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Ceftolozane-Tazobactam Activity against Phylogenetically Diverse Clostridium difficile Strains

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Ceftolozane-tazobactam (C/T) is approved for the treatment of complicated intra-abdominal and urinary tract infections and has varied activity against anaerobic bacteria. Here, we evaluate the activity of C/T against a phylogenetically diverse collection of Clostridium difficile isolates and report uniformly high MICs (≥256 μg/ml) to C/T.

In December of 2014, ceftolozane-tazobactam received U.S. Food and Drug Administration (FDA) approval for the treatment of complicated intra-abdominal infections (cIAI) and complicated urinary tract infections (cUTI) (http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm427534.htm). Ceftolozane-tazobactam is a novel β-lactam/β-lactamase inhibitor combination with activity against Pseudomonas aeruginosa, extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, and members of the Streptococcus anginosus group (1). The activity of ceftolozane-tazobactam against anaerobic bacteria is varied. For example, the MIC<sub>90</sub> of 244 Bacteroides fragilis isolates, 86 Bacteroides thetaiotaomicron isolates, and 12 Fusobacterium species isolates was 4 μg/ml, 32 μg/ml, and 0.25 μg/ml, respectively (2).

Clostridium difficile infection (CDI) is well documented as a potential adverse consequence of antimicrobial therapy with a cephalosporin (3–5). The incidence and severity of CDI have increased in recent years, resulting in significant morbidity, mortality, and cost to the health care system (6, 7). C. difficile is classified as one of only three urgent, or highest priority, multidrug-resistant microbial threats by the Centers for Disease Control and Prevention (http://www.cdc.gov/drugresistance/threat-report-2013/). To date, limited clinical data regarding ceftolozane-tazobactam therapy exist, and the risk of CDI following therapy with this specific agent is relatively understudied. In two of the clinical trials that evaluated ceftolozane-tazobactam treatment of cIAI (8) and cUTI (9), 3 cases of CDI were documented (<1% incidence).

In light of the varied antianaerobic activity of ceftolozane-tazobactam, an assessment of the antimicrobial resistance profile for ceftolozane-tazobactam against C. difficile could be important for understanding the potential risk of developing CDI during therapy with this agent. A recent study by Snydman et al. (2) evaluated the activity of ceftolozane-tazobactam against a large collection of anaerobic bacteria using agar dilution; this collection included 30 isolates of C. difficile. The MIC range of ceftolozane-tazobactam against the C. difficile isolates was 0.25 μg/ml to ≥256 μg/ml, and the MIC<sub>90</sub> was ≥256 μg/ml (2). In contrast, the MIC range and MIC<sub>90</sub> documented for Clostridium perfringens (n = 11) and other Clostridium spp. (n = 13) were lower (MIC<sub>90</sub> 0.25 μg/ml and 16 μg/ml, respectively) (2). Currently, there are no categorical interpretative criteria for ceftolozane-tazobactam MICs for Clostridium species. It is well documented that the overall antimicrobial resistance profiles of C. difficile strains can vary widely among different strain types (10–12). Unfortunately, no C. difficile strain typing data were available as part of the Snydman et al. study (2), and thus it was unknown if this profile was generalizable to different C. difficile strain types.

The objective of our study was to evaluate the antimicrobial activity of ceftolozane-tazobactam against a diverse collection of C. difficile strain types. The isolates investigated were recovered from fecal specimens or rectal swab specimens from patients at Barnes-Jewish Hospital from January 2010 to July 2012. Specimens were collected as part of other ongoing studies to assess C. difficile diagnostic assays, C. difficile infection, or asymptomatic colonization (3, 10, 13, 14). C. difficile isolates were recovered in culture (15) and ribotyped (16), as previously described.

All isolates were recovered from frozen stocks and were subcultured twice on preduced 5% sheep blood agar (Hardy Diagnostics, Santa Maria, CA) in an anaerobic environment prior to testing. The identity of each C. difficile isolate was confirmed by matrix-assisted laser desorption ionization—time of flight mass spectrometry (MALDI-TOF MS) using the Vitek MS (IVD version 2.3.3; bioMérieux, Durham, NC) (17). Ceftolozane-tazobactam susceptibility testing was performed using a gradient diffusion method (Etest; bioMérieux, Durham, NC), according to the manufacturer’s recommendations. In brief, a 1 McFarland standard suspension of each isolate was prepared in 0.9% saline and plated as a lawn onto brucella blood agar with hemin and vitamin K (Hardy Diagnostics), and ceftolozane-tazobactam Etest strips were applied. The plates were incubated under anaerobic conditions and read at 24 and 72 h of incubation. During each day of analysis, the quality control strain B. fragilis ATCC 25285 (acceptable quality control [QC] range, 0.12 to 1 μg/ml) was tested.

Eighty-one C. difficile isolates were tested, representing 15 different ribotypes (Table 1). Of note, our test set included 26 C. difficile ribotype 027 (NAP1/BI/ST-1) strains and 3 C. difficile ri-
bototype 078 isolates. These strain types are currently among the most common ribotypes in North America and parts of Europe (18, 19); the toxigenic status of each ribotype was determined by phenotypic (i.e., toxin enzyme immunoassays) and/or genotypic methods (3, 10, 13, 14).

Ceftolozane-tazobactam demonstrated no activity against any of the isolates investigated. All isolates tested (n = 81) had ceftolozane-tazobactam MICs of ≥256 µg/ml at both the 24- and 72-h incubation time points. This phenotype appears to be distinct from that of other Clostridium species (2).

A potential limitation of this study is that all of the isolates investigated were recovered at a single medical center. However, this limitation is mitigated by the careful characterization of the isolates, which demonstrated that a phylogenetically diverse population of strains was tested, and this is a strength of this investigation. The impact of ceftolozane-tazobactam on the gastrointestinal microbiota is unknown at this time. Similar to other investigations of strains was tested, and this is a strength of this investigation. The impact of ceftolozane-tazobactam on the gastrointestinal microbiota is unknown at this time. Similar to other investigations. The impact of ceftolozane-tazobactam on the gastrointestinal microbiota is unknown at this time. Similar to other investigations. The impact of ceftolozane-tazobactam on the gastrointestinal microbiota is unknown at this time. Similar to other investigations. The impact of ceftolozane-tazobactam on the gastrointestinal microbiota is unknown at this time. Similar to other investigations.