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In Vitro Activity of Ceftolozane-Tazobactam against Anaerobic Organisms Identified during the ASPECT-cIAI Study

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The *in vitro* activities of ceftolozane-tazobactam, meropenem, and metronidazole were determined against anaerobic organisms isolated from patients with complicated intraabdominal infections (cIAI) in global phase III studies. Ceftolozane-tazobactam activity was highly variable among different species of the *Bacteroides fragilis* group, with MIC_{90} values ranging from 2 to 64 μg/ml. More-potent *in vitro* activity was observed against selected Gram-positive anaerobic organisms; however, small numbers of isolates were available, and, therefore, the clinical significance of these results is unknown. Variable activity of ceftolozane-tazobactam against anaerobic organisms necessitates use in combination with metronidazole for the treatment of cIAI.

Ceftolozane-tazobactam is a novel antibacterial with activity against *Pseudomonas aeruginosa*, including drug-resistant strains, and other common Gram-negative pathogens, including most extended-spectrum-β-lactamase–producing *Enterobacteriaceae* (1, 2). Ceftolozane-tazobactam has been approved by the U.S. Food and Drug Administration for the treatment of complicated intraabdominal infections (cIAI) in combination with metronidazole (3). The *in vitro* activity of ceftolozane-tazobactam was determined against anaerobic pathogens identified at baseline during the global phase III studies of ceftolozane-tazobactam plus metronidazole versus meropenem in cIAI from 2011 to 2013 (ASPECT-cIAI; NCT01445665 and NCT01445678). In ASPECT-cIAI, ceftolozane-tazobactam plus metronidazole met its primary endpoint of noninferiority to meropenem for clinical cure at the test-of-cure visit in the microbiological intent-to-treat (MITT) population (4). The MITT population consisted of 806 patients, and 389 patients received treatment with ceftolozane-tazobactam. Of these, 137 patients (35.2%) had at least one Gram-negative anaerobic organism identified at baseline, and all but 3 patients had polymicrobial infections that included at least one aerobic organism (4).

Local laboratories from clinical study sites were responsible for the primary identification of all pathogens. All pathogens were required to be shipped to the central laboratory (ICON Laboratories, Farmingdale, NY, USA) for confirmation of identification and susceptibility testing. Anaerobic bacterial strains were shipped frozen in tryptic soy broth with 15% glycerol. Susceptibility testing was performed by broth microdilution in brucella broth supplemented with hemin, vitamin K_1, and 5% lysed horse blood for *Bacteroides fragilis* group strains. Antibiotic susceptibility testing of *B. fragilis* group isolates and quality control (QC) were performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines (5). Anaerobic isolates that were not *B. fragilis* group were shipped from ICON to JMI Laboratories (North Liberty, IA, USA) for susceptibility testing. Isolate MIC values for the non-*fragilis* group *Bacteroides* organisms were determined under anaerobic conditions using the reference CLSI agar dilution method (6). Agar dilution plates were produced by JMI Laboratories using brucella agar supplemented with hemin, vitamin K_1, and 5% laked sheep blood. The *B. fragilis* ATCC 25285 QC strain was tested daily, and inoculum density was monitored by colony counts. All QC data were in range for both broth and agar dilution testing.

The *in vitro* activity of ceftolozane-tazobactam and comparators against anaerobic isolates identified in the ASPECT-cIAI study is presented in Table 1. Ceftolozane-tazobactam activity against *Bacteroides fragilis* group isolates was variable. The MIC_{90} against *Bacteroides thetaiotaomicron* and *Bacteroides vulgatus* was 64 μg/ml in each case, while *Bacteroides fragilis* and *Bacteroides stercoris* each displayed a ceftolozane-tazobactam MIC_{90} of 2 μg/ml. Other Gram-negative anaerobes identified in the study were *Fusobacterium* spp., *Parabacteroides distasonis*, and *Prevotella* spp. Ceftolozane-tazobactam was active against *Prevotella* spp., with an MIC_{90} of 0.06 μg/ml against the 10 isolates obtained.

Ceftolozane-tazobactam was also active *in vitro* against the most prevalent Gram-positive anaerobic species identified, with an MIC_{90} of 0.5 μg/ml against the 45 isolates of *Clostridium perfringens*. Activity was variable against the remaining Gram-positive anaerobic organisms, each with fewer than 9 isolates available from the clinical trial.

Among *Bacteroides fragilis* group isolates, the ceftolozane-tazobactam MIC_{90} values were within one dilution of those published in an earlier study (8). However, the MIC_{50} values were generally lower in the surveillance study than those observed with ASPECT-cIAI isolates. Activity levels of ceftolozane-tazobactam against non-*B. fragilis* Gram-negative anaerobes were generally similar between the two studies. The activities of metronidazole and meropenem were unchanged relative to the surveillance study.

The *in vitro* results support the clinical findings of the phase III ASPECT-cIAI study, demonstrating the utility of ceftolozane-tazobactam in combination with metronidazole for the treatment of patients with cIAI. An application for approval of ceftolozane-

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TABLE 1 *In vitro* activity of ceftolozane-tazobactam against anaerobes isolated at baseline from patients in ASPECT-cIAI

Baseline pathogen (no.)	Antibiotic	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% susceptible[a]
Gram-negative anaerobes					
Bacteroides fragilis (107)[b]	Ceftolozane-tazobactam	0.06 to >64	0.5	2	96.3
	Meropenem	0.06 to 64	0.125	2	95.3
	Metronidazole	0.125 to 64	1	2	99.1
Bacteroides ovatus (87)[b]	Ceftolozane-tazobactam	0.06 to >64	16	64	NA[c]
	Meropenem	0.06 to 8	0.25	0.5	98.9
	Metronidazole	0.125 to 8	1	2	100
Bacteroides thetaiotaomicron (68)[b]	Ceftolozane-tazobactam	0.06 to >64	32	64	NA
	Meropenem	0.06 to 1	0.25	0.25	100
	Metronidazole	0.125 to 64	1	2	98.5
Bacteroides vulgatus (39)[b]	Ceftolozane-tazobactam	1 to >64	16	64	NA
	Meropenem	0.06 to 1	0.125	0.5	100
	Metronidazole	0.06 to 2	0.5	1	100
Parabacteroides distasonis (27)[d]	Ceftolozane-tazobactam	16 to >64	64	>64	NA
	Meropenem	0.12 to 2	0.25	1	100
	Metronidazole	0.25 to 2	1	2	100
Bacteroides uniformis (15)[b]	Ceftolozane-tazobactam	0.5 to 64	16	32	NA
	Meropenem	0.06 to 1	0.125	0.5	100
	Metronidazole	0.125 to 8	0.5	4	100
Bacteroides stercoris (12)[b]	Ceftolozane-tazobactam	0.06 to 2	0.125	2	NA
	Meropenem	0.06 to 0.25	0.125	0.25	100
	Metronidazole	0.5 to 4	1	4	100
Fusobacterium spp. (11)[d]	Ceftolozane-tazobactam	≤0.03 to >64	≤0.03	>64	NA
	Meropenem	≤0.015 to 1	0.06	1	100
	Metronidazole	≤0.12 to 1	0.25	0.5	100
Prevotella spp. (10)[d]	Ceftolozane-tazobactam	≤0.03 to 16	≤0.03	0.06	NA
	Meropenem	0.03 to 0.25	0.06	0.12	100
	Metronidazole	≤0.12 to >16	1	4	90.0
Bacteroides spp. (6)[d]	Ceftolozane-tazobactam	0.5 to >64	1	NA	NA
	Meropenem	0.12 to 1	0.12	NA	100
	Metronidazole	0.25 to 1	0.5	NA	100
Gram-positive anaerobes					
Clostridium perfringens (45)[d]	Ceftolozane-tazobactam	≤0.03 to 16	0.06	0.5	NA
	Meropenem	≤0.015 to 0.12	≤0.015	0.03	100
	Metronidazole	≤0.12 to >16	1	2	97.8
Propionibacterium acnes (8)[d]	Ceftolozane-tazobactam	≤0.03 to 16	2	NA	NA
	Meropenem	≤0.015 to 1	0.06	NA	100
	Metronidazole	>16	>16	NA	0
Eggerthella lenta (6)[d]	Ceftolozane-tazobactam	>64	>64	NA	NA
	Meropenem	0.25	0.25	NA	100
	Metronidazole	0.25 to 2	0.25	NA	100
Eubacterium spp. (5)[d]	Ceftolozane-tazobactam	0.5 to 4	1	NA	NA
	Meropenem	0.03 to 0.25	0.06	NA	100
	Metronidazole	0.25 to 8	0.5	NA	100
Parvimonas micra (5)[d]	Ceftolozane-tazobactam	≤0.03 to 4	≤0.03	NA	NA
	Meropenem	≤0.015 to 0.5	≤0.015	NA	100
	Metronidazole	0.25 to 0.5	0.25	NA	100
Slackia exigua (3)[d]	Ceftolozane-tazobactam	≤0.03 to 16	0.06	NA	NA
	Meropenem	0.03 to 0.12	0.06	NA	100
	Metronidazole	0.25 to 0.5	0.5	NA	100
Other (6)[d,d]	Ceftolozane-tazobactam	0.5 to 2	1	NA	NA
	Meropenem	0.06 to 0.25	0.12	NA	100
	Metronidazole	0.5 to >16	4	NA	50.0

[a] Ceftolozane-tazobactam susceptibility of *Bacteroides fragilis* isolates was calculated based on the U.S. Food and Drug Administration breakpoint of 8 μg/ml for *Bacteroides fragilis*. Susceptibilities of meropenem and metronidazole were calculated based on breakpoints published by the Clinical and Laboratory Standards Institute (M100-S22) (7).
[b] Broth microdilution, ICON Laboratories.
[c] NA, not available.
[d] Agar dilution, JMI Laboratories.
[e] Other Gram-positive pathogens consisted of *Bifidobacterium* spp. ($n = 2$), *Peptoniphilus asaccharolyticus* ($n = 2$), *Collinsella aerofaciens* ($n = 1$), and *Peptostreptococcus anaerobius* ($n = 1$).

tazobactam in combination with metronidazole has also been submitted to the European Medicines Agency.

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