex	
Male	38 (48.7)
Age, years	
≤50	16 (20.5)
51–65	14 (18.0)
>65	48 (61.5)
Classification of disease	
Mild	49 (62.8)
Moderate	22 (28.2)
Severe	5 (6.4)
Complicated clinical course[a]	25 (32.1)
Death (within 30 days)	7 (9.0)
Follow-up[b]	
Recurrence	3 (3.8)
Reinfection	2 (2.6)
Development of diarrhoea	
Community-onset (prior or within 2 days)	2 (2.6)
Hospital associated (between 2–14 days)	43 (55.1)
Hospital associated (after 14 days)	32 (42.3)
Underlying disease	
Rehabilitation	20 (25.6)
Neoplasm	12 (15.4)
Infectious disease	11 (14.1)
Cardiovascular system disease	6 (7.7)
Respiratory disease	3 (3.8)
Others	26 (33.3)
Duration of hospitalisation before onset of diarrhoea, days (median [IQR])	11.5 (5–22)
Previous use of antibiotics	
Any antibiotic	71 (91.0)
Penicillins+betalactamase inhibitor	42 (53.8)
Cephalosporins	12 (15.4)
Quinolones	18 (23.1)
Macrolides	4 (5.1)
Clindamycin	1 (1.3)
Other	18 (23.1)
Duration of antibiotic therapy, days (median [IQR])	8 (6–12)
Use of protone pump inhibitors	47 (60.3)
Laboratory parameters at time of diagnosis	
White blood cell count, >10^9/l	41 (52.6)
Albuminaemia, <35 g/l	60 (76.9)
C-reactive protein, >40 mg/l	49 (62.8)
Fever >38.5 °C	24 (30.8)
McCabe score	
No fatal disease	54 (69.2)
Fatal disease in the following 5 years	17 (21.8)
Fatal disease in the following 0.5 year	7 (9.0)
Charlson index (mean ± SD)	2.6 (±1.9)
Predisposing factors	
Previous surgery (1 month)	26 (33.3)
Cancer	12 (15.4)
Nasogastric tube	12 (15.4)
Chemotherapy	12 (15.4)
Immunosupression	9 (11.5)

Table 2 (continued)

Characteristic	*n* (%)
Hospital ward	
Internal medicine	33 (42.3)
Surgical department	10 (12.8)
Intensive care unit	7 (9.0)
Geriatric	24 (30.8)
Others	4 (5.1)

IQR interquartile range, *SD* standard deviation
[a] Defined as admission to an intensive care unit, surgical intervention, or death
[b] At least one or more than one recurrence or reinfection

(39-bp deletion), tcdC-B (18-bp deletion), and tcdC-sc1 (18-bp deletion plus single nucleotide deletion at position 117) correspond to genotypes previously described [9, 16] whereas the sequence of tcdC-UHBS2 with a 54-bp deletion is newly recognized (GeneBank accession no. EU075381). All strains with ribotype 027 were moxifloxacin resistant (MIC >32 mg/l, sensitivity 100%), and the frequency of resistance to moxifloxacin among non-027 strains was 9.4% (specificity 90.6%) (Table 1). Among toxigenic strains, MIC_{50} and MIC_{90} for metronidazole and vancomycin were 0.064 mg/l and 0.25 mg/l, 0.5 mg/l and 1.5 mg/l, respectively, and therefore interpreted as susceptible.

Epidemiological data and patient characteristics

Among the microbiologically analysed single-patient isolates during the study period, 78 fulfilled the case definition of CDAD and complete data from chart review was available. Among these, ten CDAD cases were toxin-negative but suffered from diarrhoea, were *C. difficile* culture positive, and were treated for CDAD. In eight cases, the strain was not available for further microbiological analysis. Overall, incidence was 2.65 per 1,000 hospitalized patients and 2.3 per 10,000 patient-days. Among all CDAD cases, 61.5% were older than 65 years, 62.8% had a mild clinical course, crude mortality was 9%, frequency of community-onset of CDAD was 2.6%, and recurrence rate was 3.8% (Table 2). The median duration of hospitalisation before onset of diarrhoea was 11.5 days; 91% of patients had documented antibiotic therapy before disease onset and most common underlying disease/diagnosis was rehabilitation, neoplasm, and infectious disease. Only four (5.1%) did not receive a specific treatment, but all of them were toxin-positive.

Table 3 Comparison of *Clostridium difficile*-associated disease (CDAD) patients infected with PCR-ribotype 027 versus non-027 strains

PCR-ribotype				
Variable	027 strains (*n*=16)	Non-027 strains (*n*=44)	OR (95% CI)	*p*
Age, years (median, IQR)	82.5 (78.5–91.5)	65.5 (53.5–77)		<0.0001
Moderate to severe CDAD 7	7 (43.8)	15(34.1)	1.4 (0.43–4.52)	0.57
Complicated clinical course[a] (*n*, %)	4 (25.0)	17 (38.6)	0.53 (0.15–1.89)	0.33
Follow-up[b]	4 (25.0)			
Recurrence	1 (6.2)	2 (4.6)	1.41 (0.12–16.67)	0.79
Reinfection (*n*, %)	1 (6.2)	1 (2.3)	2.86 (0.17–47.62)	0.45
Death (within 30 days)	3 (18.8)	4 (9.1)	2.3 (0.46–11.11)	0.31
Duration of hospitalisation before onset of diarrhoea (median days, IQR)	15.5 (12.5–50.5)	9 (5–18)		0.014
Duration of antibiotic therapy, days (median, IQR)	9.5 (6.5 - 13)	7.5 (5–13)		0.3
Use of protone pump inhibitors (*n*, %)	9 (56.3)	27 (61.4)	0.81 (0.25–2.56)	0.72
Previous use of antibiotics (n, %)				
Beta-lactam antibiotics	12 (75.0)	31 (70.5)	1.26 (0.34–4.63)	0.73
Quinolones	6 (37.5)	9 (20.5)	2.33 (0.67–8.14)	0.18
Macrolides	3 (18.8)	1 (2.3)	9.92 (0.95–103.70)	0.054
Laboratory parameters at time of diagnosis (n, %)				
White blood cell count > 10^9/l	9 (56.3)	22 (50.0)	1.28 (0.41–4.0)	0.67
Albuminaemia < 35 g/l	10 (62.5)	34 (77.3)	0.49 (0.14–1.69)	0.26
C-reactive protein > 40 mg/l 8	8 (50.0)	28 (63.6)	0.57 (0.18–1.82)	0.34
Toxin A/B positive directly from stool [c] (*n*, %)	9 (56.3)	23 (52.3)	1.18 (0.37–3.70)	0.79

95% CI 95% confidence interval, *IQR* interquartile range
[a] Defined as admission to an intensive care unit, surgical intervention, or death
[b] At least one or more than one recurrence or reinfection
[c] All cases were toxin-positive either directly from stool or by toxigenic culture

Clinical and laboratory features in patients infected with *C. difficile* PCR-ribotype 027 and non-027

Comparing 60 patients for whom PCR-ribotyping results were available, we found that patients infected with *C. difficile* ribotype 027 (16 patients, or 20.5% of total CDAD cases) were significantly older than non-027 patients (median age 82.5 vs. 65.5 years, $p<0.0001$, Table 3) and were hospitalised longer before onset of diarrhoea (median 15.5 vs. 9 days, $p=0.014$). Although statistically not significant, there was a trend towards more cases of moderate to severe CDAD in the 027 group (43.8% vs. 34.1%, $p=0.57$), higher crude mortality (18.8% vs. 9.1%, $p=0.31$), longer duration of antibiotic therapy before CDAD (median 15.5 vs. 9.0 days, $p=0.31$), as well as previous use of quinolones (37.5% vs. 25.7%, $p=0.18$) and macrolides (18.8% versus 2.3%, $p=0.054$). Recurrence and reinfection rate was generally low (2.3–6.2%). Toxin A/B was detected in only half of the patients directly in stool specimens and would have been missed if toxin assay was performed alone (Table 3), but repeated toxin testing from *C. difficile* cultures from specimens that were toxin-negative directly from stool considerably increased sensitivity. In the non-027 group, seven patients underwent chemotherapy compared to zero patients in the 027 group.

Disease severity according to strain and patient characteristics

Disease severity of CDAD according to strain and patients' characteristics is summarized in Table 4. For two patients, the severity of disease was not assessable. The univariate analysis showed that moderate to severe CDAD is statistically significant associated with use of a nasogastric tube and with a shorter hospital stay before onset of diarrhoea (Table 4). Toxin A/B positivity directly detected from stool (instead of positive only from culture isolate testing) was significantly associated with moderate to severe disease ($p=0.048$). We did not observe an association between severity of disease and PCR-ribotype 027, *tcdC* genotype, as well as presence of binary toxin A/B. There was a trend towards more frequent recurrences and reinfection in the more severe disease group; however, the sample size was too small to infer an association.

Table 4 Disease severity of *Clostridium difficile*-associated disease (CDAD) cases according to strain and patients' characteristics

Variable	No. of patients with available information	Disease severity of CDAD		OR (95% CI)	*p*
		Moderate to severe	Mild		
Age, years (median, IQR)	76	70 (55–83)	73 (53–85)		0.88
Gender (*n*, %)	76				
Male		14 (51.9)	23 (46.9)	1.22 (0.48–3.13)	0.68
Follow-up[a]					
Recurrence (*n*, %)	76	2 (7.4)	1 (2.0)	3.85 (0.33–50.0)	0.25
Reinfection (n, %)	76	1 (3.7)	1 (2.0)	1.85 (0.11–33.33)	0.665
Death (within 30 days) (*n*, %)		4 (14.8)	3 (6.1)	2.63 (0.55–12.5)	0.22
Predisposing factors (*n*, %)					
Previous surgery (within 3 months)	76	3 (11.1)	5 (10.2)	1.09 (0.24–5.0)	0.9
Cancer	76	3 (11.1)	9 (18.4)	0.56 (0.14–2.27)	0.4
Nasogastric tube	76	7 (25.9)	4 (8.2)	4 (1.03–14.29)	0.03
Chemotherapy	76	1 (3.7)	11 (22.4)	0.13 (0.02–1.09)	0.06
Laboratory parameters at time of diagnosis (*n*, %)					
Albuminaemia <35 g/l	67	23 (95.8)	35 (81.4)	5.26 (0.62–50.0)	0.09
C-reactive protein >40 mg/l	73	20 (74.0)	27 (58.7)	2.01 (0.71–5.69)	0.19
Duration of hospitalisation before onset of diarrhoea, days (median, IQR)	76	8 (4–13)	15 (8–26)		0.002
Duration of antibiotic therapy, days (median, IQR)		6 (3–11)	9 (7–12)		0.08
PCR-ribotype 027 (*n*, %)	58	7 (31.8)	9 (25.0)	1.4 (0.43–4.52)	0.57
Binary toxin positive (*n*, %)	68	8 (36.4)	14 (30.4)	1.31 (0.45–3.82)	0.63
Toxin A/B positive directly from stool (*n*, %)	60	17 (70.8)	16 (44.4)	3.13 (1.02–9.09)	0.048
tcdC genotype (*n*, %)	58				
tcdC-A (39-bp deletion)		1 (4.5)	4 (11.1)	0.43 (0.05–4.0)	0.45
tcdC wildtype (no deletion)		14 (63.6)	22 (61.1)	1.32 (0.52–3.45)	0.56

95% CI 95% confidence interval, *IQR* interquartile range

[a] At least one or more than one recurrence or reinfection

Discussion

Overall, the incidence of 2.65 CDAD/1,000 hospitalized patients or 2.3/10,000 patient-days was comparable to a study out of the Netherlands in 2005 (0.1–4.6, median 1.6/1,000 patient admissions) [23] and from a recent European survey (0.13–7.1/10,000 patient-days) [6]. The incidence of CDAD remained stable at the University Hospital, but increased during an outbreak with *C. difficile* PCR-ribotype 027 in January 2007 at the affiliated geriatric hospital [13]. The slightly higher incidence compared to the Netherlands may be partly explained by the definitions used. The new guidelines such as those issued by the European Centre for Disease Prevention and Control (ECDC) define CDAD more stringently [4, 24] than what was used in our study. These new guidelines require the detection of toxin that was negative in ten (13%) of our cases, but fulfilled the Centers for Disease Control and Prevention (CDC) surveillance definition [22].

CDAD cases were similar to those of other European countries regarding age, sex, and severity of disease [10, 23]. In contrast to these studies, community-onset CDAD was rare (only 2.6%) in this study and the recurrence rate was lower.

Among all *C. difficile* strains isolated at the Microbiology Laboratory during the study period, rates of resistance to moxifloxacin and clindamycin were overall rather low, but in the range of a recent European survey [6]. Moxifloxacin resistance was highly associated with the presence of PCR-ribotype 027; however, these strains were isolated from an outbreak, potentially biasing the strength of this association.

Comparing CDAD due to PCR-ribotype 027 with non 027, ribotype 027 was significantly associated with older age and longer duration of hospital stay before onset of disease. Although a trend was seen towards higher crude mortality, a more severe clinical course, previous use of macrolides, and a longer duration of antibiotic therapy in the 027 patient group, these findings did not reach a statistically significant level, possibly due to small sample size. However, results from previous reports support the observed trends [6, 7]. In contrast to the latter studies, our 027 strains were all clindamycin-resistant. It is noteworthy that previous use of macrolides was more strongly associated with PCR-ribotype 027 than use of quinolones, but both were statistically not significant.

Severe to moderate disease correlated with use of a nasogastric tube and shorter duration of hospitalisation before onset of diarrhoea. Strikingly, the clinical course of CDAD in patients under chemotherapy was mild; these patients were predominantly cared for in a special haematological unit where immediate treatment with metronidazole after onset of any kind of diarrhoea is standard of care. Disease severity was not linked to the presence of *tcdC* deletions or binary toxin in contrast to previous findings [6, 10]. However, the percentage of binary-toxin positive strains among CDAD cases was quite high (37%) compared to the average among all strains isolated at the laboratory (22%) from patients with and without clinical symptoms. Patients with toxin-positive stool specimens experienced a more severe disease (compared to patients with toxin-negative faeces but with a toxigenic culture result), possibly due to higher stool toxin levels. The disease is mediated by toxins A and B, but toxin levels and bacterial counts in stool are probably affected by many factors and there seems to be no correlation between severity of disease and faecal toxin level [4, 25]. Surprisingly, two strains were toxin A positive but toxin B negative by the PCR method we used. They were isolated from two patients without CDAD diagnosis. This is a rare observation that requires confirmation [11].

A limitation of our study is the relatively small number of total CDAD cases and the small subgroup of patients infected with ribotype 027 during the 12-month period. However, all consecutive cases were included, clinical data were complete and comprehensive, and isolates were genotypically and phenotypically well characterised. The follow-up period varied but was at least one month, and we cannot rule out that recurrences were treated in the outpatient setting. But seriously ill patients requiring hospitalization are unlikely to go elsewhere since the University Hospital is the only tertiary care hospital in the region.

Based on our study, we propose the introduction of laboratory-based and epidemiological surveillance systems, at least periodically and in populations at high risk. Susceptibility testing of culture isolates for moxifloxacin may serve as a basic screening marker for the epidemic ribotype 027 strains. Incidence and clinical presentation of CDAD cases must be monitored combined with molecular characterisation of strains to detect emergence of new epidemic strains.

Acknowledgements We are indebted to Dr Ed J. Kuijper for helpful discussion and for confirmation of PCR-ribotype 027. We thank the infection control team and the microbiology laboratory team at University Hospital Basel for excellent assistance.

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