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Research note

An outbreak of *Clostridium difficile* infections due to new PCR ribotype 826: epidemiologic and microbiologic analyses☆

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ABSTRACT

Objectives: To investigate an unusual outbreak of five patients with a total of eight episodes of a *Clostridium difficile* infection on a gastrointestinal surgical ward of a Dutch tertiary-care, university-affiliated hospital.

Methods: Clinical case investigations and laboratory analyses were performed. Laboratory analyses included PCR ribotyping, multiple-locus variable-number tandem repeat analysis typing, toxin typing, antimicrobial susceptibility testing and whole genome sequencing.

Results: The outbreak was associated with recurrent and severe disease in two of five patients. All episodes were due to a unique ribotype that was not recognized in the collection of an international network of reference laboratories and was assigned PCR ribotype 826. PCR ribotype 826 is a toxin A−, toxin B− and binary toxin−positive ribotype which according to molecular typing belongs to clade 5 and resembles the so-called hypervirulent ribotype 078. The presence of a clonal outbreak was confirmed by whole genome sequencing, yet the source of this newly identified ribotype remained unclear.

Conclusions: This newly identified *C. difficile* PCR ribotype 826 is part of clade 5 and might also have increased virulence. The recognition of this outbreak highlights the need for ongoing *C. difficile* infection surveillance to monitor new circulating ribotypes with assumed increased virulence. **M.J.T. Crobach, Clin Microbiol Infect 2018;24:309.e1−309.e4**

Introduction

We identified an outbreak of eight episodes of *Clostridium difficile* infection (CDI) in five patients within a 4-month period (1 December 2015 to 31 March 2016). The outbreak occurred on a gastrointestinal surgical ward of a Dutch tertiary-care hospital. In this case series, we describe the clinical characteristics of affected patients and microbiologic investigations that were performed on the identified strain.

Methods

The case series was conducted at a gastrointestinal surgical ward of the Erasmus University Medical Center in Rotterdam, the Netherlands. The facility participates in the national sentinel CDI surveillance program and therefore sends all samples from hospitalized CDI patients to the national Reference Laboratory for PCR ribotyping (I. K. Sanders *et al.*, paper presented at the 25th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID 2015), abstract P0793, 2015) [1]. In case of an outbreak (defined as more than two isolates of the same type detected fewer than 7 days apart in one hospital either with onset of symptoms on the same ward or accompanied by an increased CDI monthly incidence within the hospital; http://www.rivm.nl/Documenten_en_publicaties/Algemeen_Actueel/Uitgaven/Infectieziekten/CDiffNL/Tenth_Annual_Report_of_the_National_Reference_Laboratory_for_Clostridium_difficile_and_results_of_the_sentinel_surveillance),

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additional analyses can be performed by the reference laboratory. These include multiple-locus variable-number tandem repeat analysis [2], PCRs for toxin genes [3], PCRs for clade-specific makers [4], antimicrobial susceptibility screening tests (Etest) and whole genome sequencing [5].

Patient information and medical history from all CDI cases during this outbreak were collected from the electronic medical records. Defined daily doses for all antibiotics used up to 3 months before development of CDI and Charlson comorbidity scores were calculated [6]. CDI was classified as severe if one or more of the following conditions were present (attributable to CDI): fever (temperature of 38.5°C or higher), rigors, haemodynamic instability, ileus, peritonitis, mental status changes, admission to intensive care unit, end organ failure, leukocytosis (>15 × 10^9), leukopenia (<2 × 10^9), hypoalbuminaemia (<30 g/L), >1.5-fold increase in creatinine level above baseline, serum lactate >2.2 mmol/L, pseudomembranous colitis, colonic wall thickening, pericolonic fat stranding or ascites. All other cases were classified as mild CDI [7,8].

Written approval to conduct the case series was obtained from the medical ethics research committee of Erasmus University Medical Center, Rotterdam, the Netherlands (MEC-2015-306).

Results

The CDI incidence rate on the gastrointestinal surgical ward was 3.3 per 10 000 patient-days (July 2009 to November 2015) and increased to 19.8 per 10 000 patient-days (December 2015 to March 2016). In total, six patients with CDI were diagnosed, five of whom had the same PCR ribotype.

The index case of this outbreak (patient A) was an 83-year-old man who underwent pancreaticoduodenectomy to treat a carcinoma of the common bile duct 1 month earlier. In December 2015, during a readmission that was due to infected ascites, he developed diarrhoeal symptoms and was diagnosed with hospital-acquired CDI. Within 1 week after the start of his symptoms, two other patients (patients B and C) on the same ward were diagnosed with hospital-acquired CDI. All three patients were treated with a 7- to 11-day oral course of metronidazole and discharged.

In January 2016, a fourth hospital-acquired CDI case (patient D) on the ward was noticed. In February 2016, patient A was readmitted because of a CDI recurrence, and a fifth case (patient E) was reported. Patient A was readmitted once more to treat a second recurrence in February, and patient D was also diagnosed with a CDI recurrence in March. In total, four of eight CDI episodes (in two patients) were classified as severe CDI. None of the patients was admitted to the intensive care unit because of CDI, and no CDI-related mortality (within 30 days) occurred. All patients had received antibiotic therapy before acquiring CDI, and total defined daily doses of antibiotics administered before the onset of CDI ranged from 21 to 63 (median, 26.9). Four of five patients had received therapy with proton pump inhibitors before the CDI diagnosis. The median Charlson comorbidity score was 2 (range, 0 to 8).

In accordance with local guidelines, all patients who had or who were suspected to have CDI were placed in a single room and were not allowed to use shared sanitation. Medical personnel wore protective disposable gowns and gloves when entering the room, and handwashing with soap and water was endorsed. Isolation precautions were discontinued 48 hours after resolution of diarrhoeal symptoms. In reaction to this CDI outbreak, additional infection prevention measures were implemented on the ward during certain time periods (Fig. 1). These additional infection

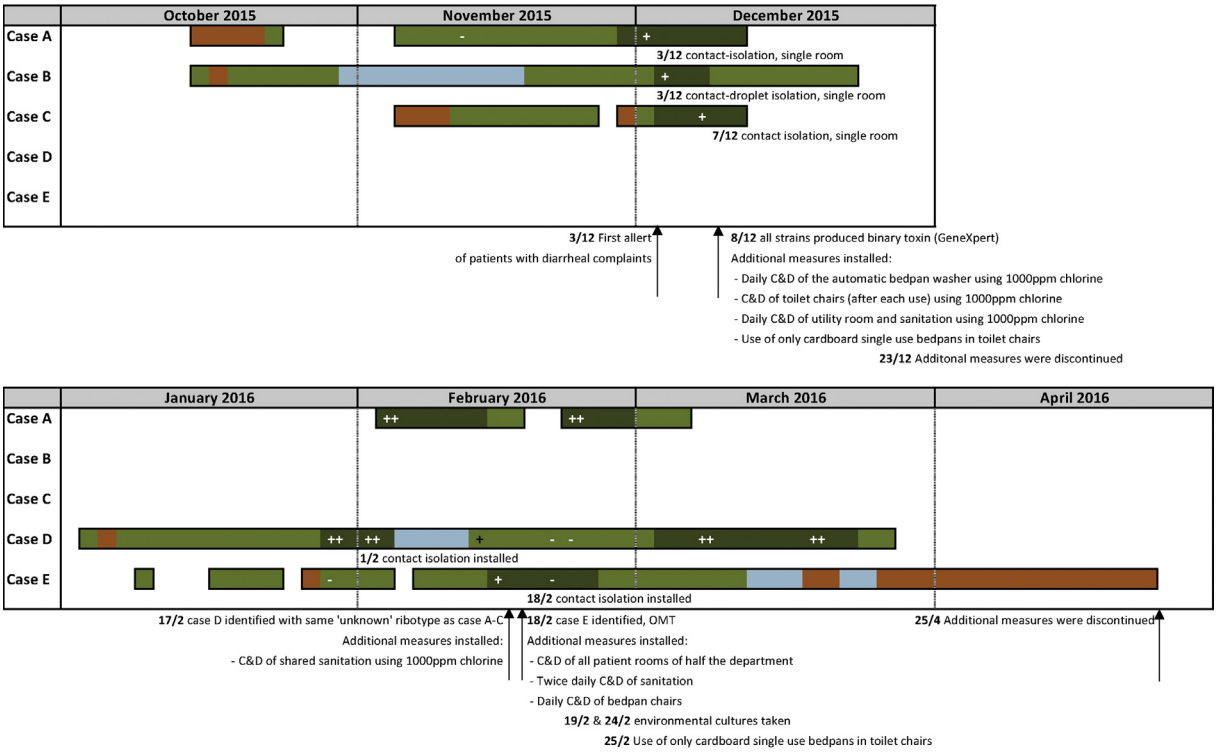

Fig. 1. Epidemic curve of five patients infected with *Clostridium difficile* caused by PCR ribotype 826. Green, outbreak, non-ICU ward; orange, other non-ICU ward; blue, ICU; dark green, diarrhoeal episode; white +, positive culture for *C. difficile* and mild *C. difficile* infection; white ++, positive culture for *C. difficile* and severe *C. difficile* infection; black +, positive *C. difficile* culture without diarrhoea; white −, negative culture for *C. difficile*. C&D, cleaning and disinfection; ICU, intensive care unit; OMT, outbreak management team.

prevention measures included cleaning and disinfection using 1000 ppm chlorine of the following items: automatic bedpan washer (daily), toilet chairs (after each use), utility room and sanitation (once or twice a day) and all patients rooms of half the department (once, after recognition of the fifth case). Additionally, the metal bedpans were replaced by cardboard single-use bedpans. Moreover, after the fifth case was diagnosed, 56 environmental swabs were taken on two different sampling days, 19 and 24 February. Samples were taken from the following sites: sink, water tap, grip of cabinet, alarm system, dustbin, chairs/tables and bed curtains of a room that had been occupied by a CDI patient (before final cleaning); the same items in a clean room (after cleaning and disinfection with 1000 ppm chlorine); and toilet, shower chair, sink, shower curtain, sack of laundry and towel dispenser of a shared bathroom (after cleaning and disinfection). Environmental swabs were inoculated in *Clostridium difficile* enrichment-modified broth (*C. difficile* enrichment broth; Mediaproducts, Groningen, The Netherlands) for 1 week and subcultured on CLO plates (*C. difficile* agar; bioMérieux, Marcy l'Étoile, France). No antibiotic restriction policy was implemented during this outbreak.

Stool samples of all five patients tested positive for toxin B and binary toxin genes in the Xpert *C. difficile* (Cepheid, Sunnyvale, CA, USA); however, the *Tcd*CΔ117 deletion specific for ribotype 027 was not identified. Investigations at the reference laboratory demonstrated the presence of *Tcd*A and confirmed the presence *Tcd*B and the binary toxin genes. In addition, a 39 bp deletion in *Tcd*C was detected.

All five isolates and one isolate obtained from an environmental culture (taken from the sack of laundry in the shared bathroom after cleaning and disinfection) displayed the same PCR ribotyping profile. The profile was not recognized in the Dutch Reference Library (which is able to recognize 221 different PCR ribotypes), but it most resembled the profile of ribotypes 078, 126 and 066 (all belonging to clade 5) (Fig. 2a). A data set of sized fragments obtained by capillary gel–based electrophoresis PCR ribotyping [1] was sent as FSA file to international *C. difficile* reference laboratories (including the Leeds collection encompassing more than 800 PCR ribotypes, the WEBRIBO system, the US Centers for Disease Control and Prevention database and databases from Sweden, Portugal, Belgium and Canada), but no match was found. The new strain was assigned as ribotype 826 by the Leeds ribotyping reference network. PCR analysis of a clade 5–specific DNA marker [4] revealed that all ribotype 826 isolates were positive for the marker, confirming that ribotype 826 is part of clade 5.

According to Clinical and Laboratory Standards Institute (CLSI) breakpoints, all isolates were susceptible for erythromycin (minimum inhibitory concentration (MIC) <2 mg/L), clindamycin (MIC <2 mg/L), metronidazole (MIC <2 mg/L) and vancomycin (MIC <2 mg/L), but resistant to ciprofloxacin (MIC >32 mg/L) and moxifloxacin (MIC >32 mg/L) [9].

The isolates were 100% identical with no summed tandem repeat differences, thereby confirming a clonal complex according to multiple-locus variable-number tandem repeat analysis.

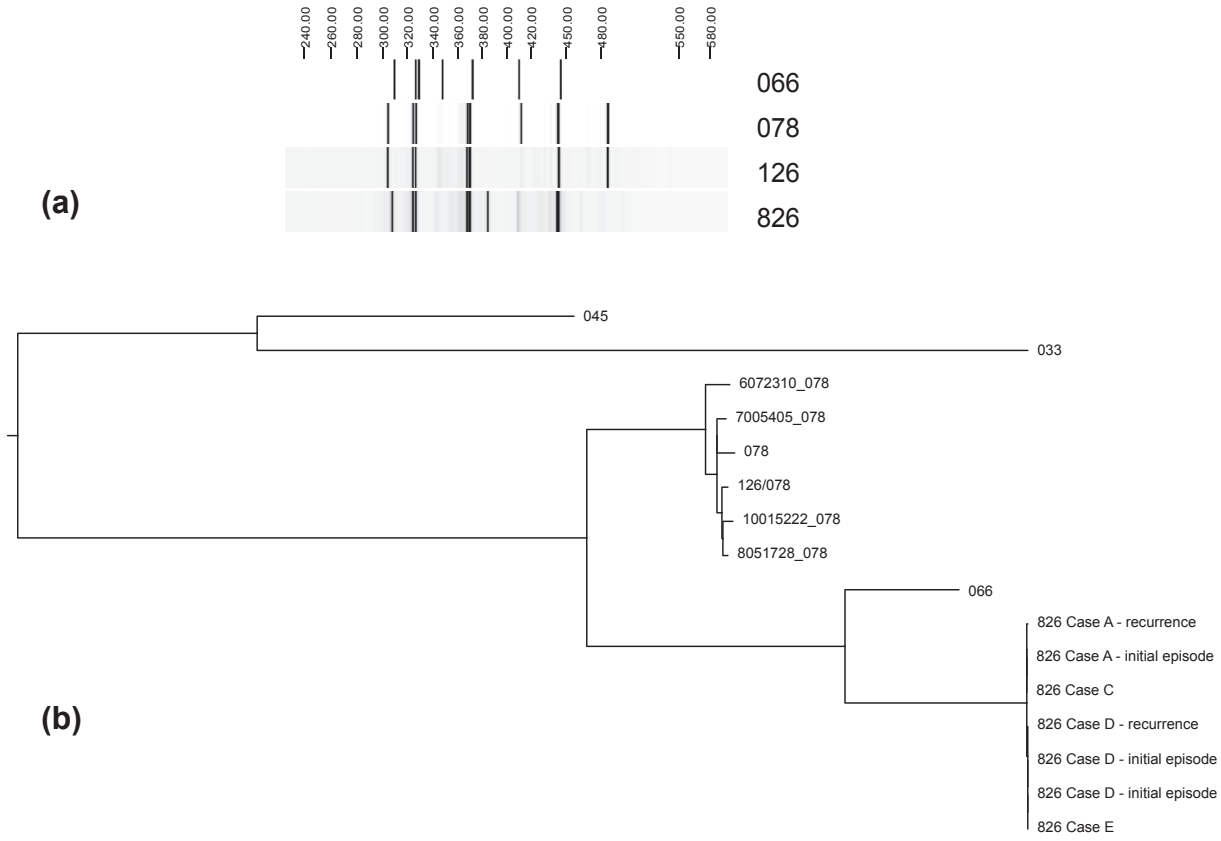

Fig. 2. (a) PCR ribotyping patterns for ribotypes 066, 078, 126 and 826. Upper row indicates fragment sizes. (b) Phylogenetic tree of ribotype 826 outbreak isolates and related ribotypes. 078, reference ribotype 078 strain; 066, reference ribotype 066 strain; 045, reference ribotype 045 strain; 126078, reference ribotype 126/078 strain 7005405_078/10015222_078; 8051728_078, 6072310_078, clinical patient CDI samples with confirmed ribotype 078; 4_826, sample from patient A (recurrent episode); 3_826; sample from patient A (initial episode), 6_826, sample from patient C; 1_826, sample from patient D (recurrent episode); 2_826, sample from patient D (initial episode); 8_826, sample from patient D (initial episode, repeat sample); 5_826, sample from patient E. Isolate from patient B could not be sequenced.

In addition, whole genome sequencing was performed (Fig. 2b). To provide phylogenetic context, reference strains 078, 126/078, 045, 033 and 066 and four patient samples from confirmed strain 078 cases were included. In total, 1678 single nucleotide polymorphisms (SNPs) were identified within this sample selection, which is the expected variation between different ribotypes of one clade. Within the outbreak isolates, only two SNPs were identified (there was one SNP difference between the isolate from the recurrence in patient A compared to the initial patient A isolate, and one SNP difference between the patient D/patient E isolates and the initial patient A isolate). Clonality of these cluster isolates was thus confirmed by whole genome sequencing, as the commonly used cutoffs for classifying isolates as clonal is zero to two SNPs [5].

Discussion

The occurrence of this CDI outbreak was uncommon, as it occurred on a ward where transmission of *C. difficile* was rare, as proven by sentinel CDI surveillance. Also, two of five patients had recurrent disease and were severely affected. Cases were due to the newly identified ribotype 826. Additional investigations showed that ribotype 826 belongs to clade 5 with a characteristic clade 5–specific DNA marker and a 39 bp deletion in *Tcd*C. Whole genome sequencing revealed that ribotype 826 resembles ribotype 078 quite well. CDI cases due to clade 5 ribotypes have been reported to be associated with the highest 14-day mortality [10]. We therefore assume that this new ribotype also has increased virulence, thus explaining the occurrence of this outbreak.

Whole genome sequencing results demonstrated clonality, thereby confirming transmission, but unanswered questions remain, including the source of this ribotype and how transmission occurred. The index patient could have introduced this ribotype into the ward, although no unusual profession, recent travel or other remarkable expositions were reported. Alternatively, an undetected asymptomatic carrier might have introduced the ribotype and spread it to other patients. Transmission could have occurred via shared items, as contamination was demonstrated in one of the environmental cultures, but unfortunately environmental swabs were only taken after the last patient was detected. The outbreak ceased with the implementation of additional infection prevention measures, suggesting that these cleaning and disinfection measures were effective, probably together with a raised awareness among the healthcare workers.

Because most PCR ribotypes of clade 5 are also found in animals, it is tempting to speculate that the newly recognized ribotype 826 derives from animals. The lack of this PCR ribotype in the databases of human collections supports this hypothesis. Unfortunately, reference laboratories for animal-associated *C. difficile* infections are not available that could be used to match our isolates. To our knowledge, no additional ribotype 826 isolates have been detected since this outbreak.

This outbreak indicates that new *C. difficile* ribotypes with increased virulence still emerge at unexpected locations and without a clear source. Given the increased virulence and still-unknown source of this newly identified ribotype, ongoing CDI surveillance remains essential, and other institutions should now be aware of ribotype 826.

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Transparency Declaration

All authors report no conflicts of interest relevant to this article.

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