ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 1991, p. 208–210
0066-4804/91/010208-03$02.00/0

Vol. 35, No. 1

Prospective Study of *Clostridium difficile* Intestinal Colonization and Disease following Single-Dose Antibiotic Prophylaxis in Surgery

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Received 15 May 1990/Accepted 12 October 1990

A total of 108 volunteers undergoing an elective surgical procedure were randomly given a single 2-g intravenous prophylactic dose of either a cephalosporin or mezlocillin. Stool samples were cultured for *Clostridium difficile* the day before the operation and later on postoperative days 4, 7, and 14. *C. difficile* was detected in 23.0% of patients who received a cephalosporin (cefoxitin, 8.3%; cefazolin, 14.3%; cefotetan, 20.0%; ceftriaxone, 25.0%; cefoperazone, 43.7%), in 3.3% of patients given mezlocillin, and in none of 15 control volunteers given no antimicrobial agent. No patient experienced diarrhea.

Some published studies show that most *Clostridium difficile* colonizations and infections of hospitalized patients are nosocomial in origin (5, 10, 17, 20, 21, 27); person-to-person transmission (17, 20, 22, 27) and carriage of the organism on the hands of health care workers have, indeed, been extensively documented (17, 19–21). Moreover, widespread contamination of the inanimate hospital environment occurs in hospital wards in which there are patients with *C. difficile*-associated disease, and, even though less extensive, environmental contamination from asymptomatic carriers has also been documented (14, 22).

An asymptomatic intestinal carrier state may follow *C. difficile*-associated diarrhea or pseudomembranous colitis, but it can also occur without any preceding clinical disease (26). The pathogenicity of *C. difficile* is related to the production of at least two distinct toxins, enterotoxin (toxin A) and cytotoxin (toxin B). Highly toxigenic strains have been isolated from patients with no detectable fecal cytotoxin as well as from asymptomatic carriers (13, 20, 23), and some asymptomatic colonized individuals can subsequently develop disease. The incidence of *C. difficile*-associated disease and asymptomatic intestinal colonization has been shown to dramatically increase in patients who are given antimicrobial agents. Apart from clindamycin, which is the first agent that was clearly associated with pseudomembranous colitis, the antimicrobial agents most commonly implicated as inciting factors of both *C. difficile* intestinal colonization and disease are cephalosporins and ampicillin (1, 2, 4, 9, 11, 15, 16, 28). *C. difficile* colonization and disease develop more commonly after prolonged antibiotic treatment, but they have also been reported after short-term (usually three doses) perioperative prophylaxis with some antimicrobial agents (3, 6, 11, 18, 25, 29).

The observations that (i) the emergence of *C. difficile* is not necessarily dependent upon long-term antibiotic treatment and (ii) cephalosporins are more likely to be associated with *C. difficile* intestinal disease (1, 2, 11, 18) and to have a significant effect on the colonization resistance of the lower gastrointestinal tract (7, 24) than are ureidopenicillins prompted us to perform a prospective study of the influence of single-dose perioperative prophylaxis with different antimicrobial agents on acquisition of the *C. difficile* carrier state during hospitalization.

A total of 123 volunteers (71 females and 52 males), with a mean age of 48.8 years (range, 17 to 86 years), who had neither received any antibiotic treatment nor reported any diarrheal disease during at least the preceding 3 months and who were hospitalized for an elective clean surgical procedure requiring general anesthesia (such as saphenectomy or hernioplasty) took part in the study. Because it seemed necessary to have a control group given no antimicrobial agent, patients undergoing operation for which antibiotic prophylaxis is mandatory, such as cardiac and orthopedic surgeries, were excluded. Subjects reporting a history of allergy to beta-lactams or at greater risk for developing *C. difficile* infection, such as those with neoplasia, concurrent infections, malnutrition, and concomitant renal diseases, were likewise excluded. Informed consent was obtained from each patient. To minimize the influence of colonization by endemic strains on the results of the study, only patients admitted to the two surgical wards of a single hospital (S. Anna Hospital) subjected to a strict surveillance program for *C. difficile* infection and environmental contamination (11) were enrolled and the length of the study was restricted to 3 months. Throughout this period cefazolin, cefoxitin, cefotetan, ceftriaxone, and cefoperazone were each randomly assigned to one of the surgical teams on the wards as the unique agent for perioperative prophylaxis and mezlocillin was assigned to two surgeons in order to include more treated patients, allowing comparison not only with each single cephalosporin but also with cephalosporins as a group. Between 15 and 20 min before surgery, eligible patients received either no antibiotic (controls) or a single 2-g intravenous bolus injection of one of the study drugs depending on the operating surgeon. This resulted in greater convenience to the surgeons, who were each using only one drug for prophylaxis; it also meant that throughout the study, the wards contained patients who had received different antimicrobial agents. The surgical teams operated in the same theaters, and after their operations the patients were not segregated in the wards according to the operating surgeon but were nursed in the same wards and by the same

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TABLE 1. Incidence of *C. difficile* and cytotoxin after injection of a single dose of different antibiotics

Antibiotic	No. of patients (%)			
	Total	Evaluable	Total colonized	Total toxin positive
Cefazolin	14	14	2 (14.3)	2 (14.3)
Cefoxitin	14	12	1 (8.3)	1 (8.3)
Cefotetan	21	20	4 (20.0)	4 (20.0)
Cefoperazone	17	16	7 (43.7)	4 (25.0)
Ceftriaxone	12	12	3 (25.0)	3 (25.0)
All cephalosporins	78	74	17 (23.0)	14 (18.9)
Mezlocillin	30	30	1 (3.3)	1 (3.3)
All antibiotics	108	104	18 (17.3)	15 (14.4)
Controls	15	15	0	0

personnel. The treatment groups did not differ significantly in age, sex ratio, or surgical procedures performed.

Stool samples were collected the day before operation and later on postoperative days 4, 7, and 14. Patients who received any antibiotic treatment during the 14 days of observation following surgery were excluded from the study.

Samples were plated both directly and after heat shock (80°C for 10 min) on CD selective blood agar (Oxoid Ltd., Basingstoke, United Kingdom) supplemented with cycloserine (250 mg/liter) and cefoxitin (8 mg/dl) and inoculated in the enrichment broth described by Buchanan (8), which was subcultured on CD selective agar after 24 h. The identification of *C. difficile* and the cytotoxin assay were performed as previously described (12). Toxin neutralization was done with *C. difficile* antiserum. Statistical analysis of the results was performed by the two-tailed Fisher exact test on a personal computer, using the SAS statistical package (SAS Institute, Cary, N.C.).

For four patients (3.2%), *C. difficile* was isolated from the sample collected before the operation. These patients were therefore excluded from further evaluation.

In the follow-up period (14 days), no patient experienced diarrhea or other gastrointestinal symptoms. The incidence of microorganism isolation and cytotoxin detection after injection of a single dose of antibiotic is shown in Table 1.

In several patients *C. difficile* was isolated from stool samples after enrichment in selective broth, but not by direct plating. This is possibly related to low bacterial counts in the feces. Seven of the 18 positive patients (38.9%) were found to be colonized at the first postoperative stool culture; 10 more patients (55.6%) were found to be colonized at the second one; and 1 patient (5.5%) was found to be colonized only at the 14-day postoperative culture.

The *C. difficile* carrier state was considered to be transient (five patients [25.4%]) or persistent (six patients [35.3%]) when the 14-day stool culture became negative or persisted in being positive, respectively. From this point of view, no conclusion could be drawn for six subjects, since the 14-day stool sample was unavailable. There was no difference in the distribution of transient or persistent colonization among the drugs.

No clustering by time or location in the wards was observed for patients colonized by *C. difficile* following drug administration during the study.

The incidence of *C. difficile* colonization following cephalosporin administration was higher than in the general patient population (the incidence in the general patient population was obtained from an incidence survey performed in the same wards during the month preceding this study). Actually, the overall incidence of *C. difficile* acquisition during hospitalization (regardless of antibiotic administration or any other recognized risk factor), evaluated by serial stool cultures in all patients admitted to the same wards, was 5.1%.

Overall, cephalosporins were found to be associated with *C. difficile* colonization significantly more often than mezlocillin was (Fisher's exact test; $P = 0.02$). The most heavy inducers of *C. difficile* colonization relative to mezlocillin were cefoperazone ($P = 0.01$) and ceftriaxone ($P = 0.06$). The comparison between mezlocillin and the other cephalosporins did not show any significant difference. Cefoperazone was also correlated with *C. difficile* colonization significantly more often than were the other cephalosporins, considered as a whole ($P = 0.04$). Nevertheless, even if cefoperazone was excluded, the other cephalosporins (ceftriaxone, cefotetan, cefoxitin, and cefazolin [pooled data]) were still correlated with colonization significantly more often than was mezlocillin ($P = 0.04$).

In general, broad-spectrum antimicrobial agents are considered to induce asymptomatic acquisition of *C. difficile* to a greater extent than are narrow-spectrum agents (26). According to the results we obtained, the ability of different parenteral antibiotics to induce the *C. difficile* carrier state would more probably be related to their pharmacokinetics (such as high biliary excretion) or to other properties rather than to their antimicrobial spectrum, which is often largely superposable.

Overall, the results we obtained suggest that in surgical prophylaxis, single-2-g-dose mezlocillin is safer than single-2-g-dose cephalosporins (especially broad-spectrum cephalosporins) as regards postoperative *C. difficile* intestinal acquisition. Although none of the colonized patients observed in this study developed diarrhea, environmental colonization and person-to-person transmission have also been documented for asymptomatic carriers.

We conclude that when a comparable efficacy in preventing postoperative infections is proven for different antimicrobial agents on the basis of controlled clinical trials, the rate of *C. difficile* colonization should be taken into account in the choice of the most suitable antibiotic for surgical prophylaxis. In our opinion, moreover, the shift to drugs less prone to induce *C. difficile* colonization might also be considered among the measures to be applied to reduce nosocomial transmission in epidemic or endemic settings in surgical wards.

REFERENCES

1. **Aronsson, B., R. Mollby, and C. E. Nord.** 1984. Diagnosis and epidemiology of *Clostridium difficile* enterocolitis in Sweden. J. Antimicrob. Chemother. **14**(Suppl. D):85–95.
2. **Aronsson, B., R. Mollby, and C. E. Nord.** 1985. Antimicrobial agents and *Clostridium difficile* in acute enteric disease: epidemiological data from Sweden, 1980–1982. J. Infect. Dis. **151:** 476–481.
3. **Arsura, E. L., R. A. Fazio, and P. C. Wickremesinghe.** 1985. Pseudomembranous colitis following prophylactic antibiotic use in primary cesarean section. Am. J. Obstet. Gynecol. **151:**87–89.
4. **Bartlett, J. G., N. S. Taylor, T. W. Chang, and J. Dzink.** 1980. Clinical and laboratory observations in *Clostridium difficile* colitis. Am. J. Clin. Nutr. **33:**2521–2526.
5. **Bender, B. S., B. E. Laughon, C. Gaydos, M. S. Forman, R. Bennet, W. B. Greenough, S. D. Sears, and G. Bartlett.** 1986. Is *Clostridium difficile* endemic in chronic-care facilities? Lancet **ii:**11–13.
6. **Block, B. S., L. J. Mercer, M. A. Ismail, and A. H. Moavad.** 1985. *Clostridium difficile*-associated diarrhea follows perioper-

ative prophylaxis with cefoxitin. Am. J. Obstet. Gynecol. **153**:835–838.

7. **Bodey, G. P., V. Fainstein, I. Garcia, B. Rosenbaum, and Y. Wong.** 1983. Effect of broad-spectrum cephalosporins on the microbial flora of recipients. J. Infect. Dis. **148**:892–897.
8. **Buchanan, A. G.** 1984. Selective enrichment broth culture for detection of *Clostridium difficile* and associated cytotoxin. J. Clin. Microbiol. **20**:74–76.
9. **Church, J. M., and V. M. Fazio.** 1986. A role for colonic stasis in the pathogenesis of disease related to *Clostridium difficile*. Dis. Colon Rectum **29**:804–809.
10. **Cumming, A. D., B. J. Thomson, J. Sharp, I. R. Poxton, and A. G. Fraser.** 1986. Diarrhoea due to *Clostridium difficile* associated with antibiotic treatment in patients receiving dialysis: the role of cross infection. Br. Med. J. **292**:238–239.
11. **de Lalla, F., G. Privitera, G. Ortisi, G. Rizzardini, D. Santoro, A. Pagano, E. Rinaldi, and P. Scarpellini.** 1989. Third generation cephalosporins as a risk factor for *Clostridium difficile*-associated disease: a four-year survey in a general hospital. J. Antimicrob. Chemother. **23**:623–631.
12. **de Lalla, F., G. Privitera, E. Rinaldi, G. Ortisi, D. Santoro, and G. Rizzardini.** 1989. Treatment of *Clostridium difficile*-associated disease with teicoplanin. Antimicrob. Agents Chemother. **33**:1125–1127.
13. **Ellis, M. E., B. K. Mandal, E. M. Dunbar, and K. R. Bundell.** 1984. *Clostridium difficile* and its cytotoxin in infants admitted to hospital with infectious gastroenteritis. Br. Med. J. **288**:524–526.
14. **Fekety, R., K. Kim, D. Brown, D. H. Batts, M. Cudmore, and J. Silva.** 1981. Epidemiology of antibiotic-associated colitis. Isolation of *Clostridium difficile* from the hospital environment. Am. J. Med. **70**:906–908.
15. **Finegold, S. M.** 1986. Clinical considerations in the diagnosis of antimicrobial agent-associated gastroenteritis. Diagn. Microbiol. Infect. Dis. **3**:S87–S91.
16. **George, W. L., R. D. Rolfe, and S. M. Finegold.** 1982. *Clostridium difficile* and its cytotoxin in feces of patients with antimicrobial agent-associated diarrhea and miscellaneous conditions. J. Clin. Microbiol. **15**:1049–1053.
17. **Heard, S. R., S. O'Farrel, D. Holland, S. Crook, M. J. Barnett, and S. Tabaqchali.** 1986. The epidemiology of *Clostridium difficile* with use of a typing scheme: nosocomial acquisition and cross-infection among immunocompromised patients. J. Infect. Dis. **153**:159–162.
18. **Keigley, M. R. B., N. S. Ambrose, D. L. Morris, and D. W. Burdon.** 1983. Evaluation of mezlocillin in elective gastrointestinal surgery. J. Antimicrob. Chemother. **11**(Suppl. C):65–69.
19. **Kim, K. H., R. Fekety, D. H. Batts, D. Brown, J. Silva, and W. Waters.** 1981. Isolation of *Clostridium difficile* from the environment and contacts of patients with antibiotic-associated colitis. J. Infect. Dis. **143**:42–50.
20. **Malamou-Ladas, H., S. O'Farrel, J. Q. Nash, and S. Tabaqchali.** 1983. Isolation of *Clostridium difficile* from patients and the environment of hospital wards. J. Clin. Pathol. **36**:88–92.
21. **McFarland, L. V., M. E. Mulligan, R. Y. Y. Know, and W. E. Stamm.** 1989. Nosocomial acquisition of *Clostridium difficile* infection. N. Engl. J. Med. **320**:205–210.
22. **Mulligan, M. E., W. L. George, R. D. Rolfe, and S. M. Finegold.** 1980. Epidemiological aspects of *Clostridium difficile*-induced diarrhea and colitis. Am. J. Clin. Nutr. **33**(Suppl. 11):2533–2538.
23. **Nakamura, S., S. Nakashio, T. Inamuatsu, N. Nishida, N. Taniguchi, and S. Nishida.** 1980. Toxigenicity of *Clostridium difficile* isolates from patients and healthy adults. Microbiol. Immunol. **24**:995–997.
24. **Nord, C. E.** 1987. Effect of antimicrobial prophylaxis on colonization resistance. Program Abstr. First Int. Conf. Hosp. Infect. Soc., abstr. S8/4. Hospital Infection Society, London.
25. **Roberts, A. P., and A. W. Hughes.** 1985. Complications with antibiotics used prophylactically in joint replacement surgery: a case of cephradine-induced pseudomembranous colitis. Int. Orthop. **8**:299–302.
26. **Rolfe, R. D.** 1988. Asymptomatic intestinal colonization by *Clostridium difficile*, p. 201–225. *In* R. D. Rolfe and S. M. Finegold (ed.), *Clostridium difficile*: its role in intestinal disease. Academic Press, Inc., San Diego, Calif.
27. **Savage, A. M., and R. H. Alford.** 1983. Nosocomial spread of *Clostridium difficile*. Infect. Control **4**:31–33.
28. **Talbot, R. W., R. C. Walker, and R. W. Beart.** 1986. Changing epidemiology, diagnosis and treatment of *Clostridium difficile* toxin-associated colitis. Br. J. Surg. **73**:457–460.
29. **Tan, J., L. H. Bayne, and P. J. McLeod.** 1979. Pseudomembranous colitis: a fatal case following prophylactic cephaloridine therapy. J. Am. Med. Assoc. **242**:749–750.