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Cefiderocol MIC against cefiderocol-resistant *Acinetobacter baumannii* can be affected by starting inoculum size

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BACKGROUND

- Cefiderocol is a siderophore cephalosporin which is effective against carbapenem-resistant Gram-negative pathogens.
- Reduced reproducibility of MICs has been reported against *Acinetobacter baumannii* with elevated cefiderocol MIC values (>2 µg/mL) [1].
- In this study, we evaluated if variations in inoculum size could affect the cefiderocol MIC and be one of the reasons of the reported poor reproducibility.

METHODS

- Clinical isolates of 27 *A. baumannii*, 8 *Pseudomonas aeruginosa*, and 8 *Klebsiella pneumoniae* were used. These strains consisted of twenty susceptible strains, four intermediate strains, and nineteen resistant strains based on the interpretive criteria of the Clinical and Laboratory Standards Institute (CLSI) [2].
- The MICs of cefiderocol were determined by the broth microdilution method using iron-depleted cation-adjusted medium [2, 3] prepared from Mueller-Hinton broth powder (BD BBL™).
- For each strain, a bacterial suspension with OD_{625} = 0.1 was prepared as 10× inoculum solution (cf. the absorbance at 625 nm of McFarland 0.5 solution should be 0.08-0.13 [4]). For MIC determinations, this suspension was diluted 1-, 3.3-, 10-, 33- and 100-fold, corresponding to 10×, 3×, 1×, 1/3×, and 1/10× of the CLSI recommended inoculum.
- Viable count of each strain was determined with the standard (1×) inoculum by counting the colonies formed after overnight incubation at 35°C on Mueller-Hinton agar plates.

RESULTS

- The number of viable cells for the standard (1×) inoculum was in the range of 2 to 8 ×10^5 CFU/mL for all strains, except for *A. baumannii* strain 1217591 with 1.6 ×10^5 CFU/mL. The number of viable cells for *A. baumannii* averaged 4.5 ± 1.7 ×10^5 CFU/mL and tended to vary more compared to *P. aeruginosa* and *K. pneumoniae*, which averaged 4.5 ± 0.8 ×10^5 CFU/mL and 4.8 ± 0.8 ×10^5 CFU/mL, respectively.

A. baumannii (Table 1)

- Among 4 highly susceptible strains with cefiderocol MIC values of 0.25 to 0.5 µg/mL, no inoculum effect on the MIC was observed.
- Among 7 susceptible strains closer to the breakpoints, with cefiderocol MIC values of 1 to 4 µg/mL, all showed MIC values >8 µg/mL (resistant) when the 10× inoculum was used, and 4 of these already showed MIC values >16 µg/mL when the 3× inoculum was used.
- Two intermediate isolates with cefiderocol MIC value of 8 µg/mL also showed an increase in MIC values to >64 µg/mL (resistant) when the 3× or 10× inoculum was used.
- Among 14 resistant strains with cefiderocol MIC values >8 µg/mL, 3 showed MIC values of 2 or 4 µg/mL (susceptible) and 3 strains showed MIC values of 8 µg/mL (intermediate) when 1/10× inoculum was used. Even when 1/3× inoculum was used, 2 strains showed MIC values of 4 µg/mL (susceptible) or 8 µg/mL (intermediate).
- There were 13 cases where the MIC changed ≥ 8 fold when a 3-fold change in the inoculum size was used, including all 7 strains near the breakpoint.

P. aeruginosa and *K. pneumoniae* (Table 2)

- For *P. aeruginosa* and *K. pneumoniae*, the inoculum effect on the MIC values was much less pronounced.

Table 1: In vitro activity of cefiderocol against *Acinetobacter baumannii* strains using various inoculum sizes

species	strain ID	β-lactamase content	Viable cell (CFU/mL) of 1× inoculum	cefiderocol MIC (µg/mL) at inoculum size of 10×	3×	1×	1/3×	1/10×
A. baumannii	1208115 (AB148)	TEM-OSBL	5.5 × 10^5	0.25	0.25	0.25	0.25	0.12
A. baumannii	993394 (AB90)	OXA-23	4.5 × 10^5	0.5	0.5	0.5	0.25	0.5
A. baumannii	1081271 (AB139)	OXA-72	7.7 × 10^5	1	1	0.5	0.5	0.5
A. baumannii	1081184 (AB146)	OXA-72	2.6 × 10^5	2	1	0.5	1	1
A. baumannii	1217591 (AB231)	ADC-25-like, OXA-23, OXA-82	1.6 × 10^5	16	2	1	1	1
A. baumannii	2092810 (AB319)	ADC-type, OXA-24, OXA-829	2.9 × 10^5	64	4	2	2	2
A. baumannii	730770 (AB230)	ADC-33, OXA-82	5.8 × 10^5	>64	4	2	2	1
A. baumannii	1081172 (AB143)	OXA-72	5.3 × 10^5	64	32	2	2	0.5
A. baumannii	1100194 (AB152)	ADC-33, OXA-23, OXA-82	5.7 × 10^5	64	32	4	4	2
A. baumannii	921969 (AB96)	No available information	5.0 × 10^5	>64	>64	4	2	2
A. baumannii	1179589 (AB97)	No available information	4.3 × 10^5	>64	>64	4	4	4
A. baumannii	2082614 (AB318)	ADC-33-like, OXA-23, OXA-82	4.4 × 10^5	>64	>64	8	4	4
A. baumannii	1950059	No available information	3.5 × 10^5	>64	>64	8	8	4
A. baumannii	1890217	No available information	2.5 × 10^5	>64	>64	16	8	2
A. baumannii	2018628	No available information	4.0 × 10^5	>64	>64	16	4	2
A. baumannii	957498 (AB87)	ADC-26, OXA-64, NDM-1	2.3 × 10^5	64	64	32	32	16
A. baumannii	1559552 (AB323)	ADC-25-like, OXA-172	3.7 × 10^5	>64	>64	64	64	64
A. baumannii	1890222	No available information	2.8 × 10^5	>64	>64	64	16	4
A. baumannii	1299176 (AB126)	ADC-33, OXA-82	6.6 × 10^5	>64	>64	>64	16	8
A. baumannii	850681 (AB84)	ADC-25, OXA-23, OXA-66, PER-1	4.7 × 10^5	>64	>64	>64	64	32
A. baumannii	1121759 (AB132)	OXA-72, PER-1	3.7 × 10^5	>64	>64	>64	64	32
A. baumannii	2079864 (AB324)	ADC-11, OXA-66, OXA-72, PER-1, TEM-1D	7.4 × 10^5	>64	>64	>64	64	32
A. baumannii	1247963 (AB125)	ADC-11, OXA-66, PER-1	5.6 × 10^5	>64	>64	>64	64	16
A. baumannii	1922968 (AB316)	ADC-25-like, OXA-66, OXA-72, PER-1, TEM-1D	4.3 × 10^5	>64	>64	>64	>64	64
A. baumannii	2097670 (AB320)	ADC-33-like, OXA-23, OXA-82	7.6 × 10^5	>64	>64	>64	32	16
A. baumannii	2079553	OXA-24 type, PER-1	6.7 × 10^5	>64	>64	>64	32	8
A. baumannii	2053094	ADC-11, OXA-66, PER-1, TEM-OSBL	2.0 × 10^5	>64	>64	>64	16	8

Color tones are based on the cefiderocol interpretive category by CLSI M100 guideline [2]: , resistant; , intermediate; , susceptible.
The box with a black frame indicates an 8-fold change in MIC due to a 3-fold or 1/3-fold difference from standard (1×) inoculum size.

Table 2: In vitro activity of cefiderocol against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* strains using various inoculum sizes

species	strain ID	β-lactamase content	Viable cell (CFU/mL) of 1× inoculum	cefiderocol MIC (µg/mL) at inoculum size of 10×	3×	1×	1/3×	1/10×
P. aeruginosa	1523365 (PSA1598)	No available information	4.3 × 10^5	2	0.5	0.25	0.5	0.12
P. aeruginosa	1606608	IMP-7	5.5 × 10^5	0.5	0.5	0.5	0.5	1
P. aeruginosa	AR Bank 1106	ACT-16, CTX-M-15, NDM-1, OXA-1	4.7 × 10^5	4	1	1	0.25	0.25
P. aeruginosa	1073848 (PSA1573)	VIM-2	3.8 × 10^5	8	2	1	0.5	0.12
P. aeruginosa	847239 (PSA1560)	PER-1	3.0 × 10^5	32	4	4	1	1
P. aeruginosa	1265553	No available information	4.4 × 10^5	32	16	16	8	8
P. aeruginosa	AR Bank 0246	NDM-1, OXA-10, OXA-50, PAO, VEB-1	5.5 × 10^5	64	16	16	16	16
P. aeruginosa	AR Bank 1116	IMP-26, OXA-488, PDC-35	4.9 × 10^5	>64	32	16	8	1

species	strain ID	β-lactamase content	Viable cell (CFU/mL) of 1× inoculum	cefiderocol MIC (µg/mL) at inoculum size of 10×	3×	1×	1/3×	1/10×
K. pneumoniae	147867	No available information	5.3 × 10^5	0.5	0.25	0.25	0.12	≤0.06
K. pneumoniae	ATCC 13833	No available information	6.1 × 10^5	0.5	0.5	0.25	0.25	0.12
K. pneumoniae	1061237 (KP528)	No available information	5.0 × 10^5	0.25	0.5	1	0.5	0.5
K. pneumoniae	1478266	No available information	4.5 × 10^5	2	1	1	1	1
K. pneumoniae	KI2	NDM-1	3.4 × 10^5	>64	16	8	4	4
K. pneumoniae	1217638 (KP534)	No available information	4.6 × 10^5	>64	32	8	8	4
K. pneumoniae	1319427 (KP549)	SHV-12, TEM-OSBL	4.6 × 10^5	>64	64	16	8	4
K. pneumoniae	NCTC 13443	NDM-1	5.3 × 10^5	>64	>64	64	8	16

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The box with a black frame indicates an 8-fold change in MIC due to a 3-fold or 1/3-fold difference from standard (1×) inoculum size.

CONCLUSIONS

- Variation in inoculum size (from 1/10- to 10-fold of standard inoculum) affected the cefiderocol MIC of *A. baumannii* isolates with cefiderocol MIC ≥ 1 µg/mL, and the interpretative category could change, in particular for *A. baumannii* strains showing MIC values near the breakpoint.
- Inoculum size should be carefully controlled for cefiderocol MIC determinations, particularly for *A. baumannii* with MIC values ≥ 1 µg/mL.
- Further investigation is required to determine the impact of different bacterial loads on in vivo efficacy.

REFERENCES

1. Simner PJ, Palavecino EL, Satlin MJ, Mathers AJ, Weinstein MP, Lewis JS, Humphries R. 2023. Potential of Inaccurate Cefiderocol Susceptibility Results: a CLSI AST Subcommittee Advisory. J Clin Microbiol 61:e01600-22.
2. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 34th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2024.
3. EUCAST. Guidance document on broth microdilution testing of cefiderocol. European Committee on Antimicrobial Susceptibility Testing; 2024.
4. ISO. Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices – broth micro-dilution reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases. ISO 20776-1. Geneva, Switzerland: International Organization for Standardization; 2019.