African Journal of Microbiology Research Vol. 6(12), pp. 2985-2992, 30 March, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR12.038
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

In vitro synergistic activity of carbapenems in combination with other antimicrobial agents against multidrug-resistant *Acinetobacter baumannii*

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Accepted 27 February, 2012

Multidrug-resistant *Acinetobacter baumannii* (MDR-AB) is emerging as a major nosocomial pathogen worldwide. In recent years, the inadequacy of antimicrobial agents available to treat infections particularly in Intensive Care Units (ICUs) due to MDR-AB, has constrained clinicians and forced them to use combination therapies. In this study, *in vitro* synergistic activities of imipenem and meropenem in combination with cefoperazone-sulbactam, ampicillin-sulbactam, polymyxin B and rifampin were tested against 34 clinical isolates of (MDR-AB), all collected from the Intensive Care Units of Süleyman Demirel University Hospital. Minimum inhibitory concentration values of all antibiotics were determined by the broth microdilution method and antibiotic interactions were analyzed by checkerboard assay. The combination of meropenem with ampicillin-sulbactam showed synergy against 94.1% of MDR-AB while the synergy rates for combinations of imipenem and ampicillin-sulbactam, imipenem and rifampin, imipenem and cefoperazone-sulbactam, imipenem and polymyxin B, meropenem and rifampin, meropenem and cefoperazone-sulbactam and meropenem and polymyxin B were 88.2, 73.5, 70.6, 38.2, 17.6, 8.8 and 2.9%, respectively. Antagonism was not observed in any of the combinations. We must emphasize the fact that evaluating the efficacy of combinations against MDR-AB by synergy tests is essential to guide the treatment.

Key words: *Acinetobacter baumannii*, antimicrobial combination, carbapenems, checkerboard assay, multidrug-resistant.

INTRODUCTION

Acinetobacter baumannii, among the most important causes of nosocomial infections, is becoming a serious threat for hospitalized patients because of increasing antibiotic resistance rates. In recently conducted surveillance studies, it has been reported that resistance is increasing among carbapenems, which are still considered as the primary treatment against these bacteria. Thus, they have become among the most difficult nosocomial Gram-negative pathogens to control and treat (Jain and Danziger, 2004; Lockhart et al., 2007). Increasing resistance rates seen in *A. baumannii* strains have necessitated administration of combination therapies as an alternative choice for the treatment of multidrug-resistant *A. baumannii* (MDR-AB) infections and consequently empirical combination therapies have become common in practice (Bonapace et al., 2000). It is anticipated that the addition of other active antimicrobials to carbapenem may save its role as a core antimicrobial to fight against infections due to MDR-AB (Pongpech et al., 2010). Previous research evaluating the activity of rifampin against *A. baumannii* isolates indicated that rifampin shows *in vitro* bactericidal activity against these bacteria (Thornsberry et al., 1983). Furthermore, synergism was observed with the combination of rifampin plus imipenem (Tripodi et al., 2007). Sulbactam is another interesting antimicrobial agent that possesses the highest intrinsic antibacterial activity among β

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lactamase inhibitors against *A. baumannii* with minimal side effects (Peleg et al., 2008). The combination of ampicillin-sulbactam or sulbactam alone with carbapenems has been reported to improve the activity of β-lactams against MDR-AB isolates significantly (Ko et al., 2004; Wang et al., 2004). With limited therapeutic options in MDR-AB infections, clinicians have returned to the use of old class of antibiotics like polymyxin B and colistin which were abandoned during previous years in most parts of the world because of their unfavorable effects. Since most of the MDR-AB isolates are found to be susceptible to polymyxins they have been accepted as savior agents and despite security concerns about toxicity, clinicians tend to use polymyxins for the treatment of infections caused by MDR-AB (Peleg et al., 2008).

Antimicrobial susceptibility testing of *A. baumannii* has shown equally high susceptibility rates for polymyxin B (95 to 99%) and for colistin (98 to 100%) (Diez et al., 2004; Landman et al., 2007; Manikal et al., 2000). However, the emergence of resistance during treatment when used alone and potentially toxic effects have given rise to the use of polymyxins in combinations instead of increasing the dose as a single agent. Frequently preferred antimicrobials for this purpose are carbapenems like imipenem, meropenem or doripenem. While there are various reports about polymyxin that synergistic activity is obtained with this drug when used in combination therapies, it is also known that the results of synergy tests with polymyxin are highly strain and method dependent and *in vitro* synergy may or may not translate into *in vivo* benefit (Pankey and Ashcraft, 2009; Wareham and Bean, 2006). In this study, the antibacterial effect of imipenem and meropenem in combination with cefoperazone-sulbactam, ampicillin-sulbactam, polymyxin B and rifampin was evaluated *in vitro* by the checkerboard microdilution method against 34 clinical isolates of MDR-AB.

MATERIALS AND METHODS

Bacterial isolates

The MDR-AB phenotype was defined as *A. baumannii* isolates resistant to at least three different antimicrobial classes of traditional antimicrobials. Thirty-four *A.baumannii* strains which were determined to be resistant to ticarcillin, cefepime, amikacin and ciprofloxacin besides imipenem and meropenem by disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) recommendations have been included in this study (Clinical and Laboratory Standards Institute, 2010). These strains were isolated from various clinical samples [tracheal aspirate cultures (50%), blood (47%) and wound (3%)], collected from different patients hospitalized in Intensive Care Units (ICUs) of Süleyman Demirel University Hospital in Isparta, Turkey between February 2009 and February 2011. All isolates were identified according to standard microbiological procedures by using BBL Crystal Identification Kits (Becton Dickenson, USA). The selected isolates subcultured on Luria-Bertani broth were stored at -80°C until use for *in vitro* testing.

Antimicrobial agents and minimum inhibitory concentration (MIC) assays

The antimicrobial agents used in combinations were: imipenem, meropenem, cefoperazone-sulbactam, ampicillin-sulbactam, polymyxin B and rifampin. All agents were purchased from individual pharmaceutical companies as standard reference powders for laboratory use and they were obtained from Sigma-Aldrich (Taufkirchen, Germany), except sulbactam (Zhejiang Xinhua Pharmaceutical Co. Ltd). Antimicrobial stock solutions were prepared by solvents and diluents according to CLSI standards and stored at -80°C (Clinical and Laboratory Standards Institute, 2010). MIC values of all isolates for each antibiotic were determined by broth microdilution method, using standard inoculum with 0.5 McFarland diluted with cation-adjusted Mueller Hinton II broth (BD, BBL) in a ratio of 1:100 and a final concentration of $5x10^5$ CFU/mL. Following this step, microtiter plates were visually read after incubation for 24 h at 37°C. The concentration range tested was 0.0625 to 128 mg/L for imipenem, 0.0625 to 128 mg/L for meropenem, 0.125 to 256 mg/L for ampicillin-sulbactam, 0.25 to 512 mg/L for cefoperazone-sulbactam, 0.031 to 64 mg/L for rifampin and 0.001 to 4 mg/L for polymyxin B. Procedures followed the descriptions of CLSI protocols (Clinical and Laboratory Standards Institute, 2010). *Escherichia coli* ATCC 25922 was used as internal quality control strain in each susceptibility test.

MIC results were interpreted according to the CLSI breakpoint criteria for *A. baumannii*. Since there are no CLSI interpretation criteria relevant to *A. baumannii* for cefoperazone-sulbactam and rifampin, the susceptibility breakpoints for these antibiotics were based on the MIC interpretive standards of CLSI for other non-enterobacteriaceae and Gram-positive bacteria, respectively (Clinical and Laboratory Standards Institute, 2010).

Checkerboard assay

The activity of the antimicrobial combinations were determined using the checkerboard assay (Pillai et al., 2005). The range of concentrations was determined according to the previously assessed MIC of each antibiotic for each isolate. Concentrations tested ranged from 0.03xMIC to 4xMIC of each antibiotic. The two-fold dilutions of antibiotics were prepared in sterilized tubes and 25 µL of each antimicrobial agent in each combination was added in 96-well checkerboard plates making up a total of 50 µL in each well. Then 50 µL of 1/100 diluted 0.5 McFarland bacterial suspension of *A. baumannii* was added to each well forming a final concentration of the test strains as approximately 5×10^5 CFU/mL. The plates then were incubated at 37°C for 20 h. Positive and negative controls were performed once for each combination. The positive control wells contained a mixture of broth and bacterial suspension and the negative control wells contained broth without bacteria. MICs and fractional inhibitory concentrations (FICs) were determined after overnight incubation by examining for turbidity. The absence of viable cells in non-turbid wells was confirmed by the addition of alamar blue reagent (Sigma-Aldrich, Germany), which turns the wells containing active bacteria to red. The interpretation of the checkerboard synergy testing results was determined with the method of Anon in which the lowest FIC index (FICI) of all the non-turbid wells along the turbidity/non-turbidity interface was used (Anon, 1992). FICIs and FICI were calculated for each antimicrobial combination using the formulas below:

FIC A: MIC of drug A in combination/ MIC of drug A alone
FIC B: MIC of drug B in combination/ MIC of drug B alone
FICI: FIC A + FIC B

FICI results for each combination were defined as synergy for

FICI≤0.5, additivity for 0.5 < FICI ≤ 1, indifference for 1< FICI < 4 and antagonism for FICI ≥ 4 (Pillai et al., 2005).

RESULTS

Specimen sources and MIC values of the study isolates are shown in Table 1. In all of the isolates the MIC values were within the resistance range for imipenem (MIC:16 to 64 mg/L), meropenem (MIC:16 to 128 mg/L), ampicillin-sulbactam (MIC:32 to 128 mg/L) and cefoperazone-sulbactam (MIC:32 to 512 mg/L), but they were susceptible to polymyxin B (MIC range 0.0078 to 0.125 mg/L). Twenty-four of the isolates (70,6%) demonstrated intermediate MIC levels (2 mg/L) and the MIC values of the other 10 were in the resistance range (29.4%) for rifampin. MIC_{50}/MIC_{90} values (mg/L) of imipenem, meropenem, ampicillin-sulbactam, cefoperazone-sulbactam, polymyxin B and rifampin against 34 A.baumannii strains were 32/64, 32/64, 32/64, 128/256, 0.0156/0.0625 and 2/4 respectively.

Table 2 demonstrates the number and percentage of isolates with synergistic, additive, indifference and antagonistic interactions for all combinations. Synergy was demonstrated more frequently when ampicillin-sulbactam was combined with a carbapenem. The rates were 94.1% for meropenem and 88.2% for imipenem**;** the rate for the combination of cephoperazone-sulbactam with imipenem was 70.6% while it was 8.8% with meropenem. Furthermore additivity were found among 23.5 and 64.7% of all isolates for the combination of cephoperazone-sulbactam with imipenem and meropenem, respectively. Synergistic interaction was detected among 73.5% of isolates with imipenem+rifampin combination and remaining 26.5% displayed additivity, whereas the combination of meropenem and rifampin showed mostly additive interaction with a rate of 76.5% and synergism was detected among only 17.6% of isolates. Synergistic interaction was detected between imipenem and polymyxin B among 38.2% of isolates, while additivity was 55.9%. Only a single isolate revealed synergistic activity with meropenem plus polymyxin B as being the lowest among all combinations (2.9%). Nevertheless, 64.7% of all isolates showed additivity for this combination and indifference was seen in 32.4% of the isolates. None of the combinations demonstrated antagonism (Table 2). FICIs of the carbapenem combinations for 34 isolates are shown in Table 3.

DISCUSSION

In this study, the highest synergy rates were observed for the combinations of meropenem plus ampicillin-sulbactam (94.1%) and imipenem plus ampicillin-sulbactam (88.2%). In accordance with our findings, high synergy rates have been reported in many previous studies investigating *in vitro* synergistic activities of carbapenems with sulbactam containing agents (Kiratisin et al., 2010; Ko et al., 2004; Pongpech et al., 2010). Moreover, among one of the few clinical studies, Lee et al. (2007) reported favorable clinical outcomes with carbapenem-sulbactam combination therapy in 4 critically ill patients with bacteremia who were infected by MDR-AB isolates showing resistance to both agents. The high rates of synergism found for carbapenems in combination with agents containing sulbactam have led us to think that these combinations may be good alternatives for the treatment of MDR-AB infections. However, while synergy was detected among 70.6% of the strains for imipenem plus cefoperazone-sulbactam, the synergistic effect of meropenem combined with cefoperazone-sulbactam was significantly lower than the other sulbactam combinations (8.8%). The reason of this discordant finding may be attributed to the fact that synergy was defined as an FICI of ≤ 0.5 in our study. As a matter of fact, 64.7% of the strains showed FICI of ≤ 1 for meropenem plus cefoperazone-sulbactam combination. Moreover, although synergy rates were generally lower for combinations with meropenem than imipenem, important proportion of the strains showed FICI of ≤ 1 for combinations with meropenem. Various reports defining synergism as FICI ≤ 1 would support our approach to this subject (Pongpech et al., 2010; Yoon et al., 2004).

In the present study, we have also searched the activity of carbapenems in combination with rifampin and we determined *in vitro* synergistic interactions between imipenem and rifampin against 73.5% of the isolates while meropenem+rifampin combination showed synergistic activity among only 17.6% of the tested strains. Antagonistic activity was detected among none of the strains for these two combinations. Several researchers investigating the activity of combination regimens against MDR-AB infections have also tested nontraditional agents like rifampin. Rifampin has been considered as inactive against Gram-negative bacteria due to its hydrophobicity, negative charge and large molecular size, all of which could decrease its outer membrane permeability (Song et al., 2009). However, in several *in vitro* studies it has been reported that the combinations of rifampin with carbapenems, ampicillin-sulbactam and polymyxins which are used against Gram-negative bacteria act synergistically against multidrug and especially carbapenem-resistant *A. baumannii* (Giamarellos-Bourboulis et al., 2001; Hogg et al., 1998; Tascini et al., 1998). Tripodi et al. (2007) reported in their study with time-kill method that regrowth was seen in isolates at 24 h when imipenem and rifampin were used alone but a significant synergism was determined between these antibiotics against all imipenem-resistant isolates. Moreover they also reported that the rapidly developing resistance in rifampin when used alone was blunted by the addition of imipenem suggesting that the combination therapies may be more effective than

Table 1. Specimen sources for thirty-four clinical *A. baumannii* isolates with MIC50 and MIC90 of each antimicrobial and MICs of each antibiotic alone.

No.	Specimen source	MICs of carbapenems (mg/L)			MICs of other antimicrobials (mg/L)		
		IPM	MEM	SAM	SCP	RIF	PB
1	Blood	16	64	64	128	2	0.125
2	Blood	16	64	64	128	2	0.0625
3	Tracheal aspirate	32	32	64	256	2	0.0078
4	Blood	16	16	32	64	2	0.0156
5	Tracheal aspirate	16	16	64	256	2	0.0156
6	Tracheal aspirate	16	16	64	256	2	0.0156
7	Blood	32	16	64	256	2	0.0156
8	Blood	16	16	32	64	2	0.0156
9	Tracheal aspirate	32	16	32	64	2	0.0078
10	Tracheal aspirate	16	16	32	64	2	0.0078
11	Tracheal aspirate	64	16	64	512	2	0.0156
12	Blood	32	32	32	64	4	0.031
13	Blood	16	16	32	64	2	0.0156
14	Blood	32	32	128	512	2	0.0156
15	Blood	32	16	32	128	4	0.0078
16	Blood	32	16	64	256	2	0.0078
17	Tracheal aspirate	16	32	32	64	2	0.0078
18	Blood	16	64	32	128	2	0.0156
19	Blood	32	64	64	128	2	0.0078
20	Blood	32	32	32	256	2	0.125
21	Tracheal aspirate	16	16	32	64	4	0.125
22	Tracheal aspirate	32	64	64	128	4	0.0156
23	Blood	32	64	32	128	2	0.0156
24	Tracheal aspirate	64	64	128	512	16	0.0156
25	Tracheal aspirate	64	128	32	32	4	0.0156
26	Tracheal aspirate	16	32	32	64	2	0.0156
27	Blood	32	64	64	128	4	0.031
28	Tracheal aspirate	32	64	64	128	2	0.031
29	Wound	32	64	64	128	4	0.0156
30	Tracheal aspirate	32	64	32	128	2	0.0156
31	Blood	32	64	32	128	2	0.0156
32	Tracheal aspirate	32	64	64	128	2	0.0156
33	Tracheal aspirate	16	16	32	256	4	0.0156
34	Tracheal aspirate	64	128	64	128	64	0.0156

Table 3. Contd.

	MIC50 = 32 MIC90 = 64	MIC50 = 32 MIC90 = 64	MIC50 = 32 MIC90 = 64	MIC50 = 128 MIC90 = 256	MIC50 = 2 MIC90 = 4	MIC50 = 0.0156 MIC90 = 0.0625

IPM, imipenem; MEM, meropenem; SAM, ampicillin-sulbactam; SCP, cefoperazone-sulbactam; RIF, rifampin; PB, polymyxin B

Table 2. Interactions for all combinations.

Drug combination	Synergy (FICI ≤ 0.5) n (%)	Additivity (0.5 < FICI≤1) n (%)	Indifference (1 < FICI<4) n (%)	Antagonism (FIC I≥ 4) n (%)
IPM+SAM	30 (88.2)	4 (11.8)	-	-
IPM+SCP	24 (70.6)	8 (23.5)	2 (5.9)	-
IPM+RIF	25 (73.5)	9 (26.5)	-	-
IPM+PB	13 (38.2)	19 (55.9)	2 (5.9)	-
MEM+SAM	32 (94.1)	2 (5.9)	-	-
MEM+SCP	3 (8.8)	22 (64.7)	9 (26.5)	-
MEM+RIF	6 (17.6)	26 (76.5)	2 (5.9)	-
MEM+PB	1 (2.9)	22 (64.7)	11(32.4)	-

Table 3. Fractional inhibitory concentration indices (FICIs) of various carbapenem combinations.

No:	Imipenem combination with				Meropenem combination with			
	SAM	SCP	RIF	PB	SAM	SCP	RIF	PB
1	0.75	1.06	0.53	0.75	0.50	1.03	0.75	0.56
2	0.50	0.50	0.53	0.53	0.50	0.53	0.53	0.53
3	0.50	0.25	0.28	0.31	0.25	0.75	0.31	0.56
4	0.28	0.28	0.50	0.50	0.18	0.53	0.53	0.53
5	0.25	0.28	0.28	0.53	0.25	0.75	0.53	1.00
6	0.37	0.50	0.56	0.53	0.15	0.56	1.00	0.75
7	0.15	0.28	0.50	0.28	0.15	0.50	1.00	0.53
8	0.15	0.28	0.28	0.31	0.15	0.53	0.53	0.53
9	0.15	0.25	0.25	0.37	0.25	0.53	0.56	0.62
10	0.15	0.50	0.31	1.50	0.15	1.03	0.53	0.62
11	0.09	0.15	0.15	0.15	0.28	0.62	1.03	1.00
12	0.15	0.28	0.25	0.28	0.12	0.53	0.28	0.37
13	0.28	0.53	0.50	1.00	0.28	1.03	0.75	1.00
14	0.28	0.50	0.53	0.62	0.15	0.53	0.53	0.53
15	0.37	0.28	0.28	0.31	0.28	1.03	0.75	1.06

Table 3. Contd.

16	0.15	0.28	0.28	0.31	0.25	0.56	0.53	0.75
17	0.28	0.50	0.37	0.56	0.15	0.37	0.50	0.56
18	0.28	0.53	0.53	0.62	0.28	0.53	0.53	0.53
19	0.53	1.03	0.53	1.06	0.53	1.03	1.00	1.06
20	0.25	0.18	0.28	0.28	0.50	0.50	1.00	0.75
21	0.25	0.37	0.31	0.53	0.25	0.75	0.37	0.56
22	0.50	0.62	0.37	0.53	0.37	1.03	0.75	1.03
23	0.28	0.53	0.50	0.53	0.28	1.00	1.03	1.03
24	0.28	0.28	0.28	0.50	0.37	1.00	0.75	1.03
25	0.37	0.28	0.28	0.53	0.37	1.00	0.50	0.53
26	0.31	0.53	0.50	0.53	0.53	1.03	1.00	1.03
27	0.53	0.62	0.50	0.56	0.50	1.00	0.62	1.03
28	0.50	0.56	0.53	0.37	0.31	1.06	1.00	1.03
29	0.31	0.37	0.37	0.53	0.37	1.03	0.53	1.03
30	0.53	0.53	0.53	0.50	0.28	0.53	0.56	0.75
31	0.28	0.50	0.50	0.53	0.28	1.00	1.00	1.03
32	0.28	0.28	0.28	0.53	0.37	1.00	0.53	0.53
33	0.15	0.37	0.37	0.53	0.25	0.56	0.37	1.03
34	0.28	0.50	0.53	0.53	0.28	0.53	0.53	0.53

FICI interpretation: ≤0.5, synergy; >0.5-≤1, additivity; >1-<4, indifference; ≥4, antagonism.

monotherapy. The observation of high synergistic activity for imipenem+rifampin combination in our study was in accordance with these reports. This high synergistic activity had possibly been related to substantial changes that might have occurred in the outer membrane of carbapenem-resistant *A. baumannii* isolates, thus they would permit rifampin to penetrate into the cell (Li et al., 2007). In our study, when we consider the high percentages of synergistic and partial synergistic effects evaluated with carbapenems and rifampin (for imipenem plus rifampin: 100% and for meropenem plus rifampin: 94.1%) we can predict that these combinations would individually be alternative therapeutic options for the treatment of MDR-AB infections. On the other hand, although it has been reported in a clinical study that 7 of 10 patients suffering from serious infections due to carbapenem-resistant *A. baumannii* isolates were cured with imipenem+rifampin combination therapy (70%), high-level rifampin-resistance developed in seven strains (70%) suggesting the limitation of this agent in clinical use (Saballs et al., 2006).

Several *in vitro* studies have tested a carbapenem with a polymyxin in combination against carbapenem-resistant *A. baumannii* isolates (Pankey and Ashcraft, 2009; Pongpech et al., 2010; Tripodi et al., 2007; Wareham and Bean, 2006; Yoon et al., 2004). In our study, synergistic activity was observed among 38.2% of tested strains for imipenem+polymyxin B combination, while this rate was 2.9% for meropenem+polymyxin B combination. Higher additive interaction rates were found for both combinations (55.9 and 64.7%, respectively). Although a four-fold reduction was detected in the MIC value of carbapenem in the combination among most of the isolates, the synergy rates were lower than the rates that we observed within the other carbapenem based combinations. The possible reason is that the isolates we used in this study were susceptible to polymyxin B with low-level MIC values (MIC ≤ 0.125 µg/ml). In fact, the synergy rate evaluated for imipenem+polymyxin B combination was at a level that should not be underestimated (38.2%) and is in accordance with the study of Wareham and Bean (2006) in which

they reported 40% synergistic activity between imipenem and polymyxin B against imipenem-resistant and polymyxin B-susceptible *A. baumannii* isolates. Pongpech et al. (2010), in their study with 30 MDR-AB isolates all resistant to imipenem and meropenem, have observed 100% synergistic activity between imipenem and colistin by the checkerboard and time-kill methods and have demonstrated major morpho-logical damages with scanning electron microscopy indicating that the synergistic effect may be related to weakening of cell wall or membrane due to actions of colistin. There are several other studies reporting higher levels of synergy relevant to this combination. In a recent study, Pankey and Ashcraft (Pankey and Ashcraft, 2009) demonstrated *in vitro* synergy between meropenem and polymyxin B against genetically unique 8 meropenem-resistant *A. baumannii* strains by time-kill assay (100%) and Etest (63%). In the time-kill study by Tripodi et al. (2007) it has been reported thatcolistin showed bactericidal activity when used both alone or in combination with imipenem however it has also been reported that combination of colistin with imipenem would not provide any additional advantage. In this regard, it has been suggested that if the bacterial isolates are susceptible to one of the agents in the combination it will be preferable to use monotherapy instead of combi-nation. However, owing to its poor diffusion into lung epithelial lining fluid, the use of polymyxin as a single agent may be inadequate especially in *A. baumannii* pneumonia (Garnacho-Montero et al., 2003). Moreover, Landman et al. (2005) reported that the increased use of polymyxin B as a single agent for the treatment of *A. baumannii* infections susceptible only to polymyxins have caused an increase in the incidence of *Pseudomonas aeruginosa* isolates resistant to polymyxins. In a recently conducted study, Falagas et al. (2010) reported that patients who were treated with colistin meropenem combination had a better outcome of infection than patients who received colistin in combination with other antimicrobials. The authors further noted that although there was no statistically significant difference in clinical outcome found between mono-therapy of colistin and colistin+meropenem combination therapy, the use of combination is preferable since heteroresistance may arise when colistin is used alone (Falagas et al., 2010). Since antagonism was not detected for both combinations in our study, it can be suggested that the use of these combinations against *A. baumannii* might be an alternative choice. Furthermore, adding a carbapenem to the treatment at maximum concentration without increasing the dosage of polymyxin, will probably enhance the bactericidal effect and reduce the toxicity of polymyxin.

In conclusion, owing to the high percentages of synergistic interactions between a carbapenem (especially imipenem) and ampicillin-sulbactam, cefoperazone-sulbactam, rifampin or polymyxin B and because no antagonism was detected in our study, these combinations are of considerable interest and may provide a rationale for innovative and effective therapeutic options for infections caused by MDR-AB. If combination therapy is preferred, the combinations which are known to be effective might be used empirically in the light of previous *in vitro* studies. However, since synergistic activity may depend on bacterial strains and susceptibility testing methods, it must be emphasized that evaluating the efficacy of combinations against isolates by synergy tests is essential to guide the treatment properly. Further comprehensive studies with clinical evidence are also warranted.

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