Performance assessment of a Cefepime-Taniborbactam Gradient Diffusion Strip for Antimicrobial Susceptibility Testing of Enterobacterales and *Pseudomonas aeruginosa*

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Background

- Cefepime-taniborbactam (FTB) is an investigational β-lactam/β-lactamase inhibitor combination intended for the treatment of patients with infections caused by multidrug-resistant (MDR) and carbapenem-resistant Enterobacterales (CRE) and *Pseudomonas aeruginosa,* including isolates producing serine β-lactamases and metallo-β-lactamases (Hamrick 2020; Hernández-García 2022; Karlowsky 2023).
- In the Phase 3 CERTAIN-1 study of adult patients with cUTI, FTB was superior to meropenem for the primary composite (microbiologic and clinical) endpoint at Test of Cure (Day 19-23) (Wagenlehner 2024).
- Development of an FTB gradient strip for antimicrobial susceptibility testing will enable health-care providers to determine the susceptibility of clinical isolates of Enterobacterales and *P. aeruginosa* and select appropriate antimicrobial therapy.

Results Summary

- The tested isolates of Enterobacterales and *P. aeruginosa* were obtained from the Phase 3 CERTAIN-1 study (clinical contemporary isolates tested within 6 months of collection), frozen clinical stocks, and selected challenge organisms encoding a diverse range of β-lactamases (Table 1).
- The Essential Agreement (EA) between MICs determined with the Liofilchem FTB MIC Test Strip (MTS) and the reference broth microdilution (BMD) MIC method was:
  - 95.2% (638/670) for all Enterobacterales (Fig.1A)
    - 95.4% (392/411) for *E. coli* (Fig.1B)
    - 93.6% (160/171) for *K. pneumoniae* (Fig. 1C)
    - 98.0% (49/50) for *E. cloacae* complex (Fig. 1D)
    - 97.4% (37/38) for *P. mirabilis* (Fig. 1E)
  - 94.7% (178/188) for *P. aeruginosa* (Fig. 1F)
- The EA for the evaluable results of the tested species ranged from 93.6% to 97.9% (Table 2).
- Using the reference BMD MIC provisional breakpoints for FTB of Susceptible ≤ 16 mg/L and Resistant ≥ 32 mg/L, the Categorical Agreement (Table 2) was:
  - 97.9% (656/670) for all Enterobacterales
    - 98.3% (404/411) for *E. coli*
    - 98.2% (168/171) for *K. pneumoniae*
    - 92.0% (46/50) for *E. cloacae* complex
    - 100% (38/38) for *P. mirabilis*
  - 92.6% (174/188) for *P. aeruginosa*
- Amongst the tested resistant organisms, the adjusted Very Major Error rates were 2.1% (1/48) and 0.0% (0/53) for Enterobacterales and *P. aeruginosa* isolates, respectively (Table 2).
- A trending bias exceeding ±30% was only observed for *E. cloacae* complex (-36.0%) and *P. mirabilis* (31.6%) (Table 2).

Conclusions

- The Liofilchem FTB MTS reliably reflects the FTB reference broth microdilution MIC of gram-negative pathogens and meets the International Standards Organisation (ISO) performance criteria.
- These results will support regulatory review of the FTB MTS as a diagnostic device for antimicrobial susceptibility testing.

Methods

- The Research Use Only FTB MTS (Liofilchem srl) contains a cefepime gradient range from 0.016 to 256 mg/L and taniborbactam fixed at 4 mg/L, reflecting its concentration in the reference broth microdilution (BMD) MIC method.
- Concurrent BMD and MTS assays were performed with the same inoculum suspension according to the ISO 20776-1:2019 standard (equivalent to CLSI M07Ed11) and the manufacturer's instructions for use.
- Performance was assessed against clinical and challenge isolates of Enterobacterales (n=660) and *P. aeruginosa* (n=188) encoding a diversity of β-lactamases. ISO criteria (ISO 20776-2:2021) used to assess FTB MTS performance included:
  - Essential Agreement (EA; FTB MTS MIC within ±1 dilution of reference BMD value) of ≥90%
  - EA for the evaluable results (only isolates with on-scale MIC values for both methods are considered) of ≥90%
  - Categorical Agreement (CA; agreement of susceptibility interpretive results between the two methods) of ≥90%
  - An adjusted Very Major Error rate (false susceptible results not within EA of the susceptibility breakpoint) of ≤2.0%
  - Trending bias (difference between the percentage of isolates with an MTS MIC of ≤1-dilution and ≥1-dilution from the reference method) of ≤ ± 30%

Results

Table 1. Source of the tested Enterobacterales and *P. aeruginosa* isolates

Pathogen	Clinical contemporary[a] (CERTAIN-1 Phase 3 study)	Clinical stock[b]	Challenge[c]	Total
E. coli	325	58	28	**411**
K. pneumoniae	72	52	47	**171**
E. cloacae complex	19	20	11	**50**
P. mirabilis	21	14	3	**38**
P. aeruginosa	29	103	56	**188**
Total	**466**	**247**	**145**	**858**

[a]Clinical contemporary isolates were tested within 6 months from isolation and were minimally sub-cultured. [b]Clinical stock refers to an isolate that has been retained, stored or ontained from a culture collection. [c]Challenge isolates were selected based on phenotypic resistance to FTB (using the provisional breakpoints) and a diversity of Ambler Class A, B, C, and D β-lactamase genotypic profiles.

Figure 1. Scatterplots of cefepime-taniborbactam BMD and MTS MIC determinations for the tested isolates of Enterobacterales and *P. aeruginosa*

A) Enterobacterales isolates (n=670)

MTS MIC (mg/L)	Reference BMD MIC (mg/L)														
	≤0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥256
≤0.016	11	15	5	1											
0.03	13	107	91	5											
0.06	3	13	84	30	1										
0.12			17	23	16	3									
0.25				16	17	20		1							
0.5				2	8	12	6								
1					2	12	16	9							
2							5	6	5						
4							1	4	7	2					
8				1				1	6	2	1	1			
16								1		7	5	4			
32											9	6	1		
64												11	6	3	
128												2	6		
≥256												1	1	4	2
Evaluable Results		120	197	78	44	47	28	22	18	11	15	25	14	3	

B) *Escherichia coli* isolates (n=411)

MTS MIC (mg/L)	Reference BMD MIC (mg/L)														
	≤0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥256
≤0.016	10	14	5	1											
0.03	11	99	78	4											
0.06	2	7	47	24	1										
0.12			5	17	5										
0.25				11	9	6									
0.5				1	4	4	1								
1					1	4	3	6							
2						2	2								
4								4							
8				1				2			1				
16									1	1	2				
32										4	2				
64											3	2	1		
128												1			
≥256											1	1			
Evaluable Results		106	135	59	20	14	6	8	6	1	5	9	4	1	

C) *Klebsiella pneumoniae* isolates (n=171)

MTS MIC (mg/L)	Reference BMD MIC (mg/L)														
	≤0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥256
≤0.016	1														
0.03	1	6	9												
0.06	1	3	20	2											
0.12			2	4	10	3									
0.25				3	4	8									
0.5				1	2	6	1								
1					1	6	11								
2						3	2	3							
4						1	4	3	1						
8							1	4	1	1					
16							1		6	3					
32										3	4	1			
64											8	4	1		
128											2	4			
≥256													3	2	
Evaluable Results		9	31	10	17	23	16	8	10	8	7	14	9	1	

D) *Enterobacter cloacae* complex isolates (n=50)

MTS MIC (mg/L)	Reference BMD MIC (mg/L)														
	≤0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥256
≤0.016		1													
0.03		2	1	1											
0.06		1	2	4											
0.12				1	1										
0.25				2	3	6									
0.5						2	4								
1						1	2	2							
2								2	2						
4										1					
8										1					
16											1	2			
32											2				
64													1		
128													1		
≥256														1	
Evaluable Results		3	3	8	4	9	6	4	2	2	3	2	1	1	

E) *Proteus mirabilis* isolates (n=38)

MTS MIC (mg/L)	Reference BMD MIC (mg/L)														
	≤0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥256
≤0.016															
0.03	1		3												
0.06		2	15												
0.12			10	1											
0.25					1			1							
0.5					2										
1						1	1								
2															
4															
8															
16															
32															
64															
128															
≥256															
Evaluable Results		2	28	1	3	1	1	1	0	0	0	0	0	0	

F) *Pseudomonas aeruginosa* isolates (n=188)

MTS MIC (mg/L)	Reference BMD MIC (mg/L)														
	≤0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥256
≤0.016															
0.03															
0.06			1												
0.12															
0.25															
0.5						1									
1							11	11							
2								18	10						
4								2	9	4					
8									11	14					
16									2	10	19	2			
32									1		10	3			
64												4	3		
128													3	1	
≥256									1			1	5	15	16
Evaluable Results		0	1	0	0	1	11	31	34	28	29	10	11	1	

The green horizontal and vertical lines represent the cefepime-taniborbactam provisional breakpoints of Susceptible ≤ 16 mg/L and Resistant ≥ 32 mg/L. The darkest shaded squares represent absolute Essential Agreement whereas the bordered horizontal rectangles show MTS MIC values within Essential Agreement (±1 dilution) of the reference BMD method. Values in green at the intersect of the green lines are within Essential Agreement of the indicated breakpoints and are not considered Very Major (false susceptible) or Major (false resistant) errors when an Intermediate susceptibility category is absent. Values in red represent Very Major Errors not within Essential Agreement.

Table 2. Summary of cefepime-taniborbactam MTS performance relative to the BMD MIC reference method

Pathogen	Essential Agreement	Essential Agreement, Evaluable	Categorical Agreement	Very Major Errors, Adjusted	Trending noted[a]
Enterobacterales	95.2% (638/670)	95.3% (593/622)	97.9% (656/670)	2.1% (1/48)[b]	No (-11.1%)
E. coli	95.4% (392/411)	95.5% (357/374)	98.3% (404/411)	7.1% (1/14)[b]	No (-21.7%)
K. pneumoniae	93.6% (160/171)	93.9% (153/163)	98.2% (168/171)	0.0% (0/29)	No (11.9%)
E. cloacae complex	98.0% (49/50)	97.9% (47/48)	92.0% (46/50)	0.0% (0/5)	Yes (-36.0%)
P. mirabilis	97.4% (37/38)	97.3% (36/37)	100% (37/37)	NA[c]	Yes (31.6%)
P. aeruginosa	94.7% (178/188)	93.6% (147/157)	92.6% (173/188)	0.0% (0/53)	No (22.1%)

[a]Trending bias is indicated when the difference between the percentage of isolates with an MTS MIC of ≤1-dilution and ≥1-dilution from the BMD MIC exceeds ±30%. [b]The Very Major Error was caused by an *E. coli* clinical stock isolate with an IMP-type β-lactamase. [c]NA=Not Applicable (no resistant isolates were tested).

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