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Comparison of Conventional and Iron-Depleted Agar Plates for Susceptibility Testing of Cefiderocol by Disk Diffusion

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ABSTRACT (Revised)

Introduction

Cefiderocol is a siderophore-conjugated cephalosporin with good activity against Gram-negative pathogens, including carbapenem- and multidrug-resistant isolates. Cefiderocol's activity can be attributed to its stability against hydrolysis by β-lactamases, being less prone to efflux, and an increased concentration in bacterial cells mediated through uptake via iron-siderophore transporters. The importance of siderophore uptake systems for cefiderocol potency was demonstrated through susceptibility measurements using broth microdilution, which showed lower minimum inhibitory concentrations for cefiderocol when cells were grown in Mueller–Hinton broth that was depleted for iron to concentrations encountered in the host and allowing for the expression of iron uptake systems. When potency of cefiderocol is assessed through disk diffusion, depletion of iron of the agar medium is not considered necessary as it has been suggested that agar media provide an iron-sequestering environment that results in the expression of iron-uptake systems. However, this has never been demonstrated for cefiderocol.

Methods

This study evaluated the potency of cefiderocol using disk diffusion with regular and iron-depleted Mueller–Hinton agar and examined the expression of iron-uptake systems when cells are grown on agar plates.

Results

Using disk diffusion, zones of inhibition for five *Escherichia coli*, five *Klebsiella pneumoniae*, 12 *Pseudomonas aeruginosa,* and 11 *Acinetobacter baumannii* isolates were within 2 mm of each other when grown on either Mueller–Hinton or iron-depleted Mueller–Hinton agar, showing that iron-depletion had little effect on the potency of cefiderocol. To determine if iron-uptake systems are already expressed in cells grown on regular agar plates, outer membrane proteins of *P. aeruginosa* PAO1 grown overnight on tryptic soy and blood agar plates were extracted and separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. Upon SYPRO Ruby staining, bands at around 82 and 75 kDa were visible, indicative of the expression of the PiuA and FptA iron-uptake transporters, respectively. Western blotting, using antibodies directed against PiuA and FptA, confirmed the expression of these two iron-uptake systems.

Conclusions

The data confirm that growth on agar plates triggers the expression of iron-uptake systems, thereby allowing for the use of regular, rather than iron-depleted, Mueller–Hinton agar to assess the susceptibility of isolates against cefiderocol using disk diffusion.

INTRODUCTION

- Cefiderocol is a siderophore-conjugated cephalosporin with good activity against Gram-negative pathogens, including carbapenem- and multidrug-resistant isolates.
- Cefiderocol penetration in the bacterial cell depends on the expression of iron-siderophore transporters under iron-limiting conditions; therefore, the *in vitro* activity of cefiderocol needs to be assessed under iron-limiting conditions.
- Agar media provide an iron-sequestering environment that results in the expression of iron-uptake systems in bacteria[1]; therefore, depletion of iron from agar medium would not be necessary for assessment of the potency of cefiderocol through disk diffusion. However, this has never been demonstrated for cefiderocol.
- This study evaluated the potency of cefiderocol through disk diffusion with regular and iron-depleted Mueller–Hinton agar and examined the expression of iron-uptake systems in *Pseudomonas aeruginosa* when grown on agar.

Figure 1: Observed growth patterns within zones of growth inhibition for *A. baumannii* strains. Red dashed circles indicate inner zone of inhibition.

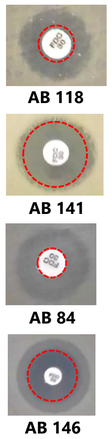


Figure 2: Correlation between cefiderocol (inner) mean zones of inhibition measured using iron-depleted (ID) and regular Mueller–Hinton agar (MHA).

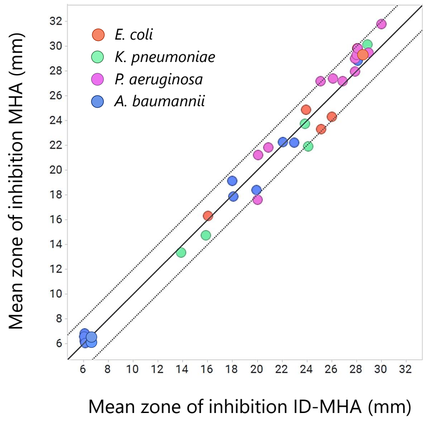
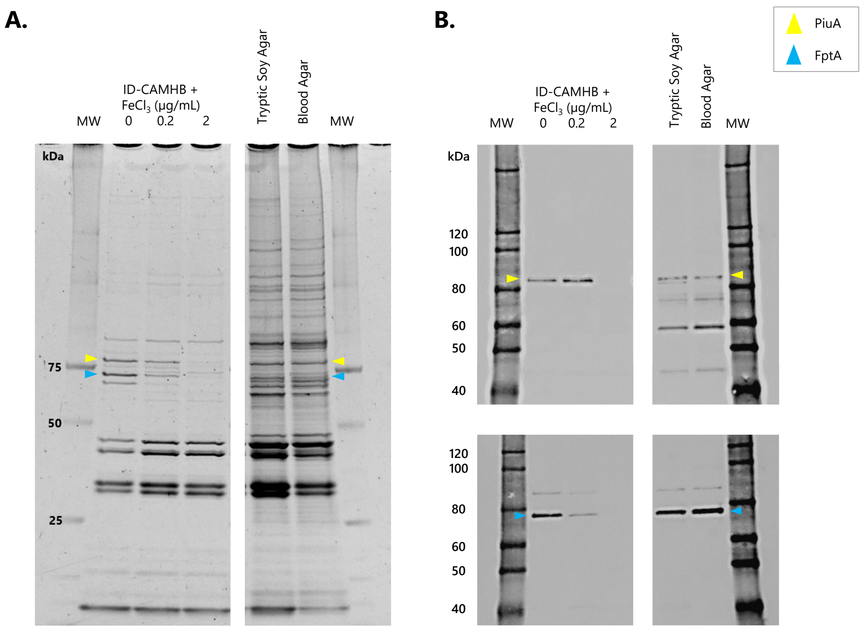


Figure 3: Expression of PiuA and FptA iron-uptake systems in *P. aeruginosa* PAO1 grown for 8 hours in iron-depleted cation-adjusted Mueller–Hinton broth (ID-CAMHB) with 0, 0.2, or 2 µg/mL of $FeCl_3$, or on tryptic soy agar, or blood agar. Outer membrane proteins of *P. aeruginosa* PAO1 were extracted and separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and stained with SYPRO Ruby (A), and Western blotting was performed with antibodies directed against PiuA (**top**) and FptA (**bottom**) (B).



CONCLUSIONS

- Growth on agar plates triggered expression of iron-uptake systems, allowing for the use of regular Mueller–Hinton agar, rather than iron-depleted agar, to assess the susceptibility of isolates against cefiderocol with disk diffusion. The data confirm the CLSI-approved method for cefiderocol disk diffusion testing.
- Use of iron-depleted Mueller–Hinton agar did not change growth patterns observed within zones of growth inhibition.

RESULTS

- Several growth patterns within the zone of growth inhibition were observed with some isolates, particularly, *A. baumannii* (**Figure 1**). These patterns were observed with both regular and iron-depleted Mueller–Hinton agar (MHA).
- Zones of inhibition determined on MHA and iron-depleted MHA were within 2 mm of each other, below the accepted standard deviation of ±3 mm (**Figure 2**).
- As expected, expression of the iron-uptake systems PiuA or FptA in Mueller–Hinton broth were dependent on the concentration of iron in the media (**Figure 3**).
- Iron-uptake systems PiuA or FptA were expressed in cells grown on regular (non-iron-depleted) agar plates (i.e. tryptic soy and blood agar) (**Figure 3**).

METHODS

- Susceptibility testing was performed with agar plates prepared with cation-adjusted Mueller–Hinton broth (CAMHB) or iron-depleted CAMHB (ID-CAMHB) (both prepared with BD-BBL MHB powder) after supplementing them with agar (BD Bacto; 1.7% final concentration).
- Zones of inhibition (inner zones) were determined in triplicate with five *Escherichia coli*, five *Klebsiella pneumoniae*, 12 *P. aeruginosa,* and 11 *Acinetobacter baumannii* isolates in the disk diffusion assay using 30 µg cefiderocol disks (Mast). Minimum inhibitory concentration values of isolates ranged from 0.12 – >32 µg/mL.
- Outer membrane proteins were extracted and separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis from *P. aeruginosa* PAO1 grown overnight on tryptic soy agar and blood agar plates and from cells grown for 8 hours in iron-depleted cation-adjusted Mueller–Hinton broth with or without the supplementation of 0.2 and 2 µg/mL of $FeCl_3$, and stained with SYPRO Ruby.
- Western blots were carried out with in-house-prepared antibodies directed against PiuA or FptA.

Conflict of interest: The authors are employees of the Shionogi GROUP. **Funding:** This study was funded by Shionogi & Co., Ltd., Osaka, Japan. **Reference:** 1. Critchley IA and Basker MJ. FEMS Micro Letters 1988;50:35–39.