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Comparison of commercial methods for evaluating susceptibility to cefiderocol

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INTRODUCTION

Cefiderocol is used for serious MDRO infections, but resistance has been reported albeit rarely so susceptibility testing is essential and must provide the clinician with a reliable result as soon as possible.

Testing cefiderocol in clinical microbiology laboratories is complex. Broth microdilution (the gold standard) is not available due to the difficulty of preparing the broth and the lack of access to the active substance.

In August 2022 EUCAST evaluated commercially available kits for testing this drug, noting that all have problems with accuracy, reproducibility, and biases. At the moment of writing this poster this warning is still active. The EUCAST recommended disk diffusion metho, but this test frequently results in ATU values, which are difficult to interpret without a confirmative test.

This study aimed to compare different commercial devices available for susceptibility testing on cefiderocol, including disks, gradient strips, and broth microdilution systems. All these tests were compared with a broth microdilution test, used as reference, performed using a homemade iron depleted cation adjusted Muller Hinton broth (ID-CAMHB).

MATERIALS AND METHODS

A collection of two hundred bacterial clinical isolates were analyzed: details are shown in table 1.

All isolates were evaluated using the disk diffusion method (30 µg disks and Mueller Hinton II Agar, both from Liofilchem, Italy), the ComASP® Cefiderocol (Liofilchem, Italy) and the UMIC® Cefiderocol BMD strips (Bruker, Germany). PA isolates were also tested using the gradient test MTS® cefiderocol (Liofilchem, Italy).

The ID-CAMHB used for the BMD considered as the comparative standard was prepared in house, according to the CLSI and the publication of Hackel et al., Diagn. Microbiol. Infect. Dis., 2019.

Briefly, cefiderocol active substance was provided by Shionogi (Japan) as lyophilized powder, resuspended in saline to a final concentration of 1024 mcg/ml. The stock vials were stored at -80°C until the different test sessions were performed. For any working session, eleven working concentrations of cefiderocol (ranging from 0.032 µg/ml to 32 µg/ml in 2-fold dilutions) were prepared in separate tubes containing ID-CAMHB, according to the dilution scheme proposed by CLSI. 100 µl of each intermediate concentration was dispensed into the wells of a microwell plates. For each strain tested a positive growth control will be included in the first well of the plate. control strains (*E. coli* ATCC-25922^T and PA ATCC-27853^T) were included in each experiment.

Isolated bacterial colonies grown from a 24h culture were suspended in saline and the turbidity adjusted to 0.5 McFarland. This suspension was then used as working solution for the BMD and for all the devices tested.

Contemporarily with the BMD tests, cefiderocol susceptibility tests were performed, using the same bacterial suspension, with all the other devices tested according to the manufacturer's instructions.

The final reading of the homemade BMD was performed according to the criteria as in Simner and Patel, J. Clin. Microbiol. 2020; The MIC results were interpreted according to 2024 EUCAST breakpoints (susceptible ≤2 mg/L and resistant >2 mg/L for Enterobacterales).

The performances of the tests were evaluated analyzing the essential agreement (EA), categorical agreement (CA) and bias according to the ISO 20776-2:2021.

RESULTS

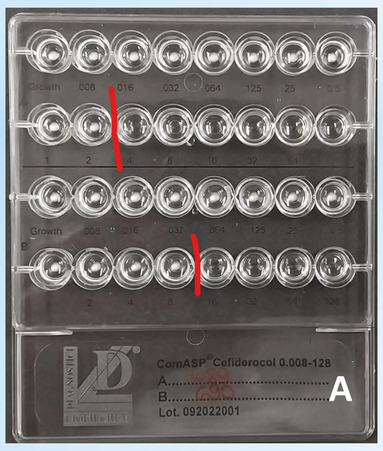
- Study results are summarized in tables 2-4.
- Considering UMIC®, the essential agreement (EA), was 76,3%, 93,8% and 96,1% for PA, AB and Enterobacterales, respectively. This test reported 7 major errors (MEs) and 3 VMEs for Enterobacterales. The test biases resulted slightly above the acceptable (±30%) for PA (32,7%).
- ComASP® showed an EA of 84,2%, 96,9% and 96,1% for PA, AB and Enterobacterales, respectively, with 9 VMEs for Enterobacterales. The test biases resulted slightly blow the acceptable (±30%) for Enterobacterales (-31,8%).
- MTS® had an EA of 89,5% for PA, with no VME or ME.
- Regarding disk diffusion, the categorical agreement (CA) was 71,8%, 93,8% and 71,9% for PA, AB and Enterobacterales, respectively. Eleven, 2 and 36 MEs resulted for PA, AB and Enterobacterales, respectively, whereas no VMEs were documented. ATU was important for both PA and Enterobacterales (7 and 30 isolates, respectively), accounting for the 18,4% of PA and 23,4% of the Enterobacterales.

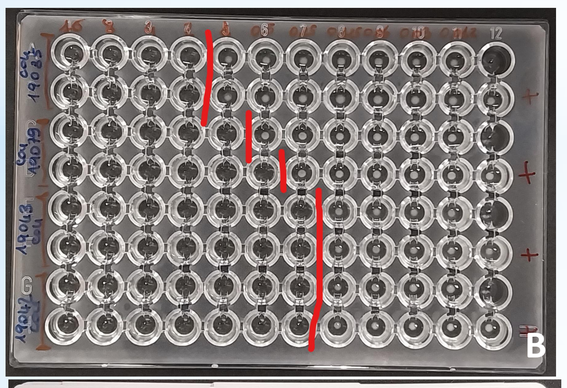
- 40 *Pseudomonas aeruginosa* (20 VIM+; 10 XDR, 10 wild-type)
- 30 *Acinetobacter baumannii* (25 OXA23+)
- 10 *Enterobacter cloacae,* (9 hyperexpressing AmpC and 1 wild-type)
- 10 *Escherichia coli* (9 ESBL+ and 1 wild-type)
- 10 *Klebsiella pneumoniae* (9 ESBL+ and 1 wild-type)
- 100 CPE (25 KPC+, 25 NDM+, 25 VIM+, 15 with more than one gene, 10 OXA48+)

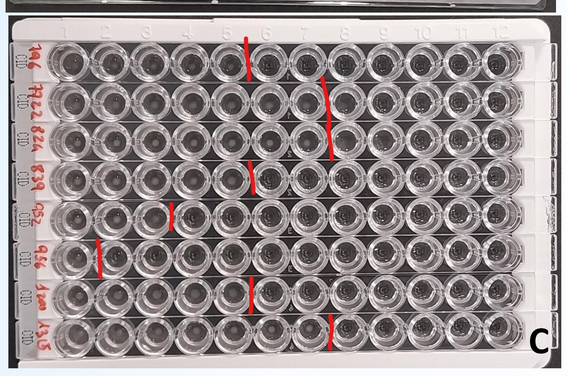
Table 1 – List of the isolates analyzed in the present study

	nr. isolates	UMIC				ComASP				MTS cefiderocol				Disk diffusion			
		EA%	VME nr.	ME nr.	test bias %	EA%	VME nr.	ME nr.	test bias %	EA%	VME nr.	ME nr.	test bias %	CA%	VME nr.	ME nr.	ATU
Pseudomonas aeruginosa	38	76,3	0	0	32,7	84,2	0	0	2,6	89,5	0	0	10,5	71,8	0	11	7
Acinetobacter baumannii	32	93,8	0	0	15,2	96,9	0	0	6,3	N/A	N/A	N/A	N/A	93,8	0	2	N/A
Enterobacterales	128	96,1	3	7	18,7	96,1	9	0	-31,77	N/A	N/A	N/A	N/A	71,9	0	36	30

Table 2 - Study results. EA = essential agreement, VME = very major errors, ME = major errors; CA = categorical agreement; ATU = areas of technical uncertainty







Figures – A, the ComASP® Cefiderocol (Liofilchem, Italy). B, homemade BMD plate. C, the UMIC® Cefiderocol BMD strips (Bruker, Germany).. Red lines at the MICs

All enterobacterales

UMIC Results	Reference BMD Results											
	≤0,016	0,03	0,06	0,125	0,25	0,5	1	2	4	8	≥16	
≤0,03		1	1									
0,06												
0,125			3	2	3							
0,25				2	5	6						
0,5			1	1	11	11	7					
1					2	9	22	2				
2						1	7	9	3			
4								7	5	1		
8									2	1		
16											3	
32												
	0	1	5	5	21	27	36	18	10	2	3	128

≤-2	-1	0	1	≥2	BIAS
0% (0)	17,97% (23)	46,09% (59)	32,03% (41)	3,91% (5)	18,69%

VME
ME

All enterobacterales

COMASP Results	Reference BMD Results											
	≤0,016	0,03	0,06	0,125	0,25	0,5	1	2	4	8	≥16	
≤0,016												
0,03		1										
0,06			2	1	1							
0,125			2	4	2							
0,25			1		12	6						
0,5					5	18	22					
1					1	2	12	10	1			
2						1	2	8	8			
4									1	2		
8											2	
16											1	
	0	1	5	5	21	27	36	18	10	2	3	

≤-2	-1	0	1	≥2	BIAS
1,56% (2)	41,41% (53)	46,09% (59)	8,59% (11)	2,34% (3)	-31,77%

VME
ME

Pseudomonas aeruginosa

UMIC Results	Reference BMD Results											
	≤0,016	0,03	0,06	0,125	0,25	0,5	1	2	4	8	≥16	
≤0,03	1	1	1									
0,06		1		3								
0,125		1	1	4								
0,25		1		1	4							
0,5					2	3	1					
1				1	3	4	1					
2						3		2				
4												
8												
16												
32												
	1	4	2	9	9	10	2	2	0	0	0	39

≤-2	-1	0	1	≥2	BIAS
0% (0)	12,82% (5)	41,03% (16)	23,08% (9)	23,08% (9)	31,45%

Pseudomonas aeruginosa

COMASP Results	Reference BMD Results											
	≤0,016	0,03	0,06	0,125	0,25	0,5	1	2	4	8	≥16	
≤0,016			1									
0,03		3		2								
0,06		1		3								
0,125			1	3	1							
0,25				1	7	2						
0,5						2						
1					1	4	2					
2						2		2				
4												
8												
16												
	0	4	2	9	9	10	2	2	0	0	0	

≤-2	-1	0	1	≥2	BIAS
7,89% (3)	15,79% (6)	50% (19)	18,42% (7)	7,89% (3)	2,63%

Tables 3-4 – Correlation between MICs of cefiderocol obtained using ComASP® and UMIC® strips compared with the reference BMD method on 128 Enterobacterales (upper) and 38 *Pseudomonas aeruginosa* (bottom) isolates. MICs were classified in grey for EA (1 $\log_2$ difference dilution)

DISCUSSION

- As the gold standard, in this comparative study was used a homemade BMD. The broth preparation and the procedures complied CLSI suggestions. Many previously published papers compared the tests with another commercial product (Thermo Fisher Sensititre™ CML1FEUD plate), which is no longer available on the market due to issues in the broth preparation.
- In our experience commercial tests showed an overall good performance and were easy to interpret. Only occasionally the commercial BMDs showed trailing (UMIC®) or well jumps (both systems). Although the reading was carried out in accordance with the criteria expressed by the literature, in our opinion read MICs at the total inhibition is more protecting for the patient (of which the possible condition of immunodepression is not known) and, of course, easier for the microbiologists.
- Considering the miniaturized BMD methods, UMIC® tends to overestimate MIC (+1 dilution), while ComASP® underestimates it (-1 dilution), which explain the high number of VMEs for this test. As expected, the broth is the crucial component of the kits. It should be noted that, if the broths of the two commercial systems are exchanged and used with the opposite lyophilized panel, the same inverted results are obtained.
- Most of the VMEs for both methods (11/12) remained within the EA: this reinforces the need to introduce a grey zone for cefiderocol MICs (similarly to the ATU for disk diffusion or to the intermediate category for CLSI). The number of isolates in ATU is however very high and unacceptable for the good clinical practice. The use of the commercial broth microdilution tests, albeit with the limits that this study has also highlighted, could be of help for clinical microbiologists as reflex in the cases of ATU.

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