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Effect Of Susceptibility Testing Conditions On The *In Vitro* Antibacterial Activity Of Sulbactam-Durlobactam

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Abstract

Background: Sulbactam-Durlobactam (SUL-DUR) is an antibiotic designed to treat serious infections caused by *Acinetobacter baumannii*, including multidrug-resistant strains, which is currently in Phase 3 clinical testing. Durlobactam (previously 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 *A. baumannii*. The goal of this study was to determine the effects of varying *in vitro* testing parameters of the broth microdilution assay on SUL-DUR activity.
Methods: Susceptibility testing was performed according to CLSI guidelines. The effect of varying testing conditions, including inoculum, temperature, atmosphere, divalent cations, pH and body fluids on the SUL-DUR MIC was measured against ten clinical isolates of A. baumannii, which were selected based on their range of susceptibilities to sulbactam alone and their β-lactamase gene content.
Results: Altering the size of the starting inoculum, temperature, atmospheric conditions, divalent cation concentration, pH or the presence of surfactant, urine, human albumin or human serum did not affect the susceptibility of SUL-DUR against a majority of the isolates tested with one exception: a 4- to 8-fold decrease in susceptibility to SUL-DUR for most of the isolates in the presence of 50% urine at pH 5.0 was observed. The MIC values under all other test conditions were within one to two dilutions of the values seen in the presence of the CLSI standard media.
Conclusions: The only experimental condition that consistently increased SUL-DUR MIC values was testing in the presence of urine at pH 5.0, where up to an 8-fold decrease in susceptibility was seen against several isolates. Changes in temperature, atmospheric conditions, starting inoculum size, concentration of divalent cations, presence of surfactant, or the presence of human serum or human albumin had little impact on *A. baumannii* susceptibility to SUL-DUR. These data suggest that slight variations in testing conditions during routine susceptibility testing should have no significant effects on SUL-DUR MIC values.

Introduction

Sulbactam-durlobactam (SUL-DUR) is an antibiotic currently in Phase 3 clinical development for the treatment of infections caused by *Acinetobacter baumannii-calcoaceticus* complex (ABC), including multidrug-resistant isolates [1]. Sulbactam (SUL) is a β-lactamase inhibitor that also has intrinsic antibacterial activity against ABC. However, degradation of SUL by a variety of β-lactamases present in most clinical ABC isolates limit its clinical use. Durlobactam (DUR, formerly ETX2514) is a diazabicyclooctane (DBO) β-lactamase inhibitor with an expanded spectrum of activity compared to other DBO inhibitors, which includes coverage of a broad range of class A, C and D β-lactamases [2].

The goal of this study was to determine the effects of experimental conditions on the SUL-DUR *in vitro* susceptibility test. The effects of varying the starting inoculum concentration, incubation temperature and atmosphere, pH of the growth medium, and concentration of divalent cations in the growth medium were evaluated. The effect of surfactant and urine over a range of pH values on bacterial susceptibility to SUL-DUR was also tested. In addition, the potential of SUL-DUR protein binding to alter antibacterial activity was measured by performing in vitro susceptibility testing in the presence of human albumin and human serum.

Materials and Methods

- MIC values were determined using the broth dilution method following CLSI guidelines [3] and MBC values were determined using CLSI methods [4]. Susceptibility tests were set up with the various additives to the media or incubated under different atmospheric conditions (ambient, 5% CO2, or anaerobic) or different temperatures (30°C, 35°C or 37°C).
- Ten clinical isolates of *A. baumannii* were tested based upon their range of susceptibilities to sulbactam and their β-lactamase gene content, determined by whole genome sequencing.
- The 5% CO_2 and anaerobic conditions were created using the BD GasPak™ EZ Container System with the CO2 Pouch System or the Anaerobe Sachets, per manufacturer's instructions.
- Three different starting inocula were assessed: standard 5 x 10^5 CFU/mL specified by CLSI [3], a lower inoculum of approximately 10^4 CFU/mL and a higher inoculum of approximately 10^7 CFU/mL. Each starting inoculum was confirmed by enumerating the actual CFU/mL.
- The MBC was interpreted to be bactericidal if a ≥3 log reduction in viable organism counts within 8-fold of the MIC within 24 hours was observed.
- To determine the effect of varying pH on SUL-DUR *in vitro* antibacterial activity, cation-adjusted Mueller Hinton Broth (MHBII) was adjusted with 0.1 N NaOH to obtain a pH of 8.0 or 0.1 N HCl to obtain a pH of 7.0, 6.0 or 5.0. After pH adjustment, the medium was filter sterilized.
- The MIC values for SUL-DUR in the presence of 50% human urine, 50% human serum, 4% human albumin, and 1%, 5% or 10% bovine surfactant in MHBII was assayed. To prepare the 50% urine and 50% human serum growth media, a 2X concentration of MHBII was diluted 1:2 with either heat-inactivated human serum or human urine (pooled urine from 5 human volunteers). The 50% human urine solution was pH adjusted to pH 5.0, 6.0, 7.0 and 8.0 as described above. Albumin from human serum was added to MHBII at 4% w/v. The 4% human albumin, 50% human serum and 50% human urine solutions were subsequently filtered sterilized. Sterile bovine surfactant was added to sterile MHBII at 1%, 5% or 10%.
- The following divalent cation concentrations were assessed: non-supplemented MHB, which contains trace amounts of divalent cations, 25 and 50 mg/L Ca^{2+}, 12.5 and 25 mg/L Mg^{2+}; and 50 mg/L Mn^{2+}. Preparation of cation stock solutions followed CLSI guidelines.
- To test the activity of SUL-DUR in the absence of iron, an iron-deficient version of MHBII was prepared [5]. Media was filter sterilized and 3.6 ml of 1 M $MgSO_4$ and 360µL of 1M $CaCl_2$ were added to supplement the chelated Mg^{2+} and Ca^{2+}.

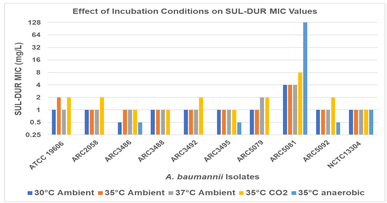
A. baumannii Strains Used in This Study

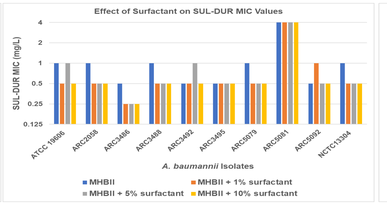
Strain Number	Description / β-lactamase genes
ATCC 19606	ADC-2-like; OXA-98
NCTC 13304	CLSI reference strain / ADC-30; TEM-1; OXA-23; OXA-66
ARC2058	ADC-3-like; OXA-259
ARC3486	ADC-30; TEM-1; OXA-66; OXA-72
ARC3488	ADC-76; OXA-68; OXA-235-like
ARC3492	ADC-52-like; TEM-1; OXA-24; OXA-132
ARC3495	ADC-30-like; OXA-24; OXA-109
ARC5079	ADC-52-like; OXA-65; OXA-72
ARC5081	ADC-80; ADC-81-like; OXA-23; OXA-94
ARC5092	ADC-5-like; OXA-23; OXA-64

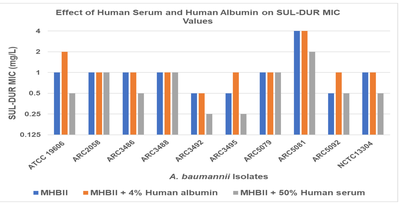
References

1 - NCT03894046: Study to evaluate the efficacy and safety of intravenous sulbactam-ETX2514 in the treatment of patients with infections caused by *Acinetobacter baumannii-calcoaceticus* complex (ATTACK). https://clinicaltrials.gov/ct2/show/NCT03894046.
2 - Durand-Reville TF *et al.* (2017) ETX2514 is a broad-spectrum β-lactamase inhibitor for the treatment of drug-resistant Gram-negative bacteria including *Acinetobacter baumannii*. Nat Microbiol 2:17104.
3 - CLSI M07-A10. (2015). Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. Volume 35. Number 2.
4 - CLSI M26-A. (1999). Clinical and Laboratory Standards Institute. Methods for Determining Bactericidal Activity of Antimicrobial Agents; Approved Guideline. Volume 19. Number 18.
5 – McPherson CJ *et al.* (2012). Clinically relevant Gram-negative resistance mechanisms have no effect on the efficacy of MC-1, a novel siderophore-conjugated monocarbam. Antimicrob. Agents and Chemother. 56: 6334-6342.
6 – Keepers TR *et al.* (2015) Effect of in vitro testing parameters on ceftazidime-avibactam minimum inhibitory concentrations. Int Sch Res Notices 2015, 489547..

Results



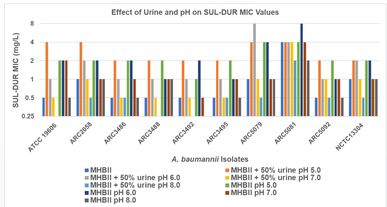


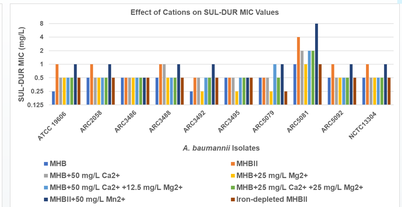


- MIC values for SUL-DUR were within 1-dilution of each other across 30ºC, 35ºC and 37ºC.
- Most of the *A. baumannii* isolates grew poorly under anaerobic conditions. ARC5081 showed decreased susceptibility to SUL-DUR under anaerobic conditions, but growth was not robust.

- Inability of a compound to penetrate the surfactant layer of the lungs can decrease antibiotic susceptibility in the treatment of lung infections
- Addition of 1%, 5% or 10% bovine surfactant had no effect on SUL-DUR susceptibility
- Conversely, there was a 32-fold decrease in *S. aureus* susceptibility to daptomycin in the presence of 1% surfactant (data not shown)

- The addition of 50% human, heat-inactivated serum or 4% human albumin had no effect on the SUL-DUR susceptibility.
- All MIC values were within one dilution compared to what was seen with MHBII.
- In contrast, a >8-fold decrease in *S. aureus* susceptibility to novobiocin in the presence of serum (data not shown)





- The presence of urine had little effect on SUL-DUR susceptibility at a pH range of 6.0-8.0
- There was a 4- to 8-fold decrease in SUL-DUR susceptibility in the presence of 50% urine at pH5.0.

- Varying the concentration of divalent cations resulted in a ≤2-fold change in SUL-DUR MIC values for most isolates
- Conversely, decreases in tigecycline and daptomycin susceptibilities were observed, depending on the cation (data not shown)

Also performed, but not shown:
- No inoculum effect was observed for SUL-DUR for 9 out of 10 *A. baumannii* isolates
- MBC values derived from the CLSI standard inoculum indicate that SUL-DUR is bactericidal, for all but one isolate.

Conclusions

- **The effects of changing experimental conditions that may influence SUL-DUR susceptibility testing were measured against ten *A. baumannii* isolates.**
- **Changes in starting inoculum concentration, temperature, atmospheric conditions, concentration of divalent cations and iron in the growth media and pH had no effect on the MIC values for SUL-DUR against most of the strains tested.**
- **The presence of pulmonary surfactant, human albumin, human serum or human urine also had little effect on SUL-DUR susceptibilities.**
- **One condition that did influence SUL-DUR MIC values was low pH (pH 5.0), particularly in the presence of 50% urine, where up to an 8-fold decrease in susceptibility was seen against seven out of the 10 isolates. This is similar to what has been reported for the ceftazidime-avibactam β-lactam / β-lactamase combination [6].**
- **These data indicate that small variations in testing parameters during routine MIC testing should have no significant impact on SUL-DUR MIC values.**

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