Can ceftazidime/avibactam and aztreonam overcome β-lactam resistance conferred by metallo-β-lactamases in Enterobacteriaceae?

Steven Marshall1*, Andrea M. Hujer1,2*, Laura J. Rojas1,2,3, Krisztina M. Papp-Wallace1, Romney M. Humphries5, Brad Spellberg5, Kristine M. Hujer1,2, Emma K. Marshall1, Susan D. Rudin1,2, Federico Perez1,2, Brigid M. Wilson1, Ronald B. Wasserman6, Linda Chikowski7, David L. Paterson8, Alejandro J. Vila9, David van Duin10, Barry N. Kreiswirth11, Henry F. Chambers12, Vance G. Fowler Jr13, Michael R. Jacobs14, Mark E. Pulse15, William J. Weiss15, and Robert A. Bonomo1,2,3,16#

1 Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Cleveland, OH; (2) Department of Medicine, Case Western Reserve University School of Medicine, Cleveland, OH; (3) Department of Molecular Biology and Microbiology, Case Western Reserve University School of Medicine, Cleveland, Ohio; (4) Department of Pathology and Laboratory Medicine, University of California, Los Angeles, CA; (5) Division of Infectious Diseases, Keck School of Medicine at USC and the Los Angeles County-USC Medical Center, Los Angeles, CA; (6) Infectious Disease Doctors Medical Group, Walnut Creek, CA; (7) John Muir Health, Walnut Creek, CA; (8) The University of Queensland, UQ Centre for Clinical Research, Brisbane, Queensland, Australia; (9) Instituto de Biología Molecular y Celular de Rosario Consejo Nacional de Investigaciones Científicas y Técnicas de Argentina, Universidad Nacional de Rosario, Argentina; (10) Division of Infectious Diseases, University of North Carolina, Chapel Hill, NC; (11) Public Health Research Institute Center, New Jersey Medical School-Rutgers University, Newark, NJ; (12) University of California, San Francisco General Hospital, San Francisco, CA; (13) Division of Infectious Diseases, Department of Medicine, and Duke Clinical Research Institute, Duke University Medical Center, Durham, NC; (14) Department of Pathology, Case Western Reserve University School of Medicine, Cleveland, OH; (15) University of North Texas Health Science Center, Fort Worth, TX; (16) Departments of Pharmacology, Biochemistry, and Proteomics and Bioinformatics, Case Western Reserve University School of Medicine, Cleveland, OH.
*S.M. and A.M.H. contributed equally to this work.

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#Corresponding Author: Robert A. Bonomo, MD, Research Service, Louis Stokes Cleveland Department of Veterans Affairs Medical Center, 10701 East Boulevard, Cleveland, OH 44106; P: 216-791-3800, x 4801

Email: robert.bonomo@va.gov
Abstract

Based upon knowledge of the hydrolytic profile of major β-lactamases found in Gram negative bacteria, we tested the effectiveness of the combination of ceftazidime/avibactam (CAZ/AVI) with aztreonam (ATM) against carbapenem-resistant enteric bacteria possessing metallo-β-lactamases (MBLs). Disk-diffusion and agar based antimicrobial susceptibility testing were initially performed to determine the *in vitro* efficacy of a unique combination of CAZ/AVI and ATM against 21 representative *Enterobacteriaceae* isolates with a complex molecular background that included *bla*IMP, *bla*NDM, *bla*OXA-48, *bla*CTX-M, *bla*AmpC, and combinations thereof. Time-kill assays were conducted, and the *in vivo* efficacy of this combination was assessed in a murine neutropenic thigh infection model. By disk diffusion assay, all 21 isolates were resistant to CAZ/AVI alone, and 19/21 were resistant to ATM. The *in vitro* activity of CAZ/AVI in combination with ATM against diverse *Enterobacteriaceae* possessing MBLs was demonstrated in 17/21 isolates, where the zone of inhibition was ≥ 21 mm. All isolates demonstrated a reduction in CAZ/AVI agar dilution MICs with the addition of ATM. At 2 h, time-kill assays demonstrated a ≥ 4 log₁₀ CFU decrease for all groups that had CAZ/AVI plus ATM (8 μg/ml) added, compared to the CAZ/AVI alone group. In the murine neutropenic thigh infection model, an almost 4 log₁₀ reduction in CFUs was noted at 24 h for CAZ/AVI (32 mg/kg q8h) plus ATM (32 mg/kg q8h) vs. CAZ/AVI (32 mg/kg q8h) alone. The data presented herein, requires us to carefully consider this new therapeutic combination to treat infections caused by MBL-producing *Enterobacteriaceae*. 
Introduction

As a consequence of the threat of rising antibiotic resistance, the Infectious Diseases Society of America has challenged the pharmaceutical industry to develop novel antibiotics (“The 10x20 initiative”, http://www.idsociety.org/10x20/). Of particular importance are carbapenem-resistant strains of bacteria, which are typically resistant to most or all commonly used therapeutic options, and cause high morbidity and mortality (1). In response, the “antibiotic pipeline” has delivered an important β-lactam β-lactamase inhibitor combination, ceftazidime/avibactam, CAZ/AVI (2).

Avibactam (AVI) is a novel diazabicyclooctane (DBO) β-lactamase inhibitor with *in vitro* activity against serine enzymes, such as the class A extended spectrum β-lactamases (ESBLs) and *Klebsiella pneumoniae* producing carbapenemases (KPCs), as well as the class D OXA-48 found in *Enterobacteriaceae*, and the class C cephalosporinases present in enteric bacteria and *Pseudomonas aeruginosa*. AVI restores the activity of CAZ against a broad array of resistance threats, making CAZ/AVI a welcome addition to the antibiotic armamentarium against Gram-negative bacteria. However, CAZ/AVI is not active against strains bearing class B metallo-β-lactamases (MBLs) such as NDM, VIM and IMP. The monobactam antibiotic aztreonam (ATM) remains stable against MBLs, but is not a therapeutic option in many cases because it is inactivated by ESBLs, KPCs, and other cephalosporinases frequently found in the background of MBL-producing bacteria (2, 3).

Even before widespread clinical use of CAZ/AVI, case reports appeared that described resistance to this novel inhibitor combination (4). As a result of these findings, we now know that CAZ/AVI MICs may be elevated in the setting of unfavorable genetic backgrounds which...
include resistance determinants that control entry and egress of antibiotics (5). Additionally, MBLs are a worldwide problem, with outbreaks reported in the United States in numbers that were previously unanticipated (6, 7).

Knowing that ATM is stable against hydrolysis by MBLs, we hypothesized that addition of ATM to the CAZ/AVI combination would result in enhanced activity by “protecting” ATM from the “attack” of ESBLs and other cephalosporinases. Therefore: 1) we tested the in vitro activity of the combination of CAZ/AVI with ATM against 21 carbapenem-resistant, MBL-producing Enterobacteriaceae isolates containing \textit{bla}_{NDM} or \textit{bla}_{IMP} genes, and a pan-susceptible control; 2) we evaluated the in vivo antibacterial efficacy of CAZ/AVI with ATM in a mouse thigh infection model using a MBL producing \textit{K. pneumoniae} strain; 3) lastly, we report a case of infection caused by an \textit{Enterobacter cloacae} containing a MBL treated with ATM in combination with CAZ/AVI. Our findings encourage further study of CAZ/AVI with ATM in experimental and clinical circumstances when therapeutic options against MBL producing strains are limited.

**Materials and methods**

\textit{Antimicrobial susceptibility testing}

\textit{In vitro} efficacy of the CAZ/AVI and ATM combination was performed on 21 previously characterized strains of diverse species of \textit{Enterobacteriaceae} (8). Susceptibility testing was initially performed by disk diffusion. Disks containing CAZ (30 µg) and CAZ/AVI (30/20 µg) were obtained from Becton Dickinson (Sparks, MD) and Actavis (Schaumburg, IL), respectively. ATM (30 µg) disks were made by adding 10 µl of a 3000 µg/ml stock ATM
(Chem-Impex Int’l Inc -Wood Dale, IL) solution to blank disks. To test the activity of the combination of CAZ/AVI with ATM, 30 ug of ATM were placed directly on the CAZ/AVI disk, and allowed to dry for 30 minutes before use. Isolates were grown overnight in cation adjusted Mueller-Hinton broth (CAMHB), diluted to a McFarland 0.5 standard and inoculated to form a lawn on cation adjusted MH agar (MHA) plates. Disks containing CAZ, CAZ/AVI, and ATM were placed onto the plates and then incubated overnight at 37° C. For CAZ and ATM, zones of inhibition were interpreted according to the Clinical Laboratory Standards Institute (CLSI) guidelines (9). For CAZ/AVI, zones of inhibition were interpreted according to FDA guidelines. (Figure 1).

Agar dilution minimum inhibitory concentrations (MICs) for CAZ, ATM, CAZ/AVI, CAZ/ATM and the CAZ/AVI-ATM combination were determined in triplicate and performed according to CLSI protocol (9). Isolates were grown overnight in CAMHB and stamped on MHA plates containing doubling dilutions of CAZ (Sigma Chemical Co. St Louis, MO), ATM (Chem-Impex Int’l Inc -Wood Dale, IL) and CAZ/AVI (Avycaz™ 2g/0.5g per vial, Allergan) using a Steers replicator that delivered $10^4$ CFU/spot. The effect of adding a fixed concentration of 8 or 16 μg/ml of ATM to CAZ or to CAZ/AVI was also assessed. Plates were incubated 18 - 20 h at 37° C and the antibiotic concentration (μg/ml) at which bacterial growth was no longer observed defined the MIC. A minimum of 3 determinations were done for each susceptibility with the most frequently observed result reported.

*Time-kill assays*
Time-kill assays were performed for isolate *K. pneumoniae* 1.41 as previously described with some modifications (10). Briefly, freshly prepared colonies were resuspended in 5 ml CAMHB and incubated overnight in a shaking incubator (37°C, 220 rpm). Cultures were then diluted 1:100 and incubated in a shaking incubator until they reached 0.5 McFarland standard (approximately $10^8$ CFU/ml). A bacterial suspension was prepared in 50 ml conical tubes containing 10 ml CAMHB so that the starting inoculum was approximately $10^6$ CFU/ml.

CAZ/AVI (Avycaz™) was added to the prepared bacterial suspensions so that the final concentration was 1×, 2×, 4× or 8× the agar MIC of the CAZ/AVI-ATM combination (1 $\mu$g/ml CAZ:0.25 $\mu$g/ml AVI to 8 $\mu$g/ml CAZ:2 $\mu$g/ml AVI with ATM held constant at 8 $\mu$g/ml). A control of CAZ/AVI alone was diluted in the prepared bacterial suspension at 8× the MIC, and a growth control without antibiotics was also included. The starting inoculum was determined from the growth control tube immediately after dilution and was recorded as the count at time zero. After addition of antibiotics, the starting inoculum was $1 \times 10^6$ - $1 \times 10^7$ CFU/ml. Tubes were incubated in a shaking incubator (37°C, 180 rpm), and viability counts were performed at 1, 2, 4, 6, and 24 h by removing 200 $\mu$l of the culture, diluting as appropriate, and plating 100 $\mu$l on MHA. Plates were incubated at 37°C for 18 -20 h, colonies were counted on plates yielding 30-300 colonies per plate, and the results were recorded as the number of CFU/ml. Three replicates were conducted for each of the conditions reported in the time kill assay.

**Polymerase chain reaction (PCR) and sequencing**

PCR amplification of *bla*$_{NDM}$ and other *bla* genes, in isolates not previously characterized, was achieved using established primers and amplified with a MJ Research Gradient Cycler Model PTC 225 using thermocycling conditions adjusted to the primer melting
temperatures (8). Positive controls included well characterized isolates (in the laboratory of RAB). Amplicons were sequenced at a commercial sequencing facility (MCLAB, San Francisco, CA). Sequence data were analyzed using Lasergene 7.2 software (DNAstar, Madison, WI), and sequences were compared with BLAST online software (http://blast.ncbi.nlm.nih.gov), using the megablast algorithm.

**Mouse thigh infection model**

For the thigh infection model, Female Hsd:ICR (CD-1) mice from Envigo were rendered neutropenic by intraperitoneal (i.p.) injection of cyclophosphamide (Cytoxan, Baxter Healthcare, Deerfield, IL) as previously described (11). Five mice per 2 control and 14 treatment groups were infected by injecting a prepared inoculum of *K. pneumoniae* 1.41, input CFU = 5.01 log$_{10}$CFU/mouse into the right hind thigh muscle contained in a volume of 0.1 mL per animal. At two hours post-infection, animals were administered subcutaneous doses (q8hr) of CAZ (Sigma, Ceftazidine hydrate), CAZ + AVI (AvyCaz™), ATM (Azactam® for injection, USP) or CAZ/AVI + ATM (1:1). All animals were euthanized 24 hours post-infection and thighs aseptically removed into chilled PBS, homogenized (Kinematica® Polytron PT2100 tissue homogenizer), 10-fold serially diluted, plated on Brain-heart infusion agar plates containing 0.5% activated charcoal and incubated for the determination of bacterial thigh titers (CFU).

**Statistical Analysis**

The 24 hour log$_{10}$ CFU counts were compared across all treatment and control groups using a One-way ANOVA. Post-hoc pairwise comparisons were performed across all treatment
Results and Discussion

As established by current and previous molecular testing (8), each of the isolates possesses an MBL with the exception of the *Escherichia coli* DH10B which served as a negative control. In Table 1, we show that isolates containing a MBL were resistant to CAZ (21/21 isolates), ATM (19/21 isolates), and CAZ/AVI (21/21 isolates) by disk diffusion (9). The combination of CAZ/AVI and ATM produced an inhibition zone ≥ 21 mm, suggesting susceptibility in 17 of the 21 MBL-producing strains that were tested (i.e., ≥ 21 mm is the S zone diameter for CAZ/AVI alone), with the measured zones being larger than with CAZ/AVI or ATM alone: 10-15 mm larger for 9 isolates, and 4-9 mm larger for 5 isolates (Table 1). In 3 cases where an increase in the inhibition zone was not found with the combination of CAZ/AVI and ATM, there was already a significant zone of susceptibility to ATM observed.

For the agar dilution MIC determinations we used a series of doubling dilutions of CAZ/AVI in the ratio that is found in the commercial preparation (Avycaz™) intended for patient use. This was a deliberate decision as we feel these dilutions reflect doses of CAZ/AVI that patients receive. The commercial preparation is formulated in a 4:1 ratio (CAZ 2g: AVI 0.5g /per vial). The values reported for CAZ/AVI in Table 1 reflect the concentration of the CAZ in the 4:1 ratio formulation. For MIC determinations where the efficacy of ATM addition was assessed, ATM was kept at a fixed concentration of either 8, 16, 32 or 64 μg/ml, and the
CAZ/AVI was added in doubling dilutions. These concentrations of ATM were chosen as they represent the expected serum concentrations of the monobactam when delivered in 1 and 2 gram doses throughout the dosing interval, particularly the 8 and 16 μg/ml doses. Single 30 minute intravenous infusions of 1 and 2 gram doses of ATM produce peak serum levels immediately after administration of 54 and 90 μg/ml, and 3 and 6 μg/ml at 8 h (end of the dosing interval), respectively (Azactam package insert).

All 21 isolates were resistant to CAZ and CAZ/AVI alone by agar dilution MIC and disk diffusion assay, and all demonstrated a reduction in CAZ/AVI MICs with the addition of ATM (Table 1) in a dose dependent fashion. Notably, one *K. pneumoniae* and four *E. coli* isolates demonstrated MIC values that remained at or above the resistance breakpoint for CAZ/AVI alone (≥ 16/4 μg/ml) after the addition of ATM; genome sequencing is being performed on these isolates to further explore the underlying mechanism of this resistance. However, addition of ATM at higher concentrations of 32 and 64 μg/ml for all 5 isolates brought them into the susceptible MIC range for CAZ/AVI alone (S ≤ 8/4 μg/ml), Table 1. Remarkably, results presented herein accurately reflect the genetic backgrounds established in these isolates, and as anticipated, the CAZ/AVI with ATM combination worked especially well in NDM producing strains.

The time-kill curves detail the bactericidal activity of the combination of CAZ/AVI and ATM against the MBL containing *K. pneumoniae* 1.41 isolate. ATM at a fixed concentration of 8 μg/ml was added to varying sub lethal concentrations of CAZ/AVI ranging from 8 μg/ml CAZ: 2 μg/ml AVI down to 1 μg/ml CAZ: 0.25 μg/ml AVI; corresponding to 4 different MIC multiples of the CAZ/AVI plus ATM agar MIC of 1 μg/ml. Time-kill kinetics showed a time...
dependent decrease in CFU/ml from 1 to 24 hours (Figure 2). A ≥ 4 log_{10} CFU decrease was observed by 2 h for all combination concentrations and by 6 h, approximately 5 log_{10} reductions were observed, when compared to the growth control to which antibiotics were not added. After 24 h there was minimal regrowth observed for all combinations, still keeping a 3 log_{10} decrease as compared to t = 0 h, with the exception of the 8x MIC concentration that showed approximately a 6 log_{10} decrease.

We next evaluated the \textit{in vivo} antibacterial efficacy of CAZ/AVI in combination with ATM in a mouse thigh infection model using the MBL producing \textit{K. pneumoniae} 1.41 isolate. The results are presented in Tables 2 and Figure 3. Both CAZ alone (256 mg/kg) and CAZ/AVI alone (256/64 and 128/32 mg/kg) resulted in 3.1-, 4.7-, and 3.7-log CFU reductions respectively, as compared to the untreated control group at 24 h. The lower dose of either CAZ or CAZ/AVI were less effective. ATM alone at doses from 64 – 256 mg/kg q8h exhibited minimal efficacy (1.16 – 1.99 log CFU reduction).

Our data in Table 2 show that in this model, the addition of ATM to the CAZ/AVI treatment regimen results in significant enhancement in the reduction of infection as measured by CFUs/thigh when compared to either agent alone and the untreated controls. Doses of 32/8 mg/kg q8h CAZ/AVI (4:1 ratio) plus 32 mg/kg q8h ATM reduced bacterial thigh titers by 3.95 log CFU below the 24 hr no antibiotic controls (p=0.0016), 3.79 log CFU below those of 32/8 mg/kg q8h CAZ/AVI alone (p=0.003, data not shown) and 2.08 log CFU below ATM 64 mg/kg alone. Dosages of ≤ 4/1 mg/kg q8h CAZ/AVI (4:1 ratio) plus 4 mg/kg q8h ATM were unable to reduce the CFU count.
Clinical Commentary

A 72-year-old woman without significant medical history fell and sustained a fractured hip on an excursion. She was treated with a total hip arthroplasty performed in Eastern Europe and returned home within 2 weeks with a painful, red surgical site. The arthroplasty was removed in the United States and all intraoperative cultures grew carbapenem-resistant \textit{Enterobacter cloacae} susceptible to colistin, intermediate to tigecycline (FDA breakpoint), and resistant to all other agents tested including fosfomycin. PCR and sequencing efforts revealed that the \textit{Enterobacter cloacae} possessed \textit{bla}\textsubscript{NDM-1}, Table 1. The culture also contained an ESBL-producing \textit{Klebsiella pneumoniae} and \textit{Enterococcus faecalis} (ampicillin and vancomycin susceptible). A hip spacer was not placed at this time, knowing further debridement would be necessary.

By day 4 of colistimethate sodium (colistin), tigecycline, and meropenem treatment, serum creatinine increased from 0.9 to 2.4 mg/dl. Colistimethate sodium was reduced to 1.5 mg/kg every 36 h and meropenem was adjusted for declining renal function. The patient underwent repeat washout with cultures again yielding the carbapenem resistant \textit{E. cloacae} and ESBL-producing \textit{K. pneumoniae}. CAZ/AVI 1.25 g thrice daily was added to the antibiotic regimen; however, CAZ/AVI was discontinued after 4 days as the carbapenem resistant \textit{Enterobacter cloacae} demonstrated in vitro resistance by disk diffusion (ATM 30 µg disk: 6 mm zone size, CAZ/AVI 50 µg disk: 18 mm zone size). Meropenem was soon discontinued due to lack of evidence of efficacy. With normalizing serum creatinine, colistimethate sodium dose was increased to 2mg/kg every 12 h.
Qualitative synergy in the *E. cloacae* isolate was demonstrated *in vitro* between CAZ/AVI and ATM (inhibition zone of CAZ/AVI disk was increased from 18 mm to 28 mm upon addition of ATM, Table 1. Therefore, the treatment regimen was modified so that the final 3 weeks of her 6-week post-operative antibiotic course were comprised of colistimethate sodium, CAZ/AVI (2.5 g every 8 h), ATM (2 g every 8 h) and vancomycin. Six months after completion of antibiotics, the patient underwent successful final reimplantation of a total hip arthroplasty, with extensive inspection and washout. Six cultures were obtained from acetabular and femoral spaces. All were negative for the 3 organisms originally isolated.

**Concluding remarks**

Based upon an understanding of the biochemical mechanism of action of these three agents, we show that the combination of ATM and CAZ/AVI is able to help overcome carbapenem and expanded-spectrum cephalosporin resistance in MBL producing strains of enteric bacteria. AVI is a very potent β-lactamase inhibitor of class A and C enzymes, and since most strains of *Enterobacter* spp. possess class C enzymes and possibly some class A ESBLs, we reasoned that AVI would prevent the hydrolysis of CAZ and ATM (12). Although AVI cannot restore susceptibility to MBL producing strains, ATM is not susceptible to hydrolysis by MBLs. By inhibiting class A and C β-lactamases with AVI, and using ATM to “bypass” the class B metallo-β-lactamase, susceptibility can be restored leading to a successful microbiological and possible clinical outcome. Our clinical observation is supported by evidence from our *in vitro* investigations.

An unexpected benefit from using CAZ and ATM may arise by the simultaneous inhibition of multiple PBPs. CAZ has a high affinity for the penicillin-binding protein-3 (PBP-3)
and moderate affinity for the PBP-1a of certain Gram-negative organisms such as *E. coli* and *P. aeruginosa*. Binding to PBPs results in spheroplast formation followed by rapid lysis. Furthermore, AVI and developmental DBO inhibitors can also bind to certain PBPs (13-15). ATM is relatively PBP3 specific. The combination (CAZ/AVI and ATM) may completely affect the “divisome” of Gram-negative bacteria and have an independent impact on its own. Of note, the use of “double β-lactams” has some precedent in the literature. Overall, the existing data suggests that the potential benefit of this approach against Gram-negative pathogens should be explored carefully, especially given the recent availability of novel inhibitors and β-lactams (16).

There are important limitations that must be considered before uniform application of this promising combination: a) further animal testing with additional isolates and PK studies along with trials in humans are required before full endorsement by clinicians; and b) results from *Enterobacteriaceae* should not be extrapolated to non-fermenters without appropriate testing (studies in progress). At present, clinical experience using this combination against *Stenotrophomonas maltophilia* proved to be successful in a single case (17). Lastly, the possibility of cumulative toxicity from “double β-lactam” combinations must be considered when administering this regimen. However, one major advantage of using ATM is its safety profile. ATM is safe to use in patients with penicillin allergies, can be administered by prolonged or continuous infusion, and it is not associated with nephrotoxicity. The data presented here require us to carefully consider CAZ/AVI combined with ATM as a “new therapeutic opportunity” to treat infections caused by MBL producing strains, while recognizing the ongoing need for new antibiotics.
Acknowledgments

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Figure Legend

Figure 1. ATM placed directly on CAZ/AVI disk to evaluate synergy, isolate *E. cloacae* 6.31 was used in this assay.

Figure 2. Time kill curve for isolate *K. pneumoniae* 1.41. ATM concentrations were held constant at 8 μg/ml for all combinations with two exceptions: 1) the growth control (no antibiotics added), and 2) the CAZ/AVI NO ATM 8X MIC. Varying ceftazidime/avibactam (CAZ/AVI) concentrations were added corresponding to 1x (1 μg/ml CAZ:0.25 μg/ml AVI), 2x (2 μg/ml CAZ:0.5 μg/ml AVI), 4x (4 μg/ml CAZ:1 μg/ml AVI) and 8x (8 μg/ml CAZ:2 μg/ml AVI) the MIC of the combination CAZ/AVI and ATM obtained by the agar dilution (1ug/ml).

Three replicates were conducted for each of the conditions reported in the time kill assay.

Figure 3. Individual and Mean Log_{10} CFU/thigh counts for various antibiotic/β-lactamase inhibitor combinations in the neutropenic thigh infection model for *K. pneumoniae* 1.41 infected mice: 5 log_{10} CFU/mouse, 5 mice per treatment group. LOQ: Limit of Quantitation; 2.35 log_{10} CFU/thigh.
Table 1. Evaluation of in vitro activity of CAZ/AVI plus ATM, by both disk diffusion and agar MIC testing. Genetic background of isolates and inhibition zone diameters (in mm) of disks with CAZ, ATM, CAZ/AVI, and CAZ/AVI disks to which ATM was added is presented. MIC values are in μg/ml.

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<th>Isolate</th>
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<th>Agar MIC Testing Results</th>
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<td></td>
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<td>CAZ 30 (≥21); ATM 30 (≥18-20); CAZ/AVI 50 (≥20)</td>
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</tr>
<tr>
<td>Kp 1.41</td>
<td>NDM-1, CTX-M-15, DHA, SHV, TEM</td>
<td>6 6 17</td>
<td>32 (15)</td>
</tr>
<tr>
<td>Kp 1.42</td>
<td>NDM-1, CTX-M-15, SHV-12</td>
<td>6 6 17</td>
<td>27 (10)</td>
</tr>
<tr>
<td>Kp 1.44</td>
<td>NDM-1, CTX-M-15, CMY-2, DHA, SHV, TEM</td>
<td>6 6 15</td>
<td>25 (10)</td>
</tr>
<tr>
<td>Kp 1.50</td>
<td>NDM-1, CTX-M-15, SHV</td>
<td>6 6 20</td>
<td>30 (10)</td>
</tr>
<tr>
<td>Kp 1.63</td>
<td>NDM-1, CTX-M-15, CMY-2, SHV TEM</td>
<td>6 6 15</td>
<td>30 (15)</td>
</tr>
<tr>
<td>Kp 6913</td>
<td>IMP, SHV</td>
<td>6 28 17</td>
<td>28 (0)</td>
</tr>
<tr>
<td>Kp 11-01-13</td>
<td>NDM-1, CTX-M-15, SHV</td>
<td>6 6 17</td>
<td>30 (13)</td>
</tr>
<tr>
<td>Pn 6348</td>
<td>NDM-1, SHV</td>
<td>6 37 6</td>
<td>34 (-3)</td>
</tr>
<tr>
<td>Pr 1.27</td>
<td>NDM-1, CMY-2, DHA</td>
<td>6 14 10</td>
<td>20 (6)</td>
</tr>
<tr>
<td>Mn 1.39</td>
<td>NDM-1, CTX-M-15, DHA</td>
<td>6 12 10</td>
<td>25 (13)</td>
</tr>
</tbody>
</table>

*Data presented in Table 1 is the zone diameter of the CAZ/AVI disk to which ATM was added. In parenthesis is the difference between this zone size and the zone of inhibition of either CAZ/AVI or ATM alone, whichever of the two was greater. Isolates in this*
Table were available from the laboratories of the authors and characterized previously (8). **Presumed AmpC. CAZ/AVI 50 =
ceftazidime 30 μg/avibactam 20 μg; ATM 30 = aztreonam 30 μg; CAZ 30 = ceftriaxone 30 μg. Interpretative criteria for CAZ and
ATM, MICs were according to the Clinical Laboratory Standards Institute (CLSI) guidelines (9). For CAZ/AVI, MICs were
interpreted according to FDA guidelines from the package insert. CAZ S ≤ 4, I = 8, R ≥ 16; ATM S ≤ 4, I = 8, R ≥ 16; CAZ/AVI S ≤
8/4, R ≥ 16/4 μg/ml. The values reported for CAZ/AVI reflect the concentration of the CAZ in the 4:1 ratio formulation. Green font
indicates susceptibility, and red font indicates resistance based upon the interpretive guidelines. Abbreviations: Ec is Escherichia coli;
Ecl is Enterobacter cloacae; Kp is Klebsiella pneumoniae; Pr is Providencia rettgeri; and Mm is Morganella morganii.
Table 2. Log10 CFU/thigh counts for various antibiotic/β-lactamase inhibitor combinations in the neutropenic thigh infection model for *K. pneumoniae* 1.41 infected mice: 5 log10 CFU/mouse, 5 mice per treatment group. Bold font indicates a statistically significant value.

<table>
<thead>
<tr>
<th>Test Article(s)</th>
<th>mg/kg SC q8h (5 mice per treatment)</th>
<th>Mean Log10 CFU/thigh ± SD</th>
<th>Change from control at 24 h no antibiotics (log10 CFU)</th>
<th>Tukey adjusted p-value (treatment vs 24-hour control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h infection control</td>
<td>na</td>
<td>8.46 ± 1.15</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>CAZ</td>
<td>256</td>
<td>5.37 ± 1.17</td>
<td>-3.08</td>
<td>0.0405</td>
</tr>
<tr>
<td></td>
<td>128</td>
<td>6.12 ± 1.34</td>
<td>-2.34</td>
<td>0.3152</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>6.35 ± 1.57</td>
<td>-2.11</td>
<td>0.4909</td>
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<tr>
<td>ATM</td>
<td>256</td>
<td>7.30 ± 1.47</td>
<td>-1.16</td>
<td>0.9912</td>
</tr>
<tr>
<td></td>
<td>128</td>
<td>6.46 ± 1.80</td>
<td>-1.99</td>
<td>0.5873</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>6.59 ± 2.02</td>
<td>-1.86</td>
<td>0.6936</td>
</tr>
<tr>
<td>CAZ/AVI</td>
<td>256/64</td>
<td>3.77 ± 0.18</td>
<td>-4.68</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>128/32</td>
<td>4.75 ± 1.00</td>
<td>-3.70</td>
<td>0.0042</td>
</tr>
<tr>
<td></td>
<td>64/16</td>
<td>6.23 ± 1.93</td>
<td>-2.22</td>
<td>0.4018</td>
</tr>
<tr>
<td></td>
<td>32/8</td>
<td>8.30 ± 0.88</td>
<td>-0.16</td>
<td>&gt; 0.9999</td>
</tr>
<tr>
<td>CAZ/AVI/ATM</td>
<td>32/8/32</td>
<td>4.51 ± 0.42</td>
<td>-3.95</td>
<td>0.0016</td>
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<tr>
<td></td>
<td>16/4/16</td>
<td>6.88 ± 1.63</td>
<td>-1.58</td>
<td>0.8818</td>
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<tr>
<td></td>
<td>8/2/08</td>
<td>6.57 ± 1.77</td>
<td>-1.88</td>
<td>0.6777</td>
</tr>
<tr>
<td></td>
<td>4/1/04</td>
<td>8.47 ± 0.85</td>
<td>0.02</td>
<td>&gt; 0.9999</td>
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