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The Effects of Colistin on Imipenem MICs in OXA-48 Producing Klebsiella pneumoniae Isolates: An In Vitro Study

Kolistinin OXA-48 Üreten *Klebsiella pneumoniae* İzolatlarında İmipenem MİK'leri Üzerindeki Etkileri: Bir *İn Vitro* Çalışma

[®] Yücel DUMAN¹, [®] Mehmet Sait TEKEREKOĞLU¹, [®] Çiğdem KUZUCU², [®] Yusuf YAKUPOĞULLARI¹

¹İnönü University Faculty of Medicine, Department of Medical Microbiology, Malatya, Turkey

²Tinaztepe University Faculty of Medicine, Department of Medical Microbiology, İzmir, Turkey

Abstract

Introduction: A new approach to carbapenem resistance–*K. pneumoniae* infections is to use combination drug therapies. However, little data are available about the effectiveness of the *in vitro* carbapenem plus colistin combination against oxacillinase–48 (OXA–48) producing *K. pneumoniae*. Therefore, the aim of this study is to assess the potential synergistic activity of imipenem plus colistin in OXA–48–producing *K. pneumoniae* strains and investigate the changes in the imipenem minimal inhibitory concentrations (MICs) to varying MICs of colistin.

Materials and Methods: Carbapenem and colistin resistance (CoIR) genes were investigated by polymerase chain reaction. In the first stage, synergistic properties were determined by the checkerboard combination method. In the second step, at varying colistin concentrations, changes in the imipenem MICs were investigated.

Results: Colistin MIC_{50} 2 μ g/ml, MIC_{90} 16 μ g/ml, and imipenem MIC_{50} 32 μ g/ml, MIC_{90} 128 μ g/ml were found, respectively. According to the fractional inhibitor concentration (FIC) formula, 62.2% of the isolates were synergistic, and 37.8% were indifference. When the colistin was fixed at 0.125 μ g/ml, 0.5 μ g/ml, 0.5 μ g/ml, 1 μ g/ml, and 2 μ g/ml, respectively. Significant decreases were observed in the imipenem MICs, especially of colistinsensitive isolates. However, imipenem MICs of CoIR isolates did not decrease to susceptible levels.

Conclusion: This information will facilitate the design of antibiotic regimens that are more suitable for treating infections due to such pathogens producing OXA-48 and prolong these antibiotics' efficacy. Further *in vitro* research is required to determine which treatment combination is best and its optimal use as combination therapy to treat these infections.

Keywords: Colistin, imipenem, combination, Klebsiella pneumoniae, OXA-48

Öz

Giriş: Karbapenem dirençli-*K. pneumoniae* enfeksiyonlarına yeni bir yaklaşım, kombinasyon ilaç tedavilerinin kullanılmasıdır. Ancak, *in vitro* karbapenem ve kolistin kombinasyonunun oksasilinaz-48 (OXA-48) üreten *K. pneumoniae*'ye karşı etkinliği hakkında çok az veri mevcuttur. Bu nedenle, bu çalışmada OXA-48 üreten *K. pneumoniae* suşlarında imipenem ve kolistinin potansiyel sinerjistik aktivitesini değerlendirmek, kolistinin değişen minimum inhibitör konsantrasyonunda (MİK) imipenem MİK'lerinin değişikliklerini araştırmak amaçlanmıştır.

Gereç ve Yöntem: Karbapenem ve kolistin direnç genleri polimeraz zincir reaksiyonu ile araştırıldı. İlk aşamada, sinerjik özellikler dama tahtası birleştirme yöntemi ile belirlenmiştir. İkinci aşamada, değişen kolistin konsantrasyonlarında imipenem MİK'lerinin değişimleri araştırılmıştır.

Bulgular: Kolistin MiK_{50} 2 μg/ml, MiK_{90} 16 μg/ml, imipenem MiK_{50} 32μg/ml, MiK_{90} 128 μg/ml bulundu. FIC formülüne göre izolatların %62,2'si sinerjik ve %37,8'i ilgisizdi. Kolistinin sırasıyla 0,125 μg/ml, 0,25 μg/ml, 0,5 μg/ml, 1 μg/ml, 2 μg/ml olarak sabitlenmesi durumunda özellikle kolistine duyarlı izolatlarda imipenem MiK'lerinde önemli düşüşler gözlendi. Bununla birlikte, kolistin dirençli izolatlarda imipenem MiK'leri, duyarlı düzeylere düsmemistir.

Sonuç: Bu çalışma, OXA-48 üreten patojenlere bağlı enfeksiyonları tedavi etmeye daha uygun antibiyotik rejimlerinin tasarlanmasını sağlayacak ve bu antibiyotiklere karşı direnç gelişimini uzatacaktır. Hangi tedavi kombinasyonunun mükemmel olduğunu ve bu enfeksiyonların tedavisi için kombinasyon tedavisinin en iyi nasıl kullanılacağını *in vitro* belirlemek için daha fazla araştırma yapılması gerekmektedir.

Anahtar Kelimeler: Kolistin, imipenem, kombinasyon, Klebsiella pneumoniae, OXA-48

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Address for Correspondence/Yazışma Adresi: Yücel Duman MD, İnönü University Faculty of Medicine, Department of Medical Microbiology, Malatya, Turkey

Phone: +90 422 341 06 60-4804 E-mail: yucel.duman@inonu.edu.tr ORCID ID: orcid.org/0000-0002-9090-2096

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Introduction

Klebsiella pneumoniae is an encapsulated Gram-negative bacillus that causes a range of infections in humans, ranging from urinary tract infections to life-threatening pneumonia, sepsis, and meningitis. Studies have indicated that it is among the top-five frequent pathogens of healthcare-associated infections, accounting for 5% to 20% of isolates^[1,2]. Recently, emerging therapeutic problems due to increasing and differentiating antimicrobial resistance in this species have been a great concern, especially carbapenem resistance (CR).

Carbapenems are clinically indispensable beta-lactam drugs used in *K. pneumoniae* infections since they have stability to the most beta-lactamases, have successful penetration to the body fluids, are available as monotherapy or combination regimes, lack an age limitation, and have relatively low side effects. However, the rising global incidence of CR-*Klebsiella* phenotypes has brought substantial difficulties in patient management. Oxacillinase-48 (OXA-48), like carbapenemases, are among the most prevalent mechanisms, particularly in Europe, the Middle East, and South America^[1,3]. Today, the European survey of carbapenemase-producing *Enterobacterales* 'EuSCAPE' working group's report classified European countries by epidemiological stage of OX-48-producing *K. pneumoniae* spread (0: cases have been reported, 1: Sporadic, 2: One hospital outbreaks, 3: Regional expansion 4: Interregional extension 5: endemic)^[3].

Colistin (polymyxin E) is a cationic polypeptide that inhibits the synthesis of the outer membrane in the Gram-negative cell wall. This drug was out of use because of its nephrotoxic side effects. It was reintroduced to clinical application against extensively drug-resistant Gram-negatives since no active agent remained. However, recent reports have shown that colistin resistance (CoIR) is increasing worldwide, especially in CR endemic areas^[4-7].

Colistin overuse produces not only nephrotoxic side effects but also increases CoIR. Studies have indicated that CoIR is directly related to the amount of colistin used^[4,6]. Therefore, increased efforts to find optimal combination regimens with colistin reduce colistin nephrotoxicity and decrease CoIR development. For this purpose, several in vitro studies, randomized controlled trials, and observational studies were performed. Though the clinical studies' outcomes remain controversial, the in vitro synergy tests have yielded promising results. The antimicrobial combination studies are often on Klebsiella pneumoniae carbapenemase (KPC), Delhi metallo-beta-lactamase (NDM), Verona integronencoded metallo-beta-lactamase (VIM)-producing CR-K. pneumoniae strains[8-12]. However, little data is available about the effectiveness of carbapenem plus colistin combination against OXA-48-like carbapenemase-producing K. pneumoniae strains, though this is a leading worldwide mechanism of CR.

Oxacillinase-48-like carbapenemases have weak hydrolytic activity on imipenem and meropenem. However, a high-level CR can emerge in strains with reduced outer membrane permeability. Therefore, we conducted this study to assess the potential synergistic activity of imipenem plus colistin, an outer membrane inhibitor, in *K. pneumoniae* strains produced mainly OXA-48.

Materials and Methods

This work was approved by the İnönü University Scientific Research and Publication Ethics Board with decision number 2020/878, date: 30.06.2020.

Bacterial Strains: Source, and Identification and Susceptibility Tests

A total of 37 clinical isolates of CR *K. pneumoniae* that were shown to be clonally unrelated with pulse-field gel electrophoresis were included in the study. All strains were prospectively collected from the patients' clinical samples' bacteriologic cultures in a 1368-bed referral tertiary care hospital between January 2017 and July 2018.

K. pneumoniae isolates were identified according to classic bacteriologic methods and with a Vitek MS (Biomérieux, France) matrix-assisted laser desorption ionization time-of-flight mass spectrometry device. Antimicrobial susceptibility tests were performed with a Vitek-2 (Biomérieux, France) automated device. Minimal inhibitory concentrations (MICs) of imipenem and colistin were studied with standard broth microdilution (BMD). Broth microdilution was performed using 96-well broth microdilution panels according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guide. The BMD MIC test range was determined as 32 μg/ml-0.06 μg/ml for colistin and 0.5-512 μg/ml for imipenem. Results were evaluated according to the EUCAST criteria^[13].

Detection of Carbapenemase and ColR Genes

The presence of carbapenemase and ColR genes were screened using in-house PCR. Carbapenem resistance genes were studied as recommended by Poirel et al.[14]. The presence of the mcr 1-5 gene regions responsible for plasmid-mediated ColR was determined using in-house multiplex PCR, as Rebelo et al.[15] suggested. The primer sets are as follows; OXA-48(F)-5'-GCGTGGTTAAGGATGAACAC-3' and OXA-48 (R)-5'-CATCAAGTTCAACCCAACCG-3' bla_{OXA-48} NDM-1 (F) 5'-GGTTTGGCGATCTGGTTTTC-3' NDM-1 (R) and 5'-CGGAATGGCTCATCACGATC-3 bla_{NDM-1}, **KPC** (F) 5'-CGTCTAGTTCTGCTGTCTTG-3' for (R) 5'-CTTGTCATCCTTGTTAGGCG-3' and **KPC** for VIM 5'-GATGGTGTTTGGTCGCATA-3' bla_{KPC} (F) and bla_{VIM} VIM (R) 5'-CGAATGCGCAGCACCAG-3'

IMP 5'-GGAATAGAGTGGCTTAAYTCTC-3' and IMP(R) 5'-CCAAACYACTASGTTATCT-3' bla_{IMP} 5'-AGTCCGTTTGTTGTGGC-3' mcr-1 mcr-1 (R) 5'-AGATCCTTGGTCTCGGCTTG-3'. mcr-2 (F) 5'-CAAGTGTGTTGGTCGCAGTT-3' mcr-2 (R) 5'-TCTAGCCCGACAAGCATACC-3', mcr-3 (F) 5'-AAATAAAAATTGTTCCGCTTATG-3' and (R) 5'-AATGGAGATCCCCGTTTTT-3'. mcr-3 mcr-4 (F) 5'-TCACTTTCATCACTGCGTTG-3' and mcr-5'-TTGGTCCATGACTACCAATG-3'. (F) (R) mcr-5 5'-ATGCGGTTGTCTGCATTTATC-3' (R) and mcr-5 5'-TCATTGTGGTTGTCCTTTTCTG-3'. For this purpose, the first DNA extraction of strains was performed with a column-based DNA isolation kit (DNA mini kit, Qiagen, Germany). The PCR run was performed using the GeneAmp PCR System 9700 device (Applied Biosystems). Amplicons were photographed with ultraviolet illumination after electrophoresis.

Antimicrobial Combination Test and the Checkerboard Method

A 96-well and U-based sterile microplate was used to perform the antimicrobial combination test and the checkerboard method described by Moody^[16]. The inoculum was prepared by putting 35 colonies in Mueller-Hinton broth from 24-hour cultures and incubating at 37 °C for 2-3 hours. A bacterial suspension of 0.5 McFarland density was prepared. For each antibiotic, double serial dilutions in separate tubes were made with cation-attached Mueller-Hinton broth according to the recommendations of EUCAST. The A1 well was used as a reproductive control, and the H12 well was used as a medium control. So, antibiotics were not added. The MICs of antibiotics were determined using the first horizontal row (an order) for imipenem from two antibiotics in combinations and the first vertical row (column 1) for colistin. Two-fold dilutions of imipenem were distributed from column 2 of the microdilution plate to column 12. Double dilutions of colistin were transferred to the B and H sequences of the microdilution plate for each dilution to be placed in one row. Next, 10 µl of the bacteria suspension was placed in all wells, and the plates were kept at 37 °C for 18 hours. Then, the fats were visually evaluated for each combination.

The MIC values obtained by the checkerboard combination method were evaluated by the fractional inhibitor concentration index (FIC) formula.

The Σ FIC was calculated as; Σ FIC = FIC A + FIC B. In this equation, FIC A is drug A MIC in combination/drug A MIC alone, and FIC B is drug B MIC in the combination/drug B alone MIC. The combination is considered synergistic when the Σ FIC index was \le 0.5, indifference was indicated by a Σ FIC index >0.5 to \le 4, whereas antagonism when the Σ FIC was >4 $^{[17]}$.

Statistical Analysis

Statistical analyses were performed using SPSS for Windows, version 17.0 (IBM-SPSS Inc., Armonk, NY). P values were determined using the Wilcoxon signed ranks test.

Results

A total of 37 CRKP clinical isolates identified from blood were included in the study. According to EUCAST, all K. pneumoniae isolates were resistant to imipenem (MICs 16 to 256 µg/ml). Imipenem MIC₅₀ and MIC₉₀ were determined as 32 μ g/ml and 128 μq/ml, respectively. The distribution of the number of isolates by imipenem MICs is shown in Figure 1. A total 9 of 37 strains were resistant to colistin. Colistin MICs for susceptible 28/37 strains ranged from 0.5-2 µg/ml and for 9 resistant strains from 8 to 32 $\mu\text{g/ml}$ (Figure 2). Colistin $\text{MIC}_{_{50}}$ and $\text{MIC}_{_{90}}$ were determined as 2 μg/ml and 16 μg/ml, respectively. The concentration of MIC values was evaluated in colistin-imipenem combinations. When colistin was fixed at 0.125 μ g/ml, 0.25 μ g/ml, 0.5 μ g/ml, 1 μ g/ml, and 2 µg/ml, significant reductions were observed in imipenem MIC values. Combinations with colistin fixed at 0.125 µg/ml in 5 isolates, at 0.25 μg/ml in 6 isolates, at 0.5 μg/ml in 9 isolates, at 1 μg/ml in 16 isolates, and at 2 μg/ml in 26 isolates imipenem MIC levels were reduced to susceptible MICs according to EUCAST (≤2 μg/ml) (Figure 3). However, imipenem MICs of ColR in seven isolates did not reduce to susceptible MIC levels, even though the colistin MIC was fixed at 2 µg/ml in the combination. But

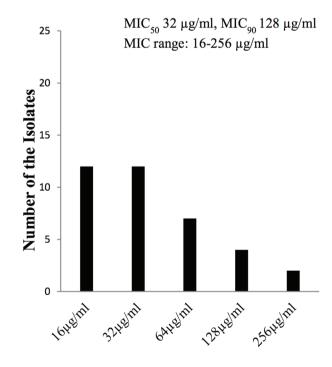


Figure 1. Distribution of the number of isolates according to imipenem MICs. MIC range, $\mathrm{MIC}_{50'}$ and MIC_{90} results

MIC: Minimal inhibitory concentrations

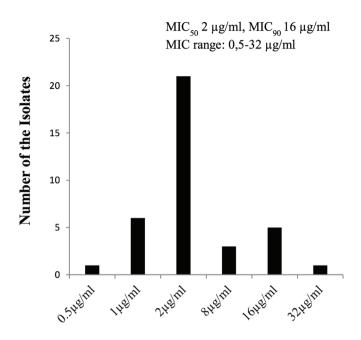


Figure 2. Distribution of the number of isolates according to colistin MICs. MIC range, MIC_{50} , and MIC_{90} results

MIC: Minimal inhibitory concentrations

these isolates imipenem MICs were decreased 2- to 3-fold log₂ dilutions of MICs. Especially in colistin-sensitive isolates, even at a low level of colistin MICs concentration, imipenem MIC levels were reduced 2- to 10-fold log₂ dilutions of MICs (Figure 3).

The 23 (62.2%) of the 37 isolates were synergistic, and 14 (37.8%) of them were determined as indifference according to the FIC formula. Σ FIC was found to range from 0.07 to 1.12, and the mean of Σ FIC was 0.467.

CR gene OXA-48 was detected in all isolates. Furthermore, only OXA-48 producing isolates were included in the study. Regarding the danger of the horizontal spread of ColR, the presence of plasmid-mediated mcr gene regions was investigated. However, the mcr gene regions (1 to 5 mcr genes) associated with plasmid-mediated ColR were not found.

Discussion

The emergence of antibiotic resistance in *K. pneumoniae* is a substantial clinical issue^[2,18]. The failure to discover new effective antibiotics further exacerbates the threat posed by CR-*K. pneumoniae*. On the other hand, few effective drug combinations exist against *K. pneumoniae*, which are extensively drug resistant^[1,2].

The mechanism of resistance to carbapenems usually involves carbapenemase enzymes. In a time-kill experiment, Tängdén et al.^[8] investigated *in vitro* activities of double and triple combinations of 10 antibiotics for 2 VIM- and NDM-producing

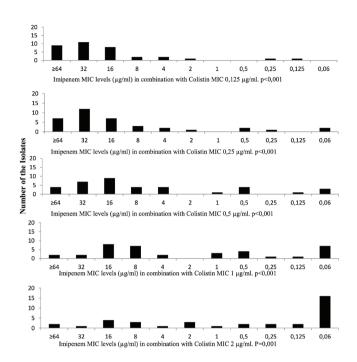


Figure 3. The number of isolates according to imipenem MIC levels in combination at varying colistin levels

MIC: Minimal inhibitory concentrations

CR-K. pneumoniae strains. They reported that the rifampinmeropenem-colistin combination was the most effective regimen with synergistic and bactericidal effects. Another study that included three KPC-producing K. pneumoniae, Lee and Burgess^[9] reported that combinations of colistin sulfate or polymyxin B with doripenem had rapid bactericidal activity for all isolates within four hours. Jernigan et al.[10] found similar results for 12 KPC-producing K. pneumoniae, even though the included strains were ColR. Sharma et al.[11] evaluated the combination of polymyxin B and meropenem in vitro on 10 KPC-producing K. pneumoniae. They found that all isolates were synergistic in combination except one isolate, which was resistant to both drugs at high levels. Machuca et al.[12] reported that combination therapy decreased the mortality in bacteremic patients due to ColR KPC-producing K. pneumoniae with highlevel CR in patients with septic shock. The size of the regions where the OXA-48 enzyme is endemic and where outbreaks occur suggests that the main cause of colistin use in these countries is CR-K. pneumoniae infections that produce OXA-48^[3,19]. However the *in vitro* efficacy of colistin-carbapenem combination therapy against OXA-48 producing K. pneumoniae has little data, and no defined treatment protocol. This requires conducting studies against OXA-48 producing bacteria. In our study, the CR gene OXA-48 was detected in all isolates.

In recent years, polymyxins have re-emerged because of increased frequency-dependent, multidrug-resistant, Gramnegative bacterial infections and inadequate treatment. During

treatment, reducing the colistin dose in combination therapy is important because it is significantly nephrotoxic when used at high doses alone. In clinical practice, colistin-associated nephrotoxicity occurs in approximately 40% of treated patients. The synergistic effectiveness of colistin and imipenem can reduce the nephrotoxic side effect by enabling the use of colistin at lower doses. Also, combination therapy can increase the efficacy of treatments. In addition, effective treatment is important because long-term use of colistin causes the selection of ColR strains in flora and increases the spread of ColR strains among patients with long-term hospitalization. Therefore, this study aims to potentiate the other drug's action dynamics by increasing the permeability to reduce outer membrane synthesis^[4,5,14]. Lee et al.^[20] reported that they used colistin alone to treat 12 patients infected with CR-K. pneumoniae. A significant increase in colistin MIC values was detected quickly, and ColR was improved in three patients. They interpreted this rapid change as reinfection due to the selection of CoIR isolates. However, they did not observe a significant change in the isolates' colistin MIC values of the patients who used the combination treatment. We assessed colistin's in vitro effect on imipenem MIC levels in the combination against OXA-48producing CoIR and/or CR-K. pneumoniae. In our study, colistin $M\dot{I}K_{50}$ 2 µg/ml, $M\dot{I}K_{90}$ 16 µg/ml, imipenem $M\dot{I}K_{50}$ 32 µg/ml, and MİK_{oo} 128 μg/ml were found. In parallel with the increased resistant of isolates, MIC levels increased against antibiotics. In another study, in intensive care units the CR-K. pneumoniae rate reached 36.5%^[21]. Significant reductions were observed in imipenem MIC values, especially in colistin-sensitive isolates. The concentration of MIC values was evaluated in the colistinimipenem combination, when colistin was fixed at 0.125 μg/ ml, 0.25 μg/ml, 0.5 μg/ml, 1 μg/ml, and 2 μg/ml. Furthermore, combinations when the colistin was fixed at 0.125 µg/ml in 5 isolates, at 0.25 μg/ml in 6 isolates, at 0.5 μg/ml in 9 isolates, at 1 μg/ml in 16 isolates, and at 2 μg/ml in 26 isolates, the imipenem MIC levels decreased to susceptible MICs (Figure 3). However, imipenem MICs of seven CoIR isolates did not decrease to susceptible MIC levels, but a 2- to 3-fold log, dilution reduction in MICs occurred. Especially in colistin-sensitive isolates, even at a low level of colistin MICs concentration, imipenem MIC levels were reduced 2- to 10-fold log, dilutions of MICs. Considering the limited number of effective antimicrobials that can be used to treat CR-K. pneumoniae infections, for a while, colistin and carbapenem combination regimens were used for treatment. However, the clinical evidence is mainly based on clinical experience and observational retrospective studies. A few research studies on the in vitro efficacy of combination therapy against CR and/or CoIR-K. pneumoniae were reported (usually on KPC-producing K. pneumoniae). Moreover, other publications report that these isolates are not restricted to only hospitals but are spread in the community^[2,22]. This rapidly growing problem

also reveals the urgent need for new therapeutic approaches to CR-*K. pneumoniae* isolates.

Studies in animal models with experimental treatments suggested using combinations to treat infections caused by CR bacteria^[22]. The US Centers for Disease Control and Prevention estimates that CR isolates are responsible for 9300 (6.6%) of approximately 140,000 nosocomial *Enterobacterales* infections in the United States each year^[23]. Many studies reported lower mortality rates among patients receiving combination therapy (0–40%) than patients receiving monotherapy (40–80%). Recently, combination therapy's positive effect is significant, especially in patients at high risk of death^[24,25].

Combination antibiotic therapy has generally been accepted for several indications, such as infective endocarditis (e.g., ampicillin and gentamicin for Enterococcus), Helicobacter pylori, and mycobacterial infections[26]. The administration of combination antibiotic treatments can also expand the spectrum of antimicrobial effects in empirical therapy. In previous studies on the benefits of combination therapy to treat Gram-negative bacterial infections before the onset of carbapenem and ColR isolates, experts report that combination therapy is beneficial to immunosuppressed patients^[24,25]. In a prospective multicenter study, including 260 patients infected or colonized with CR-K. pneumoniae, Hauck et al.[27] found that 39% of patients died due to sepsis or pneumonia, and hospital stays were prolonged for 5-10 days. In our study, 62.2% of the in vitro combination of imipenem and colistin was determined as synergistic and 37.8% of indifference according to the FIC formula. Also, we found that Σ FIC ranged from 0.07 to 1.12 and had a mean of Σ FIC was 0.467. In their study, Tzouvelekis et al.^[28] 48.6% of patients used combination therapy, 38.1% used monotherapy, and 11% used inappropriate antibiotics (an ineffective antimicrobial used). They reported that the mortality rate of patients receiving monotherapy (carbapenem, tigecycline, or colistin) was not different from patients receiving inappropriate treatment. In their study, Qureshi et al. [29] reported that the most used treatment combinations were carbapenem with colistin or tigecycline combination and found that the mortality of combination therapy (12.5%) decreased significantly compared with monotherapy mortality (66.7%). They also reported that combination therapy was associated with longer survival in carbapenemase-producing *K. pneumoniae* bacteremia.

The antimicrobial activity of colistin on the cell membrane is the displacement of calcium (Ca 2+) and magnesium (Mg 2+), which stabilize the lipopolysaccharides (LPS) membrane in the bacterial cell membrane, leading to irregularity in the cell membrane. This irregularity causes an increase in cell membrane permeability, cell content leakage, and consequently cell death^[30]. Resistance to colistin is related to chromosomal mutations [mutations in the paras/pry two-component system that regulates the

synthesis and structure of LPS)] and plasmid-mediated resistance genes mcr^[15]. Reports on CoIR are rising, and CoIR-K. pneumoniae strains emerge in hospital settings. In recent studies, Liu et al.[5] and Wang et al. [6] reported that the plasmid-mediated CoIR genes prevalence in Enterobacterales is increasing. Colistin resistance prevalence of *Enterobacterales* was reported to range from 0.05% to 1% in Europe^[31]. The studies showed higher prevalence rates in Asian countries compared with Europe. A report from the People's Republic of China described a high prevalence value of 3.5%^[32]. Regardless, the increase in resistance to colistin is terrible. Our study did not find plasmid-mediated resistance genes mcr 1-5, which are particularly important for horizontal transmission. Therefore, we believe resistance to colistin in our isolates was most likely due to chromosomal mutations. However, we could not perform further molecular analyses to identify which specific mutation existed.

While metallo-beta-lactamases have very high hydrolytic activity against carbapenems, OXA-48 enzyme is required, an outer membrane protein defect, and the activity of efflux pumps, to provide clinically significant CR. Therefore, knowing the CR mechanism carried by the strain will help investigate the combination efficacy against resistant Gram-negatives and select antimicrobials for combination in the clinic.

The limitation of the study is that it did not demonstrate the effect of combination therapy in human patients or animals.

Conclusion

In conclusion, these findings demonstrate that imipenem is effective even at very low levels combined with colistin, although isolates are resistant to carbapenem. In addition, colistin is effective at lower MIC levels when used in combination than when used alone. These findings might help clinicians determine the appropriate treatment dose in the combination of carbapenem-colistin. This study may guide the development of more suitable antibiotic regimens to treat infections caused by OXA-48 producing pathogens. Further *in vitro* research is required to determine which treatment combination is best and its optimal use as combination therapy to treat these infections.

Ethics

Ethics Committee Approval: This work was approved by the İnönü University Scientific Research and Publication Ethics Board with decision number 2020/878, date: 30.06.2020.

Informed Consent: Retrospective study.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: Y.D., Concept: Y.D., Ç.K., Design: Y.D., Ç.K., Data Collection or Processing: M.S.T., Y.Y., Analysis or Interpretation: Y.D., Y.Y., Literature Search: Y.D., Y.Y., Writing: Y.D.

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

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References

- Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers among *Enterobacteriaceae* worldwide. Clin Microbiol Infect. 2014;20:821-30.
- 2. Van Duin D, Doi Y. The global epidemiology of carbapenemase-producing *Enterobacteriaceae*. Virulence. 2017;8:460-9.
- Albiger B, Glasner C, Struelens MJ, Grundmann H, Monnet DL, European Survey of Carbapenemase-Producing Enterobacteriaceae (EuSCAPE) working group. Carbapenemase-producing Enterobacteriaceae in Europe: assessment by national experts from 38 countries, May 2015. Euro Surveill. 2015:20.
- Pogue JM, Lee J, Marchaim D, Yee V, Zhao JJ, Chopra, T, Lephart P, Kaye KS. Incidence of and risk factors for colistin-associated nephrotoxicity in a large academic health system. Clin Infect Dis. 2011;53:879-84.
- Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis. 2016;16:161-8.
- Wang Y, Tian GB, Zhang R, Shen Y, Tyrrell JM, Huang X, Zhou H, Lei L, Li HY, Doi Y, Fang Y, Ren H, Zhong LL, Shen Z, Zeng KJ, Wang S, Liu JH, Wu C, Walsh TR, Shen J. Prevalence, risk factors, outcomes, and molecular epidemiology of mcr-1-positive *Enterobacteriaceae* in patients and healthy adults from China: an epidemiological and clinical study. Lancet Infect Dis. 2017;17:390-9.
- Guducuoglu H, Gursoy NC, Yakupogullari Y, Parlak M, Karasin G, Sunnetcioglu M, Otlu B. Hospital Outbreak of a Colistin-Resistant, NDM-1- and OXA-48 Producing Klebsiella pneumoniae: High Mortality from Pandrug Resistance. Microb Drug Resist. 2018;24:966-72.
- Tängdén T, Hickman RA, Forsberg P, Lagerbäck P, Giske CG, Cars O. Evaluation of double- and triple-antibiotic combinations for VIM- and NDM-producing Klebsiella pneumoniae by in vitro time-kill experiments. Antimicrob Agents Chemother. 2014;58:1757-62.
- Lee GC, Burgess DS. Polymyxins and Doripenem Combination Against KPC-Producing Klebsiella pneumoniae. J Clin Med Res. 2013;5:97-100.
- Jernigan MG, Press EG, Nguyen MH, Clancy CJ, Shields RK. The combination of doripenem and colistin is bactericidal and synergistic against colistinresistant, carbapenemase-producing *Klebsiella pneumoniae*. Antimicrob Agents Chemother. 2012;56:3395-8.
- Sharma R, Patel S, Abboud C, Diep J, Ly NS, Pogue JM, Kaye KS, Li J, Rao GG. Polymyxin B in combination with meropenem against carbapenemaseproducing *Klebsiella pneumoniae*: pharmacodynamics and morphological changes. Int J Antimicrob Agents. 2017;49:224–32.
- 12. Machuca I, Gutiérrez-Gutiérrez B, Gracia-Ahufinger I, Rivera Espinar F, Cano Á, Guzmán-Puche J, Pérez-Nadales E, Natera C, Rodríguez M, León R, Castón JJ, Rodríguez-López F, Rodríguez-Baño J, Torre-Cisneros J. Mortality Associated with Bacteremia Due to Colistin-Resistant Klebsiella pneumoniae with High-Level Meropenem Resistance: Importance of Combination Therapy without Colistin and Carbapenems. Antimicrob Agents Chemother. 2017;61:e00406-17.
- European Committee on Antimicrobial Susceptibility Testing. Last Accessed Date: 22.01.2021. Available from: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_8.1_Breakpoint_Tables.pdf

- Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis. 2011;70:119-23.
- Rebelo AR, Bortolaia V, Kjeldgaard JS, Pedersen SK, Leekitcharoenphon P, Hansen IM, Guerra B, Malorny B, Borowiak M, Hammerl JA, Battisti A, Franco A, Alba P, Perrin-Guyomard A, Granier SA, De Frutos Escobar C, Malhotra-Kumar S, Villa L, Carattoli A, Hendriksen RS. Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5 for surveillance purposes. Euro Surveill. 2018;23:17-00672.
- Moody J. Synergism testing; broth microdilution checkerboard and broth macrodilution methods. In: Isenberg HD (ed). Clinical Microbiology Procedures Handbook. Washington, DC: ASM Press, 1992.
- Orhan G, Bayram A, Zer Y, Balci I. Synergy Tests by E test and Checkerboard methods of antimicrobial combinations against *Brucella melitensis*. J Clin Microb. 2005;43:140-3.
- Duman Y, Kuzucu C, Cuglan SS. Bacteria Isolated from Blood Cultures and Their Antimicrobial Susceptibility. Erciyes Med J. 2011;33:189-96.
- Duman Y, Ersoy Y, Gursoy NC, Altunisik Toplu S, Otlu B. A silent outbreak due to Klebsiella pneumoniae that co-produced NDM-1 and OXA-48 carbapenemases, and infection control measures. Iran J Basic Med Sci. 2020;23:46-50.
- Lee CR, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH. Global Dissemination of Carbapenemase-Producing *Klebsiella pneumoniae*: Epidemiology, Genetic Context, Treatment Options, and Detection Methods. Front Microbiol. 2016;13:895.
- Duman Y, Kuzucu C, Tekerekoğlu MS, Cakil B, Yakupoğullari Y, Kaysadu H. Changing trends of carbapenem resistance of *Escherichia coli* and *Klebsiella pneumoniae* strains isolated from intensive care units, inpatient services and outpatient's clinics: a five years retrospective analysis. Med Scien. 2018;7:536-9.
- Woodford N, Wareham DW, Guerra B, Teale C. Carbapenemase-producing *Enterobacteriaceae* and non-*Enterobacteriaceae* from animals and the environment: an emerging public health risk of our own making? J Antimicrob Chemother. 2014;69:287-91.
- US Centers for Disease Control and Prevention. Antibiotic/antimicrobial resistance: biggest threats. (2013) Atlanta (GA): CDC. Available from: https://www.cdc.gov/drugresistance/threat-report-2013/pdf/arthreats-2013-508.pdf

- 24. Giannella M, Trecarichi EM, Giacobbe DR, De Rosa FG, Bassetti M, Bartoloni A. Effect of combination therapy containing a high-dose carbapenem on mortality in patients with carbapenem-resistant *Klebsiella pneumoniae* bloodstream infection. Int J Antimicrob Agents. 2018;51:244-8.
- Jacobs DM, Safir MC, Huang D, Minhaj F, Parker A, Rao GG. Triple combination antibiotic therapy for carbapenemase-producing *Klebsiella* pneumoniae: a systematic review. Ann Clin Microbiol Antimicrob. 2017;16:76.
- Petrosillo N, Giannella M, Lewis R, Viale P. Treatment of carbapenemresistant *Klebsiella pneumoniae*: the state of the art. Expert Rev Anti Infect Ther. 2013;11:159–77.
- Hauck C, Cober E, Richter SS, Perez F, Salata RA, Kalayjian RC, Watkins R, Scalera NM, Doi Y, Kaye KS, Evans S, Fowler Jr VG, Bonomo RA,van Duin D, Antibacterial Resistance Leadership Group. Spectrum of excess mortality due to carbapenem-resistant *Klebsiella pneumoniae* infections. Clin Microbiol Infect. 2016;22:513–9.
- Tzouvelekis LS, Markogiannakis A, Piperaki E, Souli M, Daikos GL. Treating infections caused by carbapenemase-producing *Enterobacteriaceae*. Clin Microbiol Infect. 2014;20:862-72.
- 29. Qureshi ZA, Paterson DL, Potoski BA, Kilayko MC, Sandovsky G, Sordillo E, Doi Y. Treatment outcome of bacteremia due to KPC-producing *Klebsiella pneumoniae*: superiority of combination antimicrobial regimens. Antimicrob Agents Chemother. 2012;56:2108-13.
- Newton BA. The properties and mode of action of the polymyxins. Bacteriol Rev. 1956;20:14–27.
- Principe L, Piazza A, Mauri C, Anesi A, Bracco S, Brigante G, Casari E, Agrappi C, Caltagirone M, Novazzi F, Migliavacca R, Pagani L, Luzzaro F. Multicenter prospective study on the prevalence of colistin resistance in Escherichia coli: relevance of mcr-1-positive clinical isolates in Lombardy, Northern Italy. Infect Drug Resist. 2018;11:377-85.
- 32. Bi Z, Berglund B, Sun Q, Nilsson M, Chen B, Tärnberg M, Ding L, Lundborg CS, Bi Z, Tomson G, Yao J, Gu Z, Yin X, Kou Z, Nilsson LE. Prevalence of the mcr-1 colistin resistance gene in extended-spectrum β-lactamase-producing *Escherichia coli* from human faecal samples collected in 2012 in rural villages in Shandong Province, China. Int J Antimicrob Agents. 2017;49:493-7.