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Comparison of the Efficacy of Colistin and Meropenem Monotherapy with Meropenem/Ertapenem Combination in an Experimental Sepsis Model of Carbapenemase-producing *Klebsiella pneumoniae*

Deneysel Karbapenemaz Üreten *Klebsiella pneumoniae* Sepsisi Modelinde Kolistin ve Meropenem Monoterapisi ile Meropenem/Ertapenem Kombinasyonunun Etkinliğinin Karşılaştırılması

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Abstract

Introduction: To investigate the efficacy of double carbapenem therapy (ertapenem + meropenem combination) in an experimental sepsis model in rats with *Klebsiella pneumoniae* (*K. pneumoniae*), which is carbapenem-resistant and colistin susceptible, and compare it with colistin and meropenem monotherapy.
Materials and Methods: *K. pneumoniae* isolate that is known to carry the *bla*OXA-48 and *bla*NDM carbapenemase genes was used, and 1-2×10^8 colony forming unit/ml was inoculated intraperitoneally to 40 rats (20 males/20 females), and a sepsis model was created. The rats were divided into four equal groups: control, colistin, meropenem, and meropenem + ertapenem combination. The rats were followed for 24 h for signs of sepsis and mortality. Euthanasia was then performed, and blood cultures were taken.
Results: After 24 h, none of the rats in the control or treatment groups died. *K. pneumoniae* growth was observed in all rats in the control, five in the colistin, seven in the meropenem, and five in the meropenem + ertapenem combination groups. A statistically significant difference was found between the control group and the colistin and meropenem + ertapenem combination groups (p=0.033 and p=0.033, respectively). No statistically significant difference was found between the control group and the meropenem monotherapy group (p=0.215). The numbers of non-growth blood cultures were comparable between the colistin group and the meropenem + ertapenem combination group, and no statistically significant difference was found between the two groups (p=1). The mean time of growth signals (minutes) were compared between the treatment groups: colistin, 642.6 ± 116.4; meropenem, 582.6 ± 107.7; and meropenem + ertapenem combination, 701.2 ± 70.4 min.
Conclusion: Meropenem + ertapenem combination treatment was comparable to colistin monotherapy, and the mean time of growth signals of blood cultures were longer than those in the colistin and meropenem monotherapy groups.
Keywords: *Klebsiella pneumoniae*, double carbapenem therapy, experimental rat sepsis model

Öz

Giriş: Karbapenem dirençli ve kolistine duyarlı *Klebsiella pneumonia* (*K. pneumoniae*) ile sıçanlarda oluşturulan deneysel sepsis modelinde ikili karbapenem tedavisinin (meropenem + ertapenem kombinasyonu) etkinliğini araştırmak ve ayrıca ikili karbapenem tedavisinin etkinliğini kolistin ve meropenem monoterapisi ile karşılaştırmak.

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Gereç ve Yöntem: *bla*OXA-48 ve *bla*NDM karbapenemaz genlerini taşıdığı bilinen *K. pneumoniae* izolatı ile 1-2x10^8 koloni oluşturan birim/ml inokülüm hazırlandı. Bu inokulum toplam 40 sıçana (20 erkek/20 dişi) intraperitoneal olarak enjekte edilerek sepsis modeli oluşturuldu. Sonrasında sıçanlar kontrol, kolistin, meropenem ve meropenem + ertapenem kombinasyon grubu olmak üzere dört eşit gruba ayrıldı, sepsis bulguları ve mortalite açısından 24 saat takip edildi. Daha sonra sıçanlara ötenazi yapıldı ve kan kültürleri alındı.
Bulgular: Yirmi dört saatin sonunda kontrol veya tedavi gruplarındaki sıçanların hiçbiri ölmedi. Kontrol grubundaki sıçanların tamamında, kolistin grubundaki beş, meropenem grubundaki yedi ve meropenem + ertapenem kombinasyon grubundaki beş sıçanda *K. pneumonia* üremesi gözlendi. Kontrol grubu ile kolistin grubu ve kontrol grubu ile meropenem + ertapenem kombinasyon grupları arasında istatistiksel olarak anlamlı fark bulundu (p=0,033 ve p=0,033). Kontrol grubu ile meropenem monoterapi grubu arasında istatistiksel olarak anlamlı fark yoktu (p=0,215). Kolistin grubu ile meropenem + ertapenem kombinasyon grubu arasında üreme olmayan kan kültürü sayıları benzerdi, istatistiksel olarak da bu iki grup arasında anlamlı fark saptanmadı (p=1). Tedavi grupları arasında ortalama üreme zamanları (dakika olarak) karşılaştırıldığında: kolistin grubu 642,6 ± 116,4, meropenem grubu 582,6 ± 107,7 ve meropenem + ertapenem kombinasyon grubu 701,2 ± 70,4 dakika olarak bulundu.
Sonuç: Meropenem + ertapenem kombinasyon tedavisi etkinlik açısından kolistin monoterapisine benzer bulundu. Kan kültürlerindeki ortalama üreme zamanı, kombinasyon grubunda kolistin ve meropenem monoterapi gruplarına gore daha uzun saptandı.
Anahtar Kelimeler: *Klebsiella pneumoniae*, ikili karbapenem tedavisi, deneysel sıçan sepsis modeli

Introduction

Multidrug resistance (MDR) in bacteria is currently occurring at an alarming rate, and resistant bacteria cause both nosocomial and community-acquired infections. *Klebsiella pneumonia (K. pneumoniae)* is one of the most common organisms that carry plasmids encoding extended-spectrum beta-lactamase and carbapenemase enzymes. Globally, carbapenem-resistant *K. pneumoniae* (CRKP) infections increased, and CRKP has become a major global health problem owing to the reported increase in the last 20 years[1,2]. In a multinational and multicenter study, CRKP was found to be the main cause of the increase in carbapenem-resistant enterobacterales (CRE), constituting 71.1% of CRE isolates[3]. In the summary report by the Turkish Ministry of Health National Health Service-Associated Infections Surveillance Network for 2020, the overall average ratio of carbapenem resistance in *K. pneumoniae* nosocomial isolates was 49%[4]. Moreover, the plasmid that is responsible for carbapenemase enzyme production also contains resistance genes against antibiotics that are not structurally related to β-lactam antibiotics, such as aminoglycosides and fluoroquinolones; therefore, carbapenemase-producing microorganisms are generally potentially resistant to nearly all antibiotics.

Currently, the most commonly used antibiotics against carbapenem-resistant bacteria are tigecycline, polymyxins, fosfomycin, and some aminoglycosides. However, some strains also develop resistance to these antibiotics, and the prevalence of resistance increases over time. Therefore, in the treatment of CRE, existing old antibiotics in high doses for prolonged infusions or combinations and newly developed antibiotics/antibiotic combinations are adopted. The most common antibiotics/antibiotic combinations for this purpose are as follows: high-dose tigecycline, high-dose and long-term carbapenem infusion, double carbapenem therapy (DCT), and various combination therapies, i.e., ceftazidime-avibactam, imipenem/cilastatin-relebactam, meropenem-vaborbactam, aztreonam-avibactam, ceftolozane tazobactam, cefiderocol, plazomycin, and eravacycline.

The best studied and most widely used practice in DCT is to place ertapenem in combinations. This combination regimen was proposed as a salvage option for carbapenemase-producing *K. pneumoniae* (KP) using an *in vitro* chemostat and *in vivo* murine infection model[5]. The rationale for this combination came from the hypothesis that ertapenem might play a sacrificial role (suicide substrate) because it is preferentially hydrolyzed owing to its greater affinity for *K. pneumoniae* carbapenemase (KPC); thus, other carbapenems are expected to be effective because the enzyme is consumed during its interaction with ertapenem[5].

This study aimed to investigate the efficacy of meropenem + ertapenem combination therapy in an experimental sepsis model induced in rats by a carbapenem-resistant *K. pneumoniae* strain, which is known to be sensitive to colistin, and to compare it with colistin and meropenem monotherapies.

Materials and Methods

Bacterial Isolate

In the study, *K. pneumoniae* isolate obtained from blood culture sample was used. The identification study and antimicrobial susceptibility of this isolate were performed using the BD Phoenix ID/AST automated system (BD Diagnostic Systems, Sparks, MD, USA). For susceptibility, traditional methods were used and evaluated according to the criteria of the "European Committee on Antimicrobial Susceptibility Testing (EUCAST)". According to EUCAST, the isolate was considered resistant to ertapenem, imipenem, and meropenem. Using gradient test strips (ETP, IMP, MEM Etest®, BioMerieux, France), MIC values were found to be >32 mg/L for meropenem, >32 mg/L for imipenem, and >32 mg/L for ertapenem. The colistin sensitivity by the liquid microdilution method was 0.25 mg/L. The carbapenemase genes of the isolate were investigated by in-house polymerase

chain reaction, and it was found to carry both the *bla*OXA-48 and *bla*NDM carbapenemase genes. Moreover, the presence of carbapenemase was detected by the carbapenem inactivation method.

Laboratory Animals

Forty Wistar albino rats (male, n=20; female, n=20), weighing between 250-290 g, were used in the study. The number of animals to be used in the experiment has been determined as the minimum number that will provide statistical significance in accordance with the ethical rules of animal experiments. Ethics committee approval was obtained from Ege University Animal Experiments Local Ethics Committee (2018-042, 30.05.2018). The rats were randomly divided into four groups containing five females and five males in each. Male and female rats were caged separately at room temperature with 50%±5% humidity, and they were provided with clean water and appropriate pellet food.

Antibiotics

In this study, colistimethate sodium (Vem, Ankara, Turkey), meropenem (KoçakFarma, İstanbul, Turkey), and ertapenem (MSD, İstanbul, France) were used as antimicrobial agents. All agents were dissolved in 0.9% sodium chloride solution according to manufacturers' recommendations. The solutions were prepared fresh on the day of the experiment and stored in a refrigerator.

Study Design and Animal Experiment (*in vivo* Study)

On the study day, a 0.5 McFarland turbid inoculum (1-2×10^8 colony forming unit/ml containing *K. pneumoniae* strain) from the colonies on the blood agar was prepared. The rats were each injected intraperitoneally (IP) with 0.1 cc of fluid from this inoculum. One hour later, 0.1 cc of solutions was also given as IP: physiological saline to the control group, colistimethate sodium 1 mg/kg every 12 h (C group), meropenem 50 mg/kg (standard dose, i.e. represents 3×1 gr) every 8 h (M group), ertapenem 15 mg/kg, and 1 h later meropenem 50 mg/kg every 8 h (EM group). These antibiotic doses were determined according to previous experimental studies[6-8].

All groups were followed for 24 h for mortality, and findings regarding sepsis such as changes in appearance (such as on fur and eyes), posture, behavior, activity, respiratory rate/quality, and body temperature were closely monitored. The plan was to immediately take the blood cultures from rats in case of exitus and stop the experiment with euthanasia after 24 h and take blood cultures from rats that had not died within this period. After 24 h, all rats were euthanized by administering sodium pentobarbital, and afterwards, an average of 5 ml of intracardiac blood was inoculated into pediatric BD BactecPed Plus blood culture bottles (BD Diagnostic Systems, Sparks, MD, USA) and placed in the BD BACTEC FX automated blood culture system. The turning out positive time was recorded as the "growth time," passed into 5% sheep blood agar, and incubated for another 24-48 h. Preparations were directly made from the positive bottles and plates and subjected to gram staining. After 5 days, which is the incubation period of the automated blood culture device, cultures without growth signal were considered "no growth detected"

Statistical Analysis

Since none of the experimental rats died within 24 h, bacteriological growth was evaluated instead of survival. The proportions of rats with growth in their blood cultures were compared using a Fisher's Exact test, as the sample size was minimal. Differences were considered significant at the 95% confidence interval.

Results

In all culture bottles taken from the control group, growth signal was obtained in six of the C group, eight of the M group, and five of the EM group. On gram staining preparations: gram-negative bacilli in all the control group, gram-negative bacilli in five of the C group, gram-positive cocci in one and gram-negative bacilli in seven of the M group, gram-positive cocci in one and gram-negative bacilli in five of the EM group were observed. Gram-positive cocci growths were considered contaminations. All gram-negative bacilli were identified as carbapenemase-KP. The growths in blood cultures, growth time, and observation finding of all rats are presented in Table 1. When blood culture growths were compared, a statistically significant difference was found between the control group and the C and EM groups (p=0.033 and p=0.033, respectively). No statistically significant difference was found between the control group and the meropenem group (p=0.215). The numbers of non-growth blood cultures were comparable between the C group and the EM group, and no statistically significant difference was found between these two groups (p=1). The efficacy evaluation between antibiotic groups according to the growths in the blood culture is shown in Table 2.

The average time of growth was as follows: 645.5±184.6, 642.6±116.4, 582.6±107.8, and 701.2±70.4 min in the C, M, and EM groups respectively. The latest and fastest growth times were recorded in the EM and M groups, respectively.

Approximately 1 h after bacterial inoculation, all rats started to demonstrate motor retardation. Decreased response to stimuli and an increase in body temperature were noticed in approximately 10 h in all rats. Three rats in the EM group had an increase in the number of defecations and diarrhea. At the end of 24 h follow-up, no exitus in the control group and treatment groups was observed.

Table 1. Blood culture growth time and observation findings of all rats

Study groups	Average weight/g	Observation findings	Growths	Average growth time/ minute	Ex
Control group (n=10)	266±10	Motor retardation 10 Increase in body temperature 10	10 *K. pneumoniae*	645.5±184.6	0
Colistin group (n=10)	273±10.4	Motor retardation 10 Increase in body temperature 10	5 *K. pneumoniae* 1 Gram-positive cocci	642.6±116.4	0
Meropenem group (n=10)	263±13.7	Motor retardation 10 Increase in body temperature 10	7 *K. pneumoniae* 1 Gram-positive cocci	582.6±107.7	0
Ertapenem/meropenem combination group (n=10)	268±13.7	Motor retardation 10 Diarrhea 3 Increase in body temperature 10	5 *K. pneumoniae*	701.2±70.4	0

K. pneumoniae: Klebsiella pneumoniae

Table 2. Activity rubric

	Absence of growth	Reproduction of *K. pneumoniae*	Contamination (Gram + coccus growth)	p			
				Control group	Colistin group	Meropenem group	Ertapenem/meropenem combination group
Control group (n=10 rats)	0	10	-	-	0.033	0.215	0.033
Colistin group (n=10 rats)	4	5	1	0.033	-	0.62	1
Meropenem group (n=10 rats)	2	7	1	0.215	0.62	-	0.35
Ertapenem/ meropenem combination group (n=10 rats)	5	5	-	0.033	1	0.35	-

K. pneumoniae: Klebsiella pneumoniae

Discussion

Carbapenemases were first discovered in *K. pneumoniae* (KPC enzyme). *K. pneumoniae* is the most common strain involving KPC and NDM-1 enzymes. Turkey is considered endemic in terms of OXA-48 and remains the most common carbapenemase in Turkey. OXA-48 and NDM enzyme is seen with increasing frequency[9]. Thus, in our experimental sepsis study, *K. pneumoniae* isolate, which carried OXA-48 + NDM *carbapenemase* genes together, was used.

Generally, infections caused by carbapenemase-producing strains are treated by carbapenems given as high doses and long-term infusions, especially meropenem, in combination with other in vitro active agents. In the combination treatment, meropenem may still be considered if the minimum inhibitory concentration (MIC) of meropenem is 8 mg/L, but a high-dose and prolonged infusion regimen should be administered. In large multicenter studies, increased survival using combinations of meropenem was observed when KPC-KP exhibited minimum inhibitory concentrations of 8 mg/L[10,11]. In the study of Tumbarello et al.[12], combination regimens containing meropenem can provide significant therapeutic benefits for *K. pneumoniae* carbapenemase-KP isolate if the meropenem MIC value is ≤8 mg/L, but no benefit is observed when the meropenem MIC exceeds 32 mg/L.

Some *in vitro* studies have shown that ertapenem-based dual carbapenem therapy has a beneficial effect on meropenem MIC≤ 128 mg/L[13]. Another study reported uncertainty about the usefulness of including meropenem in combination regimens when KPC-KP strains had a meropenem MIC value of >16 mg/L[14]. In the study by Pea et al.[15], high-dose continuous infusion of meropenem with the method based on real-time therapeutic drug monitoring has been found to be potentially useful to improve clinical outcomes in the treatment of infections caused

by KPC-KP with a meropenem MIC up to 64 mg/L. In the present study, *K. pneumoniae* isolate with a meropenem MIC of >32 mg/L was used, and when the meropenem group was compared with the control group, growth found was numerically less in the meropenem group (7/10) than in the control group (10/10), but no statistically significant difference was found (p=0.215).

Dual carbapenem therapy, which continues to be researched and applied in clinical practice in the treatment of carbapenem-resistant enterobacterales, was first introduced as a rescue treatment against MDR chronic rhinosinusitis epidemiology study in a study by Bulik and Nicolau[5]. In the present study, the efficacy of the combination of ertapenem and doripenem was evaluated both as an in vitro chemostat model and an *in vivo* rat experiment, and the combination of two carbapenems increased the efficacy compared with both agents used alone[5]. An observational cohort study by Souli et al.[16] investigating ertapenem + meropenem as a rescue therapy in 27 patients with KPC-KP infection [mostly complicated urinary tract infections (59.3%) and blood stream infections (48.2%) reported a high clinical success rate of 77.8%]. Moreover, pan-resistant infections subgroup had a successful clinical and microbiological result of 78.5%. In the study by Venugopalan et al.[17], 36 patients with CRKP bacteremia were enrolled, including 18 patients receiving doripenem + ertapenem and 18 receiving doripenem + colistin, and the treatment success rate was higher (p=0.049) and 30-day mortality was lower (p=0.087) in the dual carbapenem group. Oliva et al.[18] enrolled 32 patients with CRKP infection, which included 18 patients receiving ertapenem + meropenem and 14 receiving ertapenem + meropenem + colistin, and found that the combination of colistin with dual carbapenem achieved faster bactericidal activity in the *in vitro* analysis; however, no significant differences were found between the two groups in terms of early response or 60-day mortality. Therefore, the addition of colistin to dual carbapenem therapy should be considered in severe cases with septic shock upon admission, and after clinical stabilization, it should be switched to dual carbapenem therapy, which is a less nephrotoxic and more stable regimen. Mashni et al.[10] compiled a critical review of existing studies investigating dual carbapenem therapy for CPKP infections, including eight case reports and six clinical trials (171 patients in total). Most patients were critically ill, and all have been treated with ertapenem + prolonged infusion of meropenem or doripenem. The clinical and microbiological success was reported in approximately 70% of patients and death in 24%. The most common side effects were seizures, disturbance in sodium metabolism, and gastrointestinal symptoms[10].

The efficacy of antibiotics or antibiotic combinations against resistant bacteria has also been investigated in relatively few experimental animal model studies. To determine the optimum dose to produce non-lethal sepsis in rats, Toledo et al.[11], in their experimental sepsis model with KPC-KP, stated 9×10^8 colony forming unit/ml as the amount of bacteria that can cause non-lethal sepsis in rats. Kosar et al.[19] used the same dose to produce experimental sepsis in rats by colistin-resistant *K. pneumoniae* and investigated the efficacy of dual carbapenem therapy, colistin monotherapy, ertapenem + meropenem and ertapenem + meropenem + colistin combination treatments. When the quantitative bacterial loads in the lung and liver tissues were evaluated, no statistically significant difference was observed between the groups (p>0.05), and all three treatment options were not found to be effective. In the group treated with only ertapenem + meropenem + colistin, the decrease in bacterial load at 48th hour was significant when compared with that at 24th hour (p<0.05)[19]. In our study, a similar dose was used, and the number of rats with growth in blood culture in the control group was higher than that in the treatment groups, and a statistically significant difference was found in the colistin and ertapenem + meropenem combination groups (p<0.05). When colistin and ertapenem + meropenem combination groups were evaluated, they have comparable number of growths, but when the time of growth was considered, ertapenem + meropenem group had the most delayed growth time among the groups.

Most studies regarding dual carbapenem therapy were conducted with class A carbapenemases or KPC-KP, and double carbapenem therapy (with or without colistin) administration appears to be a promising rescue treatment in infections caused isolates containing serine-carbapenemases; however, the results obtained with B and D class carbapenemases have been inconsistent[20,21]. An *in vitro* study demonstrated DCT's synergistic activity against OXA-48-producing MDR *K. pneumoniae*[22]. Another in vitro study reported variable synergistic patterns of carbapenems between producers of KPC and OXA-48, whereas no synergy was observed in NDM-producing strains, and only *K. pneumoniae* carbapenemase, not metallo-β-lactamase (MBL) enzymes, responded to the maneuver in which ertapenem acts as a suicide substrate, and in this case, various combinations of carbapenems were successful *in vitro*[23]. However, Wiskirchen et al.[24] suggested that carbapenems are still a valid treatment option in NDM-1-producing Enterobacterales infections. Despite the adverse in vitro MICs of NDM-KP, recent *in vivo* studies have demonstrated the efficacy of carbapenems against NDM-1-producing isolates in immunocompetent rats and neutropenic mouse thigh infection models[24-26]. Despite the lack of solid evidence, some clinicians have favored double carbapenem therapy for the treatment of infections caused by MBLs. Rosa et al.[27] published two case reports on patients who underwent kidney transplantation with complicated urinary tract infections caused by NDM-producing Enterobacterales. Both patients received oral fosfomycin (3 g q48-72 h 21 days) and renally adjusted meropenem (1 g q12 h) plus ertapenem (1

g per day) for 14 days, achieving complete clinical recovery and microbiological clearance[27].

In our study, OXA-48 and NDM co-KP strain was used, for which the effect of DCT was relatively less studied in previous studies, and DCT was thought to be also used in the treatment of bacteria-producing OXA-48 and NDM, since DCT and colistin have similar efficacy against this strain. However, more randomized controlled trials are needed to evaluate DCT as a rescue treatment for infections caused by MDR pathogens that demonstrate various mechanisms of resistance to carbapenems.

Study Limitations

This study has some limitations. First, the model has a short duration. Our study was terminated in 24 h, and we did not maintain it until 48 h or beyond. We followed the global 3R rules of experimental animal study (replacement, reduction, and refinement) to give less pain, suffering, or distress to the subjects. Second, the results should have been confirmed not only by blood culture but also by post-mortem biopsy findings from the liver or spleen. A strength point was that most studies regarding dual carbapenem therapy were conducted with class A carbapenemases or KPC-KP and not with strains with class B and D carbapenemases, which are the most prominent in our country.

Conclusion

The prevalence of infections caused by CRKP is increasing worlwide possessing a significant threat to public health. These isolates also produce high levels of resistance to other classes of antibiotics, thus limiting treatment options. Treatment recommendations are based on case reports and in vitro results, and the optimal treatment of carbapenem-resistant gram-negative bacterial infections is still not fully established. The majority of the studies on the use of dual carbapenem therapy are limited because of their retrospective observational design, small sample size, and non-specific evaluation of KPC-producing organisms.

In our study, in the experimental sepsis model performed on rats with carbapenem-resistant (meropenem minimum inhibitory concentration >32 mg/L) colistin-sensitive and OXA-48 and NDM co-KP, colistin and meropenem + ertapenem combination treatments were found to have comparable efficacy and the mean time of growth in blood culture was longer in the meropenem + ertapenem combination group, suggesting a lesser bacterial load.

As a result, in the treatment of carbapenemase-KP, DCT can be used, especially when colistin cannot be administered because of some reasons such as nephropathy. However, randomized controlled studies are needed to determine the true benefit of DCT and evaluate its efficacy against strains producing different carbapenemase enzymes and CRKP that do not produce carbapenemases.

Ethics

Ethics Committee Approval: Ethics committee approval was obtained from Ege University Animal Experiments Local Ethics Committee (2018-042, 30.05.2018).

Informed Consent: Animal experiment.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: D.Y.Y., A.A., R.Y., F.E.S., Concept: D.Y.Y., A.A., R.Y., F.E.S., S.T., Design: D.Y.Y., A.A., R.Y., F.E.S., S.T., Data Collection or Processing: D.Y.Y., Analysis or Interpretation: D.Y.Y., A.A., R.Y., S.T., Literature Search: D.Y.Y., Writing: D.Y.Y., A.A., S.T.

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