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BRIEF REPORT

In Vitro Activity of Ceftolozane/Tazobactam vs Nonfermenting, Gram-Negative Cystic Fibrosis Isolates

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Ceftolozane/tazobactam (C/T) was tested and compared against 93 nonfermenting, Gram-negative clinical isolates from cystic fibrosis specimens. Based on current breakpoints for intra-abdominal and urinary tract infections (which may not be appropriate for pulmonary infections), C/T was found to be the most active agent against *P. aeruginosa* (95.7% susceptible), followed by piperacillin/tazobactam (89.4% susceptible). For other Gram-negative pathogens included, C/T had varying activity.

Keywords. *Achromobacter* species; ceftolozane/tazobactam; cystic fibrosis; *Pseudomonas aeruginosa*; *Stenotrophomonas maltophilia*.

Pseudomonas aeruginosa is the most frequently isolated pathogen from patients with cystic fibrosis (CF), and by age 25, more than 70% of patients with CF are colonized with this bacterium [1, 2]. Due to chronic colonization and repeated courses of antibiotics, multidrug-resistant (MDR) *P. aeruginosa* is a common problem in this population. As limited therapeutic options exist, treatment often includes combination antibiotic therapy with potentially toxic medications (eg, polymyxins). Additionally, other nonfermenting, Gram-negative pathogens, such as *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, and *Achromobacter* species, are increasingly being isolated from patients with CF, likely due to selective antibiotic pressure. These pathogens have numerous potential resistance mechanisms, such as β-lactamases (including AmpC for *P. aeruginosa*), efflux pumps, and porin modifications.

Ceftolozane is a novel cephalosporin antibiotic that has enhanced activity against *P. aeruginosa*; it also has improved stability vs AmpC β-lactamases compared with other cephalosporins, such as ceftazidime [3]. Ceftolozane is available in combination with tazobactam (Zerbaxa, Merck & Co., Inc), a β-lactamase inhibitor that inhibits many enzymes, including extended-spectrum β-lactamases (ESBLs). By combining with tazobactam, the activity of ceftolozane against certain Gram-negative pathogens is augmented. A potential advantage of ceftolozane/tazobactam (C/T) for the CF population is that it provides potent Gram-negative activity, including MDR *P. aeruginosa*, while sparing carbapenem usage; this is desirable for antimicrobial stewardship programs as the overuse of carbapenems may lead to an increase in carbapenem-resistant *Enterobacteriaceae* (CRE) [4]. The objective of this study was to determine the in vitro activity of C/T compared with other antibiotics vs nonfermenting, Gram-negative CF isolates.

METHODS

Nonfermenting, Gram-negative clinical isolates from CF respiratory specimens obtained from a single academic medical center were included in this study. Mucoid strains of *P. aeruginosa* and duplicate isolates, which were defined as the same species from the same patient over the study period, were excluded from the study. Isolates were identified via matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) mass spectrometry and then frozen at –70°C until susceptibilities could be performed in batches. Antimicrobial susceptibility testing was performed by broth microdilution via custom panels obtained from Remel Microbiology Products (Thermo Scientific, Lenexa, KS). At the time of testing, panels were inoculated according to the manufacturer's instructions (final concentration in the well of $2–7 \times 10^5$ colony-forming units) and incubated aerobically for 18–24 hours at 35°C. All procedures were performed in accordance with methodology established by the Clinical Laboratory Standards Institute (CLSI) or manufacturer labeling. Susceptibility testing was performed once for each isolate. For C/T, minimum inhibitory concentrations (MICs) for *P. aeruginosa* were used to determine clinical category by CLSI breakpoints. Because there are currently no breakpoints established by either the Food and Drug Administration or the CLSI for C/T for the other Gram-negative pathogens included in this study, MIC values with no interpretative category were reported. CLSI-established breakpoints were used for all other antibiotics and bacteria if available.

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RESULTS

Ninety-three Gram-negative clinical isolates collected over an 8-month period were included in this study. The isolates included were primarily *P. aeruginosa* (n = 47, 50.5%), followed by *S. maltophilia*, *Achromobacter* species, and *B. cepacia* (Table 1). Other nonfermenting Gram-negative organisms included *Acinetobacter baumannii* (2), *Pseudomonas fluorescens/putida* (2), *Ralstonia* species (2), *Delftia acidovorans* (1),

Table 1. Minimum Inhibitory Concentration Distribution by Broth Microdilution (mcg/mL)

Isolate/Antibiotic	≤0.25	0.5	1	2	4	8	16	32	64	128	≥256	% Susceptible	MIC_{90}
P. aeruginosa (n = 47)													
C/T	***8***	***22***	***9***	***4***	***2***	1	0	0	1	0	0	95.7	2
P/T	4	5	4	9	8	5	**7**	0	0	2	3	89.4	128
Cefepime	0	0	4	9	12	**12**	5	1	2	1	1	78.7	32
Meropenem	18	10	5	**3**	3	3	3	1	0	1	0	76.6	16
Tobramycin	1	11	15	6	**5**	4	2	0	1	2	0	80.9	16
Amikacin	0	0	0	6	5	16	**5**	5	4	4	2	68.1	128
Levofloxacin	2	5	6	**11**	7	7	4	3	1	0	1	51.1	32
S. maltophilia (n = 13)													
C/T	***5***	***2***	***0***	***0***	***4***	1	0	1	0	0	0	N/A	8
P/T	0	0	0	0	0	1	3	3	2	1	3	N/A	≥256
Cefepime	1	0	0	1	2	1	4	3	1	0	0	N/A	32
Meropenem	0	0	0	1	1	0	0	2	2	5	2	N/A	≥256
Tobramycin	0	1	3	0	0	0	2	1	2	0	4	N/A	≥256
Amikacin	1	0	0	2	2	1	1	0	1	1	4	N/A	≥256
Levofloxacin	3	2	4	**2**	1	1	0	0	0	0	0	84.6	4
Achromobacter spp. (n = 9)													
C/T	***0***	***0***	***0***	***1***	***0***	2	1	0	5	0	0	N/A	N/A
P/T	3	0	4	0	0	0	**1**	1	0	0	0	88.9	N/A
Cefepime	0	0	0	0	1	**2**	1	1	1	2	1	33.3	N/A
Meropenem	3	0	2	1	**0**	0	0	1	1	1	0	66.7	N/A
Tobramycin	0	0	0	1	**1**	0	0	0	1	0	6	22.2	N/A
Amikacin	0	0	0	0	0	1	**1**	1	0	0	6	22.2	N/A
Levofloxacin	0	2	1	**0**	2	1	0	1	2	0	0	33.3	N/A
B. cepacia (n = 7)													
C/T	***0***	***1***	***0***	***0***	***2***	0	1	1	2	0	0	N/A	N/A
P/T	0	0	1	1	1	0	2	0	0	1	1	N/A	N/A
Cefepime	0	0	0	0	0	1	1	2	1	1	1	N/A	N/A
Meropenem	0	0	0	3	**1**	3	0	0	0	0	0	57.1	N/A
Tobramycin	0	0	0	2	0	0	1	1	1	0	2	N/A	N/A
Amikacin	0	0	0	0	1	1	0	1	1	0	3	N/A	N/A
Levofloxacin	0	0	2	**1**	0	2	0	2	0	0	0	42.9	N/A
Chryseobacterium species (n = 6)													
C/T	***1***	***2***	***2***	***1***	***0***	0	0	0	0	0	0	N/A	N/A
P/T	1	0	2	0	0	2	**0**	0	0	0	1	83.3	N/A
Cefepime	1	0	2	2	1	**0**	0	0	0	0	0	100	N/A
Meropenem	0	0	1	0	**0**	1	1	2	1	0	0	16.7	N/A
Tobramycin	0	0	0	0	**0**	0	0	0	0	1	5	0	N/A
Amikacin	0	0	0	0	0	0	**0**	2	3	0	1	0	N/A
Levofloxacin	3	0	1	**1**	0	1	0	0	0	0	0	83.3	N/A
Other nonfermenting Gram-negative organisms (n = 11)													
C/T	***4***	***2***	***0***	***0***	***0***	0	4	0	1	0	0	N/A	N/A
P/T	1	0	2	2	4	1	**0**	0	1	0	0	90.9	N/A
Cefepime	0	2	1	4	1	**1**	2	0	0	0	0	81.8	N/A
Meropenem	3	1	2	0	**0**	1	0	0	3	1	0	54.5	N/A
Tobramycin	3	2	0	2	**0**	0	0	0	1	2	1	63.6	N/A
Amikacin	0	2	2	1	2	0	**1**	0	0	0	3	72.7	N/A
Levofloxacin	6	3	1	**0**	0	1	0	0	0	0	0	90.9	N/A

Bold indicates the Clinical Laboratory Standards Institute susceptible breakpoint; bold italics highlighting indicates the *P. aeruginosa* ceftolozane/tazobactam breakpoint applied to all organisms.

Abbreviations: C/T, ceftolozane/tazobactam; N/A, not available; P/T, piperacillin/tazobactam.

Elizabethkingia menigoseptica (1), *Myroides odoratus* (1), *Pandorea pulmonicola* (1), and *Pseudomonas mendocina* (1). The MIC distribution is presented in Table 1. For *P. aeruginosa*, C/T was the most potent of the antibiotics tested (MIC_{90} = 2 mcg/mL); the 3 most active antibiotics against *P. aeruginosa* isolates were C/T (95.7% susceptible), piperacillin/tazobactam (89.4% susceptible), and tobramycin (80.9% susceptible). If applying *P. aeruginosa* breakpoints to the 46 non–*P. aeruginosa* isolates, C/T appears to have some in vitro activity against *Chryseobacterium* sp. (100% "susceptible"), *S. maltophilia* (84.6% "susceptible"), and *B. cepacia* (42.8% "susceptible"), as shown in Table 1.

DISCUSSION

Based on in vitro testing, C/T has the potential to be a valuable therapeutic option for CF respiratory pathogens, specifically *P. aeruginosa*. For the 47 *P. aeruginosa* isolates included in our study, C/T was the most active antibiotic tested, with 95.7% of isolates being susceptible (MIC ≤ 4 mcg/mL) with an MIC_{90} of 2 mcg/mL. There was only 1 intermediate and 1 resistant isolate in our study. However, despite being clinical CF isolates, most isolates retained susceptibility to antipseudomonal beta-lactams, specifically piperacillin/tazobactam. Other in vitro studies have also found enhanced activity of C/T vs *P. aeruginosa*, including MDR strains from patients with CF [5–12]. In a very large multicenter in vitro study of non-CF *P. aeruginosa* from all culture sites (n = 3737), 97.3% of isolates were susceptible to C/T (MIC_{90} = 2 mcg/mL); for the MDR isolates (n = 783), which was defined as resistance to at least 1 agent in ≥3 antimicrobial classes, 88.6% were susceptible (MIC_{90} = 8 mcg/mL) [11]. This study also included extensive drug-resistant isolates (n = 348), with drug resistance defined as resistance to at least 1 agent in all but 2 or fewer antimicrobial classes, and C/T retained substantial activity (77.6% susceptible, MIC_{90} = 32 mcg/mL). Studies have determined that high-level resistance to C/T in *P. aeruginosa* occurs primarily in strains that have acquired multiple mutations, which leads to overexpression and structural modifications of AmpC [6, 13, 14].

For the other nonfermenting, Gram-negative isolates included in our study, it was found that C/T had varying activity. We observed relatively potent activity against *S. maltophilia,* with an MIC_{90} of 8 mcg/mL, but only 13 isolates were included. However, other larger studies have not found significant activity of C/T for *S. maltophilia,* with the MIC_{90} being >32 mcg/mL [5, 8]. For *B. cepacia*, our study included only 7 isolates, which were found to have varying MICs (range, 0.5–64 mcg/mL). Other studies have found that ceftolozane had activity similar to ceftazidime for *B. cepacia*, which is generally considered to be one of the most active β-lactams against this pathogen [9, 10]. An additional study found that C/T had an MIC_{90} of 4 mcg/mL for *B. cepacia* (n = 22) [15]. Lastly, C/T appears to have limited activity vs *Achromobacter* species, which has been observed in another in vitro study as well [5].

It has been determined that the pharmacokinetic–pharmacodynamic target associated with ceftolozane efficacy is time above the MIC of ~30% of the dosing interval [16]. The current CLSI breakpoint of ≤4/4 mcg/mL is based on the approved intra-abdominal and urinary tract infection dosing regimen of 1.5 g (1 g ceftolozane/0.5 g tazobactam) intravenously every 8 hours for adult patients with normal renal function. However, antimicrobial concentrations are much lower in lung epithelial lining fluid than in serum (48% of serum concentration for ceftolozane) and easily penetrable sites of infection, such as urine [17]. Therefore, given the likelihood of reduced ceftolozane concentrations in the lung, susceptibility results for pathogens from respiratory specimens based on the current breakpoints should be interpreted with caution, and consideration should be given for dose optimization. For nosocomial pneumonia, Monte Carlo simulations have shown that a higher dose (3 g [2 g ceftolozane/1 g tazobactam] intravenously every 8 hours) is required to achieve greater than 90% probability of target attainment against pathogens with an MIC ≤8 mcg/mL [18]. Currently, this more aggressive C/T dosing regimen is being evaluated for pulmonary infections [19]. A small study of 20 adult patients with CF found that a short course using this dosing regimen was well tolerated with minimal adverse events [20]. Based on this information, if using C/T off-label for treatment of CF pulmonary exacerbations, clinicians may consider using the higher dose of C/T.

It is recognized that our study has several limitations, such as the limited number of isolates included. Additionally, susceptibility testing was only performed once on each isolate due to laboratory space constraints; however, this practice is routine in most clinical microbiology laboratories.

In conclusion, C/T appears to be a potentially useful agent for the treatment of CF pulmonary exacerbations based on in vitro activity, particularly for *P. aeruginosa*. Further studies are needed to determine the clinical efficacy of this medication in this population with infections with *P. aeruginosa* and other nonfermenting, Gram-negative rods.

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