P1583

In vitro assessment of the novel antibiotic combination meropenem/ANT3310 against Gram-negative bacteria: CLSI Tier 1 study examining effects of varying MIC parameters and equivalency between agar dilution and broth microdilution methodologies

IHMA Europe
Rte. de l'Ile-au-Bois 1A,
1870 Monthey/VS
Switzerland
www.ihma.com

Stephen Hawser[1], Nimmi Kothari[1], Thomas Valmont[1], Magdalena Zalacain[2], Agustina Llanos[2]

[1]IHMA Europe, Monthey (Valais), Switzerland; [2]Antabio, Labège, France

INTRODUCTION

The novel serine-beta-lactamase (SBL) inhibitor ANT3310 in combination with meropenem (MEM) demonstrates potent activity against Gram-negative pathogens including carbapenem-resistant Enterobacterales (containing OXA or KPC β-lactamases) and *Acinetobacter baumannii* [1].

The combination MEM/ANT3310 is currently in Phase I clinical development.

This study investigated the effects of MIC parameters and equivalency of MICs by CLSI broth microdilution and agar dilution, as required by FDA/CLSI for the development of antimicrobial susceptible testing (AST) methods for a new compound.

METHODS

The effect of 31 MIC parameters: standard CLSI MIC, addition of Tween 80, reading at different incubation time (18, 20, 24 and 48h), frozen MIC plates, calcium supplement (50 and 100 mg/L), magnesium supplement, zinc supplement, different pH (5.8, 6.5 and 8.5), different inoculum concentration ($5x10^4$, $5x10^6$ and $5x10^7$), incubation at 30 and 40°C, addition of human serum (25 and 50%), addition of 25% human plasma, addition of bovine serum albumin (0.001, 0.02, 0.05 and 0.1%), addition of lung surfactant (1 and 5%), 50% artificial urine, use of plate seal for incubation, use of CO_2 in incubation and use of different medium suppliers, on the activity of MEM/ANT3310, with ANT3310 at a fixed concentration of 8 mg/L, against seven quality control (QC) strains was investigated.

The equivalency study using 618 clinical isolates from a global collection (Figures 1 and 2) assessed MIC differences when MEM/ANT3310 was tested using broth microdilution and agar dilution with media from different suppliers and were expressed as a doubling dilution difference (DDD) [2,3]. The CLSI QC strains *A. baumannii* NCTC 13304 (OXA-27), *K. pneumoniae* BAA-1705 (KPC-2) and *K. pneumoniae* BAA-2814 (KPC-3) were tested 20 times in equivalency studies.

RESULTS SUMMARY

- The activity of MEM/ANT3310 was only affected by high inoculum size (6 isolates) and partially by acidic pH (3 isolates). Evaluation of MEM/ANT3310 MIC by agar dilution showed data comparable to that obtained by broth microdilution. MEM/ANT3310 only showed ≥2DDD in MIC under seven conditions (see Table 1), when compared with the reference CLSI condition.
- In the equivalency study, a difference in MICs of ≥3DDD was considered relevant. Of the total of 618 isolates, 90% of the isolates showed equivalency of MEM/ANT3310 activity between MIC broth and MIC agar methods (Figures 3 and 4).
- When tested in an equivalency study, the four QC strains demonstrated reproducible data between broth and agar MIC, with 95% at ≤ 1DDD for *A. baumannii* ATCC 13304 and *P. aeruginosa* ATCC 27853 and 100% at ≤ 1DDD for *E. coli* ATCC 25822 and *K. pneumoniae* BAA-2814.

CONCLUSIONS

- MEM/ANT3310 activity was affected by 7 of the 31 test parameters, the most common being high inoculum size and acidic pH, although less common, the time of incubation (48h) also affected the activity of the compound. Any other effect was largely strain specific for other parameters tested.
- 90% of the isolates tested showed equivalency of MEM/ANT3310 between broth and agar MIC methods.
- The CLSI QC strains *A. baumannii* NCTC 13304 (OXA-27), *K. pneumoniae* BAA-1705 (KPC-2) and *K. pneumoniae* BAA-2814 (KPC-3) appear suitable for routine QC testing for MEM/ANT3310.

REFERENCES

1.Zalacain M., et al (2024), Meropenem-ANT3310, a unique β-lactam-β-lactamase inhibitor combination with expanded antibacterial spectrum against Gram-negative pathogens including carbapenem-resistant *Acinetobacter baumannii, Antimicrob Agents Chemoter,* 68(3):e01120-23

2.Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standards – Eleventh Edition*. CLSI document M07-Ed11. 2018. CLSI, Wayne, PA.

3.Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing – 33rd ed*. CLSI Supplement M100. 2023. CLSI, Wayne, PA.

DISCLOSURES

Part of this work was supported by Grants to Antabio from the Région Occitanie contrat Innovation (contract number 20006316) and from Bpi France (project ARPEGE, PSPC-AAP-9.0)

RESULTS

Table 1. Mode or Median MIC Data for MEM/ANT3310 Obtained Under the Variable Test Conditions where MIC Changed by more than One Double-Dilution (DDD) with at least one of the QC strains tested

Test condition showing ≥2 DDD	Mode or Median MEM/ANT3310 MIC (mg/L)						
	A. baumannii NCTC 13304 (OXA-27)	*K. pneumoniae* ATCC 700603	*P. aeruginosa* ATCC 27853	*K. pneumoniae* BAA-1705 (KPC-2)	*K. pneumoniae* BAA-2814 (KPC-3)	*E. coli* ATCC 25922	*E. coli* ATCC 35218 (TEM-1)
CLSI reference method	**0.25**	**0.015**	**0.25**	**0.03**	**0.12**	**0.015**	**0.03**
48h incubation	1	0.03	2	0.06	0.12	0.03	0.03
Media at pH 5.8	1	0.06	0.5	0.06	0.25	0.06	0.06
Media at pH 8.5	0.25	0.03	1	0.03	0.25	0.03	0.03
Target inoculum: $5x10^6$ CFU/mL	0.25	0.03	0.5	1	2	0.03	0.03
Target inoculum: $5x10^7$ CFU/mL	0.5	0.06	1	16	64	4	4
CA-MHB medium 1**	0.06	0.03	0.5	0.03	0.12	0.015	0.015
Agar medium 3**	0.25	0.008	0.25	0.008	0.06	0.008	0.015

MIC was 2 dilutions lower than the "CLSI conditions" MIC
MIC was 2 dilutions higher than the "CLSI conditions" MIC
MIC more than 2 dilutions higher than the "CLSI conditions" MIC
** 1 and 3 refers to different manufacturers for the dehydrated media

Figure 1. Distribution of Clinical Isolates by Geographical Location

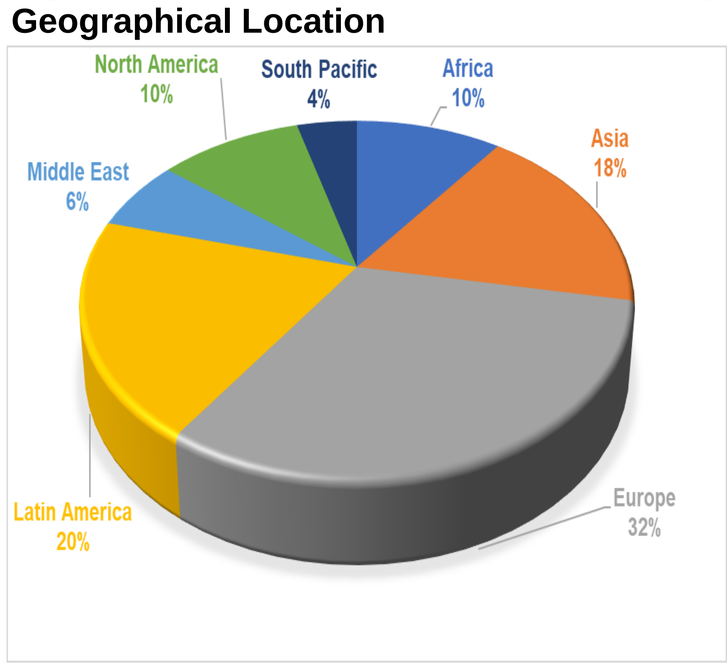
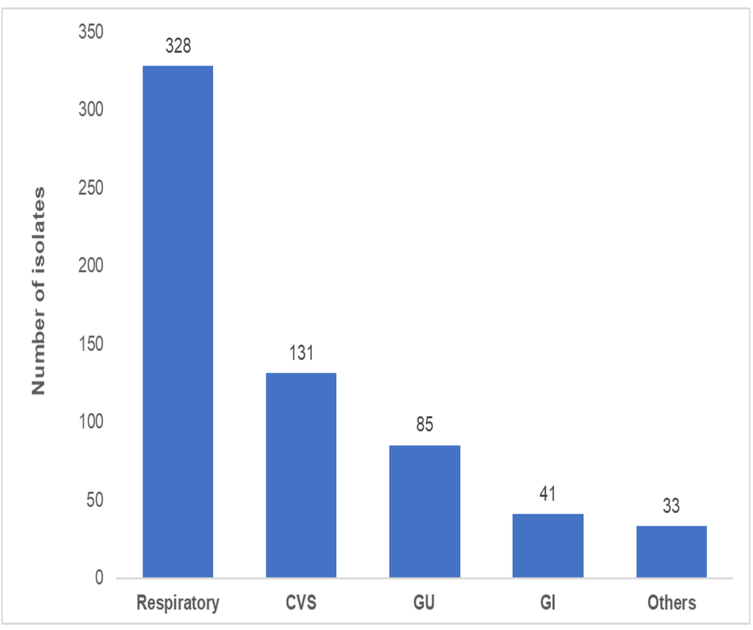


Figure 2. Distribution of Clinical Isolates by Infection Source



CVS, cardio-vascular; GU, genito-urinary; GI, gastro-intestinal

Figure 3. Doubling dilution difference for MEM/ANT3310 MIC determined by broth microdilution and agar dilution methodologies against 119 *A. baumannii*

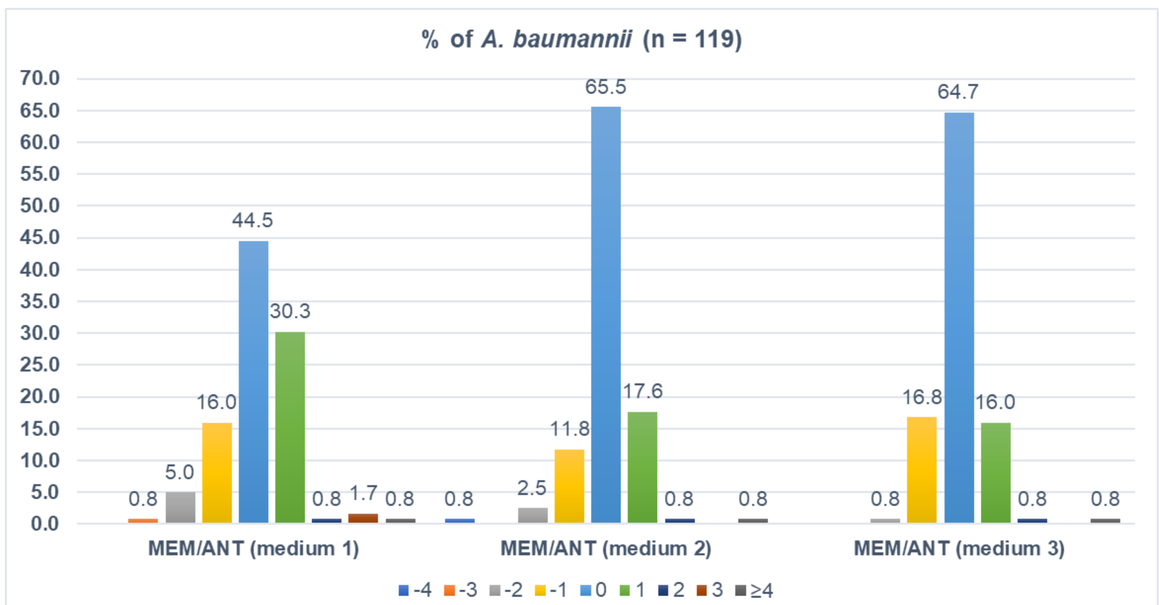


Figure 4. Doubling dilution difference for MEM/ANT3310 MIC determined by broth microdilution and agar dilution methodologies against 299 Enterobacterales