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Emergence of *Clostridium difficile* Infection Due to a New Hypervirulent Strain, Polymerase Chain Reaction Ribotype 078

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Background. Since 2005, an increase in the prevalence of *Clostridium difficile* infection (CDI) due to polymerase chain reaction ribotype 078 has been noticed in The Netherlands. This strain has also been identified as the predominant strain in pigs and calves.

Methods. CDI caused by type 078 was studied in relation to CDI caused by the hypervirulent type 027 and by types other than 027 and 078. Human and porcine isolates were further investigated and characterized by multilocus variable number tandem repeat analysis.

Results. From February 2005 through February 2008, the incidence of type 078 among isolates obtained from 1687 patients increased from 3% to 13%. Compared with patients infected with type 027, patients infected with type 078 were younger (67.4 vs. 73.5 years; $P < .01$) and more frequently had community-associated disease (17.5% vs. 6.7%; odds ratio, 2.98; 95% confidence interval, 2.11–8.02); rates of severe diarrhea (38.9% vs. 40.0%) and attributable mortality (3.8% vs. 4.0%) were similar in both groups. Compared with patients infected with other types, patients infected with type 078 more frequently received fluoroquinolone therapy (29.4% vs. 19.8%; odds ratio, 2.17; 95% confidence interval, 1.06–4.44). Type 078 isolates contained genes for toxin A, toxin B, binary toxin, and a 39–base pair deletion in toxin regulator gene *(tcdC),* as well as a point mutation at position 184, resulting in a stop codon. Multilocus variable number tandem repeat analysis of 54 human and 11 porcine isolates revealed 4 clonal complexes containing both porcine and human isolates.

Conclusions. CDI due to type 078 and CDI due to type 027 present with similar severity, but CDI due to type 078 affects a younger population and is more frequently community associated. *C. difficile* type 078 isolates from humans and pigs are highly genetically related.

Clostridium difficile infection (CDI) can present as severe disease, particularly when it is caused by a hypervirulent strain characterized as North American pulsed-field type 1, restriction-endonuclease analysis group type BI, and PCR ribotype 027; this strain has caused outbreaks of CDI in the United States, Canada, and Europe [1–6]. The first reports of outbreaks of CDI due to type 027 originated from Canada, where Quebec was most severely affected. In the United States, CDI due to type 027 has been reported from at least 38 states [7]. In addition, the European Centre for Disease Prevention and Control reported infections due to type 027 in 16 European countries [8]. The hypervirulent strain belongs to toxinotype III, harbors the toxin genes *tcdA* and *tcdB* and binary toxin genes, and has an 18–base pair deletion in the toxin regulatory gene *tcdC* and a deletion at position 117. The latter results in a frame-shift and a premature stop codon, leading to a truncated TcdC protein. The increased virulence of type 027 is assumed to be associated with higher amounts of toxin production attributable to a lack of regulatory control of intact TcdC [9, 10].

Recently, we observed an increase in the prevalence of human CDI caused by PCR ribotype 078, toxinotype V, which has been reported as the predominant ribotype

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in calves and pigs [11]. In The Netherlands, type 078 was also found at pig farms as a causative agent of diarrhea in piglets [12]. This report provides a clinical overview of human CDI caused by type 078, compared with CDI caused by type 027 and with CDI caused by types other than 027 and 078. In addition, we investigated the microbiological characteristics, virulence factors, and relatedness of human and porcine type 078 isolates.

METHODS

Definitions

Definitions proposed by the European and American Centers for Disease Control and Prevention were used [13, 14]. Health care–associated CDI was defined as development of CDI >2 days after admission to the hospital or within 4 weeks after discharge from the hospital. Community-associated CDI was defined as development of CDI on day 0, 1, or 2 after admission to the hospital or >12 weeks after discharge from the hospital. When CDI developed 4–12 weeks after hospital discharge, the association was indeterminate.

Diarrhea was defined as ⩾3 unformed stools per 24 h. Patients were considered to have CDI if they had diarrhea and a stool sample positive for *C. difficile* toxin A and/or B by laboratory assay. A complicated course of CDI was defined as CDI requiring admission to the intensive care unit or surgical intervention or that was associated with death. A recurrence was defined as an episode occurring within 8 weeks after the onset of a previous episode. An outbreak in a health care facility was defined as the occurrence of ⩾2 epidemiologically linked cases within 1 week. We defined severe diarrhea as bloody diarrhea and/or diarrhea with hypovolemia or hypoalbuminemia (albumin level, <20 g/L), fever (temperature, >38.0°C) and leukocytosis (WBC count, $>12 \times 10^9$ cells/L), and/or pseudomembranous colitis. Mortality was considered to be attributable to CDI when a patient died of the consequences of CDI during hospitalization.

Strain Collection

All *C. difficile* isolates from patients with CDI that were submitted to the National Reference Laboratory at the Leiden University Medical Center (Leiden, The Netherlands) from January 2005 through January 2008 were included. This collection consisted of 2 groups. Most of the isolates (85%) originated from health care facilities that regularly submitted isolates on a monthly basis. The other isolates (15%) originated from facilities that only submitted isolates from patients with severe CDI or when there was an increased incidence of CDI.

A random selection of human type 078 isolates from other countries was also investigated. Isolates derived from Belgium ($n = 2$), Italy ($n = 1$), France ($n = 2$), Germany ($n = 4$), the United Kingdom ($n = 1$), and Ireland ($n = 1$). These isolates were available from a recent study by Barbut et al. [15]. Porcine type 078 isolates were derived from a collection of 11 pig isolates from 2 farms (located in central and eastern areas of The Netherlands) with pigs that experienced neonatal diarrhea for >1 year. In this collection, type 078 was the exclusively found type [12].

Clinical Analysis

Collection of clinical and demographic data. A standardized questionnaire was designed to obtain information on patients' age, sex, unit where CDI was diagnosed, health care– or community-associated CDI, severity of diarrhea, clinical course, and mortality. Furthermore, data were collected on hospital admissions and use of antibiotics during the 3 months before CDI. Comorbidity was established according to the *International Classification of Diseases, Tenth Revision.* Medical microbiologists were requested to provide these clinical data along with the submission of each fecal isolate to the National Reference Laboratory.

Statistical analysis. The distribution of risk factors and clinical outcome parameters among patients with CDI due to type 078 was compared with that among patients with CDI due to type 027 and with that among patients with CDI due to types other than 027 and 078. Continuous data were compared between groups by analyses of variance. A Yates-corrected χ^2 test was used for the analysis of proportions. If a cell value was <5 in the 2-by-2 table, Fisher's exact test was used. A multiple logistic regression model was used to study the association of putative risk factors with CDI due to type 078 and CDI due to type 027. Relative risks were estimated as ORs and were presented with 95% CIs. Both crude relative risks and relative risks after adjustment for the possible confounders are provided. All risk factors were adjusted for age, sex, and confounders that were both associated with the risk factor and the target variable with a univariate $P \leqslant .20$. All analyses were performed using SPSS for Windows, version 13.0 (SPSS).

Microbiological Analysis

***Isolation and characterization of* C. difficile.** 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* [16]. All *C. difficile* strains were further investigated by PCR ribotyping [17] and toxinotyping [18]. The presence of *tcdA, tcdB,* and binary toxin genes was investigated according to standardized techniques [19–21]. Deletions in *tcdC* were determined by PCR using in-house designed primers [22].

***Sequencing of* tcdC.** Sequencing was performed for a random selection of human and porcine type 078 strains. For amplification of *tcdC,* a forward primer that was designed in-house was used: 5′ TTTTCATATGTTTTCTAAAAAAAATGA-

GGG 3′ (TcdC1s). The reverse primer was developed by Cohen et al. [23]: 5′ GCACCTCATCACCATCTTCAA 3′ (957CDas). PCR reactions were performed with Pfu polymerase, using standard conditions. Purified PCR products were used as templates in the cycle sequencing reaction. The generated data were analyzed with Vector NTI advance, version 10 (Invitrogen).

Antimicrobial susceptibility testing. A random selection of type 078 isolates was tested for the presence of the *ermB* gene, which confers resistance to clindamycin and erythromycin [24]. In addition, E-tests (bioMérieux) were performed to determine the MICs to ciprofloxacin, moxifloxacin, erythromycin, and clindamycin, using the breakpoints described elsewhere [15].

Multilocus variable number tandem repeat analysis (MLVA). Molecular genotyping by MLVA was performed for a random selection of type 078 strains as described elsewhere [25], with 1 alteration: a new reverse primer was developed for marker *Cd*G8 (5′ ACCAAAAATTTCTAACCCAAC 3′). Minimum spanning tree analysis of MLVA types was performed to determine the genetic distance between isolates, using the number of differing loci and the summed tandem repeat difference (STRD) as coefficients for the genetic distance in BioNumerics, version 4.6 (Applied Maths) (A. Goorhuis, M. C. Legaria, R. J. Van den Berg, C. Harmanus, C. H. W. Klaassen, J. S. Brazier, G. Lumelsky, E. J. Kuijper, unpublished data) [25, 26]. Isolates with a STRD $\leqslant$10 were defined as genetically related. Clonal complexes were defined by an STRD $\leqslant$2 [26].

RESULTS

From February 2005 through February 2008, we received isolates from 2039 patients with CDI; isolates from 1687 patients were available for further typing. Only the first isolate per patient was analyzed. The isolates originated from 75 health care facilities, including 49 hospitals (1400 isolates; 83%), 14 nursing homes (34; 2%), and 12 regional laboratories (253; 15%). The most frequently encountered types were 027 (in 289 isolates; 17%), 014 (173; 10%), 078 (150; 9%), and 001 (29; 2%). During the study period, the proportion of isolates with type 078 increased from 3% to 13%, and the proportion of isolates with type 027 decreased from 27% to 1%. During the second half of 2007, type 078 was the second most frequently found type (in 39 [13%] of 308 isolates), after type 014 (41 [113%] of 308). Outbreaks of CDI due to type 027 occurred in 14 facilities, whereas sporadic cases were detected in 20 facilities. In contrast, only 1 outbreak of CDI due to type 078 (among 4 patients) was observed in a nursing home in the northeastern area of The Netherlands. A total of 27 cases occurred in another hospital; however, the cases were not epidemiologically linked. Sporadic cases were found in another 37 facilities. The spread of CDI due to types 027 and 078 is depicted in figure 1. Both types were most frequently encountered in the western and central part of the country, but type 078 showed a wider distribution to the more-peripheral and rural areas.

Clinical Analysis

We received questionnaires from 715 (42%) of 1687 patients with CDI; questionnaires were from 57 (38%) of 150 patients with CDI due to type 078, 129 (45%) of 289 patients with CDI due to type 027, and 529 (42%) of 1248 patients with CDI due to types other than 078 and 027. In table 1, risk factors for and outcomes of CDI due to types 078, 027, and types other than 078 and 027 are shown. Differences between the groups are expressed as percentages of the total number of patients for whom information was available. Unless stated otherwise, all mentioned differences in the following comparisons were statistically significant in multivariate analysis (table 2).

Comparison of CDI due to type 078 and CDI due to types other than 078 and 027. Compared with patients with CDI due to types other than 078 and 027, patients with CDI due to type 078 less frequently had other diseases and more frequently received fluoroquinolone therapy.

Comparison of CDI due to type 078 and CDI due to type 027. Compared with patients with CDI due to type 027, patients with CDI due to type 078 were younger (a lower percentage of patients was aged >80 years) and more frequently had community-associated CDI and indeterminate CDI (whereas health care–associated CDI was observed less frequently). The incidence of severe diarrhea and attributable mortality were similar in both groups of patients. Patients with CDI due to type 078 less frequently had a complicated course, compared with patients with CDI due to type 027.

Comparison of CDI due to type 027 and CDI due to types other than 078 and 027. Compared with patients with CDI due to types other than 078 and 027, patients with CDI due to type 027 were older (a higher percentage of patients was aged $\geqslant$65 years) and more frequently had health care–associated CDI. Patients with CDI due to type 027 more frequently received cephalosporins, second-generation cephalosporins, and fluoroquinolones and less frequently used macrolides, clindamycin, and aminoglycosides. Severe diarrhea occurred more frequently among patients with CDI due to type 027 than among patients with CDI due to types other than 078 and 027. Although a complicated course, overall mortality, attributable mortality, and recurrences were significantly associated with CDI due to type 027 in univariate analysis, none of these differences were statistically significant in multivariate analysis.

Microbiological Analysis

A random selection of 51 *C. difficile* type 078 isolates (43 human and 8 porcine) was available for investigation of *tcdA* and *tcdB,* binary toxin genes, toxinotyping, sequencing of *tcdC,* and susceptibility testing. All of these contained *tcdA, tcdB,* and binary

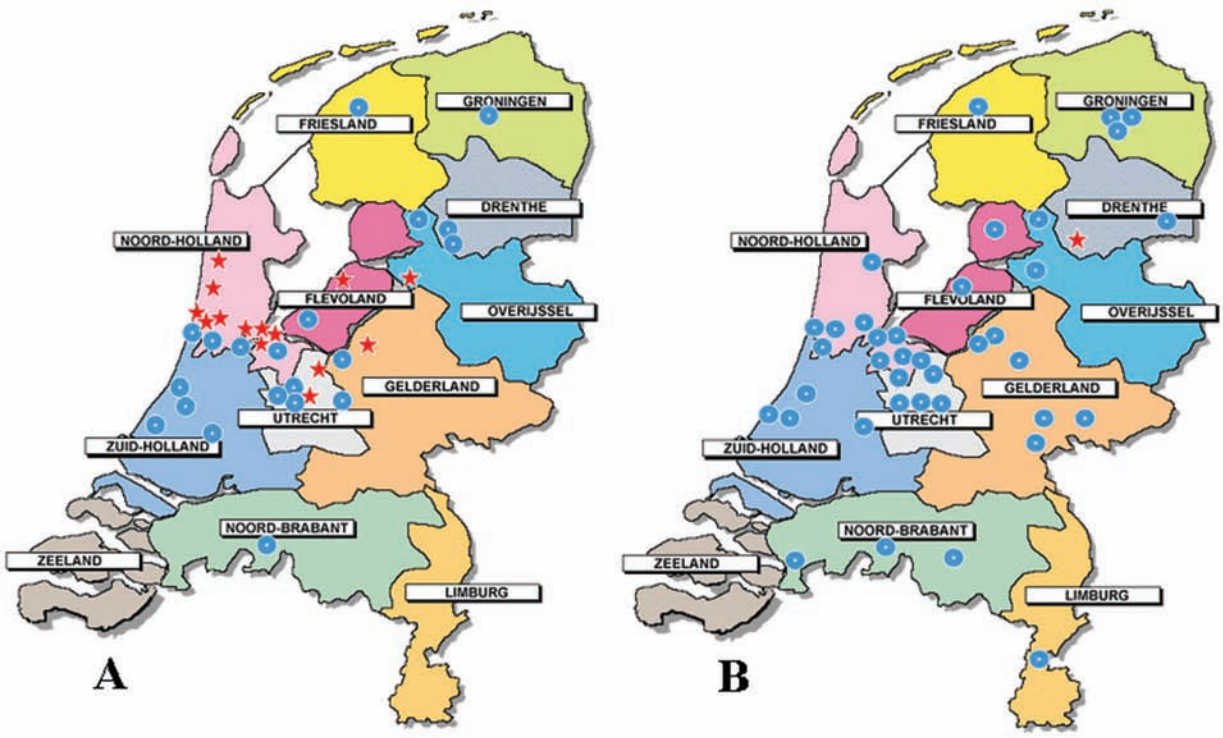

Figure 1. *A,* Spread of PCR ribotype 027 across The Netherlands. *B,* Spread of PCR ribotype 078 across The Netherlands. Circles represent health care facilities where *Clostridium difficile* infection (CDI) due to the respective type was endemic, and stars represent facilities that have experienced outbreaks of CDI due to the respective type.

toxin genes and were toxinotype V. Sequencing of *tcdC* identified in all isolates a 39–base pair deletion (from nucleotides 341 to 379) and a point mutation at position 184 (C184T), resulting in a premature stop codon (TAA). Susceptibility testing was performed on 49 isolates, because 2 human isolates were not available. Of these, 46 (94%) were resistant to ciprofloxacin (MIC, ⩾4 mg/L), and 38 (78%) were resistant to erythromycin (MIC, ⩾4 mg/L). Forty-three isolates (88%) were susceptible to moxifloxacin (MIC, <4 mg/L), and 28 (57%) were susceptible to clindamycin (MIC, <4 mg/L). The *ermB* gene was found in 7 (14%) of 49 isolates. No statistically significant differences in antimicrobial susceptibility were found between human and porcine isolates.

For MLVA, the collection of 51 isolates was expanded with 11 human type 078/toxinotype V isolates that were available from other countries and another 3 Dutch type 078/toxinotype V porcine isolates; this provided a total collection of 65 isolates (54 human and 11 porcine). Figure 2 shows the minimum spanning tree analysis of these 65 isolates. All were genetically related (STRD, ⩽10), and 4 clonal complexes (CCs) with an STRD ⩽2 were recognized (boxed CC-A to CC-D). CC-A and CC-B both contained human and porcine isolates. Eight (73%) of 11 porcine isolates were found in these 2 CCs. The remaining 3 porcine isolates were 100% homologous to each other and were related to CC-B. Of 14 human isolates belonging to CC-A and CC-B, 4 were from other countries (ITA1 in CC-A and GBR1, BEL1, and GER2 in CC-B).

DISCUSSION

In a 2.5-year period, we experienced the emergence of CDI caused by type 078, which has been reported as the predominant type in pigs and calves [11]. To date, *C. difficile* type 078 is the second-most frequently encountered type (13%). Important findings were that, compared with patients with CDI due to type 027, patients with CDI due to type 078 were younger and had a higher proportion of community-associated disease; both groups had equal proportions of severe diarrhea and attributable mortality. Similar to CDI due to type 027, use of fluoroquinolones was found to be an independent risk factor for CDI due to type 078. Compared with the other types of CDI, infection with type 027 was associated with more recurrences and the highest risk of a complicated course, as has been described elsewhere [2, 5].

In 2005, type 078 was the eleventh-most frequently found type in Europe [15]. Recently, it was increasingly recognized in Belgium, Spain, and Germany, although exact data are missing [27, 28]. Cases of CDI due to type 078 were more widely distributed across the country than were cases of CDI due to type 027. Because type 078 is mainly found at pig farms, we

Table 1. Risk factors for and outcomes of *Clostridium difficile* infection (CDI), by PCR ribotype.

Risk factor	Proportion of patients with CDI (%)		
	Type 078	Type 027	Other types
Age, years			
0–64	47/145 (32.4)	59/270 (21.9)	418/1148 (36.4)
65–79	55/145 (37.9)	111/270 (41.1)	391/1148 (34.1)
⩾80	43/145 (29.7)	100/270 (37.0)[a]	339/1148 (29.5)
Male sex	68/133 (51.1)	115/254 (45.3)	463/1052 (44.0)
Place of transmission			
Health care setting	41/57 (71.9)	112/120 (93.3)[a]	385/491 (78.4)
Community	10/57 (17.5)	8/120 (6.7)[a]	78/491 (15.9)
Indeterminate	6/57 (10.5)	0/120 (0.0)[a]	28/491 (5.7)
Underlying disease			
Any	47/51 (92.2)	114/126 (90.5)	462/504 (91.7)
Solid tumor	6/47 (12.8)	16/120 (13.3)	71/486 (14.6)
Hematologic malignancy	8/49 (16.3)	12/121 (9.9)	46/488 (9.4)
Endocrine disease	8/48 (16.7)	13/119 (10.9)	81/480 (16.9)
Respiratory diseases	16/48 (33.3)	51/121 (42.1)	129/478 (27.0)
Digestive diseases	11/49 (22.4)	31/120 (25.8)	133/482 (27.6)
Cardiovascular diseases	17/49 (34.7)	42/121 (34.7)	178/483 (36.9)
Genitourinary diseases	13/49 (26.5)	37/120 (30.8)	135/477 (28.3)
Other[b]	11/46 (23.9)[c]	43/119 (36.1)	177/471 (37.6)
Antibiotic therapy			
Any	44/52 (84.6)	110/123 (89.4)	425/501 (84.8)
Penicillins	23/51 (45.1)	55/122 (45.1)	236/478 (49.4)
Cephalosporins			
All	22/51 (43.1)	68/121 (56.2)[d]	201/477 (42.1)
First generation	1/48 (2.1)	8/121 (6.6)	34/456 (7.5)
Second generation	9/48 (18.8)	36/112 (32.1)[d]	85/456 (18.6)
Third generation	10/48 (20.8)	21/112 (18.8)	91/456 (20.0)
Fluoroquinolones	15/51 (29.4)[c]	37/122 (30.3)[d]	95/480 (19.8)
Macrolides and clindamycin	6/51 (11.8)	15/121 (12.4)[c]	94/480 (19.6)
Aminoglycosides	9/51 (17.6)	6/123 (4.9)[a]	52/481 (10.8)
Carbapenems	4/51 (7.8)	4/120 (3.3)	23/473 (4.9)
Vancomycin	4/51 (7.8)	18/123 (14.6)[c]	43/479 (9.0)
Metronidazole	6/51 (11.8)	16/121 (13.2)	41/480 (8.5)
Sulfonamides and trimethoprim	7/51 (13.7)	11/121 (9.1)	68/478 (14.2)
Outcome			
Severe diarrhea	21/54 (38.9)[c]	48/120 (40.0)[d]	134/476 (28.2)
Complicated course	5/52 (9.6)	22/124 (17.7)[d]	54/501 (10.8)
Recurrent infections	6/38 (15.8)	21/68 (30.9)[c]	62/333 (18.6)
Overall mortality	3/52 (5.8)	16/124 (12.9)[d]	35/501 (7.0)
Attributable mortality	2/52 (3.8)	5/124 (4.0)[d]	5/501 (1.0)
Contributable mortality	0/52 (0.0)	3/124 (2.4)	13/501 (2.6)

[a] Statistically significant ($P \leqslant .05$) when compared with both CDI due to type 078 and CDI due to other types.
[b] Other diseases are diseases not belonging to any of the other disease groups and include trauma; neurological, cerebrovascular, mental, and behavioral diseases; HIV infection; and infectious diseases and disorders involving the immune mechanism, musculoskeletal system, and connective tissue.
[c] Trend ($P \leqslant .10$) when compared with CDI due to other types.
[d] Statistically significant ($P \leqslant .05$) when compared with CDI due to other types.

compared the distribution of pig farms in The Netherlands with the occurrence of human type 078 infections. We noticed an overlap in the eastern part of the country, where >90% of all pig farms are located. Here, type 078 was found in 22.4% of all submitted isolates. It is tempting to speculate that this overlap is attributable to a common source for human and pigs isolates, but surveillance data on pig farms are lacking, and a zoonotical transmission has never been demonstrated.

All *C. difficile* type 078 isolates belonged to toxinotype V; had virulence characteristics similar to those of type 027, such

Table 2. Univariate and multivariate analyses of risk factors and outcome parameters for which statistically significant differences or trends between any of the 3 groups of patients with *Clostridium difficile* infection (CDI) were found.

	OR (95% CI)					
	CDI due to type 078 vs. CDI due to other types		CDI due to type 078 vs. CDI due to type 027		CDI due to type 027 vs. CDI due to other types	
Risk factor	Univariate analysis	Multivariate analysis	Univariate analysis	Multivariate analysis	Univariate analysis	Multivariate analysis
Age, years						
0–64	Reference	Reference	Reference	Reference	Reference	Reference
65–79	1.25 (0.83–1.89)	1.23 (0.80–1.89)	0.62 (0.38–1.03)	0.67 (0.39–1.12)	2.01 (1.43–2.84)[a]	1.80 (1.26–2.56)[a]
⩾80	1.13 (0.73–1.75)	1.13 (0.71–1.80)	0.54 (0.32–0.91)[a]	0.57 (0.33–0.98)[a]	2.09 (1.47–2.97)[a]	1.94 (1.35–2.79)[a]
Place of transmission						
Health care setting	0.71 (0.38–1.31)	…	0.18 (0.07–0.46)[a]	…	3.85 (1.82–8.15)[a]	…
Indeterminate	1.95 (0.77–4.92)	…	No OR[b]	…	No OR[b]	…
Community	1.13 (0.55–2.32)	…	2.98 (1.11–8.02)[a]	…	0.38 (0.18–0.81)[a]	…
Underlying disease						
Hematologic malignancy	1.87 (0.83–4.24)	2.22 (0.89–5.55)[c]	1.77 (0.68–4.65)	…	1.06 (0.54–2.07)	…
Respiratory system diseases	1.35 (0.72–2.55)	…	0.69 (0.34–1.38)	…	1.97 (1.30–2.98)[a]	1.21 (0.73–2.01)
Other diseases	0.52 (0.26–1.05)[c]	0.44 (0.20–0.95)[a]	0.56 (0.26–1.20)	0.51 (0.21–1.28)	0.94 (0.62–1.43)	…
Antibiotic therapy						
Cephalosporins						
All	1.04 (0.58–1.87)	…	0.59 (0.31–1.14	0.78 (0.37–1.66)	1.76 (1.18–2.63)[a]	1.66 (1.03–2.67)[a]
Second generation	1.01 (0.47–2.16)	…	0.49 (0.21–1.11)	0.56 (0.24–1.29)	2.07 (1.30–3.28)[a]	1.87 (1.07–3.28)[a]
Fluoroquinolones	1.69 (0.89–3.21)	2.17 (1.06–4.44)[a]	0.96 (0.47–1.96)	…	1.76 (1.13–2.76)[a]	1.69 (1.00–2.88)[a]
Macrolides and clindamycin	0.55 (0.23–1.32)	0.45 (0.17–1.19)	0.94 (0.34–2.58)	…	0.58 (0.32–1.04)[c]	0.38 (0.19–0.77)[a]
Aminoglycosides	1.77 (0.81–3.84)	1.78 (0.73–4.32)	4.18 (1.40–12.4)[a]	4.66 (1.54–14.1)[a]	0.42 (0.18–1.00)[a]	0.31 (0.11–0.85)[a]
Vancomycin	0.86 (0.30–2.51)	…	0.50 (0.16–1.55)	…	1.74 (0.96–3.14)[c]	1.85 (0.91–3.79)[c]
Outcome						
Severe diarrhea	1.62 (0.91–2.91)[c]	1.67 (0.89–3.13)[c]	0.95 (0.49–1.84)	…	1.70 (1.12–2.58)[a]	1.65 (1.02–2.67)[a]
Complicated course	0.88 (0.34–2.31)	…	0.49 (0.18–1.38)	0.20 (0.04–0.91)[a]	1.79 (1.04–3.06)[a]	1.53 (0.81–2.88)[c]
Recurrent infections	0.82 (0.33–2.05)	…	0.42 (0.15–1.15)[c]	0.77 (0.25–2.35)	1.95 (1.09–3.50)[a]	1.80 (0.92–3.53)[c]
Overall mortality	0.82 (0.24–2.75)	…	0.41 (0.12–1.48)	0.14 (0.02–1.11)[c]	1.97 (1.05–3.69)[a]	1.57 (0.74–3.35)
Attributable mortality	3.97 (0.75–21.0)[c]	2.23 (0.24–20.4)	0.95 (0.18–5.07)	…	4.17 (1.19–14.6)[a]	3.54 (0.90–14.0)[c]

[a] Statistically significant ($P \leqslant .05$).
[b] No OR could be calculated, because the number in the group infected with CDI due to type 027 is 0 ($P = .001$ for CDI due to type 078 vs. CDI due to type 027 and $P = .003$ for CDI due to type 027 vs. CDI due to other types).
[c] Trend ($P \leqslant .10$).

as the presence of *tcdA, tcdB,* and binary toxin genes; and had a mutation (C184T) in the regulatory *tcdC* gene that resulted in a premature stop codon. This mutation in toxinotype V strains has been reported elsewhere [29, 30] but has never been ascribed to type 078. The antimicrobial susceptibility patterns of type 078 differed from those of type 027; 88% of type 078 isolates were susceptible to moxifloxacin, 43% were resistant to clindamycin, and 14% harbored the *ermB* gene.

Our study has a few limitations. First, not all Dutch health care facilities submitted isolates. Of those that did, 85% (13 hospitals and 4 regional laboratories) regularly submitted isolates on a monthly basis, and 15% only submitted isolates from patients with severe CDI or when there was an increased incidence of CDI. To investigate potentially introduced bias, these 2 subgroups were analyzed separately (data not shown). Because no statistically significant or relevant differences were found, all data were analyzed together. We estimated the expected number of CDIs from the 13 hospitals that regularly submitted samples on the basis of admission records (available on the hospital Web sites) and incidence of CDI per hospital or, when this was not known, the mean incidence reported in The Netherlands [16]. Using these expected numbers of CDIs, we estimated that we received 81% of all CDI isolates from these hospitals. Similarly, using the total number of hospital admissions in The Netherlands [31], we estimated that we received isolates from 30% of all patients with CDI in The Netherlands. The second limitation is that clinical information was available for only 715 (42%) of 1687 CDIs. The distribution of types 078, 027, and types other than these among 715 patients was, however, similar to the distribution among all 1687 patients; the distribution of age and sex was also similar. Furthermore, the result of typing was not known in advance; thus, no selection could have occurred through knowledge of this result.

A remarkable finding was that all human and animal type 078 isolates were related by MLVA to the point of clonality. This finding strongly supports the hypothesis that no inter-

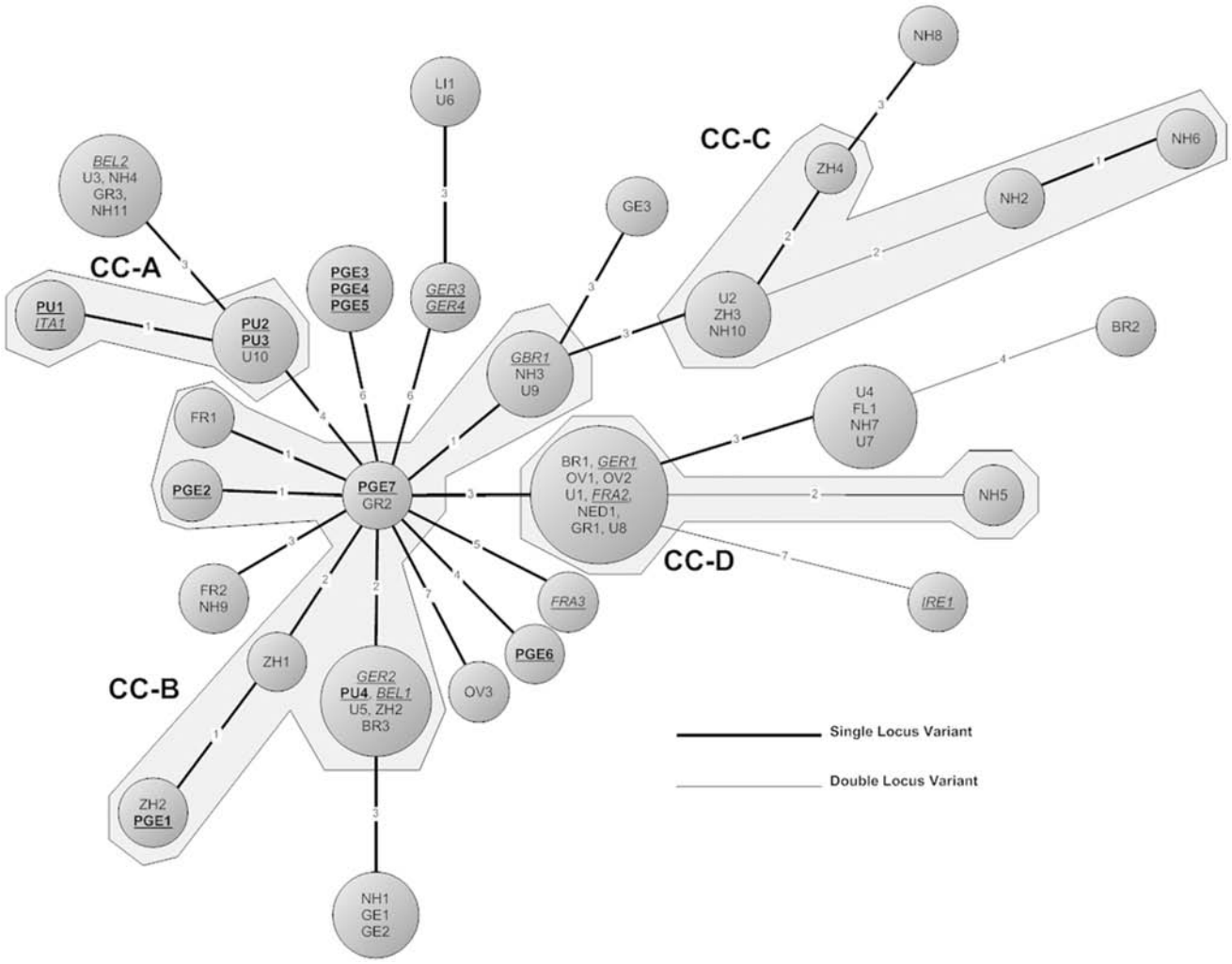

Figure 2. Minimum spanning tree analysis of 65 *Clostridium difficile* type 078 isolates (54 human isolates and 11 porcine isolates) typed by multilocus variable number tandem repeat analysis (MLVA). Porcine isolates are in boldface type and begin with "P." Of the 54 human isolates, 11 are from countries other than The Netherlands (these are underlined and italic). The countries are Belgium (BEL), Germany (GER), Italy (ITA), France (FRA), the United Kingdom (GBR), and Ireland (IRE). Each circle represents either a unique isolate or isolates that are 100% homologous. The numbers between the circles represent the summed tandem repeat difference (STRD) between MLVA types. Within the spanning tree, 4 boxed clonal complexes (CC-A to CC-D) with a STRD ≤ 2 are depicted.

species barrier exists for CDI type 078. The high degree of genetic relatedness among type 078 isolates is surprising, because previous studies revealed a high variation among types 017 and 027 [25, 32, 33]. This could indicate that type 078 has not been part of the spectrum of human CDI for a long enough time to develop a more-remote relatedness to porcine strains or that it is very stable with regard to mutations. The latter is not likely, because Stabler et al. [34] recently revealed that *C. difficile* readily undergoes genetic exchange. Using whole genome analysis, they also hypothesized that human strains arose from those found in pigs on the basis of identification of a toxinogenic clade containing porcine, bovine, and human isolates. It is not likely that the high genetic relatedness detected by MLVA is caused by a lack of discriminatory power of MLVA, because Killgore et al. [35] recently revealed that MLVA (and restriction endonuclease analysis) has superior discriminatory power, compared with other typing methods.

An important question is: what is causing the emergence of the type 078 strain? One explanation could be the increased use of fluoroquinolones by patients with CDI due to type 078. However, because 70% of patients with CDI due to type 078 did not use fluoroquinolones, this cannot be the only reason. It is probable that another yet unknown selection mechanism favors the emergence of this hypervirulent genotype. Another explanation could be that type 078 has emerged from the cattle breeding industry. This hypothesis is supported by the facts

that type 078 is predominant in porcine and bovine diarrheal samples [11], that *C. difficile* has been found in retail meat (with 078 as the predominant type in 1 study [36, 37]), and that patients with CDI due to type 078 more frequently inhabit rural areas of the country with pig farms in the vicinity, as was found in our study. The link between human and porcine CDI has also been recently suggested by Jhung et al. [38].

In conclusion, compared with CDI due to type 027, CDI type 078 causes severe disease in a younger population and causes a higher frequency of community-associated disease. The clinical and microbiological spectrum of CDI type 078 suggests that, similar to type 027, it is a new emerging hypervirulent strain that is genetically indistinguishable from porcine type 078 strains.

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