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In-Vitro Evaluation & Validation of the Custom Liofilchem® Broth Microdilution Synergy Panel Containing Aztreonam Plus Ceftazidime/Avibactam for Clinical Use.

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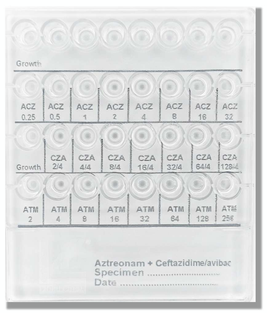
Background

Ceftazidime/avibactam (C/A) and aztreonam (AZT) combination has been proposed against metallo-βlactamase (MBL) carrying organisms; it is based on the low affinity of MBL against AZT and the inhibition provided by avibactam against non-MBL. Although the new IDSA guidance on Gram-negative infections recommends its use, there is not practical commercial in-vitro synergy testing and interpretative guidance available. Current in-vitro synergy methods include checkerboard, time-kill curve, and double gradient strips. In this study, we validated a custom broth microdilution (BMD) synergy panel designed in collaboration with Liofilchem®, combining AZT and C/A to provide a practical and quantifiable method for testing against MBL carrying organisms including the intrinsic L1 in *S. maltophilia*.

Antimicrobials and concentrations (Liofilchem®)

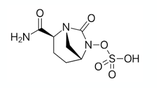
Antimicrobial								
Aztreonam + fixed CAZ/AVI (8/4 µg/mL)	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**
CAZ/AVI (µg/mL)	2/4	4/4	8/4	16/4	32/4	64/4	128/4	
Aztreonam (µg/mL)	2	4	8	16	32	64	128	256

A fixed concentration of CAZ/AVI (8/4 µ/mL) is present on each Aztreonam + CAZ/AVI well as part of the synergy testing

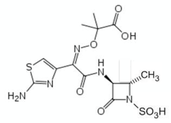


Methods

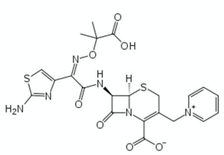
44 MBL MDR isolates were either obtained from clinical samples at AdventHealth Orlando and from the CDC AR Isolate Bank. *S. maltophilia* (n=17), *P. aeruginosa* VIM (n=9), and Enterobacterales NDM (n=14), confirmed by PCR (Cepheid Carba-R®) were included. All isolates were resistant to C/A & AZT and AZT breakpoints for *P. aeruginosa* were applied to *S. maltophilia*. The BMD panel contains dried-up antimicrobial in increasing two-fold dilutions (TfD) in separate wells for AZT (2 to 256 µg/ml) & C/A (2 to 128 µg/ml) as reference, and a combination of aztreonam (0.25 to 32 µg/ml) plus a fixed concentration of C/A (8/4 µg/ml) on each well for synergistic effect. The BMD setup was performed based on package insert. After 18-20 hours of incubation, the result was read as the MIC of AZT in presence of C/A and interpreted based on AZT breakpoints. The ability of C/A to restore the activity of AZT was compared against our in-house validated double strips test.



Avibactam



Aztreonam



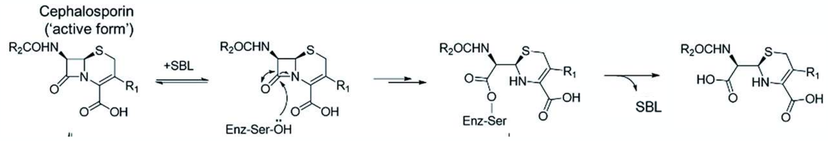
Ceftazidime

Results

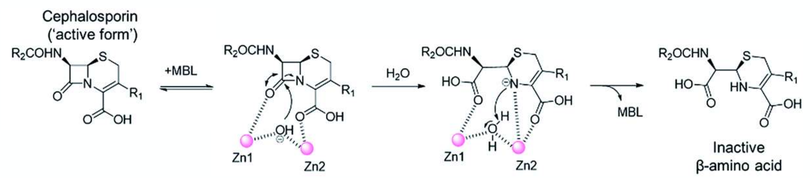
The validation agreements were, categorical 98% (43/44) and essential 95% (42/44). The mean TfD recovery of AZT MIC plus C/A by organism was *S. maltophilia* 7 TfD; *P. aeruginosa* 2 TfD & Enterobacterales 8 TfD. All Enterobacterales isolates reverted from resistant to susceptible except for one intermediate *E. coli* NDM (AZT MIC >256 to 8 µg/ml). The AZT MIC + C/A in *S. maltophilia* were recovered to ≤4 µg/ml. The presence of porins and efflux pumps mediated mechanisms in *P. aeruginosa*, seems to limit the action of AZT beyond the potential rescue provided by C/A.

Average MIC and recovered dilutions in MBL-carrying organisms

Organism	CAZ/AVI (µg/mL)	AZT (µg/mL)	AZT + CAZ/AVI (µg/mL)	AZT recovered (2-fold dilutions)
S. maltophilia	128	**256**	**4**	**7**
P. aeruginosa VIM	64	**64**	**16**	**2**
Enterobacterales NDM	256	**256**	**1**	**8**



Cephalosporin hydrolysis by serine βlactamase



Cephalosporin hydrolysis by metallo βlactamase

Conclusion

The in-vitro activity of AZT in presence of C/A is promising for NDM-carrying Enterobacterales and *S. maltophilia*. The Liofilchem® synergy panel provides a practical and quantifiable laboratory method to test for this combination that is currently in clinical use.

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