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Research Paper

Synergistic effects of sulbactam in multi-drug-resistant *Acinetobacter baumannii*

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Abstract

Acinetobacter baumannii is a frequently isolated etiologic agent of nosocomial infections, especially in intensive care units. With the increase in multi-drug resistance of *A. baumannii* isolates, finding appropriate treatment alternatives for infections caused by these bacteria has become more difficult, and available alternate treatments include the use of older antibiotics such as colistin or a combination of antibiotics. The current study aimed to evaluate the in vitro efficacy of various antibiotic combinations against multi-drug resistant *A. baumannii* strains. Thirty multi-drug and carbapenem resistant *A. baumannii* strains isolated at the Ankara Training and Research Hospital between June 2011 and June 2012 were used in the study. Antibiotic susceptibility tests and species-level identification were performed using conventional methods and the VITEK 2 system. The effects of meropenem, ciprofloxacin, amikacin, tigecycline, and colistin alone and in combination with sulbactam against the isolates were studied using Etest (bioMérieux) in Mueller-Hinton agar medium. Fractional inhibitory concentration index (FIC) was used to determine the efficacy of the various combinations. While all combinations showed a predominant indifferent effect, a synergistic effect was also observed in 4 of the 5 combinations. Synergy was demonstrated in 43% of the isolates with the meropenem-sulbactam combination, in 27% of the isolates with tigecycline-sulbactam, and in 17% of the isolates with colistin-sulbactam and amikacin-sulbactam. No synergy was detected with the sulbactam-ciprofloxacin combination and antagonism was detected only in the sulbactam-colistin combination (6.66% of the isolates). Antibiotic combinations can be used as an alternative treatment approach in multi-drug resistant *A. baumannii* infections.

Key words: acinetobacter, antimicrobial drugs, drug resistance, drug synergism.

Introduction

Acinetobacter species are prevalent agents of nosocomial infections (Bergogne-Berezin and Towner, 1996) as they are resistant to environmental conditions and are capable of easily acquiring resistance to antibiotics; and the most commonly isolated species is *Acinetobacter baumannii* (Roberts *et al.*, 2001).

In the recent years, due to the increase in the use of wide-spectrum antibiotics, *Acinetobacter* species has acquired resistance against these antibiotics. This resistance causes serious problems in providing effective treatment, particularly in intensive care units where antibiotic use is high and interventional procedures (*e.g.*, intubation and urinary and intravenous catheters) are frequently performed (Chastre, 2003).

Alternate treatment protocols are being researched as the rate of resistance is increasing. These alternates include regimens using combination of antibiotics and synthesis of new antibiotics. Antimicrobial drug combinations are used to achieve a wider spectrum; they prevent the emergence of drug-resistant mutants, minimize toxicity, and achieve a synergistic effect.

In vitro synergy tests are used to evaluate drug interaction in antibiotic combinations, and include the checkerboard, time-kill, and Etest diffusion methods (Moody, 2004; Haddad *et al.*, 2005).

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The current study aimed to determine in vitro effects of a combination of sulbactam (SUL) with tigecycline (TGC), meropenem (MP), amikacin (AK), ciprofloxacin (CL), or colistin (CT) against multi-drug resistant nosocomial *A. baumannii* species.

Materials and Methods

Thirty multi-drug and carbapenem resistant *A. baumannii* species isolated from blood cultures of patients hospitalized in the Ankara Training and Research Hospital between June 2011 and June 2012 were included in the study. Antibiotic susceptibility testing and species-level identification were carried out using conventional methods and the VITEK 2 system (bioMérieux SA, France). Although no current standard definition exists for the term "multi-drug resistance" with respect to *Acinetobacter* sp., resistance to three or more antibiotic classes used in the treatment of Acinetobacter infections is currently accepted as multi-drug resistance. The isolates used in this study were obtained from different clinics and patients hospitalized on different dates, and only one clinical isolate from each patient was included. All isolates were stored at -20 °C in brain-heart medium (Oxoid, UK) containing glycerol until use.

The effects of meropenem, ciprofloxacin, amikacin, tigecycline, and colistin alone, and in combination with sulbactam, against the 30 isolates were studied using the Etest method (bioMérieux, France) in Mueller-Hinton agar medium (Oxoid, UK) according to the manufacturer's instructions. The susceptibility breakpoint of the antibiotics used against *A. baumannii* is shown in Table 1 (Henwood *et al.*, 2002; Kahlmeter *et al.*, 2006; CLSI, 2007).

The fractional inhibitory concentration (FIC) index was used to determine the efficacy of each combination (Sopirala *et al.*, 2010). To determine the FIC index using the Etest, the minimum inhibitory concentration (MIC) of the two antibiotics (denoted as A or B) involved in the combination was first calculated and recorded. To establish the MIC value of the combination, the B strip was placed on the medium and incubated at 37 °C for 1 h. Then, the B strip was removed and the A strip was placed such that it completely overlapped the concentration lines of the B strip. After incubation at 37 °C for 24 h, the intersection of the inhibition zone diameter at the edge of the Etest band was recorded as the MIC value of A in the combination. The same procedure was repeated with each antibiotic and for all combinations. To determine the efficacy of the combination, the FIC index was calculated according to the following equation:

$$\text{FIC A} = \frac{\text{MIC of drug A in combination}}{\text{MIC of drug A alone}}$$

$$\text{FIC B} = \frac{\text{MIC of drug B in combination}}{\text{MIC of drug B alone}}$$

Σ FIC index = FIC A+ FIC B.

The Σ FIC index values were interpreted as follows:

Σ FIC index ≤ 0.5 = synergistic

Σ FIC index > 0.5 to 1 = additive

Σ FIC index > 1 to < 4 = indifferent (ineffective) and

Σ FIC ≥ 4 = antagonistic interaction

Pseudomonas aeruginosa American Type Culture Collection (ATCC) 27853 and *Escherichia coli* ATCC 25922 were used as control species.

Table 1 - CLSI (2007) MIC values of antimicrobials against *A. baumannii* strains

Antibiotic	MIC (μg/mL) breakpoints		
	Sensitive	Intermediate	Resistant
Colistin	≤ 2		≥ 4
Ciprofloxacin	≤ 1	2	≥ 4
Meropenem	≤ 4	8	≥ 16
Amikacin	≤ 16	32	64
Tigecycline*	≤ 2	2-8	≥ 8
Sulbactam**	≤ 4	8	≥ 16

*Standards determined by FDA for *Enterobacteriaceae*.
**Sulbactam values were used according to CLSI (2007) MIC breakpoints for ampicillin-sulbactam.

Results

Table 2 shows the MIC values (μg/mL), MIC50 values, MIC90 values (μg/mL), and the susceptibility ratios of the multi-drug and meropenem-resistant isolates.

As shown in the Table 2, all of the isolates were resistant to meropenem and ciprofloxacin but susceptible to colistin. Resistance to amikacin (56%) but susceptibility to tigecycline were also high (47%).

A total of 150 Σ FIC values were calculated for the five antibiotic combinations (CT-SUL, MP-SUL, CL-SUL, AK-SUL, TGC-SUL) for all isolates. Our interpretation of the Σ FIC values (synergistic, additive, ineffective (indifferent), and antagonistic) are shown in Table 3, and data on the percentage of the interaction are presented in Table 4.

In the current study, for all drug combinations, the most common interaction observed was indifferent or additive. CL-SUL had the highest indifferent effect at 90% (27/30 isolates), while TGC-SUL had the highest additive effect at 43% (13/30). MP-SUL showed the highest synergistic effect at 43% (13/30) followed by TGC-SUL at 27% (8/30). Antagonistic interference was observed only in the CT-SUL combination (6.66%, 2/30 isolates).

Table 2 - MIC intervals, MIC 50 values, and MIC90 values and susceptibility ratios against *A. baumannii* isolates that were determined with Etest.

Antibiotics	Bacteria (n = 30)	MIC range (μg/mL)	MIC (μg/mL) 50%	MIC (μg/mL) 90%	Susceptibility ratios					
					Susceptible		Intermediate susceptibility		Resistant	
					Number	%	Number	%	Number	%
Colistin	30	0.16-8	0.032	0.19	30	100	0	0	0	0
Meropenem	30	16-32	32	32	0	0	0	0	30	100
Amikacin	30	1.5-256	96	256	5	17	8	27	17	56
Tigecycline	30	0 75-32	3	32	14	47	9	30	7	23
Sulbactam	30	2-256	12	32	2	8	14	46	14	46
Ciprofloxacin	30	32-32	32	32	0	0	0	0	30	100

Discussion

The emergence of high antibiotic resistance in *A. baumannii* isolates has impeded the successful treatment of these infections, thus necessitating alternative treatment options. Among the available options, the use of a combination of antibiotics is currently the most preferred treatment approach (Falagas and Kasiakou, 2005).

Combination treatment is mainly used to prevent the development of antibiotic resistance and decrease dose-dependent side effects, and to treat polymicrobial infections. Furthermore, it is also used to treat severe infections with high rates of mortality as a combination of antibiotics provides a synergistic effect against the multi-drug-resistant isolates (Kiffer *et al.*, 2005).

While a combination of antimicrobial agents with different mechanisms of action may provide better pharmacokinetic effects or synergy, they may also cause antagonism. The absence of antagonistic interaction among antibiotics has clinical importance, and therefore, many studies have emphasized the need to determine the interactive effects of antibiotic combinations in vitro. A synergistic effect is especially beneficial (is especially beneficial (Fass *et al.*, 1990; Haddad *et al.*, 2005). It is known that the combined administration of carbapenem and aminoglycoside group of antibiotics, the most frequently used combination in the empiric treatment of *Acinetobacter* infections, generally demonstrates an in vitro synergistic effect (Marques *et al.*, 1997; Bonapace *et al.*, 2000). However, due to the prevalence of high resistance to both the groups of drugs, as shown in the present study and by previous reports in recent years, colistin appears to be the only viable treatment option. However, as colistin is associated with high nephrotoxicity and neurotoxicity current research is focused on using other treatment options, including use of different antibiotic and drug combinations (Falagas and Kasiakou, 2006; Dinc *et al.*, 2013). Thus, the present study aimed to investigate the in vitro interactions between sulbactam and available antibiotics as possible treatment options.

Sulbactam alone has demonstrated direct antimicrobial activity against *Bacteroides fragilis* and Acinetobacter spp., and has intrinsic bactericidal activity against multi-drug-resistant *Acinetobacter* spp., as it inhibits the penicillin-binding proteins (Allen & Hartman 2010). Tazobactam and clavulanate are less effective compared to sulbactam, but there are no well-documented clinical practice guidelines. In the current study, the MIC50 and MIC90 values of sulbactam were found to be 12 μg/mL and 32 μg/mL, respectively and these values are in agreement with previously reported values. Swenson *et al.* (2004) have evaluated the efficacy of sulbactam against 195 *A. baumannii* isolates and reported MIC50 and MIC90 values of 8 μg/mL and 128 μg/mL, respectively, while Hawley *et al.* (2007)

Table 3 - The list of FIC values for antibiotic combinations against multi-drug resistant *A. baumannii* isolates.

(n = 30)	CT-SUL		MP-SUL		CL-SUL		AK-SUL		TGC-SUL	
	Σ FIC	Effect	Σ FIC	Effect	Σ FIC	Effect	Σ FIC	Effect	Σ FIC	Effect
1	0.0166	S	0.13	S	0.546	ADD	0.148	S	0.09	S
2	0.123	S	0.156	S	0.697	ADD	0.156	S	0.421	S
3	0.124	S	0.18	S	0.843	ADD	0.421	S	0.5	S
4	0.251	S	0.27	S	1.011	ID	0.43	S	0.5	S
5	0.338	S	0.281	S	1.015	ID	0.5	S	0.5	S
6	1	ID	0.281	S	1.023	ID	0.523	ADD	0.5	S
7	1.001	ID	0.39	S	1.024	ID	0.523	ADD	0.5	S
8	1.005	ID	0.39	S	1.062	ID	0.546	ADD	0.5	S
9	1.015	ID	0.401	S	1.125	ID	0.546	ADD	0.56	ADD
10	1.392	ID	0.406	S	1.25	ID	0.593	ADD	0.59	ADD
11	1.393	ID	0.421	S	1.335	ID	0.729	ADD	0.593	ADD
12	1.453	ID	0.468	S	1.335	ID	0.781	ADD	0.625	ADD
13	1.469	ID	0.5	S	1.337	ID	0.781	ADD	0.625	ADD
14	1.47	ID	0.729	ADD	1.337	ID	0.796	ADD	0.625	ADD
15	1.48	ID	0.78	ADD	1.338	ID	0.843	ADD	0.687	ADD
16	1.484	ID	0.796	ADD	1.364	ID	1.01	ID	0.729	ADD
17	2	ID	0.843	ADD	1.502	ID	1.02	ID	0.833	ADD
18	2	ID	0.875	ADD	1.505	ID	1.03	ID	0.84	ADD
19	2	ID	1	ID	1.507	ID	1.031	ID	0.843	ADD
20	2	ID	1.01	ID	1.511	ID	1.031	ID	0.87	ADD
21	2	ID	1.03	ID	1.523	ID	1.05	ID	0.916	ADD
22	2	ID	1.04	ID	1.625	ID	1.166	ID	1	ID
23	2	ID	1.04	ID	2	ID	1.166	ID	1.031	ID
24	2.01	ID	1.06	ID	2.003	ID	1.375	ID	1.056	ID
25	2.95	ID	1.06	ID	2.005	ID	1.375	ID	1.125	ID
26	2.985	ID	1.125	ID	2.009	ID	1.523	ID	1.25	ID
27	3	ID	1.52	ID	2.031	ID	1.546	ID	1.341	ID
28	3.91	ID	1.523	ID	2.039	ID	1.56	ID	1.5	ID
29	5.7	AG	1.562	ID	2.062	ID	2.04	ID	1.5	ID
30	7.813	AG	2.02	ID	2500	ID	2.729	ID	1.666	ID

CT: Colistin; MP: Meropenem; AK: Amikacin; TGC: Tigecycline; SUL: Sulbactam; CL: Ciprofloxacin; S: Synergistic; ADD: Additive; ID: Indifferent; AG: Antagonist; ΣFIC: Fractional inhibitory concentration index.

have reported MIC50 and MIC90 values of 16 μg/mL and 64 μg/mL, respectively.

A synergistic effect against *A. baumannii* species was observed when sulbactam was combined with ampicillin, carbapenem, or cefoperazone (Chu *et al.*, 2013). Pongpech *et al.* (2010) also reported synergistic effects when a combination of meropenem-sulbactam (70%) or colistin-sulbactam (53%) was used against carbapenem, and multi-drug-resistant *A. baumannii* isolates. While Kiffer *et al.* (2005) reported 29% synergy (14/48 isolates) and 58.4% additivity (28/48 isolates) with a sulbactam-meropenem combination against MDR *A. baumannii* isolates using the CB method, conversely, Santimaleeworagun *et al.* (2011), using the same method, reported no synergistic interaction when a combination of sulbactam and colistin. We also demonstrated considerable synergistic effects when sulbactam

Table 4 - Interpretation for interaction results of antibiotic combinations against multi-drug resistant *A. baumannii* isolates.

Combinations	Observed effect							
	Synergistic		Additive		Indifferent		Antagonist	
	Number	%	Number	%	Number	%	Number	%
CT-SUL	5	17	0	0	23	76	2	7
MP-SUL	13	43	5	17	12	40	0	0
CL-SUL	0	0	3	10	27	90	0	0
AK-SUL	5	17	10	33	15	50	0	0
TGC-SUL	8	27	13	43	9	30	0	0

CT: Colistin; MP: Meropenem; AK: Amikacin; TGC: Tigecycline; SUL: Sulbactam; CL: Ciprofloxacin.

was combined with meropenem (43%, 13/30), tigecycline (27%, 8/30), colistin (17%, 5/30), or amikacin (17%, 5/30), but no synergistic interaction was observed with ciprofloxacin. Importantly, to the best of our knowledge, this is the first report on a synergistic effect between tigecycline and sulbactam, and this result warrants further investigation.

Although Santimaleeworagun *et al.* (2011) reported no antagonism with a combination of sulbactam and colistin, we observed a small antagonistic effect (6.66% (2/30) which prompted a reconsideration of the usefulness of this combination and its subsequent rejection.

Although there are several methods for detecting in vitro interactions between combinations of antibiotics, but none is standard. Synergy testing by Etest is an easy-to-perform method that does not obscure the effects of the active drug when used in combination with other drugs (Sopirala *et al.*, 2010). Even though we used sulbactam as the main antibiotic in the Etest, it is not possible to comment on the exact effect of sulbactam as we obtained different results with the various antibiotics tested. While the findings reported here demonstrate similarities with many previous studies in the literature, some differences also exist which could be due to the differences in methodology or resistance patterns of the bacteria tested.

Given the possibility of antibiotic resistance patterns being different for different isolates of *A. baumannii*, any effect observed with a given combination is expected to be strain-specific. This implies that synergy testing for various combinations of antibiotics should be carried out against each patient-based isolate of MDR *A. baumannii*.

Furthermore, as in vitro studies do not accurately represent in vivo conditions, the data obtained from such in vitro studies should be supported by similar results from adequately controlled clinical studies.

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