RESEARCH ARTICLE / ARAŞTIRMA

DOI: 10.4274/mjima.galenos.2020.2021.51
Mediterr J Infect Microb Antimicrob 2021;10:51
Erişim: http://dx.doi.org/10.4274/mjima.galenos.2020.2021.51

In Vitro Evaluation of Synergy in Carbapenem-resistant *Klebsiella pneumoniae* Strains with Antibiotic Combinations of Meropenem, Fosfomycin, Colistin, and Tigecycline

Meropenem, Fosfomisin, Kolistin ve Tigesiklin Antimikrobiyal Kombinasyonları ile Karbapeneme Dirençli *Klebsiella pneumoniae* Suşlarında Sinerjinin *In Vitro* Değerlendirilmesi

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Abstract

Introduction: Carbapenem-resistant (CR) *Klebsiella pneumoniae* is one of the most common CR *Enterobacteriaceae* species and is resistant to many antibiotics. Generally, combined use of antibiotics is preferred during infections of these microorganisms. This study aimed to demonstrate the *in vitro* synergy between meropenem (MEM), fosfomycin (FOS), colistin (CS), and tigecycline (TGC) in CR-*K. pneumoniae* (Kp) strains.

Materials and Methods: A total of 52 CR-Kp strains were included. MicroScan WalkAway (Beckman Culture) plus system were used to identify strains. Antibiotic susceptibilities of the isolates were detected in the MicroScan WalkAway device (Beckman Culture) using MicroScan Gram-negative panel type 44 (Beckman Culture), and the minimal inhibitory concentrations (MIC) of antibiotics and extended-spectrum beta-lactamases (ESBL) formation were detected. Synergy studies were performed by the microdilution checkerboard method. A fractional inhibitory concentration index was used to interpret the results.

Results: Of 52 strains, 11 had only carbapenem (MEM) resistance. Twenty-three of strains were also resistant to FOS, 27 to CS, and eight to TGC. When antibiotic combinations were compared, MEM + FOS, MEM + CS, and CS + TGC showed the best synergy in our strains. By contrast, the lowest synergy was observed with MEM + TGC, and the difference was significant ($p<0.005$). In addition, when the antibiotic combinations were compared in terms of MIC values, the lowest MIC concentration was observed with MEM + FOS. Ten of our strains had ESBL positivity besides CR. No combination showed >50% synergistic effect in these strains.

Conclusion: This study showed that high FOS and CS resistance was also present in our CR-Kp strains. However, even in case of resistance to one or more of the antibiotics mentioned in this study, the results showed that, in combination with these antibiotics, especially MEM + FOS, MEM + CS, and CS + TGC, a significant amount of in vitro synergy can be achieved.

Keywords: *K. pneumoniae*, fosfomycin, colistin, tigecycline, synergy

Cite this article as: Kandemir Ö, Öksüz Z, Delialioğlu N, Aslan DC, Serin MS. *In Vitro* Evaluation of Synergy in Carbapenem-resistant *Klebsiella pneumoniae* Strains with Antibiotic Combinations of Meropenem, Fosfomycin, Colistin, and Tigecycline. Mediterr J Infect Microb Antimicrob. 2021;10:51.

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Received/Geliş Tarihi: 01.12.2020 **Accepted/Kabul Tarihi:** 23.08.2021 **ORCID ID:** orcid.org/0000-0002-2602-5826

Published: 31 August 2021

Öz

Giriş: Karbapenem-dirençli (CR) *Klebsiella pneumoniae*, CR *Enterobacteriaceae* (CRE) türlerinin en yaygın olanlarından biridir ve birçok antibiyotiğe dirençlidir. Bu mikroorganizmaların enfeksiyonlarında genellikle kombine antibiyotik kullanımı tercih edilmektedir. Bu çalışmanın amacı CR *K. pneumoniae* suşlarında meropenem (MEM), fosfomisin (FOS), kolistin (CS) ve tigesiklin (TGC) arasındaki *in vitro* sinerjiyi göstermektir.
Gereç ve Yöntem: Toplam 52 CR-Kp suşu dahil edildi. Suşları tanımlamak için MicroScan WalkAway plus Sistemi (Beckman Coulter) kullanıldı. İzolatların antibiyotik duyarlıkları MicroScan Gram-olumsuz panel tip 44 (Beckman Culture) kullanılarak MicroScan WalkAway cihazında (Beckman Culture) antibiyotiklerin minimal inhibitör konsantrasyonları (MIC) ve ESBL oluşumu tespit edildi. Sinerji çalışmaları mikro seyreltme dama tahtası yöntemi ile yapıldı. Sonuçları yorumlamak için fraksiyonel bir inhibitör konsantrasyon indeksi kullanıldı.
Bulgular: Elli iki suşun 11'i sadece karbapenem (MEM) direncine sahipti. Suşların 23'ü aynı zamanda FOS'ye, 27'si CS'ye ve sekizi TGC'ye dirençliydi. Antibiyotik kombinasyonları karşılaştırıldığında MEM + FOS, MEM + CS ve CS + TGC suşlarımızda en iyi sinerjiyi gösterdi. Diğer taraftan MEM + TGC ile en düşük sinerji gözlendi ve fark istatistiksel olarak anlamlı idi (p<0,005). Ek olarak, antibiyotik kombinasyonları MIC değerleri açısından karşılaştırıldığında, en düşük MIC konsantrasyonu MEM + FOS kombinasyonunda gözlenmiştir. Suşlarımızın 10'unda karbapenem direncinin yanı sıra ESBL pozitifliği vardı. Bu suşlarda hiçbir kombinasyon %50'den fazla sinerjistik etki göstermedi.
Sonuç: Bu çalışma CR-Kp suşlarımızda yüksek FOS ve CS direncinin de mevcut olduğunu göstermiştir. Bununla birlikte, burada belirtilen bir veya daha fazla antibiyotiğe direnç durumunda bile, bu antibiyotiklerle, özellikle MEM + FOS, MEM + CS ve CS + TGC ile kombinasyon halinde önemli oranda *in vitro* sinerji elde edilebileceği gösterilmiştir.
Anahtar Kelimeler: *K. pneumoniae*, fosfomisin, kolistin, tigesiklin sinerji

Introduction

Therapeutic options in the treatment of multidrug-resistant (MDR) and extensively drug-resistant (XDR) *Enterobacteriaceae* infections, which are life-threatening and frequently encountered in our daily practice, are quite limited[1]. *Klebsiella pneumoniae* is an important member of this group, and the frequency of carbapenem-resistance (CR) has been increasing in this microorganism recently. Although new antibiotics are needed to fight infections caused by these microorganisms, developing new molecules effective in antibiotic therapy is not easy. Until recently, the use of antibiotics, which are generally available as a treatment for such infections, in high doses, for long-term/continuous infusion, or in combinations of 2-3 to provide synergistic/additive effect and decrease resistance, has been implemented in practice. In addition, the re-use of old antibiotics [such as polymyxins and fosfomycin (FOS)] that have been used and released in previous years has now become a rapid solution for such infections due to difficulties in developing new molecules.

At present, carbapenems, polymyxins, and tigecycline (TGC) are widely used in the treatment of these infections in double or triple combinations. The intravenous (IV) form of FOS has been developed for use in some infections that are desperate for treatment, taking into account the pharmacokinetic and pharmacodynamic properties of orally used FOS. Clinical experience has increased in recent years in combination with other antibiotics effective in infections caused by CR *Enterobacteriaceae* spp. (CRE). FOS inhibits cell wall synthesis through the inactivation of the pyrivyl transferase enzyme[2].

The combination of FOS with other antibiotics with different mechanisms of action (TGC, with bacteriostatic effect by inhibiting bacterial protein translation; colistin (CS), which binds to lipopolysaccharide and phospholipid on the outer membrane of Gram-negative microorganisms, allowing penetration through the outer membrane; meropenem (MEM), which binds to PBP 2, 3, and 4 like other beta-lactams, inhibiting peptidoglycan synthesis) is thought to increase the effect on bacterial death[2-4].

Some studies have reported the synergistic activity of IV FOS with various antibiotics, but only a few studies are investigating the synergy between FOS and CS, MEM, and TGC in CR-*K. pneumoniae*.

In this study, we aimed to investigate the antibacterial activity of FOS in our CR-*K. pneumoniae* strains isolated from various clinical samples by conducting an *in vitro* synergy study with MEM, CS, and TGC. Synergies between MEM and TGC, MEM and CS, and CS and TGC were also studied in these strains.

Materials and Methods

Collection of Isolates

A total of 52 CR-*K. pneumoniae* strains were isolated from patients hospitalized and receiving outpatient treatment in Mersin University Hospital (Turkey). Isolates were collected in the Medical Microbiology Department of this hospital between January 2019 and July 2020. Ten of the isolates were also extended-spectrum beta-lactamase positive [ESBL (+)]. These isolates were obtained from various clinical samples. MicroScan WalkAway plus System (Beckman Coulter, USA) automated systems were used to identify strains.

Determination of Extended-spectrum Beta-lactamases

Isolates were tested for antibiotic susceptibility using MicroScan Gram-negative panel 44 (Beckman Coulter). This panel includes ceftazidime and ceftazidime-clavulanic acid, cefotaxime, and cefotaxime-clavulanic acid. In accordance with the manufacturer's instructions, the synergy of ceftazidime and cefotaxime with clavulanic acid was evaluated in isolates according to the minimum inhibitory concentration (MIC) values, and ESBL was detected (MicroScan)[5].

Synergy Studies (Microdilution Checkerboard Method)

Antibiotic susceptibility tests of CR-*K. pneumoniae* isolates were examined by Kirby-Bauer disk diffusion test, automated system (MicroScan), and microbroth dilution. MicroScan Gram-negative [Neg MIC 44 European Committee on Antimicrobial Susceptibility Testing (EUCAST)] panel was used to determine the breakpoint MIC with the automated system and analyzed with the MicroScan WalkAway plus System (Beckman Coulter). The imipenem and ertapenem MIC values of all CR isolates, except MEM, were investigated with an automated system. In the microdilution method, MIC values were determined according to EUCAST guidelines for FOS, CS, and MEM[6]. The MIC for TGC was determined according to the Food and Drug Administration since it is not in the EUCAST guidelines (Table 1)[7]. *Escherichia coli* ATCC 25922 was used as the quality control strain.

Experiments were performed using the checkerboard method in 96-well plates. Antibiotic agents FOS, CS, TGC, and MEM were obtained from Koçak Pharma (Turkey). The stock solution of each antibiotic was prepared by dissolving in sterile distilled water to obtain a concentration of 1024 µg/ml. First, 50 µl of cation-adjusted Mueller-Hinton Broth (Becton Dickinson, USA) was distributed to each well of the microdilution plates. In the microdilution test for FOS, 25 mg/l glucose-6-phosphate was added to the medium. Then, the first antibiotic (50 µl from stock solution) of the combination was serially diluted along the rows to obtain final concentrations of 0.5, 1, 2, 4, 8, 16, 32, 64, 128, 256, and 512 µg/ml. The second antibiotic (50 µl from stock solution) was also serially diluted along the columns. An inoculum equal to a 0.5 McFarland turbidity standard was prepared from each CR-*K. pneumoniae* isolate in cation-adjusted Mueller-Hinton Broth (Becton Dickinson). After mixing 50 µl of each dilution of antibiotics from rows and columns, each microtiter well containing a total of 100 µl of the antibiotic combination was inoculated with 5 µl of a bacterial inoculum (diluted 1:30 with Mueller-Hinton Broth) given a 5×10^5 final cell suspension. Then, the 96-well microtiter plates were sealed and incubated for 22-24 h at 35 °C in a non-CO_2 incubator. The MIC value was read as the least dilution without any turbidity[8]. First, a total of 50 µl of cation-adjusted Mueller-Hinton Broth (Becton Dickinson) was distributed to each well of the microdilution plates. Then, the 96-well microtiter plates were sealed and incubated for 22-24 h at 35 °C in a non-CO_2 incubator. The MIC value was read as the least dilution without any turbidity[8].

Six different combinations of antibiotics were tested for synergistic activity by the checkerboard test: MEM + FOS, MEM + CS, MEM + TGC, FOS + CS, FOS + TGC, and CS + TGC. The activity of each antibiotic combination was assessed at least twice using each of the 52 CR-*K. pneumoniae* isolates. The concentration ranges from 0.5 µg to 256 µg MIC.

A fractional inhibitory concentration index (FICI) was used to interpret the results. Different antibiotic agent combinations were defined according to the FICI as the MIC of drug A or B in combination/the MIC of drug A or B alone. Accordingly, FICI was defined as follows: synergy ≤0.5, no interaction >0.5 to ≤4, and antagonism >4[8].

Statistical Analysis

Student's t-test was used to determine the difference between antibiotic combinations. In addition, MEM + TG, in which the lowest synergy was determined, and other antibiotic combinations were compared using the chi-square test. P values of <0.05 were considered significant.

Results

In this study, 52 CR-*K. pneumoniae* isolates were examined. These isolates were obtained from urine (n=23), urine catheter (n=6), tracheal aspirate (n=12), sputum (n=3), blood (n=2), wound swab (n=5), and tissue (n=1). In addition, 5 of the 52 isolates were obtained from children, while 22 were isolated

Table 1. MIC ranges according to EUCAST and FDA for carbapenem-resistant *K. pneumoniae*[6,7]

MIC		
Antibiotic	**Sensitive**	**Resistant**
MEM	S≤2	R>8
FOS	S≤32	R>32
CS	S≤2	R>2
TGC	S≤2	R≥8

MEM: Meropenem, CS: Colistin, FOS: Fosfomycin, TGC: Tigecycline, MIC: Minimum inhibitory concentration, EUCAST: European Committee on Antimicrobial Susceptibility Testing, FDA: Food and Drug Administration

from patients with immune suppression such as those with cancer, those admitted in the intensive care unit, and those with hematological conditions. The remaining 25 isolates were obtained from patients with immune suppression who were admitted in other units.

Only 11 of these strains had CR. Other strains had resistance to one or both of FOS, CS, and TGC. Furthermore, 23 of these strains were also resistant to FOS, 27 to CS, and 8 to TGC. In addition, 12 of 52 isolates were resistant to both FOS and CS, 4 to both FOS and TGC, and 1 to both CS and TGC. Otherwise, of the 52 CR-*K. pneumoniae* isolates, 13 were susceptible to imipenem, 16 were moderately susceptible, and 23 were resistant, and all 52 isolates are also resistant to ertapenem. When the synergistic effects of the combinations of MEM + FOS, MEM + CS, MEM + TGC, FOS + CS, FOS + TGC, and CS + TGC to the isolates were investigated, the broadest synergistic effect of the antibiotic combination in 52 isolates was determined in MEM + FOS (63.5%) and MEM + CS (63.5%). However, the lowest synergy was observed in the combination of MEM + TGC (23.1%) (Table 2).

In addition, the combinations of CS + TGC, FOS + TGC, and FOS + CS were found to be 53.8% (28/52), 44.2% (23/52), and 38.5% (20/52) synergistic, respectively (Table 3).

When antibiotic combinations were compared in terms of synergies, a significant difference was found between MEM + TGC (23.1%) and others [MEM + FOS (63.5%), MEM + CS (63.5), and COL + TGC (53.8%)] ($p<0.005$).

However, no significant difference was found when other antibiotic combinations were compared. In addition, when the antibiotic combinations were compared in terms of MIC values, the lowest MIC was observed in the combination MEM + FOS (Figure 1).

Synergy rates of the antibiotic combinations according to the FOS, CS, and TGC resistance status of strains are shown in Table 4.

As shown in the table, MEM + CS (74%) in CS-resistant strains, MEM + FOS (69%), and CS + TGC (69%) in FOS-resistant strains, and MEM + FOS (87.5%) in TGC-resistant strains showed the highest synergy. In FOS + CS-resistant strains, the best synergy was seen with MEM + CS (75%). The number of strains resistant to FOS + TGC and CS + TGC was found to be low for healthy interpretation.

Ten of our strains had ESBL positivity besides CR. In these strains, the broadest synergistic effect of the antibiotic combinations

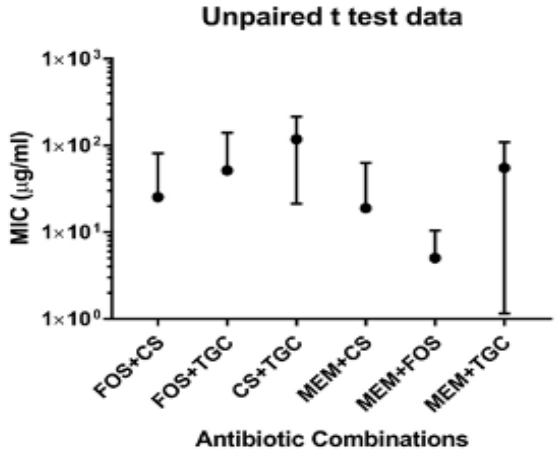

Figure 1. Average minimum inhibitory concentration value of the antibiotic combinations for carbapenem-resistant *K. pneumoniae*. Only fractional inhibitory concentration values <0.5 were considered synergy

MIC: Minimum inhibitory concentration, MEM: Meropenem, CS: Colistin, FOS: Fosfomycin, TGC: Tigecycline

Table 2. Synergistic effect of meropenem with colistin, fosfomycin, and tigecycline

Antibiotics in combination with MEM	Synergistic effects n/n (%)
CS	33/52 (63.5)
FOS	33/52 (63.5)
TGC	12/52 (23.1)

MEM: Meropenem, CS: Colistin, FOS: Fosfomycin, TGC: Tigecycline

Table 3. Synergistic effects among FOS, CS, and TGC

Antibiotic combinations of FOS, CS, and TGC	Synergistic effects n/n (%)
CS + TGC	28/52 (53.8)
FOS + TGC	23/52 (44.2)
FOS + CS	20/52 (38.5)

CS: Colistin, FOS: Fosfomycin, TGC: Tigecycline

was determined in MEM + CS, MEM + TGC, MEM + FOS, and FOS + COL (50%). The synergistic effects of other antibiotics in ESBL (+) isolates are as shown in Table 5. In addition, more than one combination of antibiotics showed synergy in most of these strains. These data are shown in Table 6.

CR-*K. pneumoniae* is one of the most common of CREs and is resistant to many antibiotics. Infections caused by these microorganisms are an important problem for our country as well as worldwide[9]. The management of CR-*K. pneumoniae* infections is difficult because of limited treatment options. In addition to carbapenems, TGC and polymyxins have been widely used in clinical practice in the form of double or triple combinations in the treatment of difficult infections caused by this microorganism. However, due to reasons such as single use or insufficient doses in CRE infections, CS resistance is also increasingly observed. There are also problems with its toxic side effects and its penetration into certain tissues (e.g., lung).

Table 4. Antibiotic combinations showing synergy according to the resistance status of strains

Synergy (+)	Drug resistance					
	FOS (n=23)	CS (n=27)	TGC (n=8)	FOS and CS (n=12)	FOS and TGC (n=4)	CS and TGC (n=1)
FOS + CS	9	10	2	4	2	1
FOS + TGC	10	13	6	4	3	1
CS + TGC	14	8	6	6	4	1
MEM + CS	13	20	4	9	2	1
MEM + FOS	14	16	7	5	4	1
MEM + TGC	5	11	1	5	1	1

MEM: Meropenem, CS: Colistin, FOS: Fosfomycin, TGC: Tigecycline

Table 5. Antibiotic synergy rates in extended-spectrum beta-lactamases-positive isolates

Antibiotic combinations	Synergistic effects n/n (%)
FOS + CS	5/10 (50)
FOS + TGC	5/10 (50)
CS + TGC	4/10 (40)
MEM + FOS	5/10 (50)
MEM + CS	5/10 (50)
MEM + TGC	3/10 (30)

MEM: Meropenem, CS: Colistin, FOS: Fosfomycin, TGC: Tigecycline

Table 6. Antibiotic combinations for extended-spectrum beta-lactamases-positive isolates demonstrating synergistic effects

ESBL (+) isolate no	Antibiotic combinations					
	FOS + CS	FOS + TGC	CS + TGC	MEM + CS	MEM + FOS	MEM + TGC
1	+		+	+	+	
2	+		+	+	+	+
3		+		+	+	
4	+				+	+
5				+		
6		+				+
7		+				
8			+	+		
9	+	+			+	
10	+	+	+			
Total	5	5	4	5	5	3

MEM: Meropenem, CS: Colistin, FOS: Fosfomycin, TGC: Tigecycline

FOS is also an old molecule like CS, and its oral form has often been used in uncomplicated urinary tract infections. Considering the pharmacokinetic/pharmacodynamic properties of this form of FOS, the IV formulation has been developed for use in infections caused by MDR and XDR microorganisms, and it has been suggested to be used in combination with other effective antibiotics. Since the mechanism of action and chemical structure of FOS is different, cross-resistance with other antibiotics is rare. Moreover, unlike CS, it is not nephrotoxic and has a good penetration into tissues. The use of combination therapy in the management of CR-*K. pneumoniae* infections is a generally accepted approach[10]. In several *in vitro* studies, the combination of antibiotic agents shows synergistic activity, and its reflection in the clinic can cure the disease. However, in terms of synergistic effect, some inconsistencies can be observed according to the strain and methods of studies[11,12]. Therefore, at present, it is still unclear which treatment is optimal in the management of these infections.

In this study, CR-K. pneumoniae strains isolated from various samples in our hospital were also resistant to FOS, CS, and TGC at certain rates (44.2%, 51.9%, and 15.3%, respectively). In the study of Süzük Yıldız et al.[13,14] reflecting Turkish data in 2018, CS and FOS resistance rates were 76% and 67% (according to EUCAST), respectively, in 147 CRE strains (91% of which were CR-K. pneumoniae). Kaase et al.[14] revealed simultaneous FOS resistance in 16 (32%) of 50 CR-K. pneumoniae strains, while another study reported 42%[1]. In a study by van Duin et al.[15], CS and TGC resistance rates were 7% and 45%, respectively, in 251 CR-K. pneumoniae strains. Worldwide, FOS, CS, and TGC sensitivity rates in CR-K. pneumoniae strains vary according to strain and geographic region[16].

However, in the present study, some strains also showed multiple drug resistance [FOS + CS in 12 (23%), FOS + TGC in 4 (7.6%), and CS + TGC in 1 (1.9%)]. In a similar study, Bakthavatchalam et al.[1] found 16% of FOS + CS resistance in CR-*K. pneumoniae* strains, and these isolates were not investigated for molecular determinants. However, in another study, the presence of *fosA* gene and mutation in *mgrB* was described for the co-occurrence of FOS and CS resistance in CR-*K. pneumoniae*[17]. In the present study, unfortunately, the presence of resistance genes by molecular methods could not be investigated. These findings show that even the combinations of a small number of antibiotics that are thought to be effective in CR-*K. pneumoniae* strains might fail.

Considering all 52 strains in the present study, the best synergy was observed in MEM + FOS and MEM + CS antibiotics.

In the study of Samonis et al.[12], the highest synergy was observed between FOS and imipenem (74%), MEM (70%), and doripenem (74%) in 50 CR-*K. pneumoniae* strains, and synergy was lower between FOS + CS and FOS + TGC (36% and 30% respectively).

In a review by Falagas et al.[18], the best synergy was observed between carbapenems and FOS (70%). In addition, synergies with FOS + CS and FOS + TGC were 36% and 30%, respectively. However, Dundar et al.[19] showed that CS + TGC (70%) combination had the best synergy in Cr-*K. pneumoniae* strains. Synergy with FOS was not considered in this study.

In addition, the present study showed that synergy can be observed even though the isolate is resistant to one or both of the antibiotics in combination.

Especially, in strains with FOS or TGC resistance, the best synergy was found in the combination of MEM + FOS. In addition, MEM + CS show the highest synergy in CS-resistant strains. Similarly, Bakthavatchalam et al.[1] revealed a good synergistic activity of MEM and FOS combination against CS- and FOS-resistant CR-*K. pneumoniae*.

Extended-spectrum beta-lactamases positivity was also present in 10 of our strains. Synergy was not >50% in any combination in these strains. The lowest synergy was found in the combination of MEM + TGC (30%). Studies of *in vitro* synergy in *K. pneumoniae* strains together with CR and ESBL positivity are very rare. In an *in vitro* synergy study performed by Ku et al.[20] in 174 ESBL (+) *K. pneumoniae* strains, only five isolates were resistant to carbapenem at the same time (imipenem or MEM), and synergies among FOS, CS, and TGC were examined in these strains. In this study, synergies were not evaluated with carbapenems. They stated that CS + TGC showed the best synergy both in these strains and only ESBL (+) strains. However, a low number of strains were both ESBL (+) and CR. Thus, extensive studies with more strains related to both ESBL (+) and CR-*K. pneumoniae* strains are needed.

In the present study, when all strains were considered, the best synergy was found with MEM + FOS and MEM + CS, the lowest synergy was observed in the combination of MEM + TGC, and the difference between these combinations was significant. In addition, the combination of CS + TGC showed good synergy, and the difference with MEM + TGC is significant.

Perdigão Neto et al.[21] reported a clinical cure rate of 70% in patients treated with FOS + MEM, who had CS-resistant Gram-negative infections. However, the reflection of our results on the clinic will be demonstrated in future clinical studies.

The present study has some limitations. First, resistance genes could not be detected by molecular methods. Thus, it will be more valuable to detect these genes and to know which combinations show better synergy at which type of resistance. Second, the presence of ESBL in our CR-*K. pneumoniae* strains was not demonstrated by molecular method. Therefore, the

synergy evaluation of CR-*K. pneumoniae* strains with ESBL in our study only provided preliminary data. In the future, we will evaluate the presence of ESBL in CR-*K. pneumoniae* strains and synergy studies by molecular methods.

Conclusion

This study showed that high FOS and CS resistance was also present in the CR-*K. pneumoniae* strains analyzed. However, even in the case of resistance to one or more of these antibiotics, the combination with these antibiotics, especially MEM + FOS, MEM + CS, and CS + TGC, can achieve a significant amount of *in vitro* synergy. Of course, these synergy rates may vary in each center depending on the strain, and the reflection of this synergy to the clinic may not always overlap because of factors such as patient population and disease severity.

In brief, options for combating MDR *Enterobacteriaceae* species, especially CR-*K. pneumoniae*, are not a lot. Thus, each unit should define its strains and establish better antibiotic use policies.

Ethics

Ethics Committee Approval and Informed Consent: Since our study is an *in vitro* synergy study, it does not require ethics committee approval and patient consent.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Ö.K., Concept: Ö.K., M.S.S., Design: Ö.K., Z.Ö., N.D., Data Collection or Processing: N.D., D.C.A., M.S.S., Analysis or Interpretation: Ö.K., Z.Ö., M.S.S., Literature Search: Ö.K., D.C.A., Writing: Ö.K., Z.Ö., M.S.S.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: We thank Koçak Pharmaceutical Company for providing the antibiotics used in the study.

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