# Comparative evaluation of Meropenem/Vaborbactam MIC determination with the new ETEST® MEV* and CLSI broth microdilution method

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

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## BACKGROUND

Carbapenem antibiotics are still a key weapon in the fight against β-lactam resistant Gram-negative infections. However, the increase of carbapenem-resistant *Enterobacteriaceae* (CRE) has become a global public health problem. This phenomenon has led to the development of new drug and inhibitor combinations, such as Meropenem/Vaborbactam. Vabomere® (Melinta Therapeutics) is FDA approved for the treatment of adults with complicated urinary tract infections (cUTI) including pyelonephritis caused by the following susceptible microorganisms : *Escherichia coli, Klebsiella pneumoniae and Enterobacter cloacae species* complex.

The new ETEST® MEV (Meropenem/Vaborbactam - MIC range 0.004/8-64/8 µg/mL) has been developed and calibrated versus the broth microdilution reference method (BMD) as described by the Clinical and Laboratory Standards Institute (CLSI). This test is intended to determine the MIC of Meropenem/Vaborbactam toward the *Enterobacteriaceae* group.

## OBJECTIVE

The aim of this study was to perform a comparative study of ETEST MEV with the CLSI Broth Microdilution method on a specific panel of 225 strains.

## METHODS

●The panel includes 198 *Enterobacteriaceae* (among them 23 resistant strains to MEV ), 22 *Pseudomonas aeruginosa*, and 5 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 ; The Medicines Company collection ; and the CDC collection (*Enterobacteriaceae* Carbapenem Breakpoint panel – Gram Negative Carbapenemase Detection Panel – *Enterobacteriaceae* Carbapenemase Diversity Panel).
● The selected panel consisted of a majority of MDR strains. Among them : 135 strains with resistant genes to carbapenem such as KPC (92 strains), NDM, VIM, IMP (25 strains), OXA-48 (16 strains). Other resistance mechanisms or combinations with carbapenem resistance are represented : ESBL, strains with impermeability, AmpC as well as wild type strains.

BMD was performed using the **2017 CLSI** recommendations for Meropenem/Vaborbactam.
ETEST MEV 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 each method, the MIC was read at complete inhibition of growth.

The FDA approved breakpoints for *Enterobacteriaceae* were applied :
**S≤4 µg/mL – I=8 µg/mL - R≥16 µg/mL**

| QC Strains | ATCC® number | CLSI 2017 MIC ranges (µg/mL) |
|---|---|---|
| *E. coli* | ATCC 25922 | 0,008 – 0,06 |
| *K. pneumoniae* | ATCC 700603 | 0,015 – 0,06 |
| *K. pneumoniae* | ATCC BAA 1705 | 0,008 – 0,06 |
| *P.aeruginosa* | ATCC 27853 | 0,12 - 1 |
| *K. pneumoniae* | ATCC BAA 2814 | 0,12 – 0,5 |

**Table 1** – CLSI QC strains and associated MIC ranges for Meropenem/Vaborbactam

| Species | Number of strains |
|---|---|
| *K. pneumoniae* | 93 |
| *E. coli* | 40 |
| *E. cloacae* | 29 |
| *P. aeruginosa* | 22 |
| *E. aerogenes* | 10 |
| *Citrobacter* | 8 |
| *Proteeae* | 12 |
| *S. marcescens* | 3 |
| *K. oxytoca* | 3 |

**Table 2** – Detail of species

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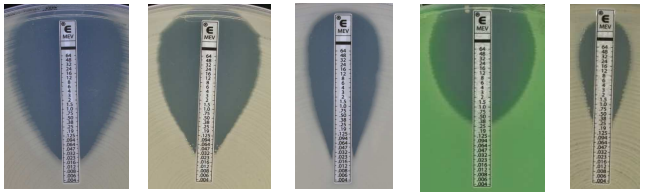
## RESULTS

**Bactericidal reading**
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 0,023 µg/mL | *K. pneumoniae* ATCC 700603 0,047 µg/mL | *K. pneumoniae* ATCC BAA-1705 0,023 µg/mL | *P. aeruginosa* ATCC 27853 0,38µg/mL | *K. pneumoniae* ATCC BAA-2814 0,38 µg/mL

The essential MIC agreement [±1 dilution] is **94.5%** without overestimation or underestimation trend between ETEST MEV and BMD (see Table 3)

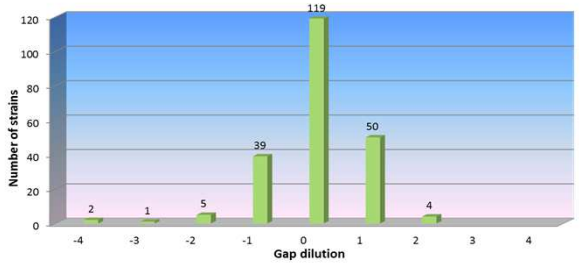



**Table 3** - Distribution of gap dilution between ETEST MEV and BMD (220 strains)

The global distribution shows that discrepancies are linked to high MIC values, without trend to over or underestimate (see Table 4)

| Etest (µg/mL) \ BMD µg/mL | <=0,004 | 0,008 | 0,016 | 0,032 | 0,064 | 0,125 | 0,25 | 0,5 | 1 | 2 | 4 | 8 | 16 | 32 | >=64 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| <=0,004 | | | | | | | | | | | | | | | |
| 0,008 | | | | | | | | | | | | | | | |
| 0,016 | | | 15 | 5 | | | | | | | | | | | |
| 0,032 | | | 5 | 38 | 3 | | | | | | | | | | |
| 0,064 | | | | 7 | 16 | 4 | | | | | | | | | |
| 0,125 | | | | | 5 | 7 | 3 | | | | | | | | |
| 0,25 | | | | | | 5 | 8 | 3 | 1 | | | | | | |
| 0,5 | | | | | | | 4 | 7 | 7 | 1 | | | | | |
| 1 | | | | | | | | 4 | 7 | 5 | 1 | 1 | 1 | | |
| 2 | | | | | | | | | 5 | 4 | 6 | 1 | | 1 | |
| 4 | | | | | | | | | 1 | 5 | | | | | |
| 8 | | | | | | | | | | | 1 | | | | |
| 16 | | | | | | | | | | | 1 | 2 | 2 | 2 | 1 |
| 32 | | | | | | | | | | | | 1 | 1 | | 1 |
| >=64 | | | | | | | | | | | | | 1 | 6 | 15 |
| Total strains | | | 20 | 50 | 24 | 16 | 15 | 14 | 21 | 15 | 9 | 5 | 5 | 9 | 17 | 220 |

Breakpoints for *Enterobacteriaceae* S≤4 µg/mL - I=8 µg/mL - R≥16 µg/mL

**Table 4** – Distribution diagram between ETEST MEV and BMD (220 strains)

The list of discrepant strains is presented in Table 5.
All the discrepancies are linked to strains with high MICs with the reference method (1µg/mL ≥ MIC ≥ 64 µg/mL). Among them 5 MBL strains and 1 KPC strain are underestimated and 3 KPC strains are overestimated. Overestimation is due to the presence of macrocolonies in the ellipse. This phenomenon is known with carbapenems and mutants in these groups.

| Species | Resistance profile | ETEST MEV MIC µg/mL | BMD MEV MIC µg/mL | ETEST MEV/BMD gap |
|---|---|---|---|---|
| *E. cloacae* | VIM-1, TEM-1B, ACT-7 | 1,5 | 8 | -2,5 |
| *E. cloacae* | Impermeability | 0,38 | 2 | -2,5 |
| *E. coli* | NDM-1,TEM-1B,CMY-6, CIT | 2 | 32 | -4 |
| *E. coli* | NDM | 1 | 8 | -3 |
| *K. pneumoniae* | NDM-1,TEM-1B,CTX-M-15, OXA-1 | 12 | 64 | -2,5 |
| *K. pneumoniae* | OXA-48,SHV-11 | >=64 | 16 | +2 |
| *K. pneumoniae* | KPC-3, TEM, SHV | 32 | 8 | +2 |
| *K. pneumoniae* | OXA-181, CTX-M-15, SHV-26 | 0,75 | 4 | -2,5 |
| *K. pneumoniae* | KPC | 3 | 1 | +1,5 |
| *K. pneumoniae* | KPC | 0,19 | 1 | -2,5 |
| *P. rettgeri** | NDM-1 | 0,75 | 16 | -4,5 |
| *P. aeruginosa* | KPC-5, OXA-50 | 12 | 4 | +1,5 |

**Table 5** – Detail of discrepant strains in term of essential agreement

The categorical agreement is **96%** with 190/198 compliant strains between both methods (see Tables 6a and 6b).
2 Very Major Errors (2 NDM-1 producing strains*), 6 minor errors and no Major Error are found (see Table 7 for details).

| ETEST MEV | | Etest S | Etest I | Etest R | Etest Total |
|---|---|---|---|---|---|
| BMD | S | **169** | 1 | 0 | 170 |
| | I | 2 | **0** | 3 | 5 |
| | R | 2 | 0 | **21** | 23 |
| | Total | 173 | 1 | 24 | 198 |

| ETEST MEV / BMD | % | Number of strain | Total |
|---|---|---|---|
| Category agreement | **96,0** | 190 | 198 |
| minor error | **3,0** | 6 | 198 |
| Major Error | **0,0** | 0 | 170 |
| Very Major Error | **8,7** | 2 | 23 |

**Tables 6a and 6b** - Categorical agreement between ETEST MEV and BMD for *Enterobacteriaceae*

| Species | Resistance profile | ETEST MEV MIC µg/mL | BMD MEV MIC µg/mL | Lower Breakpoint ≤ | Upper Breakpoint ≥ | Category ETEST MEV | Category BMD |
|---|---|---|---|---|---|---|---|
| *E. cloacae* | VIM-1, TEM-1B, ACT-7 | 1,5 | 8 | 4 | 16 | S | I |
| *E. coli* | NDM-1,TEM-1B,CMY-6, CIT | 2 | 32 | 4 | 16 | **S** | **R** |
| *E. coli* | NDM | 1 | 8 | 4 | 16 | S | I |
| *K. pneumoniae* | KPC | 16 | 8 | 4 | 16 | R | I |
| *K. pneumoniae* | KPC-3, TEM, SHV | 32 | 8 | 4 | 16 | R | I |
| *K. pneumoniae* | non-CP-CRE | 12 | 8 | 4 | 16 | R | I |
| *K. pneumoniae* | OXA-48 | 8 | 4 | 4 | 16 | I | S |
| *P. rettgeri** VME in red | NDM-1 | 0,75 | 16 | 4 | 16 | **S** | **R** |

**Table 7** – Detail of discrepant strains in term of category agreement

*note : the *Proteeae* group could be removed from the ETEST MEV claims because of reading difficulties linked to the specific swarming of these strains.

## CONCLUSION

**In this study, the new ETEST MEV is found to be substantially equivalent to the CLSI reference method. MIC end points are easy to read. With a 15-dilution range and simplicity of use, ETEST MEV represents a valuable tool for MIC determination and is an alternative to the BMD reference method. ETEST MEV is currently under clinical studies in order to be IVD cleared (FDA and CE).**