In Vitro Activity of ß-Lactam Antimicrobial Agent Combinations with Aztreonam When Testing Metallo-ß-Lactamase-Producing *Pseudomonas aeruginosa* and *Acinetobacter* spp.

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ABSTRACT

Objective: To evaluate the interactions between aztreonam (AZT) and selected ß-lactams when tested against metallo-ß-lactamase (MßL)-producing *P. aeruginosa* (PSA) and *Acinetobacter* spp. (ASP) strains. AZT often displays on-scale MIC results when tested against MßL-producing strains.
Methods: Ten PSA strains, including 9 well-characterized MßL-producers (IMP-1, -2, -13, -16, VIM-1, -2, -7, SPM-1 and GIM-1) and 5 ASP strains, including 3 MßL-producers (IMP-1 and -2) were tested using time kill/bactericidal activity methods. AZT at 4, 8 and 16 mg/L was combined with 4 ß-lactams (cefepime [CPM], ceftazidime [CAZ], meropenem [MEM] and piperacillin/tazobactam [P/T] for PSA or ampicillin/sulbactam [A/S] for ASP), which were tested at the NCCLS susceptible breakpoint concentration. Bacterial counts were determined at time 0, 4, 8 and 24 hours. Enhanced activity was defined as ≥ 2 log_{10} reduction in the CFU/ml compared to the result of the most active antimicrobial tested alone. Antagonism (ANT) was defined as ≥ 2 log_{10} increase in CFU/ml compared to the most active drug.
Results: All MßL-producing PSA were resistant to tested ß-lactams, except for AZT on IMP-16 (MIC, 1 mg/L), SPM-1 (8 mg/L) and GIM-1 (16 mg/L), and for P/T on IMP-16 (4/4 mg/L), VIM-2 (16/4 mg/L) and IMP-2 (32/4 mg/L). Enhanced activity was observed with 4 PSA strains (IMP-16, VIM-2, SPM-1 and GIM-1) and 4 ASP, while ANT was observed with 1 PSA (IMP-16) with MEM and 1 ASP (non-MßL-producing). All other strains showed indifferent interactions (CFU/ml variation of +/- 1 log_{10}) with any combination evaluated. Results of CFU/ml variation observed when AZT at 8 mg/L was combined with the ß-lactams are listed in the Table:

Organism	CFU/ml variation (log_{10}) + AZT				
	CPM (8 mg/L)	CAZ (8 mg/L)	MEM (4 mg/L)	P/T (64/4 mg/L)	A/S (8/4 mg/L)
PSA IMP-16	-3.8[a]	-2.6	+2.5	-3.8	-
PSA VIM-2	+0.3	-1.0	-0.6	-2.7	-
PSA SPM-1	-0.4	+1.4	+0.8	-1.2	-
PSA GIM-1	-1.6	+0.1	0.0	+0.1	-
ACB IMP-2	+0.1	+0.2	0.0	-	-2.2
ACB 4-575 (non-MßL)	+0.3	+0.4	0.0	-	-2.7

a. Underline values indicate SYN.

Enhanced activity was also observed when AZT at 4 mg/L was combined with P/T (PSA IMP-16, VIM-2 and SPM-1), MEM (PSA SPM-1), CPM (PSA GIM-1) or A/S (ASP IMP-1 and non-MßL) and when AZT at 16 mg/L was combined with CPM, MEM and P/T (PSA GIM-1), CAZ (ASP IMP-1) or A/S (ASP non-MßL). Among PSA strains, ANT was observed only with the IMP-16 strain when AZT at 4 and 8 mg/L was combined with MEM or when AZT at 4 mg/L was combined with CAZ, while among ASP ANT was observed when AZT at 4 mg/L was combined with CPM.
Conclusions: MßL-producing PSA and ASP strains are usually resistant to most ß-lactams except AZT. AZT can favorably interact with other ß-lactam agents against some multi-drug resistant isolates for possible chemotherapy.

INTRODUCTION

The emergence of serious infections caused by multidrug-resistant (MDR) Gram-negative organisms poses difficult treatment challenges. The carbapenems, meropenem and imipenem, are usually active against these organisms; however, resistance to these ß-lactam compounds has been increasing in some geographic regions, especially among *Pseudomonas aeruginosa* and *Acinetobacter* spp. isolates. Acquired resistance to carbapenems can be caused by the production of metallo-ß-lactamases (MßLs, Ambler class B), and the recognized clinically important acquired MßL types among Gram-negative bacteria are IMP, VIM and the recently described SPM and GIM ß-lactamases. The treatment of infections caused by MßL-producing strains is very complex since these isolates are usually resistant not only to all ß-lactams (except aztreonam in some cases), but also to the vast majority of antimicrobial agents available for clinical use.

Combination therapy can be used to expand the antimicrobial spectrum, to prevent the emergence of resistant mutants and to obtain synergistic antimicrobial activity. Aztreonam is a synthetic monocyclic ß-lactam antimicrobial agent, which belongs to the monobactam family and remains exclusively active against the aerobic Gram-negative bacilli, including many *P. aeruginosa*. It is neither ototoxic nor nephrotoxic and has been used as an alternative to the aminoglycosides in a variety of clinical infection situations. The objective of the present study is to explore the in vitro activity of aztreonam when tested in combination with other ß-lactams against well-characterized MßL-producing *P. aeruginosa* and *A. baumannii* strains.

MATERIALS AND METHODS

Bacterial isolates. Ten *P. aeruginosa* and five *A. baumannii* recent clinical strains were evaluated. The *P. aeruginosa* strains included nine well-characterized MßL-producing strains (IMP-1, -2, -13, -16, VIM-1, -2, -7, SPM-1 and GIM-1) and one multidrug-resistant (MDR; resistant to imipenem, meropenem, cefepime, ceftazidime, aztreonam, ciprofloxacin and gentamicin) non-MßL-producing strain; while the five *A. baumannii* strains, including three MßL-producing strains (IMP-1 and IMP-2), a non-MßL-producing MDR strain (resistant to aztreonam, ceftazidime, ciprofloxacin, and tobramycin and intermediate to cefepime, imipenem, meropenem and amikacin) and a wild-type strain (resistant only to aztreonam among the ß-lactams evaluated) were selected for the study.

Baseline antimicrobial susceptibility tests. All isolates were tested for antimicrobial susceptibility by reference broth microdilution tests as described by the National Committee for Clinical Laboratory Standards (NCCLS) methods. Etest strips (AB BIODISK, Solna, Sweden) were also utilized to extend the dilution range for some antimicrobial agents. All strains were tested against aztreonam, cefepime, ceftazidime and meropenem. In addition, *P. aeruginosa* strains were tested against piperacillin/tazobactam (fixed 4 mg/L concentration) while *A. baumannii* strains were tested against ampicillin/sulbactam (2:1 ratio). Concurrent susceptibility testing of *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 assured quality control.

Time-kill combination testing. Each *P. aeruginosa* and *A. baumannii* strain was tested using time-kill/bactericidal activity methods against aztreonam at three fixed concentrations (4, 8 and 16 mg/L) and four additional antimicrobial agents at the breakpoint of susceptibility for non-fermentative Gram-negative bacilli; i.e. meropenem at 4 mg/L, cefepime at 8 mg/L, ceftazidime at 8 mg/L and piperacillin/tazobactam at 64/32 mg/L for *P. aeruginosa* or ampicillin/sulbactam at 8/4 mg/L for *A. baumannii* strains. To assess possible synergy or antagonism between these antimicrobial agents, combinations were prepared for each ß-lactam or penicillin/ß-lactamase inhibitor combination agent with each of the three aztreonam concentrations. The bacterial concentration of each test condition or combination was sampled at T_0, T_4, T_8 and T_{24} by removing a 100 µL aliquot from each tube and performing serial dilutions in 0.85% saline followed by plating to drug-free blood agar plates. After 24 and 48 hours of incubation, viable colonies were counted to determine the survival rate of each bacterial strain for each test condition.

The surviving colony forming unit counts per milliliter (CFU/ml) for each antimicrobial agent and each antimicrobial combination at each sampled time point was converted to log_{10} CFU/ml. Time kill-curves were then plotted over a 24 hour time interval. Synergism was defined as a ≥ two log_{10} reduction in the CFU/ml, while additive effect was defined as ≥ one and < two log_{10} reduction in the CFU/ml compared to result of the most active antimicrobial agent tested alone. Antagonism was defined as a ≥ two log_{10} increase in CFU/ml while a suggestion toward antagonism was defined as a ≥ one and < two log_{10} increase in CFU/ml relative to the most active compound alone. A reduction or an increase of < one log_{10} dilution in the CFU/ml was considered as indifferent.

RESULTS

- MßL-producing *P. aeruginosa* strains were categorized as resistant to all agents tested alone, except aztreonam for strains producing IMP-16 (MIC, 1 mg/L), SPM-1 (MIC, 8 mg/L), and GIM-1 (MIC, 16 mg/L) and piperacillin/tazobactam for strains producing IMP-16 (MIC, 4/4 mg/L), VIM-2 (MIC, 16/4 mg/L), and IMP-2 (MIC, 32/4 mg/L).
- MßL-producing *A. baumannii* strains were resistant to aztreonam, cefepime, ceftazidime and meropenem. However, two of these strains were categorized as susceptible to ampicillin/sulbactam according to current NCCLS breakpoints (8/4 mg/L).
- Against the IMP-16-producing strain (Table 1, Figure 1a), synergism was observed when aztreonam at 8 mg/L was combined with cefepime (-3.8 log_{10} CFU/ml) and ceftazidime (-2.6 log_{10} CFU/ml). Synergy was also observed when aztreonam at 4 and 8 mg/L was combined with piperacillin/tazobactam (-3.1 and -3.8 log_{10} CFU/ml).
- Testing aztreonam at 8 mg/L, antagonism (increase of ≥ two log_{10} CFU/ml) was only observed with meropenem against the IMP-16 strain (+2.5 log_{10} CFU/ml), while a suggestion of antagonism was observed with ceftazidime against the SPM-1 producing strain (+1.4 log_{10} CFU/ml; Table 1).

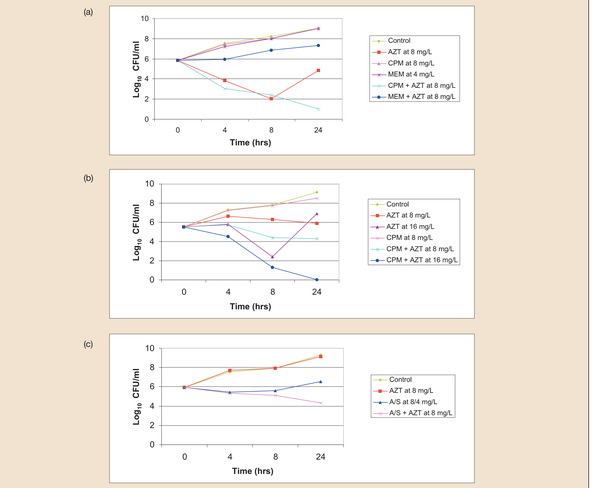
Table 1. Comparative activity of five ß-lactam compounds tested in combination with aztreonam (8 mg/L) against 10 *P. aeruginosa* and five *Acinetobacter* spp. strains.

Organism/isolate (MßL type)	CFU/ml variation (log_{10}) relative to the most active agent tested alone (conc.):				
	Cefepime (8 mg/L)	Ceftazidime (8 mg/L)	Meropenem (4 mg/L)	Piperacillin/Tazobactam (64/4 mg/L)	Ampicillin/Sulbactam (8/4 mg/L)
P. aeruginosa					
BB 319 (IMP-1)	-0.1	+0.1	-0.2	0.0	-[d]
205-5344E (IMP-2)	+0.1	+0.4	-0.5	-0.1	-
86-14571A (IMP-13)	+0.1	+0.1	-0.1	+0.1	-
101-4704C (IMP-16)	-3.8[a]	-2.6[a]	+2.5[b]	-3.8[a]	-
75-3677C (VIM-1)	-0.4	0.0	+0.1	-0.5	-
49-4583C (VIM-2)	+0.3	-1.0[a]	-0.6	-2.7[a]	-
7-408 (VIM-7)	+0.1	+0.2	+0.2	+0.1	-
48-1997A (SPM-1)	-0.4	+1.4[b]	+0.8	-1.2[a]	-
73-15553A (GIM-1)	-1.6[a]	+0.1	0.0	+0.1	-
7-9279A (MDR)[c]	+0.1	-0.2	0.0	-0.5	-
A. baumannii					
39-13622 (IMP-1)	-0.1	-0.7	+0.4	-[d]	+0.1
48-694D (IMP-1)	-0.2	-0.1	-0.2	-	-0.3
AC54/97 (IMP-2)	+0.1	+0.2	0.0	-	-2.2[a]
6-1657 (MDR)[c]	-0.3	+0.3	-0.2	-	-0.2
4-575 (MDR)[c]	+0.3	+0.4	0.0	-	-2.7[a]

a. Enhanced killing (synergy or partial synergy).
b. Antagonist or decreased killing effects.
c. MDR = multidrug-resistant and - = not tested.
d. ⇒ not tested.

- The combination of aztreonam (4 or 8 mg/L) and piperacillin/tazobactam exhibited enhanced activity (synergism or additive effect) against VIM-2 and SPM-1-producing strains (Table 1). Additive effect was also observed when piperacillin/tazobactam (-1.9 log_{10} CFU/ml) or ceftazidime (-1.0 log_{10} CFU/ml) were combined with aztreonam at 16 mg/L against the VIM-2-producing strain.
- Further studies performed with checkerboard method in non-clonal, distinct SPM-1-producing *P. aeruginosa* strains showed that cefepime and piperacillin/tazobactam MIC values might decrease to clinically achievable levels (susceptible or intermediate range) when these antimicrobials are combined with aztreonam (data not shown).
- The combination of aztreonam and cefepime showed excellent results against the unique GIM-1-producing strain (Germany) with a CFU/ml reduction of 1.4 to 6.9 log_{10} depending on the aztreonam concentration used in the test (Figure 1b).
- Only indifferent interactions were observed with IMP-1, -2, -13, VIM-1 and -7 producing isolates as well as with the MDR, non-MßL-producing *P. aeruginosa* strain evaluated.
- Against the *A. baumannii* strains, synergism was observed when aztreonam was combined with ampicillin/sulbactam against three strains: 48-694D (only with aztreonam at 4 mg/L), AC 54/97, and 4-575 (Figure 1c). Enhanced activity (partial synergism) was also documented when aztreonam (16 mg/L) was combined with ceftazidime against the 39-13622A IMP-1-producing strain (-1.9 log_{10} CFU/ml); while antagonism (+2.2 log_{10} CFU/ml) was observed only with aztreonam (16 mg/L) plus cefepime against the 4-575 *A. baumannii* strain.

Figure 1. Time-kill curve plots for aztreonam (at 8 or 16 mg/L) in combination with other ß-lactam antimicrobial agents (at the susceptible breakpoint) tested against selected MßL-producing strains: a) IMP-16-producing *P. aeruginosa* (strain 101-4704C; b) GIM-1-producing *P. aeruginosa* (strain 73-15553A); and c) IMP-2-producing *A. baumannii* (strain AC54/97). Abbreviations: AZT, aztreonam; CPM, cefepime; MEM, meropenem; and A/S, ampicillin/sulbactam.



CONCLUSIONS

- The degree of synergism observed in the present study varied according to the type of MßL produced. The best results were achieved with the IMP-16, VIM-2, SPM-1 and GIM-1-producing *P. aeruginosa* strains.
- Against the *A. baumannii* strains the best results were observed with ampicillin/sulbactam, where enhanced activity (synergism or additive effect) was routinely observed.
- The results presented here indicate that aztreonam could enhance the activity of other ß-lactams, especially cefepime and piperacillin/tazobactam against some MßL-producing *P. aeruginosa*, and ampicillin/sulbactam against *A. baumannii* strains. Clinical evaluation of these combinations appears warranted directed by the understanding of which metallo-ß-lactamase types are prevalent in a geographic area.

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