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

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2   **Intervention Bundle**

3

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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.

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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 15th, 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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Conflict of interest

None.

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Disclosure

All authors have approved the final article.

311

Authors' contribution

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

Laboratory method harmonization: CC, RS

Whole genome sequencing and analysis: HSS, AE

Writing of manuscript: AK, JM

Critical reviewing of the manuscript: all authors.

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320   None

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394

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.
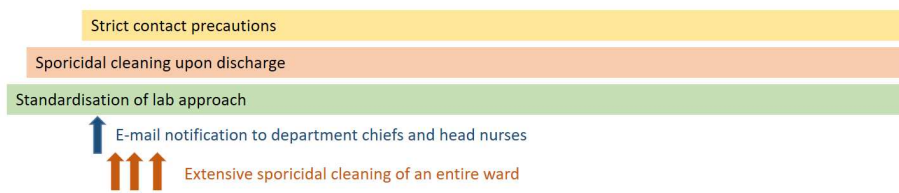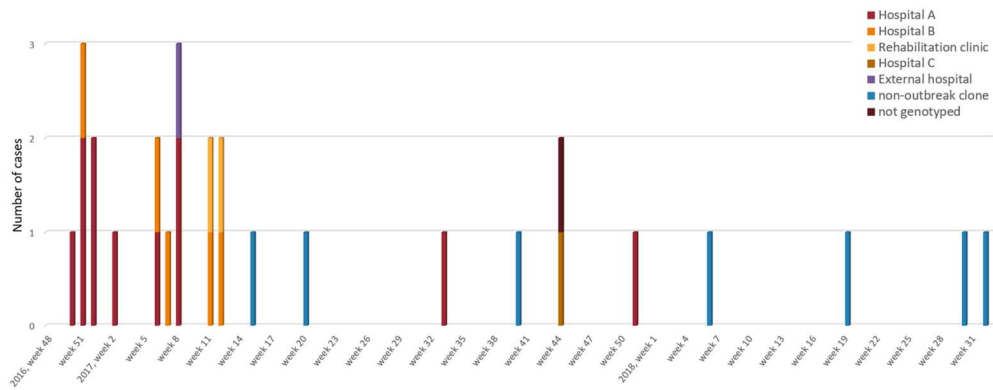
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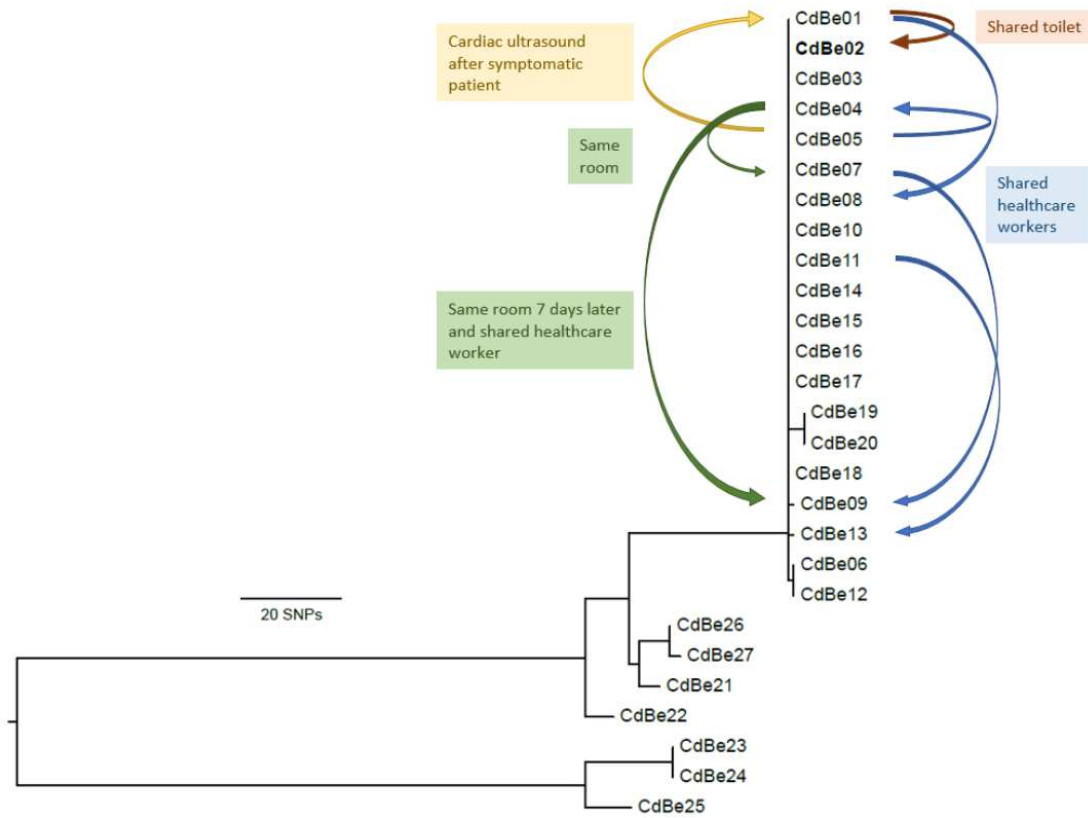
417   **Figure 1**

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423   **Figure 2**

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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.

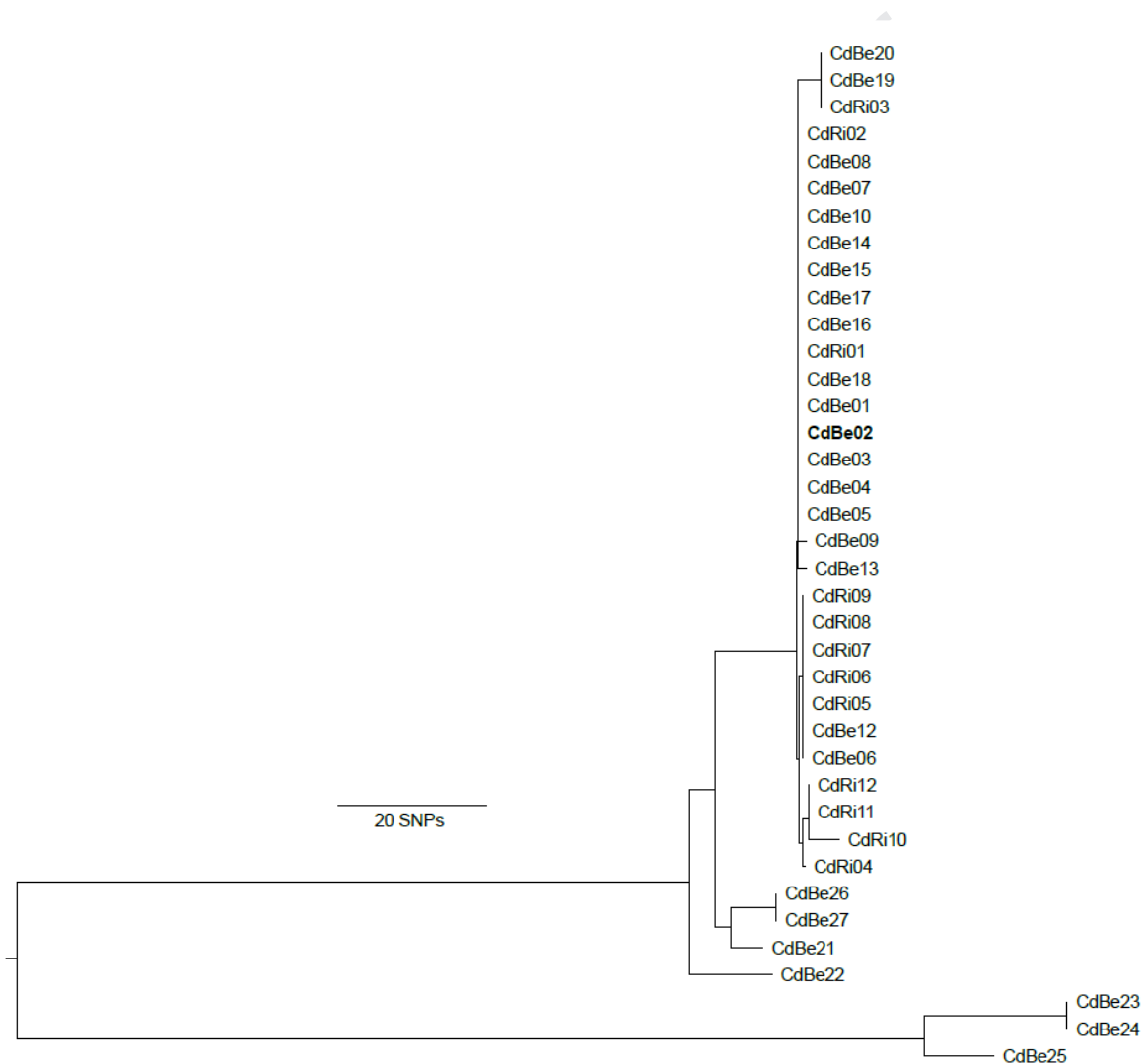450   Samples from the external laboratory (CdRi01-12) are shown in Figure S1.

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453   **Figure S1**

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