In Vitro Activities of Ceftazidime-Avibactam and Aztreonam-Avibactam against 372 Gram-Negative Bacilli Collected in 2011 and 2012 from 11 Teaching Hospitals in China

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Avibactam is a member of a class of inhibitors called diazabicyclooctanes (DBOs) that does not contain a β-lactam core but maintains the capacity to covalently acylate its β-lactamase targets (1, 2, 3). Avibactam alone has very little intrinsic antimicrobial activity but has been shown to efficiently restore the in vitro activities of cephalosporins (including ceftazidime and cephalotline) (4, 5) against Ambler class A, class C, and some class D β-lactamases (6, 7, 8, 9, 10), excluding metallo-β-lactamases (MBLs) and Acinetobacter OXA carbapenemases (2). The combination of aztreonam and avibactam has been proposed as a principal candidate for the treatment of infections with MBL-producing Gram-negative organisms (8, 11). To date, few data have been available in China describing the in vitro activities of ceftazidime-avibactam and aztreonam-avibactam against clinical Gram-negative bacilli, especially Enterobacteriaceae. In this study, we evaluated the in vitro activities of ceftazidime and aztreonam alone and combined with a fixed concentration of 4 mg/liter of avibactam against routinely collected clinical Gram-negative bacilli, including Enterobacteriaceae, Acinetobacter spp., and Pseudomonas aeruginosa, from two national surveillance programs in China, the Chinese Meropenem Susceptibility Surveillance (CMSS) program in 2012 and the Chinese Antimicrobial Resistance Surveillance of Nosocomial Infections (CARES) in 2011.

A total of 372 nonrepetitive, routinely collected isolates (from 2011 and 2012) were obtained from 11 teaching hospitals representing the south, north, northwest, east, and middle districts of mainland China. The 372 organisms included the following. (i) Two hundred ninety-one routinely collected but otherwise unselected Enterobacteriaceae isolates (from CMSS) and 26 carbapenem-nonsusceptible Enterobacteriaceae isolates (meropenem MIC, ≥2 mg/liter; 19 isolates from CARES and the remainder from CMSS) were tested. Of the 26 carbapenem-nonsusceptible Enterobacteriaceae isolates, 10 produced KPC-2, 11 produced IMP β-lactamases (10 isolates produced IMP-4 and 1 isolate produced IMP-8), and 3 produced NDM-1. The remaining 2 isolates possessed TEM-1 and CTX-M, enzymes that are not regarded as carbapenemases. Most of the carbapenemase producers coharbored other β-lactamases (ACT-14/15, CMY-2, DHA-1, and SHV-12/11/107) (Table 1). (ii) Thirty routinely collected but otherwise unselected Acinetobacter isolates (from CMSS), including 11 carbapenem-nonsusceptible isolates in which coexisting genes were detected encoding OXA-23-like, OXA-51-like, and TEM-1 β-lactamases, were tested. (iii) Twenty-five routinely collected but otherwise unselected P. aeruginosa isolates (from CMSS), including 11 carbapenem-nonsusceptible bacteria that harbored genes encoding OXA-50-like and/or TEM-1 β-lactamases, were tested. All the isolates, obtained from intra-abdominal, urinary tract, respiratory tract, or bloodstream infections, were sent to the central laboratory (Laboratory Medicine, Peking University People’s Hospital, Beijing, China) for reidentification and antibiotic susceptibility testing. The Vitek GNI system (bioMérieux Vitek Inc., Hazelwood, MO) or API20E or API20NE (bioMérieux, Marcy l’Etoile, France) was used for bacterial identification.

MIC measurements were performed by the reference broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI) M7-A9 (2012) (12). MICs of ceftazidime and aztreonam alone and in combination with avibactam (AstraZeneca Pharmaceuticals) at a fixed concentration of 4 mg/liter (CLSI M100-S23, 2013) (13) were measured. Antibiotic solutions for susceptibility testing were prepared fresh; i.e., dried panels were not used in this study. MICs of ceftazidime, aztreonam, and comparator agents were interpreted according to CLSI criteria in M100-S23, 2013 (13).

For meropenem-nonsusceptible Gram-negative bacilli (Table 1), PCR was used to amplify carbapenemase genes (blaKPC, blaNDM, blalM, blaOXA-24-like, blaoxa-24-like, blaoxa-51-like, blaoxa-58-like, and blaoxa-50-like) and...
Table 1. In vitro activities of ceftazidime-avibactam, aztreonam-avibactam, and comparators against meropenem-nonsusceptible beta-lactamase-produc

<table>
<thead>
<tr>
<th>Isolate Description</th>
<th>MIC (mg/L)</th>
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<tr>
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Note: MIC values represent the minimum inhibitory concentration in milligrams per liter.
In the present study, both clavulanic acid (data not shown) protected by clavulanic acid (with no MIC90 reduction; data not shown) was 8 mg/liter for 9 of 25 isolates (36%) (data not shown). For 18 of 25 isolates (72%) (data not shown). For 18 of 25 isolates (72%), and the MIC of aztreonam-avibactam was 8 mg/liter for 9 of 25 isolates (36%) (data not shown). For 18 P. aeruginosa isolates that were ceftazidime and/or aztreonam nonsusceptible, avibactam restored the activities of ceftazidime and aztreonam against 3 of these isolates with 128- and 256- to 1,024-fold MIC90 reduction, respectively. The MIC of ceftazidime-avibactam for 25 unselected clinical isolates of P. aeruginosa was 8 mg/liter (Table 2), as was found previously in studies from Canada (18) and France (19). The MIC of ceftazidime-avibactam was ≤8 mg/liter for 24 of 25 isolates (96%) (data not shown). For 18 P. aeruginosa isolates that were ceftazidime and/or aztreonam nonsusceptible, the addition of avibactam reduced the MIC90 of ceftazidime from 16 to 8 mg/liter (Table 2; Fig. 1). The MIC of aztreonam alone was ≤8 mg/liter for 7 of 25 P. aeruginosa isolates (28%), and the MIC of aztreonam-avibactam was ≤8 mg/liter for 9 of 25 isolates (36%) (data not shown). Eleven P. aeruginosa isolates were phenotypically identi-
fied as meropenem nonsusceptible; however, the mechanisms of nonsusceptibility were not clarified by \textit{bla} gene analysis. The only nonresident \textit{\beta}-lactamase gene identified was \textit{bla}_{\text{TEM-1}}, in 3 of the isolates (Table 1). Regardless of mechanism, more than 90% of ceftazidime-avibactam MICs for these 11 carbapenem-nonsusceptible \textit{P. aeruginosa} isolates were $\leq 8$ mg/liter (Table 1).

MIC$_{90}$s of ceftazidime and aztreonam against \textit{Acinetobacter baumannii} were lowered by $>4$-fold and $>2$-fold, respectively, upon combining with avibactam at 4 mg/liter (Table 2). For the 11 meropenem-nonsusceptible \textit{A. baumannii} isolates that harbored nonresident genes encoding OXA-23-like and TEM-1 \textit{\beta}-lactamases, the ranges of MICs of ceftazidime-avibactam and aztreonam-avibactam were relatively high, at 16 to 128 and 32 to 64 mg/liter, respectively, even though avibactam reduced the MIC$_{90}$s somewhat (Tables 1 and 2).

In conclusion, the \textit{in vitro} activity of ceftazidime-avibactam against bacteria isolated from patients in China supports further evaluation of ceftazidime-avibactam in clinical studies against ESB$L^-$, AmpC-, and serine carbapenemase-producing \textit{Enterobacteriaceae} isolates. In \textit{in vitro}, ceftazidime-avibactam showed more activity than a carbapenem against carbapenem-nonsusceptible and KPC-producing isolates. At the same time, aztreonam-avibactam could serve as a candidate for the treatment of infections with MBL-producing \textit{Enterobacteriaceae}, especially NDM-producing organisms (Table 1) (19). In both cases, additional studies are needed to establish what the potential roles of ceftazidime-avibactam and aztreonam-avibactam might be as substitutes for carbapenems to reduce the dissemination of carbapenemases in the future.

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**REFERENCES**


