

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

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



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BACKGROUND

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

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 89 strains comprising 69 Enterobacterales and 20 *Enterococci*.

METHODS

• The panel includes 89 strains (among them 69 Enterobacterales and 20 *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 Tetrphase pharmaceuticals collection.

• The selected panel consisted of 21% of resistant strains and 18% of strains with MIC at the breakpoint.

Enterobacterales include strains with various beta-lactam resistance mechanisms including ESBL (13), AmpC high level (8), Carbapenemase (8) 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)	Species	Number of strains
<i>E. coli</i>	ATCC 25922	0.03 – 0.12	<i>Klebsiella pneumoniae</i>	13
<i>E. faecalis</i>	ATCC 29212	0.016 – 0.06	<i>Klebsiella oxytoca</i>	6
			<i>Klebsiella aerogenes</i>	7
			<i>Enterobacter cloacae</i>	10
			<i>Escherichia coli</i>	15
			<i>Citrobacter koseri</i>	5
			<i>Citrobacter freundii</i>	8
			<i>Serratia marcescens</i>	5
			<i>Enterococcus faecalis</i>	11
<i>P. aeruginosa</i>	ATCC 27853	2 – 16	<i>Enterococcus faecium</i>	9

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

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 $S \leq 0.5 \mu\text{g/mL}$ and for *Enterococci* $S \leq 0.064 \mu\text{g/mL}$

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% without overestimation or underestimation trend between ETEST ERV and BMD (see Table 3).

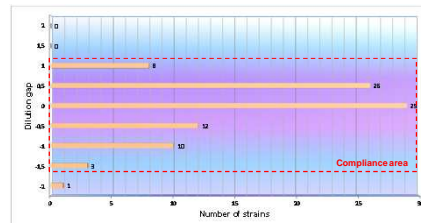


Table 3 – Distribution of gap dilution between ETEST ERV and BMD - 89 strains

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

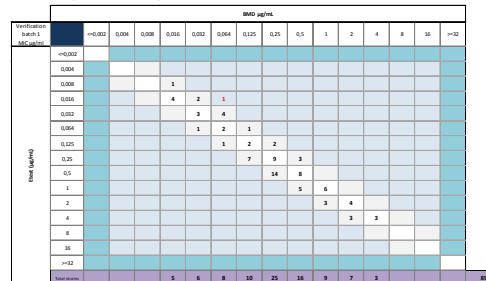


Table 4 – Distribution diagram between ETEST ERV* and BMD (89 strains)

* 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
<i>Enterococcus faecalis</i>	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 93.3% 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 interpretive criteria FDA MIC breakpoints (µg/mL)	
	Susceptible S	Non Susceptible NS
Enterobacterales	≤ 0.5	-
<i>Enterococci</i>	≤ 0.064	-

Table 6 – FDA Breakpoints

Categories (all strains)	Etest ERV			Etest ERV / BMD (all strains)	%	Number of strains	Total
	S	NS	Total				
BMD	S	63	6	69	93.3	83	89
	NS	0	20	20			
	Total	63	26	89			
Category agreement					93.3	83	89
Major Error					8.7	6	69
Very Major Error					0.0	0	20

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 $S \leq (\mu\text{g/mL})$	Category	
						Etest ERV	BMD
<i>E. coli</i>	Acquired Pse, Tetracycline resistant (tet A)	0.75	0.5	+0.5	0.5	R	S
<i>K. pneumoniae</i>	Carbapenemase (KPC) Tetracycline resistant	1	0.5	+0.5	0.5	R	S
<i>K. pneumoniae</i>	ESBL Tetracycline resistant	0.75	0.5	+0.5	0.5	R	S
<i>K. aerogenes</i>	Carbapenemase (KPC) Tetracycline wild	0.75	0.5	+0.5	0.5	R	S
<i>C. koseri</i>	ESBL (ctx-M like) Tetracycline resistant	0.75	0.5	+0.5	0.5	R	S
<i>E. faecalis</i>	VSE	0.094	0.064	+0.5	0.064	R	S

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

CONCLUSION

In this study, the new ETEST ERV strip has been found to be substantially equivalent to the CLSI reference method. MIC end-points are easier to read in comparison to the reference method. With a 15-dilution MIC range and simplicity of use, ETEST ERV could represent a valuable tool for MIC determination and an alternative to the BMD reference method. ETEST will undergo clinical studies to seek IVD FDA clearance and CE marking.