

Assessment of aztreonam-avibactam (ATM-AVI) CLSI 30/20 µg disk content against carbapenemase-producing Enterobacterales (CPEs)

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ATM-AVI is a combination drug that retained activity against metallo-β-lactamases (MBL) and serine carbapenemases as KPC/OXA-48-like. There is currently no approved and practical method for testing ATM-AVI susceptibility in clinical laboratories. Only CLSI has issued guidelines on the disk mass for ATM-AVI (30µg of ATM plus 20µg of AVI) and has defined a validation range for quality control organisms in the disk diffusion (DD) test. Breakpoints for clinical isolates have not been established to date per CLSI/EUCAST. Aim: to compare reference MIC with the DD for ATM-AVI, utilizing the recommended CLSI 30/20µg disks across a set of CPEs.

200 clinical isolates (3 *Citrobacter* spp., 22 *Enterobacter cloacae*, 21 *Escherichia coli*, 3 *Klebsiella aerogenes*, 3 *K. oxytoca*, 124 *K. pneumoniae*, 2 *Morganella morganii*, 2 *Providencia rettgeri*, 9 *P. stuartii*, and 11 *Serratia marcescens*) sourced from the National Reference Laboratory repository, and ATCC strains *K. pneumoniae* 700603, *Pseudomonas aeruginosa* 27853, and *E. coli* 25922 were included. The molecular characterization of β-lactamases was carried out using PCR/sequencing or/and WGS. The carbapenemases included were: 98 *bla*NDM, 2 *bla*IMP, 82 *bla*KPC, 9 *bla*OXA-48-like, and 9 *bla*KPC+*bla*OXA-48-like. The DD and agar dilution methods, following CLSI standardization, were performed in duplicates, while ATCC strains were tested four-times. To create CLSI-recommended 30/20ug ATM-AVI disk, 10µl of 2 mg/ml AVI powder solutions were added to 30µg ATM disks (Oxoid). MICs were interpreted with PK/PD breakpoints provided in phase 3 studies NCT03329092/NCT03580044: susceptible ≤8mg/L, non-susceptible >8mg/L.

14 isolates exhibited ATM-AVI MICs >8mg/L: 3 *bla*KPC+*bla*PER-2, while 11 carried *bla*NDM-5 associated with inhibitor-resistant *bla*SHV-231. All replicated zones fell within ±2mm (97.2% ±1mm). ATCC strains yielded results within the expected range. DD revealed a correlation coefficient (R^2) of 0.954-0.9628 (Fig.1-3). Zones ≥18 or ≥19 mm indicated susceptibility, while ≤17mm classified as resistant, resulting in 100% agreement with MIC-based categories.

ATM-AVI DD employing CLSI 30/20µg disks demonstrated a global R^2 of 0.954 with MIC values, when applying PK/PD cut-off points. Wild and non-wild-type populations of MBL producers displayed significant divergence, which would facilitate the choice of the breakpoints for DD.

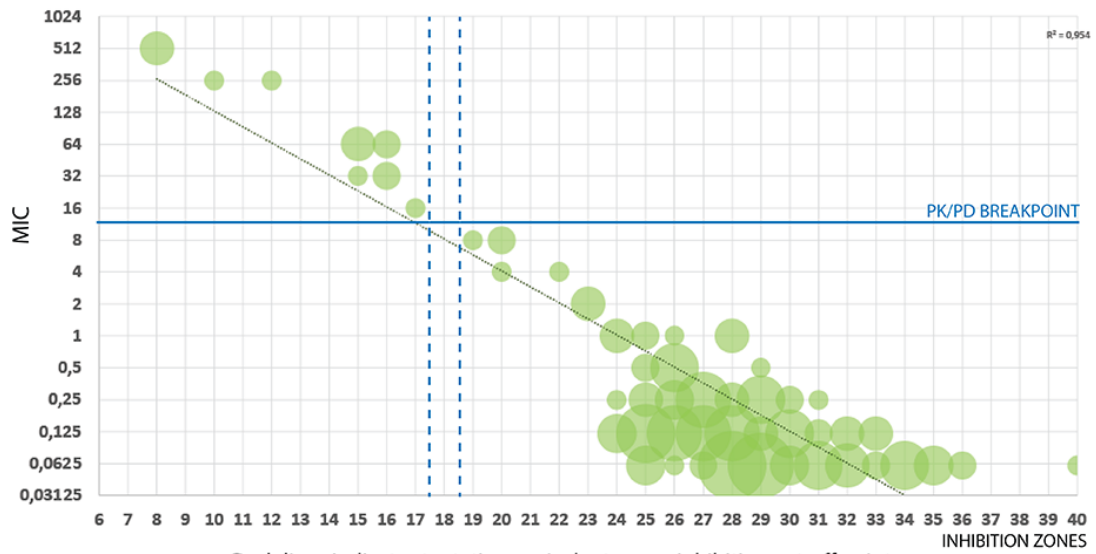
FIG.1: Correlation between MIC (mg/L) vs inhibition zones (mm). Complete panel (n:200)

FIG.2: Correlation between MIC (mg/L) vs inhibition zones (mm). MBL panel (n:100)

FIG.3: Correlation between MIC (mg/L) vs inhibition zones (mm). SCP panel (n:100)

FIGURE 1. Correlation between MIC (mg/L) vs inhibition zones (mm) obtained with 30/20ug ATM-AVI disks

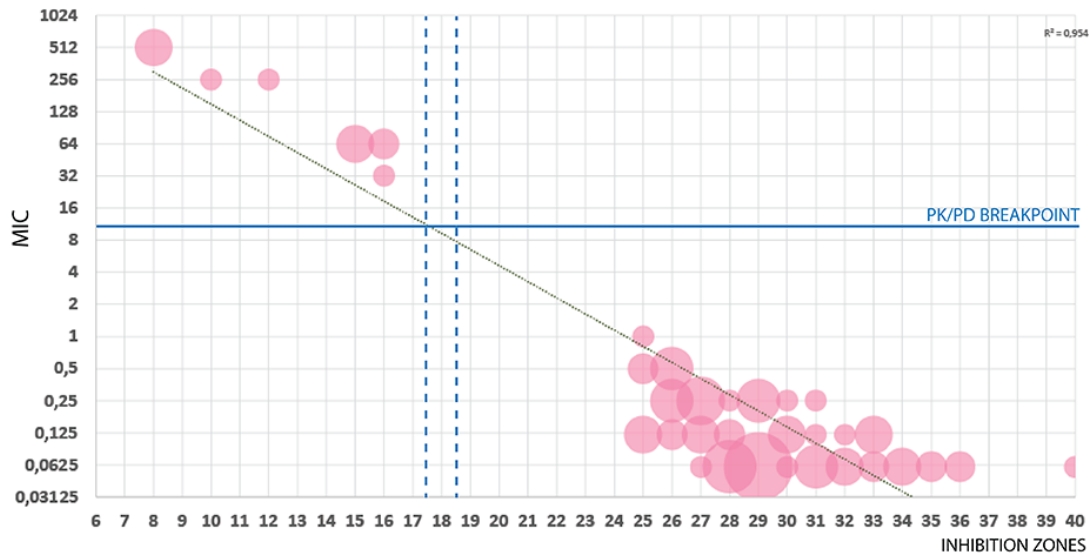
Panel of carbapenemase-producing Enterobacterales (n 200)



Dash lines indicates tentative equivalente zone inhibition cut-off points.
The size of the sphere is proportional to the numbers of events. Scale: ● 1 strain

FIGURE 2. Correlation between MIC (mg/L) vs inhibition zones (mm) obtained with 30/20ug ATM-AVI disks

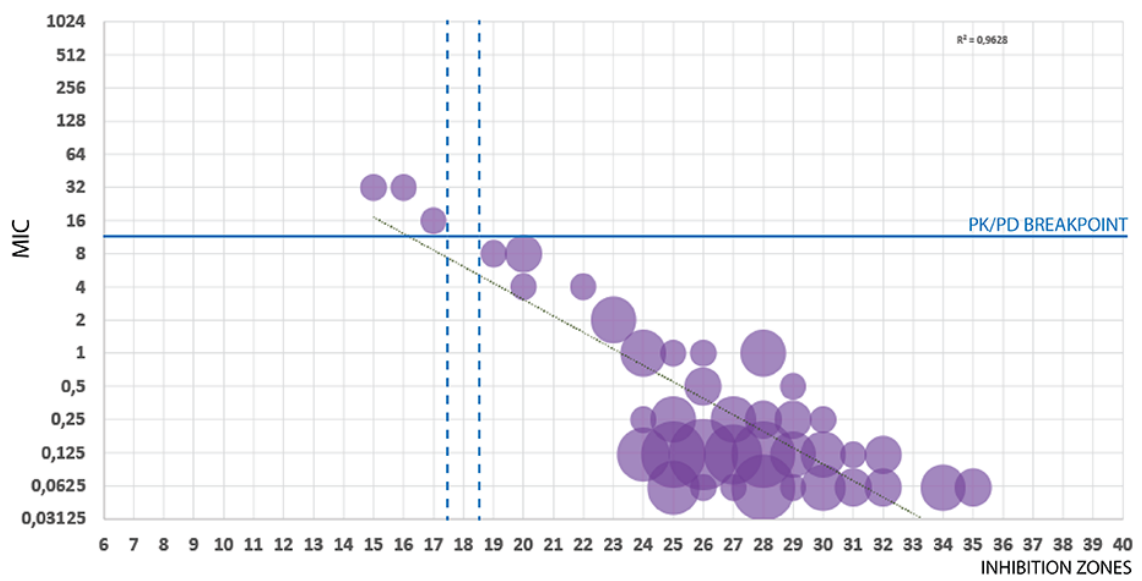
Panel of metallo- β -lactamase-producing Enterobacterales (n 100)



Dash lines indicates tentative equivalente zone inhibition cut-off points.
The size of the sphere is proportional to the numbers of events. Scale: ● 1 strain

FIGURE 3. Correlation between MIC (mg/L) vs inhibition zones (mm) obtained with 30/20ug ATM-AVI disks

Panel of serine carbapenemase-producing Enterobacterales (n 100)



Dash lines indicates tentative equivalente zone inhibition cut-off points.
The size of the sphere is proportional to the numbers of events. Scale: ● 1 strain