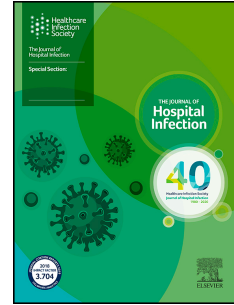


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Successful Management of a *Clostridioides difficile* Ribotype 027 Outbreak with a Lean Intervention Bundle

Andrea B. Kuenzli, Silvie Burri, Carlo Casanova, Rami Sommerstein, Niccolo Buetti, Helena MB. Seth-Smith, Thomas Bodmer, Adrian Egli, Jonas Marschall



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1 **Successful Management of a *Clostridioides difficile* Ribotype 027 Outbreak with a Lean**  
2 **Intervention Bundle**

3  
4 Andrea B. Kuenzli<sup>1</sup>, Silvie Burri<sup>1</sup>, Carlo Casanova<sup>2</sup>, Rami Sommerstein<sup>1</sup>, Niccolo Buetti<sup>1,3</sup>,  
5 Helena MB Seth-Smith<sup>4,5</sup>, Thomas Bodmer<sup>6</sup>, Adrian Egli<sup>4,5</sup>, Jonas Marschall<sup>1</sup>

6  
7 <sup>1</sup> Department of Infectious Diseases, Inselspital, Bern University Hospital, University of Bern,  
8 Switzerland;

9 <sup>2</sup> Institute for Infectious Diseases, University of Bern, Switzerland;

10 <sup>3</sup> IAME, DeSCID team, INSERM, Université Paris Diderot and Sorbonne Paris Cité, Paris,  
11 France; <sup>4</sup> Division of Clinical Bacteriology and Mycology, University Hospital Basel,  
12 Switzerland;

13 <sup>5</sup> Applied Microbiology Research, Department of Biomedicine, University of Basel, Switzerland;

14 <sup>6</sup> labormedizinisches zentrum Dr. Risch, Liebefeld, Switzerland

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21  
22  
23 Corresponding author:

24 Jonas Marschall, MD, Department of Infectious Diseases, Bern University Hospital,  
25 Freiburgstrasse, PH6 D05, 3010 Bern, Switzerland.

26 Telephone: +41 31 632 06 46.

27 E-mail: [jonas.marschall@insel.ch](mailto:jonas.marschall@insel.ch)

28

29 Alternate corresponding author:

30 Andrea Kuenzli, MD, Department of Infectious Diseases, Hôpital de Pourtalès, Rue de la  
31 Maladière, 2000 Neuchâtel, Switzerland

32 Telephone: +41 79 559 54 66

33 E-mail: [andrea.kunzli@gmail.com](mailto:andrea.kunzli@gmail.com)

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36

37 **Abstract**

38 Background: In a 2015 point prevalence study, *Clostridioides difficile* 027, a hypervirulent  
39 ribotype, was absent from healthcare institutions in Switzerland. In late 2016, we detected an  
40 outbreak of *C. difficile* infection (CDI) with ribotype 027 occurring across several hospitals in the  
41 same hospital network.

42 Methods: The first cases of CDI due to ribotype 027 triggered an outbreak investigation,  
43 including whole genome sequencing (WGS) to identify outbreak strains.

44 Findings: We identified 28 patients with CDI caused by ribotype 027 between December 2016  
45 and December 2017, out of which twenty were caused by a single clone. Commonalities among  
46 these patients were hospitalization in the same room or on the same ward, receiving care from the  
47 same healthcare workers, and shared toilet areas. In addition to the epidemiological links  
48 suggesting possible transmission pathways between cases, WGS confirmed the clonality of this  
49 *C. difficile* 027 outbreak. The outbreak was contained by isolation precautions, raising awareness  
50 among healthcare workers, harmonizing diagnostic algorithms, and switching to a sporicidal  
51 agent for environmental disinfection. Of note, neither default gowning and gloving nor  
52 handwashing with water and soap were implemented.

53 Conclusions: This *C. difficile* 027 outbreak was recognized belatedly due to lack of screening for  
54 this ribotype in some hospitals, and was contained by a swift response with simple infection  
55 prevention measures and adapting the laboratory approach. In order to have a better  
56 understanding of *C. difficile* epidemiology, diagnostic approaches should be standardized, CDI  
57 declared notifiable, and longitudinal data on prevalent ribotypes collected in countries where this  
58 is not established.

## 59 **Introduction**

60 *Clostridioides difficile* infection (CDI) is a common healthcare-associated infection and often  
61 causes outbreaks. These outbreaks can be difficult to manage because transmission not only  
62 occurs via contact but also through the environment, where *C. difficile* spores may survive for  
63 extended periods of time [1]. Certain ribotypes of *C. difficile* have been found to be more virulent  
64 and more likely to sporulate than others. The ribotype 027/NAP1/B1 is considered the most  
65 prominent hypervirulent ribotype [2]. It first came to attention in 2000 when an outbreak with  
66 unusually poor clinical outcomes was reported from Philadelphia [3]. Since then, *C. difficile* 027  
67 has caused numerous outbreaks in healthcare settings around the world and is feared both for its  
68 effect on mortality and the increased risk of recurrent CDI in affected patients [4]. Accordingly,  
69 the knowledge on how to best prevent CDI cases and outbreaks has been assembled in practice  
70 guidelines such as in the HAI compendium by the Society for Healthcare Epidemiology of  
71 America (SHEA) [5].

72 In a 2015 point-prevalence study, *Clostridioides difficile* 027 was absent from healthcare  
73 institutions in Switzerland [6], although rare cases had been reported previously [7]. Within one  
74 week in December 2016, we detected three unrelated cases of patients affected by *C. difficile* 027  
75 in our university hospital. Subsequently, an outbreak of *C. difficile* 027 occurred across several  
76 hospitals in the same network, which continued until December 2017. Here, we report on this  
77 outbreak, and how we investigated and managed it. A special focus is placed on the lean  
78 intervention measures used to halt the outbreak.

79

80

## 81 **Methods**

82 In December 2016, our central microbiology laboratory identified a potential hypervirulent *C.*  
83 *difficile* 027. Within four days, two other patients were found to be affected, and an outbreak  
84 investigation was started, including a detailed line list and an epidemic curve.

### 85 *Case definitions*

86 All patients from our hospital group with stool samples indicative for *C. difficile* 027 (see section  
87 on laboratory analysis) were included in this outbreak report, without any exclusion criteria,  
88 resulting in 28 patients since December 2016. Twenty patients were affected by the outbreak  
89 clone, as confirmed by WGS.

### 90 *Setting*

91 Our hospital group consists of a 950-bed tertiary care hospital, a city hospital, three regional  
92 hospitals, and a rehabilitation clinic, together caring for approximately 60,000 inpatients per year,  
93 and with a catchment area of approximately 1,000,000 inhabitants. Patients are transferred to  
94 another site within the hospital group according to their medical needs. Each hospital has its own  
95 staff, which are not shared with other sites.

### 96 *Infection control measures*

97 We noticed that initially, some of the laboratories within our hospital group only used a rapid  
98 enzyme immunoassay for the detection of *C. difficile* toxins A and B. These assays do not  
99 identify putative ribotype 027 strains. Therefore, starting the third week of the outbreak, all stool  
100 samples with a positive screening test for *C. difficile* were analyzed in the central lab using a PCR  
101 method that indicates hypervirulent strains (Figure 1).

102 In addition to the standard of care requiring contact isolation and a separate restroom for every  
103 patient with diarrhoea, known case patients were admitted to single rooms only. Rooms of  
104 affected patients were disinfected with a sporicidal agent (Pentapotassium

105 bis(peroxymonosulphate) bis(sulphate), Perform1%™, Schuelke, Hamburg, Germany) upon  
106 patient discharge. Despite these measures, additional patients tested positive, and fomites of some  
107 of those rooms were suspected to be the source of ongoing transmission. Therefore, isolation  
108 precautions and cleaning procedures were stepped up: 1) Sporicidal cleaning of rooms of *C.*  
109 *difficile* 027 positive patients was performed once daily; 2) Wards with more than two affected  
110 patients and therefore suspicion of transmission were at one time cleaned entirely with sporicidal  
111 agents, starting the third week of the outbreak.

112 Daily contact between the teams from the affected wards and the infection prevention team  
113 ensured understanding of the need for enhanced preventive measures and may have led to  
114 improved compliance with hand hygiene. The division chiefs and head nurses of the entire  
115 hospital group were notified of the outbreak and an information sheet on infection control  
116 measures for this pathogen was distributed by e-mail. In addition, clinicians were encouraged to  
117 test for *C. difficile* in any patient with new onset of diarrhoea during hospitalization, which  
118 resulted in a 31% increase of tests for *C. difficile* in the third month of the outbreak.

119 Thus, our lean intervention bundle consisted of three elements: 1) ensuring that patients were  
120 correctly diagnosed by harmonizing the lab approach and promoting *C. difficile* testing in all  
121 patients with diarrhoea; 2) daily ward rounds by the IPC team to raise awareness of the  
122 importance of hand hygiene using alcohol-based solutions; and 3) sporicidal environmental  
123 cleaning.

#### 124 *Laboratory analysis*

125 In the tertiary care hospital of the group, stool samples are screened for *C. difficile* using  
126 glutamate dehydrogenase ELISA (GDH ELISA; C-DIFF CHEK-60®, Techlab, Blacksburg VA,  
127 USA), followed by real-time PCR for toxins and suspected hypervirulence (GeneXpert® *C.*  
128 *difficile*, Cepheid, Sunnyvale CA). A combination of positive toxin B gene, *tcdCΔ117* deletion (a

129 regulator gene of toxin synthesis), and positive binary toxin gene, is highly suspect of the 027  
130 ribotype. However, in the other four hospitals of our group, stool samples were initially only  
131 tested for the presence of *Clostridioides* toxin A and B (Immunocard Toxins A&B, Meridian  
132 Bioscience Inc., Memphis TN, USA), without any further analysis. As this approach does not  
133 detect potential ribotype 027, from the third week of the outbreak on, all stool samples that  
134 screened positive for *C. difficile* were analyzed in the main microbiology laboratory using  
135 GeneXpert®. Stool samples suspected to contain *C. difficile* 027 were sent for culture, ribotyping  
136 and whole genome sequencing (MiSeq, Illumina, San Diego CA, USA) to the University  
137 Hospital Basel, starting December 2016.

138 PCR-ribotyping was performed using high-resolution capillary gel-based electrophoresis [8] as  
139 described elsewhere [9]. Capillary electrophoresis used the ABI-3500 Genetic Analyzer (Applied  
140 Biosystems [Life Technologies], Foster City, CA). Fragments were analysed using GeneMapper  
141 v 5.0 (Applied Biosystems) and Bionumerics v 7.6.2 (Applied Maths, Sint-Martens-Latem,  
142 Belgium) software to compare fragment profiles against the standard set of the ECDC Brazier  
143 strain collection of PCR ribotypes, obtained from the European *Clostridium difficile* infection  
144 study network (ECDIS-NET).

#### 145 *Whole-Genome Sequencing*

146 All suspected 28 *C. difficile* 027 isolates underwent DNA extraction using EZ1 Advanced XL  
147 (Qiagen, Hilden, Germany), except for one sample which did not show growth. Resulting DNA  
148 was sequenced on the Illumina MiSeq (300 bp paired end reads) or NextSeq (150 bp paired end  
149 reads) platforms following Nextera XT or Nexteraflex library creation. The genome of isolate  
150 CdBe2 was assembled in CLC Genomics Workbench 9.5.3 giving 575 contigs totalling 4.2Mb.  
151 All data were mapped within CLC Genomics Workbench 12.0.3 against this reference genome



152 giving mean read depth over 52x in all cases but one (37x). All WGS data is available from the  
153 European Nucleotide Archive (<https://www.ebi.ac.uk/ena/>) under project PRJEB37809.

#### 154 *Ethical considerations*

155 Given the fact that this outbreak investigation was conducted as part of the portfolio of duties by  
156 our intervention prevention unit and considered quality assurance, institutional review board  
157 approval was not required.

158

## 159 **Results**

### 160 *Outbreak description*

161 The first detection of a potential ribotype 027 in stool samples of three patients within one week  
162 triggered an outbreak investigation. Infections with the outbreak clone affected twenty patients,  
163 with a mean age of 77 years (range, 56 to 88 years); all were inpatients, and all had received  
164 antibiotics before presenting with CDI. Three patients (15%) died as a result of the infection; a  
165 fourth patient died of sepsis of unknown cause two weeks after in-patient treatment for CDI.  
166 Three patients (15%) suffered from relapses (in total, seven episodes), requiring five  
167 readmissions for colitis in two of those patients. One patient was treated with two fecal  
168 microbiome transplantations from her son, as relapses occurred despite several courses of  
169 antibiotic treatment. The subsequent length of stay was 13 days once CDI had been diagnosed  
170 (median; range 7.25 to 20 days), compared to an overall average stay of 6 days in our hospital  
171 group.

172 Four out of five hospitals of our hospital group were involved in the outbreak, across eleven  
173 individual wards. We noted clustering of cases, with one specific ward in hospital A witnessing  
174 six patients and another ward in hospital B having seven patients, with few patients being

175 transferred between hospitals. A spatio-temporal investigation revealed shared restrooms, shared  
176 rooms and care provided by the same healthcare workers as the most likely sources of  
177 transmission. Being admitted to the same ward as a CDI patient, but not the same area within that  
178 ward, for a time-period of only 20 hours proved sufficient for transmission in one case. However,  
179 for a few patients, the transmission route could not be established, *e.g.*, one patient had no other  
180 feature in common with a symptomatic patient other than having a cardiac ultrasound performed  
181 using the same equipment a few hours later. Certain other institutions use the Bern University  
182 microbiology laboratory for processing their samples; the revised algorithm allowed us to detect  
183 one further case in a regional hospital outside our network. This patient had never visited our  
184 hospital network before being diagnosed with CDI, but was transferred to one of our  
185 rehabilitation clinics afterwards.

186 Most cases were detected within a three-month period after the beginning of the outbreak. In our  
187 hospital network, no new infections due to this strain were identified after December 2017, and  
188 this remains the case as of July 15<sup>th</sup>, 2020 (Figure 1).

#### 189 *Outbreak strain characterization by WGS*

190 In all stool samples highly suspect of ribotype 027 by GeneXpert®, this hypervirulent ribotype  
191 was confirmed by ribotyping, with the exception of one sample from which *C. difficile* could not  
192 be cultured.

193 Ribotyping may show limited information in terms of resolution, as outbreak and non-outbreak  
194 related isolates with the same ribotype cannot be differentiated. Therefore, we conducted an  
195 analysis using whole genome sequencing. Phylogenetic analysis of all *C. difficile* 027 isolates  
196 confirmed that all outbreak isolates (samples CdBe01-20) are very closely related, being identical  
197 across the whole genome with the exception of 1-3 SNP differences, seen in six isolates (Figure  
198 2). Seven further ribotype 027 isolates were identified during 2017 (samples CdBe21-27), which

199 showed over 30 SNP differences to the outbreak strain, suggesting that these are unlikely to be  
200 direct transmissions; they also were not epidemiologically linked.

201

## 202 **Discussion**

203 Several hospitals in our network were affected by this outbreak caused by a single clone of *C.*  
204 *difficile* 027, a ribotype not identified in a nationwide point-prevalence study the year before.

205 In order to facilitate implementation, we opted for a lean intervention bundle to counter this  
206 outbreak: focusing on raising awareness of this hypervirulent ribotype, harmonizing the  
207 diagnostic approach, strict hand hygiene, and sporicidal cleaning.

208 Stool samples of two of the earliest patients had tested positive for *C. difficile* by Immunocard  
209 toxin testing three weeks before their confirmation as ribotype 027. Most likely they would have  
210 been identified as suffering from *C. difficile* 027, had adequate diagnostic methods been  
211 employed. This delayed the recognition of the outbreak and thus enabled spreading of the  
212 hypervirulent ribotype, as indicated by missing epidemiological links among some of the first  
213 patients. Standardizing the lab diagnostic procedure allowed identification of stool samples with  
214 a possible 027 strain, which was confirmed by WGS in all cases but one. Detailed phylogenetic  
215 analysis using WGS based data revealed that 20 isolates fell within three SNPs of the reference  
216 case, which is highly suggestive of transmission of the outbreak clone between individual cases.

217 Transmission most probably occurred through contaminated hands of healthcare workers, as few  
218 patients had direct contact among each other. In several cases, being admitted to the same unit as  
219 an infected patient, but not in the same room, even for less than 24 hours, was sufficient for  
220 transmission. Residual spores not eliminated by terminal cleaning may have been another way of  
221 transmission.

222 Unfortunately, we could not determine how and when this pathogen was introduced into our  
223 healthcare system. Ribotype 027 is the most common *C. difficile* ribotype reported in European  
224 countries besides Switzerland [10]. However, the standard screening of repatriated patients  
225 arriving in our hospital currently does not include *C. difficile*, so we have insufficient insight into  
226 transmission dynamics.

227 In order not to undermine adherence to our modified contact precautions (which does not require  
228 gloves or gowning unless if anticipating contact with bodily fluids [11]), we did not require glove  
229 use for every contact with a CDI patient, nor did we enforce hand washing with soap and water  
230 instead of our alcoholic handrub. This decision was taken despite the fact that handrub alcohol  
231 does not kill *C. difficile* spores.

232 According to the 2018 IDSA clinical practice guidelines for *C. difficile* infection, in endemic  
233 settings, either soap and water or an alcohol-based hand hygiene product can be used (strong  
234 recommendation, moderate quality of evidence), whereas in outbreaks, hand hygiene with soap  
235 and water should be given preference (weak recommendation, low quality of evidence) [12].

236 Likewise, the European Society of Clinical Microbiology and Infectious Diseases Study Group  
237 for *C. difficile* recommends switching from alcohol-based handrub to hand washing in outbreak  
238 settings (conditional recommendation, very low quality of evidence), as well as using gloves and  
239 gowns (strong recommendation, very low quality of evidence) [13].

240 Despite these recommendations, we felt that there was no need for stepping up and propagating  
241 general glove use or hand washing with water and soap prior to leaving the patient room, as the  
242 installed bundle halted the outbreak.

243 Daily sporicidal cleaning of affected patients' rooms and one-time sporicidal cleaning of entire  
244 wards with possible transmission proved to be sufficient to substantially reduce hospital-acquired  
245 CDI, as described in one other report [14].

246 Other reported *C. difficile* 027 outbreaks were controlled with: terminal cleaning [15] or cleaning  
247 of an entire facility [16] including adjacent rooms upon discharge of a CDI patient [17]; efficient  
248 case identification and treatment [18-20]; isolating CDI patients in single rooms [19, 20] or on a  
249 dedicated ward [18, 21]; isolating patients with diarrhoea until *C. difficile* was ruled out [21]; and  
250 restricting fluoroquinolone use [18-21]. Some reports describe the successful use of hydrogen  
251 peroxide for environmental disinfection [19], also as a vaporized preparation [17, 20], or  
252 chlorine-containing disinfectants [20, 21]. In contrast, daily cleaning of a CDI patient's room and  
253 of the bedpan cleaning area with non-sporicidal disinfectants (chloride concentration < 1000  
254 p.p.m.) actually *increased* CDI incidence in one report [22]. Selective decontamination of the  
255 digestive tract in ICU patients (using oropharyngeal and intestinal applications of colistin,  
256 tobramycin and amphotericin in combination with systemic cefotaxime during the first two to  
257 four days) during an outbreak also increased CDI risk [19]. Information campaigns to medical  
258 personnel were key in several reports [16, 20, 21, 23]. Most reports, however, stressed reinforcing  
259 hand hygiene [19-21], some also by the affected patients themselves [18, 20], and wearing gloves  
260 and gowns [19, 20].

261 To our knowledge, so far no outbreak has been managed by continuing the usual hand hygiene  
262 with alcoholic solutions and by explicitly refraining from both handwashing with soap and water  
263 as well as default gloving and gowning. Our approach was to facilitate compliance with hand  
264 hygiene by maintaining the usual hand hygiene using alcoholic handrub, as studies suggest that  
265 this approach ensues higher compliance compared with hand washing with soap and water [24].  
266 Alcoholic handrub can be made more easily available and its application is less time-consuming.  
267 Potential surface contamination with spores was addressed by sporicidal environmental cleaning.  
268 This is what we decided to label as "lean intervention bundle", as it was a minimalistic outbreak  
269 management strategy that resorted to few but highly effective measures. Further, given the low

270 level of fluoroquinolone utilization in our inpatient setting, we opted against including an  
271 antibiotic stewardship intervention in the bundle of measures to contain this outbreak.

272

273 As for the time being, not all stool samples positive for *C. difficile* are tested for ribotype 027 in  
274 our country, and because CDI is not a notifiable disease in Switzerland, individual cases may be  
275 missed and the spread of potentially hypervirulent strains underestimated. Therefore, we  
276 recommend establishing a nationwide screening for hypervirulent ribotypes of all *C. difficile*  
277 positive stool samples, as well as mandatory notification of health authorities. In case of  
278 clustering of *C. difficile* cases, WGS is to be employed to check for clonality.

279 Limitations of our study include the fact that, because of possible lack of clinical vigilance, and  
280 due to the previous absence of testing for ribotype 027 in peripheral hospitals of our network,  
281 related cases prior to the identified index case may have been missed.

282

### 283 **Conclusion**

284 In conclusion, this *C. difficile* 027 outbreak was caused by a single strain with an unknown  
285 source. Ribotyping alone did not allow strains to be recognized as outbreak clones; this resolution  
286 was achieved by WGS only. The response to this outbreak without gloving and gowning or using  
287 soap and water for hand hygiene, but with sporicidal cleaning, proved to be efficient, and  
288 suggests that such lean intervention bundles may save resources while achieving their goals.

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305

**306 Conflict of interest**

307 None.

308

**309 Disclosure**

310 All authors have approved the final article.

311

**312 Authors' contribution**

313 Outbreak investigation and management: SB, AK, JM, NB

314 Laboratory method harmonization: CC, RS

315 Whole genome sequencing and analysis: HSS, AE

316 Writing of manuscript: AK, JM

317 Critical reviewing of the manuscript: all authors.

318

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320 None

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395 **Figure legends**

396

397 **Figure 1**398 **Epidemiological curve of 027 isolates and interventions**

399

400 **Figure 2**401 **Phylogeny of *C. difficile* 027 isolates from this study.**

402 This neighbour joining single nucleotide polymorphism (SNP) phylogeny used the assembly of  
403 isolate CdBe02 as a reference (shown in bold), rooted using unrelated 027 isolates. It was  
404 generated in CLC Genomics Workbench 12.0.3 with parameters that differed from the default as:  
405 variant calling with 10x minimum coverage, 10 minimum count and 70% minimum frequency,  
406 and SNP tree creation with 10x minimum coverage, 10% minimum coverage, 0 prune distance  
407 and including multi-nucleotide variants (MNVs). Outbreak isolates show a diversity of up to  
408 three SNPs from the reference. Specific examples of epidemiological links between outbreak  
409 isolates are superimposed.

410

411 **Figure S1**

412 **Phylogeny of *C. difficile* 027 isolates including the external laboratory samples.** This  
413 phylogeny used the assembly of isolate CdBe02 as a reference (shown in bold), rooted using  
414 unrelated 027 isolates. Outbreak isolates show a diversity of up to six SNPs from the reference.

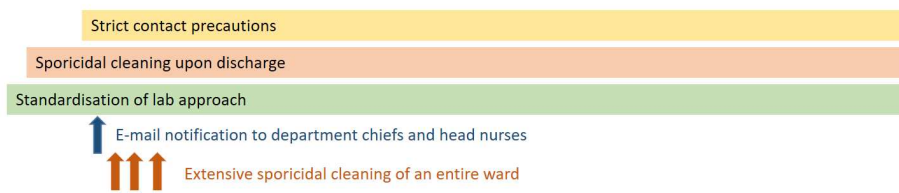
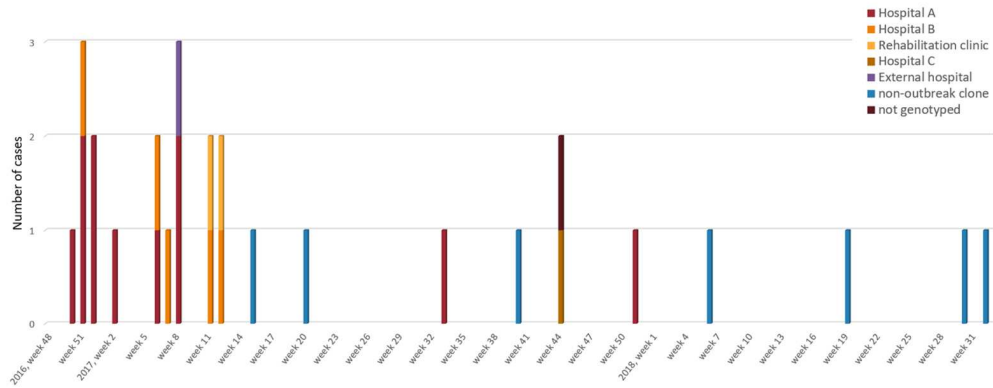
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417 **Figure 1**

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426 **Supplement**

427 In order to better understand the outbreak, we collaborated with a private laboratory,  
428 labormedizinisches zentrum Dr. Risch, serving private hospitals and practices in our region. In  
429 this laboratory, stools are screened for the presence of toxigenic *Clostridoides difficile* using the  
430 algorithm proposed by Fenner *et al.* [25]. Reactive screening tests are confirmed by PCR  
431 (GeneXpert CDIF®). In case of suspected ribotype 027, the stool specimens are sent to an expert  
432 laboratory for confirmation (AE) using ribotyping and whole genome sequencing. In hospitalized  
433 patients, positive test results prompt timely, automated alerts to the sender as well as the  
434 respective hospital hygiene teams.

435 Stool samples analysed at the external private laboratory revealed 14 further patients (median age  
436 82.5 years, range 53 to 93 years) belonging to this cluster. As these outpatients' charts could not  
437 be accessed, we were unable to analyze the outpatients' outcomes and epidemiological links  
438 outside of our hospital group.

439 Only two of these patients had been hospitalized in our hospital group: one patient was admitted  
440 six days after a symptomatic patient into an adjacent ward, the second was managed on the same  
441 ward as another of our patients five months earlier, making a transmission on that ward rather  
442 unlikely. Of these, one patient had diarrhoea after receiving antibiotic therapy as an inpatient, but  
443 was tested for *C. difficile* only after discharge two weeks later; the second patient was diagnosed  
444 with CDI nine months later. Two other outpatients had been seen at our cardiology outpatient  
445 clinic in late 2016 two and seventeen days after a symptomatic inpatient of this cluster did,  
446 respectively. Of note, the ultrasound examinations were performed by different physicians, so  
447 possible transmissions are suspected to have occurred via fomites. However, these outpatients  
448 were diagnosed with the outbreak strain 14 months and 17 months after the clinic visit, so an  
449 epidemiological link is uncertain.

450 Samples from the external laboratory (CdRi01-12) are shown in Figure S1.

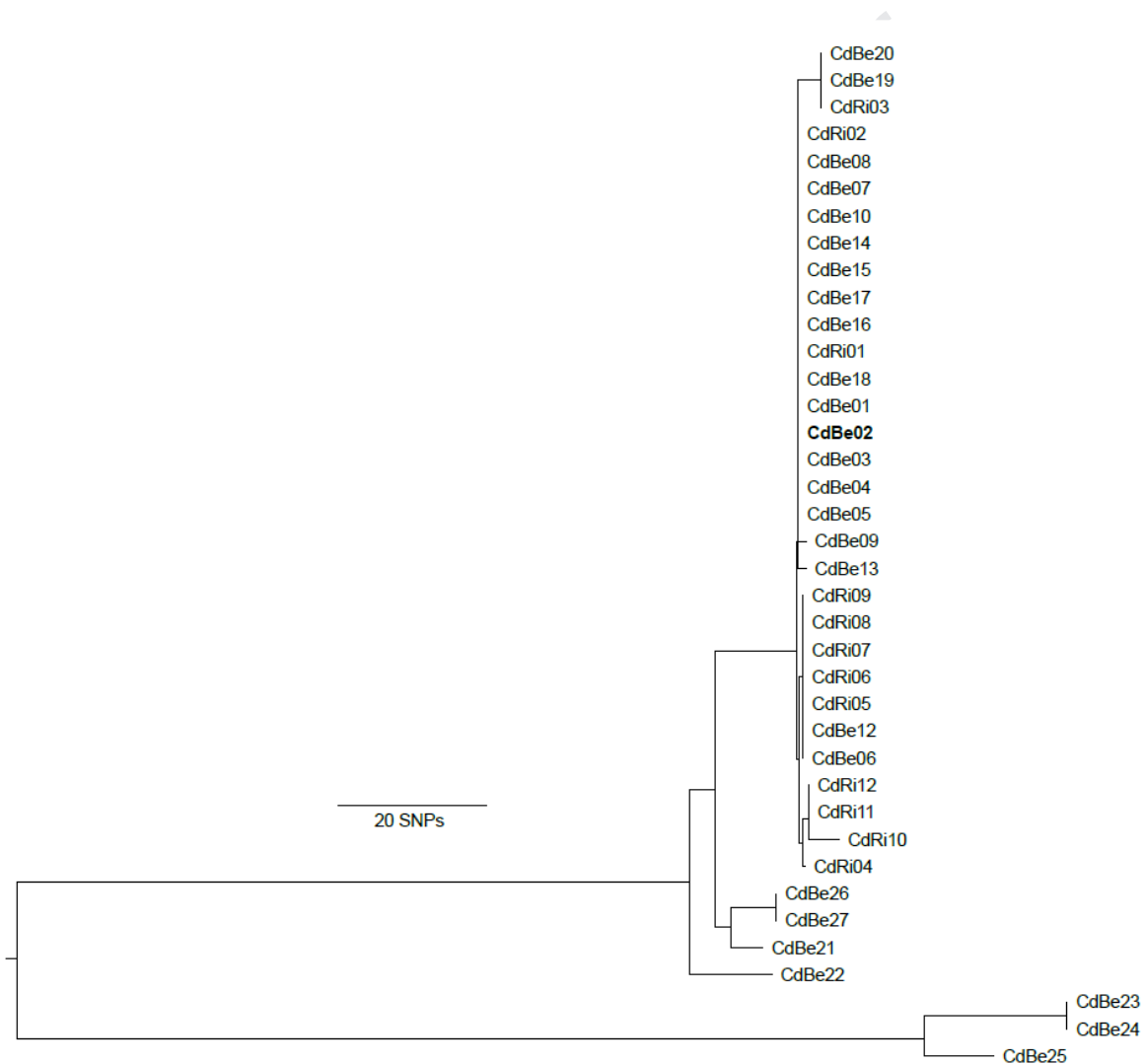
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453

**Figure S1**

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