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# Epidemiological explorations on Clostridium difficile Infection

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# Chapter 2

## Spread and Epidemiology of *Clostridium difficile* Polymerase Chain Reaction Ribotype 027/Toxinotype III in The Netherlands

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## Abstract

### Background

After reports of emerging outbreaks in Canada and the United States, Clostridium difficile-associated disease (CDAD) due to polymerase chain reaction ribotype 027 was detected in 2 medium-to-large hospitals in The Netherlands in 2005.

### Methods

National surveillance was initiated to investigate the spread and the epidemiology of CDAD. Microbiologists were asked to send strains recovered from patients with a severe course of CDAD or recovered when an increased incidence of CDAD was noted. A standardized questionnaire was used to collect demographic, clinical, and epidemiological patient data. Strains were characterized by polymerase chain reaction ribotyping, toxinotyping, the presence of toxin genes, and antimicrobial susceptibility.

### Results

During the period from February 2005 through November 2006, 1175 stool samples from 863 patients were sent from 50 health care facilities. Of these patients, 218 (25.3%) had CDAD due to ribotype 027, and 645 patients (74.7%) had CDAD due to other ribotypes, mainly 001 (17.8%) and 014 (7.2%). Polymerase chain reaction ribotype 027 was more frequently present in general hospitals than in academic hospitals (odds ratio [OR], 4.38; 95% confidence interval [CI], 1.60–12.0). Outbreaks of CDAD were observed in 10 hospitals and in 1 nursing home. Patients infected with ribotype 027 were significantly older (OR, 2.18; 95% CI, 1.43–3.33), and significantly more patients used fluoroquinolones (OR, 2.88; 95% CI, 1.01–8.20), compared with those who were infected with other ribotypes. Clear trends were observed for more severe diarrhea (OR, 1.99; 95% CI, 0.83–4.73), higher attributable mortality (6.3% vs. 1.2%; OR, 3.30; 95% CI, 0.41–26.4), and more recurrences (OR, 1.44; 95% CI, 0.94–2.20).

**Conclusions**

Ribotype 027 was found in 20 (18.3%) of 109 hospitals in The Netherlands, with a geographic concentration in the western and central parts of the country. The clinical syndrome in patients with CDAD differed on the basis of ribotype. Thus, early recognition of the ribotype has benefits.

## Introduction

Since the emergence of a new virulent strain of *Clostridium difficile* characterized as toxinotype III, North American pulsed-field type 1, restriction-endonuclease analysis group type BI and PCR ribotype 027, various outbreaks of infection have been reported in North America and Europe [1-4]. It has been suggested that disease due to this strain is associated with higher morbidity and mortality rates, but the published reports were based on the historical evolution of mortality [2,3,5], with the exception of 1 recent Canadian study [6]. The increased virulence is assumed to be associated with higher amounts of toxin production [7].

In June 2005, the first outbreak of infection due to ribotype 027 was detected in The Netherlands [8,9]. In response to these outbreaks, the Leiden University Medical Centre and the Centre for Infectious Disease Control at the National Institute for Public Health and the Environment in Bilthoven initiated a national surveillance program. This report describes the results of analyses of bacterial samples that were submitted to the reference laboratory during the period from February 2005 through November 2006. Clinical and epidemiological data were collected to compare patients who had *C. difficile*–associated disease (CDAD) due to ribotype 027 with those who had CDAD due to non-027 ribotypes.

## Methods

### Definitions.

Definitions proposed by the European Society of Clinical Microbiology and Infectious Diseases [10] and by McDonald et al. [11] were used. CDAD was defined as diarrhea and a stool sample positive for *C. difficile* toxin A and/or B, as determined using a laboratory assay. A complicated course of CDAD was defined as admission to an intensive care unit, surgical intervention, or death associated with CDAD. A case was considered to have been nosocomially acquired if the diarrhea started > 48 h after admission to the hospital. Community-onset CDAD was defined as diarrhea that started before hospital admission.

Health care-associated CDAD was defined as the development of CDAD 2 days after admission or within 4 weeks after discharge, community-associated CDAD was defined as development of CDAD on day 0, 1, or 2 after hospital admission. Recurrence was defined as an episode that occurred > 8 weeks after the onset of a previous episode. An outbreak in a health care facility was defined as a significant increase in the incidence of CDAD over a defined period, taking into account the background rate of CDAD. At least a doubling of the incidence and/or > 2 epidemiologically linked cases from 1 ward were considered to be significant increases. We defined severe diarrhea as bloody diarrhea or as diarrhea with hypovolemia and/or with fever and leukocytosis and/or with hypoalbuminemia and/or with pseudomembraneous colitis. Mortality was considered to be attributable to CDAD when a patient died of the consequences of CDAD during hospitalization.

**Submission of stool samples or bacterial strains.**

Health care facilities and microbiological laboratories were asked to send stool samples or bacterial strains when they encountered a severe case of CDAD or an increased incidence of CDAD in a health care facility. Four laboratories had stored samples obtained from patients with severe diarrhea from 7 health care facilities for cases that had occurred during the period from February 2005 through June 2005; these were also submitted.

**Isolation and characterization of *C. difficile*.**

Isolation and identification of *C. difficile* was performed as described previously [2,9]. All isolates were genetically identified as *C. difficile* by an in-house PCR for the presence of the *gluD* gene, encoding the glutamate dehydrogenase specific for *C. difficile* [9]. Glutamate dehydrogenase is a protein produced by *C. difficile* only [12]. The *C. difficile* strains were investigated by PCR ribotyping [13]. Toxinotyping was performed as described by Rupnik et al. [14]. The presence of tcdA, tcdB, and binary toxin genes was investigated in accordance with standardized techniques [15-17]. Deletions in tcdC were determined by PCR using in-house designed primers [8].

**Susceptibility tests.**

Ribotype 027 isolates from each facility were tested for the presence of the *ErmB* gene, which confers resistance to clindamycin and erythromycin [18]. In addition, Etests (bioMérieux) were performed to determine the MICs of ciprofloxacin, moxifloxacin, vancomycin, metronidazole, penicillin, erythromycin, and clindamycin using supplemented Brucella blood agar medium [19].

**Collection of clinical and demographic data**.

A standardized questionnaire was designed to obtain information on patients' age, sex, previous hospital admissions, ward of acquisition, origin of CDAD, disease severity, clinical course, and attributable mortality and on whether the patient had undergone surgery or received antibiotic treatment in the 3 months preceding a positive test result. Comorbidities were established on the basis of the International Classification of Diseases, 10 Edition, classification.

**Statistical analysis.**

The distribution of risk factors and clinical outcome parameters in patients infected with ribotype 027 was compared with the distribution in patients infected with other ribotypes. Continuous data were compared between groups using analyses of variance. A Yates-corrected $\chi^2$ test was used for the analysis of proportions. If a cell value was <5 in the 2 x 2 table, Fisher's exact test was used. A multiple logistic regression model was used to study the association of putative risk factors with ribotype 027. Relative risks were estimated as ORs and are presented with 95% CIs. Both crude relative risks and relative risks after adjustment for the possible confounders of age and sex (confounders for all risk factors), hospital (confounder for comorbidity, antibiotics used, and severity of disease), and comorbidities (confounder for antibiotic use) are provided. Only risk factors with a univariate P value < .20 were tested in multivariate analysis. Given the large number of hospitals in this study, correction for hospital as possible confounder resulted in a high number of degrees of freedom in the regression

model and, thus, a decreased accuracy of risk estimations. Therefore, only the results without adjustment for hospital are shown, and the effect of adjustment is discussed in the text. All analyses were performed using the SPSS for Windows software package, version 13.0 (SPSS).

## Results

### Samples received at the reference laboratory.

During the period from February 2005 through November 2006, a total of 1175 specimens were received from 50 health care facilities and laboratories (36 hospitals, 9 nursing homes, and 5 regional laboratories) in The Netherlands without information on the institution involved (table 1). Of 1175 samples, 1055 (89.8%) contained *C. difficile*.

### Distribution and characteristics of *C. difficile* in health care facilities.

Ribotype 027 was found in 279 (26.5%) of 1055 samples, which were obtained from 863 patients: 218 patients (25.3%) with CDAD due to ribotype 027 and 645 patients (74.7%) with CDAD due to other ribotypes (most frequently ribotypes 001 [17.8%] and 014 [7.2%]). Ribotype 027 was present in 22 health care facilities (17 hospitals and 5 nursing homes). Outbreaks of CDAD were observed in 10 hospitals and in 1 nursing home; the other facilities (7 hospitals and 4 nursing homes) only experienced sporadic cases (table 1 and figure 1).

**Table 1. Data on ribotyping results, health care facilities, and outbreaks of *Clostridium difficile*–associated disease per province in The Netherlands.**

| Province | No. (%) of samples | | | No. of facilities | No. of facilities with ribotype 027 present | |
|---|---|---|---|---|---|---|
| | All[a] | Ribotype | | | | |
| | | 027 | Other | | Outbreaks | Sporadic cases |
| Flevoland | 44 | 18 (40.9) | 26 (59.9) | 2 | 1 | 1 |
| Noord Holland | 335 | 116 (34.6) | 219 (65.4) | 14 | 7 | 3 |
| Gelderland | 96 | 22 (22.9) | 74 (77.1) | 7 | 1 | 1 |
| Utrecht | 251 | 55 (21.9) | 196 (78.1) | 8 | 2 | 2 |
| Noord-Brabant | 14 | 1 (7.1) | 13 (92.9) | 3 | 0 | 1 |
| Zuid Holland | 85 | 5 (5.9) | 80 (94.1) | 6 | 0 | 2 |
| Groningen | 18 | 1 (5.6) | 17 (94.4) | 5 | 0 | 1 |
| Overijssel | 2 | 0 (0.0) | 2 (100) | 1 | 0 | 0 |
| Drenthe | 3 | 0 (0.0) | 3 (100) | 2 | 0 | 0 |
| Limburg | 15 | 0 (0.0) | 15 (100) | 2 | 0 | 0 |
| Friesland | 0 | 0 (0.0) | 0 (0.0) | 0 | 0 | 0 |
| Zeeland | 0 | 0 (0.0) | 0 (0.0) | 0 | 0 | 0 |
| Total | 863 | 218 (25.3) | 645 (74.4) | 50 | 11 | 11 |

[a] Data are no. of samples per province sent to the reference laboratory during the period from February 2005 through November 2006.

In June 2005, soon after detection of the first outbreaks associated with ribotype 027 in 2 hospitals from the same region, outbreaks were detected in 5 more hospitals. The Centre for Infectious Disease Control started monitoring the CDAD incidence in these institutions and formulated guidelines for surveillance, infection control, and treatment of CDAD [NO STYLE for: ]. Attention to CDAD was increased through symposia at scientific meetings and in publications in Dutch medical journals. In 2 hospitals, the incidence had already increased (since 2002 at one hospital and since 2004 at the other). The others experienced sharp increases in incidence in the period March–June 2005. All hospitals increased attention to existing infection-control measures or introduced new measures, such as isolation of patients in private rooms or cohort nursing, increased cleaning and disinfection with hypochlorite, and advisement to restrict the use of certain antibiotics (predominantly fluoroquinolones). Of the

10 hospitals with outbreaks of CDAD due to ribotype 027, 4 also restricted the use of clindamycin and cephalosporins. Although the incidence thereupon decreased, several hospitals continued to experience new, although often less extensive, outbreaks during the following year. From fall 2005 onwards, 12 additional institutions detected ribotype 027 (figure 2A). However, only 3 more hospitals ascertained that the presence of ribotype 027 was concurrent with an increase in the CDAD incidence, which did not reach the high levels found in hospitals that were already affected, probably because of increased awareness. By the end of 2006, the incidence in most hospitals had decreased to pre-outbreak levels. In addition to the 22 facilities where ribotype 027 was found, 3 hospitals experienced isolated cases involving ribotype 027 before the start of the surveillance period, all in 2005.

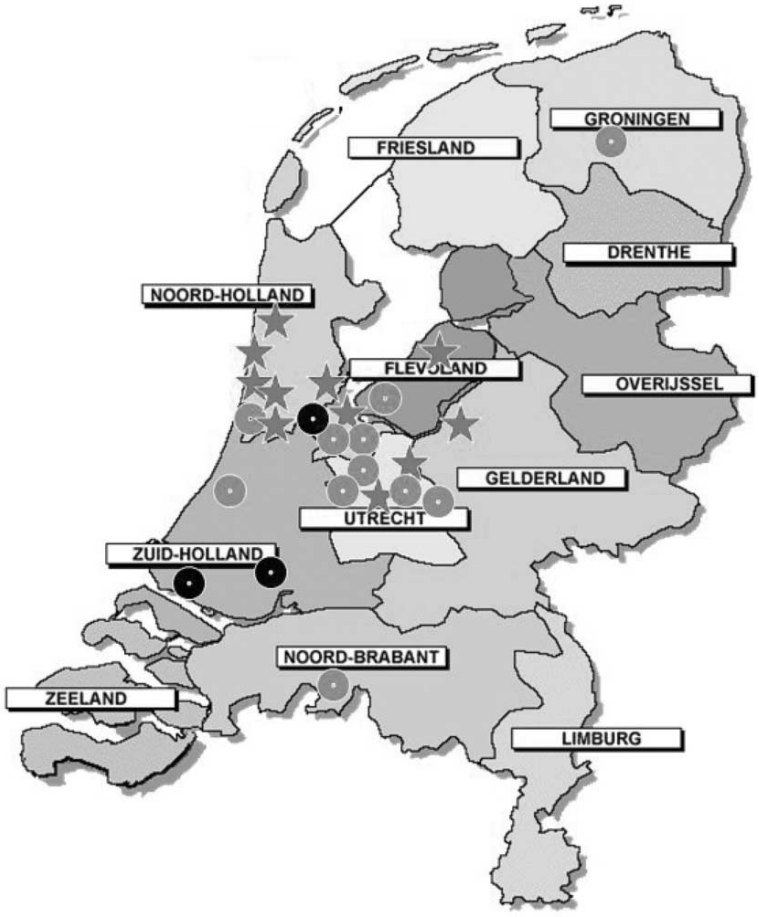


**Figure 1.** Health care facilities with outbreaks ($n = 11$; *stars*) and sporadic cases ($n = 11$; *light circles*) of *Clostridium difficile*–associated disease due to ribotype 027. *Black circles,* earlier sporadic cases ($n = 3$) that were found in samples obtained before February 2005.

One strain, which dated from 2002, from a hospital that also experienced an outbreak involving ribotype 027 during the surveillance period was retrospectively characterized as ribotype 027; this was the first known ribotype 027 strain of C. difficile in The Netherlands. At present, 25 facilities are known to have encountered ribotype 027. In total, 20 (18.3%) of 109 hospitals have been affected since 2002. Figure 2B depicts the monthly number of patients with CDAD. After the first peak in June 2005, another peak occurred during January–April 2006. Samples from February-May 2005 originated from patients with a high suspicion of infection with ribotype 027 and were retrospectively tested after recognition of the first outbreak in June 2005. After this period, 9 (53%) of 17 affected hospitals started submitting all toxin-positive samples.

Molecular characterization of each first ribotype 027 strain per facility (n = 22) confirmed the presence of binary toxin, toxin A, and toxin B genes, as well as an 18-bp *tcdC* deletion. All ribotype 027 strains were of toxinotype III, were *ErmB* negative, and were resistant to erythromycin (MIC, > 256 mg/ L), ciprofloxacin (MIC, > 32 mg/L), and moxifloxacin (MIC, > 32 mg/L) and susceptible to clindamycin (MIC, 2 ml/L), metronidazole (MIC, 0.19 mg/L), vancomycin (MIC, 0.38 mg/L), and penicillin (MIC, 0.50 mg/L). Ten random samples from each of the 2 most frequently circulating non-027 ribotypes (ribotypes 001 and 014) were also tested. All ribotype 001 and 014 strains were resistant to ciprofloxacin (MIC, 132 mg/L). None of the ribotype 014 strains and 40% of the ribotype 001 strains were resistant to moxifloxacin (MIC, 132 mg/L). The first known ribotype 027 strain from 2002 was susceptible to ciprofloxacin (MIC, 0.19 mg/L), moxifloxacin (MIC, 0.125 mg/ L), clindamycin (MIC, 0.064 mg/L), and erythromycin (MIC, 0.25 mg/L).

**Risk factors for CDAD due to ribotype 027 versus CDAD due to other ribotypes.**

Of 863 requests for questionnaires, 229 (26.5%) were completed and received at the reference laboratory. Fifty (22.9%) of 218 patients with CDAD due to ribotype 027 and 179 (27.8%) of 645 patients with CDAD due to other ribotypes submitted questionnaires.

Questionnaires were received from 30 of the 50 health care institutions. Of these, 18 returned ≥ 50% of the questionnaires (n = 169), and 12 returned < 50% (n = 60).

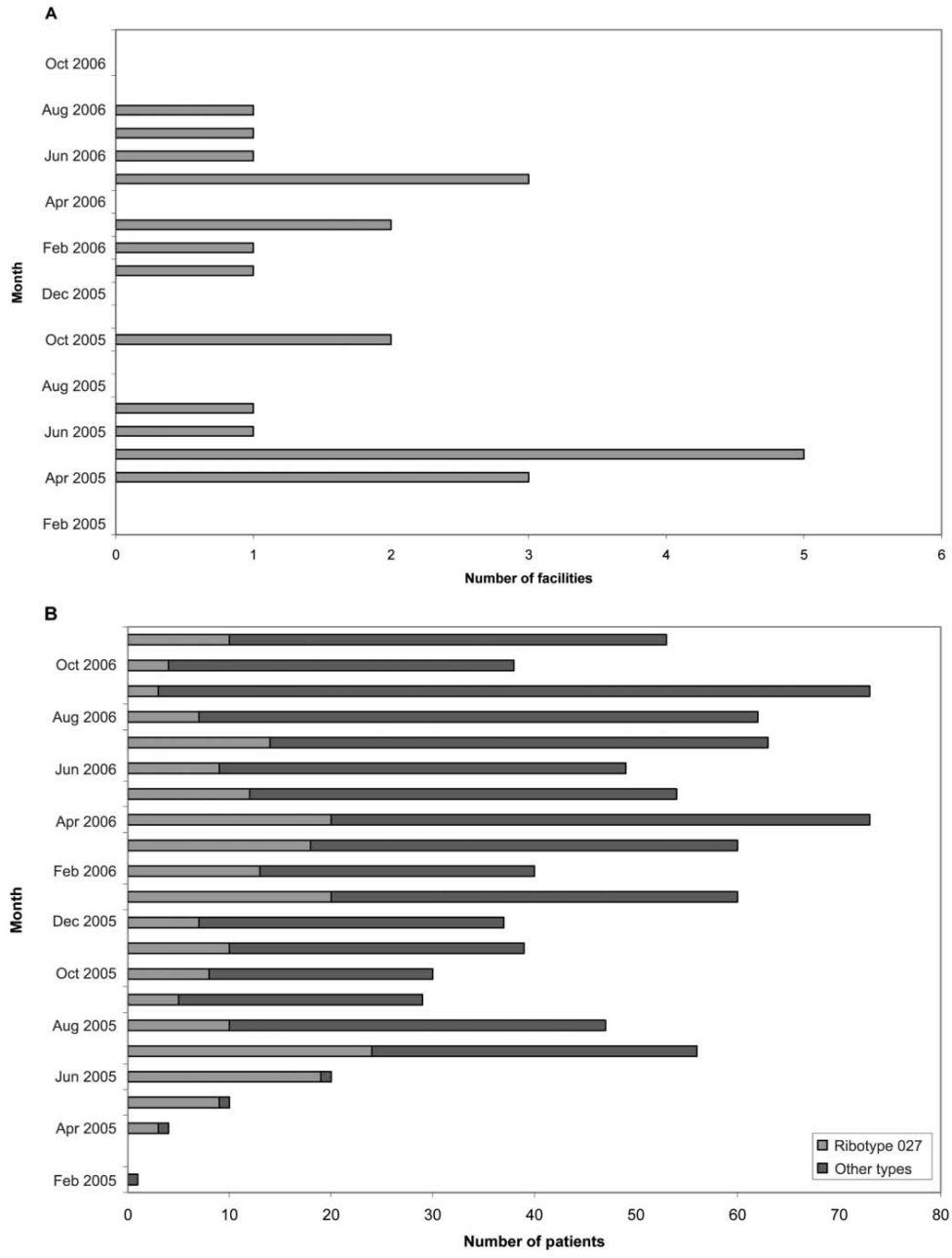


**Figure 2.** *A,* Number of health care facilities (*n* = 22) with their first case *Clostridium difficile*–associated disease (CDAD) due to ribotype 027 per month. *B,* Monthly number of patients with CDAD due to ribotype 027 versus patients with CDAD due to other ribotypes (calculated from samples sent to the reference laboratory).

Table 2 presents the demographic data and clinical characteristics of patients. There were more female patients than male patients in both groups. The mean age was higher for patients infected with ribotype 027 (74 vs. 67 years), because that group contained a

significantly higher number of patients aged > 80 years (43% vs. 29%; OR, 2.18; 95% CI, 1.43–3.33). The most prevalent comorbidities were respiratory system disease (37.8% vs. 20.4%; crude OR 2.37; 95% CI, 1.15–4.87), neoplasms, and cardiovascular disease (table 2). None of these differences were found to be statistically significant in multivariate analysis.

One hospital experienced an outbreak of infection due to ribotype 027 on a pulmonary ward. When this hospital was excluded from the analysis, the overall association between respiratory system disease and ribotype 027 disappeared (OR, 1.12; 95% CI, 0.43–2.96). Health care- and community-onset CDAD were equally distributed among patients infected with ribotype 027 and those infected with non-027 ribotypes. The mean durations of hospital stay before the development of CDAD were 15 and 13 days, respectively (P = not significant). The 2 groups did not differ with regard to previous surgical interventions.

Of 17 hospitals at which ribotype 027 was found, 3 had an academic status (with 822, 882, and 1002 beds), and 14 were general hospitals (with 255–913 beds). In academic hospitals, ribotype 027 accounted for 21.3% of all CDAD cases, whereas it accounted for 33.3% of cases at general hospitals (OR adjusted for age and sex, 4.38; 95% CI, 1.60–12.0).

The overall use of antibiotics was high in both groups of patients, but significantly more patients who were infected with ribotype 027 were prescribed fluoroquinolones (23.9% vs. 15.4%; OR, 2.88; 95% CI, 1.01–8.20). When adjusted for hospital, the association became stronger (OR, 4.54; 95%, CI 1.08– 19.0).

**Outcome of CDAD due to ribotype 027 versus CDAD due to non-027 ribotypes.**

In multivariate analysis, diarrhea tended to be more severe in patients with CDAD due to ribotype 027 than in those with CDAD due to other ribotypes (22.4% vs. 13.9%; OR, 1.99; 95% CI, 0.83–4.73) (table 3). When also adjusted for hospital, the association became stronger and statistically significant (OR, 3.96; 95% CI, 1.05–15.0). Adjustment for comorbidities and antibiotics did not have significant effects.

**Table 2. Demographic data and risk factors for patients with *Clostridium difficile*–associated disease (CDAD) due to ribotype 027, compared with patients with CDAD due to non-027 ribotypes.**

| Characteristic | No. of patients with available information | Strain type[a] | | OR (95% CI) | |
|---|---|---|---|---|---|
| | | Ribotype 027, toxinotype III | Other | Univariate analysis | Multivariate analysis[b] |
| Sex | | | | | |
| All | 697 | 178 | 519 | … | … |
| Male | … | 79 (44.4) | 226 (43.5) | 0.97 (0.69–1.36) | … |
| Female | … | 99 (55.6) | 293 (56.5) | … | … |
| Age, years | | | | | |
| All | 793 | 199 | 594 | … | … |
| 0–17 | … | 0 (0) | 20 (3.4) | 0.00 (0.00) | … |
| 18–64 | … | 42 (21.1) | 184 (31.0) | Reference | … |
| 65–79 | … | 72 (36.2) | 219 (36.9) | 1.44 (0.94–2.21) | … |
| ⩾80 | … | 85 (42.7) | 171 (28.8) | 2.18 (1.43–3.33) | … |
| Mean years | … | 74.2 | 66.8 | … | … |
| Main comorbidity[c] | | | | | |
| All | 209 | 48 | 161 | … | … |
| Any underlying disease | 188 | 43/48 (89.6) | 145/161 (90.1) | 0.95 (0.33–2.74) | … |
| Neoplasm | 41 | 12/45 (26.7) | 29/151 (19.2) | 1.53 (0.71–3.32) | … |
| Diabetes | 19 | 3/45 (6.7) | 16/150 (10.6) | 0.60 (0.17–2.15) | … |
| Respiratory system disease | 48 | 17/45 (37.8) | 31/152 (20.4) | 2.37 (1.15–4.87) | 2.06 (0.91–4.65)[d] |
| Digestive system disease | 35 | 7/47 (14.9) | 28/153 (18.3) | 0.78 (0.32–1.92) | … |
| Cardiovascular system disease | 45 | 9/45 (20.0) | 36/153 (23.5) | 0.81 (0.36–1.85) | … |
| Genitourinary system disease | 36 | 5/46 (10.9) | 31/150 (20.7) | 0.47 (0.17–1.28) | 0.65 (0.23–1.84)[e] |
| Other | 44 | 7/45 (15.6) | 37/146 (25.3) | 0.54 (0.22–1.32) | 0.61 (0.23–1.61)[f] |
| Community-onset CDAD | | | | | |
| All | 50 | 9 (20.0) | 41 (25.6) | … | … |
| Health care associated[g] | 13 | 4 (44.4) | 9 (22.0) | … | … |
| Unknown association[h] | 37 | 5 (45.6) | 32 (78.0) | … | … |
| Health care–onset CDAD | | | | | |
| All | 155 | 36 (80.0) | 119 (74.4) | 0.73 (0.32–1.64) | … |
| Health care associated[g] | 133 | 33 (91.7) | 100 (84.0) | … | … |
| Unknown association[i] | 22 | 3 (8.3) | 19 (16.0) | … | … |
| Surgery in 3 months before onset of CDAD | | | | | |
| All | 201 | 45 | 156 | … | … |
| Yes | 56 | 11 (24.4) | 45 (28.8) | 0.80 (0.37–1.71) | … |
| No | 145 | 34 (75.6) | 111 (71.2) | … | … |
| Duration of hospitalization before onset of diarrhea, mean days | … | 15.4 | 12.8 | … | … |
| Use of antibiotics in 3 months before onset of CDAD | | | | | |
| All | 205 | 46 | 159 | … | … |
| Any antibiotic | 180 | 39/46 (84.4) | 141/159 (88.7) | 0.71 (0.28–1.83) | … |
| Penicillins | 77 | 17/46 (37.0) | 60/147 (40.8) | 0.85 (0.43–1.68) | … |
| Cephalosporins | 77 | 17/46 (37.0) | 60/147 (40.8) | 0.85 (0.43–1.68) | … |
| Any quinolone | 34 | 11/46 (23.9) | 23/149 (15.4) | 1.72 (0.77–3.87) | 2.88 (1.01–8.20)[j] |
| Clindamycin | 13 | 1/46 (2.2) | 12/149 (8.1) | 0.25 (0.32–2.00) | 0.33 (0.03–3.22)[k] |
| Macrolides | 18 | 3/46 (6.5) | 15/149 (10.1) | 0.62 (0.17–2.26) | … |
| Sulfonamides and/or trimethoprim | 9 | 1/46 (2.2) | 8/148 (5.4) | 0.39 (0.47–3.19) | … |
| Aminoglycosides | 17 | 3/46 (6.5) | 14/149 (9.4) | 0.67 (0.19–2.45) | … |

[a] Data are no. (%) of patients or proportion of patients (%) with data available, unless otherwise indicated.
[b] Only risk factors with a *P* value <.20 on univariate analysis were used in the multivariate analysis.
[c] Determined using the *International Classification of Diseases, 10 Edition.*
[d] When also adjusted for hospital, the OR was 1.68 (95% CI, 0.58–4.82); after exclusion of 1 hospital with an outbreak on a pulmonary ward, the OR was 1.12 (95% CI, 0.43–2.96).
[e] When also adjusted for hospital, the OR was 0.60 (95% CI, 0.15–2.47).
[f] When also adjusted for hospital, the OR was 0.93 (95% CI, 0.29–2.99).
[g] Defined as development of CDAD >2 days after hospital admission or ⩽4 weeks after discharge.
[h] Defined as an unknown time interval between the onset of CDAD and prior discharge from the hospital.
[i] Defined as the development of CDAD on day 0, 1, or 2 after hospital admission.
[j] When also adjusted for hospital, the OR was 4.64 (95% CI, 1.97–18.0).
[k] When also adjusted for hospital, the OR was 0.45 (95% CI, 0.03–8.27).

A complicated course of CDAD was observed in 12.5% of patients with CDAD due to ribotype 027, compared with 8.0% of patients with CDAD due to non-027 ribotypes (P = not significant). Attributable mortality was higher among patients infected with ribotype 027 (6.3% vs. 1.2%), but the total number of patients was low.

**Table 3. Disease severity, clinical course, and recurrence of disease in patients with *Clostridium difficile*–associated disease (CDAD) due to ribotype 027, compared with patients with CDAD due to other ribotypes.**

| | No. of patients with available information | | | | |
|---|---|---|---|---|---|
| | | Strain type | | OR (95% CI) | |
| Characteristic | All | Ribotype 027, toxinotype III | Other | Univariate analysis | Multivariate analysis |
| Severity of diarrhea[a] | | | | | |
| All | 215 | 49 | 166 | … | … |
| Mild | 181 | 38 (77.6) | 143 (86.1) | … | … |
| Severe | 34 | 11 (22.4) | 23 (13.9) | 1.80 (0.81–4.02) | 1.99 (0.83–4.73)[b] |
| Clinical course[c] | | | | | |
| All | 211 | 48 | 163 | … | … |
| Not complicated | 192 | 42 (87.5) | 150 (92.0) | … | … |
| Complicated | 19 | 6 (12.5) | 13 (8.0) | 1.65 (0.59–4.60) | … |
| Death due to CDAD | 5 | 3 (6.3) | 2 (1.2) | 5.37 (0.87–33.1) | 3.30 (0.41–26.4) |
| Recurrence | | | | | |
| All | 863 | 218 | 645 | … | … |
| Yes | 141 | 45 (20.6) | 96 (14.9) | 1.49 (1.00–2.20) | 1.44 (0.94–2.20) |
| 1 recurrence | … | 32 (14.7) | 74 (11.5) | 1.32 (0.85–2.08) | 1.27 (0.79–2.06) |
| >1 recurrence | … | 13 (6.0) | 22 (3.4) | 1.80 (0.89–3.63) | 1.80 (0.84–3.85) |
| No | 722 | 173 (79.4) | 549 (85.1) | … | … |

[a] Severe diarrhea was defined as bloody diarrhea or diarrhea with hypovolemia, fever, and leukocytosis; with hypoalbuminemia; or with pseudomembraneous colitis.
[b] When adjusted for hospital, the OR was 3.97 (95% CI, 1.05–15.0).
[c] A complicated course was defined as admission to an intensive care unit, surgical intervention, or death associated with CDAD.

Among the 141 patients with ≥ 1 recurrence, 45 recurrences (20.6%) occurred among the 218 patients with CDAD due to ribotype 027, and 96 recurrences (14.9%) occurred among the 645 patients with non–ribotype 027 CDAD (OR, 1.49; 95% CI, 1.00–2.20). In multivariate analysis,

the association became weaker (OR, 1.44; 95% CI, 0.94–2.20). Of 218 patients infected with ribotype 027, 32 (14.7%) had 1 recurrence, and 13 (6.0%) had 11 recurrence, compared with 74 (11.5%) and 22 (3.4%)of patients in the non–ribotype 027 group, respectively (OR, 1.27 and 1.80; P = not significant).

## Discussion

During the period from February 2005 through November 2006, 22 health care facilities (5 nursing homes and 17 hospitals) were affected by cases of CDAD due to ribotype 027. Outbreaks were observed in 10 hospitals and 1 nursing home. In total, 20 (18.3%) of 109 Dutch hospitals have been affected since 2002. Ribotype 027 was more frequently found in general hospitals than in academic hospitals. The 3 affected academic hospitals represent 38% of all academic hospitals in The Netherlands but were all located in the western and central parts of the country. Of all general hospitals in The Netherlands, 13% were affected by CDAD due to ribotype 027, but the hospitals were also located in these areas, suggesting that transfer of patients with CDAD plays an important role in the introduction of ribotype 027 into a new facility. Such an exchange was observed in 1 academic hospital and 2 general hospitals where outbreaks occurred after transfer of a patient with diarrhea.

Patients infected with ribotype 027 were significantly older and used more fluoroquinolones, compared with patients infected with non-027 ribotypes. The mortality among patients infected with ribotype 027 was higher, although the numbers were too low to make any firm statement. A clear trend was found for more severe diarrhea and more recurrences in patients with CDAD due to ribotype 027. Our findings are in accordance with those of Hubert et al. [6], who found an association between ribotype 027 and severe diarrhea in a prospective surveillance study from 88 Quebec hospitals that encompassed 478 consecutive patients with nosocomial CDAD. As is the case for ribotype 027 strains from the United States, Canada, and United Kingdom, ribotype 027 strains in our study belonged to toxinotype III; had

genes for binary toxin, toxin A, and toxin B; contained an 18-bp *tcdC* deletion; and were *ErmB* negative [3,4,7]. The antimicrobial resistance to fluoroquinolones and erythromycin was also similar.

The inclusion of patients with severe diarrhea could have resulted in selection bias. However, 53% of the affected hospitals and 18% of all facilities submitted all of their toxin-positive samples. In total, 60% of all received samples were submitted by these facilities. Although the response rate for the questionnaires was 27%, the distributions of age and sex for patients who submitted a questionnaire were similar to those for patients who did not submit a questionnaire. Finally, we received comparable numbers of questionnaires from patients infected with ribotype 027 (23%) and from those infected with non-027 ribotypes (28%), allowing a comparison between the 2 groups. Because facilities that submitted samples did not know the typing result in advance, no selection could have occurred through knowledge of this result.

Prior use of fluoroquinolones was noted for 34 patients, 21 of whom (62%) had used ciprofloxacin. For the remaining 13 patients, the drug class was provided, but not the specific fluoroquinolone. In The Netherlands, gatifloxacin has not yet been officially approved by the authorities, and use moxifloxacin is scarce [NO STYLE for: ]. Interestingly, available data from 8 of the 10 outbreak hospitals revealed an increase in pre-epidemic use of fluoroquinolones in 4 hospitals (hospitals A–D; 50%). The rate of use increased from 119 to 632 defined daily doses per 10,000 patient-days (a 431% increase) in hospital A, from 1152 to 2059 defined daily doses per 10,000 patient-days (a 79% increase) in hospital B, from 1128 to 1264 defined daily doses per 10,000 patient-days (a 12% increase) in hospital C, and from 1958 to 2516 defined daily doses per 10,000 patient-days (a 28% increase) in hospital D.

Several studies have concluded that exposure to fluoroquinolones is a major risk factor for development of CDAD due to ribotype 027 strains [22-24]. To date, these studies only included matched control patients who did not have CDAD. In contrast, we compared patients infected

with ribotype 027 with patients infected with non-027 ribotypes, and we found that ribotype 027 was more frequently associated with previous fluoroquinolone use than were non-027 ribotypes. An explanation may be a higher level of fluoroquinolone resistance in ribotype 027 [6] that is probably associated with a single transition mutation in gyrA [25]. We found that the 2 most frequently circulating non-027 ribotypes (ribotypes 001 and 014) were all resistant to ciprofloxacin, but they less frequently displayed resistance to moxifloxacin. Interestingly, the first known (sporadic) ribotype 027 strain from 2002 was susceptible to the fluoroquinolones, clindamycin, and erythromycin.

Previous studies found that larger hospital size (1100 beds) was associated with the presence of ribotype 027 [26,27]. In The Netherlands, all hospitals with ribotype 027 had < 1250 beds. Interestingly, nonacademic hospitals had a significantly higher proportion of patients with CDAD due to ribotype 027 than did academic hospitals. This difference may be caused by the fact that academic hospitals have fewer patients per ward and stronger separation between different specialties than do non-academic hospitals.

Patients infected with ribotype 027 had a trend towards more severe diarrhea that became statistically significant after adjustment for the hospital. Although the differences were not statistically significant, patients infected with ribotype 027 had higher attributable mortality and recurrence rates (6.3% and 21%, respectively) than did patients infected with non-027 ribotypes (1.2% and 15%, respectively). The attributable mortality rate for patients infected with ribotype 027 is lower than the attributable mortality rates found earlier in the United States and Canada [5,28,29] but are in agreement with data from the most recent surveillance study performed in Quebec, Canada (8.4%) [6]. This may be explained by a higher awareness about the ribotype.

Recurrences have been found to be associated with patient age, duration of hospitalization after the onset of CDAD, and treatment with metronidazole instead of

vancomycin [29,30]. Because we were not informed about the treatment and the duration of hospitalization in our study, these associations could not be investigated.

A high percentage of cases of community-onset CDAD were found both in patients infected with ribotype 027 (20%) and in those infected with non-027 ribotypes (25.6%). Because we knew about previous hospitalizations for only a few patients, we were mostly unable to determine whether there was a health care or community association. These recently proposed epidemiological definitions [10] are important, because severe CDAD has also been described in populations in the community that were previously thought to be at low risk [31].

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