MONDAY-003

Broth Microdilution Testing of S-649266, Siderophore Cephalosporin, Against *Acinetobacter baumannii* and Appropriate Interpretation of MIC Endpoints

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Revised Abstract

Background: S-649266 is a novel parenteral catechol-substituted siderophore cephalosporin that is active against carbapenem-resistant Gram-negative bacteria. Accurate *in vitro* testing of S-649266 by broth microdilution (BMD) requires the use of iron-depleted (ID) conditions to mimic the condition in mammalian hosts. Indistinct MIC endpoints (trailing) have been observed when testing *Acinetobacter baumannii.* This study was designed to ascertain the reproducibility of MIC endpoints, and to establish proposed guidelines for determining MIC values for S-649266.

Methods: All MICs were determined by BMD according to CLSI guidelines except cation-adjusted Mueller Hinton broth (CAMHB) was replaced with ID-CAMHB made iron deficient by pretreatment with Chelex-resin (Bio-Rad Laboratories) with replenishment of Ca, Mg and Zn. Technologists were instructed to confirm that there was strong growth control (i.e. a button of > 2 mm) before proceeding to read the MICs. MIC endpoints were defined as the first well in which growth was significantly reduced (i.e. a button of < 1 mm or light/faint turbidity) relative to control. Trailing was noted when present. To determine the reproducibility of interpretation of the MIC endpoint, 20 *A. baumannii* were read by 8 technologists, who were instructed to follow the proposed guidelines.

Results: When read by 8 different technologists, 17/20 (85%) *A. baumannii* MICs were within one doubling dilution.

Conclusions: Trailing occurs when testing *A. baumannii*, however the proposed interpretive criteria guidelines for reading S-649266 MIC endpoints are effective when trailing is encountered.

Introduction

S-649266 is a novel parenteral catechol-substituted siderophore cephalosporin that is active against carbapenem-resistant Gram-negative bacteria. Accurate *in vitro* testing of S-649266 by broth microdilution requires the use of iron-depleted (ID) conditions to mimic the condition in mammalian hosts. Indistinct minimum inhibitory concentration (MIC) endpoints (trailing) have been observed when testing *Acinetobacter baumannii.* This study was designed to determine how well different technologists follow the recommended interpretive criteria (summarized below) when reading broth microdilution MICs of S-649266, and whether following the criteria results in consistent S-649266 MICs being generated across different laboratory personnel.

Materials & Methods

MICs for 20 *A. baumannii* were read by eight technologists who were instructed to follow the proposed guidelines to determine whether they are effective in interpreting the S-649266 MIC endpoint.

All MICs were determined by broth microdilution according to CLSI guidelines [1] except cation-adjusted Mueller Hinton broth (CAMHB) was replaced with iron deficient CAMHB (ID-CAMHB) made iron deficient by pretreatment with Chelex-resin (Bio-Rad Laboratories) with replenishment of Ca, Mg and Zn. CAMHB was used for a control drug (meropenem).

Growth controls were included for both ID-CAMHB and CAMHB. The panels were incubated at 35°C for 20 hours before reading the MIC endpoints.

Quality control (QC) testing was performed each day of testing using *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. All meropenem QC results were within specified CLSI ranges [2].

Guidelines for reading S-649266 endpoints are shown in Table 1:

Results

Table 1. Proposed Guidelines for Reading S-649266 Endpoints

Step 1 - Confirmation of Strong Growth in CT-MHB Growth Control Well

- Prior to reading MICs the technologist will examine the ID-CAMHB growth control well and confirm that there is strong growth (i.e. a button of > 2 mm or heavy turbidity).
- If strong growth is confirmed, reading of S-649266 MIC can proceed.
- If strong growth is not obtained, the reading of the S-649266 MIC should not proceed. The test can be repeated and if strong growth still is not obtained, the MIC should be recorded as NA (not available due to poor growth).

Step 2 - Reading the S-649266 MIC

- Reading the MIC is contingent on the presence of strong growth in the growth control as per Step 1 above.
- The S-649266 MIC is read as the first drug well in which the growth is significantly reduced (i.e. a button of < 1 mm or light/faint turbidity) relative to the growth observed in the growth control.

Figure 1. Broth Microdilution Panel Showing *A. baumannii* #1103193.

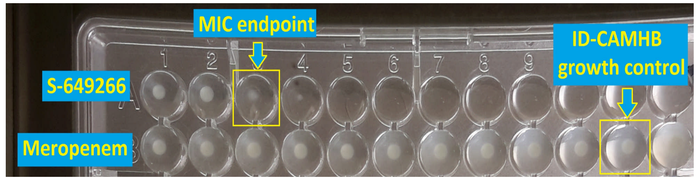

Figure 2. Broth Microdilution Panel Showing *A. baumannii* #1065051.

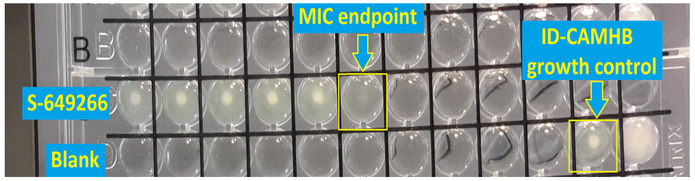

Table 2. Blinded Interpretation of MIC values for S-649266 against *A. baumannii* by Eight Technologists

IHMA No.	Tech #1	Tech #2	Tech #3	Tech #4	Tech #5	Tech #6	Tech #7	Tech #8	Trailing Yes/No
1010939	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	N
1065042	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	N
1065046	0.25	0.12	0.25	0.12	0.25	0.25	0.25	0.25	Y
1065051	1	1	1	1	1	1	1	1	N
1070594	0.12	0.12	0.12	0.12	0.25	0.12	0.25	0.25	N
1073418	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	Y
1073445	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	Y
1098736	0.06	0.06	0.06	0.06	0.12	0.12	0.12	0.12	N
1103193	0.12	0.12	0.25	0.12	0.25	0.25	0.12	0.25	Y
1120696	0.25	0.25	0.25	1	0.25	0.25	0.25	0.25	Y
1136535	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	Y
1136536	4	4	8	4	8	8	8	8	Y
1136539	0.5	0.5	0.25	1	0.25	1	0.5	1	Y
1145091	0.12	0.12	0.25	0.25	0.25	0.25	0.25	0.25	N
1145223	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	Y
1163910	0.12	0.12	0.25	0.12	2	0.12	0.12	0.25	Y
1217593	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	Y
1217598	0.12	0.12	0.12	0.12	0.12	0.12	0.12	0.12	N
1217599	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	N
1217604	≤0.03	≤0.03	≤0.03	≤0.03	0.12	0.12	0.12	0.12	N

All MIC values in µg/mL

Results Summary

- Examples of S-649266 endpoints following proposed guidelines are shown in Figure 1 and Figure 2.
- When read by eight different technologists, 17/20 (85%) *A. baumannii* MICs were within one doubling dilution (Table 2).

Conclusions

- Proposed guidelines allow for accurate and reproducible reading of S-649266 MICs
- Trailing occurs inconsistently when testing *A. baumannii*, however the proposed interpretive criteria guidelines for reading S-649266 MIC endpoints are effective when trailing is encountered.

References

1. Clinical Laboratory Standards Institute (CLSI), 2015. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standards -- Tenth Edition.* CLSI document M07-A10 (ISBN 1-56238-988-2). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

2. Clinical and Laboratory Standards Institute (CLSI), 2015. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement.* CLSI Document M100-S25 (ISBN 1-56238-990-4). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.