Exploring Aztreonam in Combination with Ceftazidime-Avibactam or Meropenem-Vaborbactam as Potential Treatments for Metallo- and Serine-β-Lactamase-Producing Enterobacteriaceae

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ABSTRACT Metallo-β-lactamase (MBL)-producing Enterobacteriaceae, particularly those that coharbor serine β-lactamases, are a serious emerging public health threat given their rapid dissemination and the limited number of treatment options. Preclinical and anecdotal clinical data support the use of aztreonam in combination with ceftazidime-avibactam against these pathogens, but other aztreonam-based combinations have not been explored. The objective of this study was to evaluate the in vitro activity and compare synergy between aztreonam in combination with ceftazidime-avibactam and meropenem-vaborbactam against serine and MBL-producing Enterobacteriaceae via time-kill analyses. Eight clinical Enterobacteriaceae strains (4 Escherichia coli and 4 Klebsiella pneumoniae) coproducing NDM and at least one serine β-lactamase were used for all experiments. Drugs were tested alone, in dual-β-lactam combinations, and in triple-drug combinations against all strains. All strains were resistant to ceftazidime-avibactam and meropenem-vaborbactam and 7/8 (87.5%) strains were resistant to aztreonam. Aztreonam combined with ceftazidime-avibactam was synergistic against all 7 aztreonam-resistant strains. Aztreonam combined with meropenem-vaborbactam was synergistic against all aztreonam-resistant strains with the exception of an OXA-232-producing K. pneumoniae strain. Neither triple combination was synergistic against the aztreonam-susceptible strain. These data suggest that aztreonam plus meropenem-vaborbactam has similar activity to aztreonam plus ceftazidime-avibactam against Enterobacteriaceae producing NDM and other non-OXA-48-like serine β-lactamases. Confirmation of these findings in future in vitro and in vivo models is warranted.

KEYWORDS metallo-β-lactamase, NDM, aztreonam, ceftazidime-avibactam, meropenem-vaborbactam, synergy

The now widespread dissemination of carbapenem-resistant Gram-negative organisms (CRO) threatens our remaining antibiotic armamentarium and poses enormous health concerns both nationally and internationally (1, 2). Infections due to CROs are associated with high mortality rates and are often treated with antimicrobials that have limited efficacy data and high toxicity, such as the polymyxins (3). The increasing prevalence of CROs has spurred the need for development of novel agents with improved activity against these pathogens and more data evaluating optimal treatment regimens (4, 5). Although several new β-lactam/β-lactamase inhibitor combination agents have been developed, neither ceftazidime-avibactam nor meropenem-vaborbactam has activity against the entire range of CROs (6, 7). Importantly, neither agent has activity against metallo-β-lactamase (MBL)-producing organisms from the VIM, IMP, or NDM groups within the Ambler class B enzymes.

NDM-producing CROs are particularly concerning given their rapid global dissemi-
TABLE 1 Genotypic and phenotypic susceptibility of tested serine and NDM-producing *E. coli* and *K. pneumoniae* isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Genotypic profile</th>
<th>MIC (mg/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM</td>
<td>ATM+AVI</td>
<td>ATM+VBR</td>
</tr>
<tr>
<td>EC-1</td>
<td>NDM, CMY-2/FOX, CTX-M-1, TEM</td>
<td>≥256</td>
</tr>
<tr>
<td>EC-2</td>
<td>NDM, TEM</td>
<td>16</td>
</tr>
<tr>
<td>EC-3</td>
<td>NDM-1, CMY-6, CTX-M-15, TEM-1</td>
<td>≥256</td>
</tr>
<tr>
<td>EC-4</td>
<td>NDM-5, OXA-1</td>
<td>0.25</td>
</tr>
<tr>
<td>KP-1</td>
<td>NDM-1, CMY-6, CTX-M-15, OXA-1, SHV-11, TEM-1B</td>
<td>128</td>
</tr>
<tr>
<td>KP-2</td>
<td>NDM-1, CTX-M-15, OXA-232, SHV, TEM</td>
<td>≥256</td>
</tr>
<tr>
<td>KP-3</td>
<td>NDM, SHV</td>
<td>128</td>
</tr>
<tr>
<td>KP-4</td>
<td>NDM, CTX-M-1 group, SHV, TEM</td>
<td>≥256</td>
</tr>
</tbody>
</table>

*Avibactam and vaborbactam were tested at fixed concentrations of 4 and 8 mg/liter, respectively.*

*ATM, aztreonam; AVI, avibactam; VBR, vaborbactam; CAZ, ceftazidime; MER, meropenem.*

nation (8) and their ability to hydrolyze all available β-lactam antibiotics, with the exception of aztreonam (9). Despite its activity against MBLs, aztreonam is readily hydrolyzed by most serine β-lactamases which are often coharbored with the MBL enzyme (10), making it ineffective as monotherapy against these pathogens. As the fixed-dose combination of aztreonam-avibactam is not yet available for clinical use (11), data have emerged supporting the use of aztreonam in combination with ceftazidime-avibactam. These are primarily limited to anecdotal clinical reports (12, 13) and in vitro data (14–16). Importantly, there are no data evaluating aztreonam in combination with meropenem-vaborbactam against pathogens coharboring serine and MBL enzymes.

While the theory behind combining aztreonam with either ceftazidime-avibactam or meropenem-vaborbactam is the same, notable differences between these combinations are present upon comparison of the penicillin-binding protein (PBP) targets of the β-lactams (ceftazidime and meropenem) and the β-lactamase affinity of the inhibitors (avibactam and vaborbactam) (17). The impact of these important differences on the activity of aztreonam-based combinations has yet to be explored.

As there is an urgent need to identify optimal treatment regimens against serine and MBL-producing CROs, the objective of this study was to evaluate and compare the *in vitro* activity of aztreonam plus ceftazidime-avibactam and aztreonam plus meropenem-vaborbactam against clinical *Escherichia coli* and *Klebsiella pneumoniae* strains coproducing NDM and one or more serine β-lactamases.

(Results of this study were presented in part at IDWeek 2018 San Francisco, CA, USA, as abstract 2443 [18] and at ECCMID 2019 Amsterdam, Netherlands as abstract 6204 [19].)

RESULTS

Genotypic and phenotypic susceptibility profiles are displayed in Table 1. All strains were resistant to ceftazidime, ceftazidime-avibactam, meropenem, and meropenem-vaborbactam. Likewise, all strains were resistant to aztreonam except for EC-4 (MIC, 0.25 mg/liter). For the seven aztreonam-resistant strains, the addition of either avibactam or vaborbactam to aztreonam reduced the MIC by 4-log₂ to 12-log₂ or 1-log₂ to 10-log₂ dilutions, respectively (Table 1).

Results of time-kill experiments of each commercially available agent alone at the highest concentration tested against each *E. coli* and *K. pneumoniae* strain are displayed in Fig. 1 and 2, respectively. Neither ceftazidime (with or without avibactam) nor meropenem (with or without vaborbactam) was bactericidal alone at 24 h regardless of the concentration tested (Fig. 1 and 2). Against the *E. coli* strains, aztreonam was not bactericidal against strains EC-1 and EC-3 (Fig. 1A and C) but was bactericidal against EC-2 at ≥2× MIC and EC-4 at ≥0.5× MIC (Fig. 1B and D). Aztreonam was not bactericidal against any *K. pneumoniae* strain at any concentration tested (Fig. 2A to D).

Based on results from individual time-kill experiments, a concentration of 1× MIC of 0.25× MIC of aztreonam was combined with ceftazidime-avibactam at the maximum concentration of free, unbound drug in serum (fCmax) or meropenem-vaborbactam at fCmax for strains EC-2 and EC-4, respectively (Fig. 3B and D). For the remaining strains
(EC-1 and EC-3) and all *K. pneumoniae* strains, aztreonam at $f_{C_{\text{max}}}$ was combined with either ceftazidime-avibactam or meropenem-vaborbactam at $f_{C_{\text{max}}}$ (Fig. 3A and C and 4A to D). Dual $\beta$-lactam combinations of aztreonam plus either ceftazidime or meropenem were synergistic against 0/8 (0%) and 1/8 (12.5%) strains, respectively (see Fig. S1 and S2 in the supplemental material). The triple-drug combinations (aztreonam plus either ceftazidime-avibactam or meropenem-vaborbactam) were synergistic against all aztreonam-resistant strains, based on the known $\beta$-lactamases present, regardless of the species (Fig. 3 and 4). The triple-drug combination of aztreonam plus ceftazidime-avibactam was synergistic against 3/4 (75%) *E. coli* strains (all except the aztreonam-susceptible strain [EC-4]) and was synergistic against 4/4 (100%) *K. pneumoniae* strains. The combination of aztreonam plus meropenem-vaborbactam was also synergistic against 3/4 (75%) *E. coli* strains (all except the aztreonam-susceptible strain [EC-4]) and was synergistic against 3/4 (75%) *K. pneumoniae* strains (all except the OXA-232-producing strain [KP-2]). Tables S1 to S4 display means ± standard deviations (SDs) of the 24-h bacterial densities ($\log_{10}$ CFU/ml) for each drug alone and in combination against each isolate at each concentration tested.
DISCUSSION

Despite the recent therapeutic advancements in the treatment of CROs, MBL-producing pathogens remain a significant challenge. While aztreonam is capable of evading MBL-mediated hydrolysis, the frequent presence of coharbored serine β-lactamases renders aztreonam ineffective, necessitating the need for combination therapy. To date, the only data available have evaluated aztreonam in combination with ceftazidime-avibactam. It is imperative that potential alternatives are evaluated in order to provide clinicians with options against this significant unmet medical need.

In this study, combining aztreonam with ceftazidime-avibactam or meropenem-vaborbactam produced synergistic interactions against 7/8 (87.5%) and 6/8 (75%) clinical Enterobacteriaceae strains coproducing NDM and at least one serine β-lactamase. The only difference in synergy observed between the two triple-drug combinations was against strain KP-2, where synergy was observed when aztreonam was combined with ceftazidime-avibactam but not with meropenem-vaborbactam. This discordance is likely attributable the inhibitory activity of avibactam (20), but not vaborbactam, against the OXA-48-like variant OXA-232 produced by this strain. Additionally, synergy was not observed with either triple combination against EC-4 (which

FIG 2 Mean log₁₀ CFU/ml versus time profiles for each drug at the highest concentration tested against the four K. pneumoniae strains. (A to D) All drugs are shown at Fₘₚₚ. Curves represent average concentrations from triplicate experiments.
was exquisitely aztreonam susceptible), presumably due to the absence of aztreonam-hydrolyzing β-lactamases susceptible to inhibition by either avibactam or vaborbactam. Together, these data suggest that combining aztreonam with either ceftazidime-avibactam or meropenem-vaborbactam may be a potential treatment option for patients with aztreonam-resistant NDM and serine-β-lactamase-producing Enterobacteriaceae infections. Furthermore, these results suggest that the combinations of aztreonam plus ceftazidime-avibactam and aztreonam plus meropenem-vaborbactam are largely interchangeable, with the exception of OXA-48-like-producing Enterobacteriaceae strains, in which case aztreonam plus ceftazidime-avibactam may be the preferred combination. Finally, our work also suggests the activity of these aztreonam-based combinations can be predicted based on the β-lactamase profile regardless of the species of Enterobacteriaceae.

A notable observation from this study is that, based on MIC test results, avibactam restored aztreonam susceptibility in aztreonam-resistant strains more consistently than
However, in time-kill experiments there were no significant differences observed in the bacterial killing of avibactam- or vaborbactam-based triple combinations, with the exception of strain KP-2 as discussed above. This may be due to differences in the $f_{\text{Cmax}}/\text{MIC}$ ratios for meropenem and ceftazidime in this study, as meropenem alone produced at least a 1-log$_{10}$ CFU/ml reduction in 7/8 (88%) strains followed by regrowth, whereas ceftazidime alone had no reduction in log$_{10}$ CFU/ml for any strain (Fig. 1 and 2). These results indicate that in vitro MICs may not accurately reflect the bactericidal activity of these triple drug combinations due to differences in their pharmacokinetics, and dynamic pharmacokinetics/pharmacodynamics (PK/PD) experiments utilizing serum-achievable drug concentrations are under way to further explore this observation.

Our work adds to the existing data suggesting synergy between aztreonam and ceftazidime-avibactam and expands on these data by evaluating meropenem-vaborbactam. Limitations of our study include the 24-h static nature of time-kill experiments and the number of strains and combinations tested. Strengths of our study...

**FIG 4** Mean log$_{10}$ CFU/ml versus time profiles for each individual drug at the highest concentration tested that demonstrated no activity and triple drug combinations against the four *K. pneumoniae* strains. (A to D) All drugs are shown alone and in combination at $f_{\text{Cmax}}$. Curves represent average concentrations from triplicate experiments.
included the use of NDM-producing strains with a range of susceptibilities to aztreo- 
nam along with a complex array of background serine β-lactamases, inclusion of both 
E. coli and K. pneumoniae clinical strains, and testing both the dual and triple combi-
nations of aztreonam plus ceftazidime (with or without avibactam) or meropenem (with 
without vaborbactam). Testing dual β-lactam combinations allowed us to separately 
assess the interactions between the backbone β-lactams and aztreonam and the 
triple-drug combinations. Our findings suggest that synergy is primarily driven by the 
interaction between aztreonam and the β-lactamase inhibitor rather than the dual 
β-lactam interaction, although pharmacokinetic differences between the backbone 
β-lactams (i.e., ceftazidime and meropenem) may play a role as previously discussed. 

In summary, the addition of aztreonam to either ceftazidime-avibactam or 
meropenem-vaborbactam was reliably synergistic against aztreonam-resistant serine-
and NDM-producing Enterobacteriaceae. Given the appropriate genotypic resistance 
profile, our findings suggest ceftazidime-avibactam and meropenem-vaborbactam 
may be interchangeably combined with aztreonam for aztreonam-resistant NDM-
producing Enterobacteriaceae infections. These results support the further investiga-
tion of aztreonam-based combinations against MBL-producing Gram-negative 
pathogens and give hope to optimizing clinical treatment regimens in the future if 
confirmed in additional in vitro and in vivo models.

MATERIALS AND METHODS

Bacteria and susceptibility testing. Eight clinical Enterobacteriaceae strains (4 E. coli [EC-1 to -4] and 
4 K. pneumoniae [KP-1 to -4]) were used for all experiments. These strains produced the following 
β-lactamases: EC-1 produced NDM, CTX-M-1 group, CMY-2/FOX, and TEM; EC-2 produced NDM and TEM; 
EC-3 produced NDM-1, CMY-6, CTX-M-15, and TEM-1; EC-4 produced NDM-5 and OXA-1; KP-1 produced 
NDM-1, CMY-6, CTX-M-15, OXA-1, SHV-11, and TEM-1B; KP-2 produced NDM-1, CTX-M-15, OXA-232, SHV, 
and TEM; KP-3 produced NDM and SHV; KP-4 produced NDM, CTX-M-1 group, SHV, and TEM. 

Strains were maintained at ~80°C in cation-adjusted Mueller-Hinton broth (CAMHB) with 20% 
glycerol and were subcultured twice on tryptic soy agar plates supplemented with 5% sheep blood prior 
to use. Analytical grade avibactam, aztreonam, ceftazidime, meropenem (Sigma-Aldrich, St. Louis, MO), 
and vaborbactam (Selleck Chemicals, Houston, TX) powders were obtained commercially. Stock solutions 
of each agent were freshly prepared as single-use aliquots at the beginning of each week and kept frozen 
at ~8°C. MIC tests were performed in triplicate by broth microdilution according to Clinical and 
Laboratory Standards Institute (CLSI) guidelines (21). E. coli ATCC 25922 and Klebsiella pneumoniae ATCC 
70603 were utilized as quality control organisms. Modal MIC values are reported.

Time-kill experiments. Time kill experiments were performed in triplicate on the same day 
according to CLSI guidelines (22) modified using a final volume of 2 ml in deep-well non-tissue-treated 
plates. A direct suspension of 3 to 4 isolated colonies was selected from a pure overnight culture and 
suspended in 5 ml of sterile saline; the resulting suspension was incubated with agitation to ensure 
log-phase growth. Suspensions were adjusted to 0.5 McFarland standard in sterile saline and diluted 
in CAMHB to a starting inoculum of ~10^6 CFU/ml. Colony counts were performed to ensure starting 
inoculum densities. Time-kill experiments proceeded stepwise as follows. First, aztreonam, ceftazi-
dime, ceftazidime-avibactam, meropenem, and meropenem-vaborbactam were tested at 0.25×, 0.5×, 
1×, 2×, and 4× the MIC unless any of these concentrations exceeded the respective drug’s IC_{max} value, 
in which case the IC_{max} was used. The IC_{max} values utilized were chosen to simulate a 2-g dose of each 
agent as follows: aztreonam, 112 mg/liter (23, 24); ceftazidime, 80 mg/liter (25, 26); meropenem, 45 mg/ 
liter (27). Next, dual β-lactam combinations of aztreonam plus ceftazidime and aztreonam plus mero-
penem were combined at the highest concentration of each drug from step 1 that showed no 
meaningful activity compared to that of the drug-free control strain (≤1 log_{10} CFU/ml decrease from the 
starting inoculum at 24 h). Finally, triple-drug combinations of aztreonam plus ceftazidime-avibactam 
and aztreonam plus meropenem-vaborbactam were combined at the highest concentration of each drug 
from step 1 that showed no activity. The concentrations of avibactam and vaborbactam were fixed at 4 
and 8 mg/liter, respectively, in all experiments. A growth control without any antibiotic was included with 
each experiment. At 0, 2, 4, 6, and 24 h, an aliquot was removed from each sample and serially diluted 
in log_{10} dilutions in sterile saline. A 50-μl aliquot was subsequently plated on Mueller-Hinton agar plates 
using an automated spiral plater (Don Whitley WASP Touch) and incubated at 35°C for 24 h. Colony 
counts were performed the following day using a colony counter (ProtoCOL 3 Plus). The theoretical lower 
limit of quantification was 100 CFU/ml. Time-kill curves were generated by plotting the average log_{10} 
CFU/ml versus time to compare the 24-h killing effects of single agents alone and in combination. 
Bactericidal activity was defined as ≥3 log_{10} CFU/ml reduction at 24 h compared to the starting 
inoculum, and synergy was defined as a ≥2-log_{10} reduction in CFU/ml between the combination and the 
most active single agent alone (22). Antagonism was defined as a ≥2-log_{10} increase in CFU/ml between the 
combination and the most active single agent alone.
SUPPLEMENTAL MATERIAL
Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01426-19.

SUPPLEMENTAL FILE 1, PDF file, 0.8 MB.

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REFERENCES