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MIC Determinations for Cefiderocol Using Broth Microdilution Can Be Affected by Mueller–Hinton Broth Manufacturer Source, But Not by Their Media Lots

Boudewijn L.M. DeJonge,[1] Christine M. Slover,[1] Sean T. Nguyen,[1] Christopher Longshaw,[2] Miki Takemura,[3] Naomi Anan,[3] Hidenori Yamashiro,[3] Yoshinori Yamano[3]

[1]Shionogi Inc., Florham Park, NJ, USA; [2]Shionogi B.V., London, UK; [3]Shionogi & Co., Ltd., Osaka, Japan

Contact: Boudewijn L.M. DeJonge, Shionogi Inc.
Email: boudewijn.dejonge@shionogi.com
Phone: 01-862-395-4327

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ABSTRACT

Introduction

Cefiderocol minimum inhibitory concentrations (MICs) with the broth microdilution reference method need to be determined in iron-depleted cation-adjusted Mueller–Hinton broth (ID-CAMHB).[1] We have observed discrepant MIC values when MICs were determined in ID-CAMHB prepared with Mueller–Hinton broth (MHB) from different manufacturers.[2] These differences could not be attributed to the iron content of the different ID-CAMHB media and appear to be manufacturer-related.[2] As only one lot of each media was investigated, it was unclear whether the observed differences could perhaps be attributed to lot-specific variations, rather than differences between manufacturers. In this study, we examined lot-to-lot variability for each of the media.

Methods

Three lots of BD-BBL and BD-Difco and two lots of Oxoid MHB were used to prepare ID-CAMHB.[1] Single MIC determinations (0.06 to 64 µg/mL) were conducted over three different days for each lot, thus collecting nine MIC values for BD-BBL and BD-Difco and six MIC values for Oxoid, with five *Klebsiella pneumoniae* and two *Pseudomonas aeruginosa* isolates that showed discrepant MIC values when measured in ID-CAMHB prepared with MHB from different manufacturers.

Results

Good reproducibility was observed for all media lots of all three manufacturers, with only one isolate (*K. pneumoniae* VA-357) showing MIC values spread across four dilutions in ID-CAMHB prepared with MHB from BD-Difco and Oxoid. All other isolates showed only a one to three dilution spread in MIC values in the different ID-CAMHB media. While reproducible MIC values (within ±1 dilution of modal MIC) were obtained with the different lots of MHB for each manufacturer, the modal MIC values obtained for each ID-CAMHB differed by MHB manufacturer; e.g., *K. pneumoniae* VA-391 showed modal MIC values of 2, 4, and 8 µg/mL in ID-CAMHB prepared with MHB from BD-BBL, BD-Difco, and Oxoid, respectively, with MIC values for each media manufacturer within ±1 dilution of its modal MIC.

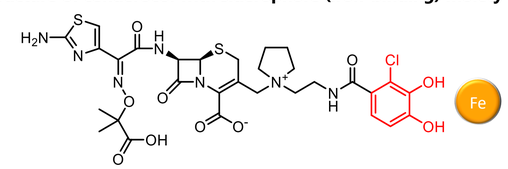
Conclusions

These results confirm that ID-CAMHB prepared with MHB from different manufacturers can result in different MIC values for cefiderocol, and that those differences are inherent to the MHB of each manufacturer and not dependent on media lot. The reason(s) for these discrepancies are unknown but are unrelated to the iron content of the media. When testing cefiderocol susceptibility with broth microdilution and/or comparing MIC results, attention needs to be paid to the origin of MHB used to prepare ID-CAMHB. Our previous study recommends cefiderocol MIC determinations with ID-CAMHB prepared with MHB from BD-BBL or BD-Difco.[2]

INTRODUCTION

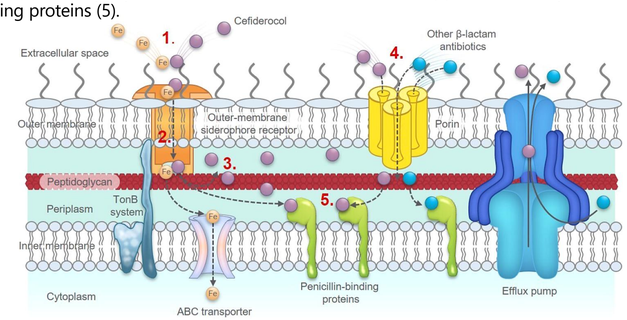
- Cefiderocol is a siderophore-conjugated cephalosporin that is actively taken up by iron transport systems of Gram-negative bacteria (**Figures 1** and **2**).
- To reflect the activity of cefiderocol *in vivo*, minimum inhibitory concentrations (MICs) for cefiderocol, using the broth microdilution reference method, need to be determined in iron-depleted cation-adjusted Mueller–Hinton broth (ID-CAMHB).[1]
- For some isolates, discrepant MIC values are obtained when MICs are determined in ID-CAMHB prepared with Mueller–Hinton broth from different manufacturers; differences that cannot be attributed to different iron contents of the different ID-CAMHB media but appear to be manufacturer related.[2]
- However, lot-to-lot variability for each medium has not been investigated and this is now specifically evaluated in the current study.

Figure 1: Structure of cefiderocol with siderophore (iron-binding) moiety shown in red



Adapted from Morgan G, Yamano Y, et al. *Nature Portfolio* 2020; https://www.nature.com/articles/d42473-020-00446-9.

Figure 2: Cefiderocol mode of cell entry into Gram-negative bacteria cells. By chelating Fe^{3+} (1), the cefiderocol-iron complex is actively transported over the outer membrane of Gram-negative bacteria via iron transport systems (2), in addition to the passive diffusion via porins (4). In the periplasm, cefiderocol dissociates from iron (3) and binds to penicillin-binding proteins (5).



Adapted from Morgan G, Yamano Y, et al. *Nature Portfolio* 2020; https://www.nature.com/articles/d42473-020-00446-9.

METHODS

- Cefiderocol MIC values were determined in ID-CAMHB prepared from three lots of Mueller–Hinton broth from BD-BBL, three lots from BD-Difco, and two lots from Oxoid according to CLSI guidelines.[1]
- MIC determinations (range: 0.06–64 µg/mL) were conducted over three different days for each lot, collecting a total of nine MIC values for BD-BBL and BD-Difco media and six for Oxoid.
- Five *K. pneumoniae* and two *P. aeruginosa* isolates that showed discrepant MIC values when measured in ID-CAMHB prepared with Mueller–Hinton broth from different manufacturers were tested.

RESULTS

- All isolates showed reproducible MIC values in the different ID-CAMHB media (one to three dilution spread in MIC values) for all media lots of all three manufacturers, except for *K. pneumoniae* VA-357, which showed MIC values spread across four dilutions in ID-CAMHB prepared from Mueller–Hinton broth from BD-Difco and Oxoid (**Table 1**).
- While reproducible MIC values (within ±1 dilution of modal MIC) were obtained with the different lots of Mueller–Hinton broth for each manufacturer, the modal MIC values obtained for each ID-CAMHB differed by manufacturer (**Table 2**).
- All isolates, except for *K. pneumoniae* VA-357, showed a different modal MIC value for each medium, with MIC values being the lowest in media prepared with Mueller–Hinton broth from BD-BBL, followed by BD-Difco and Oxoid (**Table 2**).

CONCLUSIONS

- MIC determinations of cefiderocol in ID-CAMHB are reproducible; however, MIC values can differ by manufacturer, but not by lot number, of Mueller–Hinton broth used to prepare ID-CAMHB. The reasons for the discrepancies are unclear but are not related to the iron content of the media.
- When testing cefiderocol susceptibility with broth microdilution and/or comparing MIC results, attention needs to be paid to the Mueller–Hinton broth origin used to prepare ID-CAMHB. It is recommended to perform cefiderocol MIC determinations with ID-CAMHB prepared with Mueller–Hinton broth from BD-BBL or BD-Difco, as those values were most predictive for *in vivo* efficacy.[2]

Table 1: Cefiderocol MIC values (by media lot and modal) and number of MIC dilutions for isolates when measured in ID-CAMHB prepared with Mueller–Hinton broth from BD-BBL, BD-Difco, and Oxoid

	BD-BBL ID-CAMHB					BD-Difco ID-CAMHB					Oxoid ID-CAMHB			
Isolate	Lot 1 MIC (µg/mL)	Lot 2 MIC (µg/mL)	Lot 3 MIC (µg/mL)	Number of MIC dilutions	Modal MIC (µg/mL)	Lot 1 MIC (µg/mL)	Lot 2 MIC (µg/mL)	Lot 3 MIC (µg/mL)	Number of MIC dilutions	Modal MIC (µg/mL)	Lot 1 MIC (µg/mL)	Lot 2 MIC (µg/mL)	Number of MIC dilutions	Modal MIC (µg/mL)
K. pneumoniae VA-357	2, 1, 2	2, **0.5**, 2	2, 1, 2	3	2	4, **0.5**, 4	4, **1**, 4	4, **1**, 4	4	4	4, **1**, 4	4, **0.5**, 4	4	4
K. pneumoniae VA-361	2, 2, 2	2, 2, 2	2, 4, 2	2	2	4, 2, 8	4, 4, 4	4, 4, 4	3	4	8, 8, 8	8, 8, 8	1	8
K. pneumoniae VA-384	2, 2, 2	4, 2, 4	4, 4, 2	2	2	8, **2**, 8	8, 4, 4	8, 4, 8	3	8	16, 8, 16	16, 16, 8	2	16
K. pneumoniae VA-391	2, 2, 2	2, 1, 2	2, 2, 1	2	2	8, 2, 4	4, 2, 4	8, 4, 4	3	4	8, 4, 8	8, 8, 8	2	8
K. pneumoniae KI2	4, 4, 4	4, 4, 4	4, 8, 4	2	4	16, 4, 8	-, 8, 8	-, 8, 8	3	8	16, 16, 16	16, 16, 16	1	16
P. aeruginosa SR27001	1, 1, 1	1, 1, 1	1, 2, 1	2	1	4, 1, 2	2, 2, 2	2, 2, 2	3	2	8, 4, 8	8, 8, 4	2	8
P. aeruginosa NCTC 13437	0.5, 0.5, 0.5	0.5, 0.5, 0.5	0.5, 1, 0.5	2	0.5	1, 0.5, 1	1, 1, 1	1, 1, 1	2	1	2, 4, 4	2, 2, 4	2	2/4

-, indicates skipped wells; underlined bold values are outliers from modal MIC (>1 dilution difference).
Green cells show all MIC values within three dilutions; yellow cells show MIC values not within three dilutions.

Table 2: Cefiderocol modal MIC values for isolates when measured in ID-CAMHB prepared with Mueller–Hinton broth from BD-BBL, BD-Difco, and Oxoid

Isolate	BD-BBL	BD-Difco	Oxoid
K. pneumoniae VA-357	2	4	4
K. pneumoniae VA-361	2	4	8
K. pneumoniae VA-384	2	8	16
K. pneumoniae VA-391	2	4	8
K. pneumoniae KI2	4	8	16
P. aeruginosa SR27001	1	2	8
P. aeruginosa NCTC 13437	0.5	1	2/4

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References: 1. CLSI. Performance standards for antimicrobial susceptibility testing; 36th Informational Supplement, M100-S36. Wayne, PA, USA: CLSI; 2026. **2.** DeJonge BLM, et al. J Clin Microbiol. 2025;63:e0047125.
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