**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 antibacterial activity but has been shown to efficiently restore the *in vitro* activities of cephalosporins (including ceftazidime and cefotaroline) (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 GN 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 (*bla*KPC, *bla*NDM, *bla*IMP, *bla*VIM, *bla*OXA-24, *bla*OXA-23-like, *bla*OXA-48, *bla*OXA-24-like, *bla*OXA-51-like, *bla*OXA-58-like, and *bla*OXA-50-like) and...
<table>
<thead>
<tr>
<th>Isolates</th>
<th>Year</th>
<th>Origin</th>
<th>Species</th>
<th>Minimum Inhibitory Concentration (MIC) (µg/mL)</th>
</tr>
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<tbody>
<tr>
<td>A. baumannii</td>
<td>2012</td>
<td>SH</td>
<td>Enterobacteriaceae</td>
<td>2012 SH ND TEM-1, ACT-15, CTX-M-31</td>
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<tr>
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<td>2011</td>
<td>BJ</td>
<td>Enterobacteriaceae</td>
<td>2011 BJ ND TEM-1, ACT-15, CTX-M-31</td>
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<td>Escherichia coli</td>
<td>2011</td>
<td>FZ</td>
<td>Enterobacteriaceae</td>
<td>2011 FZ ND IMP-8, DHA-1</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>2011</td>
<td>BJ</td>
<td>Enterobacteriaceae</td>
<td>2011 BJ 876 IMP-4, DHA-1</td>
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<td>2011 BJ 1100 IMP-4, SHV-1</td>
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<td></td>
<td></td>
<td>2011 BJ 876 IMP-4, TEM-1</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>2011</td>
<td>GZ</td>
<td>Enterobacteriaceae</td>
<td>2011 GZ 11 KPC-2, CMY-2, SHV-11, CTX-M-14</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2011 SH 11 KPC-2, TEM-1, SHV-12</td>
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<tr>
<td>CAZ-AVI and ATM-AVI against Gram-Negative Bacilli</td>
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</table>

Table 1: Minimum Inhibitory Concentration (MIC) of CAZ-AVI and ATM-AVI against Gram-Negative Bacilli.
other β-lactamase genes (blaTEM, blaSHV, blaCTX-M, bla AmpC, blaOXA, and blaCMY) according to procedures described in previous studies (14). All PCR products were sequenced with an ABI7500 sequencer (Applied Biosystems, Foster City, CA).

In this study, avibactam potentiated the activities of ceftazidime and aztreonam in vitro against most species of clinical isolates of Enterobacteriaceae tested. For isolates of Enterobacteriaceae that were nonsusceptible to ceftazidime and/or cefotaxime (33%), 97 of 291), the addition of 4 mg/liter avibactam greatly increased the activities of ceftazidime and aztreonam against most species (64–1,024-fold MIC90 reduction) (Table 2). For the ceftazidime- and/or cefotaxime-nonsusceptible isolates of Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, and Providencia rettgeri in the present study, both clavulanic acid (data not shown) and avibactam restored the activity of ceftazidime. For the isolates of Citrobacter freundii, Enterobacter aerogenes, Enterobacter cloacae, and Morganella morganii that were nonsusceptible to ceftazidime and/or ceftaxime, the activity of ceftazidime could not be protected by clavulanic acid (with no MIC90 reduction; data not shown) but avibactam retained its protection of ceftazidime and aztreonam, with clear MIC90 reductions (64- to 256-fold). It is possible that a large proportion of the ceftazidime/cefotaxime-nonsusceptible isolates of these species produced stably derepressed AmpC β-lactamases (15–17).

Avibactam potentiated the activities of ceftazidime and aztreonam against carbapenem-nonsusceptible Enterobacteriaceae that coharbored genes encoding KPC-2 and other class A enzymes (CTX-M-14/3, SHV-11/12, and TEM-1/1b) or class C enzymes (ACT-15, and CMY-2) (Table 1). Against carbapenem-nonsusceptible Enterobacteriaceae that carried genes encoding MBLs, such as IMP-4/8 and NDM-1, avibactam did not restore the activity of ceftazidime; however, the aztreonam-avibactam combination remained active (8).

The MIC90 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 bla gene analysis. The only nonresident β-lactamase gene identified was blaTEM-1, in 3 of the isolates (Table 1). Regardless of mechanism, more than 90% of ceftazidime-avibactam MICs for these 11 carbapenem-nonsusceptible P. aeruginosa isolates were ≤8 mg/liter (Table 1).

MIC₉₀s of ceftazidime and aztreonam against 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 A. baumannii isolates that harbored nonresident genes encoding OXA-23-like and TEM-1 β-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 MIC₉₀s somewhat (Tables 1 and 2).

In conclusion, the in vitro activity of ceftazidime-avibactam against bacteria isolated from patients in China supports further evaluation of ceftazidime-avibactam in clinical studies against ESBL-, AmpC-, and serine carbapenemase-producing Enterobacteriaceae isolates. 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 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.

ACKNOWLEDGMENTS

We express our appreciation to the staff members of the Department of Clinical Laboratory of Peking University People’s Hospital for assistance in performing the study.

REFERENCES


FIG 1 Ceftazidime, ceftazidime-avibactam, aztreonam, and aztreonam-avibactam MIC distributions for 291 Enterobacteriaceae isolates (excluding meropenem-nonsusceptible isolates), 30 Acinetobacter isolates, and 25 P. aeruginosa isolates.