Impact of different empirical antibiotic treatment regimens for community-acquired pneumonia on the emergence of *Clostridium difficile*

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**Background:** Treatment of community-acquired pneumonia (CAP) with newer fluoroquinolones may contribute to selection for *Clostridium difficile*. We studied the prevalence of *C. difficile* carriage and *C. difficile* infection (CDI) on admission, and nosocomial acquisition rates in patients hospitalized for CAP and compared different empirical treatment strategies.

**Methods:** In a prospective study among patients admitted for antibiotic treatment of CAP, consecutive stool and skin samples were collected and cultured for *C. difficile*. Cultured isolates were typed by PCR ribotyping and characterized for toxinogenicity.

**Results:** In total, 20 of 107 (18.7%) patients included carried *C. difficile*. Various ribotypes were found and 14 (70%) isolates were toxinogenic. On admission, prevalence of *C. difficile* carriage was 9.4% (*n* = 9), of which 22% also carried *C. difficile* on the skin and one patient had mild CDI with persistent positive cultures. The overall nosocomial acquisition rate of *C. difficile* carriage was 11.2%. No nosocomially acquired CDI occurred. Acquisition rates of *C. difficile* were 11.9% (5/45) in moxifloxacin-, 11.1% (5/47) in β-lactam- and 9.0% (1/14) in β-lactam plus macrolide- or fluoroquinolone-treated patients (*P* = 0.84). Risk factors for *C. difficile* carriage were intravenous antibiotic treatment >7 days [odds ratio (OR) 3.89; 95% confidence interval (CI) 1.30 to 11.79] and hospitalization during the past 3 months (OR 4.08; 95% CI 1.40 to 11.90).

**Conclusions:** In a non-outbreak setting with a low endemic rate, the prevalence of *C. difficile* carriage in patients admitted because of CAP is high and nosocomial acquisition rates for *C. difficile* colonization are 11%. Fluoroquinolones were not associated with increased acquisition rates for *C. difficile* as compared with other empirical regimens for CAP.

**Keywords:** colonization, lower respiratory tract infections, fluoroquinolones, resistance

**Introduction**

The emergence of *Clostridium difficile* as a major cause of nosocomial infection is largely facilitated by excessive antibiotic use.¹ Management of respiratory tract infections accounts for 75% of all antibiotic use worldwide, and consequently plays a major role in the spread and development of *C. difficile* infections.²

For treatment of patients hospitalized because of community-acquired pneumonia (CAP) several equivalent empirical antibiotic strategies are recommended. Current guidelines advise for patients not needing intensive care unit (ICU) admission fluoroquinolone monotherapy, β-lactam monotherapy or β-lactam plus a macrolide or fluoroquinolone combination therapy, which are all considered equally effective.³,⁴ However, the individual impact of these antimicrobial regimens on selection of *C. difficile* is less clear. Historically, third-generation cephalosporins and broad-spectrum penicillins have been implicated as risk factors for *C. difficile* infection (CDI)¹,⁵ whereas, in the past few years, increasing use of newer fluoroquinolones, particularly moxifloxacin and gatifloxacin, may have promoted...
several large-scale outbreaks of CDI caused by various hypervirulent strains.6–10 Nowadays, although not evidenced by all studies, the use of fluoroquinolones is considered to be an important risk factor for selection of *C. difficile*, especially in outbreak situations. Reduced susceptibility to fluoroquinolones of epidemic and non-epidemic strains and facilitation of germination and toxin production are regarded as underlying mechanisms.11 Consequently, some institutions have also restricted the use of fluoroquinolones in non-outbreak settings.12 However, the attributable risk of CDI of fluoroquinolones compared with other empirical strategies for CAP has not been established yet.13 In addition to proven CDI, undetected *C. difficile* carriers may represent a reservoir for disease transmission and play an important role in the emergence of *C. difficile*. Moreover, asymptomatic carriers may facilitate skin and environmental contamination that contributes to the transmission of *C. difficile*.14–16

Therefore, we prospectively evaluated colonization dynamics of *C. difficile* as measured by consecutive stool and skin cultures in patients hospitalized for CAP in a non-epidemic setting. The prevalence of *C. difficile* carriage and CDI upon admission was determined and nosocomial acquisition rates of *C. difficile* were compared between the different empirical antibiotic regimens for CAP.

**Materials and methods**

**Study design and population**

We conducted a prospective observational cohort study among patients admitted for antibiotic treatment of CAP. From July 2008 to October 2009, all adult patients admitted to the University Medical Center Utrecht, a 1042 bed tertiary care hospital, and the Diakonessenhuis Utrecht, a 559 bed teaching hospital, in the Netherlands were eligible for inclusion. Patients with bronchiectasis and colonized with Gram-negative species in their respiratory tract, patients with severe neutropenia (neutrophils <0.5×10⁹/L or a CD4 cell count <200/mm³) and patients admitted directly to an ICU were excluded from participation.

The choice of empirical antibiotic regimen for CAP was left to the discretion of the treating physician considering the Dutch guidelines for antimicrobial therapy.17

Information on demographics, co-existing illnesses, previous hospitalizations, clinical characteristics, risk factors for *C. difficile*, outcomes and re-admissions was obtained.1 Renal disease, heart failure, liver disease, cerebrovascular disease, neoplasm and chronic obstructive pulmonary disease were considered to be relevant co-existing illnesses.

The aetiology of CAP was determined by isolation of a bacterial pathogen, described as the possible cause of CAP, from sputum or blood culture and/or by a positive urinary antigen test.

Stool samples and skin swabs were collected from all patients included, on admission, Day 5, 3 days after discontinuation of antibiotics and Day 30, and subsequently cultured for *C. difficile*. Since stool cultures cannot be taken immediately, a delay of 24 h was accepted. Skin cultures were obtained by swabbing a 5×10 cm region in the groin with a moistened swab and the samples were collected in Stuart medium. When stool cultures were obtained but not in the case of skin cultures, a delay of 24 h was accepted. Skin cultures and stool samples were collected in Stuart medium.

All patients were followed for 30 days after admission with careful monitoring for development of diarrhoea. An episode of diarrhoea was defined as three or more loose or liquid stools per day or more frequently than normal for the individual, for at least 48 h.18 In cases of diarrhoea, additional tests for *C. difficile* were performed according to the hospital guidelines for *Clostridium* diagnostics including a faeces toxin test (ImmunoCard Toxins A&B (ICTAB), Meridian® or *C. difficile* toxin/antitoxin test, Techlab®) and subsequent culture for *C. difficile* in the case of a positive faeces toxin test. CDI was defined by diarrhoea with a positive faeces toxin test.18 The study protocol was approved by the medical ethics committee of the University Medical Center Utrecht. The present research was conducted in accordance with the declaration of Helsinki and national and institutional standards.

**Microbiological analysis**

**Stool and skin cultures for *C. difficile***

Stool samples were transferred into anaerobic jars, alcohol shocked and plated onto cycloserine-cefoxitin-fructose agar (CCFA) and colistin-nalidixic acid agar (CNA) plates for culture. Skin swabs from the groin were incubated for 48 h in cycloserine-cefoxitin–fructose broth containing taurocholic acid and lysozyme and then plated onto CCFA and CNA plates and incubated for an additional 48 h. The first step in microbiological determination of isolates was based on typical odour and appearance of colonies. Final confirmation was performed at the Leiden reference laboratory for *C. difficile* (E. J. Kuijper), where all isolates were genetically identified by PCR for the gltA gene encoding the glutamate dehydrogenase specific for *C. difficile*.8

**Characterization of *C. difficile* isolates**

In the case of confirmed *C. difficile*, stools were tested for the presence of toxins using rapid immunocard assays (ICTAB, Meridian®). All *C. difficile* isolates were further characterized by PCR ribotyping at the Leiden reference laboratory.19,20 In addition, the presence of the tcdA, tcdB or binary toxin genes determined by molecular methods as described previously was considered to be a marker of toxigenicity.8

**Antimicrobial susceptibility**

Etests® (AB Biodisk, Sweden) were performed to determine the MICs for isolates of selected antimicrobials. Resistance rates were determined according to the breakpoints listed in Table 3.21–23 MICs for 50% (MIC₅₀) and 90% (MIC₉₀) of organisms were calculated.24

**Analytical approach and statistical analysis**

Based on empirical treatment for CAP initiated within 24 h of admission and continued for at least 5 days, all patients included were classified into one of three treatment groups: (i) moxifloxacin monotherapy; (ii) β-lactam monotherapy; or (iii) β-lactam plus macrolide or fluoroquinolone combination therapy. Patients’ characteristics were tested for comparability between the antibiotic treatment groups. Differences in continuous variables were estimated using Student’s t-test or the Mann–Whitney U-test, when appropriate, and differences in categorical variables using χ² tests. Baseline prevalence of *C. difficile* colonization, CDI and nosocomial acquisition rates of *C. difficile* were determined. Differences in acquisition rates for *C. difficile* between antibiotic regimens were studied using χ² tests and two-sample Z tests to compare sample proportions.

Cases of *C. difficile* acquisition were closely reviewed for switches in antimicrobial treatment preceding the date of acquisition. Multivariate logistic regression analysis was used to assess parameters associated with carriage and acquisition of *C. difficile*. A two-sided P value of <0.05 was considered statistically significant. Statistical analysis was performed with the statistical software package SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

**Results**

Serial stool and skin cultures were collected from 117 patients admitted because of CAP. Ten (8.5%) patients with missing cultures at more than one timepoint were excluded from further
analysis. None of these 10 patients had any cultures positive for *C. difficile*. Eighty-eight out of 107 (82.2%) patients included completed follow-up till Day 30 (Figure 1). The endemic rate of CDI in the participating hospitals was low: 0.6/1000 admissions a year. No outbreak occurred during the study period.

**Patient characteristics**

Table 1 shows the baseline characteristics of the 107 patients included in the study. Overall, the mean (±SD) age was 63 ± 17.5 years and 84 (78.5%) patients had one or more co-morbidities. Forty-five (42.1%) patients were initially treated with moxifloxacin, 47 (43.9%) with β-lactam monotherapy and 15 (14.0%) with β-lactam plus macrolide or plus fluoroquinolone (n=6) combination therapy. Patients treated with β-lactam plus macrolide/fluoroquinolone combination therapies were more likely to be residents of a nursing home (26.4% versus 2.2% and 17.0%, respectively) and to have faecal incontinence (26.7% versus 6.7% and 25.5%) than those treated with moxifloxacin or β-lactam monotherapy. No differences in other known risk factors for CDI or total duration of antibiotic treatment were observed between the treatment groups.

Patients treated with β-lactam plus macrolide/fluoroquinolone more often received intravenous antibiotics for >7 days as compared with patients receiving moxifloxacin or β-lactam monotherapy (60.0% versus 8.9% and 21.3%, respectively) (Table 1). Causative pathogens of CAP were identified in 58 (54.2%) patients. In eight (7.5%) patients empirical treatment was switched based upon results of microbiological exams. One of these patients acquired *C. difficile* during antibiotic treatment. In this particular case, ceftriaxone monotherapy was switched to ceftazidime monotherapy. In the other cases, β-lactam plus macrolide or fluoroquinolone combination therapy was narrowed to β-lactam monotherapy (n=4) or β-lactam monotherapy was extended to β-lactam plus macrolide or fluoroquinolone combination therapy (n=3).

**Prevalence of *C. difficile* carriage on admission**

On admission, the prevalence of *C. difficile* colonization was 9.4% (9/96). Of those, six (66.7%) patients carried toxigenic strains. Seven of nine (77.8%) carriers were asymptomatic on admission. One patient, a nursing home resident, carried a non-toxigenic strain and had symptoms of diarrhoea, and one patient had mild CDI with persistent positive cultures (Figure 2 and Table 2). Patients who received empirical treatment with β-lactam plus macrolide/fluoroquinolone antibiotics were more frequently carriers of *C. difficile* prior to antimicrobial treatment for CAP as compared with patients who received moxifloxacin or β-lactam monotherapy [26.7% (n=4) versus 4.8% (n=2) and 7.7% (n=3), respectively; *P* =0.04)

In two of nine (22.2%) patients colonized with *C. difficile* in stools at baseline, skin cultures of the groin were positive for *C. difficile*. Of those, one was a resident of a nursing home and the other had tube feeding. No positive skin cultures were retrieved from patients without *C. difficile* carriage in stools.

**Nosocomial acquisition of *C. difficile* carriage**

In total, 11 patients, stool culture negative for *C. difficile* on admission, acquired *C. difficile* during or after antibiotic treatment for CAP. The overall nosocomial acquisition rate of *C. difficile* colonization was therefore calculated as 11.2% (11/98). In eight (72.7%) patients toxigenic strains were identified. Of all 11 patients who acquired *C. difficile* during the study period, eight (72.7%) remained symptom-free, three patients had symptoms of diarrhoea but ICTAB tests were negative for the presence of toxins. The 3 patients were not treated for CDI and diarrhoea resolved spontaneously. No acquired CDI or relapse of previous CDI occurred. No acquisition of *C. difficile* carriage on the skin was observed (Figure 3 and Table 2). *Clostridium* diagnostics performed in 12 (11.2%) patients with diarrhoea according to the hospital guidelines revealed no additional cases of CDI.

Acquisition of *C. difficile* was 11.9% (n=5) in moxifloxacin-, 11.1% (n=5) in β-lactam- and 9.0% (n=1) in β-lactam plus macrolide/fluoroquinolone-treated patients and did not differ significantly between the treatment groups (*P* =0.84). Moreover, no significant differences in nosocomial acquisition of toxigenic strains were identified between the treatment groups (*P* =0.51); mean differences, moxifloxacin versus β-lactam [0.8%; 95% confidence interval (CI) –10.1 to 11.7]; moxifloxacin versus β-lactam plus macrolide (2.9%; 95% CI –15.5 to 21.3);
β-lactam versus β-lactam plus macrolide (2.1%; 95% CI –15.8 to 20.0) (Figure 3).

In patients who acquired C. difficile, no switches between the three antimicrobial regimens were identified preceding the first positive culture for C. difficile, except for one patient empirically treated with β-lactam monotherapy who had received five classes of antimicrobials during hospitalization and acquired C. difficile by Day 30. Figure 4 shows the overall and acquired colonization rates in stools at the four timepoints of culturing. Data indicate that during antibiotic treatment overall carriage rates for C. difficile are relatively low and carriage rates rise after cessation of antibiotics to a level higher than the admission carriage rate. Rates for acquisition of C. difficile are slightly higher on Day 3 after antibiotic treatment as compared with Day 30.

Isolate characteristics and antimicrobial susceptibility

In total, during the study period, C. difficile was cultured from 18.7% (n=20) of patients. All isolates were confirmed to be C. difficile by positive tests for gluD. Five intrinsic non-toxinogenic strains (ribotypes 140, 010) and 12 strains of toxinogenic ribotypes were identified by PCR ribotyping. In 3 strains, unknown ribotypes were identified. Tests for tcdA, tcdB or binary toxin genes were positive in 14 strains. One patient had faecal toxin production (Table 2).

All isolates were susceptible to metronidazole. Susceptibility to moxifloxacin was 90%. Two strains, ribotypes 012 (MIC 10 mg/L) and 075 (MIC 8 mg/L), were resistant to moxifloxacin. Resistance to amoxicillin/clavulanic acid, ceftriaxone and erythromycin was

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Table 1. Comparison of characteristics of the patients included in the study for empirical antibiotic regimens

<table>
<thead>
<tr>
<th></th>
<th>All</th>
<th>Moxifloxacin</th>
<th>β-Lactam</th>
<th>β-Lactam+macrolide or fluoroquinolone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>107 (100)</td>
<td>45 (42.1)</td>
<td>47 (43.9)</td>
<td>15 (14.0)</td>
</tr>
<tr>
<td>Patient characteristics/demographics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>age, years, mean ± SD</td>
<td>62.6±17.5</td>
<td>60.0±17.6</td>
<td>65.2±16.9</td>
<td>61.6±19.8</td>
</tr>
<tr>
<td>male</td>
<td>80 (74.8)</td>
<td>36 (80.0)</td>
<td>33 (70.2)</td>
<td>11 (73.3)</td>
</tr>
<tr>
<td>pneumonia severity index score ≥90</td>
<td>79 (73.8)</td>
<td>29 (64.4)</td>
<td>37 (78.7)</td>
<td>13 (86.7)</td>
</tr>
<tr>
<td>co-morbid illness</td>
<td>84 (78.5)</td>
<td>29 (64.4)</td>
<td>41 (87.2)</td>
<td>14 (93.3)</td>
</tr>
<tr>
<td>previous episode of pneumonia</td>
<td>44 (41.1)</td>
<td>19 (42.2)</td>
<td>17 (36.2)</td>
<td>8 (53.3)</td>
</tr>
<tr>
<td>nursing home resident</td>
<td>13 (12.1)</td>
<td>1 (2.2)</td>
<td>8 (17.0)</td>
<td>4 (26.7)</td>
</tr>
<tr>
<td>Risk factors for C. difficile</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tube feeding</td>
<td>20 (18.7)</td>
<td>7 (15.6)</td>
<td>8 (17.0)</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>faecal incontinence</td>
<td>19 (17.8)</td>
<td>3 (6.7)</td>
<td>12 (25.5)</td>
<td>4 (26.7)</td>
</tr>
<tr>
<td>gastrointestinal surgery</td>
<td>2 (1.9)</td>
<td>0 (0.0)</td>
<td>1 (2.1)</td>
<td>1 (6.7)</td>
</tr>
<tr>
<td>use of probiotics</td>
<td>3 (2.8)</td>
<td>1 (2.2)</td>
<td>2 (4.3)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>hospitalization in the past 3 months</td>
<td>26 (24.3)</td>
<td>9 (20.0)</td>
<td>12 (25.5)</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>use of steroids or chemotherapeutics</td>
<td>63 (58.9)</td>
<td>24 (53.3)</td>
<td>33 (70.2)</td>
<td>6 (40.0)</td>
</tr>
<tr>
<td>use of NSAIDS</td>
<td>45 (42.1)</td>
<td>18 (40.0)</td>
<td>22 (46.8)</td>
<td>5 (33.3)</td>
</tr>
<tr>
<td>antacids/proton pump inhibitors</td>
<td>59 (55.1)</td>
<td>24 (53.3)</td>
<td>26 (55.3)</td>
<td>9 (60.0)</td>
</tr>
<tr>
<td>antibiotic treatment in past 3 months</td>
<td>48 (44.9)</td>
<td>18 (40.0)</td>
<td>22 (46.8)</td>
<td>8 (53.3)</td>
</tr>
<tr>
<td>Antibiotic treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>duration of antibiotic treatment, days, mean ± SD</td>
<td>12.5±7.8</td>
<td>12.4±9.2</td>
<td>11.7±6.6</td>
<td>15.2±6.7</td>
</tr>
<tr>
<td>antibiotic treatment &gt;14 days</td>
<td>23 (21.5)</td>
<td>7 (15.6)</td>
<td>10 (21.3)</td>
<td>6 (40.0)</td>
</tr>
<tr>
<td>intravenous antibiotic treatment &gt;7 days</td>
<td>23 (21.5)</td>
<td>4 (8.9)</td>
<td>10 (21.3)</td>
<td>9 (60.0)</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>readmission within 30 days</td>
<td>14 (13.1)</td>
<td>6 (13.3)</td>
<td>7 (14.9)</td>
<td>1 (6.7)</td>
</tr>
<tr>
<td>mortality within 30 days</td>
<td>10 (9.3)</td>
<td>4 (8.9)</td>
<td>4 (8.5)</td>
<td>2 (13.3)</td>
</tr>
</tbody>
</table>

Data are n (%) unless otherwise indicated.

β-Lactam antibiotics include cephalosporins (n=22, 46.8%), amoxicillin+clavulanic acid (n=22, 46.8%), piperacillin/tazobactam (n=2, 4.3%) and penicillin (n=1, 2.1%).
observed in 5%, 20% and 55%, respectively. All strains were fully resistant to ciprofloxacin (Table 3).

### Parameters associated with C. difficile carriage

In multivariate logistic regression analysis, risk factors for C. difficile carriage at any time were intravenous antibiotic treatment for 7 days (odds ratio (OR) 3.89; 95% CI 1.30 to 11.76) and hospitalization in the past 3 months (OR 4.08; 95% CI 1.40 to 11.90). The use of non-steroidal anti-inflammatory drugs (NSAIDs) was associated with a lower risk of nosocomial C. difficile acquisition (OR 0.09; 95% CI 0.01 to 0.78), whereas tube feeding was associated with an increased risk of nosocomial acquisition (OR 4.35; 95% CI 1.00 to 18.87) (Table 4).
Discussion

Of patients admitted to the hospital for treatment of CAP, 9.4% carried *C. difficile* in their intestinal tract, of which 22% were also colonized in the groin. In this non-outbreak setting, we found an 11.2% nosocomial acquisition rate of *C. difficile* carriage. Neither acquisition on skin nor episodes of acquired CDI were identified during the study period. Patients receiving moxifloxacin monotherapy had no increased risk for *C. difficile* acquisition as compared with patients receiving treatment with β-lactam monotherapy or β-lactam plus macrolide or fluoroquinolone combination therapy.

Antibiotic treatment for CAP accounts for major antibiotic use in hospitals and consequently plays an important role in nosocomial selection for *C. difficile*.2 Interestingly, to the best of our knowledge, this is the first study prospectively comparing the recommended empirical antibiotic regimens for CAP for selection for *C. difficile*. In contrast to several previous alarming reports, we did not find a risk attributable to fluoroquinolones of increased acquisition of *C. difficile* in an endemic setting.6–9 Reduced susceptibility of fluoroquinolones for *C. difficile* has been mentioned as facilitating an underlying mechanism for the association between fluoroquinolone use and acquisition of *C. difficile*.11 Yet, in the present study most strains characterized were susceptible to moxifloxacin, except for two toxinogenic strains, ribotypes 012 and 075, suggesting that these proposed mechanisms are not applicable to most of the non-epidemic *C. difficile* strains here identified.

However, all previous studies were conducted in an outbreak setting which probably explains the contrast with present findings. Present data clarify that reports on fluoroquinolones as a risk factor for CDI in outbreaks cannot easily be extrapolated to common non-outbreak healthcare situations. More specifically, multivariate analysis showed that intravenous antibiotics for >7 days and hospitalization in the past 3 months were associated with an increased risk of *C. difficile* colonization. This further indicates that the risk of *C. difficile* colonization cannot be ascribed to one particular class of antibiotics but that duration of antibiotic treatment for CAP accounts for major antibiotic use in hospitals and consequently plays an important role in nosocomial selection for *C. difficile*.2 Interestingly, to the best of our knowledge, this is the first study prospectively comparing the recommended empirical antibiotic regimens for CAP for selection for *C. difficile*. In contrast to several previous alarming reports, we did not find a risk attributable to fluoroquinolones of increased acquisition of *C. difficile* in an endemic setting.6–9 Reduced susceptibility of fluoroquinolones for *C. difficile* has been mentioned as facilitating an underlying mechanism for the association between fluoroquinolone use and acquisition of *C. difficile*.11 Yet, in the present study most strains characterized were susceptible to moxifloxacin, except for two toxinogenic strains, ribotypes 012 and 075, suggesting that these proposed mechanisms are not applicable to most of the non-epidemic *C. difficile* strains here identified.

Table 3. Antimicrobial susceptibility of the 20 isolated *C. difficile* strains

<table>
<thead>
<tr>
<th>Antibiotic (breakpoint of resistance)</th>
<th>Resistance (%)</th>
<th>MIC₅₀ (mg/L)</th>
<th>MIC₉₀ (mg/L)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole (&gt;32 mg/L)</td>
<td>0</td>
<td>0.14</td>
<td>0.29</td>
<td>0.03 – 0.75</td>
</tr>
<tr>
<td>Moxifloxacin (&gt;8 mg/L)</td>
<td>10</td>
<td>0.50</td>
<td>7.03</td>
<td>0.002 – 27.5</td>
</tr>
<tr>
<td>Ciprofloxacin (&gt;2 mg/L)</td>
<td>100</td>
<td>32.0</td>
<td>30.44</td>
<td>0.50 – 64.0</td>
</tr>
<tr>
<td>Amoxicillin/clavulanic acid (&gt;16 mg/L)</td>
<td>5</td>
<td>0.38</td>
<td>47.44</td>
<td>0.06 – 256.0</td>
</tr>
<tr>
<td>Ceftriaxone (&gt;64 mg/L)</td>
<td>20</td>
<td>32.0</td>
<td>93.36</td>
<td>0.19 – 256.0</td>
</tr>
<tr>
<td>Erythromycin (&gt;2 mg/L)</td>
<td>55</td>
<td>32.0</td>
<td>168.47</td>
<td>0.25 – 256.0</td>
</tr>
</tbody>
</table>

*aBreakpoints according to the 15th version of the CSLI standards.

Table 4. Multivariate analysis for parameters associated with *C. difficile* carriage in stools at baseline, nosocomial acquisition and overall carriage

<table>
<thead>
<tr>
<th>C. difficile carriage in stools</th>
<th>yes, n (%)</th>
<th>no, n (%)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall carriage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>iv antibiotics &gt;7 days during admission or past 3 months</td>
<td>9 (45.0)</td>
<td>14 (16.1)</td>
<td>3.89 (1.30–11.76)</td>
<td>0.02</td>
</tr>
<tr>
<td>hospitalization in the past 3 months</td>
<td>10 (50.0)</td>
<td>16 (18.4)</td>
<td>4.08 (1.40–11.90)</td>
<td>0.01</td>
</tr>
<tr>
<td>Acquisition</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>use of NSAIDs</td>
<td>1 (9.1)</td>
<td>44 (45.8)</td>
<td>0.09 (0.01–0.78)</td>
<td>0.03</td>
</tr>
<tr>
<td>tube feeding</td>
<td>4 (36.4)</td>
<td>16 (16.7)</td>
<td>4.35 (1.00–18.87)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

iv, intravenous.

Parameters investigated in the univariate analysis were: antibiotic treatment regimen in the 3 months prior to hospitalization; patient characteristics (age, gender, co-morbid illnesses); pneumonia severity index; multilobar pneumonia; duration of total and iv antibiotic treatment; tube feeding; nursing home residence; use of NSAIDs; previous hospitalizations; previous antibiotic use; gastrointestinal surgery; faecal incontinence; use of probiotics; use of proton pump inhibitors; and use of steroids or chemotherapeutics.

Parameters with a P value <0.1 in the univariate analysis that were included in the multivariate logistic regression analysis were: use of NSAIDs; tube feeding; nursing home residence; hospitalization in the past 3 months; use of antibiotics in the past 3 months and during hospitalization; antibiotic treatment for >14 days or iv antibiotics for >7 days; and class of empirical antibiotic treatment.
of antibiotic use and frequent hospitalizations, demonstrated by others as well, seem to be more important risk factors.\textsuperscript{13,16} The majority of patients (75%) colonized with \textit{C. difficile} remained symptom free. Implications of asymptomatic carriage have been extensively debated in literature. Previously, it was hypothesized that asymptomatic carriers themselves do not have a higher risk of developing CDI, and colonization is paradoxically associated with a decreased risk of CDI, possibly due to a boost in serum antitoxin A antibody levels.\textsuperscript{25,26} However, a recent hypothesis suggests that most episodes of nosocomial CDI are propagated by newly admitted undetected asymptomatic carriers, representing a reservoir for transmission of \textit{C. difficile}.\textsuperscript{15} Moreover, asymptomatic carriage of \textit{C. difficile} may facilitate skin and environmental contamination which in turn contributes to the transmission of \textit{C. difficile}.\textsuperscript{16} Our results, in a population of patients at risk of CDI (mean age 62.6 years) in a non-outbreak setting show that rates of asymptomatic carriage of toxigenic \textit{C. difficile} strains are considerable, i.e. 78.6\% of all carriers remain symptom-free. Since most asymptomatic carriers remain undiagnosed in routine clinical practice, these are a potential source of increased spread of \textit{C. difficile}.

Implications of carriage of non-toxin producing strains are less clear. Nevertheless, it is possible that, even though actual toxin production could not be proved and the risk of transmission is reduced in the absence of diarrhoea, toxigenic strains are potentially harmful.\textsuperscript{27–29}

The findings in the present study may have two important clinical implications. First, given the high and mostly asymptomatic baseline prevalence (9.4\%) of \textit{C. difficile} carriage in patients admitted for CAP, screening on admission for undetected carriage in this particular group of patients may help to reduce nosocomial acquisition rates of \textit{C. difficile}.\textsuperscript{30} This high carriage rate at admission may be related to the composition of the study group of elderly CAP patients with frequent co-morbidities, transfers from nursing homes and antibiotic use.

Secondly, in a non-epidemic setting, not one class of antibiotics specifically, but rather longer duration of (intravenous) antibiotic treatment seems to be implicated in \textit{C. difficile} colonization. Therefore, general measures to optimize antimicrobial usage such as short courses, early switch to oral treatment allowing early discharge and individual risk assessment for \textit{C. difficile} seem more beneficial in reducing \textit{C. difficile} acquisition than abolition of one particular class of antibiotics. Moreover, abolition of fluoroquinolones for the treatment of CAP seems impractical as it would most probably lead to the increased use of other \textit{C. difficile} precipitants, such as \(\beta\)-lactams and macrolides.\textsuperscript{31}

A limitation of this study is that many patients have received multiple antimicrobials prior to admission and during hospitalization, complicating analysis for association of one particular agent with nosocomial acquisition of \textit{C. difficile}. In the present study, 45\% of patients had received antibiotics prior to hospitalization and in 7.5\% empirical treatment was switched based upon results of microbiological exams. However, due to consecutive cultures and strict follow-up in our study, the moment of acquisition of \textit{C. difficile} could be determined with knowledge of the antimicrobial regimen prior to acquisition. All patients who acquired \textit{C. difficile} had negative cultures for \textit{C. difficile} preceding the date of acquisition, minimizing the potential influence of prior antibiotic treatment on newly acquired \textit{C. difficile}. Moreover, treatment with a specific class of antibiotics in the 3 months prior to hospitalization was not a predictor of \textit{C. difficile} acquisition in multivariate analysis. In one patient who was treated with \(\beta\)-lactam monotherapy initially and was \textit{C. difficile} positive by Day 30, it was not possible to determine empirically whether one specific antimicrobial was the cause of \textit{C. difficile} acquisition. The patient was severely ill and had received five classes of antimicrobials during hospitalization. Excluding this patient from our analysis did not change the study results (\(P=0.85\)). The study is also limited by its observational design, leading to baseline differences in the study groups such as site of treatment, route of administration of antibiotics and baseline carriage rate of \textit{C. difficile}. All these parameters may have influenced the results and need to be considered in future studies. Nevertheless, the present study is one of the first with careful, prospective and serial specimen collection and it is highly unlikely that there is a difference of >20\% in nosocomial \textit{C. difficile} acquisition rates between all three groups of empirical antibiotic treatment with an alpha of 0.05\%.

In conclusion, in a non-epidemic setting, the prevalence of undetected (asymptomatic) \textit{C. difficile} carriage among patients admitted because of CAP is high. Nosocomial acquisition of \textit{C. difficile} colonization during treatment for CAP is ~11\% and is not associated with CDI. No specific empirical antibiotic regimen for CAP was associated with increased acquisition for \textit{C. difficile}. Therefore, management strategies such as screening for \textit{C. difficile} in patients at risk on admission, minimizing the duration of antibiotic treatment and an early switch to oral treatment allowing early discharge seem more likely to reduce nosocomial acquisition rates of \textit{C. difficile} than avoiding one particular class of antibiotics.

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