Vol. 55, No. 5

# Effect of Ceftobiprole Treatment on Growth of and Toxin Production by *Clostridium difficile* in Cecal Contents of Mice[▽]

Michelle M. Nerandzic[1] and Curtis J. Donskey[2]*

*Research Service, Cleveland VA Medical Center, Cleveland, Ohio 44106,[1] and Geriatric Research, Education and
Clinical Center, Cleveland VA Medical Center, Cleveland, Ohio 44106[2]*

**Ceftobiprole and ceftobiprole medocaril did not promote growth of or toxin production by *Clostridium difficile*
in mouse cecal contents, whereas ceftazidime, cefoxitin, ceftriaxone, cefotaxime, and ertapenem did. The
relatively low propensity of ceftobiprole to promote *C. difficile* was attributable to inhibitory activity against *C.
difficile* and sparing of anaerobic microflora.**

Antimicrobial therapy promotes *Clostridium difficile* infection (CDI) due to disruption of the indigenous microflora of the colon, thereby allowing *C. difficile* to grow to high concentrations with production of toxin (13, 15). Nearly all classes of antibiotics have been associated with CDI, but clindamycin, broad-spectrum cephalosporins, and penicillins have traditionally been considered the agents that pose the greatest risk (3, 6, 13). With the emergence of the North American pulsed-field gel electrophoresis type 1 (NAP1) epidemic strain of *C. difficile* with increased resistance to fluoroquinolone antibiotics, fluoroquinolones have also been associated with CDI in multiple studies (6, 13). However, there remains some controversy regarding the relative importance of fluoroquinolones as a risk factor for CDI because these agents cause only minor disruption of intestinal anaerobes (1, 6, 13). There is some evidence that antibiotics with inhibitory activity against *C. difficile* (e.g., piperacillin-tazobactam and tigecycline) may be less likely to promote CDI, presumably because they inhibit growth of *C. difficile* in the colon during therapy (1, 6, 8, 9, 16). In mice, we demonstrated that piperacillin-tazobactam prevented the establishment of colonization by *C. difficile* during treatment but facilitated growth and toxin production when exposure to spores occurred after treatment during the period of recovery of the indigenous microflora (15).

Although cephalosporins are considered high-risk agents for promotion of *C. difficile*, there are significant differences among cephalosporins with regard to biliary excretion and anti-anaerobic activity (18). In general, the greatest risk for promotion of *C. difficile* has been associated with extended-spectrum cephalosporins that are excreted in significant concentrations into the intestinal tract (e.g., ceftriaxone and ceftazidime) (3, 18). In mice, we found that ceftriaxone promoted overgrowth and toxin production by *C. difficile*, whereas cefepime, an agent that is almost entirely excreted via the kidneys, did not (15). Because of the differences in routes of elimination and *in vitro* activity among cephalosporins, it is important that new cephalosporins be evaluated to assess their propensity to promote *C. difficile* and other healthcare-associated pathogens.

Ceftobiprole is a novel, broad-spectrum cephalosporin with activity against methicillin-resistant *Staphylococcus aureus* (2, 5, 7, 10). Ceftobiprole has the potential to promote *C. difficile* infection to a lesser degree than many other broad-spectrum cephalosporins for two reasons. First, ceftobiprole is primarily excreted via the kidneys, resulting in relatively low levels of intestinal exposure and only minor disruption of intestinal anaerobes (2, 10). Second, in comparison to other cephalosporins, ceftobiprole demonstrated relatively good activity against *Clostridium species* (MIC$_{90}$ for *Clostridium* spp. = 4 μg/ml for ceftobiprole, 64 μg/ml for cefepime, >128 μg/ml for ceftazidime, 32 μg/ml for cefotaxime, and 16 μg/ml for cefoxitin), including some strains of *C. difficile* (5, 7). Here, we used a mouse model to test the hypothesis that ceftobiprole is less likely than other cephalosporins to promote establishment of colonization by *C. difficile*.

## MATERIALS AND METHODS

***C. difficile* strains.** Four strains of *C. difficile* were studied. ATCC 43593 was a nontoxigenic strain from the American Type Culture Collection. The other strains were cultured from patients with CDI in Cleveland, OH. VA 17 and VA 20 were epidemic North American pulsed-field gel electrophoresis type 1 (NAP1) strains. VA 11 was a restriction endonuclease analysis (REA) J-type strain. Table 1 shows the MICs of the test antibiotics, as determined by broth dilution (11), for each of the strains.

**Growth of *C. difficile* in mouse cecal contents.** The mouse model was adapted from the hamster model of colonization resistance to CDI developed by Borriello et al. (4). These investigators demonstrated that antibiotics that promoted growth of and toxin production by *C. difficile* in cecal emulsions of hamsters also caused CDI in hamsters, whereas antibiotics that did not promote growth and toxin production in cecal contents did not cause disease (4). We have previously found that this model yields similar results in mice (1, 9, 15). The Animal Care Committee of the Cleveland Veterans Affairs Medical Center approved the experimental protocol.

Female CF-1 mice (8 per group) weighing 25 to 30 g (Harlan Sprague-Dawley, Indianapolis, IN) were housed in individual cages. Mice received daily subcutaneous injections (0.2-ml total volume) of saline, ceftobiprole (0.75 mg/day), ceftobiprole medocaril (i.e., the prodrug of ceftobiprole) (1.5 mg/day), ceftriaxone (2 mg/day), ceftazidime (3 mg/day), cefoxitin (3 mg/day), ertapenem (0.5 mg/day), clindamycin (1.4 mg/day), or piperacillin-tazobactam (8 mg/day) for 5 days. In some experiments, mice received daily ceftobiprole or ceftobiprole medocaril in combination with clindamycin. The antibiotic doses were equal to the usual human doses administered over a 24-h period (milligrams of antibiotic per gram of body weight).

Mice were killed by $CO_2$ asphyxiation 6 h after the final antibiotic dose. The

---

* Corresponding author. Mailing address: Geriatric Research, Education and Clinical Center (111C(W)), Cleveland VA Medical Center, 10701 East Blvd., Cleveland, OH 44106. Phone: (216) 791-3800, ext. 4788. Fax: (216) 231-3482. E-mail: curtisd123@yahoo.com.

TABLE 1. MICs for the four *C. difficile* test strains

| Antibiotic | MIC (μg/ml)[a] | | | |
| --- | --- | --- | --- | --- |
| | ATCC 43599 | VA 11 | VA 17 | VA 20 |
| Piperacillin-tazobactam | 2 | 2 | 8 | 8 |
| Ertapenem | 4 | 8 | 8 | 8 |
| Ceftobiprole | 1 | 1 | 2 | 1 |
| Ceftobiprole medocaril | 1 | 2 | 2 | 2 |
| Ceftazidime | 128 | >256 | 64 | 64 |
| Ceftriaxone | 8 | 128 | 64 | 64 |
| Cefotaxime | 16 | 128 | 64 | 64 |
| Cefoxitin | 128 | >256 | >256 | >256 |
| Clindamycin | >256 | >256 | >256 | >256 |

[a] MICs were determined by broth dilution.

ceca were removed and opened longitudinally, and the contents were collected and transferred to an anaerobic chamber (Coy Laboratories, Grass Lake, MI) within 5 min. The cecal contents were diluted 3-fold (volume/volume) with sterile prereduced phosphate-buffered saline (PBS). A final concentration of $10^4$ CFU/ml of each *C. difficile* strain was added to separate aliquots of cecal contents from individual mice. The *C. difficile* strains were prepared for inoculation by serially diluting 72-h broth cultures in sterile prereduced PBS. To quantify *C. difficile*, after incubation for 24 h, samples were diluted in sterile PBS and plated on prereduced cycloserine-cefoxitin-brucella agar containing 0.1% taurocholic acid and lysozyme at 5 mg/ml (12). To determine toxin production in cecal contents, a *C. difficile* Tox A/B II (Wampole Laboratories, Princeton, NJ) test kit was used according to the manufacturer's instructions.

**Effect of antibiotic treatment on the concentrations of total anaerobes.** Aliquots of cecal contents collected 6 h after completion of the final dose of 4 days of subcutaneous antibiotic treatment were transferred to the anaerobic chamber. The contents were serially diluted in prereduced PBS and plated onto brucella agar (Becton Dickinson) to measure concentrations of total anaerobes. The lower limit of detection was ~4 $\log_{10}$ CFU/g of stool.

**Statistical analysis.** Data analyses were performed with Stata software (version 6.0; Stata, College Station, TX). A one-way analysis of variance was performed to compare the groups with *P* values adjusted for multiple comparisons using the Scheffe correction. Because each of the four strains yielded similar results, the data for the four strains were pooled for analysis for each antibiotic group.

## RESULTS

**Growth of *C. difficile* in mouse cecal contents.** Figure 1 shows the effects of antibiotic treatment on growth of *C. difficile* in

cecal contents collected 6 h after the final antibiotic dose. In comparison to saline controls, ceftazidime, ceftriaxone, cefoxitin, cefotaxime, ertapenem, and clindamycin promoted growth of *C. difficile* ($P \leq 0.01$), whereas ceftobiprole, ceftobiprole medocaril, and piperacillin-tazobactam did not ($P \geq 0.68$). For the toxigenic strains, *C. difficile* toxin was detected in ≥75% of all cecal content samples of mice treated with antibiotics that promoted growth of *C. difficile*, whereas none of the mice in the ceftobiprole, ceftobiprole medocaril, or piperacillin-tazobactam groups had detectable levels of toxin in cecal contents.

As shown in Fig. 2, administration of ceftobiprole and ceftobiprole medocaril in combination with clindamycin reduced clindamycin-induced overgrowth of *C. difficile* in cecal contents collected 6 h after the last dose of antibiotics ($P < 0.001$). Concentrations of *C. difficile* were significantly reduced in the ceftobiprole medocaril plus clindamycin versus the ceftobiprole plus clindamycin group ($P < 0.01$).

Figure 3 shows the effects of antibiotic treatment on concentrations of total anaerobes in cecal contents. The concentrations of total anaerobes were reduced significantly in each of the antibiotic treatment groups in comparison to the saline control group ($P \leq 0.02$). In comparison to the ceftobiprole and ceftobiprole medocaril groups, concentrations of total anaerobes were significantly reduced in the ceftriaxone, cefoxitin, piperacillin-tazobactam, ertapenem, and clindamycin groups ($P < 0.003$), but not in the cefotaxime or ceftazidime groups ($P \geq 0.08$).

## DISCUSSION

We found that ceftobiprole and the prodrug ceftobiprole medocaril did not promote growth of or toxin production by *C. difficile* in cecal contents of mice, whereas ceftazidime, cefoxitin, ceftriaxone, cefotaxime, and ertapenem did. In comparison to the other cephalosporins, ceftobiprole and ceftobiprole medocaril had much lower MICs for the *C. difficile* test strains (1 to 2 μg/ml versus 8 to >256 μg/ml). In addition, when ceftobiprole or ceftobiprole medocaril were combined with clinda-
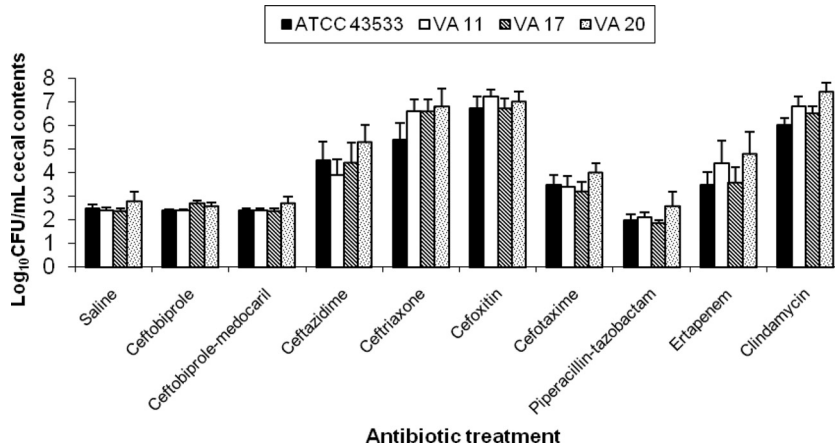


FIG. 1. Effect of antibiotic treatment on growth of *C. difficile* in the cecal contents of mice. Mice received daily subcutaneous antibiotic treatment for 5 days. At 6 h after the final antibiotic dose, the cecal contents were collected and inoculated with $10^4$ CFU of the *C. difficile* strains/ml. Samples were incubated anaerobically for 24 h, and then serial dilutions were plated onto selective media for quantification of *C. difficile* and assayed for toxin production. If *C. difficile* was not detected, the lower limit of detection (~2 $\log_{10}$ CFU/g) was assigned. Error bars represent standard errors.
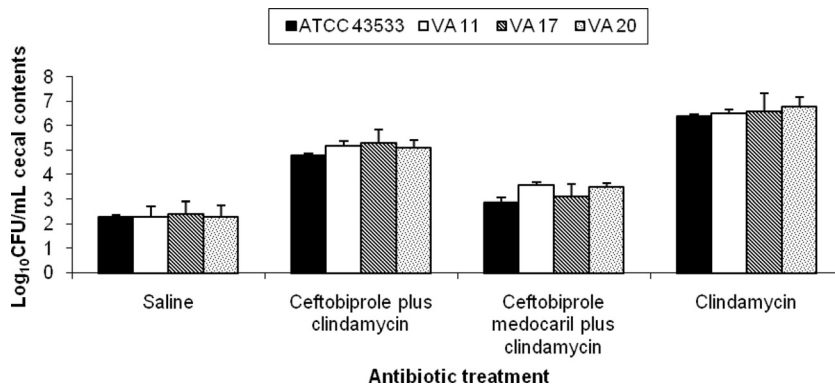
FIG. 2. Effect of antibiotic treatment on growth of *C. difficile* in the cecal contents of mice. Mice received daily subcutaneous antibiotic treatment for 5 days. At 6 h after the final antibiotic dose, the cecal contents were collected and inoculated with $10^4$ CFU of the *C. difficile* strains/ml. Samples were incubated anaerobically for 24 h, and then serial dilutions were plated onto selective media for quantification of *C. difficile* and assayed for toxin production. If *C. difficile* was not detected, the lower limit of detection ($\sim 2 \log_{10}$ CFU/g) was assigned. Error bars represent standard errors.

mycin, clindamycin-induced overgrowth of *C. difficile* was significantly reduced. Ceftobiprole and ceftobiprole medocaril also caused less suppression of total anaerobes in the cecal contents than did cefoxitin or ceftriaxone. These findings suggest that ceftobiprole may have a relatively low propensity to promote CDI in comparison to many other broad-spectrum cephalosporins due to greater inhibitory activity against *C. difficile* and reduced disruption of the anaerobic microflora.

The effects of the antibiotics studied on total anaerobes in the intestinal tract are consistent with findings of previous studies in human volunteers and patients (18). Cefoxitin and ceftriaxone achieve higher concentrations in the intestinal tract and/or have greater anti-anaerobic activity than do cefotaxime and ceftazidime and cause greater suppression of intestinal anaerobes measured in stool samples from volunteers or patients (18). The effects of ertapenem, piperacillin-tazobactam, and clindamycin on concentrations of anaerobes are consistent with previous studies in humans and in mice (9, 15, 18). Cefto-biprole was not detectable in stool of human volunteers and caused only minor suppression of intestinal anaerobes (2).

Our study has several limitations. First, growth of *C. difficile* in cecal contents was assessed at a single time point 6 h after the final dose of a 5-day course of treatment. The risk for establishment of colonization by *C. difficile* may vary depending on the timing of exposure in relationship to antibiotic dosing, the duration of antibiotic treatment, and the time required for recovery of the indigenous microflora (6, 13). Therefore, it is possible that our results could differ in clinical settings where the timing of exposure to *C. difficile* spores is unpredictable and duration of treatment is variable. Second, the pharmacokinetics of antibiotics differs significantly in mice and humans such that higher and more frequent dosing of mice is required to achieve equivalent systemic drug exposures (15). However, we have previously found that equivalent doses (mg/kg of body weight) result in similar levels of antibiotics in stool of mice and humans with similar effects on the indigenous microflora (1, 9, 15, 17). Third, the effects of antibiotics on *C. difficile* colonization and infection depends on multiple factors, including the antibacterial spectrum of activity against the indigenous microflora, the amount of excretion into the intestinal tract,
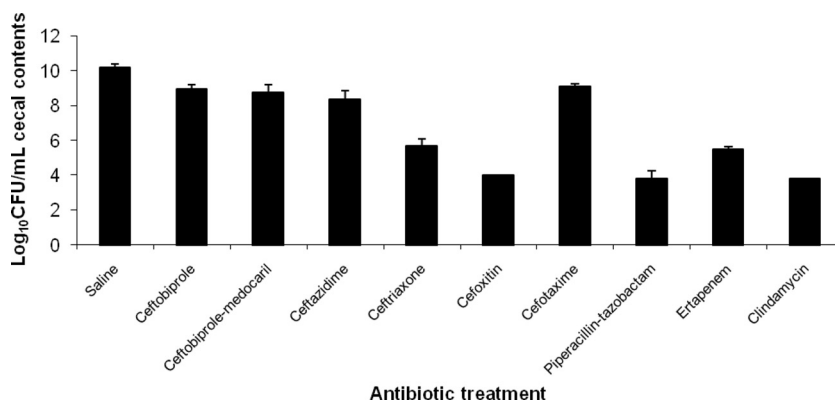


FIG. 3. Effect of subcutaneous antibiotic treatment on concentrations of total anaerobes in cecal contents of mice. Mice received daily subcutaneous antibiotic treatment for 5 days. At 6 h after the final antibiotic dose, the cecal contents were collected, transferred to an anaerobic chamber, and plated onto brucella agar to determine bacterial densities. Identical aliquots were plated onto brucella agar and incubated in room air to confirm than anaerobes were being measured. If organisms were not detected in stool, the lower limit of detection (4 $\log_{10}$ CFU/g) was assigned.

the half-life of the agent, and the agent's activity against *C. difficile* (6, 13). The relative importance of each of these factors in patients has not been determined conclusively. In particular, data are needed to clarify whether systemic antibiotics with *in vitro* inhibitory activity against *C. difficile* (e.g., piperacillin-tazobactam, tigecycline, and ceftobiprole) are able to inhibit colonization by *C. difficile* in patients. Finally, the commercial enzyme immunoassay for toxins A and B used to evaluate toxin production in cecal contents has suboptimal sensitivity in comparison to cell culture cytotoxicity assays and also has less than ideal specificity (14).

In summary, we found that ceftobiprole was less likely than other broad-spectrum cephalosporins to promote the growth of and toxin production by *C. difficile* in the cecal contents of mice. If ceftobiprole is licensed for clinical use, further studies will be needed to evaluate its impact on colonization and infection with *C. difficile* in patients.

### REFERENCES

1. **Adams, D. C., M. M. Riggs, and C. J. Donskey.** 2007. Effect of fluoroquinolone treatment on growth of and toxin production by epidemic and non-epidemic *Clostridium difficile* in the cecal contents of mice. Antimicrob. Agents Chemother. **51:**2674–2678.
2. **Backstrom, T., et al.** 2010. Effect of ceftobiprole on the normal human intestinal microflora. Int. J. Antimicrob. Agents **36:**537–541.
3. **Bignardi, G. E.** 1998. Risk factors for *Clostridium difficile* infection. J. Hosp. Infect. **40:**1–15.
4. **Borriello, S. P., F. E. Barclay, and A. R. Welch.** 1988. Evaluation of the predictive capability of an in-vitro model of colonization resistance to *Clostridium difficile* infection. Microb. Ecol. Health Dis. **1:**61–64.
5. **Ednie, L., S. Shapiro, and P. C. Appelbaum.** 2007. Antianaerobe activity of ceftobiprole, a new broad-spectrum cephalosporin. Diagn. Microbiol. Infect. Dis. **58:**133–136.
6. **Gerding, D. N.** 2004. Clindamycin, cephalosporins, fluoroquinolones, and *Clostridium difficile*-associated diarrhea: this is an antimicrobial resistance problem. Clin. Infect. Dis. **38:**646–648.
7. **Goldstein, E. J. C., et al.** 2006. In vitro activity of ceftobiprole against aerobic and anaerobic strains isolated from diabetic foot infections. Antimicrob. Agents Chemother. **50:**3959–3962.
8. **Johnson, S., et al.** 1999. Epidemics of diarrhea caused by a clindamycin-resistant strain of *Clostridium difficile* in four hospitals. N. Engl. J. Med. **341:**1645–1651.
9. **Jump, R. L., Y. Li, M. J. Pultz, G. Kypriotakis, and C. J. Donskey.** 2011. Tigecycline exhibits inhibitory activity against *Clostridium difficile* in the colon of mice and does not promote growth of or toxin production. Antimicrob. Agents Chemother. **55:**546–549.
10. **Murthy, B., and A. Schmitt-Hoffmann.** 2008. Pharmacokinetics and pharmacodynamics of ceftobiprole, and anti-MRSA cephalosporin with broad-spectrum activity. Clin. Pharmacokinet. **47:**21–33.
11. **National Committee for Clinical Laboratory Standards.** 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow anaerobically, 5th ed. Approved standard M7–A5. National Committee for Clinical Laboratory Standards, Wayne, PA.
12. **Nerandzic, M. M., and C. J. Donskey.** 2009. An effective and reduced cost modified selective medium for isolation of *Clostridium difficile*. J. Clin. Microbiol. **47:**397–400.
13. **Owens, R. C., C. J. Donskey, R. P. Gaynes, V. G. Loo, and C. A. Muto.** 2008. Antimicrobial-associated risk factors for *Clostridium difficile* infection. Clin. Infect. Dis. **46**(Suppl. 1)**:**S19–S31.
14. **Peterson, L. R., and A. Robicsek.** 2009. Does my patient have *Clostridium difficile* infection? Ann. Intern. Med. **151:**176–179.
15. **Pultz, N. J., and C. J. Donskey.** 2005. Effect of antibiotic treatment on growth of and toxin production by *Clostridium difficile* in the cecal contents of mice. Antimicrob. Agents Chemother. **49:**3529–3532.
16. **Settle, C. D., M. H. Wilcox, W. N. Fawley, O. J. O. J. Corrado, and P. M. Hawkey.** 1998. Prospective study of the risk of *Clostridium difficile* diarrhoea in elderly patients following treatment with cefotaxime or piperacillin-tazobactam. Aliment. Pharmacol. Ther. **12:**1217–1223.
17. **Stiefel, U., N. J. Pultz, and C. J. Donskey.** 2007. Effect of carbapenem administration on establishment of intestinal colonization by vancomycin-resistant enterococci and *Klebsiella pneumoniae* in mice. Antimicrob. Agents Chemother. **51:**372–411.
18. **Sullivan, A., C. Edlund, and C. E. Nord.** 2001. Effect of antimicrobial agents on the ecological balance of human microbiota. Lancet Infect. Dis. **1:**101–114. 15.