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## **Toxigenic Clostridium difficile carriage in general practice: results of a laboratory-based cohort study**

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2 **Toxigenic *Clostridium difficile* carriage in general practice: results of a laboratory-based cohort**  
3 **study**

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21 **Running title:** *Clostridium difficile* in general practice

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40 disease in Amsterdam, The Netherlands (8-12 April 2016) (EP022)

41

42 **Summary of the article's main point:**

43 Stool samples from 2541 patients attending their general practitioner (GP) for diarrhoea or other  
44 gastrointestinal disorders were systematically tested for *C. difficile* irrespective of physician request.

45 The results show that toxigenic *C. difficile* carriage is frequent in general practice but remains under-  
46 recognized.

47 **Objectives**

48 The reported rates of community-acquired *Clostridium difficile* infections (CDIs) have been  
49 increasing. However, the true burden of the disease in the general practice is still unknown in France.  
50 Our objective was to determine the incidence of toxigenic *C. difficile* (CD) carriage and the  
51 percentage of stool samples prescribed by general practitioners (GPs) which contained free *C. difficile*  
52 toxins .

53 **Methods**

54 During an eleven-month period, all stool samples submitted for any enteric pathogen detection to 15  
55 different private laboratories in Paris and the surrounding areas were tested for *C. difficile*,  
56 irrespective of the general practitioners' request. A clinical questionnaire was completed for each  
57 patient. Stool samples were screened using a rapid simultaneous GDH and toxins A/B detection test:  
58 any positive result (GDH or toxin) was further confirmed by the stool cytotoxicity assay (CTA) on  
59 MRC-5 cells and by toxigenic culture (TC) at a central laboratory. *C. difficile* isolates were  
60 characterized by PCR ribotyping.

61 **Results**

62 2541 patients (1295 female, 1246 male) were included. The incidence of patients with a positive  
63 toxigenic culture and a positive CTA was 3.27% [CI95% 2.61-4.03] and 1.81% [CI95% 1.33-2.41],  
64 respectively. GPs requested CD testing in only 12.93% of the stool samples, detecting 52.30% of all  
65 TC-positive patients. The 83 toxigenic *C. difficile* strains belonged to 36 different PCR-ribotypes.

66 **Conclusions**

67 Toxigenic *C. difficile* carriage is frequent in general practice but remains under-recognized. It may  
68 affect young patients without previous antimicrobial therapy or hospitalisation.

69

70

## 71 **Introduction**

72 *Clostridium difficile* is a major enteropathogen which is responsible for 10-25% of antibiotic-  
73 associated diarrhoea and for virtually all cases of pseudomembranous colitis (PMC) [1, 2]. It is the  
74 most frequent agent of hospital acquired diarrhoea in adults [3], and *Clostridium difficile* infections  
75 (CDIs) are associated with prolonged hospital stay and excess mortality [4]. The clinical epidemiology  
76 and frequency of healthcare-associated CDI is well described [5, 6] whereas little is known about the  
77 true burden of the disease in the general practice. Currently, more than a quarter of all CDIs are  
78 estimated to be community-acquired [3, 7]. Therefore, our aim was to determine the incidence of  
79 toxigenic *C. difficile* carriage in patients who submitted a faecal stool sample for routine  
80 microbiological testing following a visit with their general practitioners (GP).

## 81 **Materials and methods**

### 82 ***Study design***

83 This prospective cohort study was conducted in 15 different private laboratories in Paris (France) and  
84 the surrounding areas. During an eleven-month period (August 10<sup>th</sup> 2015 to June 30<sup>th</sup> 2016), all  
85 patients who submitted a faecal stool sample for routine microbiological testing following a visit with  
86 their GP for diarrhoea or other gastrointestinal disorders were consecutively included. All stool  
87 samples were systematically tested for *C. difficile*, irrespective of GP request. Duplicate stool  
88 samples (defined as a patient with a second stool sample) were excluded. For each patient, a  
89 standardized questionnaire including demographic data (gender, age, hospitalization in the previous  
90 12 weeks) and clinical data (diarrhoea, CDI in the previous 8 weeks) was completed. Information  
91 about antimicrobial treatment in the month prior to obtaining the stool sample was also recorded.  
92 The study protocol was approved by the local Research Ethics Committee and by the Comité  
93 consultatif sur le Traitement de l'Information en matière de Recherche dans le domaine de la Santé

94 (CCTIRS n° 15.1021). Study information was given to the patients, and the patients signed an  
95 informed consent form.

96

### 97 **Microbiological analysis**

98 Stool samples were collected in 15 different laboratories and were sent within two hours to a central  
99 laboratory, which used a rapid simultaneous GDH and toxins A/B detection assay (C. Diff Quik Chek  
100 complete®, TechLab, Alere) for analyses according to the manufacturer's instructions: any positive  
101 result (GDH or toxin) was further confirmed by the stool cytotoxicity assay (CTA) and the toxigenic  
102 culture (TC) at the National Reference Laboratory for *C. difficile*.

103 CTA was performed using MRC-5 cells as described elsewhere [8]. Toxigenic culture was performed  
104 on the selective chromogenic medium ChromID *C. difficile* (bioMérieux, Marcy l'Etoile, France). Plates  
105 were incubated for 48 hours at 37°C in an anaerobic atmosphere. Black colonies or suspicious non-  
106 black colonies (based on the morphological aspect) were analysed using Matrix Assisted Laser  
107 Desorption Ionisation-Time Of Flight (MALDI-TOF) mass spectrometry. DNA extraction from colonies  
108 of *C. difficile* was performed with the InstaGene Matrix™ kit (Bio-Rad Laboratories, California, USA).  
109 The *in vitro* determination of the cytotoxicity of *C. difficile* isolates was performed by an in-house  
110 multiplex PCR assay detecting *tpi*, *tcdA*, *tcdB*, *tcdC*, *cdtA* and *cdtB* genes coding for triose phosphate  
111 isomerase, toxin A, toxin B, TcdC and the two components of the binary toxin, respectively  
112 **(supplementary Table).**

113 *C. difficile* isolates were characterized by capillary gel electrophoresis-based PCR-ribotyping on the  
114 ABI 3500 sequencer (Applied Biosystems), using primers described by Bidet *et al.* [9]. PCR ribotypes  
115 (PR) were assigned by comparing profiles to European reference collection strains including PR 001,  
116 002, 003, 005, 012, 014/020, 015, 017, 018, 019, 023, 027, 029, 046, 050, 053, 056, 070, 077, 075,  
117 078, 081, 087, 106, 117, 126, 130, 131 and 430.

118 At each sampling site, stool samples were also introduced into 2-ml Cary Blair broth using the Faecal  
119 Transwab (Medical wire mwe, Corsham, England) and into a selenite broth (Elitech, France). The  
120 broth samples were then sent to a central laboratory. Swabs were automatically plated (Kiestra,  
121 Becton Dickinson) on selective media, including Campyloset (bioMérieux), Hektoen (bioMérieux),  
122 Drigalski (bioMérieux), CIN (bioMérieux), and Sabouraud (bioMérieux) which were incubated for 24h  
123 at 37°C in an aerobic atmosphere (48h in microaerophilic atmosphere for Campyloset). Selenite  
124 broth samples were first incubated for 24 hours at 37°C, then plated on Hektoen. Identification of  
125 *Salmonella* spp., *Shigella* spp., *Campylobacter* spp. and *Yersinia* spp. was confirmed by MALDI-TOF  
126 mass spectrometry (Andromas, bioMérieux). *Shigella* sp. was also confirmed by biochemical strip  
127 (API 20E, bioMérieux) and latex agglutination (Bio-Rad).

128 Stool samples were also tested for the presence of Rotavirus and Adenovirus upon a physician's  
129 request using the Rapid Immunochromatographic Assay Rota/Adeno (Meridian Bioscience Europe)  
130 according to the manufacturer's instructions.

131

### 132 **Parasitological stool examination**

133 Parasitological stool examination was also performed upon a physician's request. Parasitic elements  
134 were observed by direct stool examination and after concentration with KOP-COLOR II (Fumouze  
135 Diagnostics, Levallois-Perret, France). Briefly, the sediment obtained after two-phase concentration  
136 (Bailenger's method) was resuspended with 1 or 2 drops of physiological saline. 25 µl of the  
137 suspension was mixed with 10 µL of KOP-COLOR II on a slide. A coverglass was placed over the  
138 sediment suspension, which was examined under a microscope using a white light (blue filter).

139

### 140 **Case definition**

141 A diarrheic stool sample was defined as a stool taking the shape of the container. Clinical diarrhoea  
142 was defined by the passage of 3 or more loose stools for at least 24 hours. *C. difficile* carriage was

143 considered community-acquired if the patient had no hospitalization in the previous 12 weeks [10].  
144 *C. difficile* carriage was considered as healthcare-associated if the patient has been hospitalized in  
145 the previous 4 weeks, as “indeterminate” when the patient has a history of hospitalisation 4-12  
146 weeks before stool sample collection and as ‘unknown’ origin when the exposure setting could not  
147 be determined because of lack of available data.

148

#### 149 ***Statistical analysis***

150 Statistical analyses were performed using the R software, version 3.3.3 (R Core Team (2017). R  
151 Foundation for Statistical Computing, Vienna, Austria). Patient demographic characteristics were  
152 described by their median and interquartile range (IQR) for quantitative variables and by the  
153 frequency for categorical variables. Quantitative and categorical variables were compared between  
154 patients with or without CDI by Mann-Whitney-Wilcoxon and Fisher exact tests. Factors  
155 independently associated with a CDI were identified in 2 steps: 1) Factors associated with CDI were  
156 first explored by performing bivariate comparisons; 2) All factors significantly associated with a CDI (p  
157 value <0.05) were then entered into a multivariate logistic regression model. A p value of <0.05 was  
158 considered significant.

159



## 160 **Results**

161 In total, 2565 patients were invited to participate in the study: 24 patients were excluded because of  
162 duplicate stool samples (n=14), lack of consent (n=7), and error in stool labelling (n=3). In total, 2541  
163 patients (1295 female, 1246 male, median age=38 years) were included. Among them, 106 (4.17%)  
164 were children less than 3 years old. Of the 2541 patients, 1264 (49.74%) reported clinical diarrhoea  
165 and 1253 (49.31%) had abdominal pain. Among the 1253 patients with abdominal pain, 331 (26.41%)  
166 did not present with clinical diarrhoea and among these 201 (16.04%) had no diarrheic stools. Only  
167 120 patients (4.72%) had a previous hospital stay either in day-care (n=42), acute care setting (n=69),  
168 or in a long-term care facility (n=2). Two patients reported a CDI episode in the previous 8 weeks.

### 169 **Comparison of EIA assay for GDH and toxins versus TC and CTA**

170 Screening for *C. difficile* was performed by a central laboratory using EIA for GDH and toxins. Of the  
171 2541 patients, 116 were positive for GDH: 112 were subsequently confirmed by culture on ChromID  
172 *C. difficile* and 4 turned out to be negative, despite several culture attempts. In addition, 33 patients  
173 were positive by EIA for toxins, of which 29 were confirmed by CTA and 4 were negative by CTA.  
174 Among the 83 samples negative for toxins by EIA but positive for GDH, 17 were positive by CTA.

### 175 ***Incidence of enteropathogens***

176 The incidences of patients with a positive TC and a positive stool CTA were 3.27% [83/2541, CI95%  
177 2.61-4.03] and 1.81% [46/2541, CI95% 1.33-2.41], respectively (**Figure 1**). Among stool samples  
178 positive for toxigenic *C. difficile*, 7 (8.4%) were positive for other enteropathogens (3 *Salmonella* sp.,  
179 1 *Campylobacter* sp., 1 *Yersinia enterocolitica*, 1 *Giardia intestinalis*, 1 *Endolimax nana*)

180 Of the 2541 patients, 2199 had a bacteriological stool culture, 2001 had a parasitological stool  
181 examination, 1663 had both, and 114 had viral testing for the presence of adenovirus and rotavirus  
182 antigens. Incidence of the different pathogens is reported in **Figure 2**. Among all the microorganisms  
183 detected, many (such as *Blastocystis*, *Endolimax*, *Entamoeba coli*, *Trichomonas intestinalis*,

184 *Chilomastix mesnili*, *Entamoeba hartmanni* or *Pseudolimax butschlii*) have a doubtful or uncertain  
185 clinical significance.

#### 186 ***Clinical characteristics of patients with toxigenic C. difficile carriage***

187 Among the 66 patients  $\geq 3$  years old with a positive TC, 59 (89.39%) had either a clinical diarrhoea or  
188 a diarrheic stool and 43 (65.15%) had no history of hospitalization in the previous 12 weeks. Among  
189 these, 17 patients (25.75 %) were less than 65 years old, had not received antibiotic treatment in the  
190 month preceding the diarrhoea, and had no enteropathogens other than *C. difficile*. When restricting  
191 the analysis to the 41 patients with a positive CTA, 37 (86.04%) had either a clinical diarrhoea or a  
192 diarrheic stool and 24 (58.53%) had no history of hospitalization in the previous 12 weeks. Among  
193 these 6/41 (14.63%) were  $< 65$  years, had not received antibiotic treatment in the month preceding  
194 the diarrhoea and had no enteropathogens other than *C. difficile*.

195 Using a multivariate logistic regression model, independent factors associated with positive CTA or  
196 positive TC in patients  $\geq 3$  years old were antibiotic treatment in the month prior to diarrhoea and  
197 hospitalization in the previous 12 weeks (**Table 1**).

#### 198 ***Optimal algorithm for CD testing in patients from general practice***

199 GPs requested CD testing in only 12.93 % of the stool samples, resulting in detection of 52.30 % of all  
200 TC-positive patients. We determined the performance of different testing algorithms based on risk  
201 factor identification (**Table 2**) and/or recommendations from the UK or the Netherlands [11]. If *C.*  
202 *difficile* testing were performed in patients  $\geq 65$  years old, with recent antibiotic use or with a history  
203 of recent hospitalization, only 29.53 % of stool samples of our population would have had to have  
204 been processed, and 70% of all toxin-positive cases would have been identified.

#### 205 ***Characterization of C. difficile strains***

206 In total, 83 *C. difficile* isolates were toxigenic and 29 were non-toxigenic. The toxigenic *C. difficile*  
207 isolates harboured both *tcdA* and *tcdB* genes and belonged to 36 different PCR-ribotypes, the

208 complex 014/020 (n=11, 13.25%) being the most predominant (**Figure 3**). Eight isolates (9.63%) were  
209 positive for binary toxin (PR 019, PR 027, PR 126, PR 244). Only two patients were colonized by the  
210 epidemic 027 clone.

211

## 212 Discussion

### 213 Incidence of toxigenic *C. difficile* carriage and other pathogens

214 Our study showed that the overall toxin-positive assay incidence in patients from general practice  $\geq 3$   
215 years old was 1.81% [CI95% 1.33-2.41] and that toxigenic *C. difficile* carriage was 3.27% [CI95%: 2.61-  
216 4.03]. This incidence was higher in elderly patients, as previously reported by others [12, 13].

217 Children under 3 years old were also frequently found positive for toxins and culture, but the  
218 presence of *C. difficile* in this population has often been considered as non-pathogenic [14, 15].

219 Toxigenic *C. difficile* represented the second most frequent bacterial pathogen isolated in patients  
220 from general practice after *Campylobacter* spp. (3.64%). Other recent studies reported a CDI  
221 incidence in the community ranging from 0.62% in a study from Kuwait using GDH detection followed  
222 by PCR [16], to 5.5% in a small study including 288 Australian GP patients, suggesting that *C. difficile*  
223 is a significant cause of community-associated diarrhoea [17]. A point prevalence multicentre  
224 European study of community-associated diarrhoea, where stool samples were investigated with the  
225 FilmArray GI Panel reported an incidence of 1.62% (BioFire Diagnostics, Salt Lake City, UT, USA) [18].  
226 In the Netherlands, the incidence of toxigenic *C. difficile* in 2710 patients who had visited their GP for  
227 gastrointestinal complaints was significantly higher compared to controls without gastrointestinal  
228 complaints matched on age and sex (4.2% versus 1.8%,  $p < 0.001$ ) [19].

229 The incidence of positive free toxins (1.81%) is consistent with that of 1.5% found by Hensgens *et al.*  
230 [11] who looked at 12 714 diarrhoeal stool samples requested by GPs and with Bauer *et al.* [20] who  
231 looked at 2443 stool samples. In UK, a study based on 2000 patients  $> 2$  years old who had visited  
232 their GP showed that the proportion of faecal samples positive for cytotoxin was 2.1% [21]. A more  
233 recent study indicated that *C. difficile* was a rare cause of community-associated diarrhoea [22]. The  
234 strength of our study lies in the large recruitment of patients from Paris and the surrounding areas  
235 and the diagnostic strategy based on GDH screening followed by a highly sensitive method for toxin  
236 detection (the stool cytotoxicity assay), as recommended by the European guidelines [23]. This

237 strategy is highly specific for CDI and the presence of free toxins in stools have been shown to better  
238 correlate to disease severity [24, 25] as compared to detection of toxigenic strain (by PCR or by  
239 culture), which detects carriage of toxigenic strain and is likely to overestimate the true incidence of  
240 CDI. In addition, 86.04% of patients  $\geq 3$  years old with a positive CTA presented with either a clinical  
241 diarrhoea or a diarrheic stool, suggesting that they were truly infected with *C. difficile* rather than  
242 simply colonized.

### 243 **Clinical features of patients with toxigenic *C. difficile* carriage**

244 The survey data of patients with a positive CTA or the presence of a toxigenic strain of *C. difficile*  
245 indicated that more than half of these patients had not been hospitalized within the 12 weeks  
246 preceding the symptoms, suggesting that *C. difficile* carriage was truly community-associated. In  
247 addition, among these patients, 14.65% (for CTA) to 25.75 % (for TC) were < 65 years old, had not  
248 received any antimicrobial treatment in the preceding month and tested negative for other  
249 enteropathogens in the stools. These data highlight that a substantial proportion of community-  
250 acquired toxigenic *C. difficile* carriage occurs in young patients without traditional risk factors for CDI,  
251 as suggested by others [21, 26].

### 252 **Under-recognized toxigenic *C. difficile* carriage in general practice**

253 In France, there are no specific guidelines for testing *C. difficile* in diarrheic patients who visit their  
254 GP. Therefore, it is not surprising that CD testing was requested by GPs in only 12.93% of cases.  
255 However, the testing which was requested was well-oriented and relevant, resulting in detection of  
256 52.3 % and 56.09 % of TC-positive and CTA-positive patients, respectively. Nevertheless, there  
257 remains an important under-recognized diagnosis of toxigenic *C. difficile* carriage. Although we did  
258 not assess the clinical outcome of these undiagnosed patients with toxigenic *C. difficile* carriage, it  
259 has been shown that missed CDI are associated with significantly increased length of diarrhoea than  
260 identified cases [27], emphasizing the risk of adverse outcomes if CDI diagnoses are missed.

261 If CD testing is restricted to patients  $\geq$  65 years or to patients with a history of antibiotic use or  
262 previous hospitalization, then only 29.5% of all stool samples would be tested for *C. difficile*, resulting  
263 in the detection of 63.07% of all TC-positive and 70.73% of all CTA -positive patients. This strategy,  
264 which is currently recommended in UK for samples from the community, would clearly outperform  
265 current practice and would represent the best cost-effective strategy for the identification of  
266 toxigenic *C. difficile* carriage. However, 30% of toxigenic *C. difficile* carriage cases would be still  
267 missed, underlining the limitation of this strategy.

### 268 **PCR ribotypes**

269 We found an increased diversity of PCR ribotypes among strains isolated in patients from general  
270 practice. There was also an overlap in the strains isolated from community-associated cases and  
271 healthcare-associated cases, suggesting an interconnection of these two settings. The most  
272 predominant PCR ribotype (PR 014/020) only represented 13.25 % of all the isolates. These 2 PCR  
273 ribotypes were also the most frequent ribotypes (28%) in a cohort of Danish patients who had visited  
274 their general practice [28] and were the 3rd most frequently found PCR ribotypes in the EUCLID study  
275 [29]. In the study published by Hensgens *et al.* [11], the most common PCR ribotypes were 002 and  
276 078 (11% each) followed by PCR ribotype 015 (9%), 014 (8%), 001 (8%), 005 (6%), and 126 (4%). In  
277 France, a multicentre study conducted in 2009 in 78 healthcare facilities showed that the most  
278 prevalent PR were 014/020 (18.7 %), followed by 078/126 (12.1 %) whereas the prevalence of PR 027  
279 strains remained low (3.1 %) [30]. In the present study, we did not identify any PR 078, which has  
280 been frequently associated with community-associated CDI in a young population. PR 078 strains  
281 found in animals and in humans have been shown to be genetically highly related, suggesting a  
282 foodborne interspecies transmission of *C. difficile* [31]. In Canada, PR 078 epidemic strains were  
283 found in vegetables from grocery stores [32] and are thought to be implicated in a substantial  
284 portion of community-acquired CDI.

285 This study has several limitations. First, we could not calculate the incidence of community-  
286 associated toxigenic *C. difficile* carriage per 100,000 inhabitants, because the catchment area was  
287 unknown. Moreover, the population included in this study lived in Paris and the surrounding areas,  
288 and the results of the study cannot be extrapolated to the whole country. Second, investigation of  
289 the incidence of enteropathogens other than *C. difficile* was not systematically performed, although  
290 1663 samples were both cultured for bacterial pathogens and submitted for parasitological  
291 examination. Third, this study was not designed to monitor patients with toxigenic *C. difficile*  
292 carriage; therefore, we could not assess the impact of carriage in the community in terms of  
293 mortality, hospitalization or quality of life.

294 In conclusion, this study shows that toxigenic *C. difficile* carriage is the second most frequent  
295 pathogen found in stool samples from patients who visited their GP but remains under-recognized.  
296 Physicians should be aware that CDI may occur outside the hospital in patients without traditional  
297 risk factors for CDI.

298

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304 management, or data analysis and interpretation. Alere provided editorial assistance with grammar  
305 and English language editing.

306

307 **Conflict of Interest:**

308 Frédéric Barbut reports grants, personal fees and non-financial support from Astellas, personal fees  
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319

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323 **Ethical approval:** The study protocol was approved by the local Research Ethics Committee.



324

325 **Informed consent:** Yes

326

327

328 **Reference**

329

- 330 [1] Leffler DA, Lamont JT. *Clostridium difficile* Infection. N Engl J Med. 2015;373(3):287-8.
- 331 [2] Bartlett JG. *Clostridium difficile* Infection. Infect Dis Clin North Am. 2017;31(3):489-95.
- 332 [3] Lessa FC, Winston LG, McDonald LC, Emerging Infections Program CdST. Burden of
- 333 *Clostridium difficile* infection in the United States. N Engl J Med. 2015;372(24):2369-70.
- 334 [4] Barbut F, Bouee S, Longepierre L, Goldberg M, Bensoussan C, Levy-Bachelot L. Excess
- 335 mortality between 2007 and 2014 among patients with *Clostridium difficile* infection: a
- 336 French Health Insurance Database analysis. J Hosp Infect. 2017.
- 337 [5] Bauer MP, Notermans DW, van Benthem BH, Brazier JS, Wilcox MH, Rupnik M, et al.
- 338 *Clostridium difficile* infection in Europe: a hospital-based survey. Lancet. 2011;377(9759):63-
- 339 73.
- 340 [6] Davies KA, Longshaw CM, Davis GL, Bouza E, Barbut F, Barna Z, et al. Underdiagnosis of
- 341 *Clostridium difficile* across Europe: the European, multicentre, prospective, biannual, point-
- 342 prevalence study of *Clostridium difficile* infection in hospitalised patients with diarrhoea
- 343 (EUCLID). Lancet Infect Dis. 2014;14(12):1208-19.
- 344 [7] Khanna S, Pardi DS, Aronson SL, Kammer PP, Orenstein R, St Sauver JL, et al. The
- 345 epidemiology of community-acquired *Clostridium difficile* infection: a population-based
- 346 study. Am J Gastroenterol. 2012;107(1):89-95.
- 347 [8] Barbut F, Surgers L, Eckert C, Visseaux B, Cuingnet M, Mesquita C, et al. Does a rapid
- 348 diagnosis of *Clostridium difficile* infection impact on quality of patient management? Clin
- 349 Microbiol Infect. 2013.
- 350 [9] Bidet P, Barbut F, Lalande V, Burghoffer B, Petit JC. Development of a new PCR-ribotyping
- 351 method for *Clostridium difficile* based on ribosomal RNA gene sequencing. FEMS Microbiol
- 352 Lett. 1999;175(2):261-6.
- 353 [10] Krutova M, Kinross P, Barbut F, Hajdu A, Wilcox MH, Kuijper EJ, et al. How to: Surveillance of
- 354 *Clostridium difficile* infections. Clin Microbiol Infect. 2018;24(5):469-75.
- 355 [11] Hensgens MP, Dekkers OM, Demeulemeester A, Buiting AG, Bloembergen P, van Benthem
- 356 BH, et al. Diarrhoea in general practice: when should a *Clostridium difficile* infection be
- 357 considered? Results of a nested case-control study. Clin Microbiol Infect. 2014;20(12):O1067-
- 358 74.
- 359 [12] Soes LM, Holt HM, Bottiger B, Nielsen HV, Torpdahl M, Nielsen EM, et al. The incidence and
- 360 clinical symptomatology of *Clostridium difficile* infections in a community setting in a cohort
- 361 of Danish patients attending general practice. Eur J Clin Microbiol Infect Dis. 2014;33(6):957-
- 362 67.
- 363 [13] Kutty PK, Woods CW, Sena AC, Benoit SR, Naggie S, Frederick J, et al. Risk factors for and
- 364 estimated incidence of community-associated *Clostridium difficile* infection, North Carolina,
- 365 USA. Emerg Infect Dis. 2010;16(2):197-204.
- 366 [14] Bolton RP, Tait SK, Dear PR, Losowsky MS. Asymptomatic neonatal colonisation by
- 367 *Clostridium difficile*. Arch Dis Child. 1984;59(5):466-72.
- 368 [15] Schutze GE, Willoughby RE, Committee on Infectious D, American Academy of P. *Clostridium*
- 369 *difficile* infection in infants and children. Pediatrics. 2013;131(1):196-200.

- 370 [16] Jamal W, Pauline E, Rotimi V. A prospective study of community-associated *Clostridium*  
371 *difficile* infection in Kuwait: Epidemiology and ribotypes. *Anaerobe*. 2015;35(Pt B):28-32.
- 372 [17] Riley TV, Wetherall F, Bowman J, Mogyorosy J, Golledge CL. Diarrheal disease due to  
373 *Clostridium difficile* in general practice. *Pathology*. 1991;23(4):346-9.
- 374 [18] Spina A, Kerr KG, Cormican M, Barbut F, Eigentler A, Zerva L, et al. Spectrum of  
375 enteropathogens detected by the FilmArray GI Panel in a multicentre study of community-  
376 acquired gastroenteritis. *Clin Microbiol Infect*. 2015;21(8):719-28.
- 377 [19] Bruijnesteijn van Coppenraet LE, Dullaert-de Boer M, Ruijs GJ, van der Reijden WA, van der  
378 Zanden AG, Weel JF, et al. Case-control comparison of bacterial and protozoan  
379 microorganisms associated with gastroenteritis: application of molecular detection. *Clin*  
380 *Microbiol Infect*. 2015;21(6):592 e9-19.
- 381 [20] Bauer MP, Veenendaal D, Verhoef L, Bloembergen P, van Dissel JT, Kuijper EJ. Clinical and  
382 microbiological characteristics of community-onset *Clostridium difficile* infection in The  
383 Netherlands. *Clin Microbiol Infect*. 2009;15(12):1087-92.
- 384 [21] Wilcox MH, Mooney L, Bendall R, Settle CD, Fawley WN. A case-control study of community-  
385 associated *Clostridium difficile* infection. *J Antimicrob Chemother*. 2008;62(2):388-96.
- 386 [22] Tam CC, Rodrigues LC, Viviani L, Dodds JP, Evans MR, Hunter PR, et al. Longitudinal study of  
387 infectious intestinal disease in the UK (IID2 study): incidence in the community and  
388 presenting to general practice. *Gut*. 2012;61(1):69-77.
- 389 [23] Crobach MJ, Planche T, Eckert C, Barbut F, Terveer EM, Dekkers OM, et al. European Society  
390 of Clinical Microbiology and Infectious Diseases: update of the diagnostic guidance document  
391 for *Clostridium difficile* infection. *Clin Microbiol Infect*. 2016;22 Suppl 4:S63-81.
- 392 [24] Polage CR, Gyorke CE, Kennedy MA, Leslie JL, Chin DL, Wang S, et al. Overdiagnosis of  
393 *Clostridium difficile* Infection in the Molecular Test Era. *JAMA Intern Med*.  
394 2015;175(11):1792-801.
- 395 [25] Planche TD, Davies KA, Coen PG, Finney JM, Monahan IM, Morris KA, et al. Differences in  
396 outcome according to *Clostridium difficile* testing method: a prospective multicentre  
397 diagnostic validation study of C difficile infection. *Lancet Infect Dis*. 2013;13(11):936-45.
- 398 [26] Gupta A, Khanna S. Community-acquired *Clostridium difficile* infection: an increasing public  
399 health threat. *Infect Drug Resist*. 2014;7:63-72.
- 400 [27] Davies K, Davis G, Barbut F, Eckert C, Spigaglia P, Barbanti F, et al. Adverse outcomes for  
401 missed cases of CDI, results from retrospective data collection on patients with samples  
402 received during the European, multi-centre, prospective bi-annual point prevalence study of  
403 *Clostridium difficile* Infection in hospitalised patients with Diarrhoea (EUCLID2). 26th  
404 European Congress of Clinical Microbiology and Infectious Diseases. Amsterdam,  
405 Netherlands; 2016.
- 406 [28] Soes LM, Holt HM, Bottiger B, Nielsen HV, Andreasen V, Kemp M, et al. Risk factors for  
407 *Clostridium difficile* infection in the community: a case-control study in patients in general  
408 practice, Denmark, 2009-2011. *Epidemiol Infect*. 2014;142(7):1437-48.
- 409 [29] Davies KA, Ashwin H, Longshaw CM, Burns DA, Davis GL, Wilcox MH, et al. Diversity of  
410 *Clostridium difficile* PCR ribotypes in Europe: results from the European, multicentre,  
411 prospective, biannual, point-prevalence study of *Clostridium difficile* infection in hospitalised  
412 patients with diarrhoea (EUCLID), 2012 and 2013. *Euro Surveill*. 2016;21(29).
- 413 [30] Eckert C, Coignard B, Hebert M, Tarnaud C, Tessier C, Lemire A, et al. Clinical and  
414 microbiological features of *Clostridium difficile* infections in France: the ICD-RAISIN 2009  
415 national survey. *Med Mal Infect*. 2013;43(2):67-74.
- 416 [31] Debast SB, van Leengoed LA, Goorhuis A, Harmanus C, Kuijper EJ, Bergwerff AA. *Clostridium*  
417 *difficile* PCR ribotype 078 toxinotype V found in diarrhoeal pigs identical to isolates from  
418 affected humans. *Environ Microbiol*. 2009;11(2):505-11.
- 419 [32] Metcalf DS, Costa MC, Dew WM, Weese JS. *Clostridium difficile* in vegetables, Canada. *Lett*  
420 *Appl Microbiol*. 2010;51(5):600-2.

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422

423 **Table 1:** Factors associated with positive CTA or positive toxigenic *C. difficile* (multivariate analysis) in424 patients  $\geq 3$  years old.

Variables	Toxigenic culture			CTA		
	OR	(95%CI)	p	OR	(95%CI)	p
Age						
3-16	0.998	0.987-1.022	0.910	1.002	0.974-1.030	0.526
17-45	ref			ref		
46-64	0.992	0.974-1.010	0.401	0.988	0.952-1.025	0.459
$\geq 65$	1.00	0.977-1.022	0.993	0.989	0.964-1.015	0.425
Gender (Male)	0.988	0.974-1.003	0.132	0.997	0.981-1.014	0.799
Abdominal pain	0.997	0.981-1.013	0.758			
Antimicrobials in the previous						
month	1.078	1.056-1.101	<0.001	1.058	1.034-1.082	<0.001
Hospitalization in the previous						
12 weeks	1.042	1.008-1.078	0.015	1.049	1.014-1.085	0.005
Diarrhoeal stools*	1.017	1.002-1.032	0.025	1.004	0.986-1.022	0.635
Stools with blood or mucus	-	-	-	1.979	0.911-1.053	0.582

425

426 \* taking the shape of the container

427 **Table 2:** Performance of different algorithms for testing diarrhoeal stool samples for *C. difficile* in  
 428 patients who visited their GP.

Test algorithm for CDI in samples from GP	No patients tested	Patients tested (% of all stool samples)	Positive results for CT (%)	Positive results for CTA (%)	Detection of	Detection of
					toxigenic CD carriage (compared to all positive CT) (%)	CD toxins (compared to all positive CTA) (%)
All ≥ 3 years	2435	100	2.67	1.68	100	100
≥ 65 years	397	16.30	4.28	3.02	26.15	29.27
Previous AB	324	13.30	9.57	6.79	47.69	53.65
Previous hospitalization	113	4.64	8.84	6.19	15.38	17.07
≥ 65 years OR previous AB	660	27.10	6.06	4.24	61.53	68.29
≥ 65 years OR previous hospitalization	482	19.79	5.19	3.73	38.46	43.90
≥ 65 years OR previous AB OR previous hospitalization	719	29.53	5.70	2.64	63.07	70.73
Physician's request	315	12.93	10.79	7.30	52.30	56.09

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431

432 **Figure 1:** Prevalence of toxigenic culture (TC) and stool cytotoxicity assay (CTA) according to patients'  
433 age group

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436

437 **Figure 2:** Incidence of the different enteropathogens found in stools submitted for routine  
438 microbiological testing from patients who visited their general practitioner (GP) for diarrhoea or  
439 other gastrointestinal disorders. Note that of 2541 patients, 2199 had a bacteriological stool culture  
440 for *Campylobacter* sp., *Salmonella* sp., *Yersinia* sp., 2001 had a parasitological stool examination,  
441 1663 had both, and 114 had viral testing for the presence of adenovirus and rotavirus antigens.

442

443

444 **Figure 3:** Distribution of PCR ribotypes isolated from healthcare-associated, community-associated *C.*  
445 *difficile* carriage and from patients < 3 years old.

446

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448

449

Figure 1

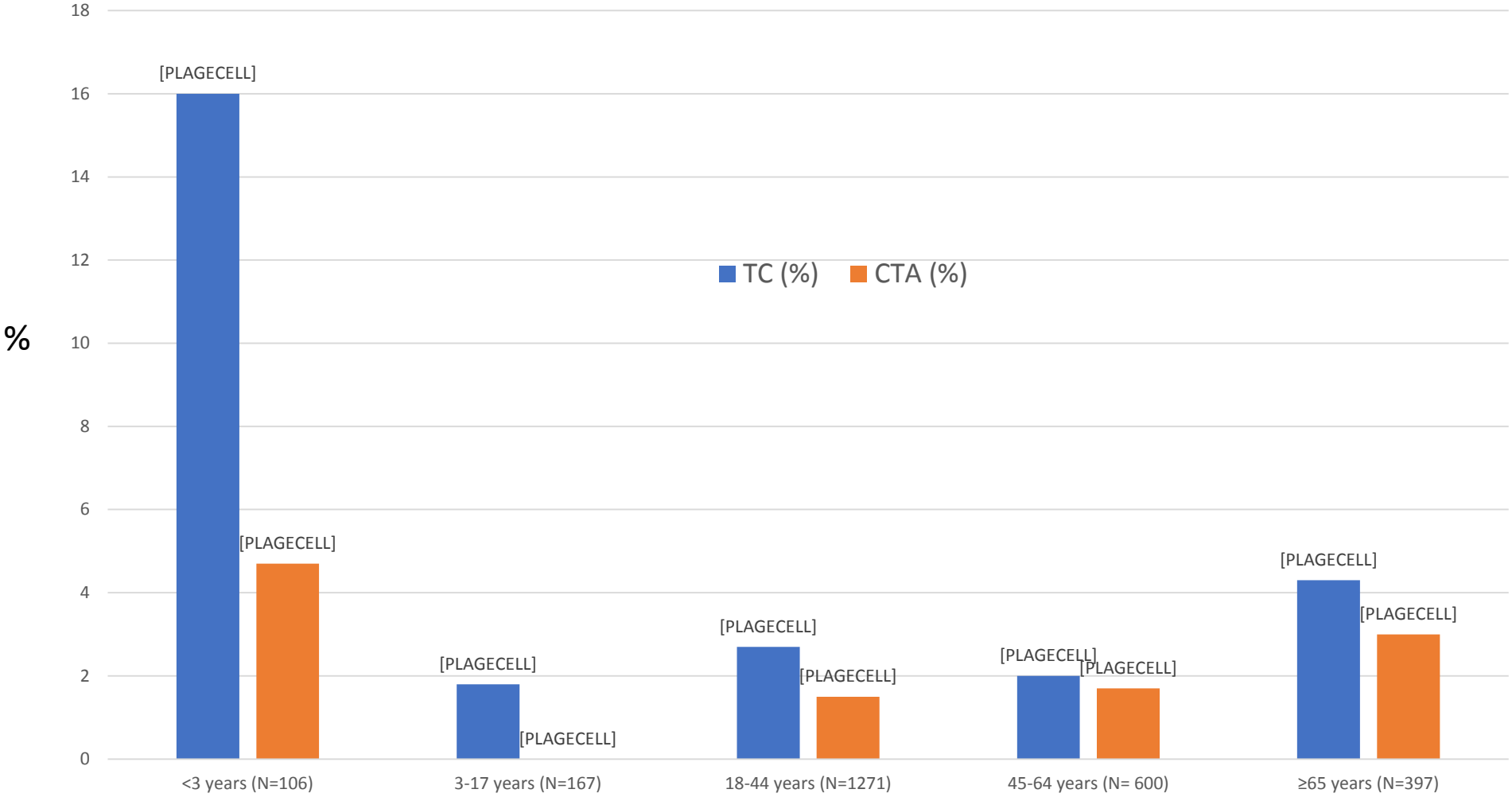
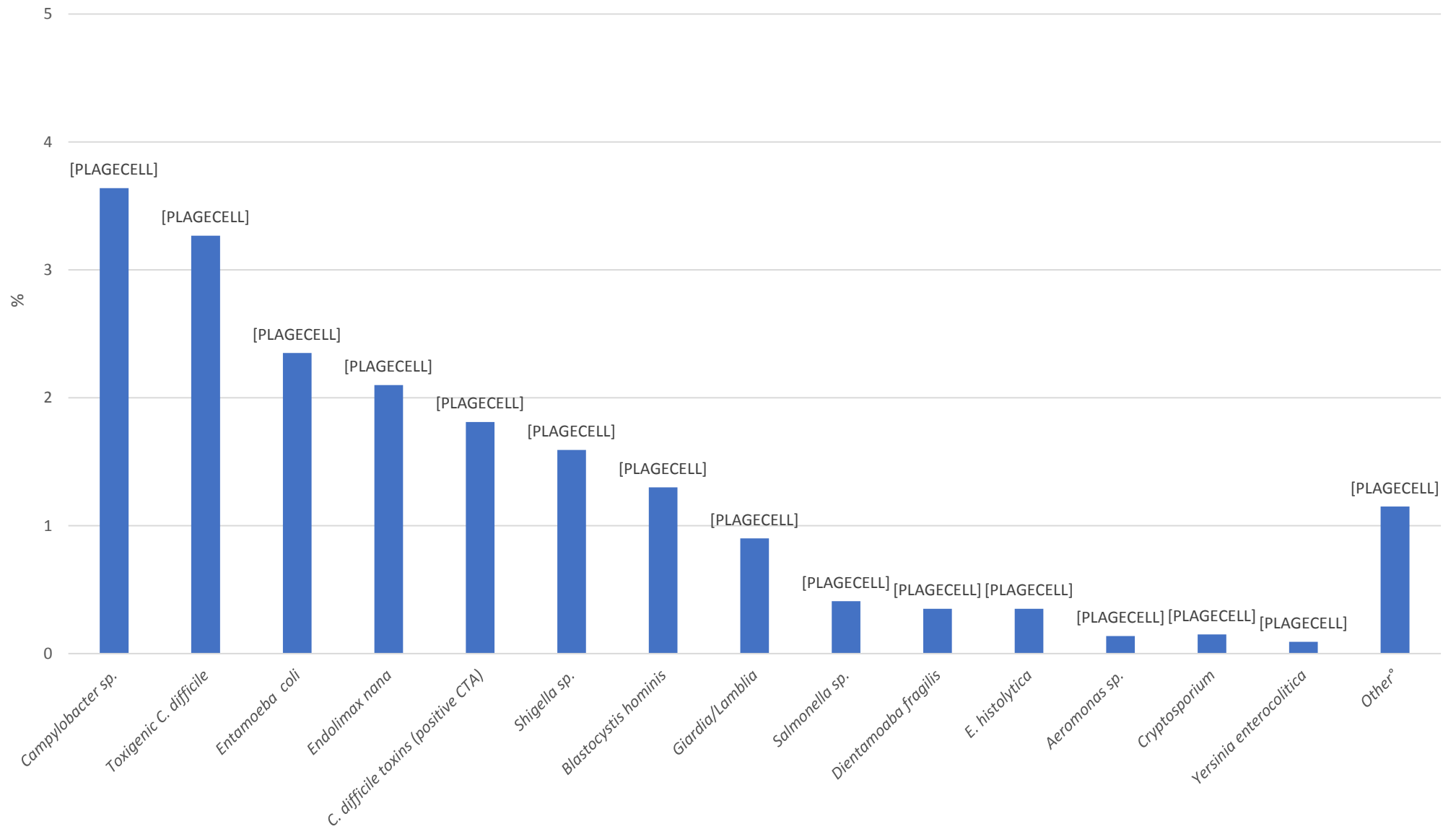


Figure 2



Other: *Trichomonas intestinalis* (n=2), *Chilomastix mesnili* (n=2), *Cyclospora cayentensis* (n=1), *Entamoeba hartmanni* (n=5), *Pseudolimax butschlii* (n=13)

**Figure 3**

