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Paper Poster Session
Antimicrobial susceptibility testing of Gram-negative bacteria

Use of Iron Depleted Mueller Hinton Broth (IDMHB) for microdilution testing of S-649266, a novel siderophore cephalosporin

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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 requires the use of iron-depleted conditions to mimic the condition in mammalian hosts. Previous MIC testing of S-649266 has used apotransferrin containing medium and Chelex-treated Isosensitest (ISB) broth. In this study, iron depleted Mueller Hinton Broth (IDMHB) was used as an alternative method to address issues of standardization.

Material/methods: Broth microdilution testing was done according to appropriate CLSI guidelines except that IDMHB was made iron deficient by treatment with Chelex-resin with subsequent replenishment of Ca, Mg and Zn. IDMHB did not affect the growth control. The S-649266 MIC was defined 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 control. The PK/PD relationship between *in vitro* MIC in IDMHB and *in vivo* efficacy was determined using rat lung infection model with SPF SD male rat (n=4-6). The free human plasma exposure profile of free S-649266 based on a 2 g dose 1 hour infusion in healthy volunteers was recreated in cannulated rats. Using the human PK profiles, the efficacy of S-649266 was evaluated against 5 strains of KPC or NDM producing *K. pneumoniae*, 2 strains of *P. aeruginosa* and 4 strains of *A. baumannii* including multi-drug-resistant (MDR) strains. In addition, to examine the effect of iron concentration on antibacterial activity of S-649266.MIC experiments were performed to correlate specific iron concentrations in IDMHB (0.02->0.2 mg/L).

Results: IDMHB was considered to be the best medium for the MIC determination of S-649266 due to the following reasons: 1)Trailing was observed in 64/200 (32%) of the *A. baumannii*, however using the established guidelines for reading the MICs, it was possible to assign a reproducible MIC value to 199/200 (99.5%) of the isolates; 2) MIC determined in IDMHB showed well correlation with the *in vivo* efficacy in rodent lung infection models caused by Enterobacteriaceae, *P. aeruginosa*, or *A. baumannii* whereas MIC in CAMHB did not correlate with *in vivo* efficacy. By using new MIC endpoint, almost same MIC values were obtained in IDMHB and Chelex-treated ISB. 3) CA-MHB with defined iron

concentration showed MIC increased depending on the iron concentration. S-649266 MIC against various Gram-negative bacteria fluctuates depending on ion concentration of medium.

Conclusions: Based on the good correlation between in vitro MICs obtained in IDMHB and *in vivo* efficacy in the animal models IDMHB is the most effective and reliable broth medium for assessing the *in vitro* activity of S-649266.