Comparative evaluation of ETEST® ERV* bioMérieux with the CLSI broth microdilution method for Eravacycline MIC determination

**** For Research Use Only. The performance characteristics of this product have not been established yet.***

V. SAUVONNET[1], C.FYFE[2], S.FONTAINE[1], M.BOUVIER[1], D. HALIMI[1], R. MARTELIN[1], G. ZAMBARDI[1].

[1] RESEARCH & DEVELOPMENT MICROBIOLOGY, BIOMÉRIEUX, LA BALME-LES-GROTTES, FRANCE.

[2] TETRAPHASE PHARMACEUTICALS, WATERTOWN, USA.

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BACKGROUND

Eravacycline (XERAVA™) is a novel, FDA and EMA approved fully-synthetic fluorocycline antibiotic developed by Tetraphase Pharmaceuticals Inc. for the treatment of complicated intra-abdominal infections (cIAI) including those caused by multidrug-resistant (MDR) pathogens. cIAI are an important cause of morbidity and are the second most common cause of infectious mortality in the intensive care unit. MDR pathogens have been highlighted as urgent public health threats by the US CDC and the WHO.

The new ETEST® ERV strip (MIC range 0.002 – 32 µg/mL) has been developed and calibrated versus the broth microdilution reference method (BMD) as described by the Clinical and Laboratory Standards Institute (CLSI) to determine the minimal inhibitory concentration (MIC) of eravacycline against Enterobacterales and Enterococci.

OBJECTIVE

The aim of the study was to compare ETEST ERV to the CLSI BMD method on a panel of 166 strains comprising 131 Enterobacterales and 35 Enterococci.

METHODS

●The panel includes 166 strains (among them 131 Enterobacterales and 35 Enterococci*)* and 3 CLSI QC strains.
The details of QC strains and panel are presented in Tables 1 and 2.
● The strains were provided by bioMérieux internal collection and Tetraphase pharmaceuticals collection.
● The selected panel consisted of 42 resistant strains and 124 susceptible strains to eravacycline.
● Enterobacterales include strains with various beta-lactam resistance mechanisms including ESBL, AmpC high level, Carbapenemase and various Tetracycline and Tigecycline resistance phenotypes as well as wild type strains.
● Enterococci strains include both Vancomycin Susceptible and Resistant Enterococci.

QC Strains	ATCC® number	CLSI 2019 MIC ranges (µg/mL)
E.coli	ATCC 25922	0.03 – 0.12
E.faecalis	ATCC 29212	0.016 – 0.06
P.aeruginosa	ATCC 27853	2 – 16

Table 1 – CLSI QC strains and associated MIC ranges for Eravacycline

Species	Number of strains
Escherichia coli	26
Klebsiella pneumoniae	27
Klebsiella oxytoca	18
Klebsiella aerogenes	10
Enterobacter cloacae	22
Citrobacter koseri	5
Citrobacter freundii	18
Serratia marcescens	5
Entrerococcus faecalis	23
Entrerococcus faecium	12

Table 2 – Details of species

BMD was performed using the **2019 CLSI** recommendations for Eravacycline.
ETEST ERV was evaluated using the standard ETEST MIC procedure for aerobic strains (inoculum 0.5 McF from 18/24h cultures on Columbia agar+5% sheep blood, testing on Mueller Hinton agar medium, incubation at 35°C during 16-20h).
For ETEST, the reading was performed using the bacteriostatic mode.
For BMD the reading was performed using the CLSI recommendations.

FDA approved breakpoints :
For Enterobacterales **S≤ 0.5µg/mL** and for *E.faecalis* and *E.faecium* **S≤ 0.06µg/mL**

RESULTS

The MICs for QC strains are within the expected CLSI ranges with reproducible results.
Ellipses are easy to read, clear, without trailing.

E. coli ATCC 25922
MIC = 0.064 µg/mL

E.faecalis ATCC 29212
MIC = 0.016 µg/mL

P.aeruginosa ATCC 27853
MIC = 4 µg/mL

The essential MIC agreement [±1 dilution] is **99.4%** without overestimation or underestimation trend between ETEST ERV and BMD (see Table 3).

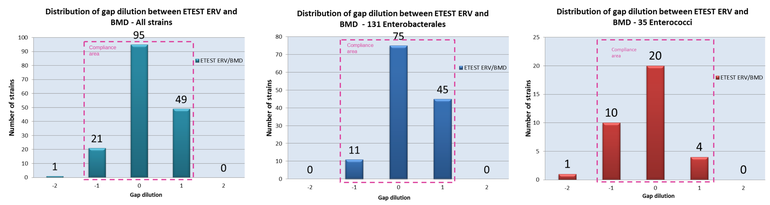

Table 3 - Distribution of gap dilution between ETEST ERV and BMD

The global distribution is homogeneous and shows only 1 discrepancy in term of Essential Agreement (see Table 4).

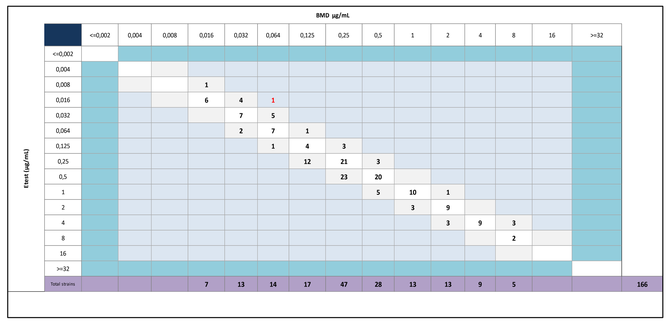

Table 4 – Distribution diagram between ETEST ERV* and BMD (166 strains)

* When the ETEST MIC is read between 2 two-fold values, the value is rounded up to the upper two-fold value

The single discrepancy is presented in Table 5.

Specie	Resistance profile	ETEST ERV MIC µg/mL	BMD ERV MIC µg/mL	ETEST ERV/BMD dilution gap
Enterococcus faecalis	VSE, Cyclines Resistant	0.016	0.064	-2

Table 5 – Discrepant strain in term of essential agreement

Following breakpoints presented in Table 6, the category agreement is **96.4%** with 6 Major Errors : 5 Enterobacterales and 1 *Enterococcus*, all at +/- 1 acceptable dilution around the single breakpoint (see details in Table 8).
No Very Major Error (VME) is observed (see Tables 7a, 7b).

	Susceptibility interpretative criteria FDA MIC breakpoints (µg/mL)	
	Susceptible S	**Non Susceptible NS**
Enterobacterales	≤ 0.5	-
E.Faecalis and *E.faecium*	≤ 0.06	-

Table 6 – FDA Breakpoints

Categories (all strains)		ETEST ERV		
		S	**NS**	**Total**
BMD	**S**	118	6	124
	NS	0	42	42
	Total	118	48	166

ETEST ERV / BMD (all strains)	%	Number of strains	Total
Category agreement	**96.4**	160	166
Major Error	**4.8**	6	124
Very Major Error	**0.0**	0	42

Tables 7a and 7b - Categorical agreement between ETEST ERV and BMD

Species	Resistance profile	ETEST ERV MIC (µg/mL)	BMD ERV MIC (µg/mL)	ETEST ERV/BMD dilution gap	Lower Breakpoint S ≤ (µg/mL)	Category	
						ETEST ERV	BMD
E. coli	Acquired Pase, Tetracycline resistant (tet A)	0.75	0.5	+0.5	**0.5**	R	S
K. pneumoniae	Carbapenemase (KPC) Tetracycline resistant	1	0.5	+0.5	**0.5**	R	S
K. pneumoniae	ESBL Tetracycline resistant	0.75	0.5	+0.5	**0.5**	R	S
K.aerogenes	Carbapenemase (KPC) Tetracycline wild	0.75	0.5	+0.5	**0.5**	R	S
C.koseri	ESBL (ctx-M like) Tetracycline resistant	0.75	0.5	+0.5	**0.5**	R	S
E. faecalis	VSE	0.094	0.064	+0.5	**0.064**	R	S

Table 8 – Detail of discrepant strains in term of category agreement

CONCLUSION

In an age of ESBL-related resistance, Eravacycline is a useful contribution to antimicrobial stewardship, as a carbapenem-sparing option. MIC determination enables to consolidate the molecule choice to treat the patient.

The new ETEST ERV strip could represent a valuable tool for eravacycline MIC determination and an alternative to the BMD reference method. ETEST will undergo clinical studies to seek IVD FDA clearance and CE marking.

Poster availability : veronique.sauvonnet@biomerieux.com