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Comprehensive In Vitro Evaluation of Cefepime Combined with Aztreonam or Ampicillin/Sulbactam Against Multi-Drug Resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp.

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ABSTRACT

Backgroud: *Pseudomonas aeruginosa* (PSA) and *Acinetobacter* spp. (ASP) are increasingly resistant to antimicrobial agents and serious infections caused by these organisms often require combination therapy.

Methods: Interactions of cefepime with either aztreonam (PSA, n=46) or ampicillin/sulbactam (ASP, n=34) were investigated by the checkerboard synergy method against isolates with various resistance phenotypes, including imipenem-resistant (IMP-R) strains (36 PSA and 19 ASP). Each tray design contained the following antimicrobial agents in combination with cefepime (MIC range, 0.06 - 64 µg/ml): aztreonam (0.5 - 16 µg/ml) for PSA strains and ampicillin/sulbactam (1/0.5 - 16/8 µg/ml) for ASP strains. The fractional inhibitory concentrations (FIC) was calculated for each agent and the summation of both FICs was used to classify the combined activity of antimicrobial agents as synergistic (≤ 0.5), partially synergistic (> 0.5 and < 1), additive (1), indifferent (> 1 and < 4) and antagonistic (≥ 4).

Results: Synergy or partial synergy interactions were observed with 56.5% of PSA and 88.2% of ASP strains examined. Among the IMI-R strains, synergy or partial synergy interactions were observed in 47.2% of PSA and 84.2% of ASP strains. Additive interactions were observed in 12 (26.1%) PSA and 4 ASP (11.8%). Antagonistic interactions did not occur. Indifferent interactions were observed only for 8 PSA isolates, 6 of which had very high MIC values (≥ 64 µg/ml) for at least one of the antimicrobials. The vast majority of IMP-R strains showed MIC values for at least one of the antimicrobials evaluted in the combination within achievable concentrations in human plasma.

Conclusions: The role of selected ß-lactam + ß-lactam combination therapy in the treatment of severe infections caused by multidrug-resistant PSA and ASP should be further evaluated to maximize favorable clinical outcomes using these safe agents.

INTRODUCTION

Cefepime is a broad spectrum fourth-generation cephalosporin. It is highly active against Gram-negative bacteria due to rapid penetration into the periplasmic space and high stability against ß-lactamases, especially AmpC and some carbapenemases, such as NmcA. Aztreonam is a synthetic monocyclic ß-lactam (monobactam) very active against aerobic Gram-negative bacilli, including *P. aeruginosa*. This monobactam can also act as an inhibitor of ß-lactamases in some species due to high affinity for active sites of AmpC enzymes. Aztreonam can be hydrolyzed by many extended-spectrum ß-lactamases (ESBLs), AmpC enzymes and metallo-ß-lactamases, but it remains stable to some metallo-ß-lactamases such as SPM-1.

Sulbactam is a ß-lactamase inhibitor with direct antimicrobial activity against *Acinetobacter* spp. Its mechanism of antimicrobial activity against *Acinetobacter* spp. may be related to its ability to bind essential PBPs or to alter the permeability of the outer membrane of Gram-negative bacilli, which results in the leakage of ß-lactamases and thus better penetration by other antimicrobial agents. In addition, sulbactam has been extensively used in combination with other ß-lactams, such as ampicillin and cefoperazone outside of North America. The objectives of the present study were to evaluate the in vitro interaction between cefepime and aztreonam against multidrug-resistant (MDR) *P. aeruginosa*, and between cefepime and ampicillin/sulbactam against MDR *Acinetobacter* spp. strains.

MATERIALS AND METHODS

Bacterial isolates. A total of 46 isolates of *P. aeruginosa* and 34 *Acinetobacter* spp. were evaluated in the present study. The isolates were selected based on their antimicrobial resistance phenotype (Table 1). Thirty-six (78.3%) *P. aeruginosa* strains were resistant to imipenem (MIC, ≥ 16 µg/ml), while 19 (55.9%) *Acinetobacter* spp. strains were resistant to imipenem, ceftazidime, piperacillin/tazobactam, ciprofloxacin and amikacin. Five *Acinetobacter* spp. strains representing distinct epidemic clones from New York City were included among the isolates tested. None of the MDR strains tested were metallo-ß-lactamase-producers. *P. aeruginosa* ATCC 27853 and a susceptible *A. baumannii* strain from New York City were included as controls.

Antimicrobial susceptibility and synergism tests. Isolates were initially tested against individual compounds using the broth microdilution method on validated dry-form panels (TREK Diagnostics, Cleveland, OH) following the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) guidelines or by Etest (AB BIODISK, Solna, Sweden) where necessary for expanded MIC range determination and results interpreted by CLSI/NCCLS criteria. Synergy tests were performed in 96-well broth microdilution panels containing two antimicrobial agents in two-fold dilutions dispensed in a checkerboard format. Each panel contained the following antimicrobial agents (MIC range tested in parenthesis) in combination with cefepime (0.06 - 64 µg/ml): aztreonam (0.5 - 16 µg/ml) for *P. aeruginosa* strains and ampicillin/sulbactam (1/0.5 - 16/8 µg/ml) for *Acinetobacter* spp. strains. Standard antimicrobial powders were supplied by respective domestic manufacturers and the checkerboard panels were prepared by JMI Laboratories. The quality control strains used for all tests were *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. All results were within published ranges.

The interpretations of the antimicrobial combinations were based on the calculation of the fractional inhibitory concentration (FIC) for each drug pair. The FIC of each agent was calculated by dividing the MIC of the drug in combination by the MIC of the drug alone. The summation of both FICs (ΣFIC = FIC of drug A + FIC of drug B) in each well was used to classify the combined activity of antimicrobial agents at the given concentrations as synergistic (ΣFIC, ≤ 0.5), partially synergistic (ΣFIC, > 0.5 and < 1), additive (ΣFIC, 1), indifferent (ΣFIC, > 1 and < 4) and antagonistic (ΣFIC, ≥ 4).

RESULTS

- Synergy (ΣFIC ≤ 0.5) or partial synergy (ΣFIC > 0.5 and < 1) interactions between cefepime and aztreonam were detected in the majority of *P. aeruginosa* strains (56.5%). Synergy was observed in five strains (10.9%) whereas partial synergy was observed in 21 strains (45.7%). Additive and indifferent interactions were observed in 12 (26.1%) and eight strains (17.4%), respectively, and none of the isolates showed an antagonistic interaction (Table 2).

Table 1. Antimicrobial susceptibility of bacterial strains utilized in the study.

Organism/antimicrobial agent (no. tested)	MIC (µg/ml) 50%	MIC (µg/ml) 90%	MIC (µg/ml) Range	Category % S	Category % R
P. aeruginosa (46)					
Aztreonam	16	>128	2->256	30.4	28.3
Cefepime	8	32	1->64	63.0	15.2
Imipenem	>8	>8	1->8	19.6	78.3
Acinetobacter spp. (34)					
Ampicillin/sulbactam	8	>16	2->16	58.9	11.8
Cefepime	8	>64	0.12->64	55.9	26.5
Imipenem	>8	>8	0.12->8	41.2	55.9

Table 2. MIC results for cefepime and aztreonam when each drug was tested alone and in combination against 46 *P. aeruginosa* isolates.

Isolate #	MIC of the drug tested alone (µg/ml) Cefepime	MIC of the drug tested alone (µg/ml) Aztreonam	MIC of the drug tested in combination (µg/ml) Cefepime	MIC of the drug tested in combination (µg/ml) Aztreonam	Category[a]
8152	4	4	1	1	SYN
19[b]	4	8	1	2	SYN
6236[b]	8	64	2	16	SYN
6344[b]	16	64	4	16	SYN
12515[b]	32	64	8	16	SYN
1172[c]	1	4	0.25	2	PSYN
10141[b]	2	2	0.5	1	PSYN
1313	2	4	0.5	2	PSYN
8017	2	8	1	2	PSYN
2237[b]	2	16	1	4	PSYN
2362	4	4	1	2	PSYN
1166	4	4	1	2	PSYN
9646	4	8	2	2	PSYN
366[b]	4	8	1	4	PSYN
6935[b]	4	16	0.5	8	PSYN
3312	4	32	1	16	PSYN
14804	8	16	2	8	PSYN
3436	8	16	2	8	PSYN
12795[b]	8	16	4	4	PSYN
154[b]	8	16	4	4	PSYN
1932[b]	8	16	2	8	PSYN
6336[b]	16	16	4	8	PSYN
7721[b]	16	64	8	16	PSYN
1314[b]	16	32	4	16	PSYN
697[b]	32	16	16	4	PSYN
6627[b]	64	256	32	16	PSYN
11375[b]	1	4	0.5	2	ADD
1486[b]	2	8	1	4	ADD
1118[b]	2	16	1	8	ADD
156	4	16	2	8	ADD
1932[c]	8	16	4	8	ADD
252[c]	8	16	4	8	ADD
8042[b]	8	16	4	8	ADD
3248[b]	8	16	4	8	ADD
10147[b]	16	16	8	8	ADD
4997[c]	16	16	8	8	ADD
4987[b]	16	16	8	8	ADD
6996[b]	16	32	8	16	ADD
8035[b]	8	8	2	8	INDIF
7760[b]	8	16	2	16	INDIF
14198[b]	16	>256	16	16	INDIF
3463[b]	16	64	16	16	INDIF
8586[b]	32	128	32	16	INDIF
9279[c]	32	>256	64	16	INDIF
641[b]	32	>256	32	16	INDIF
12656[b]	>64	8	>64	4	INDIF

a. SYN, synergy; PSYN, partial synergy; ADD, additive; and INDIF, indifferent as calculated by ΣFIC results.
b. Isolates resistant to imipenem.

- Thirty-six *P. aeruginosa* isolates (78.3%) were resistant to imipenem (MIC, ≥ 16 µg/ml). Among isolates in this group, synergism was demonstrated in four isolates (11.1%), partial synergism in 13 (36.1%) and additive effect in 11 (30.6%). In addition, 30 of 36 (83.3%) imipenem-resistant isolates tested had cefepime and/or aztreonam MIC values in the susceptible range (≤ 8 µg/ml) when these two compounds were tested in combination.

- No antagonistic interactions were observed among *Acinetobacter* spp. isolates (Table 3). Nine isolates (26.5%) showed synergistic interaction (ΣFIC, ≤ 0.5), while 21 isolates (61.8%) showed partial synergism. Four isolates (11.8%) showed an additive effect. Cefepime MIC values were ≤ 8 µg/ml for 85.3% of strains, while ampicillin/sulbactam MIC values were ≤ 16/8 µg/ml (intermediate) for all isolates when these compounds were tested in combination.

- Nineteen of 34 (55.9%) *Acinetobacter* spp. isolates evaluated were imipenem-resistant (MIC, ≥ 16 µg/ml). Among these carbapenem-resistant strains, synergy (five isolates) and partial synergy (11 isolates) interactions were obtained in 84.2% of isolates.

- The MIC result for cefepime and/or ampicillin/sulbactam was within the susceptible range for 18 of 19 (94.7%) imipenem-resistant *Acinetobacter* spp. strains when these two drugs were tested in combination.

- Two of five clonally distinct MDR *Acinetobacter* spp. isolates from New York City showed synergistic interactions while three showed partial synergy. No metallo-ß-lactamase production was detected in these isolates and cefepime MIC values ranged from 0.5 to 16 µg/ml (only one isolate at 16 µg/ml) when this antimicrobial was combined with ampicillin/sulbactam.

Table 3. MIC results for cefepime and ampicillin/sulbactam when each drug was tested alone and in combination against 34 *Acinetobacter* spp. isolates.

Isolate #	MIC of the drug tested alone (µg/ml) Cefepime	MIC of the drug tested alone (µg/ml) Ampicillin/Sulbactam (2:1)[a]	MIC of the drug tested in combination (µg/ml) Cefepime	MIC of the drug tested in combination (µg/ml) Ampicillin/Sulbactam (2:1)[a]	Category[b]
13645	2	4	0.5	0.5	SYN
575[c,d]	4	4	1	0.5	SYN
140	4	8	1	2	SYN
522[d]	8	2	2	0.5	SYN
13642	8	8	2	2	SYN
15706[c]	16	>16	4	8	SYN
3473[c]	32	16	8	4	SYN
7027[c]	>64	16	16	4	SYN
7041[c]	>64	>16	32	8	SYN
4984	0.12	8	≤0.06	0.25	PSYN
15816	2	2	0.5	1	PSYN
12109	2	2	0.5	1	PSYN
2856	2	2	0.25	1	PSYN
2245	2	2	1	0.5	PSYN
15758	4	2	1	1	PSYN
16604	4	4	1	2	PSYN
2852	8	4	2	2	PSYN
2826	8	8	0.25	4	PSYN
13628	8	16	4	4	PSYN
1767[c]	8	16	4	4	PSYN
12457[c]	8	16	2	8	PSYN
13213[c]	16	8	2	4	PSYN
1134[c,d]	16	8	0.5	4	PSYN
6364[c]	16	16	2	8	PSYN
15182[c]	16	>16	8	8	PSYN
11270[c]	32	4	8	2	PSYN
2961[c]	32	8	8	2	PSYN
187[c,d]	32	8	8	2	PSYN
1145[c,d]	64	>16	16	16	PSYN
7467[c]	>64	16	32	8	PSYN
2016	4	2	2	1	ADD
13348[c]	8	16	4	8	ADD
16605[c]	16	16	8	8	ADD
2145[c]	>64	16	64	8	ADD

a. Values indicate the concentration of the ampicillin component.
b. SYN, synergism; PSYN, partial synergism; and ADD, additive as calculated by ΣFIC results.
c. Isolates resistant to imipenem, ceftazidime, piperacillin/tazobactam, tobramycin, amikacin, gentamicin and ciprofloxacin.
d. Isolates from the five New York City endemic clones [5].

CONCLUSIONS

- Most of the isolates evaluated in the present study, e.g. 78.3% of *P. aeruginosa* and 55.9% of *Acinetobacter* spp., were resistant to imipenem and many other antimicrobial agents used to treat severe invasive infections caused by these organisms.

- The fact that the vast majority of imipenem-resistant organisms (83.3% of *P. aeruginosa* and 94.7% of *Acinetobacter* spp.) had MIC values within the susceptible range for at least one of the antimicrobials when they were tested in combination shows the clinical potential of these antimicrobial combinations for the treatment of infections caused by MDR *P. aeruginosa* and MDR *Acinetobacter* spp. strains.

- In an era of increasing antimicrobial resistance, and with very few promising drugs targeting Gram-negative bacilli on the horizon, it is important to seek ways to reduce the rate of resistance development and effectively use existing antimicrobials currently on hospital formularies. Utilization of these two combination therapies should be explored further given the limited choices in therapy currently available.

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