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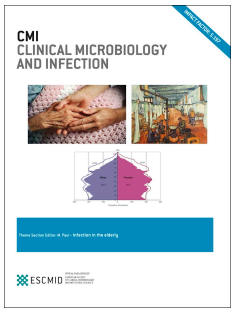

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PII: S1198-743X(17)30460-3

DOI: 10.1016/j.cmi.2017.08.014

Reference: CMI 1046

To appear in: *Clinical Microbiology and Infection*

Received Date: 20 June 2017

Revised Date: 15 August 2017

Accepted Date: 16 August 2017

Please cite this article as: Crobach MJT, Voor in 't holt AF, Knetsch CW, van Dorp SM, Bras W, Harmanus C, Kuijper EJ, Vos MC, An outbreak of *Clostridium difficile* infections due to a new PCR ribotype 826: epidemiological and microbiological analyses, *Clinical Microbiology and Infection* (2017), doi: 10.1016/j.cmi.2017.08.014.

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Intended category: Research note

An outbreak of *Clostridium difficile* infections due to a new PCR ribotype 826: epidemiological and microbiological analyses

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Running title: outbreak of CDI caused by ribotype 826

Keywords: *Clostridium difficile*, epidemiology, outbreaks, infection control, surveillance

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Abstract

Objectives. The aim was to investigate an unusual outbreak of 5 patients with in total 8 episodes of a *Clostridium difficile* infection (CDI) on a gastro intestinal surgical ward of a Dutch tertiary care university affiliated hospital.

Methods. Clinical case investigations and laboratory analyses were performed. Laboratory analyses included PCR ribotyping, MLVA typing, toxinotyping, antimicrobial susceptibility testing and whole genome sequencing.

Results. The outbreak was associated with recurrent and severe disease in 2 out of 5 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.

Conclusion. This newly identified *C. difficile* PCR ribotype 826 is part of clade 5 and might as well have increased virulence. The recognition of this outbreak highlights the need of ongoing CDI surveillance to monitor new circulating ribotypes with assumed increased virulence.

Introduction

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

Methods

The case series was conducted at a gastro-intestinal surgical ward of the Erasmus University Medical Center in Rotterdam, the Netherlands. The Erasmus MC 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 (1, 2). In case of an outbreak (defined as >2 isolates of the same type detected less 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 (3)) additional analyses can be performed by the Reference Laboratory. These include multiple-locus variable-number tandem-repeat analysis (4) , PCRs for toxin genes (5), PCRs for clade specific makers (6), antimicrobial susceptibility screening tests (E-test) and whole genome sequencing (7).
Patient information and medical history from all CDI cases during this outbreak were collected from the electronical medical records. Defined daily doses for all antibiotics used up to three months before development of CDI and Charlson co-morbidity scores were calculated. (8). CDI was classified as severe if one or more of the following conditions were present (attributable to CDI): fever (equal or above 38.5°C), rigors, hemodynamic instability, ileus, peritonitis, mental status changes, admission to ICU, end organ failure, leukocytosis (>15 x 10^9), leukopenia (<2 x 10^9), hypoalbuminemia (<30g/L), >1.5-fold increase in creatinine level above baseline, serum lactate >2.2mmol/L, pseudomembranous colitis, colonic wall thickening, pericolonic fat stranding or ascites. All other cases were classified as mild CDI. (9, 10).

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

Results

The CDI incidence rate on the gastro-intestinal surgical ward was 3.3 per 10,000 patient days (July 2009-November 2015) and increased to 19.8 per 10,000 patient days (December 2015-March 2016). In total, 6 patients with CDI were diagnosed of which 5 had the same PCR ribotype.

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

In January 2016, a fourth hospital-acquired CDI case (D) on the ward was noticed. In February 2016, case A was readmitted because of a CDI recurrence and a fifth case (E) was reported. Case A was readmitted once more due to a second recurrence in February and case D was also diagnosed with a CDI recurrence in March. In total, 4 out of 8 CDI episodes (in 2 patients) were classified as severe CDI. None of the patients were admitted to the ICU due to CDI, and no CDI-related mortality (within 30 days) occurred. All patients had used antibiotics before acquiring CDI and total defined daily doses of antibiotics used before onset of CDI ranged from 21 to 63 (median 26.9). Four out of 5 patients had used proton pump inhibitors before the CDI diagnosis. The median Charlson co-morbidity score was 2, ranging from 0 to 8.

In accordance with local guidelines, all patients suspected of or having 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 48hours after resolution of diarrheal symptoms. In reaction to this CDI outbreak, additional infection prevention measures were implemented on the ward during certain time periods (see Figure 1).These additional infection prevention measures included cleaning and disinfection using 1000ppm chlorine of the following items: automatic bedpan washer (daily), toilet chairs (after each use), utility room and sanitation (daily or twice daily) 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 2 different sampling days: February 19th and February 24th. Samples were taken from: 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 1000pppm); 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 *C. difficile* enrichment modified broth (*Clostridium difficile* enrichment broth, Mediaproducts BV, Groningen, The Netherlands) for 1 week and subcultured on CLO plates (Clostridium difficile agar, Biomerieux, Marcy l'Etoile, France). No antibiotic restriction policy was implemented during this outbreak.

Stool samples of all 5 patients tested positive for toxin B and binary toxin genes in the Xpert *C. difficile*; however, the *TcdCΔ117* deletion specific for ribotype 027 was not identified. Investigations at the Reference Laboratory demonstrated the presence of *TcdA*, and confirmed the presence *TcdB* and the binary toxin genes. In addition, a 39-bp deletion in *TcdC* was detected.

All 5 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 resembled the profile of ribotypes 078, 126 and 066 most (all belonging to clade 5) (Figure 2a). A dataset of sized fragments obtained by capillary gel-based electrophoresis

PCR ribotyping (2) 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 CDC 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 (6) 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 (MIC<2), clindamycin (MIC<2), metronidazole (MIC=<2) and vancomycin (MIC<2), but resistant to ciprofloxacin (MIC>32) and moxifloxacin (MIC>32)(11).

The isolates were 100% identical with 0 summed tandem-repeat differences (STRD), thereby confirming a clonal complex according to MLVA.

In addition, whole genome sequencing was performed (Figure 2b). To provide phylogenetic context, reference strains 078, 126/078, 045, 033 and 066 and 4 patient samples from confirmed 078 cases were included. In total, 1678 SNPs were identified within this sample selection which is the expected variation between different ribotypes of one clade. Within the outbreak isolates, only 2 SNPs were identified (there was 1 SNP difference between the isolate from the recurrence in case A compared to the initial case A isolate and 1 SNP difference between the case D /case E isolates and the initial case A isolate). Clonality of these cluster isolates was thus confirmed by whole genome sequencing as the commonly used cut-off for classifying isolates as clonal is 0-2 SNPs (7).

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 out of five patients had recurrent disease and were severely affected. Cases were due to a newly identified ribotype 826. Additional investigations showed that ribotype 826 belongs to clade 5 with a characteristic clade 5 specific DNA marker and a 39bp deletion in *TcdC*. Whole genome sequencing revealed that ribotype

826 resembles the ribotype 078 quite well. CDI cases due to clade 5 ribotypes have been reported to be associated with the highest 14-day mortality (12). We therefore assume that this new ribotype also has increased virulence, explaining the occurrence of this outbreak.

Whole genome sequencing results demonstrated clonality thereby confirming transmission, but still unanswered questions are what the source of this ribotype was 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. Since most PCR ribotypes of clade 5 are also found in animals, it is tempting to speculate that the newly recognised 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 the best of our knowledge, no additional 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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Part of this work was presented at ECCMID 2017, Vienna (oral presentation #OS0223).

Funding

No financial support was received for this study.

Acknowledgments

We thank Alexander Indra (Webribo, AGES-Institut für medizinische Mikrobiologie und Hygiene Wien, Austria), Thomas Akerlund (Public Health Agency, Sweden), Monica Oleastro (Laboratoria Nacional de Referencia das Infecoes Gastrintestinais, Portugal), Johan van Broeck (National Reference Center Clostridium difficile Bruxelles, Belgium), George Golding (Antimicrobial Resistance and Nosocomial Infections, National Microbiology Laboratory, Winnipeg, Canada) and Brandi Limbago (CDC, US) for comparing the dataset of sized fragments of this new ribotype to profiles in their (reference) databases.

We thank Mark Wilcox (Leeds Teaching Hospitals & University of Leeds, United Kingdom) for assigning a new ribotype.

We thank the European Study Group of *Clostridium difficile* (ESCMID-ESGCD) for European activities.

We thank employees of the diagnostic laboratory and the infection control practitioners from the Department of Medical Microbiology and Infectious Diseases and the treating physicians and all other health care workers involved on the department of gastro-intestinal surgery from the Erasmus MC University Medical Center, Rotterdam, the Netherlands.

Declarations of Interest

MC, AV, CK, SvD, WB, CH, EK, MV: Nothing to declare.

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195 Author's contribution

196 Epidemiological investigation and analysis: MC, AV, WB, EK, MV. Whole genome sequencing: CK.
197 Outbreak management: WB, MV. All other laboratory investigations: CH. Surveillance data: SvD.
198 Coordinated and supervised the study: EK, MV. All authors commented and agreed upon the final
199 manuscript.

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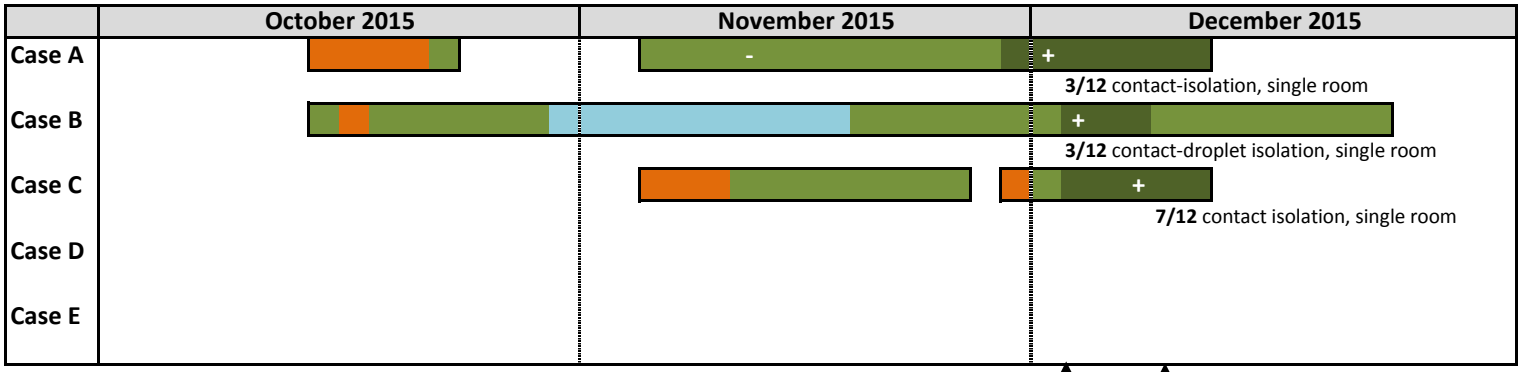
Figure legends.

Figure 1. Epidemic curve of the 5 case patients infected with *C. 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*. Abbreviations: C&D= cleaning and disinfection, ICU= intensive care unit, OMT= outbreak management team

Figure 2a. PCR ribotyping patterns for ribotype 066, 078, 126 and 826.The upper row indicates the fragment sizes.

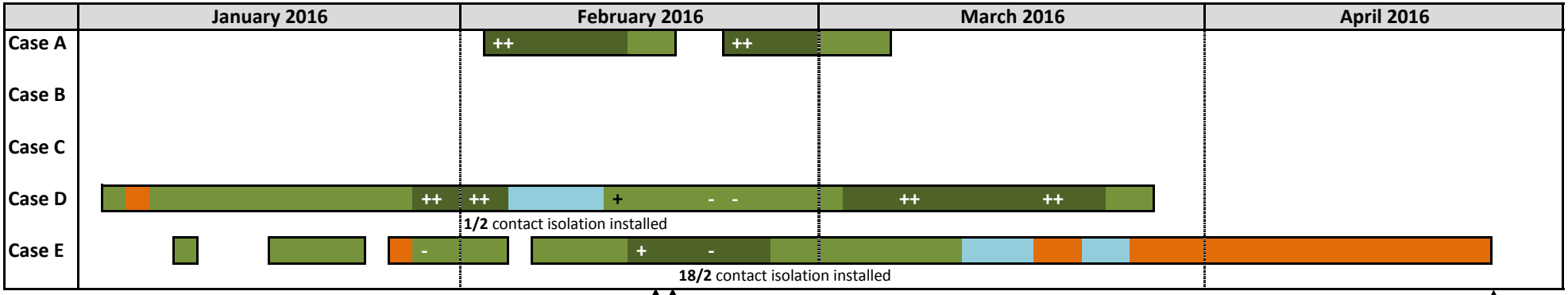
Figure 2b. Phylogenetic tree of ribotype 826 outbreak isolates and related ribotypes. 078; reference ribotype 078 strain; 066 reference ribotype 066 strain; 045; reference ribotype 045 strain; 126078 reference ribotype 126/078 strain; 7005405_078/10015222_078, 8051728_078, 6072310_078; clinical patient CDI samples with confirmed ribotype 078 . 4_826; sample from case A (recurrent episode); 3_826; sample from case A (initial episode); 6_826; sample from case C; 1_826; sample from case D (recurrent episode); 2_826; sample from case D (initial episode); 8_826; sample from case D (initial episode, repeat sample), 5_826; sample from case E. The isolate from case B could not be sequenced.



3/12 First allert
of patients with diarrheal complaints

8/12 all strains produced binary toxin (GeneXpert)
Additional measures installed:
- Daily C&D of the automatic bedpan washer using 1000ppm chlorine
- C&D of toilet chairs (after each use) using 1000ppm chlorine
- Daily C&D of utility room and sanitation using 1000ppm chlorine
- Use of only cardboard single use bedpans in toilet chairs

23/12 Additonal measures were discontinued



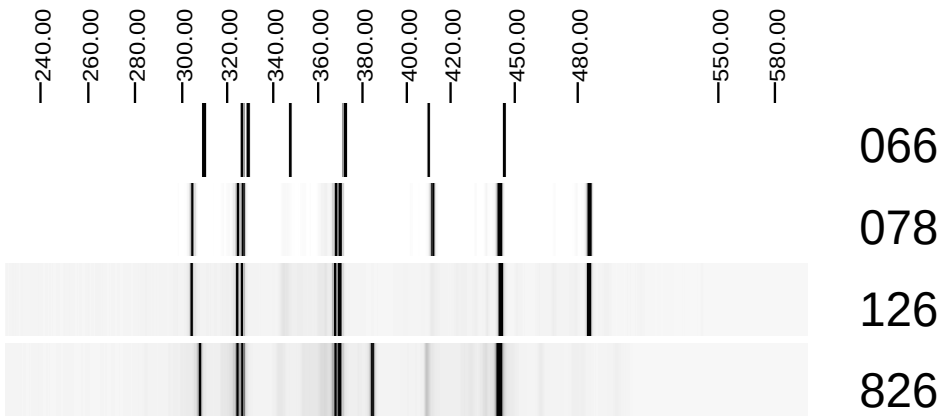
17/2 case D identified with same 'unknown' ribotype as case A-C
Additional measures installed:
- C&D of shared sanitation using 1000ppm chlorine

18/2 case E identified, OMT
Additional measures installed:
- C&D of all patient rooms of half the department
- Twice daily C&D of sanitation
- Daily C&D of bedpan chairs

19/2 & **24/2** environmental cultures taken

25/2 Use of only cardboard single use bedpans in toilet chairs

25/4 Additional measures were discontinued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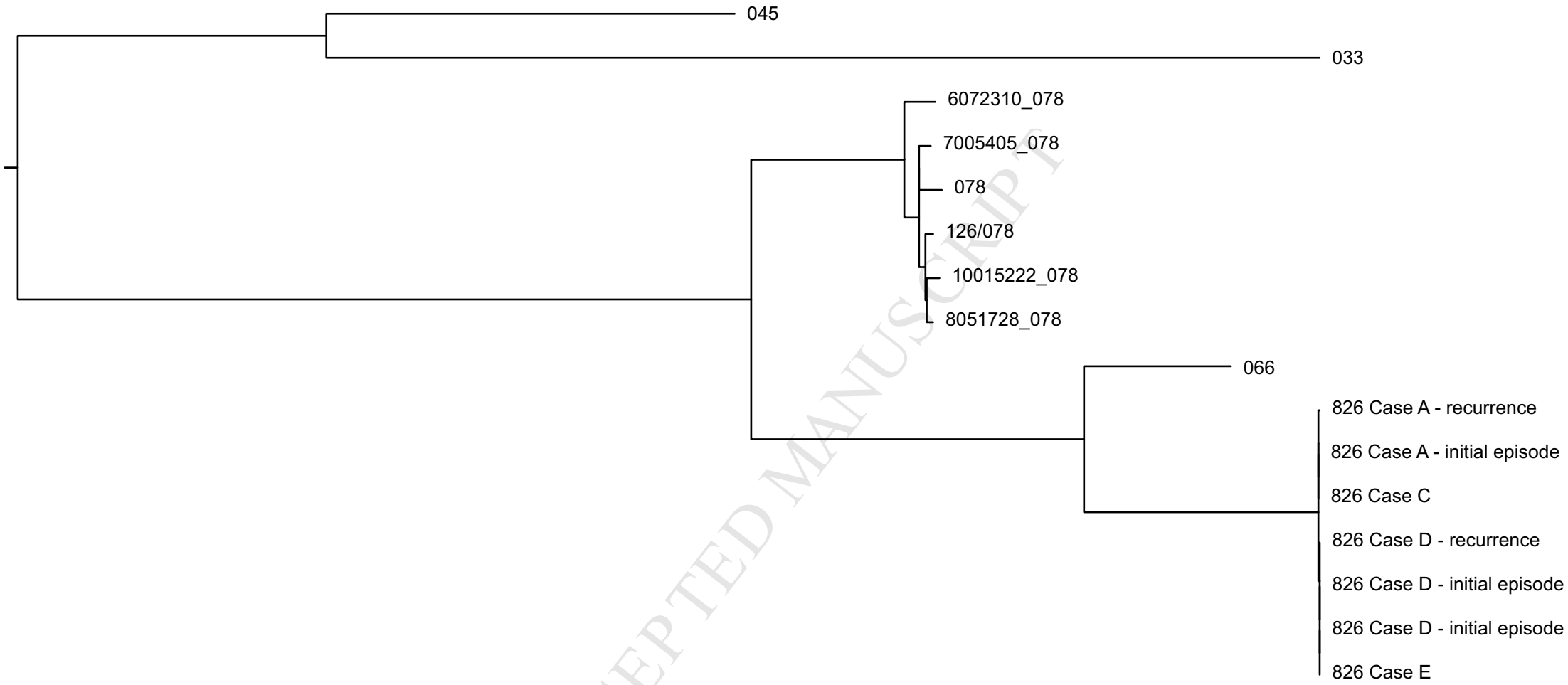

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