# Emergence of Avibactam Resistance in Multidrug-Resistant *Enterobacteriaceae*

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## Introduction

- Avibactam (AVI) is a non-β-lactam β-lactamase inhibitor used clinically with the β-lactam antibiotic ceftazidime.
- In addition to its β-lactamase inhibitor activity, AVI is known to bind penicillin-binding protein 2 (PBP2). [1]
- We have observed intrinsic *in vitro* antibacterial activity of AVI against multidrug-resistant *Enterobacteriaceae*.
- Here we describe emergence of AVI resistance during treatment and persistence in the absence of selective pressure.

## Methods

### Bacterial strains

- Two multidrug-resistant, carbapenem-resistant isolates:
  - **AR-0636** (*Klebsiella pneumoniae*; pan-resistant Nevada strain) [3]
  - **ARLG 2829/MCR1_NJ** (*E. coli)* [2]
- Two broadly susceptible clinical strains from our institution:
  - **BIDMC 22** (*K. pneumoniae*)
  - **BIDMC 49A** (*E. coli*)

### Emergence and duration of resistance

- ARLG 2829 and AR-0636 were grown in liquid culture with 128 µg/mL AVI (16x MIC) for 24 hours
- AVI MICs were then tested daily for 17 days following serial passage on antibiotic-free media

### Bacterial cell morphology

- Serial Gram stain images were obtained during AVI exposure

### Cross-resistance between AVI and other β-lactamase inhibitors

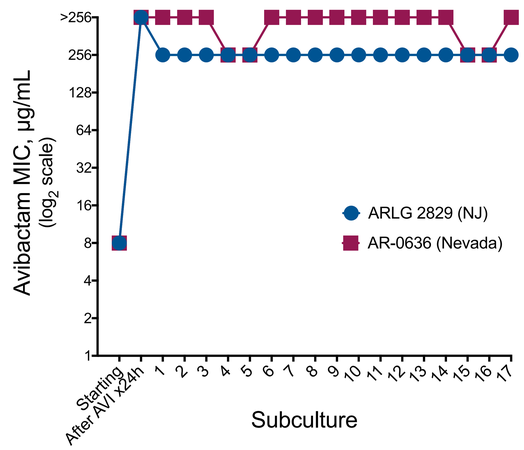
- BIDMC 22 and BIDMC 49A were grown in liquid culture with 128 µg/mL AVI for 24 hours
- MICs of AVI and 6 β-lactam antibiotics with different penicillin-binding protein (PBP) affinities were tested before and after AVI exposure

### Mouse thigh infection model

- Groups of 5 mice were infected with 1x10$^8$ CFU/thigh of *K. pneumoniae* AR-0636
- Mice were treated with AVI 250 mg/kg or saline every 8 hours for 24 hours then sacrificed for bacterial colony enumeration

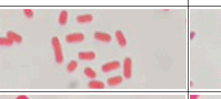
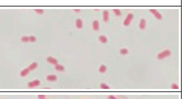
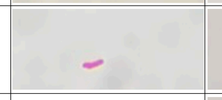
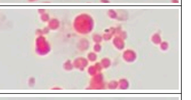
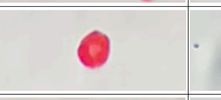
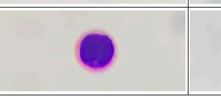
## Results

### Emergence and duration of resistance




AVI MIC before and after AVI exposure
Subcultures performed on antibiotic-free media

### Bacterial cell morphology

| AVI concentration | Time since addition of AVI to starting culture | | | |
|---|---|---|---|---|
| | 1 hr | 3 hr | 5 hr | 24 hr |
| None |  |  |  |  |
| 8 µg/mL |  |  |  |  |
| 32 µg/mL | No cells seen |  |  |  |
| 128 µg/mL | No cells seen |  |  |  |

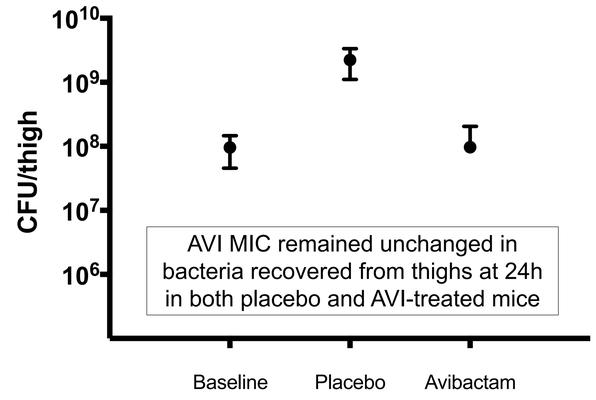
Rounded, enlarged appearance of cells is consistent with PBP2 treatment effect.
Gram stain images of AR-0636 viewed at 100x under oil immersion.

### Cross-resistance between AVI and other β-lactamase inhibitors

| Drug | PBP target(s) | BIDMC 22 (*K. pneumoniae*) | | | BIDMC 49A (*E. coli*) | | |
|---|---|---|---|---|---|---|---|
| | | Initial MIC | MIC after AVI | Doubling dilution change | Initial MIC | MIC after AVI | Doubling dilution change |
| Avibactam | 2 | 16 | 128 | | 8 | 128 | |
| Mecillinam | 2 | 0.25 | >16 | + | 0.063 | 1 | |
| Meropenem | 2>4>3>1 | 0.063 | 0.063 | 0 | 0.016 | 0.031 | |
| Amoxicillin | 4>2>3 | 8 | 32 | | 4 | 2 | |
| Cefepime | 3>2>1>4 | 0.063 | 0.063 | 0 | 0.031 | 0.125 | |
| Ceftazidime | 3>1 | 0.25 | 0.25 | 0 | 0.25 | 0.25 | 0 |
| Aztreonam | 3 | 0.063 | 0.063 | 0 | 0.063 | 0.125 | |

MICs before and growth for 24 hours with AVI 128 µg/mL
PBP: Penicillin-binding protein

### Mouse thigh infection model




CFU/thigh (AR-0636) at 1h after infection (baseline) and after 24h of treatment with saline or avibactam 250 mg/kg q8hr; 5 mice/group

## Conclusions

- AVI resistance emerged rapidly *in vitro* and persisted for >2 weeks in the absence of selective pressure.
- The observed morphological changes and the co-emergence of mecillinam resistance suggest that AVI resistance may be mediated by alterations in PBP2 or related pathways.
- In a mouse model, AVI reduced bacterial growth at 24 hours compared to placebo; resistance was not observed.
- These results have important implications for new β-lactamase inhibitors (nacubactam, zidebactam) with structural similarities to AVI that are likely to play an important future role in treatment of multidrug-resistant Gram negative bacteria. Future studies will include sequencing of resistant isolates to identify causative mutations.

**References**

[1] Sutaria DS et al. First Penicillin-Binding Protein Occupancy Patterns of β-Lactams and β-Lactamase Inhibitors in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother*. 2018 May 25;62(6):e00282-18.
[2] Chen L et al. Notes from the field: pan-resistant New Delhi metallo-beta-lactamase-producing *Klebsiella pneumoniae* – Washoe County, Nevada, 2016. *MMWR* 2017; 66:33.
[3] Mediavilla JR et al. Colistin-and carbapenem-resistant *Escherichia coli* harboring mcr-1 and blaNDM-5, causing a complicated urinary tract infection in a patient from the United States. *mBio* 2016; 7:e01191-16.

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