

## EPIDEMICS OF DIARRHEA CAUSED BY A CLINDAMYCIN-RESISTANT STRAIN OF *CLOSTRIDIUM DIFFICILE* IN FOUR HOSPITALS

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### ABSTRACT

**Background** Large outbreaks of diarrhea caused by a newly recognized strain of *Clostridium difficile* occurred in four hospitals located in different parts of the United States between 1989 and 1992. Since frequent use of clindamycin was associated with the outbreak in one of the hospitals, we examined the resistance genes of the epidemic-strain isolates and studied the role of clindamycin use in these outbreaks.

**Methods** Case-control studies were performed at three of the four hospitals to assess the relation of the use of clindamycin to *C. difficile*-associated diarrhea. All isolates of the epidemic strain and representative isolates of other strains identified during each outbreak were tested for susceptibility to clindamycin. Chromosomal DNA from these representative isolates was also analyzed by dot blot hybridization and amplification with the polymerase chain reaction (PCR) with the use of probes and primers from a previously described determinant of erythromycin resistance — the erythromycin ribosomal methylase B (*ermB*) gene — found in *C. perfringens* and *C. difficile*.

**Results** In a stratified analysis of the case-control studies with pooling of the results according to the Mantel-Haenszel method, we found that the use of clindamycin was significantly increased among patients with diarrhea due to the epidemic strain of *C. difficile*, as compared with patients whose diarrhea was due to nonepidemic strains (pooled odds ratio, 4.35; 95 percent confidence interval, 2.02 to 9.38;  $P < 0.001$ ). Exposure to other types of antibiotics or hospitalization in a surgical ward was not significantly associated with the risk of *C. difficile*-associated diarrhea due to the epidemic strain. All epidemic-strain isolates were highly resistant to clindamycin (minimal inhibitory concentration,  $>256 \mu\text{g}$  per milliliter). DNA hybridization and PCR analysis showed that all these isolates had an *ermB* gene, which encodes a 23S ribosomal RNA methylase that mediates resistance to macrolide, lincosamide, and streptogramin antibiotics. Only 15 percent of the nonepidemic strains were resistant to clindamycin.

**Conclusions** A strain of *C. difficile* that is highly resistant to clindamycin was responsible for large outbreaks of diarrhea in four hospitals in different states. The use of clindamycin is a specific risk factor for diarrhea due to this strain. Resistance to clindamycin further increases the risk of *C. difficile*-associated diarrhea, an established complication of antimicrobial use. (N Engl J Med 1999;341:1645-51.)

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SINCE its etiologic role in pseudomembranous colitis was discovered 21 years ago,<sup>1</sup> *Clostridium difficile* has been recognized as a major nosocomial pathogen throughout the world.<sup>2</sup> A wide variety of strains are capable of causing disease,<sup>3,4</sup> and outbreaks or epidemics of *C. difficile*-associated diarrhea are often linked to a single strain, but the relatedness of these strains among different institutions and geographic regions is not clear. A recent collaborative typing study demonstrated that a newly recognized strain of *C. difficile* was responsible for outbreaks of diarrhea in four hospitals in different parts of the United States that occurred between 1989 and 1992.<sup>5</sup> We evaluated the association of diarrhea from this strain with the use of clindamycin, the resistance of this strain to clindamycin, and the genetic basis for resistance to clindamycin. Three of these outbreaks were reported previously as unrelated events,<sup>6-8</sup> but we now know that the outbreaks were caused by one strain with an apparent propensity to cause epidemics.

### METHODS

#### Outbreaks of Diarrhea Associated with *C. difficile* Infection

The clinical aspects of the previously reported outbreaks in New York, Arizona, and Massachusetts<sup>6-8</sup> and the outbreak in Florida are summarized in Table 1. Criteria for case definitions varied between investigations but were based on clinical symptoms of diarrhea and the detection of *C. difficile* cytotoxin in the stool of affected patients in each instance. In the Arizona outbreak diarrhea was defined as four or more loose or unformed stools in a period of 24 to 36 hours, but it was not defined on the basis of frequency or a specific period in the other outbreaks.

#### New York

In 1989 there was an abrupt increase in cases of *C. difficile*-associated diarrhea in a 460-bed Veterans Affairs facility in upstate New York.<sup>6</sup> The incidence of *C. difficile*-associated diarrhea

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**TABLE 1.** OUTBREAKS OF DIARRHEA ASSOCIATED WITH *CLOSTRIDIUM DIFFICILE* INFECTION IN FOUR HOSPITALS.

LOCATION	SIZE AND TYPE OF FACILITY*	NO. OF CASES	DATE OF REPORTED OUTBREAK	DURATION OF REPORTED OUTBREAK (MO)	INCIDENCE	COMMENTS
New York <sup>6</sup>	460-Bed VA hospital	174	1989–1990	18	20/1000 admissions	Incidence 10 times as high as in previous 2 years; outbreak continued through 1993
Arizona <sup>7</sup>	300-Bed VA hospital	101	1990–1991	13	15.8/1000 discharges	Incidence 5 times as high as in previous 21 months; outbreak resolved abruptly with restriction of clindamycin use
Florida	786-Bed community hospital	106	1990–1991	2.5	19/1000 discharges	Incidence decreased to 7/1000 discharges 2 months after the end of the outbreak
Massachusetts <sup>8</sup>	431-Bed teaching hospital	98	1992	6	16/1000 discharges	Overall incidence unchanged from previous year, but focal outbreaks occurred on two wards for 2 months

\*VA denotes Veterans Affairs.

during this period (20 per 1000 admissions) was 10 times as high as in the previous two years. The reason for the marked increase in the number of cases was not reported, but two case-control studies conducted early in the outbreak (from December 1988 to May 1989) identified antimicrobial therapy, particularly with second- and third-generation cephalosporins, as the chief risk factor. Criteria for the use of antimicrobial therapy were adopted by the hospital, but the epidemic continued at least through the spring of 1993.

#### Arizona

In July 1990 a Veterans Affairs facility in Arizona noted an abrupt increase in cases of *C. difficile*-associated diarrhea.<sup>7</sup> The incidence of disease during this outbreak (15.8 per 1000 discharges) was five times as high as in the previous 21 months. However, three months after clindamycin was removed from the hospital formulary, the incidence decreased to rates documented before the outbreak.

#### Florida

A 786-bed community hospital in southwest Florida documented 106 cases of *C. difficile*-associated diarrhea between November 12, 1990, and January 28, 1991. The incidence during the outbreak was 19 per 1000 discharges, and it had decreased to 7 per 1000 discharges by March 1991. At the time, this decrease was attributed to a change in housekeeping procedures.

#### Massachusetts

A 431-bed tertiary-care teaching hospital in a large city in eastern Massachusetts documented 98 cases of *C. difficile*-associated diarrhea between June and December 1992.<sup>8</sup> The overall incidence during this period (16 per 1000 discharges) was unchanged from the previous year, but focal outbreaks were recognized on two hospital wards over a two-month period. These focal outbreaks resolved without specific intervention.

#### Identification of Strains Associated with the Outbreaks

Isolates from all four outbreaks were systematically compared by three methods<sup>5</sup>: restriction-endonuclease analysis of whole-cell DNA with the use of *Hind*III,<sup>7,8</sup> a polymerase-chain-reaction (PCR) assay with the use of arbitrary primers,<sup>9</sup> and pulsed-field gel electrophoresis with *Sma*I restriction-enzyme analysis.<sup>8</sup> The predominant, epidemic-associated strain at each hospital was either a single strain (on the basis of PCR analysis) or two highly related types (J7 and J9) that were only distinguished by one *Hind*III-derived

genomic fragment on the basis of restriction-endonuclease analysis.<sup>5</sup> Type J7 isolates were recovered only from the Arizona outbreak. Since this difference between J7 and J9 was most likely the result of a single genetic event,<sup>10</sup> these strains were determined to be part of a single genetic lineage,<sup>5</sup> which we refer to as the epidemic strain.

The epidemic strain accounted for 66 percent of isolates (27 of 41) typed at the New York hospital,<sup>9</sup> 52 percent of isolates (33 of 63) at the Arizona hospital,<sup>7</sup> 33 percent of isolates (6 of 18) at the Florida hospital (unpublished data), and 33 percent of isolates (30 of 90) at the Massachusetts hospital.<sup>8</sup> During the two-month outbreak in the medical and surgical wards at the Massachusetts hospital, the epidemic strain accounted for 62 percent of the isolates (16 of 26).

#### Patients

Investigators at three of the hospitals reviewed data bases and patients' charts for clindamycin use in the patients with *C. difficile*-associated diarrhea. Data linking patients to the *C. difficile* typing results were not available from the Florida hospital. For patients who had more than one episode of *C. difficile*-associated diarrhea, only the first episode was analyzed. The records of all patients for whom the recovered *C. difficile* isolate was typed were analyzed to determine whether clindamycin had been given at any time during the two months before the illness. Patients were classified as having *C. difficile*-associated diarrhea due to the epidemic strain or due to nonepidemic strains. In the New York outbreak there were 29 episodes of *C. difficile*-associated diarrhea for which antibiotic histories and typing data were available; the epidemic strain was recovered from 20 patients and other strains were recovered from 9. These data were available for 63 episodes of *C. difficile*-associated diarrhea in the Arizona hospital (33 related to the epidemic strain and 30 related to other strains) and for 90 episodes in the Massachusetts hospital (30 related to the epidemic strain and 60 to other strains). Data on exposure to antibiotics other than clindamycin and the type of ward (surgical or other) the patient was in at the time of the episode were available for 160 of the 183 episodes: 28 episodes in New York (21 related to the epidemic strain and 7 related to other strains), 42 episodes in Arizona (25 related to the epidemic strain and 17 to other strains), and 90 episodes in Massachusetts (30 related to the epidemic strain and 60 to other strains). Odds ratios and confidence intervals for three variables (clindamycin use, use of other antibiotics, and hospitalization in a surgical ward) were calculated for individual institu-

tions and combined according to the Mantel-Haenszel method.<sup>11</sup> All P values are two-sided.

### Susceptibility Testing of *C. difficile* Isolates

We used the E test (AB Biodisk, Solna, Sweden) to assess all 85 epidemic-strain isolates for susceptibility to clindamycin, including 16 isolates from New York, 33 from Arizona, 6 from Florida, and 30 from Massachusetts. Two representative isolates of the epidemic strain from each of the four outbreaks were identified by restriction-endonuclease analysis and selected for additional testing for susceptibility to erythromycin, ciprofloxacin, ampicillin, and tetracycline and were identified as follows: type J9 (isolates 5602 and 5610) from New York, type J7 (isolates 4224 and 4290) from Arizona, type J9p2 (isolates 5644 and 5650) from Florida, and type J9 (isolates 4478 and 5627) from Massachusetts.

Three toxigenic isolates, identified by restriction-endonuclease analysis, served as controls; two strains were susceptible to clindamycin (type K12p [isolate 5672], an endemic strain from Cook County Hospital, Chicago,<sup>12</sup> and type Y4 [isolate 1323], an endemic strain from the Minneapolis Veterans Affairs Medical Center<sup>13</sup>), and one strain was resistant to clindamycin (type B1 [isolate 832], an epidemic-associated strain from the Minneapolis Veterans Affairs Medical Center<sup>14</sup>).

In addition, representative *C. difficile* isolates of the nonepidemic strains from each of the four outbreaks were also tested for susceptibility to clindamycin. One isolate of each nonepidemic strain was chosen from each outbreak for analysis. In New York, 3 of the 9 types identified on PCR (from 9 nonepidemic cases of *C. difficile*-associated diarrhea) were available for susceptibility testing, whereas 1 isolate of each of the 17 identified by restriction-endonuclease analysis (from 30 nonepidemic cases) was available from Arizona, 1 isolate of 6 of the 7 types identified by PCR (from 12 nonepidemic cases) was available from Florida, and 1 isolate of each of the 20 strains identified by pulsed-field gel electrophoresis (from 60 nonepidemic cases) was available from Massachusetts. In brief, we performed the E test as directed by the manufacturer, using reduced brucella agar plates supplemented with 5 percent defibrinated sheep's blood, 1 mg of vitamin K per liter, and 5 mg of hemin per liter (Remel, Lenexa, Kans.).<sup>15</sup> The isolates were incubated overnight in reduced tryptic soy broth, the amount of the inoculum of *C. difficile* was standardized, and the bacteria were inoculated onto plates and grown to confluency. Antibiotic-impregnated strips were then placed on the inoculated plates, and the plates were incubated anaerobically at 37°C for 48 hours. The minimal inhibitory concentration was measured at the intercept of the inhibition ellipse.

### Genetic Analysis of Strains' Resistance to Clindamycin

For the following analyses *C. difficile* strains were grown in brain-heart infusion medium with iron sulfate<sup>16</sup> in an anaerobic glove chamber in an atmosphere of 80 percent nitrogen, 10 percent hydrogen, and 10 percent carbon dioxide at 37°C. When appropriate, the medium was supplemented with erythromycin (50 µg per milliliter).

DNA was extracted from 100-ml broth cultures of *C. difficile* that had been grown to the late log phase. The cells were harvested and lysed according to the sarkosyl lysis procedure,<sup>17</sup> and chromosomal DNA purified by dye buoyant density-gradient ultracentrifugation at 260,000×g for 20 hours at 20°C. The chromosomal DNA was extracted from the gradient, dialyzed against TRIS-EDTA buffer (0.01 mM EDTA and 0.1 mM TRIS, pH 7.5), and concentrated by evaporation.

PCR assays were conducted with a GenAmp2400 thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.) in volumes of 100 µl that contained approximately 100 ng of template DNA. Two oligonucleotide primers specific for the erythromycin ribosomal methylase B (*ermB*) gene of *C. difficile* strain 630<sup>18</sup> — 2980 (5'AATAAGTAAACAGGTAACGTT3') and 2981 (5'GCTCCTTGGAAGCTGTCTAGTAG3') — were included at a concentration of approximately 0.7 µM per reaction. The PCR assay consisted of 30 cycles of amplification at 95°C for one minute, two minutes of

annealing at 55°C, and three minutes of extension at 72°C. The products were separated by electrophoresis on 0.8 percent agarose gels.

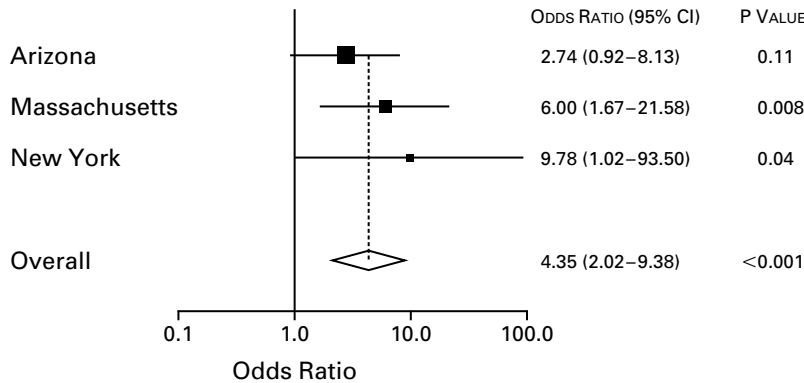
Samples of chromosomal DNA (10 µg) from each strain were blotted onto nylon membranes (Hybond N<sup>+</sup>, Amersham, Arlington Heights, Ill.), and cross-linked to the membrane by exposure to ultraviolet light for five minutes at 312 nm. A 688-bp *ermB*-specific probe labeled with digoxigenin-11-deoxyuridine triphosphate was prepared by PCR with use of the primers 2980 and 2981 and allowed to hybridize to DNA immobilized on the membrane at 65°C overnight. The membrane was washed twice at room temperature in 2× sodium citrate buffer (SSC) (300 mM sodium chloride and 30 mM sodium citrate), pH 7.5, containing 0.1 percent sodium dodecyl sulfate, and twice at 65°C in 0.2× SSC, containing 0.1 percent sodium dodecyl sulfate. Bound probe was detected with use of an anti-digoxigenin-specific, chemiluminescent substrate (CDP-Star, Roche Diagnostics Australia, Castle Hill, Australia) according to the manufacturer's specifications.

## RESULTS

### Association of the Epidemic Strain of *C. difficile* with Clindamycin Use

Case-control studies were performed at the New York, Arizona, and Massachusetts hospitals to evaluate the relation between exposure to clindamycin and diarrhea due to the epidemic strain of *C. difficile* (Fig. 1). The frequency of exposure to clindamycin among patients with diarrhea due to nonepidemic strains ranged from 7 percent in Massachusetts to 23 percent in Arizona, which is indicative of variation in the overall frequency of the use of clindamycin among the institutions. Yet, within each institution, clindamycin use was a more frequent cause of diarrhea due to the epidemic strain than of diarrhea due to nonepidemic strains. In the New York hospital, 11 of 20 cases of diarrhea due to the epidemic strain were associated with clindamycin use (55 percent), as compared with 1 of 9 cases of diarrhea due to nonepidemic strains (11 percent); the respective values for the Arizona hospital were 15 of 33 (45 percent) and 7 of 30 (23 percent), and the respective values for the Massachusetts hospital were 9 of 30 (30 percent) and 4 of 60 (7 percent). Overall, 35 of the 83 cases of diarrhea due to the epidemic strain were associated with clindamycin use (42 percent), as compared with 12 of 99 cases due to nonepidemic strains (12 percent, P<0.001). The odds ratio for the use of clindamycin ranged from 2.74 to 9.78 (Fig. 1). The pooled odds ratio for the association between clindamycin use and diarrhea due to the epidemic strain was 4.35 (95 percent confidence interval, 2.02 to 9.38; P<0.001) (Fig. 1).

In contrast, the use of other antibiotics was not associated with diarrhea due to the epidemic strain. The pooled odds ratio was 1.13 (95 percent confidence interval, 0.53 to 2.41; P=0.74) for cefazolin, 1.02 (95 percent confidence interval, 0.45 to 2.32; P=0.95) for third-generation cephalosporins (ceftazidime, ceftriaxone, and cefotaxime), 0.43 (95 percent confidence interval, 0.16 to 1.20; P=0.10) for ampicillin, 1.09 (95 percent confidence interval, 0.49 to 2.45; P=0.83) for vancomycin, and 1.10 (95 percent con-



**Figure 1.** Odds Ratio for the Use of Clindamycin before Becoming Ill among Patients with Diarrhea Due to the Epidemic Strain of *Clostridium difficile* as Compared with Patients with Diarrhea Due to Non-epidemic Strains.

The size of each symbol is proportional to the weight of the corresponding study. The overall odds ratio was obtained with use of the Mantel–Haenszel method; the dotted line indicates the point estimate of this odds ratio.  $P=0.5$  for the test of homogeneity. CI denotes confidence interval.

confidence interval, 0.45 to 2.71;  $P=0.83$ ) for aminoglycosides (gentamicin and tobramycin). Similarly, hospitalization in a surgical ward was not a risk factor for diarrhea due to the epidemic strain (pooled odds ratio, 0.75; 95 percent confidence interval, 0.38 to 1.49;  $P=0.42$ ).

#### Susceptibility of Epidemic and Nonepidemic Strains of *C. difficile* to Clindamycin

All 85 isolates of the epidemic strain of *C. difficile* were highly resistant to clindamycin (minimal inhibitory concentration of clindamycin,  $>256 \mu\text{g}$  per milliliter). The representative isolates of epidemic strains (type J9, J7, or J9p2) from each hospital outbreak were also highly resistant to erythromycin, as was the clindamycin-resistant strain (type B1) that was used as a control (Table 2). Both clindamycin-susceptible control strains (types K12p and Y4) were susceptible to clindamycin and erythromycin. The majority of nonepidemic strains from each outbreak were susceptible to clindamycin. High-level resistance to clindamycin (minimal inhibitory concentration,  $>256 \mu\text{g}$  per milliliter) was present in only 15 percent of the nonepidemic strains (7 of 46 strains; 1 of 3 in New York, 3 of 17 in Arizona, 0 of 6 in Florida, and 3 of 20 in Massachusetts). The minimal inhibitory concentration of clindamycin for the remaining isolates of nonepidemic strains was  $4 \mu\text{g}$  per milliliter or less in the case of 34 strains and  $6 \mu\text{g}$  per milliliter in the case of the other 5 strains.

#### Genetic Basis of Clindamycin Resistance in the Epidemic Strain

Resistance to macrolide–lincosamide–streptogramin (MLS) antimicrobial agents such as erythromycin and clindamycin is often mediated by a 23S ribosomal RNA methylase encoded by one of a group of highly

related *erm* genes that have been found in gram-positive and gram-negative organisms. Two of these genes, one from *C. perfringens* and one from *C. difficile*, belong to the ErmB–ErmAM hybridization class and have been referred to as the *ermBP* and *ermZ* genes, respectively.<sup>18,19</sup> However, in accordance with a newly proposed nomenclature for the *erm* genes (unpublished data), these genes are both referred to here as *ermB* genes. DNA dot blot hybridizations were carried out on chromosomal DNA prepared from the epidemic strain of *C. difficile* and control strains under highly stringent conditions, with use of an *ermB*-specific probe derived from *C. difficile* strain 630. DNA from all the representative isolates of the clindamycin-resistant epidemic strain at each hospital showed strong hybridization with the probe, indicating that the isolates contained an *ermB* gene (Fig. 2). The control strains K12p and Y4, which are susceptible to MLS antibiotics, did not hybridize to the probe.

Next, we conducted PCR assays with primers 2980 and 2981 and each of the isolates analyzed by dot blot hybridization to confirm that the gene present in the epidemic strains was closely related to that of strain 630. Analysis of each of the MLS-resistant epidemic strains revealed PCR products of the expected size (688 bp), indicating that the gene present in the epidemic strains was an *ermB*-like gene (data not shown). No PCR products were obtained from the MLS-susceptible control strains. In addition, Southern blots carried out on DNA from each of the resistant strains indicated that there may have been more than one copy of the *ermB* gene in each of those strains (data not shown).

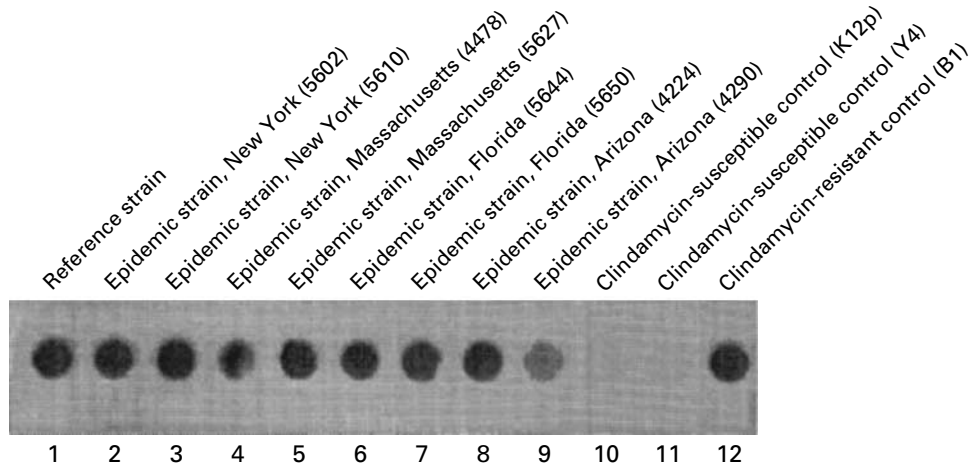
#### DISCUSSION

This study demonstrates that large outbreaks of diarrhea in four hospitals in separate regions of the Unit-

**TABLE 2.** ANTIMICROBIAL-RESISTANCE PROFILES OF REPRESENTATIVE ISOLATES OF THE EPIDEMIC STRAIN OF *CLOSTRIDIUM DIFFICILE* AT EACH HOSPITAL AND CONTROL STRAINS.\*

DRUG	EPIDEMIC STRAIN				CONTROL STRAIN		
	J9 (NEW YORK)	J7 (ARIZONA)	J9p2 (FLORIDA)	J9 (MASSACHUSETTS)	CLINDAMYCIN- SUSCEPTIBLE (K12p)	CLINDAMYCIN- SUSCEPTIBLE (Y4)	CLINDAMYCIN- RESISTANT (B1)
	minimal inhibitory concentration (micrograms per milliliter)						
Clindamycin	>256	>256	>256	>256	0.75	0.75	>256
Erythromycin	>256	>256	>256	>256	0.5	0.50	>256
Ciprofloxacin	>32	>32	>32	>32	>32	>32	>32
Ampicillin	0.75	0.75	0.75	0.75	3.0	1.0	1.5
Tetracycline	0.06	0.06	0.06	0.06	0.09	0.05	12

\*Each isolate was identified by restriction-endonuclease analysis; P indicates the presence of plasmids.<sup>5</sup> Groups of closely related *C. difficile* strains are designated by uppercase letters, whereas unique types are designated by numbers. The minimal inhibitory concentrations of each drug are shown for the epidemic strain from each of the four hospitals (two isolates were tested from each outbreak) and for the control strains.



**Figure 2.** DNA Dot Blot Analysis of Strains of *Clostridium difficile*.

Each analysis used 10  $\mu$ g of chromosomal DNA and an *ermB*-specific probe. Lane 1 shows reference strain 630, lanes 2 and 3 epidemic-strain isolates from New York, lanes 4 and 5 epidemic-strain isolates from Massachusetts, lanes 6 and 7 epidemic-strain isolates from Florida, lanes 8 and 9 epidemic-strain isolates from Arizona, lanes 10 and 11 clindamycin-susceptible control strains, and lane 12 a clindamycin-resistant control strain.

ed States were all caused by a specific, highly clindamycin-resistant strain of *C. difficile* and that the use of clindamycin was a specific risk factor. The relation between clindamycin use and infection with the epidemic strain was consistent among the institutions, which justifies our pooled analysis and strengthens our findings. Although other virulence factors associated with this particular strain may affect its epidemic potential, resistance of specific *C. difficile* strains to clindamycin may partially explain the well-known propensity of this agent to precipitate outbreaks and epidemics of diarrhea.

These results cast new light on the relation between antibiotic use and *C. difficile*-associated diarrhea. The role of the antimicrobial agent has been assumed to be to disrupt the normal intestinal flora, particularly anaerobes, of the host, which is an important resistance factor with respect to infection with *C. difficile*. The antimicrobial agent precipitating a particular episode of *C. difficile*-associated diarrhea has been thought to have no direct association with the pattern of resistance of the infecting strain.<sup>2</sup> For example, most strains of *C. difficile* are susceptible to ampicillin, yet historically, this agent has com-

monly been implicated in episodes of diarrhea. Although *C. difficile* isolates are routinely resistant to cephalosporins such as cefoxitin, resistance to clindamycin is less common. High-level resistance to clindamycin was present in only 15 percent of the non-epidemic strains in our study. Our results indicate that the relatively high likelihood of *C. difficile*-associated diarrhea after exposure to clindamycin is not just a consequence of effects on the resident flora; it may also be linked to the susceptibility profile of the organism.

Hospital-wide use of clindamycin has been identified as the chief factor responsible for the outbreak of *C. difficile*-associated diarrhea at the Arizona hospital,<sup>7</sup> but this association was not apparent or was not assessed in the initial investigations of the other outbreaks.<sup>6,8</sup> The outbreak in Arizona was abruptly terminated by the removal of clindamycin from the hospital formulary.<sup>7</sup> The use of cephalosporins was identified as the chief risk factor for *C. difficile*-associated diarrhea early in the New York outbreak, on the basis of multivariate analyses of two case-control studies in which ward controls and diarrhea controls, respectively, were used.<sup>6</sup> The use of clindamycin, however, was also a risk factor in the ward study and showed a trend in the diarrhea study.<sup>6</sup> Stool culture, with typing of the recovered *C. difficile* isolates, was not performed in New York until one year after the original case-control studies.<sup>9</sup> We used the later cohort of cases (identified between March and October 1990) and found that clindamycin use was a specific risk factor for diarrhea due to the epidemic strain of *C. difficile*. A subsequent comparative typing study of *C. difficile* isolates from this same hospital documented persistence of the epidemic strain two years later (January to November 1992).<sup>20</sup> The risk of *C. difficile*-associated diarrhea associated with the use of specific antibiotics had not been reported previously for the outbreak at the Massachusetts hospital,<sup>8</sup> and data on antibiotic use were not available from the Florida outbreak.

There is evidence that this strain or genetically related strains may have an even broader geographic distribution than is suggested by the distribution of these four outbreaks. Preliminary results obtained with use of PCR ribotyping indicate that the epidemic strain from the Massachusetts hospital designated as type J9 on the basis of restriction-endonuclease analysis or type D1 on the basis of pulsed-field gel electrophoresis is PCR ribotype 1.<sup>21</sup> PCR ribotype 1 was the most common strain among hospitalized patients in England and Wales, accounting for 57 percent of all isolates in one survey,<sup>21</sup> and was responsible for a large outbreak in northwest England involving 175 patients and 17 deaths at one hospital.<sup>22</sup> A formal comparison of restriction-endonuclease analysis and PCR ribotyping methods should clarify whether these European epidemic strains are related to the epidem-

ic strains we studied. We have also used restriction-endonuclease analysis to analyze two *C. difficile* isolates of the clonal strain associated with another clindamycin-related epidemic of diarrhea that was recently reported in Virginia.<sup>23</sup> Neither of these isolates (kindly provided by Michael Climo and Edward Wong) was type J9 or J7.

Each of the epidemic-strain isolates contained an *erm* gene<sup>19</sup> which, on the basis of its ability to hybridize under highly stringent conditions with an *ermB*-specific probe, belongs to the ErmB class of erythromycin-resistance determinants. This conclusion was supported by PCR analysis, which showed that a product of the same size as the *ermB* determinant from *C. difficile* strain 630 was amplified from each of the epidemic isolates with use of *ermB*-specific primers. These data provide evidence that the resistance to MLS antibiotics of each of the epidemic-strain isolates results from the presence of an *ermB* gene.

MLS-resistance genes from the ErmB hybridization class have been detected in both *C. perfringens* and *C. difficile*.<sup>24-26</sup> The *ermB* gene from *C. perfringens* is located on a large nonconjugative but mobilizable plasmid, pIP402.<sup>27</sup> By contrast, the *ermB* gene from *C. difficile* strain 630, which is 99 percent homologous to the *C. perfringens* gene (unpublished data), appears to be located on the chromosome.

In strain 630, transfer of erythromycin resistance occurs in the absence of detectable plasmids. The *ermB* gene has been postulated to reside on the as yet uncharacterized conjugative transposon Tn5398.<sup>28</sup> It is possible that the *ermB* gene detected in the epidemic strain that we studied is also associated with Tn5398, or with a related mobile genetic element located on the chromosome. Such elements are likely to represent an important method for the dissemination of resistance to MLS antibiotics among clinical isolates of *C. difficile*, especially in hospitals. It is also possible that the *ermB* gene in the epidemic strain is located on the chromosome but is not associated with a transposable element or that *ermB* is located on a plasmid. However, no antibiotic-resistance plasmids have ever been reported in *C. difficile*.

Taken together, these observations suggest that a single erythromycin-clindamycin resistance gene, present in specific strains of *C. difficile*, is associated with a significantly increased risk of *C. difficile*-associated diarrhea in widely dispersed U.S. hospitals, especially in association with clindamycin use. *C. difficile*-associated diarrhea is virtually unknown in the absence of use of antimicrobial agents, and the risk of this illness among hospitalized patients increases with the use of clindamycin and the presence of clindamycin-resistant strains of *C. difficile*. *C. difficile*-associated diarrhea is yet another example of the increasing number of nosocomial infections caused by organisms resistant to antimicrobial agents. It is encouraging that in two well-described outbreaks

caused by clindamycin-resistant *C. difficile*, there was rapid resolution of the epidemic with restriction of the use of clindamycin.<sup>7,23</sup>

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