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Investigating the in vitro synergistic activities of several antibiotic combinations against carbapenem-resistant *Acinetobacter baumannii* isolates

Sevim YAVAŞ[1,*], Meltem Arzu YETKİN[2], Bircan KAYAASLAN[2], Aliye BAŞTUĞ[2], Halide ASLANER[2], Ayşe BUT[2], Dilek KANYILMAZ[3], Berrin SARI[4], Esragül AKINCI[2], Hürrem BODUR[2]

[1]Department of Infectious Diseases and Clinical Microbiology, Kelkit State Hospital, Gümüşhane, Turkey
[2]Department of Infectious Diseases and Clinical Microbiology, Ankara Numune Research and Training Hospital, Ankara, Turkey
[3]Infection Control Commitee, Ankara Numune Research and Training Hospital, Ankara, Turkey
[4]Department of Medical Microbiology and Clinical Microbiology, Ankara Numune Research and Training Hospital, Ankara, Turkey

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Background/aim: *Acinetobacter baumannii (A. baumannii)* is one of the most common healthcare-associated infectious agents worldwide. The aim of this study was to investigate the in vitro synergistic activities of several antibiotic combinations against carbapenem-resistant (CR) *A. baumannii* isolates.
Materials and methods: Eighteen CR *A. baumannii* strains were isolated from the patients who were hospitalized in the intensive care unit between June 2012 and August 2012. The in vitro effects of single and binary combinations of meropenem (MEM), colistin (CST), tigecycline (TGC), and sulbactam (SUL) on these isolates were determined using the Epsilometer test (E-test) method.
Results: All 18 isolates were resistant to MEM and SUL and susceptible to CST. TGC was detected as susceptible in two of the isolates and intermediate susceptibility results were observed in the remaining isolates. With MEM-CST and MEM-TGC combinations, synergism was determined against all isolates. The synergistic and/or additive effect ratios were detected in MEM-SUL, CST-SUL, TGC-SUL, and CST-TGC combinations as 16.7%, 38.9%, 16.7%, and 5.6%, respectively.
Conclusion: Among the tested antimicrobial combinations, the in vitro combination of MEM with TGC or CST was most effective against the CR *A. baumannii* strains.

Key words: *Acinetobacter baumannii,* drug resistance, synergy test, E-test method

1. Introduction

Acinetobacter baumannii (A.baumannii) has emerged as an important pathogen that can cause outbreaks in intensive care units (1). Its ability to acquire resistance to many antibiotic classes and to maintain its vitality on nonviable and dry surfaces for long periods of time makes it clinically significant. Carbapenems, sulbactam (SUL), tigecycline (TGC), and colistin (CST) are antibiotics whose activities have been proven against *Acinetobacter* spp.-induced infections. However, resistance of *Acinetobacter* strains against antibiotics has been increasingly reported worldwide (2).

Due to the high morbidity and mortality rates of severe *A. baumannii* infections, combination therapies, as opposed to monotherapy, are suggested (3). A synergistic effect may be developed when antibiotics are used in combination. Through this synergistic effect, treatment efficacy can be improved and resistance can be prevented (4). In vitro synergy tests can reveal combination therapies that can be used to treat carbapenem-resistant (CR) *A. baumannii* infections (5). Three methods to detect in vitro synergy have been described: the time-kill assay, checkerboard, and the Epsilometer test (E-test) method. The E-test method is simple to use and time efficient (6).

The primary objective of this study was to determine the in vitro synergistic activities of meropenem (MEM), CST, TGC, and SUL in binary combinations using the E-test method against CR *A. baumannii* isolates. The secondary objectives were: contributing to the development of new therapy protocols and decreasing the development of antibiotic resistance.

2. Materials and methods

2.1. Microorganisms

A total of 18 CR *A. baumannii* isolates, including carbapenem resistance, were included in this prospective study. These isolates were evaluated with VITEK 2

* Correspondence: drsevimyavas@gmail.com

(bioMérieux S.A., Craponne, France) for antibiotic susceptibility and resistance pattern and identifications at the species level. All bacteria were isolated from the endotracheal aspirates of individual patients in whom ventilator associated pneumonia was detected between June 2012 and August 2012 at Ankara Numune Research and Training Hospital in Ankara, Turkey.

2.2. Antimicrobial agents

The meropenem, colistin, tigecycline, and sulbactam E-test (bioMérieux S.A.) were utilized.

2.3. Minimum inhibitory concentration (MIC) determination and synergy test

In our study, the E-test method was used to determine the single MIC values of MEM, CST, TGC, and SUL. We also used it to determine the effects of the binary combination of these antibiotics against 18 *A. baumannii* isolates in a synergy test. The MIC values of the selected antimicrobial agents were detected separately and their fractional inhibitory concentrations (FIC) were calculated to allow for an interpretation (i.e. synergism) (7). For each of the 18 isolates, the single MIC values of A and B antibiotics in binary combination were determined.

According to the Clinical and Laboratory Standards Institute (CLSI) antibiotic susceptibility standards, for *Acinetobacter* spp., if the MEM MIC is ≤4 μg/mL, then it is accepted as susceptible (S); if it is 8 μg/mL, then it is accepted as intermediately susceptible (IM); and if it is ≥16 μg/mL, then it is accepted as resistant (R). If the CST MIC is ≤2 μg/mL, then it is accepted as susceptible (S) and if it is ≥4 μg/mL, then it is accepted as resistant (R). Due to lack of reference values for single SUL, an adaptation was made by taking the MIC ranges (≤8/4 susceptible; 16/8 intermediate susceptible; and ≥32/16 resistant) specified for SUL in the ampicillin-sulbactam combination as a reference in the CLSI guideline, according to other studies in the literature (8–10). For the MIC values of TGC against *Acinetobacter spp*, as in many studies, the standards specified for the *Enterobacteriaceae* family by the U.S. Food and Drug Administration were used. According to these standards, if the MIC reference values of TGC are MIC ≤ 2 μg/mL, then it is accepted as susceptible (S); if MIC is >2 < 8, then it is accepted as intermediate susceptible (IM); and if it is ≥ 8 μg/mL, then it is accepted as resistant (R) (11–13).

In our study, the E-test prediffusion method was used as a synergy test method, which was also utilized in previously published studies (6,14,15).

The fractional inhibitory concentration (FIC) index (Σ FIC) was calculated using the following formula to determine the efficacy of the combination.

$$\text{FIC [A]} = \frac{\text{The MIC numerical value of [A] in the presence of [B]}}{\text{The MIC numerical value of the single [A]}},$$

$$\text{FIC [B]} = \frac{\text{The MIC numerical value of [B] in the presence of [A]}}{\text{MIC numerical value of the single [B]}},$$

Σ FIC index = FIC [A] + FIC [B].

If Σ FIC ≤ 0.5, then it is considered synergistic.

If Σ FIC > 0.5 and ≤ 1, then it is considered additive.

If Σ FIC > 1 and ≤ 4, then it is considered indifferent.

If Σ FIC > 4, then it is considered antagonist (6,16).

Σ FIC values have been calculated for 6 different antibiotic combinations (MEM-CST, MEM-TGC, MEM-SUL, CST-TGC, CST-SUL, and TGC-SUL) using E-test in 18 CR *A. baumannii.*

2.4. Statistics

The results obtained from the in vitro interactions of the antibiotic combinations applied to 18 *A. baumannii* strains were examined. The results were analyzed using SPSS; the interactions in the antibiotics combinations were grouped as the presence of synergistic and/or additive interactions and the absence of any of the two interactions. In addition, the statistical analysis was evaluated with McNemar's test in SPSS.

3. Results

According to the MIC values, all isolates were resistant to MEM (MIC ≥ 32 μg/mL) and SUL (MIC 16–96 μg/mL); however, all isolates were susceptible to CST (MIC 0.38–1 μg/mL). TGC was found to be sensitive in 2 of the isolates (MIC 0.75/0.75 μg/mL), and intermediate susceptibility results were observed for the remaining isolates (MIC 3–6 μg/mL). The ΣFIC values were calculated for six antibiotic combinations (MEM-CST, MEM-TGC, MEM-SUL, CST-TGC, CST-SUL, and TGC-SUL), and their synergistic, additive, indifferent, and antagonistic interactions are shown in the Table. The synergistic effects between MEM and CST and between MEM and TGC were detected against all the tested microorganisms. Between CST and TGC, antagonism was detected in two isolates, and an indifferent effect was observed in the remaining isolates. In seven isolates, an additive effect was observed for the TGC-SUL combination. An additive effect was also detected in two isolates for the MEM-SUL and TGC-SUL combinations, and indifference was detected for the remaining isolates.

3.1. Statistical comparison of antibiotic combinations in terms of the synergistic effect

The rate of the presence of one synergistic and/or additive interaction was greater in the MEM-CST and MEM-TGC combinations compared with the CST-SUL, MEM-SUL, and TGC-SUL combinations; this result was statistically significant ($P < 0.05$).

4. Discussion

A. baumannii infections are difficult to treat and combination antibiotics therapy is often required.

Table. Results of antimicrobial synergistic activities for binary combinations of MEM-CST, MEM-TGC, MEM-SUL, CST-TGC, CST-SUL, and TGC-SUL.

	MEM-CST		MEM-TGC		MEM-SUL		CST-TGC		CST-SUL		TGC-SUL	
	ΣFIC		ΣFIC		ΣFIC		ΣFIC		ΣFIC		ΣFIC	
1	0.32	S	0.25	S	2.1	ID	5	AG	1.05	ID	2.05	ID
2	0.38	S	0.43	S	2.17	ID	4.33	AG	1.13	ID	1.41	ID
3	0.15	S	0.31	S	0.5	S	1.83	ID	0.6	ADD	1	ADD
4	0.23	S	0.44	S	1.13	ID	1.7	ID	1.45	ID	1.13	ID
5	0.5	S	0.23	S	2.1	ID	2.5	ID	1.06	ID	0.72	ADD
6	0.15	S	0.25	S	2.1	ID	1.58	ID	0.66	ADD	0.77	ADD
7	0.09	S	0.25	S	1.13	ID	1.7	ID	1	ADD	1.13	ID
8	0.1	S	0.32	S	0.85	ADD	2	ID	1	ADD	1.1	ID
9	0.2	S	0.25	S	1.13	ID	3.17	ID	1.35	ID	1.08	ID
10	0.14	S	0.23	S	1.75	ID	1.57	ID	1.35	ID	1.19	ID
11	0.27	S	0.23	S	1.69	ID	2.22	ID	1.34	ID	1.1	ID
12	0.18	S	0.28	S	10.6	AG	1.99	ID	1.33	ID	2	ID
13	0.32	S	0.25	S	1.63	ID	3.67	ID	1.06	ID	1.46	ID
14	0.16	S	0.25	S	1.13	ID	0.89	ADD	1	ADD	1.17	ID
15	0.25	S	0.19	S	0.58	ADD	1.33	ID	0.68	ADD	2	ID
16	0.2	S	0.27	S	1.63	ID	1.99	ID	1.35	ID	1.46	ID
17	0.2	S	0.5	S	1.13	ID	1.75	ID	1.5	ID	1.13	ID
18	0.27	S	0.38	S	2.13	ID	1.75	ID	1	ADD	1.06	ID

MEM: meropenem, CST: colistin, TGC: tigecycline, SUL: sulbactam, S: synergy, ID: indifference, ADD: additive, and AG: antagonism.

However, a definitive consensus on which combination therapy is effective in the treatment of *A. baumannii* infections is not available. In our study, we evaluated the synergistic activity of different antimicrobial combinations against CR *A. baumannii* isolates.

We examined the studies on this subject in the literature to compare the results of our study. Sopirala et al. (16) evaluated the activity of binary combinations of TGC, CST, and imipenem (IPM) against 8 pan-drug resistant *A. baumannii* isolates. The authors observed synergistic activity in all isolates for the IPM-TGC and IPM-CST combinations.

Similarly, Pongpech et al. (17) evaluated the activities of binary combinations of MEM with CST, MEM with SUL, and CST with SUL against 32 IPM- and MEM-resistant *A. baumannii* isolates. The synergistic activity rates detected by the authors in binary MEM-SUL and MEM-CST combinations were 70% and 73.3%, respectively.

In a study by Pankey and Ashcraft (18), the synergy was evaluated using the E-test method for the combination of MEM with polymyxin B against 8 MEM-resistant clinical *A. baumannii* isolates. The authors observed synergistic activity in five isolates for the combination of MEM with polymyxin B.

In our study, synergistic activity was detected for the MEM-CST and MEM-TGC combinations against strains that we found to be resistant to MEM and moderately susceptible to TGC, which is in line with the literature. The results of our study and similar studies in the literature indicate that the activity of MEM increases when it is used with TGC and CST against MEM-resistant strains. This result may be related to the bactericidal effect, as well as to the fact that these drugs have different effect mechanisms.

The synergistic activity of the combination CST-TGC was examined in our study. Indifference and antagonistic activity were detected for the CST-TGC combination. In a study by Tan et al. (19), 40% of isolates demonstrated synergistic activity according to the combination of polymyxin B and TGC against *A. baumannii* isolates. In other studies evaluating the synergistic activity of the CST and TGC combination, synergy and indifference were reported, but no antagonistic effects were demonstrated (16,20–22). Regarding the combination of CST with TGC, some differences were found between our study results and

other studies in the literature, most likely resulting from the different methods used in the synergy tests. The E-test prediffusion method was used in our study; however, other studies have used variations of that method or different methods entirely.

The synergistic activity of sulbactam with MEM, CST, and TGC was also examined in our study, and indifferent activity was observed. Based on the synergistic activity of SUL with various other antibiotics in the literature, synergistic, additive, and indifferent effects were detected in some other studies (23–25). The differences between the study results are based on the absence of a certain MIC value specified by CLSI for SUL; therefore, there is uncertainty concerning the acceptable limits of the measurements. In addition, the SUL MIC values in the *A. baumannii* strains included in the study have high values, such as 16–96 µg/mL. These high MIC values may have affected these results.

Additionally, in our study, we sought to determine the combination with which we could achieve the most successful results for practical use by comparing the activities of antimicrobial drug combinations for which we have identified synergistic/additive activity. As a result of these comparisons, the best in vitro synergistic activity was achieved with the MEM-CST and MEM-TGC combinations.

There were some disadvantages related to our study. One great disadvantage was that all the isolates were derived from the same hospital. In addition, the value of the overall results could have been increased if we studied a larger number of isolates. Another limitation of this study was that the limited scale of the antibiotic concentration in the E-test strips did not allow the recording of higher MIC values. The absence of standardization in all synergy test methods could be considered a limitation as well.

In conclusion, until new agents are developed against resistant microorganisms, the only alternative therapy option appears to be the use of combination therapies (26). According to our study, the combination of MEM with CST or TGC offers an alternative option by increasing the activity of MEM when treating CR *Acinetobacter* strains.

Prospective clinical comparison studies are needed to understand the potential benefits of combination therapies against monotherapy in treating CR Gram-negative bacteria infections.

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