Evaluation of the In Vitro Activity of Ceftazidime-Avibactam and Ceftolozane-Tazobactam against Meropenem-Resistant Pseudomonas aeruginosa Isolates


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We compared ceftazidime-avibactam, ceftolozane-tazobactam, ceftazidime, cefepime, and piperacillin-tazobactam MICs for 38 meropenem-resistant Pseudomonas aeruginosa isolates. No isolates harbored carbapenemases; 74% were oprD mutants. Ceftazidime-avibactam and ceftolozane-tazobactam were active against 92% of the isolates, including 80% that were resistant to all three β-lactams. Forty-three percent of ceftazidime-avibactam-susceptible isolates and 6% of ceftolozane-tazobactam-susceptible isolates exhibited MICs at the respective breakpoints. Ceftolozane-tazobactam and ceftazidime-avibactam are therapeutic options for meropenem-resistant P. aeruginosa infections that should be used judiciously to preserve activity.

Pseudomonas aeruginosa has a remarkable propensity to develop antibiotic resistance (1). β-Lactam resistance in P. aeruginosa is mediated through several mechanisms, including β-lactamase production, altered membrane permeability, MexA-MexB-OprM efflux pump overexpression, and penicillin-binding protein alterations. Inducible extended-spectrum AmpC cephalosporinases may confer reduced susceptibility to all cephalosporins (2, 3). Carbapenemases are stable to AmpC cephalosporinases alone, but activity may be attenuated by combinations of Ambler class A or B β-lactamases, AmpC production, efflux pump up-regulation, and oprD porin mutations (4, 5). Carbapenem-resistant P. aeruginosa strains are often resistant to antipseudomonal agents such as ceftazidime, cefepime, and piperacillin-tazobactam (6).

Avibactam, a new non-β-lactam β-lactamase inhibitor, inactivates extended-spectrum β-lactamases (ESBLs), AmpC cephalosporinases, and class A (including Klebsiella pneumoniae carbapenemases [KPC]), class C, and some class D β-lactamases (7). Ceftazidime-avibactam, an agent recently approved by the U.S. Food and Drug Administration (FDA), shows promising activity against carbapenem-resistant Enterobacteriaceae (CRE) strains, such as KPC-producing K. pneumoniae and Escherichia coli strains. Carbapenemase production is the primary determinant of carbapenem resistance among CRE strains. Ceftazidime-avibactam is less certain to be active against carbapenem-resistant P. aeruginosa, since resistance mechanisms are multifactorial. Ceftriaxone, a novel cephalosporin, has less affinity for hydrolysis by Amp C cephalosporinases, is a weak substrate for drug efflux systems, and is not affected by OprD loss (4, 8–10). The addition of the β-lactamase inhibitor tazobactam broadens the activity of ceftriaxone to include most ESBL-producing Gram-negative bacilli (11). In this study, we measured ceftazidime-avibactam and ceftriaxone-tazobactam activities in vitro against meropenem-resistant P. aeruginosa isolates that exhibited a range of susceptibilities to ceftazidime, cefepime, and piperacillin-tazobactam.

Bloodstream (n = 20) and respiratory tract (n = 18) isolates were collected from unique patients at the University of Pittsburgh Medical Center. The MICs of all agents except ceftolozane-tazobactam were determined in triplicate by reference broth microdilution methods (12). Avibactam was tested at a fixed concentration (4 µg/ml). Ceftazidime-tazobactam MICs were measured by Etest (bioMérieux), according to the manufacturer’s recommendations. MICs were interpreted using CLSI reference breakpoints for ceftazidime, cefepime, piperacillin-tazobactam, and meropenem; isolates classified as intermediate or resistant by CLSI criteria were defined as resistant. FDA-approved susceptibility breakpoints of ≤4 µg/ml and ≤8 µg/ml were used for ceftolozane-tazobactam and ceftazidime-avibactam, respectively. E. coli ATCC 25922 and P. aeruginosa ATCC 27853 were used for quality controls. PCR was used to detect Amber class A (TEM, SHV,CTX-M, GES, PER, VEB, and KPC), class B (metallo-β-lactamases VIM, IMP, and NDM), class C (CMY, MOX, FOX, ACT, and DHA), and class D (OXA) β-lactamases; oprD mutations were detected by PCR and DNA sequencing. The contributions of efflux to ceftazidime-avibactam and ceftolozane-tazobactam resistance were assessed with the efflux pump inhibitor carbonyl cyanide m-chlorophenylhydrazone (CCCP), at a fixed concentration of 12.5 µg/ml. Comparisons involving categorical or continuous variables were made using the Fisher exact test or the Mann-Whitney test, respectively.

We tested 38 meropenem-resistant P. aeruginosa isolates (Table 1). None of the isolates harbored class A, B, C, or D β-lactamases. Seventy-four percent (28/38) of isolates carried oprD mutations, compared to reference strain PAO1, including non-


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P. aeruginosa tazobactam MICs were moderately to strongly correlated with ceftolozane-tazobactam MICs were strongly correlated (Spearman’s *r* ranges of 0.86 to 0.93 and 0.84 to 0.98, respectively) (Table 2). The median ceftazidime-avibactam and ceftolozane-tazobactam MICs were 4 g/ml and 4 g/ml, respectively. Ceftazidime-avibactam and ceftolozane-tazobactam MICs were strongly correlated (Spearman’s *r* = 0.91; *P* < 0.0001). The MIC₉₀s for ceftazidime-susceptible vs ceftazidime-resistant isolates 0.0009. Ceftazidime-avibactam and ceftolozane-tazobactam MICs were at or above the susceptibility breakpoint (8 g/ml). In contrast, 6% (2/35) of isolates that were susceptible to ceftolozane-tazobactam exhibited MICs at the susceptibility breakpoint (4 g/ ml; 15/35 versus 2/35, *P* = 0.0005). Overall, 47% (18/38) and 13% (5/38) of isolates exhibited ceftazidime-avibactam and ceftolozane-tazobactam MICs that were at or above the respective breakpoints (*P* = 0.003).

All isolates that were resistant to either ceftazidime-avibactam or ceftolozane-tazobactam were also resistant to all 3 β-lactam agents. Ceftazidime-avibactam MICs were at or above the susceptibility breakpoint for 0% (0/10), 57% (4/7), 67% (4/6), and 67% (10/15) of isolates that were resistant to 0, 1, 2, and 3 β-lactam agents, respectively (Fig. 1). The corresponding rates for ceftolozane-tazobactam MICs at or above the susceptibility breakpoint were 0% (0/10 isolates), 0% (0/7), 0% (0/6), and 33% (5/15) of isolates, respectively (Fig. 1). Isolates that were resistant to ≥2 β-lactams were significantly more likely to exhibit MICs at or above the breakpoint for ceftazidime-avibactam (67% [14/21]) than that for ceftolozane-tazobactam (24% [5/21]; *P* = 0.03). Isolates that were resistant to 3 β-lactams were also more likely to exhibit MICs at or above the breakpoint for ceftazidime-avibactam (67% [10/15]) than that for ceftolozane-tazobactam (33% [5/15]; *P* = 0.14).

Sixty-four percent (18/28) and 30% (3/10) of *oprD* mutants and wild-type isolates, respectively, were resistant to cephalosporins (P = 0.08); 57% (16/28) and 20% (2/10) of isolates, respectively, were resistant to ceftazidime (P = 0.07). There was no difference

### Table 1 In vitro susceptibility data for 38 meropenem-resistant *P. aeruginosa* isolates

<table>
<thead>
<tr>
<th>Antimicrobial agent(s)</th>
<th>Median MIC (µg/ml)</th>
<th>MIC₉₀ (µg/ml)</th>
<th>Resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meropenem</td>
<td>8 (4–512)</td>
<td>32</td>
<td>100</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>16 (2 to &gt;1,024)</td>
<td>256</td>
<td>55</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>32 (8 to &gt;1,024)</td>
<td>256</td>
<td>66</td>
</tr>
<tr>
<td>Ceftolozane-avibactam</td>
<td>4 (2 to &gt;32)</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Ceftolozane-tazobactam</td>
<td>1 (0.25–64)</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

**TABLE 2 Correlations between ceftazidime-avibactam and ceftolozane-tazobactam MICs and MICs for other β-lactam agents**

<table>
<thead>
<tr>
<th>Agent and parameter for correlation</th>
<th>Ceftazidime-avibactam</th>
<th>Ceftolozane-tazobactam</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cefepime</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIC (median) (µg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefepime-susceptible isolates (n = 20)</td>
<td>4</td>
<td>0.75</td>
</tr>
<tr>
<td>Cefepime-resistant isolates (n = 18)</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td><em>P</em> for cefepime-susceptible vs cefepime-resistant isolates</td>
<td>0.0048</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Correlation between cefepime and indicated agents&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.93</td>
<td>0.98</td>
</tr>
<tr>
<td><strong>Ceftazidime</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIC (median) (µg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefazidime-susceptible isolates (n = 17)</td>
<td>4</td>
<td>0.75</td>
</tr>
<tr>
<td>Cefazidime-resistant isolates (n = 21)</td>
<td>8</td>
<td>1.5</td>
</tr>
<tr>
<td><em>P</em> for cefazidime-susceptible vs cefazidime-resistant isolates</td>
<td>0.0009</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>Correlation between cefazidime and indicated agents&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.86</td>
<td>0.93</td>
</tr>
<tr>
<td><strong>Piperacillin-tazobactam</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIC (median) (µg/ml)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piperacillin-tazobactam-susceptible isolates (n = 13)</td>
<td>2</td>
<td>0.75</td>
</tr>
<tr>
<td>Piperacillin-tazobactam-resistant isolates (n = 25)</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td><em>P</em> for piperacillin-tazobactam-susceptible vs piperacillin-tazobactam-resistant isolates</td>
<td>&lt;0.0001</td>
<td>0.0012</td>
</tr>
<tr>
<td>Correlation between piperacillin-tazobactam and indicated agents&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.86</td>
<td>0.84</td>
</tr>
</tbody>
</table>

<sup>a</sup> Correlations between agents were determined using Spearman’s correlation coefficient.
in piperacillin-tazobactam resistance between oprD mutant isolates (71% [20/28]) and wild-type isolates (50% [5/10]; P = 0.26). There were also no differences in ceftazidime-avibactam and cef­tolozane-tazobactam MICs or resistance rates between oprD mutant and wild-type isolates.

To assess efflux, we tested all isolates that were resistant to ceftazidime-avibactam and cef­tolozane-tazobactam, as well as randomly selected susceptible isolates. The median change in the ceftazidime-avibactam MIC was 0-fold (range, 0- to 2-fold) with the addition of CCCP, and median changes did not differ between resistant (n = 3) and susceptible (n = 4) isolates. Similarly, among cef­tolozane-tazobactam-resistant (n = 3) or -susceptible (n = 4) isolates, MICs were not significantly reduced in combination with CCCP (median change, 0-fold [range, 0- to 2-fold]).

To our knowledge, this is the first study to compare the in vitro activities of ceftazidime-avibactam and cef­tolozane-tazobactam against P. aeruginosa clinical isolates. Our most encouraging finding was that each agent was active against 92% of meropenem-resistant P. aeruginosa isolates (35/38), including 80% of isolates (12/15) that were resistant to all three antipseudomonal β-lactams. Overall, at least one of the new agents retained activity against 92% (23/25), 90% (19/21), and 89% (16/18) of isolates that were resistant to piperacillin-tazobactam, ceftazidime, and cefepime, respectively. At the same time, our data provide important cautionary notes. Rates of resistance to ceftazidime-avibactam and cef­tolozane-tazobactam were 8% (3/38 isolates) prior to the introduction of these agents to our hospital. Moreover, ceftazidime-avibactam and cef­tolozane-tazobactam MICs correlated with each other and with ceftazidime, cefepime, and piperacillin-tazobactam MICs, consistent with some degree of cross-resistance. The MICs of both ceftazidime-avibactam and cef­tolozane-tazobactam were significantly higher as isolates became resistant to more β-lactams. These results corroborate recent reports of decreased susceptibility to ceftazidime-avibactam and cef­tolozane-tazobactam among P. aeruginosa isolates that are resistant to other β-lactams, compared with isolates that are susceptible (3, 13–15). Taken together, the data demonstrate that ceftazidime-avibactam and cef­tolozane-tazobactam are important additions to the antimicrobial armamentarium, but findings suggest that the agents will need to be used judiciously to preserve their activity.

Our data suggest that cef­tolozane-tazobactam may be more active than ceftazidime-avibactam against meropenem-resistant P. aeruginosa strains. Isolates were significantly more likely to exhibit MICs at or above the susceptibility breakpoint for ceftazidine-avibactam than that for cef­tolozane-tazobactam. In particular, significantly greater percentages of isolates that were resistant to ≥2 antipseudomonal β-lactams exhibited MICs at or above the ceftazidime-avibactam breakpoint, compared with the cef­tolozane-avibactam breakpoint. These results should be interpreted with the understanding that definitive breakpoint MICs have not been determined for either agent. The ceftazidime-avibactam breakpoint, for example, is based on a ceftazidime dosing regimen of 1 g every 8 h as a 30-min infusion (12), whereas the drug has been administered as 2 g of ceftazidime and 500 mg of avibactam over 2 h in clinical trials (16).

It is also important to appreciate that the superior performance of cef­tolozane-tazobactam may reflect the particular resistance mechanisms of our P. aeruginosa isolates. The isolates in this study did not carry ESBLs or carbapenemases, which is consistent with previous reports from the United States (17, 18), and efflux was not a significant contributor to either ceftazidime-avibactam or cef­tolozane-tazobactam resistance. On the other hand, a sizeable majority of isolates exhibited oprD mutations. Avibactam restores the activity of ceftazidime against Gram-negative bacilli with resistance mediated through ESBLs, class A and some class D β-lactamases, and chromosomal and acquired AmpC class C enzymes (19–22). It is reasonable to hypothesize that avibactam restores susceptibility to ceftazidime through inhibition of AmpC enzymes. Indeed, a recent study showed that 91% of P. aeruginosa strains with unique AmpC sequences (31/34 isolates) demonstrated restored susceptibility to ceftazidime following the addition of avibactam (23). Tazobactam extends the activity of cef­tolozane against most class A and some class C β-lactamases, but the combination is less active than ceftazidime-avibactam against ESBL- or KPC-producing Gram-negative bacteria. Therefore, the isolates in this study were likely better suited to inhibition by cef-

FIG 1 Correlations between ceftazidime-avibactam (A) and cef­tolozane-tazobactam (B) MICs against meropenem-resistant P. aeruginosa isolates and the number of inactive β-lactam agents. Horizontal lines intersecting the y axis, FDA-approved susceptibility breakpoints. The median MICs for isolates that were resistant to 0, 1, 2, or 3 β-lactam agents were compared.
tolozane-tazobactam than ceftazidime-avibactam; results may differ at centers where ESBLs or carbapenemases are more prominent. Clinicians must understand susceptibility patterns at their institutions. Since resistance mechanisms among *P. aeruginosa* strains are complex and multifactorial, detailed molecular character-
ization of isolates should be incorporated into future studies of antimicrobial regimens (24).

We anticipate that ceftolozane-tazobactam will be most useful at our center against infections caused by *P. aeruginosa* strains that are resistant to carbapenems and all β-lactams, as the agent is likely to be more active than ceftazidime-avibactam and less toxic than colistin or gentamicin. We anticipate that ceftazidime-avibactam will be most useful against infections caused by CRE, for which β-lactamases and carbapenemases are predominant resis-
tance determinants. Indeed, ceftazidime-avibactam was more active against ESBL- and KPC-producing, carbapenem-resistant *K. pneumoniae* isolates from our center (MIC range, 0.125 to 4 μg/ml) than reported here for meropenem-resistant *P. aeruginosa* isolates (MIC range, 1 to >32 μg/ml) (25). Further studies are needed to understand how ceftolozane-tazobactam and ceftazi-
dime-avibactam can be best incorporated into clinical practice, in a manner that optimizes effectiveness while minimizing the emer-
gence of resistance.

ACKNOWLEDGMENTS

This project was supported by funding provided to the XDR Pathogen Laboratory by the University of Pittsburgh Medical Center. The content of this article is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

FUNDING INFORMATION

This work, including the efforts of Ryan K Shields, was funded by HHS | National Institutes of Health (NIH) (K08AI114883). This work, including the efforts of Cornelius J. Clancy, was funded by NIH (R21AI222037).

REFERENCES

1. Zilberberg MD, Shorr AF. 2013. Prevalence of multidrug-resistant *Pseudo-


5. Castanheira M, Deshpande LM, Costello A, Davies TA, Jones RN. 2014. Epidemiology and carbapenem resistance mechanisms of carbapenem-


donor CXA-101 (FR624205) against *Pseudomonas aeruginosa* and Burk-


12. Clinical and Laboratory Standards Institute. 2014. Performance stan-
ards for antimicrobial susceptibility testing: 24th informational supple-
ment. M100-S24. Clinical and Laboratory Standards Institute, Wayne, PA.


14. Sutherland CA, Nicolau DP. 2015. Susceptibility profile of ceftolo-
zone/tazobactam and other parenteral antimicrobials against Esche-


16. Sader HS, Castanheira M, Flamm RK, Farrell DJ, Jones RN. 2014. Antimicrobial activity of ceftazidime-avibactam against Gram-


20. Hall JM, Corca E, Sanjeevaneni HD, Inglis TJ. 2014. Molecular mecha-

isms among *Acinetobacter baumannii*- *Acinetobacter calcoaceticus* com-
plex and *Enterobacteriaceae* isolates collected in European and Mediterra-

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