03378 | 03378 Validation of a rapid predifussion disk assay to determine aztreonam plus avibactam (ATM-AVI) susceptibility for carbapenemase producing Enterobacterales (CPE)

03. Bacterial susceptibility & resistance

3c. Susceptibility testing methods (incl assay validation and comparative studies, excl TB)

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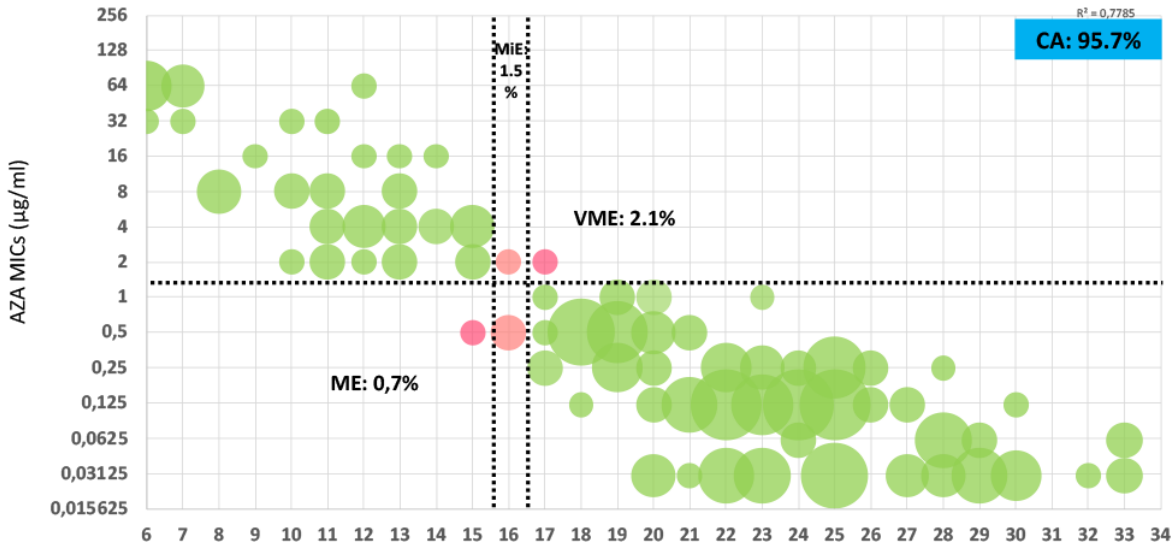
Background Combination therapy with ceftazidime/avibactam (CZA) and aztreonam (ATM) is a potential treatment option for metallo-b-lactamase (MBL) producers. ATM-AVI is also active against KPCs. There is no approved, feasible, susceptibility testing method for clinical labs to guide clinical decision making. Here, we validate a rapid predifussion assay (RaPDA) using commercial disks.

Methods Susceptibility to ATM-AVI (4ug/ml of avibactam) was performed by agar dilution (AD) against CPEs (NRL, 2019-20). Due to the absence of ATM-AVI breakpoints, we used the EUCAST criteria for ATM alone. RaPDA: a Mueller-Hinton agar plate (BD) was inoculated with the tested strains using a 0.5 McFarland inoculum, by swabbing. Subsequently, a 10/4µg CZA disk (Britania) was placed on the plate. After 15 minutes incubation at room temperature, CZA disk was aseptically removed and, in the same spot, a 30µg ATM disk (Oxoid) was placed. Plates were incubated aerobically overnight at 35°C and inhibition evaluated. Colonies within halos were considered for reading the inhibition zone. Data is the mean of 2 replicates. Categorical agreement (CA), very major (VME), major (ME) and minor errors (MiE) were calculated relative to AD. Species identification was performed by MALDI-TOF and molecular characterization of β-lactamases, by PCR/sequencing.

Results 194 clinical isolates were included: 100 MBL, 40 KPC, 30 OXAs, 24 CRE non-CPE. Species depicted in Figure 1. An ATM-AVI MIC >1.0µg/ml was associated to PER ESBL co-production. To validate the RaPDA, the potency of the CZA disk, evaluated in parallel with K. pneumoniae ATCC 700603, must result in a halo within the range recommended by EUCAST (18-24 mm). Colonies within the inhibition zones were only observed for PER coproducers (18/24, 75%). Breakpoints of susceptible ≥17mm and resistant ≤15mm produced the best CA (95,7%): 2.1% VME, 0.7% ME, 1.5% MiE (Fig.1)

Conclusions RaPDA is a simple, inexpensive and reproducible test for ATM-AVI susceptibility. A brief avibactam predifusion ensures inhibitory concentrations without additional testing delays. RaPDA also allows the screening of strains with potential hetero-resistance. Users of other disk/media brands that those used here should considered these results provisional. RaPDA could be a valuable tool to guide clinical decisions in those Institutions where treatment of MBL is a challenge.

FIG. 1. Scattergram showing aztreonam + avibactam (AZA) susceptibility results obtained with agar dilution vs. RaPDA



The size of the sphere is proportional to the number of observations. Dotted lines indicate propsed breakpoints
Species distribution (n): 30 *E. coli,* 30 *Enterobacter* spp,, 10 *K. oxytoca,* 93 *K. pneumoniae,* 16 *Providencia* spp, 10 *S. marcescens*, 5 other spp.

Keyword 1
MBL
Keyword 2
Aztreonam
Keyword 3
Avibactam

Conflicts of interest