#P0186

28th ECCMID
Madrid, Spain
21-24 April, 2018

Correlations between Cefiderocol Broth Microdilution MICs and Disk Diffusion Inhibitory Zone Diameters among Target Gram-Negative Organisms

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ABSTRACT

Background: Cefiderocol (S-649266) is a novel siderophore cephalosporin with potent activity against a wide variety of Gram-negative bacteria, including carbapenem-resistant strains. In order to evaluate the utility of disk diffusion for in vitro susceptibility testing of cefiderocol, this study was conducted to determine the correlation between broth microdilution MICs and disk diffusion inhibitory zone diameters for this drug.

Materials and Methods: In all, 1319 Gram-negative clinical isolates were included in this study (807 Enterobacteriaceae, 173 *Pseudomonas aeruginosa*, 254 *Acinetobacter baumannii*, 3 *Burkholderia cepacia*, and 82 *Stenotrophomonas maltophilia*). The study isolates represented a global collection obtained during 2014 and 2015. All isolate identifications were confirmed and susceptibility testing was performed centrally (IHMA, Inc., USA). Susceptibility testings were conducted according to the Clinical and Laboratory Standard Institute guidance. For broth microdilution, iron-depleted CAMHB (ID-CAMHB) was used. Disk diffusion tests using the disk containing 30 µg cefiderocol were performed on unsupplemented standard Mueller-Hinton agar (MHA). In addition, the potential effect of the addition of iron into MHA for disk diffusion testing was evaluated with two QC strains (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853).

Results: For the two QC strains (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853) the addition of iron at 1 mg/L to MHA did not notably impact the zone diameters obtained. Because the iron content in MHA is approximately 0.3 mg/L, it was determined that disk diffusion studies could be conducted using standard MHA. Correlation between MIC values in ID-CAMHB and disk zones was relatively high for all test isolates. The R^2 values observed for Enterobacteriaceae, *A. baumannii, P. aeruginosa*, and *S. maltophilia* were 0.70, 0.81, 0.52, and 0.65, respectively. The relatively low R^2 values observed for *P. aeruginosa* and *S. maltophilia* may not reflect true correlation due to the lack of resistant strains.

Conclusions: The disk diffusion studies using a 30 µg of cefiderocol disk and standard MHA showed that the zone diameters obtained in these studies have a good correlation with the MIC determined using ID-CAMHB. Disk diffusion test was validated as an alternative method for testing cefiderocol MIC for Gram-negative bacteria.

INTRODUCTION

Cefiderocol is a novel parenteral catechol-substituted siderophore cephalosporin that is active against carbapenem-resistant Gram-negative bacteria (1, 2, 3). This study was performed to determine the effect of iron on the *in vitro* antibacterial activity and for correlation of cefiderocol MIC and disk diffusion method.

MATERIALS AND METHODS

Test organisms
In all 1319 Gram-negative bacteria isolates (807 Enterobacteriaceae, 254 *Acinetobacter baumannii,* 173 *Pseudomonas aeruginosa,* 82 *Stenotrophomonas maltophilia,* and 3 *Burkholderia cepacia*) were analyzed. These clinical isolates were obtained from IHMA, Inc. Two ATCC quality control (QC) organisms were tested: *Escherichia coli* ATCC2522 and *P. aeruginosa* ATCC27853.

Compound
Cefiderocol and cefepime were used. Cefiderocol disks were manufactured by MAST (Merseyside, UK) and Bio-Rad (Marnes-la-Coquette,FR).

MIC determination
The appropriate medium for the disk diffusion study of cefiderocol has been approved by Clinical and Laboratories Standard Institute (CLSI) guidelines to use standard MHA. A standardized inoculum of bacteria is swabbed onto the surface of a Mueller-Hinton agar plate. Filter paper disks with cefiderocol are placed on the agar. After overnight incubation in a 35-37°C ambient incubator for 18-20 hours, the diameter of the zone of inhibition is measured around each disk. CLSI approved cefiderocol disk QC range was used as follows: *E. coli* ATCC2522 (25-31 mm) and *P. aeruginosa* ATCC27853 (22-31 mm). To check the correlation of both MIC determination methods, broth microdilution testing was done by using iron-depleted cation-adjusted Muller-Hinton Broth (ID-CAMHB) according to CLSI (4, 5).

RESULTS

Table 1. List of tested isolates

Species	N	Percent of total (%)
Enterobacteriaceae	**807**	**61.2**
Citrobacter freundii	26	2.0
Citrobacter koseri	14	1.1
Enterobacter aerogenes	44	3.3
Enterobacter asburiae	17	1.3
Enterobacter cloacae	149	11.3
Escherichia coli	167	12.7
Klebsiella oxytoca	45	3.4
Klebsiella pneumoniae	250	19.0
Proteus mirabilis	2	0.2
Serratia marcescens	93	7.1
Non-Enterobacteriaceae	**512**	**38.8**
Acinetobacter baumannii	254	19.3
Burkholderia cepacia	3	0.2
Pseudomonas aeruginosa	173	13.1
Stenotrophomonas maltophilia	82	6.2
Total	**1319**	**100**

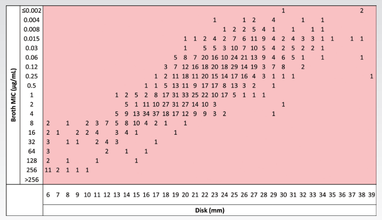
Table 2. Effect of iron on cefiderocol disk zone diameter against Gram-negative bacteria

Test Medium	Diameters (mm)			
	E. coli ATCC 25922		*P. aeruginosa* ATCC 27853	
	Cefiderocol	Cefepime	Cefiderocol	Cefepime
MHA Only	30 – 31	37	29 – 30	30 – 31
MHA + $FeCl_3$ (10 mg/L)	27 – 29	37	18 – 19	31
MHA + $FeCl_3$ (3 mg/L)	28 – 29	37	21 – 22	30 – 31
MHA + $FeCl_3$ (1 mg/L)	30	37 – 38	26 – 28	30 –31
MHA + $FeCl_3$ (0.3 mg/L)	30	37	28 – 30	31

Cefiderocol disk: 30 µg/disk (Bio-rad). CFPM disk 30 µg/disk (Becton, Dickinson and Company).
Medium: Difco MHA, BBL MHA (Becton, Dickinson and Company)

Little effect of iron concentration on disk diameter of cefiderocol was observed even when 10 mg/L of iron for E. coli ATCC 25922 and 1 mg/L of iron for *P. aeruginosa* ATCC 27853 were added to the MHA.

Figure 1. Cefiderocol MIC values compared to cefiderocol disk zone of inhibition (30 µg/disk) against 1,319 Gram-negative bacteria



There is good correlation between disk diffusion and BMD method for cefiercol MIC when multiple bacterial species (a total of 1,319 strains including 807 Enterobacteriaceae, 254 *A. baumannii*, 173 *P. aeruginosa*, 82 *S. maltophilia*, 3 *B. cepacia*) were used.

Figure 2. Cefiderocol MIC values compared to cefiderocol disk zone of inhibition (30 µg/disk) against 807 Enterobacteriaceae

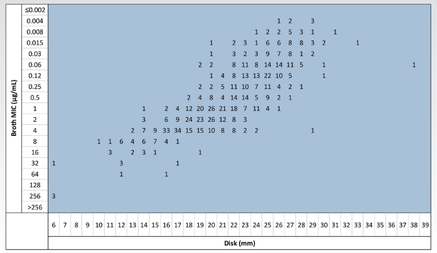


Figure 3. Cefiderocol MIC values compared to cefiderocol disk zone of inhibition (30 µg/disk) against 173 *P. aeruginosa*

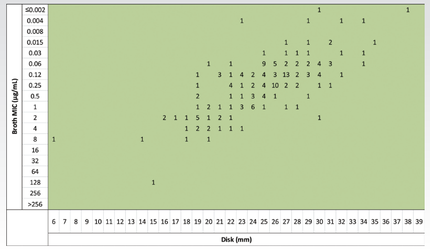


Figure 4. Cefiderocol MIC values compared to cefiderocol disk zone of inhibition (30 µg/disk) against 254 *A. baumannii*

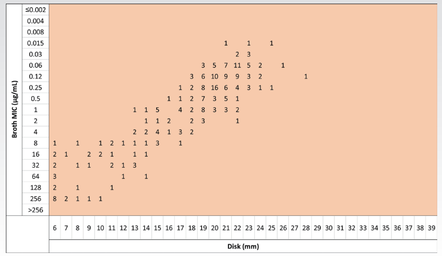


Figure 5. Cefiderocol MIC values compared to cefiderocol disk zone of inhibition (30 µg/disk) against 82 *S. maltophilia*

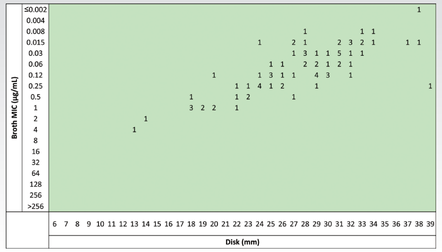


Table 3. Correlation between cefiderocol MIC values in BMD method and disk zones diameter.

Organism group	R2 values
Enterobacteriaceae	0.70
Acinetobacter baumannii	0.81
Pseudomonas aeruginosa	0.52
Stenotrophomonas maltophilia	0.65

- Correlation between MIC values in ID-CAMHB and disk zones was high for Enterobacteriaceae and *A. baumannii*, with R^2 values of 0.70 and 0.81, respectively, indicating that a disk test is very feasible for use with this organism group.
- R^2 values were reduced for *P. aeruginosa* (R^2=0.52) and *S. maltophilia* (R^2=0.65). These lower values may not reflect true correlation due to the lack of resistant strains.

CONCLUSIONS

The disk diffusion studies using a 30 µg of cefiderocol disk and standard MHA showed that the zone diameters obtained in these studies have a good correlation with the MIC determined using ID-CAMHB.

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