Saturday - Poster # 2797

# Sulbactam-Durlobactam is Bactericidal Against Clinical Isolates of *Acinetobacter baumannii*

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## Abstract

**Background**: Sulbactam-durlobactam (SUL-DUR) is an antibiotic being developed for treatment of infections caused by *Acinetobacter baumannii-calcoaceticus* complex (ABC), including multidrug-resistant and carbapenem-resistant strains. Durlobactam (ETX2514) is a diazabicyclooctane β-lactamase inhibitor with potent activity against Ambler classes A, C and D serine β-lactamases that effectively restores sulbactam antibacterial activity against ABC. The goal of this study was to determine the rate of bacterial cell death in the presence of SUL-DUR for clinical isolates of *Acinetobacter baumannii*.

**Methods**: Susceptibility testing was performed by broth microdilution according to CLSI guidelines. The clinical isolates of *A. baumannii* used in this study were selected based on their range of antibiotic susceptibilities and their β-lactamase gene content. The SUL-DUR minimal bactericidal concentration (MBC) and static time kill experiments were performed on ten *A. baumannii* clinical isolates using CLSI methodology. The static time kill was performed in the presence of 4x and 8x the SUL-DUR MIC and was measured over a period of 24 hours by removing samples at various time points to determine cell viability on compound-free media. For the MBC method, bactericidal was defined as an MBC/MIC ratio ≤4. For the time kills, bactericidal was defined as a ≥3-log reduction in viable cells.

**Results**: Using the MBC method, SUL-DUR was bactericidal against the majority of isolates tested. For 9 of 10 isolates tested, SUL-DUR MBCs were within one dilution of the MIC with an MBC/MIC ratio < 4. For one isolate the MBC/MIC ratio was 16 (64/4 μg/ml). Using the static time kill method, SUL-DUR was bactericidal against a majority of the ten *A. baumannii* isolates tested. At 8x MIC, 6 isolates demonstrated a >3-log reduction in viable cells and 3 additional isolates showed a 2.4-2.9 log reduction by 24 hours. At 4x MIC, 4 isolates resulted in >3-log reduction and 3 isolates showed a 2.4-2.8 log reduction in viable cell counts. At 4x MIC, 3 isolates had some rebound of bacterial growth at 24 hours.

**Conclusions**: Overall, sulbactam-durlobactam demonstrated bactericidal, time-dependent killing of *A. baumannii* clinical isolates. These data support the potential utility of SUL-DUR, if approved, for the treatment of infections caused by ABC organisms.

## Introduction

The Gram-negative organisms collectively named the *Acinetobacter baumannii-calcoaceticus* complex (ABC) have emerged as serious pathogens[1]. The ABC complex includes *A. baumannii*, *A. nosocomialis*, *A. pittii* and *A. calcoaceticus*. *A. baumannii* is considered the most clinically important species of the complex due to its association with nosocomial outbreaks. Globally, the susceptibility of ABC to all antimicrobial agents has declined over the last 20 years[2].

Sulbactam-durlobactam (SUL-DUR) recently completed a Phase 3 clinical trial for the treatment of infections caused by carbapenem-resistant ABC organisms. Sulbactam (SUL) is an approved β-lactamase inhibitor (BLI) with antibacterial activity against *Acinetobacter* spp. due to its inhibition of PBP3, an enzyme required for cell wall biosynthesis[3]. However, degradation of SUL by the β-lactamases present in most contemporary ABC isolates limits its clinical use. Durlobactam (DUR) is a diazabicyclooctane BLI with potent activity against class A, C and D serine β-lactamases[4]. DUR protects SUL from degradation, restoring antibacterial activity against ABC organisms.

The goal of this study was to determine the rate of bacterial cell death of *A. baumannii* in the presence of sulbactam in combination with durlobactam. Ten different *A. baumannii* clinical isolates were selected for testing based on their range of susceptibilities to sulbactam and their β-lactamase gene content, which had been previously determined by whole genome sequencing.

## *A. baumannii* isolates used in this study

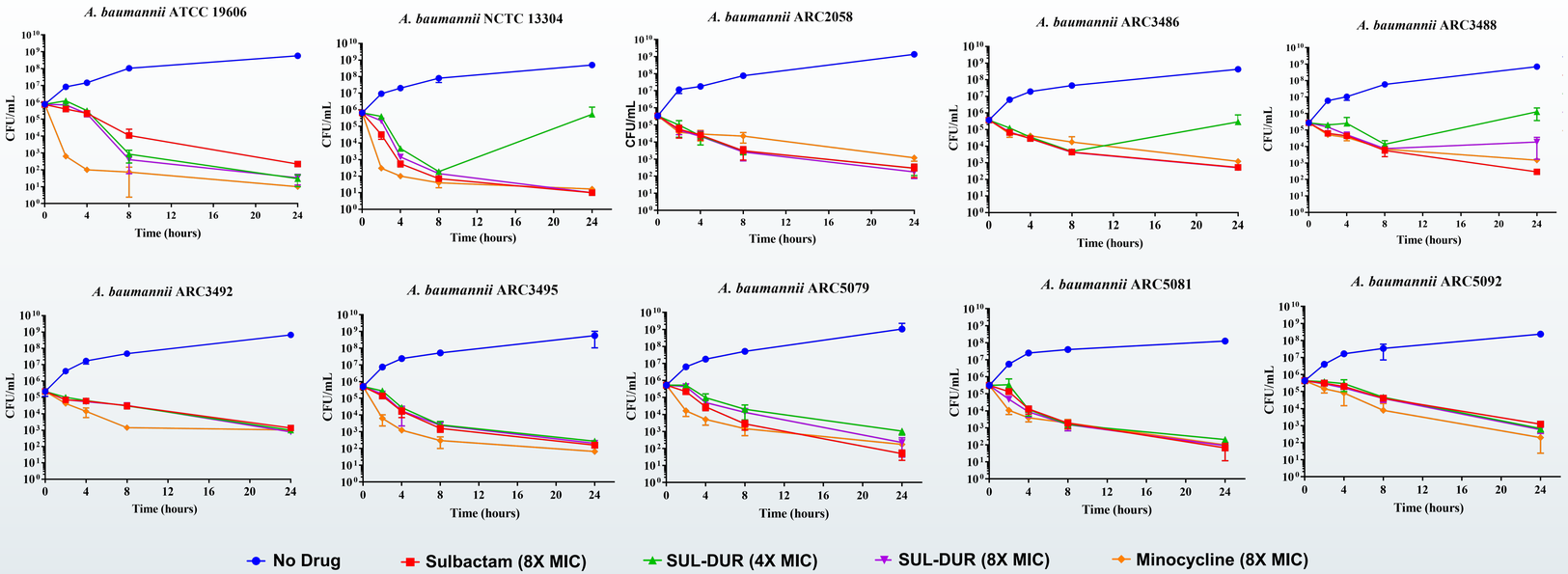
| Isolate ID | β-lactamase genes | Sulbactam MIC (μg/mL) | SUL-DUR MIC (μg/mL) | Minocycline MIC (μg/mL) |
|---|---|---|---|---|
| ATCC19606 | ADC-2-like; OXA-98 | 2 | 1 | 0.5 |
| NCTC13304 | OXA-23; OXA-66; TEM-1 | 32 | 1 | 4 |
| ARC2058 | ADC-3-like; OXA-95 | 2 | 1 | 0.5 |
| ARC3486 | ADC-30; OXA-72; OXA-66; TEM-1 | 32 | 1 | 4 |
| ARC3488 | ADC-76; OXA-235-like; OXA-68 | 8 | 1 | 2 |
| ARC3492 | ADC-15-like; OXA-24; OXA-132; TEM-1 | 16 | 1 | 2 |
| ARC3495 | ADC-30-like; OXA-24; OXA-109 | 64 | 1 | 2 |
| ARC5079 | ADC-52-like; OXA-72; OXA-65 | >64 | 2 | 1 |
| ARC5081 | ADC-80; OXA-94; OXA-23 | 16 | 4 | 0.5 |
| ARC5092 | ADC-5-like; OXA-23; OXA-64 | 16 | 1 | 8 |

SUL-DUR = sulbactam-durlobactam. MIC testing was performed as doubling dilutions of sulbactam in the presence of a fixed concentration of 4 μg/mL durlobactam.

## Minimal Bactericidal Concentration (MBC) Results

| *A. baumannii* Isolate | Inoculum (CFU/mL) | Sulbactam-Durlobactam | | | Sulbactam | | | Imipenem | | | Minocycline | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | MIC (μg/mL) | MBC (μg/mL) | MBC/MIC ratio | MIC (μg/mL) | MBC (μg/mL) | MBC/MIC ratio | MIC (μg/mL) | MBC (μg/mL) | MBC/MIC ratio | MIC (μg/mL) | MBC (μg/mL) | MBC/MIC ratio |
| ATCC19606 | $1.5 \times 10^6$ | 1 | 1 | 1 | 2 | 2 | 1 | 0.5 | 0.5 | 1 | 0.5 | 1 | 1 |
| NCTC13304 | $1.5 \times 10^6$ | 1 | 1 | 1 | 32 | 32 | 1 | 32 | 32 | 1 | 4 | 0.06 | 0.02 |
| ARC2058 | $7.8 \times 10^5$ | 1 | 2 | 2 | 2 | 4 | 2 | 0.25 | 0.25 | 1 | 0.5 | 0.06 | 0.12 |
| ARC3486 | $8.4 \times 10^5$ | 1 | 2 | 2 | 32 | 32 | 1 | >64 | ND | ND | 4 | 32 | 8 |
| ARC3488 | $5.8 \times 10^5$ | 1 | 1 | 1 | 8 | 8 | 2 | 8 | 8 | 1 | 2 | 32 | 16 |
| ARC3492 | $5.2 \times 10^5$ | 1 | 1 | 1 | 16 | 32 | 2 | 64 | 64 | 1 | 2 | 16 | 8 |
| ARC3495 | $7.7 \times 10^5$ | 1 | 1 | 1 | 64 | 64 | 1 | >64 | ND | ND | 2 | 2 | 1 |
| ARC5079 | $9.3 \times 10^5$ | 2 | 2 | 1 | >64 | ND | ND | 64 | 64 | 1 | 1 | 2 | 2 |
| ARC5081 | $9.0 \times 10^5$ | 4 | 64 | 16 | 16 | 16 | 1 | 16 | 16 | 1 | 1 | 32 | 32 |
| ARC5092 | $1.0 \times 10^6$ | 1 | 1 | 1 | 16 | 16 | 1 | 16 | 32 | 2 | 8 | >64 | ≥16 |

## Static Time Kill Studies on 10 *A. baumannii* Clinical Isolates




## Methods

**MIC/MBC Testing:** MIC values were determined using the broth dilution method following CLSI guidelines.[5] Minimal bactericidal concentration (MBC) values were measured according to CLSI guidelines.[6] MBC/MIC ratios ≤4 were interpreted as bactericidal. SUL-DUR was tested as a dilution of SUL in the presence of 4 μg/mL DUR.

**Static time-kill measurements**. Bacterial cultures were grown overnight at 35°C on blood agar plates (Remel). 5-10 colonies were resuspended in 2 mL of MHBII (Becton Dickinson) and grown overnight at 35°C with shaking at 200 rpm. The overnight culture was diluted 1/10 and incubated for ~2 hours at 35°C with shaking at 200 rpm until the cells reached exponential phase growth ($OD_{600}$=0.1 to 0.3). The exponential-phase bacterial culture was added to 18 x 150-mm glass tubes to obtain a final density of ~5 x $10^5$ CFU/mL in a volume of 10 mL. SUL-DUR was added at 4- and 8-fold of the MIC. Sulbactam alone and minocycline were tested as controls at 8-fold the MIC against the same set of strains. Each strain/drug combination was tested in triplicate. Test tubes were incubated without shaking at 35°C for 24 hrs. Samples were taken at various time points and a dilution series of the samples were plated onto MHBII agar plates. Colony forming unit (CFU) determinations were made after overnight incubation at 35°C to determine the number of viable cells. Data points are the averages and standard deviations of 3 independent measurements.

## Conclusions

- Ten *A. baumannii* clinical isolates were used to profile cell killing in the presence of SUL-DUR and comparator compounds.
  - 8 of 10 isolates were sulbactam- and carbapenem-resistant.
  - These isolates encode for a variety of β-lactamases
- SUL-DUR was bactericidal against 9 of 10 *A. baumannii* isolates tested (MBC/MIC ratio ≤4).
- In static time kill experiments, SUL-DUR demonstrated a significant reduction in cell viability over 24 hours at compound concentrations 4-fold the MIC and higher.
- These data support development of SUL-DUR for the potential treatment of multidrug-resistant *A. baumannii* infections.

## References

**1.** El Chakhtoura *et al.* (2018) *Expert Rev Anti Infect Ther* 16: 89-110
**2.** Gales *et al.* (2019) *Open Forum Infect Dis* 6:S34-S46
**3.** Penwell *et al.* (2015). *Antimicrob Agents Chemother* 59: 1680-1689
**4.** Durand-Reville *et al.* (2017) *Nature Microbiol* 2:171045.
**5.** CLSI, M07-A10, 2015).
**6.** CLSI, M26A, 1999.

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