# Things that go Bump in the Night: Combating *Klebsiella pneumoniae* Co-producing New Delhi Metallo-beta-lactamase (NDM) and Mobile Colistin Resistance (MCR)

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## ABSTRACT

**Background:** The scourge of MBLs among Gram negatives, such New Delhi Metallo-beta-lactamase-producing Klebsiella pneumoniae, has resulted in an overwhelming need for new treatmens. Worryingly, additional acquisition of plasmid-mediated polymyxin resistance through the mcr gene can produce strains resistant to all last line agents. The novel combination of aztreonam (ATM, which is not an MBL substrate) with avibactam (AVI, to inhibit extended spectrum beta-lactamases that inactivate ATM) has been proposed to restore ATM activity.

**Materials/methods:** K. pneumoniae SZ04 harboring blaNDM-5, blaCTX-M-55, and mcr-1 ($MIC_{ATM}$ = 128 mg/L, $MIC_{Polymyxin\ B}$ = 4 mg/L, $MIC_{Amikacin}$ = 1 mg/L, $MIC_{ceftazidime/avibactam}$ > 16/4 mg/L) was studied at two initial inoculum ($10^6$ and $10^8$ cfu/mL) over 24h in static time kills (SCTK). Concentration arrays of 2-, 3-, and 4-drug combinations of low- and package insert-dose polymyxin B (PMB) ± low- and package insert-dose amikacin (AMI) ± package insert dosing of ATM/AVI were simulated in > 200 individual arms of SCTK. Data were summarized using the Log Ratio Area (LRA) which is calculated by integrating the area under the bacterial killing curve, normalizing to the growth control, then log-transforming. A Hill-type function was fit to the data in order to determine the maximum effect ($E_{max}$) and drug concentration for 50% effect ($EC_{50}$).

**Results:** When simulating the maximum free concentration of amikacin 15 mg/kg (52 mg/L) in combination with package insert concentrations of ATM/AVI, there was a marked reduction of 3.22 in the LRA compared to the growth control for the $10^8$ cfu/mL starting inocula. Combining ATM with low amikacin (0.813 mg/L) and polymyxin B (0.125 mg/L) resulted in a reduction in LRA of 4.32 at $10^6$ cfu/mL. Model fitting results showed a statistically significant difference in $EC_{50}$ to amikacin between low and high inocula at 1.24 and 18.1 mg/L, respectively. Combination of AMI with low-concentration PMB (0.125 mg/L) resulted in an increase in the Emax of amikacin to -5.68.

**Conclusion:** The use of ATM/AVI combinations is a promising option against MBL and MCR co-producing K. pneumoniae. Low-dose strategies of polymyxin or amikacin dosing in combination with ATM/AVI is merits further testing for future translation to the clinical setting to improve efficacy and optimize treatment.

## BACKGROUND

- Gram negative bacteria harboring $bla_{NDM}$ and $bla_{CTX-M}$ genes can inactivate all commercially available beta-lactam antibiotics. [1,2]
- Aztreonam (ATM) is not inactivated by metallo-beta-lactamases (i.e. NDM) and benefits from use of a latest generation inhibitor, avibactam (AVI), to inhibit extended spectrum beta-lactamase (ESBL) activity of CTX-M. [3-5]
- Previous Hollow Fiber Infection Model studies have shown the benefit of the combination *Enterobacteriaceae*, but have yet to explore synergy with non-beta-lactams. [6]
- Non-beta-lactam antibiotics, such as the aminoglycosides or polymyxins, have been previously shown to produce synergistic killing when given in combination with bet-lactams.

## OBJECTIVE

Evaluate the killing effect of aztreonam/avibactam (ATM/AVI)-based combinations with polymyxin B and amikacin against NDM-, CTX-M-, and MCR-co-producing *K. pneumoniae*.

## METHODS

### Bacterial Isolate, Media, and Drug

- K. *pneumoniae* isolate (SZ04) with $bla_{NDM-5}$, $bla_{CTX-M-55}$, and *mcr-1* ($MIC_{ATM}$ = 128 mg/L, $MIC_{PMB}$= 4 mg/L, $MIC_{AMI}$ = 1 mg/L, and $MIC_{CAZ/AVI}$ > 16/4 mg/L) was used.
- All experiments were conducted using cation-adjusted Mueller-Hinton Broth and freshly prepared drug stocks.

### Static Time Kill Studies (STKS)

- STKS were conducted using clinically relevant concentrations in order to inform hollow fiber infection model studies.
- Samples were collected at 0, 1, 2, 4, 6, 8, 24h

### Mathematical Model

- STKS data were integrated using the log-trapezoidal rule and the log ratio area was calculated using the following formula:

$$LRA = \log\left(\frac{AUCFU_{Tx}}{AUCFU_{GC}}\right)$$

- Hill-type functions were fit to the LRA as a function of drug concentration using the nlme package in R (version 4.0)

### Hollow Fiber Infection Model Studies (HFIM)

- A HFIM was then run to explore the pharmacodynamics of therapy using the full humanized pharmacokinetic profiles. Approaches were identical to previous. [6]
- Samples for total counts and population analysis profiles were collected over 7 days at 0, 1, 2, 4, 6, 24, 26, 28, 30, 48, 50, 52, 54, 72, 120, and 168h.
- In order to quantify the number of resistant subpopulations, population analysis profiles were determined by plating diluted sample on drug-containing agar and incubating for 48h.

## RESULTS

- Amikacin was highly potent against this strain in time kill. In monotherapy, killing was significant at the lowest concentrations tested, which resulted in an inability to generate sufficiently diverse data to inform the mathematical model.
- Mathematical modeling of STKS data was able to describe bacterial killing effects as a function of both amikacin and PMB. Parameters were better identified as a function of amikacin due to an observed killing effect, whereas the parameters for polymyxin B killing effect were not well defined due to lack of activity.
- In the HFIM, the ATM/CAZ/AVI combination showed bactericidal activity (≥99.9% reduction); with a nadir of $4.00 \cdot 10^4$ cfu/mL.
- In the HFIM, the effects of β-lactam-only therapy were not enhanced with the addition of polymyxin B, which followed a similar trajectory. This matches static time kill observations, which showed killing effects independent of polymyxin B concentration (graphs appear as horizontal lines).
- Despite the absence of observed killing in bacterial counts, spectrophotometry readings for the ATM/CAZ/AVI combination were higher than the ATM/CAZ/AVI + PMB combination.

## RESULTS

**Figure 1: Static Time Kill Studies** For each time kill (each dot is a single 24h time kill), the area under the killing curve was obtained and normalized to the growth control. Data were fit as a function of either amikacin (AMI, left two panel) or Polymyxin B (PMB, right two panels) using Hill type functions. The model fit results are overlaid as solid lines, with each color representing different combinations. Because of the number of experiments performed, an automated, software-assisted approach was utilized to automatically pool similar experiments and fit the data to a Hill function. These data were then used to design a HFIM study.

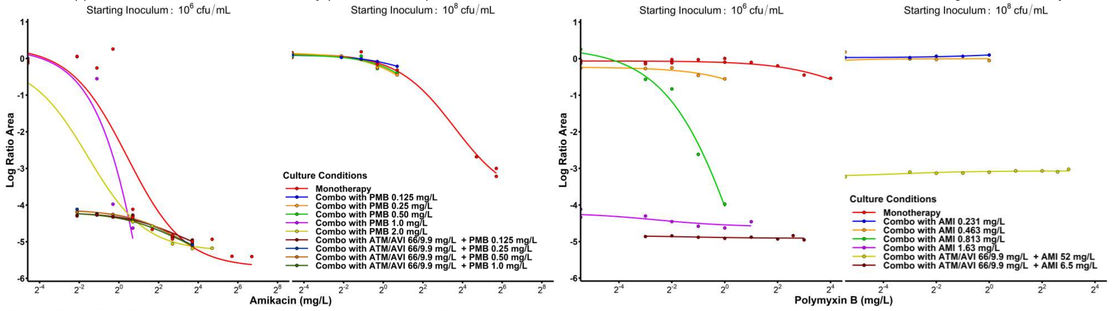


**Figure 2: Hollow Fiber Infection Model Pharmacodynamics** The total bacterial counts (black) overlaid with the resistant subpopulations from population analysis profiles (PAPs) using polymyxin B (PMB, green), ceftazidime (CAZ, blue), or aztreonam/avibactam (ATM/AVI, red). The shades of the PAPs indicate concentration with light shades being lower concentrations and dark shades being higher concentrations. All negative controls (non-treatment and monotherapies) showed a complete lack of antimicrobial activity, whereas the two combination therapies showed bactericidal effects.

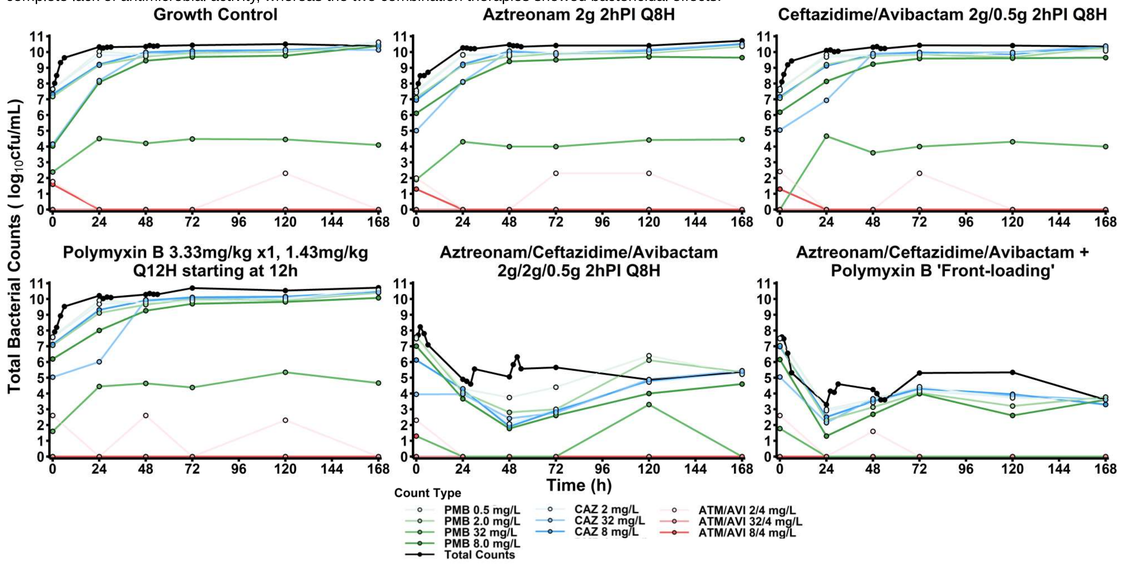


**Table 1: LRA vs. Amikacin Parameter Estimates** For time kill studies performed at $10^6$ cfu/mL starting inoculum, Hill-type functions were fit to the log-ratio area as a function of amikacin concentration alone and in combination with other agents. Because the killing as a function of PMB was nominal, the data were not able to inform a model properly and parameters were not estimated.

| Treatment | $E_{max}$ (95%CI) | $EC_{50}$ (95%CI) |
|---|---|---|
| Amikacin Monotherapy | 6.03 (4.82, 7.245) | 1.34 (0.243, 2.43) |
| + PMB 0.125 mg/L | 1.34 (-0.121, 2.80) | 8.98 (-14.6, 32.6) |
| + PMB 0.25 mg/L | 1.61 (0.678, 2.55) | 8.55 (-3.74, 20.8) |
| + PMB 0.50 mg/L | 1.42 (0.784, 2.05) | 7.48 (-1.53, 16.5) |
| + PMB 1.0 mg/L | 1.89 (0.455, 3.32) | 11.3 (-6.84, 29.5) |
| + PMB 2.0 mg/L | 5.15 (4.79, 5.52) | 0.338 (0.165, 0.511) |
| + ATM/AVI+PMB 0.125 mg/L | 4.02 (3.56, 4.48) | 12.0 (5.69, 18.3) |
| + ATM/AVI+PMB 0.25 mg/L | 21.6 (-101, 144) | 5.14 (-32.7, 43.0) |
| + ATM/AVI+PMB 0.50 mg/L | 1.89 (0.455, 3.32) | 11.3 (-6.84, 29.5) |
| + ATM/AVI+PMB 1.0 mg/L | 5.16 (4.79, 5.52) | 0.338 (0.165, 0.511) |

## CONCLUSIONS

- Gram negative bacteria harboring both a metallo-beta-lactamase (such as NDM), and an extended-spectrum beta-lactamase (such as CTX-M) are able to resist all forms of beta-lactam monotherapy.
- Additionally, co-expression of polymyxin resistance via the *mcr* gene eliminates the critically important polymyxins as a last-line therapeutic option.
- The combination of aztreonam + ceftazidime/avibactam was a able to produce bacterial killing and restore activity of the beta-lactams against SZ04, a clinical isolate of *K. pneumoniae* co-producing NDM, CTX-M, and MCR.
- The effects of MCR-mediated resistance were such that clinically relevant concentrations of polymyxin B failed to produce synergy.
- Mathematical modeling approaches of many static time kill studies are useful in identifying potent combinations and for designing more robust, translational studies in the Hollow Fiber Infection Model.

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