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The Changing Epidemiology of *Clostridium difficile* Infections

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INTRODUCTION

Since the description of *Clostridium difficile* as a cause of antimicrobial-associated diarrhea, colitis, and pseudomembranous colitis (PMC) in 1978, interest in this pathogen was predominantly because of its association with health care and its impact on morbidity and mortality in the elderly. There has been an explosion of reports on *C. difficile* infection (CDI) in the new millennium, driven by large increases in disease incidence, stark changes in clinical presentation and epidemiology, and descriptions of new risk factors. This review focuses on how these new issues have impacted the epidemiology of CDI.

A study of 187 isolates from multiple U.S. states revealed that a particular *C. difficile* strain was most closely associated with the increased incidence and altered clinical presentation of CDI. Prior to the year 2000, this strain (BI) had accounted for fewer than 1% of U.S. *C. difficile* isolates (130). The strain was characterized as toxinotype III and had an 18-bp deletion within the *tcdC* gene (putative negative regulator for the production of toxins A and B) as well as a deletion at position 117. The latter results in a frameshift and a premature stop codon, leading to a truncated TcdC protein. These strains have been shown to produce significantly more toxin *in vitro*, and it was postulated that this virulence factor is associated with increased severity (203), although other complex gut model data partly contradict these findings (68). In the gut model, this epidemic *C. difficile* strain (ribotype 027) produces toxin for longer periods of time, as opposed to more toxin per unit time, which may be expected considering the absence of a functional negative regulation mechanism (68). These strains have also been shown to produce binary toxin, although the role of this toxin in CDI pathogenesis remains largely unclear (75). A recent report mentioned that binary toxin induces microtubule protrusions that form a dense meshwork at the cell surface of intestinal epithelial cells, which wrap and embed bacterial cells, thereby increasing the adherence of clostridia (174). In addition, these epidemic strains have reduced susceptibility to fluoroquinolone antibiotics compared with both older isolates of the same type and contemporary nonoutbreak strains of *C. difficile* (130) (see below). This profile has since been assigned North American pulsed-field type 1 (NAP1), restriction endonuclease analysis (REA) group BI, and PCR ribotype 027 (sometimes referred to as BI/NAP1/027). This diverse set of bacterial typing techniques reflects, at present, the lack of a common global currency for *C. difficile* typing studies, which would help to facilitate a clearer epidemiological understanding of the disease (101). In this review, for simplicity, we have favored the use of the term "ribotype 027" when referring to this strain group.

Two key issues have hindered our understanding of the epidemiology of CDI. First, although there have been improvements in the surveillance of CDI, notably driven by the increased recognition of serious infection and outbreaks, the ascertainment of infection is still variable both within and between the majority of countries. Following early outbreaks caused by *C. difficile* ribotype 027 in Europe, the European Centre for Disease Prevention and Control (ECDC) convened a group of experts with input from the U.S. Centers for Disease Control and Prevention (CDC). The ECDC working group produced the first background paper on the emergence of CDI in Europe that included interim case definitions for CDI as well as other recommendations for surveillance (109). Very similar recommendations for surveillance were also reported by a CDC working group (132). However, surveillance systems

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for CDI tended to be initiated only after disease patterns have changed, meaning that there is often poor recognition and documentation of the early phases of changes in epidemiology. Furthermore, as interest in CDI has increased, there is no doubt that ascertainment bias has affected incidence data. Thus, the magnitudes of reported increases in CDI incidence are likely exaggerated, as improved systems to diagnose, enumerate, and analyze cases have been put in place. A good example here is the clear difference in recorded cases of CDI when mandatory data are compared with those provided on a voluntary basis; there were approximately 25% more cases in the mandatory data set from England in 2008 (40,705 versus 32,602) (81, 82). In order to be best placed to control CDI and especially to react to new shifts in disease patterns and presentations, we need to heed the lessons learned during this millennium and optimize surveillance.

A second key impediment to accurate data on epidemiology is the many different approaches to the laboratory diagnosis of CDI (45, 59, 158). Full details of this controversial area are outside the scope of this review. However, it is important to acknowledge that given the different targets and combinations that can be sought when detecting *C. difficile* (bacterium, glutamate dehydrogenase, toxins, and toxin genes), it is inevitable that the measured incidence of infection may vary according to laboratory method. The most common laboratory test used to diagnosis CDI is an enzyme immunoassay (EIA) to detect *C. difficile* toxins A and B. It is clear, however, that these tests generally have suboptimal sensitivity and specificity (45, 59, 158).

The surge in interest in *C. difficile* is typified by a wealth of genome studies (117, 123, 184), including full-genome sequencing, that have recently been reported. For example, the epidemic 027 strain has acquired a complete set of Agr genes compared with earlier strains (184). Also, a *C. difficile* strain whose *tcdB* gene was knocked out lost its pathogenicity in hamsters, whereas *tcdA* knockout strains were as virulent as wild-type bacteria (123). However, before drawing the conclusion that *tcdB* is the key factor in virulence, this observation needs confirmation, particularly as the mutants generated in this study were inherently unstable. Recently, a research group from Nottingham, United Kingdom, was unable to reproduce this observation (S. Kuehne and N. Minton, personal communication) using a different knockout technique, which generated stable mutations using ClosTron technology (83). Such approaches, especially when married with high-quality epidemiology, will likely provide key information about disease pathogenesis and potential interventions in CDI.

CHANGING *C. DIFFICILE* EPIDEMIOLOGY IN EUROPE

The first Europe-wide data on the incidence of CDI became available from a survey involving 212 hospitals that was performed by the European Study Group of *C. difficile* (ESGCD) in the United Kingdom, France, Belgium, Denmark, Germany, Italy, the Netherlands, and Spain in 2002 (13). The incidence of CDI varied considerably, depending partly on the diagnostic testing approaches in use. The mean incidence of CDI was 11 cases per 10,000 admissions. In 2005, the ESGCD performed a second 2-month Europe-wide survey of 38 hospitals in 14 countries (14). All inpatients with CDI were included; a case was defined as a patient with diarrhea and a positive toxinogenic culture. The mean incidence of CDI was 2.45 cases per 10,000 patient days but ranged from 0.13 to 7.1 cases/10,000 patient days. Comparison with the study performed in 2002 was not possible due to differences in design and data analyses. In total, 354 toxinogenic *C. difficile* isolates were collected and characterized. More than 66 different PCR ribotypes were recognized, and their distribution varied markedly among hospitals and countries. Of all toxinogenic isolates, ribotype 001 was the most common (13%), followed by ribotype 014 (9%); ribotypes 002, 012, 017, 020, and 027 were each found in 6% of toxinogenic isolates, whereas ribotype 078 was found in 3% of toxinogenic isolates. In this survey, ribotype 027 was reported from Ireland, Belgium, and the Netherlands. Patients infected with ribotype 027 were more likely to have more severe disease and to have been treated with metronidazole or vancomycin than patients infected by another PCR ribotype.

In the period between October 2003 and June 2004, the first known large United Kingdom outbreak caused by ribotype 027 occurred at the Stoke Mandeville Hospital (Buckinghamshire Hospitals NHS Trust), involving 174 cases and 19 (11%) deaths that were definitely or probably due to *C. difficile* (177). A second outbreak occurred between October 2004 and June 2005 in the same hospital, involving 160 new cases and 19 (12%) further deaths. A subsequent United Kingdom Healthcare Commission investigation concluded that the outbreaks were a consequence of a poor environment for patient care, poor practice in the control of infection, lack of facilities to isolate patients, and insufficient priority given to the control of infection by senior managers (44).

In England there is mandatory reporting of all CDI cases for each hospital, and quarterly data have been made publicly available since 2004. There has been a marked decrease in the incidence of CDI, after years of steady increases, since the introduction of enhanced surveillance in 2007 and a centrally funded scheme that provides rapid access to ribotyping (*C. difficile* Ribotyping Network [CDRN]). In 2008 to 2009 the CDRN processed 4,682 samples, meaning that the ribotypes are known for approximately 1 in 8 to 9 of all CDI cases in England (80). The most commonly identified ribotypes in England were ribotypes 027 (36%), 106 (13%), and 001 (7%). Notably, compared with data from 2007 to 2008, there was a 19% decrease in the prevalence of ribotype 027. Of the eight ribotypes most commonly seen across Europe (16), six were the top eight most prevalent ribotypes in England in 2008 to 2009 (the exceptions being ribotypes 012 and 018); the most prominent ribotype that is seen in England, but rarely elsewhere, is ribotype 106. Multivariate analysis of CDRN data showed that only ribotype 027 was significantly independently associated with mortality (odds ratio [OR] = 1.9; $P < 0.001$). There was a quadrupling in the numbers of death certificates where *C. difficile* was mentioned in England and Wales between 2004 and 2007, whereas there was a 29% decrease in 2008 (144). The successful control of ribotype 027 is likely to explain this marked reduction in CDI-related deaths.

In July 2005, *C. difficile* ribotype 027 was found in an outbreak in a hospital in Harderwijk, Netherlands (108, 199). The outbreak was noticed because of a clustering of severe CDI cases and a rapid increase in incidence (from 4 to 83 cases per 10,000 patient admissions from 2004 to April to July 2005, respectively). The outbreak ended only after the implementa-

tion of the restricted use of cephalosporins and a complete ban on fluoroquinolones in addition to general hygienic measures, cohorting of patients in a separate ward, education of staff, and intensified environmental cleaning (50). In September 2005, a ribotype 027 strain was isolated from four patients with CDI in Leper, Belgium, where the incidence had increased from 10 to 33 cases per 10,000 admissions (93). Soon after the first outbreaks in the Netherlands in 2005, a national surveillance program for *C. difficile* was initiated by the Leiden University Medical Centre (LUMC) and the Centre for Infectious Disease Control of the National Institute for Public Health and the Environment. Also, a prospective, 4-year surveillance study of the incidence of CDI and the distribution of *C. difficile* ribotypes was started in 14 Dutch hospitals in June 2006. The mean incidence of CDI measured in 14 hospitals remained stable throughout the study period (18 cases per 10,000 admissions in 2007 and 2008). Between April 2005 and June 2009, 2,788 samples were available for ribotyping. A decrease was seen in the prevalence of ribotype 027 after the second half of 2006. In the first half of 2009, the proportion of ribotype 027 isolates among all CDI cases decreased to 3.0%, whereas the proportion of ribotype 001 isolates increased to 27.5%. It was concluded that currently, there is a significant decrease in ribotype 027-associated CDI in the Netherlands (85).

An update on the spread of *C. difficile* ribotype 027 in Europe was published in 2007 (110). At this point ribotype 027 had affected health care facilities in 11 European Union member states and in Switzerland. The proportions of hospitals where ribotype 027 had been detected varied from 11% (Scotland) to 100% (Ireland), but large differences in surveillance methodology were noted. Interestingly, the Netherlands and France reported a lower attributable mortality rate associated with CDI caused by ribotype 027 (6.3% and 4%, respectively) than had been reported for the United States and Canada (121, 130, 152, 153).

One year later (July 2008), *C. difficile* ribotype 027 was detected in 16 European countries, causing outbreaks in Belgium, Germany, Finland, France, Ireland, Luxembourg, the Netherlands, Switzerland, and the United Kingdom (111). Ribotype 027 was also reported on a smaller scale in Austria, Denmark, Sweden, Norway (90), Hungary (191), Poland (157), and Spain, although Austria and Denmark later reported outbreaks of CDI (8, 89). The proportion of affected hospitals in each country varied considerably (0 to 76%), but systematic comprehensive surveillance was performed only in England (110/145 hospitals [76%]), Wales (10/16 hospitals [63%]), and Belgium (32/74 hospitals [43%]). Three countries reported the importation of patients with CDI caused by ribotype 027. In Switzerland, an outbreak of *C. difficile* ribotype 027 was observed in a geriatric hospital in Basel in 2006 (64). The index case was an 82-year-old female patient who returned from a hospitalization during her holidays abroad. The outbreak involved 15 other patients between October 2006 and May 2007. In 2007 in Spain, a patient who was transferred from a hospital in the United Kingdom was admitted with a severe form of CDI at a large tertiary-care hospital in Madrid (111). Although no spread was found among patients in the hospital, a laboratory technician working with this *C. difficile* isolate developed CDI shortly after receiving antibiotic treatment (25). In Austria, *C. difficile* ribotype 027 was reported in 2006 in a British tourist suffering from pseudomembranous colitis who was treated with antibiotics shortly before traveling to Austria (88).

A new finding was the occurrence of outbreaks due to clindamycin-resistant ribotype 027 isolates in Switzerland (64), Ireland (54), France, and, more recently, Denmark (8). Clindamycin resistance was defined as *ermB*-positive isolates with a clindamycin MIC of >256 mg/liter. Until this finding, the use of clindamycin was relatively "protective" with regard to the development of CDI due to ribotype 027 (76). The emergence of resistance to clindamycin in ribotype 027 isolates may increase the risk of CDI in patients receiving this agent, and its use may be an important factor contributing to strain persistence and spread. Unpublished observations from the National Reference Laboratory at Leiden University Medical Center indicated that clindamycin-resistant ribotype 027 isolates from these three countries were unrelated to each other, as determined by multilocus variable-number tandem-repeat analysis (MLVA) (Fig. 1). In addition, reports of erythromycin-susceptible and clindamycin-susceptible ribotype 027 isolates in Germany and Denmark indicated that antimicrobial resistance to macrolides, lincosamides, and fluoroquinolones varies for *C. difficile* ribotype 027 isolates.

Recent reports of *C. difficile* ribotype 027 infection in Finland are of special interest, notably as this country has always been considered a country with a very low incidence of CDI, and comprehensive data mapping the increased incidence of CDI are now available. A first case of fatal *C. difficile* ribotype 027-associated disease was detected in October 2007 (124). Neither the index case nor two additional cases had connections with foreign countries or each other. Since then, the National Public Health Institute intensified the surveillance and control of CDI. Other ribotype 027 isolates were detected retrospectively, indicating that this strain had previously been circulating in Finland. To determine whether the rate of CDI and related deaths was increasing in Finland, registry data from 1996 through 2004 were investigated (125). Those discharged with a CDI diagnosis doubled from 810 patients (16 patients/100,000 population) in 1996 to 1,787 patients (34 patients/100,000 population) in 2004. The increase was most prominent for patients aged >64 years, and the age-standardized mortality rate associated with CDI increased from 9 per million in 1998 to 17 per million in 2004. In January 2008, a new surveillance module for CDI started as a part of the Finnish Hospital Infection Program (SIRO). A total of 740 cases of CDI were reported from 12 hospitals. The overall rate, nosocomial rate, and prevalence at admission were 0.71 per 1,000 patient days, 0.49 per 1,000 patient days, and 0.71 per 1,000 admissions, respectively. Of the 12 hospitals with reported cases of CDI, 8 had sent isolates for genotyping. Strains of PCR ribotype 027 were detected in three of the eight hospitals (126).

In order to determine the epidemiology of CDI across Europe, the ECDC commissioned a prospective incidence survey of cases in one to seven hospitals per country (according to population size) during November 2008 (16). The study aimed to determine the baseline incidence of hospital- and community-acquired CDI, causative ribotypes, risk factors, and outcomes and to establish a network of 106 laboratories capable of isolating *C. difficile* strains in 34 European countries. The CDI incidence varied across hospitals (mean, 5.5 cases per 10,000 patient days; range, 0 to 36.3) and countries (range, 0 to 19.1).

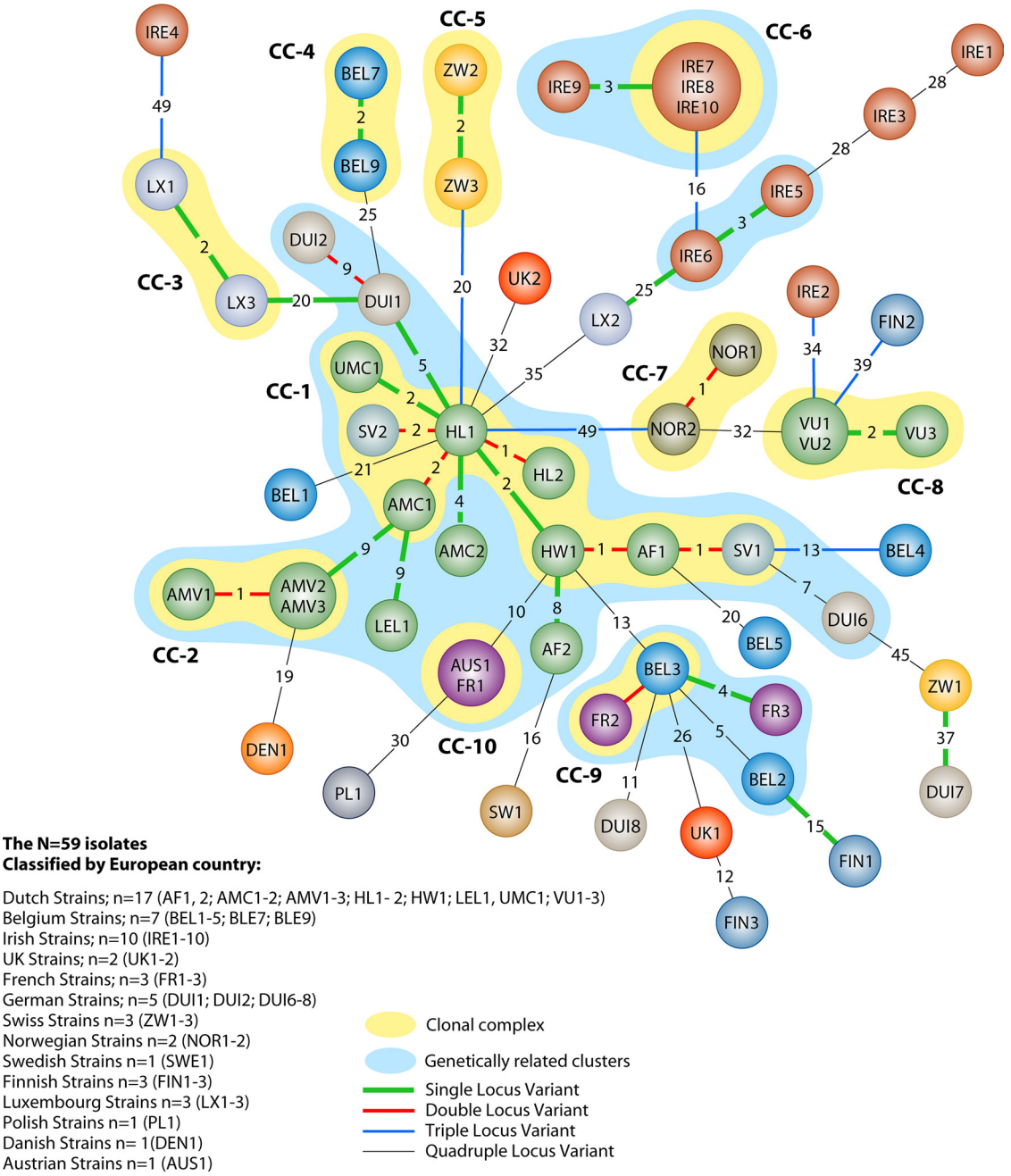

FIG. 1. Minimum-spanning-tree analysis of 59 *C. difficile* ribotype 027 isolates from 27 different regions of 14 countries typed by MLVA. Each circle (colored coded by country of origin [see key]) represents either a unique isolate or more isolates that are 100% homologous. The Dutch strains (green circles) (n = 17) are labeled by the submitting hospital plus a number. The other isolates are labeled by country and a number. The numbers between the circles represent the STRDs between MLVA types. The different-colored lines between the circles are indicative of one (green), two (red), three (blue), or four (gray) locus variants. Clindamycin-resistant isolates (n = 9) are IRE1, IRE5, IRE6, IRE8, IRE9, IRE10, FR1, BEL4, and BEL5. (Courtesy of National Reference Laboratory, Leiden, Netherlands; created by Celine Harmanus and used with permission.)

Sixty-four different *C. difficile* PCR ribotypes were found, among which ribotypes 014/020 (15%), 001 (10%), and 078 (8%) were most prevalent; the prevalence of ribotype 027 was 5%. Most patients had the classical risk profile of an elderly patient with comorbidity and recent antibiotic use. At follow-up, 22% of patients had died, and for 40% of these cases, CDI was deemed to have played a role. It was concluded that the incidence of health care-associated CDI varied widely between hospitals and countries and was generally higher than documented in 2005.

Highly discriminatory fingerprinting techniques, including restriction endonuclease analysis and MLVA, can distinguish among individual ribotypes, including ribotype 027 strains from different outbreaks (63, 101, 198). For example, MLVA

distinguished 23 types within 91 isolates of *C. difficile* ribotype 027, providing hitherto unrecognized information about related clusters of hospital cases (63). MLVA of 59 *C. difficile* ribotype 027 isolates from 27 different regions of 14 different European countries is shown in Fig. 1. To study phylogeny, minimum-spanning-tree (MST) analysis of MLVA types was performed by using the number of differing loci and the summed tandem-repeat difference (STRD) as coefficients for the genetic distance. Isolates with an STRD of ≤10 were defined as genetically related, and clonal complexes were defined by an STRD of ≤2. Of seven loci used for MLVA, two did not show variation in any of the isolates, three showed a large variation, and two remained "intermediate"-variability loci. In total, the 59 isolates clustered into 54 unique MLVA types. Four types contained more than one isolate, which were 100% identical: three types contained two isolates, and one type contained three isolates. Among the MLVA types, four genetically related clusters (GCs) and 10 clonal complexes (CCs) were recognized. The largest GC contained isolates from four different countries, two GCs were country specific (Ireland), and one GC contained isolates from Belgium and France only. Among the 10 CCs, 8 were country specific (CC-1 through CC-8), whereas two clonal complexes contained isolates from two different countries (CC-9 and CC-10). In one country, three clonal complexes (CC-1, CC-2, and CC-8) were identified. Two of these clonal complexes were region specific (CC-2 and CC-8). Of the nine clindamycin-resistant ribotype 027 strains, six (67%) originated from Ireland, and five of these strains clustered into two GCs. The four clindamycin-resistant strains that did not show clustering originated from three countries (two from Belgium and one each from Ireland and France). This analysis demonstrates the power of highly discriminatory fingerprinting techniques to provide information on isolate relatedness.

CHANGING *C. DIFFICILE* EPIDEMIOLOGY IN NORTH AMERICA

There have been well-documented changes in the incidence and severity of CDI across Canada and the United States over the last decade. A retrospective study of all cases of CDI over a 13-year period in Quebec, Canada, reported an increase in incidence from 35.6 cases per 100,000 population in 1991 to 156.3 cases per 100,000 population in 2003 (152). The proportion of infections that were complicated increased from 7.1% to 18.2% in this period, and the 30-day mortality rate increased from 4.7% to 13.8%. A later study established similar rates across the entire Sherbrooke, Canada, and Montreal, Canada, regions. The reported incidence was 22.5 cases per 1,000 admissions, which was associated with an unexpectedly high attributable mortality rate of 6.9% (121). In the United States, concurrent studies in Pittsburg, PA, established a similar epidemiological picture and reported increased incidence (2.7 cases per 1,000 discharges in 1997 to 6.8 cases per 1,000 discharges in 2001) and severity (0.15 to 0.60 cases per 1,000 discharges) of CDI (46, 139). More recently, U.S. studies have continued to report increases in CDI incidence and severity in specific institutions or states (37, 98, 190). Population-based data studies have strengthened these reports. The National Hospital Discharge Survey, representing 500 hospitals across the United States, revealed that the number of hospital discharges where CDI was listed had doubled between 1996 and 2003 (131). A 20% subset of data from the Nationwide Inpatient Sample also concluded that the incidence, case fatality rate, total mortality rate, and colectomy rate for CDI all significantly increased over the same period (159).

The increase in numbers of CDI in Canada and the United States has been attributed largely to the spread of the BI/NAP1/027 (ribotype 027) strain. Indeed, this type has now been found in all Canadian provinces and in at least 40 states in the United States (143). In Canada, the highest incidence of severe CDI and ribotype 027-associated infection has remained focused in Quebec. A 6-month prospective surveillance study (November 2004 to April 2005) covering nine Canadian provinces recently reported an overall CDI rate of 4.6 cases per 1,000 patient admissions but with a higher rate for hospitals in Quebec than for those in the rest of Canada. Additionally, CDI-attributable mortality remained more than four times higher in Quebec than that for the rest of Canada combined. A subsequent molecular analysis of a subset of *C. difficile* isolates demonstrated the presence of ribotype 027 in all Canadian provinces studied but most frequently in Quebec, Ontario, and British Columbia (79). In a study representing three different Canadian health regions from 2001 to 2004, ribotype 027 represented 75.2% of hospital isolates from the Montreal area (absent in 2000 and 2001), compared with 7.4% and 5.9% in Alberta and British Columbia, respectively. Further analysis of a subset of ribotype 027 isolates established a predominant subtype common to all three regions (127).

The evidence that ribotype 027 has been solely responsible for the increased incidence is not definitive. Studies have conflicted when comparing outcome data for patients with CDI due to ribotype 027 strains with data from those infected with other *C. difficile* types. A definitive Canadian study showed that the 30-day mortality rate for patients with ribotype 027 CDI was twice that for those infected with a toxinotype 0/ribotype 001 strain (114). Conversely, Chopra et al. recently reported no significant differences in risk factors, severity of disease, and outcome in a comparison of infections caused by BI/NAP1/027 and non-ribotype 027 strains (41). One study found that 30-day mortality was twice as likely in CDI caused by ribotype 027 but that this difference was not significant when the data were controlled for age (87). Confounding factors such as unrecognized independent risk factors, temporal changes in patient mix, and infection control practices make studies of this nature very hard to compare with confidence.

Progress in the understanding of CDI epidemiology in the United States in recent years has been slowed by the lack of standardized surveillance methods and reporting definitions. As a consequence of the increased or mandatory surveillance of CDI, guidelines and CDI case definitions have been reexamined. A study in Ohio reported that the definitions for health care-associated and community-associated CDI drawn up by the Ohio Department of Health in 2006 were discordant with those formulated locally and agreed in only 71% of cases (65). An *ad hoc C. difficile* surveillance working group reported recommended definitions for (i) health care- and community-associated disease, (ii) severity of disease, and (iii) standardized methods for reporting (132). These definitions matched those reported previously for Europe (109). Subsequent sur-

veillance definition studies have helped to consolidate these recommendations, and they have become widely adopted (58, 112). U.S. studies have continued to report increased CDI rates (33), and projected estimates for CDI in the U.S. by 2010 have reached 450,000 to 750,000 cases per annum (134, 135, 219). A recent analysis detected a 23% annual increase in CDI-related hospitalizations between 2000 and 2005, associated with an increased age-adjusted, annual case-fatality rate of 0.2% over the study period (218). However, a subsequent extension of that study reported a reduced rate of increase of CDI-associated hospitalizations in the following year (only a 6.7% rise in cases for 2005 to 2006). This was associated with a reduction in CDI-associated hospitalizations in the northeastern United States and other U.S. states (220). Decreased rates of infection associated with those states originally at the epicenter of the outbreak may signal a turning point in the pattern of spread in the United States.

Recent U.S. studies have suggested that the epidemiology of CDI in patients thought previously to be of low risk is also changing. Increased rates of infection in several peripartum women and patients from the community setting have been recorded in multiple states (35) (see below). The true extent of CDI in children remains debatable due to the increased potential for the asymptomatic carriage of *C. difficile* in this patient group (116). However, U.S. studies have recently reported increased rates of infection in this cohort, although such reports should be treated with caution because of potential ascertainment bias secondary to increased or method changes in laboratory testing. Klein et al. reported an unexpectedly high *C. difficile* culture rate (26%) for children who presented with diarrhea via a hospital emergency room between 1998 and 2001 (105). In Washington, the proportion of children with toxin-positive stool tests increased from 46% in 2001 to 64% in 2006. In that same study, severe gastrointestinal disease was seen in 91% of neonates with CDI in the intensive-care setting (21). An analysis of data from 22 hospitals across the United States reported marked increases in pediatric cases of CDI but with no increase in severity of disease or complications (103). A more recent study of all U.S. pediatric hospitalizations due to CDI (which were included in the National Hospital Discharge Survey between 1997 and 2006) reported a rate increase of 7.24 to 12.80 cases per 10,000 discharges. Incidences in newborns, nonnewborns, 1- to 4-year-old patients, and 5- to 9-year-old patients were 0.5, 32.01, 44.87, and 35.27 cases per 10,000 discharges, respectively. More studies are clearly needed to understand the true significance of *C. difficile* in children.

Data on the epidemiology of U.S. endemic and outbreak *C. difficile* strains in the last few years are fragmented. Many reports have either not included typing data at all or analyzed only a subset of implicated strains, often from only their own institutions. For example, in an investigation of an outbreak potentially associated with a switch to moxifloxacin use, only 12% (6/50) of strains were characterized. Equal numbers of toxinotype III (ribotype 027-associated) and toxinotype 0 strains were identified (23). Toxinotype 0 strains have continued to represent significant proportions of non-ribotype 027 type strains implicated in disease. This most likely represents continuing endemicity from outbreaks associated with REA type J in the 1990s (92). According to more recent studies where adequate molecular characterization has been performed, ribotype 027 is now firmly associated with hospital-associated CDI in nonoutbreak settings. A study in Michigan recently examined 30 *C. difficile* isolates from symptomatic stem cell transplant recipients and patients with and without cancer. Ribotype 027 was found in 33% patients overall. Seven different pulsotypes were found among the remaining isolates in that study (41). A recent study used REA to characterize a collection of 548 *C. difficile* isolates representing CDI cases from 45 locations in 25 U.S. states between 2006 and 2009. REA group BI (ribotype 027) represented 54% of the collection and was identified in 80% of U.S. states and 82% of individual hospitals; the isolates were submitted voluntarily and, thus, could be biased toward more severe CDI. Ribotype 027 strains were further divided into five REA subtypes, three of which (subtypes 6, 8, and 17) predominated. The next most commonly identified groups (groups Y [8%] and J [4%]) were considerably less common (38). The proportion of ribotype 027 isolates in this statewide collection is comparable to that recorded (51%) in an earlier report of a U.S. ribotype 027 outbreak in 2000 to 2003 (130). Clearly, ribotype 027 has persisted as the dominant *C. difficile* epidemic strain in the United States, although the lack of systematically collected data obscures our understanding of real-time changes and the success of control measures. Interestingly, it was recently reported that ribotype 106, hitherto known primarily as a United Kingdom epidemic strain, has been detected in North American isolates collected in 2008 to 2009; however, subtyping using REA demonstrated no overlap with strains from Europe (171).

CHANGING CDI EPIDEMIOLOGY IN THE REST OF THE WORLD

As interest in CDI has increased, we have a better appreciation of the differences in ribotype distributions in some countries. The travel and transport of patients with CDI may introduce new PCR ribotypes that can replace other types or cause new outbreaks. The first patient in Australia with CDI due to ribotype 027 was a 43-year-old woman with a permanent ileostomy who appears to have been infected while traveling in the United States (164). Unfortunately, few data were available from Australia on the incidence of CDI and the distribution of the various ribotypes before the introduction of ribotype 027. Although the incidence was high during the 1980s in hospitals in Western Australia, a significant decrease was observed, from 2.09 cases per 1,000 discharges in 1998 to 0.87 cases per 1,000 discharges in 1999 ($P < 0.0001$); this decrease persisted into 2000 and was assumed to correlate with a decreased use of broad-spectrum cephalosporins (193).

C. difficile ribotype 027 has also been detected in Japan. The first reported patient was a 30-year-old Japanese woman hospitalized for ulcerative colitis who developed pseudomembranous colitis 1 month after admission (97). In contrast to ribotype 027 strains circulating in North America and Europe, this isolate was susceptible to the newer fluoroquinolones, as was reported for those strains obtained before 2001 in North America. The epidemiological changes in PCR ribotypes in Japan are illustrated by the results of a 5-year study in a teaching hospital, where a shift was observed from predominant ribotype a in 2000 (15/33 cases [45%]) to ribotype f in 2004 (18/28 cases [64%]). Ribotype f is identical to the PCR

ribotype designated "smz," which was reported to have caused multiple outbreaks in Japan and is suspected to correspond with ribotype 001 (173). This shift was not associated with clear changes in the clinical characteristics of CDI. Another interesting finding was that of the 148 isolates examined, 33 (22%) were TcdA negative, TcdB positive, and cytotoxin negative. Most toxin A-negative, toxin B-positive (A^- B^+) *C. difficile* isolates belong to ribotype 017 (196), which is found more frequently in Asia than in some other continents. The clinical manifestations of and risk factors for CDI caused by ribotype 017 were retrospectively investigated in a small study in a Japanese hospital (106). Three factors were found to be associated with infection by A^- B^+ isolates: exposure to antineoplastic agents, the use of nasal feeding tubes, and care in a particular medical ward. There was no statistically significant difference in temperature, serum C-reactive protein (CRP) levels, white cell count, frequency of diarrhea, or type of underlying disease in cases compared with toxin A-positive toxin B-positive (A^+ B^+) patients (106).

A recent shift in the distribution of various *C. difficile* PCR ribotypes has also been observed in South Korea. Although the prevalence of A^- B^+ *C. difficile* strains in six hospitals was <5% before 2000, it began to increase in 2003 (13%) and peaked in 2004 (50%). In 2005, the mean prevalences of A^+ B^+ and A^- B^+ strains were 48% (31 to 60%) and 27% (11 to 55%), respectively (175). Among 29 cases of pseudomembranous colitis, 21 (72%) were associated with A^- B^+ strains; the authors of that study concluded that these variant strains could evoke a higher rate of PMC than A^+ B^+ strains (176), but this observation has not been confirmed by others. Kim et al. reported a similar increased prevalence pf A^- B^+ strains in South Korea (102). The first case of CDI due to ribotype 027 in South Korea was recently described (188).

Since the first reports on emerging CDI associated with ribotype 027 appeared in the literature, several laboratories and hospitals in China initiated surveillance studies. In a collection of 75 clinical isolates from Shanghai, China, 33% were ribotype 017 A^- B^+ strains, but no ribotype 027 strains were present (86). The detection of *C. difficile* ribotype 027 in Hong Kong was recently reported for a case of locally acquired health care-associated CDI; no secondary cases occurred (39). From June to December 2008, the Chinese Center for Disease Control performed a survey among hospitalized patients who were older than 18 years and who developed diarrhea. Of 112 fecal samples, 12 (11%) contained *C. difficile*, 8 of which were toxinogenic, including 3 A^- B^+ strains, but ribotype 027 was not found (40; E. Kuijper, personal communication).

Only a few reports of CDI in Latin America are available. A recent literature review using medical databases of Latin American countries identified seven recent papers in which clinical characteristics and risk factors were analyzed (32). The attributable mortality rate was lower than that reported for hospitals in other developed countries, but there is clearly a need for better prospective studies to determine the epidemiology of CDI in more detail. In a recent study in Brazil, *C. difficile* strains from fecal and hospital environmental samples were characterized. Although ribotype 027 was not reported, strains belonging to ribotype 106 were detected; this appears to be the first report of this ribotype outside the United Kingdom (11). A recent survey of a 200-bed Argentinean general hospital revealed CDI incidence rates of 37 to 84 cases per 10,000 admissions between 2000 and 2005 (78). The increased incidence prompted the initiation of systematic surveillance, which showed a high prevalence of ribotype 017. Interestingly, when multiple-locus variable-number tandem-repeat analysis (MLVA) was applied to 56 Argentinean isolates and 15 isolates from seven other countries, country-specific clonal complexes were found. This finding was in contrast with data from a previous report in which 39 A^- B^+ isolates from seven countries were investigated by use of amplified fragment length polymorphism (AFLP) and two PCR ribotyping methods. It was concluded that clindamycin-resistant ribotype 017 had a clonal worldwide spread, but this conclusion was not supported by data obtained by using the highly discriminatory MLVA technique (197).

No recent information on CDI prevalence rates or microbiological characteristics of *C. difficile* strains isolated from humans is available for Africa. CDI data from Middle East countries are sparse. *C. difficile* isolates ($n = 113$) from patients and the environment of intensive therapy units (ITUs) of four teaching hospitals in Kuwait were of 32 different ribotypes. The predominant ribotypes of clinical isolates were ribotypes 097 and 078, and eight strains did not match any strains in the PCR ribotype library established at the Anaerobe Reference Unit, Cardiff, United Kingdom (167). A recent prospective study among hospitalized adult Jordanian patients did not provide data on CDI incidence or strain characteristics (141).

COMMUNITY-ACQUIRED CDI

Although *C. difficile* is a ubiquitous microorganism that may be found in the environment, animals, and food, its prevalence is thought to be so much higher in health care facilities that, for a long time, the acquisition of CDI was thought to occur almost exclusively during or shortly after admission to such establishments. The high incidence of CDI in health care facilities compared with the community presumably results from the high density of individuals prone to CDI, classically, elderly patients with comorbidity, who may serve as a reservoir in which *C. difficile* can amplify. However, it is increasingly being recognized that some CDI cases are acquired outside health care facilities. Several studies have shown various proportions of CDI cases to be "community acquired," but crucially, the definitions of community acquisition differ. Some studies did not systematically examine previous admissions to health care facilities (120, 161, 162, 163, 210). Others variably defined an "exclusion" period to distinguish patients recently admitted to a health care facility.

An *ad hoc C. difficile* surveillance working group (109, 132) advocated a division of CDI into health care-associated and community-associated CDI. The latter is defined as CDI with onset in the community (or in a health care facility within 48 h after admission) for a patient who has not been discharged from a health care facility in the previous 12 weeks. If the patient was discharged 4 to 12 weeks previously, such a case should be classified as having an indeterminate association. A further difficulty concerning studies of community-acquired CDI is patient selection. The studied patient population may strongly influence the extrapolated incidence in the community. An estimation of incidences may also be hampered by the uncertainty of the denominator size, i.e., the population from

which stool samples were collected (for example, a laboratory's community catchment area). The issue of the important effect of diagnostic methods on reported CDI epidemiology is discussed in the Introduction. Poor assay specificity will result in overestimates of CDI cases, particularly in low-incidence settings such as the community. This may be compounded if other enteropathogens are not ruled out systematically as potential causes of community-associated diarrhea.

In 1995, Karlström et al. (96) conducted a nationwide survey of CDI in Sweden. Among 1,888 CDI cases, defined as unique patients with toxin-positive fecal samples from 13 of Sweden's 31 microbiological laboratories, 28% were reported as community acquired, defined as community onset without hospitalization in the preceding 4 weeks. Patients with community-acquired CDI were younger than patients with health care-associated CDI. A study in Ireland reported that 11% of 73 new CDI cases (diarrhea with positive stool cytotoxicity test) were community acquired, defined as occurring upon or within 72 h of admission without hospitalization in the previous 60 days (113). In 1999, a Swedish study (142) investigating all cytotoxin-positive diarrheal stool samples submitted to three major county hospitals found 22% of 267 cases of first episodes of CDI to be community acquired (without hospitalization in the previous 60 days). Again, patients with community-acquired CDI were younger than their counterparts with nosocomial CDI. Similar findings were confirmed by another Swedish study (187). A hospital-based study conducted in 2005 in the Netherlands (147) found 20% of 81 CDI cases (diarrhea and toxin-positive stool) to be cases of community-onset (without hospitalization in the preceding 4 weeks) CDI. After the implementation of a statewide surveillance system in Connecticut in 2006, 60% of 400 evaluable community-onset CDI cases (gastrointestinal symptoms and a positive toxin assay) reported that year by acute-care hospitals met the definition of community acquisition (no admissions to health care facilities in the previous 3 months) (36). A recent European surveillance study of 29 European countries used the above-described *C. difficile* surveillance working group definitions and found that the ratio of community-associated CDI and health care-associated CDI varied among participating hospitals, although it was mostly low, with only 8/83 hospitals reporting more community- than health care-associated cases (17).

A study based on data from the United Kingdom General Practice Research Database (52) evaluated the incidence of community-acquired CDI (defined as patients with toxin-positive feces or a clinical diagnosis of CDI who had not been hospitalized the previous year, divided by the number of inhabitants registered with general practitioners providing data for this database). The incidence increased from 0 to 18 cases per 100,000 persons per year between 1994 and 2004. Dial et al. (52) found that inflammatory bowel disease (relative risk [RR], 4.1; 95% confidence interval [CI], 2.6 to 6.6), irritable bowel syndrome (RR, 3.4; 95% CI, 2.3 to 5.0), hospitalizations in the previous 2 years (RR, 2.2; 95% CI, 2.0 to 2.4), and renal failure (RR, 1.7; 95% CI, 1.2 to 2.2), and the use of proton pump inhibitors (PPI) (RR, 1.6; 95% CI, 1.3 to 2.0), but not histaminic receptor-blocking gastric acid suppressants, were significantly associated with CDI. Wilcox et al. estimated the incidences of community-associated CDI in 2000 in two distinct settings in England, one urban (29.5 cases per 100,000 population) and the other rural (20.2 cases per 100,000 population), by examining fecal samples ($n = 2{,}000$) submitted by general practitioners from individuals with diarrhea in the community (214). In both settings, 2.1% of samples were cytotoxin positive. Exposure to antibiotics in the previous 4 weeks, particularly multiple agents ($P < 0.001$), aminopenicillins ($P < 0.05$), and oral cephalosporins ($P < 0.05$), was significantly more frequent among CDI cases than among age- and sex-matched cytotoxin-negative diarrheal controls from the same general practices. Notably, hospitalization in the preceding 6 months was significantly associated with CDI (45% versus 23%; $P = 0.02$). However, almost half of the cases had not received antibiotic therapy in the previous month, and approximately one-third had neither exposure to antibiotics nor recent hospitalization. Interestingly, contact with infants aged <2 years was significantly associated with CDI (14% versus 2%; $P = 0.02$). Weil and coworkers (206) also examined fecal samples for evidence of CDI irrespective of the requested diagnostic test but used a toxin EIA (and therefore, the specificity of the results is questionable). Those researchers found that 9.2% of 703 stool samples from individual patients were EIA positive; 53% of these patients had not been hospitalized in the previous 4 weeks. A recent large study in the Netherlands (17) examined all fecal samples submitted to three regional microbiological laboratories by using a toxin EIA. The proportion of positive fecal samples (1.5% of 2,443 samples) was remarkably similar to that seen in the English study (214). Among those patients with toxin-positive stool samples for whom information was available, 20 (65%) had not been admitted to a health care institution in the previous year, 13 (42%) had not used antibiotics during the 6 months previous, and 8 (26%) had neither risk factor. In the English study, the most common bacterial enteropathogens were ruled out, while the Dutch study examined only those pathogens originally requested by the general practitioner. Thus, these studies demonstrate a striking difference in studies on nosocomial CDI, which usually show that the great majority of patients have received antibiotics. The studies allow more reliable estimates of community-associated CDI to be made, but selection bias may remain. For instance, it is possible that only more prolonged or severe cases led to sample submission. Evidence of this phenomenon comes from a French study (19) in which ambulatory patients who were prescribed an antibiotic by their general practitioner for the first time in at least 2 months and who had not been hospitalized in the previous 6 months were monitored prospectively to examine the development of CDI. Of 260 evaluable patients, 1 was an asymptomatic carrier at baseline and remained so during 14 days of follow-up; 7 patients acquired a toxigenic *C. difficile* strain, and 4 of these patients developed diarrhea, which was self-limiting in all cases.

If the incidence of community-acquired CDI is indeed increasing and rising awareness does not account for this increase, then what could be the source of exposure in the community? Of course, it can be argued that increased pressure from hospitals could propagate the increased circulation of strains in the community. However, in the Dutch study, 29 isolates underwent PCR ribotyping, revealing many uncommon ribotypes, and notably, ribotype 027 was not found (despite this ribotype having recently caused major nosocomial

outbreaks in all regions investigated), which might argue against increased pressure from hospitals (17). In contrast, two earlier Swedish studies (142, 187) found similar distributions of PCR ribotypes among nosocomial and community-acquired cases. Animals are obvious candidates for a community reservoir, since *C. difficile* may be a commensal and a pathogen in animals, and farm animals are often exposed to antibiotics. Spores might be spread either by direct contact with animals and their feces or by the consumption of contaminated meat. Several studies, which will be discussed below, have demonstrated the presence of *C. difficile* in animal feces and meat. Evidence for farm animals as a potential reservoir for *C. difficile* may come from epidemiological associations and typing studies. In the Dutch study (17), the proportion of positive stool samples was highest in more rural communities, but here and in an earlier Australian study (162), there were no patients who reported contact with farm animals. However, in a more rural part of the Netherlands, 4 out of 10 CDI cases in which ribotyping was performed were caused by ribotype 078, whereas this ribotype was found in neither of the other regions. This ribotype is frequently found in farm animals and has been associated with community-associated CDI in another Dutch study (77). Multilocus variable-number tandem-repeat analysis of ribotype 078 isolates in that study revealed four clonal complexes to which both porcine and human strains belonged. Epidemiological data on *C. difficile* in animals and food is discussed in more detail below (see New Information on Etiology of and Risk Factors for CDI).

NEW INFORMATION ON ETIOLOGY OF AND RISK FACTORS FOR CDI

Gastric Acid Suppressants

The use of gastric acid suppressants, especially PPI, has been proposed as a risk factor for acquiring CDI. Theoretically, these PPI could decrease the colonization barrier against vegetative forms of *C. difficile* by elevating the gastric pH. However, *C. difficile* spores are resistant to gastric acid, and it is likely that spores represent the main mode of acquisition. A large number of studies have associated the use of gastric acid suppressants with the acquisition of CDI. A systematic review (119) found the use of H2 receptor antagonists (H2RA) and PPI to be significantly associated with having CDI (pooled ORs, 1.48 [95% CI, 1.06 to 2.06] and 2.05 [95% CI, 1.47 to 2.85], respectively), but the included studies were very heterogeneous. Notably, most studies have investigated such associations in the setting of a CDI epidemic in the hospital, which may not be a reliable setting given the potential for confounding variables. Two studies examined the association of PPI with CDI in a hospital setting where the disease is endemic. Dalton and colleagues (47) found several variables, including 10 drug classes apart from antibiotics, to be associated with the development of CDI in a univariate analysis. A logistic regression model that included the use of antidepressants, number of medicines used, days of antibiotic use, age of patient, length of hospital stay, and admission to a medical ward versus a surgical ward was constructed. The odds of CDI increased by 1.01 (95% CI, 1.00 to 1.02) for each day of PPI use; H2RA use increased the odds by 1.7 (95% CI, 1.09 to 2.64). A study by Dubberke et al. (55) found significant ORs of 1.6 and 2.0 for PPI and H2RA, respectively, using a logistic regression model that incorporated previous admissions, age of patient, hematological malignancy, albumin level, mechanical ventilation, several antibiotics, and "*C. difficile*-associated diarrhea (CDAD) pressure" (exposure to other CDI cases), a variable that has been the subject of some debate and will be discussed elsewhere. Concerning community-acquired CDI, PPI, but not H2RA, was found to be a risk factor in one study, as discussed previously (53), but this was not found by two other studies of patients with community-acquired CDI (122, 214). Some studies have examined the prognostic significance of gastric acid suppression in CDI. H2RA have been associated with a higher chance of recurrence (189), and PPI have been associated with refractory CDI (1). However, a recent study found no association between either group of gastric acid suppressants and a more severe course of CDI (84).

In spite of the extent of the literature, it is still a matter of ongoing debate whether there is a causal association between PPI or H2RA and CDI. The main reason for this is the fact that gastric suppressants may be given frequently to patients with certain comorbidities as a prophylaxis (e.g., those who use anticoagulants or nonsteroidal anti-inflammatory drugs [NSAIDs] and severely ill patients in intensive-care units [ICUs]). These factors may themselves be risk factors for or associated with other risk factors for CDI. Hence, it may be extremely difficult, if not impossible, to correct for these confounders in case-control studies. The only definitive answer might come from a randomized controlled trial that systematically evaluates both colonization with *C. difficile* and the development of CDI in patients taking gastric acid suppressants.

Animals and Foods as Potential Sources of *C. difficile*

C. difficile is ubiquitous in the environment. In a large and classical study of the distribution of *C. difficile* in the environment of South Wales, United Kingdom, a total of 2,580 samples were taken, with 184 (7.1%) yielding isolates (2). The highest yield of *C. difficile* was obtained from river (87.5%) and seawater (44%) samples, but it was also isolated from swimming pools (50%) and main tap water (5.5%). In private residences, the organism was present in 12 (2.2%) of 550 samples, whereas 2.4% of 300 raw-vegetable samples were positive. The rate of carriage of *C. difficile* in 524 fecal samples from assorted farm animals was 1%, while rates were 10% in dogs and 2% in cats. *C. difficile* has also been found in calves, ostriches, chickens, elephants, dogs, horses, and pigs, but its role in infection and its pathogenesis in animals are largely poorly understood and possibly underestimated (168). Similarly, any association between antibiotic usage and *C. difficile* colonization or diarrhea in animals is less well documented than that in humans, although the acquisition of *C. difficile* in dogs and cats during hospitalization in an ICU was associated with the development of diarrhea (43). In an experimental study, it was also demonstrated that erythromycin can induce severe colitis associated with the proliferation of *C. difficile* in mature horses (18).

Several *C. difficile* ribotypes have been found in animals, although the heterogenicity appears to be much less than that found for human CDI (168). Several indistinguishable strains may be found in multiple species (6), which suggests that there

is a potential for the interspecies transmission of *C. difficile*. *C. difficile* ribotype 027 is not an emerging pathogen of disease in animals, since no documented outbreaks have been reported, and only occasional isolates have been described (181). However, *C. difficile* is known as an important pathogen of horses, and it has been reported to infect piglets and calves. In a Canadian study of calves from 102 dairy farms, fecal samples from 144 and 134 diarrhea and control animals, respectively, were cultured for *C. difficile* and tested by using an EIA for *C. difficile* toxins A and B. Toxins were detected in 40% of calves with diarrhea but also in 21% of healthy controls. Eight different ribotypes were found, seven of which have been shown to cause CDI in humans, including outbreaks of severe disease (ribotypes 017 and 027) (165). A search for *C. difficile* in various animals in the United Kingdom examined neonatal pigs, male dairy calves (<2 months of age), horses, and dogs. The 232 isolates obtained comprised 19 ribotypes, with the predominant one being ribotype 078 in calves and pigs (99). Recently, two herds of piglets suffering from diarrhea in the Netherlands proved to be excreting *C. difficile* toxinotype V, PCR ribotype 078 (49). Thirty-one *C. difficile* isolates were obtained from 48 animals. *C. difficile* was not detected in mother sows or in 272 healthy, weaned piglets on seven farms. None of the farm workers and none of the family members of the farm owners had CDI. *C. difficile* ribotype 078 isolates from humans and pigs were highly genetically related; as shown by MLVA, all the isolates contained a 39-bp *tcdC* deletion and a point mutation at position 184, and most produced binary toxin. These findings suggest a common source and/or that recycling is common. Jhung et al. (91) also found that food animal and human isolates had a high degree of similarity, as determined by REA and pulsed-field gel electrophoresis (PFGE) subtyping. While ribotype 078 appears to be a pathogen in some piglets, a study of *C. difficile* in Spain including 780 animals from 13 pig farms found that 26% of newborn piglets but no 1- to 2-month-old piglets were *C. difficile* positive, irrespective of the presence of diarrhea (4). Similar findings were reported for piglet cohorts in a recent study in England, where despite the frequent presence of *C. difficile* ribotype 078, no animals had overt evidence of infection (L. Brunton, J. S. Knapp, J. Heritage, S. D. Baines, J. Freeman, W. N. Fawley, M. H. Wilcox, and H. M. Miller, submitted for publication). A recent study performed in Canada evaluated *C. difficile* colonization in dogs and cats in a longitudinal manner and found that *C. difficile* is commonly, albeit sporadically, found in the feces of healthy pets. *C. difficile* was isolated from 14/139 (10%) dogs and 3/14 (21%) cats. Thirteen (93%) of the dogs and all of the cats that tested positive were positive only by one of the five samples. All toxigenic strains identified in pets have been isolated from humans in Ontario, Canada. Although the prevalence of *C. difficile* in dogs and cats is low, the fact that all toxigenic strains are recognized human pathogens raises concern about interspecies transmission (205).

Spores of *C. difficile* can also be detected in meat products and ready-to-eat salads (10, 182, 201, 204), but there is no conclusive evidence that *C. difficile* contamination of food has led to clinical CDI in humans. Additionally, no food-borne disease outbreaks of *C. difficile* have been reported. In a recent Canadian study, *C. difficile* was isolated from 14/115 (12%) ground-beef samples and 14/115 (12%) ground-pork samples (204). Ribotype 078 predominated, consistent with data from some previous studies of *C. difficile* in food animals. Of cooked and uncooked meat products sold in Arizona, 42% were *C. difficile* positive, with toxigenic strains (ribotype 078, 73%; ribotype 027, 27%) (182). Following the emergence of ribotype 078 in humans, a pilot study was carried out in the Netherlands by the Food and Consumer Product Safety Authority to determine the occurrence of *C. difficile* in 500 consumable-meat samples from calves, pigs, sheep, turkeys, and chickens. The only positive samples were four samples from chicken (ribotypes 001, 003 [two samples], and 087). Similarly, of 82 meat samples collected from randomly selected retail shops in Sweden, *C. difficile* was isolated from only 2 (2.4%) (201). These results are clearly different from experiences reported for the United States and Canada. Notably, recent data from Canada show a very low prevalence of *C. difficile* (204), and a recent report suggested possible seasonal variation, with the highest prevalence in winter (166). It is clear that the epidemiology and significance of *C. difficile* in animals and food, and notably any implications for human CDI, require further investigation.

Antibiotic Risk Factor and Prescribing Intervention Studies

Prior exposure to antimicrobials is the main risk factor for CDI development. In general, expanded-spectrum and broad-spectrum cephalosporins and clindamycin are the most frequently implicated antibiotics in CDI, whereas the role of fluoroquinolones is less clear. However, the implication of almost every antibiotic in CDI development reflects widespread use and also the difficulty in ascribing the relative risk of CDI to specific antibiotics. The retrospective nature of many studies combined with noted risk factors such as polypharmacy, number of doses, and treatment duration (22, 29, 72, 133, 192, 194, 213, 215) may lead to interpretive problems. Crucially, exposure to *C. difficile*, which may be affected not only by where the patient is managed (including the relation of the place to that of other CDI cases) but also by the duration of hospital stay and infection control interventions, is also frequently overlooked when conclusions are drawn. The effects of exposure to *C. difficile* may well be exaggerated in the outbreak setting, which is typically when data on antimicrobial prescribing and CDI rates are collected. For all these reasons, odds ratios for the risk of CDI with individual antibiotics should be treated with caution (Table 1). Given the many potential confounding factors when determining CDI risk, it is not surprising that the use of antibiotics measured at the hospital level may not correlate with the likelihood of occurrence of CDI outbreaks (198, 208). When reviewing antibiotic risk data, it is important to distinguish between studies that identify apparent antibiotic risk factors for CDI and those where antimicrobial intervention strategies, typically comprising restrictive prescribing, have been employed to reduce the rate of infection.

C. difficile ribotype 027 strains appear to have acquired resistance to newer fluoroquinolones (moxifloxacin, levofloxacin, and gatifloxacin) by mutations in GyrA and GyrB, as historical (1980s to 1990s) *C. difficile* ribotype 027 isolates are susceptible to these antibiotics (130). Fluoroquinolone resistance can also be induced *in vitro* (183), and increased MICs of levofloxacin and/or moxifloxacin are associated with specific substitutions in GyrA and/or GyrB (183). It is plausible that the acquisition of

TABLE 1. Studies examining possible associations between fluoroquinolones and CDI[a]

Study (reference)	Study yr	Setting	*C. difficile* ribotype involved	Quinolone(s)	Study design (no. of positive cases/ total no. of cases)	Logistic regression analysis result(s)	Reported relative contribution to CDI cases (%)
Yip et al. (217)	1998	300-bed U.S. tertiary-care hospital	Unknown	Ciprofloxacin	Retrospective case-control study (27/54)	Ciprofloxacin OR = 9.5 Cephalosporin OR = 6.7	
McCusker et al. (129)	2001	778 beds, Veterans Affairs hospital, Baltimore, MD	Unknown	Levofloxacin, ciprofloxacin, gatifloxacin	Retrospective case-control study (30/60)	Fluoroquinolone OR = 12.7 Cephalosporin OR = 0.4 Clindamycin OR = 2.2	
Gaynes et al. (71)	2002	173-bed acute-care U.S. hospital	Unknown	Switch levofloxacin to gatifloxacin	Retrospective case-control study (37/59)	Clindamycin and increased duration of gatifloxacin therapy	
Muto et al. (139)	2000–2001	Pittsburgh, PA	Polyclonal	Levofloxacin	Retrospective case-control study (203/203)	Levofloxacin OR = 2.0 Ceftriaxone OR = 5.4 Clindamycin OR = 4.8	31 6.7 10
Loo et al. (121)	2004	12 hospitals in Quebec (8 university and 4 community)	027	Ciprofloxacin, gatifloxacin, moxifloxacin	Prospective matched case-control study (237/237)	Fluoroquinolone OR = 3.9 Cephalosporin OR = 3.8	
Pepin et al. (154)	2003–2004	Teaching hospital, Sherbrook, Canada	027	Ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin	Retrospective cohort (293/5,619)	Fluoroquinolone adjusted hazard ratio = 3.44 Cephalosporin adjusted hazard ratio = 1.56-1.89 Clindamycin adjusted hazard ratio = 1.77	35.9 10 1.5
Kazakova et al. (98)	2002–2003	Community acute-care hospital in Maine	027	Levofloxacin, ciprofloxacin	Matched case-control study (68/127)	Fluoroquinolone OR = 3.22 Cephalosporin OR = 5.19	
McFarland et al. (134)	2004	400-bed Veterans Affairs hospital, Seattle, WA	Unknown	Gatifloxacin	Retrospective matched case-control study (184/184)	Clindamycin OR = 29.9 Penicillin OR = 4.1	
Biller et al. (23)	2003	320, acute-care, nonteaching U.S. hospital	027	Switch levofloxacin to moxifloxacin	Matched case-control study (50/100)	Moxifloxacin OR = 3.14 Cephalosporin OR = NS Clindamycin OR = NS	
Weiss et al. (207)	2007	Two 600-bed tertiary-care hospitals	027	Ciprofloxacin, levofloxacin, moxifloxacin	Case-control study (15/31)	Fluoroquinolone OR = 36.2 Cephalosporin OR = 19.1	
Debast et al. (50)	2005	341-bed community hospital	027	Ciprofloxacin	Prospective case-control study (45/90), including extra control group with non-CDI diarrhea (109)	Fluoroquinolone OR = 28.8 Cephalosporin OR = 7.8 Combination OR = 57.5	33 56

[a] NS, not significant.

fluoroquinolone resistance contributed to the increased spread of *C. difficile* ribotype 027 and possibly other *C. difficile* strains via selection pressure. Loo et al. (121) conducted a prospective case-control study in 12 Quebec, Canada, hospitals and found that CDI patients were more likely to have received fluoroquinolones (OR, 3.9; 95% CI, 2.3 to 6.6) or cephalosporins (OR, 3.8; 95% CI, 2.2 to 6.6) (121). Associations between CDI and fluoroquinolones, including ciprofloxacin, were described previously (31), but the emergence of ribotype 027 resulted in a number of new studies (summarized in Table 1). The odds ratio for fluoroquinolones calculated from the 11 studies shown in Table 1 was 12.8, whereas the odds ratio for cephalosporins was 5.1. *C. difficile* ribotype 027 was involved in six of the studies. No association with a specific fluoroquinolone was found, but most studies did not perform this analysis. A recent prospective case-control study reported a high risk of CDI due to ribotype 027 in patients who received a combination of a cephalosporin and a fluoroquinolone (OR, 57.5; 95% CI, 6.8 to 483.6) (50). The relative contribution of an antibiotic or class to causing CDI may be assessed by determining the proportion of cases that were attributable to the use of specific agents (i.e., the etiological fractions). However, as depicted in Table 1, only 3/11 studies provided such data, and therefore, the magnitude of the effect of specific antibiotics in selecting for *C. difficile* or in inducing CDI remains unclear. Pepin et al. found that long-duration fluoroquinolone therapy enhanced the risk of CDI (154). A recent case-control study also reported that ciprofloxacin usage for >7 days was a significant risk factor for CDI (adjusted OR, 3.72; 95% CI, 1.38 to 10.02; $P = 0.019$) (186).

The restricted use of certain high-risk antimicrobials is a commonly used strategy for reducing CDI rates. Antibiotic prescribing intervention studies have been used to examine prospectively the effects of a prescribing change upon CDI rates, although there are relatively few of these studies. A 2005 review of interventions to improve antibiotic prescribing practices found five studies designed to assess the impact of prescribing changes upon rates of CDI (48). Four studies showed that prescribing changes were associated with significant decreases in CDI rates, while the remaining study demonstrated a trend toward decreased CDI rates (34, 42, 100, 136, 148). Two studies examined the effect of restricting cephalosporin prescribing in planned interventions. Khan and Cheesbrough described a financially driven change in prescribing policy from cefotaxime to ceftriaxone, which was associated with an increase in CDI rates. A subsequent restriction of ceftriaxone use produced a modest decrease in CDI cases. However, a reduction in the proportion of *C. difficile* toxin-positive samples from 14.5 to 8.6% was observed when levofloxacin was substituted for ceftriaxone (100). Similarly, Carling et al. described the implementation of an antibiotic management program designed to minimize the inappropriate prescribing of broad-spectrum cephalosporins and reported sustained reduced CDI rates (34). The introduction of a low-risk antimicrobial to a formulary may not in itself produce a reduction in CDI rates. Wilcox et al. (212) described how the number of CDI cases did not decrease significantly after the introduction of piperacillin-tazobactam to a medicine formulary for the elderly until its use was accompanied by the restriction of cefotaxime. Numbers of CDI cases then decreased by 52% ($P < 0.008$). That study and one other study noted that increased cephalosporin prescribing in response to a shortage of piperacillin-tazobactam was associated with increases in CDI rates (3, 212). Those studies underline the necessity of removing or restricting the use of high-risk antibiotics as opposed to simply adding lower-risk agents to the formulary (212).

Similar methodological issues have affected studies performed since the emergence of *C. difficile* ribotype 027. Gaynes et al. described a CDI outbreak in a long-term-care facility caused by this strain that was apparently precipitated by a formulary switch from levofloxacin to gatifloxacin, with a decline in infection after reversion to levofloxacin treatment (71). In contrast, Biller et al. found that an outbreak apparently precipitated by a formulary change from levofloxacin to moxifloxacin was not controlled by reversion to levofloxacin (23). However, both studies described the implementation of infection control measures during the study period, which may have affected *C. difficile* exposure among patients and, therefore, CDI rates. McFarland et al. found that an increase in numbers of CDI cases thought to have been associated with a formulary change from levofloxacin to gatifloxacin was in line with seasonal variations in CDI rates (134). Interestingly, although fluoroquinolones had been identified retrospectively as a significant risk factor during a large outbreak of CDI in Quebec, Canada (155), CDI rates subsequently decreased in association with the antimicrobial restriction of narrow-spectrum (−21%), expanded-spectrum (−93%), and broad-spectrum (−79%) cephalosporins; clindamycin (−87%); macrolides (−78%); and ciprofloxacin (−29%); however, prescribing of respiratory fluoroquinolones (predominantly moxifloxacin) and piperacillin-tazobactam increased by +79% and +114%, respectively (195). It has been suggested that the combined implementation of improved infection control measures and judicious antimicrobial use, as opposed to the restriction of a specific antimicrobial, was responsible for eventually controlling this outbreak (20, 208). A recent interesting study examined the risk factors for CDI in cases due to both epidemic (*C. difficile* NAP1) and nonepidemic strains (95). CDI cases due to the epidemic strain (but not nonepidemic strains) were more likely than control patients to have received a fluoroquinolone upon univariate analysis. However, fluoroquinolone use was not significantly associated with CDI in a multivariable analysis. Also, while the outbreak was controlled after the complete restriction of fluoroquinolone prescribing, other factors, including a change in environmental-services contractors, may have played a key role. Thus, these contradictory data mean that it remains unclear what is the true contribution of fluoroquinolones (in the absence of other risk factors) to outbreaks of CDI caused by ribotype 027.

Prior antimicrobial therapy is a major risk factor for CDI because of the selection pressure for *C. difficile* strains and/or the induction of toxin production. Reports continue to emphasize the importance of multifaceted approaches (i.e., antimicrobial stewardship and enhanced infection control procedures) to the successful control of CDI outbreaks (50, 140, 209).

Alcohol-Based Hand Hygiene and Risk of CDI

C. difficile, like all sporulating bacteria, may initiate a conversion from metabolically active vegetative cells to dormant endospores when environmental conditions become adverse.

Sporulation may occur potentially in response to nutrient limitation, exposure to antimicrobial disinfectants, or therapeutic antimicrobial agents (62, 67, 180, 211). The transmission of *C. difficile* in the nosocomial environment is believed to be due primarily to the ingestion of spores, which may have been acquired from other patients directly via the hands of health care workers or indirectly from inanimate objects. Thus, hand hygiene practices targeted at minimizing health care-associated infections (HCAI) are complicated by the intrinsic resistance of *C. difficile* spores to many disinfectants. Current World Health Organization (WHO) guidelines on hand hygiene (156) recommend a two-pronged approach to hand disinfection: (i) if exposure to sporulating pathogens is suspected, hand washing with soap and water is preferred, and (ii) in all other clinical situations, if hands are not visibly soiled, alcohol-based hand rubs (ABHR) are preferred for routine hand antisepsis (156). ABHR application is faster and easier than washing with soap and water and is easily accessible at the point of care; therefore, compliance with hand hygiene measures are potentially improved with this approach. Unfortunately, if patients are culture positive for *C. difficile* but asymptomatic, the implementation of the appropriate WHO hand hygiene strategy by health care workers is not straightforward. In this context, it has been shown, at least in the setting of a long-term-care facility affected by *C. difficile* ribotype 027, that skin and associated environmental contamination by the bacterium is commonly seen in asymptomatic carriers (160).

Given the inherent lack of activity of alcohol on spores, some have speculated that the enhanced use of ABHR may facilitate the transmission of *C. difficile* (15, 74). However, there are no robust data in the literature to support this hypothesis. Oughton and colleagues (145) performed an *in vitro* study using 10 volunteers whose hands were inoculated with a standard culture of a nontoxigenic *C. difficile* strain (1.4×10^5 CFU/ml, comprising 62% spore forms of the organism). Volunteers' hands were subsequently assigned randomly to one of five hand hygiene interventions, including an ABHR containing 70% (vol/vol) isopropanol, and *C. difficile* viable counts from hand washings were determined. The ABHR intervention was ineffective at reducing *C. difficile* counts compared with several of the other hand hygiene interventions (145). Reductions in viable counts of *C. difficile* spores have been demonstrated following ABHR application in laboratory studies (118). Leischner et al. evaluated the efficacies of chlorhexidine soap and water and three ABHR in removing *C. difficile* spores from artificially contaminated hands of volunteers. ABHR were less effective than chlorhexidine soap and water in reducing the *C. difficile* spore burden, but a decline in viable counts of 1.7 to 1.9 $\log_{10}$ CFU/ml was observed.

Despite the poor activity of ABHR against *C. difficile* spores in volunteer studies and their extensive implementation in the clinical setting, there are no convincing *in vivo* data to support the hypothesis of a resultant increased incidence of CDI. King (104) reported the results of a 3-month pilot study where ABHR were implemented in a 28-bed acute-care hospital ward. Interestingly, the *C. difficile* incidence increased following ABHR implementation (five new cases compared to zero cases in the preceding 12 months), but that study was limited to one small ward in a single institution and, therefore, was insufficiently powered to allow firm conclusions. Several more recent and larger *in vivo* studies evaluating CDI incidence following ABHR implementation have been performed (26, 94, 170, 200, 184, 199). Stone and colleagues reported the results of the first national hand hygiene campaign ("Clean Your Hands" campaign [CYHC]) involving 187 United Kingdom hospitals (185). The procurement of soap and ABHR and hospital bed occupancy data were monitored in relation to quarterly mandatory reports of methicillin-resistant *Staphylococcus aureus* (MRSA)/methicillin-sensitive *S. aureus* (MSSA) bacteremia and CDI. Interventions significantly associated with reduced CDI incidence were hand washing with soap and water and visitation of a Department of Health improvement team. The CDI incidence did not increase despite increased ABHR usage (185). In a recent study, Kaier et al. built a multivariate regression model to identify the dynamic relationship between antibiotic exposure, ABHR, and the incidence of MRSA and CDI (94). The increased usage of ABHR over the 44-month study period negatively impacted MRSA incidence but did not correlate (positively or negatively) with CDI incidence (94). ABHR are convenient for both health care workers and visitors and have been clearly proven to be efficacious in enhancing hand hygiene compliance and in reducing incidences of nosocomial infections. At an institutional level, the benefit obtained from increased hand hygiene compliance likely exceeds any risks related to the resistance of *C. difficile* spores to alcohol.

C. difficile Transmission Pressure

Increased length of hospital stay and number of hospital admissions continue to be identified as risk factors for CDI (7, 50, 70, 114, 134, 146). This could be explained simply by the increased risk of exposure to *C. difficile* (transmission pressure) via, for example, the hands of health care workers or indirectly from the hospital environment. Several studies have reported the presence of *C. difficile* in the hospital environment (61, 74, 160), and one study demonstrated increased environmental levels of *C. difficile* in a ward after it first opened to patient admissions despite there being no demonstrable *C. difficile* beforehand (61). In a recent whole-hospital-based study, the median *C. difficile* transmission pressure (termed "CDAD pressure" by those authors) was significantly higher for case patients with CDI than for controls (1.4 versus 0.3; $P < 0.001$) and notably remained an independent risk factor after adjustment for key potential confounders, including antimicrobial use. Of the 382 case patients examined, only one had a CDAD pressure of zero (56). However, "CDAD pressure" may be an uncertain measure because it lacks precision with respect to the exact time of potential exposure within the time frame of the length of stay. Thus, identical pressure indexes may be reached under conditions that represent quite distinct risks for acquiring CDI, for example, the timing of *C. difficile* exposure relative to other risk factors, such as admission/discharge day and antibiotic therapy (77).

Asymptomatic *C. difficile* carriage was not measured in the above-described studies, and thus, its contribution to *C. difficile* transmission pressure is unknown. It is arguable that a symptomatic CDI patient likely represents more of a cross-infection risk than an asymptomatic carrier. However, a recent study suggested that the asymptomatic *C. difficile* carriage rate for

elderly patients in a long-term-care facility may be as high as 52% in outbreak settings, and crucially, such individuals outnumbered CDI cases 7-fold (160); these findings may have been exaggerated by the frequent occurrence of rectal incontinence. The skin of *C. difficile* carriers and their associated environmental surfaces were often contaminated by the bacterium, and spores were easily transferred to investigators' hands. Asymptomatic carriers could therefore represent a significant *C. difficile* transmission pressure, but corroboratory studies are needed here. It is nevertheless prudent to strive to optimize universal patient and environmental hygiene as a control measure to reduce *C. difficile* transmission pressure.

A detailed discussion of control measures to reduce *C. difficile* transmission pressure methods (effective barrier precautions during patient contact, use of isolation rooms, good hand hygiene practice, use of gloves and gowns, and appropriate environmental cleaning) is outside the limit of this review but can be found in United Kingdom Department of Health/Health Protection Agency (HPA) guidelines (51), Society for Healthcare Epidemiology of America/Infectious Diseases Society of America practice recommendations (57), and a European *C. difficile* Infection Control Group/ECDC report (202).

EVIDENCE FOR CHANGING CLINICAL VIRULENCE AND OUTCOME OF CDI

Increases in the frequency, severity, and recurrence rate of CDI were recognized in cases from Quebec, Canada, occurring since 2002 (121, 152, 153, 154). More complications were observed, such as toxic megacolon, severe disease requiring colectomy, shock, and death. These complications were associated with an age of >65 years, acquisition of infection in a hospital, peripheral leukocyte count of $>20 \times 10^9$ leukocytes/liter, renal failure, and severe immunosuppression. The proportion of patients dying within 30 days after diagnosis increased from 4.7% in 1991 to 1992 to 13.8% in 2003 ($P < 0.001$). The cumulative mortality rate 12 months after diagnosis was significantly higher among patients with CDI (37%) than among controls (21%) (153). An impressive 1-year prospective surveillance study in 88 Quebec hospitals, performed in 2005, revealed that more severe disease was associated with ribotype 027 than with other types (87). Ribotype 027 was associated with both increased CDI incidence and more severe disease. CDI was the attributable cause of death in 2.6% of cases and contributed to death in another 4.5% of cases. The observation of more severe CDI associated with ribotype 027 was also made in Europe. In the Netherlands, 218 CDI cases caused by ribotype 027 were compared with 645 cases with CDI due to other ribotypes, mainly ribotypes 001 (18%) and 014 (7%) (76). Clear but nonsignificant trends were observed for more severe diarrhea (OR, 1.99; 95% CI, 0.83 to 4.73), higher attributable mortality (6.3% versus 1.2%; OR, 3.30; 95% CI, 0.41 to 26.4), and more recurrences (OR, 1.44; 95% CI, 0.94 to 2.20) in ribotype 027 cases. Lambert et al. compared the clinical characteristics of 211 patients with CDI due to ribotype 027 with 679 patients who had CDI due to other ribotypes in a large prospective study in Belgium (115); an age of over 80 years (OR, 1.6; $P = 0.01$) and the severity of the underlying condition, but not sex, were independent predictors of CDI-related death. Infection with ribotype 027 was associated with an OR of 1.9 ($P = 0.041$) for CDI-related death. In England, crude (death within 28 days) and early (death within 72 h) mortality rates were 23% and 11%, respectively, for CDI ribotype 027 cases, in comparison with 11% and 3%, respectively, for ribotype 106 cases (186). In Leeds, United Kingdom, 35 CDI cases caused by ribotype 027 were compared with 35 non-ribotype 027, age-, sex-, and location-matched cases (60). The duration or extent of diarrhea and frequency of abdominal pain, pyrexia, hypoalbuminemia, or leucocytosis did not differ between the two groups. However, there were more first-line metronidazole treatment failures in ribotype 027 cases (29% versus 3%; $P = 0.03$), and these were more likely to have an acute increase (>20 μM) in serum creatinine levels (20% versus 3%; $P = 0.055$). Crude mortality up to day 30 (25% in ribotype 027 cases versus 14% in controls), day 60 (29% versus 31%), and day 90 (34% versus 37%) appeared to reflect comorbidities. One small study was unable to demonstrate more severe CDI due to ribotype 027, but this was a retrospective analysis with no adjustment for comorbidities (137).

Given the experience of the changing clinical characteristics of CDI associated with ribotype 027, it is important to be alert for the potential emergence of new *C. difficile* strains. *C. difficile* ribotype 078 isolates carry known virulence markers similar to those present in ribotype 027 strains: the *tcdA* and *tcdB* binary toxin genes and a 39-bp deletion in *tcdC* in combination with a point mutation at position 184, resulting in a stop codon (8, 28). There is some evidence that the rate of CDI caused by ribotype 078 is increasing; a 2008 European surveillance study (16) found that 8% of cases were due to ribotype 078, compared with 3% in a 2005 study (14). In the Netherlands, the proportion of ribotype 078 increased from 3% in 2005 to 13% at the end of 2007, whereas the proportion of ribotype 027 decreased from 27% to 1%. An increase in the number of cases due to ribotype 078 has also been reported from France (169). Data from the National Reference Laboratory in the Netherlands indicate a clear trend for more severe diarrhea associated with ribotype 078 (77). Compared to CDI caused by ribotype 027, patients with CDI caused by ribotype 078 were significantly younger and had community-associated disease significantly more often. The presence of severe CDI (38.9% versus 40.0%) and the attributable mortality rate (3.8% versus 4.0%) were similar, whereas a complicated course was found significantly less frequently (9.6% versus 17.7%; OR, 0.20). In contrast to ribotype 027, outbreaks due to ribotype 078 were only sporadically found. Further data on potential emerging *C. difficile* ribotypes are needed. Clearly, this requires effective surveillance, including *C. difficile* culture, to be in place, which is underpinned by increased vigilance.

SUSCEPTIBILITY OF *C. DIFFICILE* TO METRONIDAZOLE AND VANCOMYCIN

In vitro antimicrobial resistance in *C. difficile* has been reported for several classes of antimicrobial agents that have been recognized as high risk for the induction of CDI (73). However, resistance to antimicrobial agents is not essential for CDI, since *C. difficile* may be susceptible to relatively high-risk antimicrobial agents, such as the aminopenicillins (66). Since the worldwide emergence of the apparently hypervirulent ribotype 027 *C. difficile* strain, many reports have documented

increased severities of infection, higher rates of symptomatic recurrence (i.e., primary treatment failure), and significantly increased mortality rates (60, 109, 138, 152, 154). Metronidazole has long been the first-line therapy for CDI, and its reduced effectiveness was reported in a prospective observational study of 207 CDI patients, in which only 50% of patients were successfully treated and 22% continued to experience symptomatic CDI despite ≥10 days of treatment. Additionally, 28% of patients experienced symptomatic recurrence within 90 days (138). Similar observations of a reduced initial response to metronidazole and increased recurrent CDI were also reported in Quebec, Canada (155). Notably, however, while there have been occasional reports of resistance to metronidazole (CLSI breakpoint of 32 mg/liter) (12, 27, 149, 150, 178, 179, 216), metronidazole treatment failure has not been linked to antimicrobial resistance in *C. difficile* (172).

Several recent reports have highlighted that the epidemiology of metronidazole susceptibility in *C. difficile* may have changed. In a retrospective evaluation of 272 recent *C. difficile* isolates (2005 to 2006) submitted to the *C. difficile* Ribotyping Network for the United Kingdom (CDRN), we reported that 24.4% of *C. difficile* ribotype 001 isolates demonstrated metronidazole MICs of ≥6 mg/liter using a spiral gradient endpoint (SGE) screening method ($P < 0.001$) (9). These observations were not noted for strains of two other epidemic *C. difficile* ribotypes (ribotypes 027 and 106). It was clearly demonstrated that the experimental method affects metronidazole MICs in *C. difficile*, but reproducible MICs of ≥4 mg/liter (range, 4 to 16 mg/liter) were observed for all of the isolates highlighted by SGE, and these were statistically significantly elevated compared to a comparator group ($n = 72$) of historic (1995 to 2001) *C. difficile* isolates (9). Multilocus variable-number tandem-repeat analysis (MLVA) of *C. difficile* with reduced metronidazole susceptibility revealed two clonal complexes of related strains. In a larger, more recent study (2008 to 2009), we evaluated metronidazole and vancomycin susceptibilities of 1,010 *C. difficile* isolates submitted from multiple hospitals in England to the CDRN using both SGE and agar incorporation techniques (69). *C. difficile* PCR ribotypes 027 (NAP1), 106, and 001 comprised >50% of the isolates evaluated. Overall geometric mean metronidazole MICs of *C. difficile* ribotypes 027, 106, and 017 (1.73, 1.56, and 1.15 mg/liter, respectively) were statistically significantly higher than those of the 10 other most commonly encountered PCR ribotype groups (0.39 to 0.54 mg/liter; $P < 0.0001$). Similar observations of higher overall metronidazole MICs for epidemic *C. difficile* ribotypes were reported previously (28, 30). Metronidazole MICs for *C. difficile* ribotype 001 were statistically significantly lower than those demonstrated for a panel of isolates from 2005 to 2006 ($P = 0.016$); the proportion of isolates with MICs of ≥4 mg/liter was 54% versus 17%, respectively. Interestingly, significant increases in metronidazole MICs for *C. difficile* PCR ribotypes 027 and 106 were observed ($P < 0.001$), with 17% and 44% of isolates, respectively, demonstrating metronidazole MICs of ≥4 mg/liter. Additionally, 12 *C. difficile* isolates with vancomycin MICs of 4 mg/liter, comprising PCR ribotypes 106 ($n = 6$), 001 ($n = 5$), and 027 ($n = 1$), were observed (69), compared with only a single isolate in our prior study (9). In a similarly large susceptibility study of 1,080 stool samples from diagnostic laboratories in Ontario, Canada, Martin et al. reported 19 (1.8%) *C. difficile* isolates with metronidazole MICs of >8 mg/liter and 4 isolates with vancomycin MICs of 4 mg/liter (128). However, the *C. difficile* isolates (including PCR ribotypes 027 and 078) were only transiently resistant by Etest, with resistance apparently being lost upon repeated subculturing, which reflects data from previously reported *in vitro* studies (151). The potential clinical significance of reduced susceptibility to metronidazole in *C. difficile* isolates is unclear, but given the pharmacokinetic profile of metronidazole in humans, only a small elevation in the MIC could have a marked effect on the treatment efficacy in CDI, although there are no published reports to confirm this hypothesis. Mean metronidazole concentrations in feces of patients range between <0.25 and 9.5 mg/kg and are dependent on the inflammatory state of the colon (5, 24, 107). Therefore, *C. difficile* isolates with metronidazole MICs of ≥4 mg/liter may be unaffected by colonic concentrations of the drug. In contrast, *C. difficile* strains with vancomycin MICs of 4 to 8 mg/liter are unlikely to be clinically significant, as concentrations ranging between 520 and 2,200 mg/liter in feces of patients have been demonstrated (218).

The mechanism(s) of reduced susceptibility to metronidazole in *C. difficile* has not yet been identified, but Brazier et al. did not detect the presence of *nim* genes in the first metronidazole-resistant *C. difficile* strain (MIC = 16 mg/liter) isolated in the United Kingdom (27). Pelaez and colleagues reported that metronidazole resistance in Spanish *C. difficile* isolates was heterogeneous, inducible, and not related to the presence of *nim* genes (151), but whether these observations are reproducible with *C. difficile* isolates from other countries remains to be determined. As the predominant strains causing CDI continue to change, and given the potential for the clonal spread of strains with reduced susceptibility (9), there is continuing surveillance by reference laboratories worldwide to detect resistance emergence.

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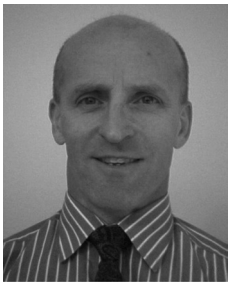

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