Cefiderocol Disc Diffusion Susceptibility Testing: CLSI vs EUCAST Discordance in NDM-Producing Gram-Negative Bacteria

Yan Sheng Tay Patrick[1], Partha Pratim De[1]

[1]Department of Laboratory Medicine, Tan Tock Seng Hospital, Singapore

Background

Cefiderocol is a siderophore-conjugated cephalosporin with activity against carbapenem-resistant Gram-negative bacteria, including metallo-β-lactamase (MBL)-producing organisms. Reliable susceptibility testing is critical given its role as a last-resort agent.

Despite using similar disc diffusion (DD) methodology - same 30 µg disc, standard Mueller-Hinton agar, and quality control strains - EUCAST and CLSI apply substantially different interpretative thresholds.

EUCAST incorporates an Area of Technical Uncertainty (ATU: 21-23 mm *Enterobacterales*, 20-21 mm *Pseudomonas aeruginosa*) for borderline zones. CLSI uses a wider susceptible category with no ATU.

This analysis seeks to review interpretation differences for various Gram-negative bacteria.

Methods

Isolates: 110 consecutive non-duplicate clinical Gram-negative isolates from a single tertiary hospital (routine diagnostic specimens)

Disc diffusion: standard Mueller-Hinton agar, 30 µg cefiderocol disc. Zone diameters interpreted independently using CLSI M100 35th Edition (2025) and EUCAST v15.0 breakpoints

Carbapenemase genotyping: all isolates were typed using Cepheid Xpert® Carba-R: NDM (n=30), OXA-48 (n=16), NDM + OXA-48 (n=11), IMP (n=8), VIM (n=1), carbapenemase-negative (n=42)

Table 1: Breakpoint Reference

	S (≥ mm)	I (mm)	R (≤ mm)	ATU (mm)	Comments
Enterobacterales					
CLSI	16	9–15	8	*NA*	—
EUCAST	23	—	22	21–23	—
Pseudomonas aeruginosa					
CLSI	18	13–17	12	*NA*	—
EUCAST	22	—	21	20–21	—
***Acinetobacter* spp.**					
CLSI	15	—	—	*NA*	—
EUCAST	—	—	—	—	≥21 mm: "mostly devoid of resistance mechanisms" <17 mm: "likely resistant"
Stenotrophomonas maltophilia					
CLSI	15	—	—	*NA*	—
EUCAST	—	—	—	—	≥28 mm: "mostly devoid of resistance mechanisms" <22 mm: "likely resistant"

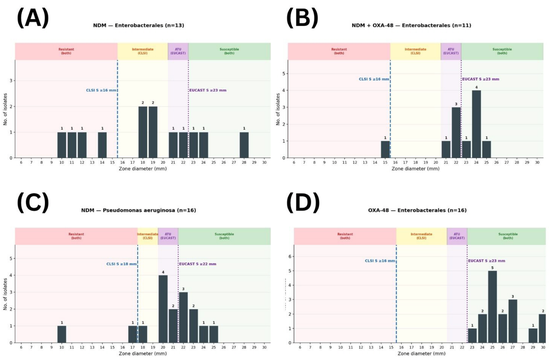
CLSI M100 35th Edition (2025) EUCAST Clinical Breakpoint Table v15.0

ATU - Area of Techinical Uncertainty; I - Intermediate; R - Resistant; S - Susceptible

Table 2: Cefiderocol Susceptibility Rates by CLSI and EUCAST DD Criteria

Group	n	CLSI %S	EUCAST %S
By Genotype			
NDM	30	87%	37%
NDM+OXA-48	11	91%	45%
Carbapenemase-negative	42	98%	74%
IMP	8	100%	88%
OXA-48	16	100%	94%
VIM	1	100%	100%
By Organism Group			
Enterobacterales	48	88%	56%
P. aeruginosa	46	96%	70%
Acinetobacter spp.	14	93%	73%
S. maltophilia	2	100%	100%
NDM producers — by organism			
NDM *Enterobacterales*	13	69%	15%
NDM+OXA-48 *Enterobacterales*	11	91%	45%
NDM *P. aeruginosa*	16	88%	44%
NDM *Acinetobacter*	1	0%	0%

Figure 1: Zone Diameter Distributions by Carbapenemase Genotypes and Organism



(A): NDM-Enterobacterales (n=13)
(B): NDM+OXA-48-Enterobacterales (n=11)
(C): NDM-*Pseudomonas aeruginosa* (n=16)
(D): OXA-48-Enterobacterales (n=16)

Results

Overall susceptibility rates showed substantial CLSI-EUCAST discordance: Enterobacterales 88% vs 56%, *P. aeruginosa* 96% vs 70%. The largest discordance occurred in NDM producers: NDM Enterobacterales (n=13) 69% vs 15% susceptibility; NDM+OXA-48 Enterobacterales (n=11) 91% vs 45%. Zone diameter distributions revealed NDM isolates cluster near EUCAST breakpoint thresholds, with many falling within the area of technical uncertainty (ATU)—a zone flagging interpretive ambiguity where disc diffusion may lack resolution to discriminate phenotypes. In contrast, OXA-48 (100% vs 94%), IMP (100% vs 88%), and carbapenemase-negative isolates (98% vs 74%) demonstrated higher concordance.

Limitations

Disc diffusion testing for cefiderocol has inherent technical challenges—Mueller-Hinton agar iron content varies between manufacturers, directly affecting zone diameters and categorical assignments. Broth microdilution (BMD) confirmation was not performed due to practical constraints: commercial kits are not widely available and reference methods require specialized iron-depleted media not routinely stocked. Without BMD data, neither standard can be validated as more accurate. This is also a single-center study and results may not be generalized to settings with different carbapenemase epidemiology. Clinical outcome data was also not assessed, precluding correlation with treatment success.

Conclusion

This study demonstrates CLSI-EUCAST interpretative discordance for cefiderocol , particularly for NDM-producing Gram-negative bacteria. The observed discordance represents interpretative label disagreement rather than confirmed resistance - clinical significance remains uncertain.

Further research should correlate findings with broth microdilution, molecular resistance mechanisms, and clinical outcomes, stratified by genotype, to establish evidence-based breakpoints that optimize patient outcomes for this antimicrobial agent.