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MEDICAL SCIENCES / DAHİLİ TIP BİLİMLERİ

Evaluation of the Effects of Different Antibiotic Combinations on Multi-Drug Resistant Gram-Negative Bacteria

Farklı Antibiyotik Kombinasyonlarının Çoklu İlaca Dirençli Gram-Negatif Bakteriler Üzerindeki Etkilerinin Değerlendirilmesi

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Abstract

Objectives: Increasing resistance to antibacterial drugs in gram-negative bacteria isolated from the community and especially from the hospital has become one of the most important health problems both in the world and in our country. *Acinetobacter* genus and *Enterobacteriacea* family have an important place among gram-negative bacteria with increased antibacterial drug resistance. In this study, we aimed to evaluate the *in vitro* effects of various antibiotic combinations with E-test method and determine synergistic effective combinations that could be used in multidrug-resistant *Acinetobacter baumannii* infections and ESBL and carbapenemase secreting gram-negative enteric bacterial infections.

Materials and Methods: It was evaluated whether the combinations of colistin with trimethoprim-sulfamethoxazole, rifampicin, doxycycline, sulbactam, imipenem, tigecycline and chloramphenicol were synergistic effective *in vitro* in 20 colistin-susceptible but multidrug-resistant, 20 colistin- and multi-drug-resistant *Acinetobacter baumannii* strains and 20 both extended-spectrum beta-lactamase and carbapenemase-producing *Klebsiella pneumoniae* strains isolated from various and different clinical samples (sputum, urine, tracheal aspirate, blood, etc.), sent from patients hospitalized in various clinics.

Results: The synergistic effect was most common in the colistin-tigecycline combination in all multi-drug resistant bacterial groups, 80% in the colistin-sensitive *Acinetobacter baumannii* group, 45% in the colistin-resistant *Acinetobacter baumannii* group, and 80% in the *Klebsiella pneumoniae* group.

Conclusion: Although further studies with more strains and different antibiotic combinations are needed for the appropriate treatment of infections due to multi-drug-resistant gram-negative bacteria, we found that colistin-tigecycline, colistin-sulbactam, colistin-rifampicin combinations are so effective for these strains *in vitro* and these results can help clinicians in treatment process.

Key Words: Multidrug Resistance, *Acinetobacter*, *Klebsiella*, Antibiotic Combination

Öz

Amaç: Toplumdan ve özellikle hastaneden izole edilen gram-negatif bakterilerde antibakteriyel ilaçlara direncin artması hem dünyada hem de ülkemizde en önemli sağlık sorunlarından biri haline gelmiştir. Artan antibakteriyel ilaç direnci açısından *Acinetobacter* cinsi ve *Enterobacteriacea* ailesi gram-negatif bakteriler arasında önemli bir yere sahiptir. Bu çalışmada, çeşitli antibiyotik kombinasyonlarının *in vitro* etkilerini E-test yöntemi ile değerlendirmeyi ve çoklu ilaca dirençli *Acinetobacter baumannii* enfeksiyonlarında ve GSBL ve karbapenemaz salgılayan gram negatif enterik bakteri enfeksiyonlarında kullanılabilecek sinerjik etkili kombinasyonları belirlemeyi amaçladık.

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Gereç ve Yöntem: Kolistinin trimetoprim-sülfametoksazol, rifampisin, doksisiklin, sulbaktam, imipenem, tigesiklin ve kloramfenikol ile kombinasyonlarının *in vitro* olarak sinerjistik etkili olup olmadığı, kolistine duyarlı ancak çoklu ilaca dirençli, kolistine ve çoklu ilaca dirençli 20'şer *Acinetobacter baumannii* ve genişlemiş spektrumlu beta-laktamaz ve karbapenemaz üreten 20 *Klebsiella pneumoniae* suşunda değerlendirildi.

Bulgular: Sinerjistik etki, kolistin-tigesiklin kombinasyonunda tüm çoklu ilaca dirençli bakteri gruplarında en yaygın etki olarak bulundu. Kolistine duyarlı *A. baumannii* grubunda %80, kolistine dirençli *Acinetobacter baumannii* grubunda %45 ve *Klebsiella pneumoniae* grubunda %80 oranında görüldü.

Sonuç: Çoklu ilaca dirençli gram-negatif bakterilere bağlı enfeksiyonların uygun tedavisi için daha fazla suş ve farklı antibiyotik kombinasyonları ile daha ileri çalışmalara ihtiyaç duyulsa da, kolistin-tigesiklin, kolistin-sulbaktam, kolistin-rifampisin kombinasyonlarının bu suşlar için *in vitro* olarak için çok etkili olduğu görüldü ve klinik uygulamalarda yol gösterici olabileceğini düşündük.

Anahtar Kelimeler: Çoklu İlaca Direnç, *Acineobacter*, *Klebsiella*, Antibiyotik Kombinasyonu

Introduction

Increasing resistance to antibacterial drugs in gram-negative bacteria isolated from the community and especially from the hospital has become one of the most important health problems both in the world and our country (1,2). The increase in nosocomial infections caused by these bacteria increases patient morbidity and mortality, as well as prolongs the hospitalization period and causes economic losses with increased costs. The increase in multi-drug resistance in gram-negative bacteria is not parallel to the increase in the development of new antibacterials. As a result, it becomes increasingly difficult to find appropriate treatment options for infections caused by these bacteria (3). Again, the problems that these bacteria cause to clinicians by creating nosocomial epidemics and developing resistance to antibiotics used during treatment have brought both the use of old antibiotics such as colistin and the combined use of antibiotics (3,4). Combined use of antibiotics can have positive results, especially in the treatment of polymicrobial infections, in the presence of two separate infections that cannot be treated with a single antibiotic, in the treatment of infections of unknown origin, in providing a synergistic effect against antibiotic-resistant isolates, in the treatment of serious infections that may progress with high mortality, and in reducing the dose-related side effects of drugs (5).

Acinetobacter genus and *Enterobacteriacea* family have an important place among gram-negative bacteria with increased antibacterial drug resistance. Both bacterial groups are frequently encountered as agents of health-care associated infections (6).

Among the genus *Acinetobacter*, *Acinetobacter baumannii* emerges as the most resistant to antibiotics and the most frequently isolated species from hospital infections (7). Among the *Enterobacteriaceae* family, *Klebsiella pneumoniae* strains, which secrete extended-spectrum beta-lactamase (ESBL) and/or are resistant to carbapenems, are the prominent species in hospital infections and the development of antibacterial resistance. It is also known that both species also cause hospital epidemics, prolong the length of hospital stay, and cause serious increases in morbidity and mortality (6). The transferability and rapid spread of resistance mechanisms between different species in these bacteria, as well as the diversity by undergoing changes, cause problems in treatment and new treatment options should be evaluated and clinical applications should be facilitated. Studies on antimicrobial treatment of these infections are based on a limited number of patient cases, therefore, appropriate treatment could not be determined. This situation necessitated the investigation of different treatment options.

In this study, we aimed to evaluate the *in vitro* effects of various antibiotic combinations with E-test method and determine synergistic effective combinations that can be used in multidrug-resistant *Acinetobacter baumannii* infections and ESBL and carbapenemase secreting gram-negative enteric bacteria infections.

Materials and Methods

It was evaluated whether the combinations of colistin with trimethoprim-sulfamethoxazole, rifampicin, doxycycline, sulbactam, imipenem, tigecycline and chloramphenicol were synergistic effective *in vitro* in 20 colistin-susceptible but multidrug-resistant, 20 colistin- and multi-drug-resistant *Acinetobacter baumannii* strains and 20 both ESBL and carbapenemase-producing *Klebsiella pneumoniae* strains isolated from various and different clinical samples (sputum, urine, tracheal aspirate, blood, etc.).

Along with the classical identification systems, 20 *Acinetobacter baumannii* isolates resistant to at least three antibiotic groups, not including colistin, identified at the species level with the Phoenix™ automated identifier system, and 20 more *Acinetobacter baumannii* isolates resistant to at least three antibiotic groups, including colistin, and 20 *Klebsiella pneumoniae* isolates, which were determined to produce ESBL by the Phoenix™ automated system, were determined to be resistant to carbapenems and were found to be carbapenemase positive by the modified Hodge test, were included in the study. The isolates were passaged into eppendorfs containing brain heart infusion broth and stored in a deep freezer (-80 °C) until the study day. *Acinetobacter baumannii* and *Klebsiella*

pneumoniae isolates removed from the deep freeze were passaged into shed eosin methylene blue agar (EMB) and sheep blood agar media before the study. Bacterial colonies obtained purely from fresh culture passages after 18-20 hours incubation at 37°C in aerobic environment, in normal atmosphere, were used in the study. *Pseudomonas aeruginosa* American Type Culture Collection (ATCC) 27853 and *Escherichia coli* ATCC 25922 were used as control strains.

Minimum inhibitory concentration (MIC) values of colistin, trimethoprim-sulfamethoxazole, rifampicin, sulbactam, imipenem, tigecycline and chloramphenicol against *Acinetobacter baumannii* and *Klebsiella pneumoniae* isolates were determined by E-test method (Liofilchem MIC Test Strip®, Italy). In line with the manufacturer's recommendations for the E-test; Suspensions equivalent to 0.5 McFarland Standard turbidity were obtained in Mueller Hinton broth of the pure bacterial colonies obtained. The prepared co-suspensions were inoculated on the surface of the pre-prepared and dried Mueller Hinton agar-containing medium plates with the help of a sterile cotton swab. It was waited for 15-20 minutes before placing the E-test strips. E-test strips of antibiotics were placed on the medium plates with the help of forceps. The point where the elliptical inhibition zone intersects with the strip was accepted as the numerical MIC value after 16-20 hours of incubation at 35±2°C in aerobic environment, in normal atmosphere. The MIC values of each antibiotic were measured in accordance with the manufacturer's and the Clinical and Laboratory Standards Institute (CLSI) recommendations, and the results were evaluated based on the breakpoints of CLSI.

In order to determine the fractional inhibitory concentration (FIC) index with the E-test, first the MIC values of the A and B antibiotics in the combination were recorded. To determine the combination MIC value, first strip B was placed in the medium and after one hour of incubation at 37 °C, strip A was placed in place of B so that the concentration lines were exactly coincident. After incubating the media at 35±2 °C for 16-20 hours, the MIC value of A was recorded in the presence of B at the point where the inhibition zone diameter cuts the edge of the E-test strip. The same procedure was repeated with A and then B antibiotics. FIC index was calculated according to the formula below to determine the effectiveness of the combination.

$$\text{FIC A} = \frac{\text{MIC of A in the presence of B}}{\text{MIC of A alone}}$$

$$\text{FIC B} = \frac{\text{MIC of B in the presence of A}}{\text{MIC of B alone}}$$

$$\text{FIC index} = \text{FIC A} + \text{FIC B}$$

If the FIC index value is 0.5 and below (≤0.5), the effect of combination is synergistic, if it is between 0.5 and one (0.5-1), it is additive, if it is greater than one but less than two (>1-<2), the combination is ineffective, two or more [(≥0.5) 2] was evaluated as antagonistic.

A total of 420 FIC values were calculated for seven antibiotic combinations evaluated in 40 multidrug-resistant *Acinetobacter baumannii* and 20 *Klebsiella pneumoniae* isolates included in the study. Not only synergistic effects but also additive, ineffective and antagonistic interactions were noted.

Statistical Analysis

Statistical analysis of the data obtained from the *in vitro* interactions of colistin-rifampicin, colistin-trimethoprim/sulfamethoxazole, colistin-doxycycline, colistin-sulbactam, colistin-imipenem, colistin-tigecycline, colistin-chloramphenicol combinations was performed using Fisher's chi-square test in SPSS (SPSS Incorporated, Chicago).

Results

While 100% of the colistin-susceptible *Acinetobacter baumannii* isolates were resistant to trimethoprim-sulfamethoxazole, 50% to rifampicin, 80% to doxycycline, 65% to sulbactam, they were 100% resistant to imipenem and chloramphenicol. All isolates were found to be susceptible to tigecycline (Table 1). It was determined that *Acinetobacter baumannii* isolates that were resistant to colistin were 5% resistant to trimethoprim-sulfamethoxazole, 70% to rifampicin, 75% to doxycycline, 35% to sulbactam, 80% to imipenem and 100% to chloramphenicol. Again, all of these isolates were found to be susceptible to tigecycline (Table 2). 15% of *Klebsiella pneumoniae* strains were found to be resistant to colistin, 85% to trimethoprim-sulfamethoxazole, 65% to rifampicin, 80% to doxycycline, 100% to sulbactam, 100% to imipenem, meropenem and ertapenem, and 75% to chloramphenicol. Again, all of these strains were found to be susceptible to tigecycline (Table 3).

Totally 420 FIC values were calculated for 60 multidrug resistant bacteria included in the study, and 28.8% (121/420) of the combinations were found synergistic, 56.2% (236/420) additive, 15% (63/420) were indifferent. There was 35% (50/140) synergistic, 50% (70/140) additive, 14.3% (20/140) indiferential interaction in colistin-sensitive *Acinetobacter baumannii* strains, and a synergistic effect of 21.4% (30/140), additive effect of 64.3% (90/140) and indifferent effect of 14.3% (20/140) in colistin-resistant strains. It was determined that the synergistic effect was 29.3% (41/140), the additive effect was 54.3% (76/140), and the indifferential effect was 16.4% (23/140) in *Klebsiella pneumoniae* strains. The additive effect was the most obtained result in all bacterial groups.

The most common effect in antibiotic combinations other than colistin-tigecycline and colistin-chloramphenicol combinations in colistin susceptible *Acinetobacter baumannii* isolates and colistin-chloramphenicol combination in colistin resistant *Acinetobacter baumannii* isolates, and colistin-sulbactam and colistin-tigecyline combinations in ESBL and carbapenemase-producing *Klebsiella pneumoniae* was additive effect. An antagonistic effect was not observed with any antibiotic combination (Tables 4-6).

The synergistic effect was most common in the colistin-tigecycline combination in all multi-drug resistant bacterial groups, 80% in the colistin-sensitive *Acinetobacter baumannii* group, 45% in the colistin-resistant *Acinetobacter baumannii* group, and 80% in the *K. pneumoniae* group (Tables 4-6).

When colistin-sensitive and resistant *Acinetobacter baumannii* isolates were compared in terms of the effects of all antibiotic combinations; 35.7% of the susceptible ones were synergistic and 50% of them were additive, while 21.4% of the resistant ones were synergistic and 64.3% of them were additive. It was determined that the additive effect was more. These differences were also found to be statistically significant ($p<0.05$) (Table 7).

When it is examined whether there is a statistical relationship between being sensitive or resistant to any antibiotic and being synergistic or additive effect of antibiotic combinations in multi-drug resistant *Klebsiella pneumoniae*, it was determined that antibiotic combinations showed a synergistic effect in 0.3% of them, and a synergistic effect was observed in 8% of those resistant to any antibiotic. This difference was found to be statistically significant ($p<0.05$) (Table 8).

Antibiotic combinations showed a synergistic effect in 83.3% of those who were sensitive to any antibiotic, while a synergistic effect was observed in 8% of those who were resistant to any antibiotic in ESBL and carbapenemase producing *Klebsiella pneumoniae*. This difference was found to be statistically significant ($p<0.05$) (Table 8).

Discussion

Colistin resistant *Acinetobacter baumannii* strains can be seen in different rates in varied countries and regions worldwide (8). In a recent publication, the data of Organization for Economic Cooperation and Development countries between 2000 and 2016 were compiled and it was determined that carbapenem resistance increased 3 times in *Enterobacteriaceae* family and non-fermentatives in 16 years period (9). During this period, colistin was considered the treatment of last resort for *Acinetobacter baumannii* infections.

In various studies conducted in our country, resistance rates for *Acinetobacter baumannii* are 77-86% for imipenem, 78-93% for ciprofloxacin, 50-63% for amikacin, 53-69% for gentamicin, 88% for trimethoprim-sulfamethoxazole, 6-8% tigecycline (10,11).

Clinical reflection of multidrug-resistant bacteria is in the form of prolonged hospital stay, nosocomial outbreaks, treatment failure, and increased mortality (6,9).

Table 1: Antibiotic susceptibility of *A. baumannii* isolates sensitive to colistin according to E-test method (n=20)

	CT	SXT	RIF	DX	SB	IMP	TIG	CL
Sensitive (%)	100	0	25	0	0	0	100	0
Intermediate (%)	0	0	25	20	35	0	0	0
Resistant (%)	0	100	50	80	65	100	0	100

CT: Colistin, SXT: Trimetoprim-sulfametoksazol, RIF: Rifampicin, DX: Doxycycline, SB: Sulbactam, IMP: Imipenem, TIG: Tigecycline, CL: Chloramphenicol

Table 2: Antibiotic susceptibility of *A. baumannii* isolates resistant to colistin according to E-test method (n=20)

	CT	SXT	RIF	DX	SB	IMP	TIG	CL
Sensitive (%)	0	95	30	0	30	0	100	0
Intermediate (%)	0	0	0	25	35	20	0	0
Resistant (%)	100	5	70	75	35	80	0	100

CT: Colistin, SXT: Trimetoprim-sulfametoksazol, RIF: Rifampicin, DX: Doxycycline, SB: Sulbactam, IMP: Imipenem, TIG: Tigecycline, CL: Chloramphenicol

Table 3: Antibiotic susceptibility of *K. pneumoniae* isolates according to E-test method (n=20)

	CT	SXT	RIF	DX	SB	IMP	TIG	CL
Sensitive (%)	85	15	0	20	0	0	100	25
Intermediate (%)	0	0	35	0	0	0	0	0
Resistant (%)	15	85	65	80	100	100	0	75

CT: Colistin, SXT: Trimetoprim-sulfametoksazol, RIF: Rifampicin, DX: Doxycycline, SB: Sulbactam, IMP: Imipenem, TIG: Tigecycline, CL: Chloramphenicol

Despite the escalating threat of multidrug-resistant gram-negative bacteria, there is so few new drugs has recently emerged that can be used to treat infections with these bacteria (3). The problems experienced by clinicians under current conditions have brought both the reuse of old antibiotics such as colistin and the combined use of antibiotics.

Although combination therapy is mainly used to prevent the development of resistance to antibiotics, it can have positive results especially in the treatment of multibacterial infections, in providing a synergistic effect against resistant isolates, in the treatment of serious infections with high mortality and in reducing the dose-related side effects of drugs. There are no randomized studies supporting monotherapy in severe infections, and combination therapy is recommended because of severe morbidity and mortality.

Combination of antimicrobial agents acting by different mechanisms may result in a better pharmacodynamic effect or synergy as well as cause antagonism. The absence of antagonistic interactions between antibiotics is of great clinical importance. For this reason, it has been emphasized in many studies that determining the *in vitro* effects of antibiotic combinations that can be used in the treatment of multi-drug-resistant microorganisms, especially the synergistic effects, may be guiding (12).

The E-test method is a method that requires less effort than other synergy tests, but shows a high correlation with the checkerboard method. In a study by Manno et al. (13), the combination of different antimicrobials in 131 *Burkholderia cepacia* isolates was compared with these two methods, and it was determined that there was a 90% correlation between

Table 4: Effects of antibiotic combinations on multidrug-resistant *A. baumannii* isolates susceptible to colistin (n=20)

	FIC					
	Synergistic		Additive		Indifferent	
	n	%	n	%	n	%
CT-SXT	7	35	13	65	0	0
CT-RIF	9	45	11	55	0	0
CT-DX	9	45	11	55	0	0
CT-SB	3	15	17	85	0	0
CT-IMP	6	30	14	70	0	0
CT-TIG	16	80	4	20	0	0
CT-CL	0	0	0	0	20	100
Total	50	35	70	50	20	14.3

CT: Colistin, SXT: Trimetoprim-sulfametoksazol, RIF: Rifampicin, DX: Doxycycline, SB: Sulbactam, IMP: Imipenem, TIG: Tigecycline, CL: Chloramphenicol

Table 5: Effects of antibiotic combinations on colistin-resistant multidrug-resistant *A. baumannii* isolates (n=20)

	FIC					
	Synergistic		Additive		Indifferent	
	n	%	n	%	n	%
CT-SXT	4	20	16	80	0	0
CT-RIF	6	30	14	70	0	0
CT-DX	5	25	15	75	0	0
CT-SB	2	10	18	90	0	0
CT-IMP	4	20	16	80	0	0
CT-TIG	9	45	11	55	0	0
CT-CL	0	0	0	0	20	100
Total	30	21.4	90	64.3	20	14,3

CT: Colistin, SXT: Trimetoprim-sulfametoksazol, RIF: Rifampicin, DX: Doxycycline, SB: Sulbactam, IMP: Imipenem, TIG: Tigecycline, CL: Chloramphenicol

Table 6: Effects of antibiotic combinations on multidrug-resistant *K. pneumoniae* isolates (n=20)

	FIC					
	Synergistic		Additive		Indifferent	
	n	%	n	%	n	%
CT-SXT	3	15	17	85	0	0
CT-RIF	7	35	13	65	0	0
CT-DX	6	30	14	70	0	0
CT-SB	0	0	0	0	20	100
CT-IMP	4	20	16	80	0	0
CT-TIG	16	80	4	20	0	0
CT-CL	5	25	12	60	3	15
Total	41	29.3	76	54.3	23	16.4

CT: Colistin, SXT: Trimetoprim-sulfametoksazol, RIF: Rifampicin, DX: Doxycycline, SB: Sulbactam, IMP: Imipenem, TIG: Tigecycline, CL: Chloramphenicol

Table 7: Comparison of the effects of being susceptible or resistant to colistin and the effects of antibiotic combinations in multidrug resistant *A. baumannii* isolates

	FIC							
	Synergistic		Additive		Indifferent		Total	
	n	%	n	%	n	%	n	%
Sensitive	50	35.7	70	50.0	20	14.3	140	100
Resistant	30	21.4	90	64.3	20	14.3	140	100
Total	80	28.6	160	57.1	40	14.3	280	100

Chi-square=7.56; SD=2; p=0.023

Table 8: Comparison of antibiotic susceptibilities and the effects of antibiotic combinations in *K. pneumoniae* isolates that are resistant to multiple drugs (n=20)

		FIC					
		Synergistic		Additive		Total	
		n	%	n	%	n	%
MIC	Sensitive	35	83.3	7	16.7	42	100
	Resistant	6	8.0	69	92.0	75	100
	Total	41	29.3	76	54.3	140	100

Chi-square=63.8; SD=1; p=0.0001

E-test and checkerboard methods. On the other hand, it has been shown by Bonapace et al. (14) that using an irregular methodology that does not comply with the manufacturer's recommendations while applying the E-test method may increase the difference.

Colistin was out of use after the 1970s due to its significant side effects and the discovery of less toxic antibiotics. However, with the emergence of multi-drug resistant gram-negative strains and reporting that these strains are susceptible to colistin, its use has come to the fore again (15). However, with the widespread use of colistin, which is thought to be very effective in the treatment of infections caused by bacteria with multiple antibiotic resistance, resistance has been reported in *Pseudomonas aeruginosa, Acinetobacter baumannii* and *Klebsiella pneumoniae* strains (15). The highest resistance rates are reported from Asia, followed by Europe. However, it is observed that colistin heteroresistant *Acinetobacter baumannii* strains are more numerous than colistin resistant strains (16). In the development of this resistance, it is very important to use colistin alone in the treatment of infections caused by resistant bacteria. In order to prevent the development of resistance, it is recommended to avoid the use of colistin alone in such infections and to prefer combination therapy (17).

Meropenem colistin combination is one of the most commonly used combinations in the clinical setting. While decreased susceptibility to imipenem is observed in some strains, resistance to meropenem can be detected at high MIC levels at the same time. Using imipenem E-tests may give an idea about meropenem combination effects but doesn't reflect the exact result of combinations. Using only imipenem is a limitation of our study (18).

In a study comparing the use of colistin alone and in combination in a group of 18 patients infected with *Klebsiella pneumoniae* that produces KPC, treatment success was found to be 14% when used alone, while 73% success was achieved with combination therapy (19).

Colistin contributes to the activity of carbapenems by increasing the outer membrane permeability of bacteria. Montero et al. (20) state that the combination of imipenem, colistin, rifampicin or tobramycin may be effective in high levels of imipenem resistance.

In our study, although most of the strains were resistant to imipenem (only four strains were moderately susceptible), 30% in the colistin-susceptible and multidrug-resistant *Acinetobacter baumannii* group, 20% in the colistin- and multidrug-resistant *Acinetobacter baumannii* group. In the ESBL and carbapenemase producing *Klebsiella pneumoniae* group, 20% synergistic effect was observed, but the additive effect was the highest. No indifferent and antagonistic effects were encountered.

Although the efficacy of rifampicin against multidrug-resistant strains is not fully known, synergistic activity of rifampicin with colistin or sulbactam has been demonstrated against these strains in *in vitro* studies. It has been reported that the combination may be beneficial in severe infections (20). Li et al. (21) found that the combination of colistin and rifampicin had a synergistic effect on colistin-sensitive *Acinetobacter baumannii* strains.

In our stduy, with the combination of colistin and rifampicin, a synergy rate of 45% in the colistin-susceptible *Acinetobacter baumannii* group, 30% in the colistin-resistant *Acinetobacter baumannii* group, and 35% in the *Klebsiella pneumoniae* group was detected. There was no differential or antagonistic effect as in imipenem.

Again, *in vitro* studies have shown that tigecycline increases the effect of colistin against *Acinetobacter* (22). In a recent study, tigecycline was found to be effective in eight KPC-producing *Enterobacteriacea* family members (four *Klebsiella pneumoniae*, two *Escherichia coli,* one *Enterobacter cloacae* and one *Serratia marcescens*). The combination of colistin and meropenem was examined *in vitro* with the time-dependent killing method, and no effective bactericidal activity was detected with the use of tigecycline, colistin and meropenem alone, while the combination of tigecycline and colistin created a bactericidal effect in all strains and the synergistic effect was found to be quite high. In *Klebsiella pneumoniae* strains producing KPC, clinical success rates were found to be 14%, 60%, and 71% with colistin monotherapy, tigecycline monotherapy, and colistin-tigecycline combination (23). In another study, early death rates (within the first 7 days of treatment) in carbapenem-resistant *Klebsiella pneumoniae* bacteremia were found to be 22% with colistin, 40% with tigecycline, and 8% with the combination of both drugs (24).

The combination of colistin-tigecycline was the combination with the most synergistic effect in our study, and it was found to show synergy at a rate of 80% in the colistin-sensitive *Acinetobacter baumannii* group and *Klebsiella pneumoniae* group, and 45% in the colistin-resistant *Acinetobacter baumannii* group. In addition, none of the bacteria used in our study were resistant to tigecycline.

Sulbactam is bactericidal to *Acinetobacter baumannii*, but its use alone should be avoided due to increased resistance. There are successful combinations of sulbactam with colistin and meropenem (25).

In our study, colistin sulbactam combination was found to be 100% ineffective in the *Klebsiella pneumoniae* group, 15% in the colistin-sensitive *Acinetobacter baumannii* group, and 10% synergistically in the colistin-resistant *Acinetobacter baumannii* group. None of these two groups were found to be ineffective.

In vitro synergistic effects have been found against multidrug-resistant *Acinetobacter baumannii* in combinations of colistin with rifampicin, minocycline, ceftazidime, imipenem or azithromycin. There are also suggestions that doxycycline or minocycline can be added to the combination of polymyxin and sulbactam in the treatment of multidrug-resistant infections (26).

In a study conducted in Turkey, the activities of antibiotic combinations that are not used routinely against multi-drug resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* strains were evaluated, and the combination of colistin and rifampicin against the four *Acinetobacter baumannii* strains used in the study showed 100% synergy. It has been determined that the combinations of colistin with meropenem and azithromycin have synergistic effects against three *Acinetobacter baumannii* strains, while the combination of colistin and doxycycline generally has a synergistic and additive effect (27).

In our study, it was determined that the colistin-doxycycline combination showed the most additive effect in all groups, 45% synergistic in the colistin-sensitive *Acinetobacter baumannii* group, 25% in the colistin-resistant *Acinetobacter baumannii* group, and 30% in the *Klebsiella pneumoniae* group, and no indifference or antagonism was encountered.

In a study evaluating the *in vitro* effect of colistin and trimethoprim-sulfamethoxazole combination against colistin-resistant *Acinetobacter baumannii, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* strains, it was found that the combination showed a synergistic effect against all strains and was bactericidal (28).

The combination of colistin and trimethoprim-sulfamethoxazole was found to have a synergistic effect of 35% against colistin-susceptible *Acinetobacter baumannii* strains, 20% against colistin-resistant *Acinetobacter baumannii* strains, and 15% against *Klebsiella pneumoniae* strains. The most detected effect against all bacteria was additive effect with the combination of colistin and trimethoprim-sulfamethoxazole.

Although there is no study in the literature about the combination of colistin and chloramphenicol, a combination of colistin and chloramphenicol was evaluated in our study, and it was determined that this combination, which was considered ineffective against both *Acinetobacter baumannii* groups, showed a 25% synergistic effect and 60% an additive effect in the *Klebsiella pneumoniae* group.

Conclusion

Dominant clones were not investigated in the isolates evaluated in our study. It does not seem possible at the moment to say how many of the multi-resistant bacteria in our center are covered by the combinations with synergistic effects due to the fact that they have a synergistic effect. However, since the effect to be achieved with combinations is more strain-specific, it would be an appropriate approach to evaluate the effect by *in vitro* research, if possible, in each patient to whom the combination will be administered.

Although further studies with more strains and different antibiotic combinations are needed for the appropriate treatment of infections due to multi-drug-resistant gram-negative bacteria, it should be known that not all *in vitro* studies reflect the *in vivo* environment, as in this study, most of the studies on combination therapies are *in vitro*. Since *in vitro* studies are based on animal experiments or case observations, the data obtained in these studies should be supported by controlled clinical studies.

Ethics

Ethics Committee Approval: No ethics committee approval is required for this study.

Informed Consent: There isn't any patient information used in this study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: G.Ç., Z.B., İ.A.K., A.Y., H.K., D.G., H.G., İ.B., Design: G.Ç., Z.B., İ.A.K., A.Y., H.K., D.G., H.G., İ.B., Data Collection or Processing: G.Ç., Z.B., İ.A.K., A.Y., H.K., D.G., H.G., İ.B., Analysis or Interpretation: G.Ç., Z.B., İ.A.K., A.Y., H.K., D.G., H.G., İ.B., Literature Search: G.Ç., Z.B., İ.A.K., A.Y., H.K., D.G., H.G., İ.B., Writing: G.Ç., Z.B., İ.A.K., A.Y., H.K., D.G., H.G., İ.B.

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