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RESEARCH ARTICLE

Molecular Investigation of Carbapenem and Colistin Resistance Mechanisms in *Klebsiella pneumoniae* Bloodstream Isolates

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SUMMARY

Carbapenem-Resistant Klebsiella pneumoniae (CRKp) infections are worrying health problems due to decreasing treatment options. This study investigates the carbapenemase (OXA-23,24, 48, 51, 55, 58, KPC, NDM-1, VIM, IMP) and mcr-1 genes of the CRKps isolates A total of 33 CRKp isolates isolated from patient blood samples from the Dicle University Medical Faculty Hospital, intensive care units (ICUs) between February 2020 and June 2020, were included in the study. The presence of carbapenemase encoding genes -including all CRKp isolates, bla OXA-23, 24, 48, 58, bla KPC, blaNDM-1, bla VIM, bla IMP- were investigated by multiplex Polymerase Chain Reaction (PCR). CRKp isolates were tested for mcr-1 gene and bla OXA-51, bla OXA-55 genes by monoplex PCR. All CRKp isolates studied with Kirby Bauer Disc Diffusion Method (DDM) (100%) were resistant to ertapenem, 9 (27.27%) resistant to imipenem, and 23 (69.70%) were resistant to meropenem. 20 (60.61%) of the isolates were found resistant to colistin. bla OXA-48, bla NDM-1 and bla OXA-24 genes were found in 75.76% (n = 25), 6.06% (n = 2) and 3.03% (n = 1) isolates, respectively. Both bla OXA-48 and bla NDM-1 genes were detected in two (6.06%) isolates and mcr-1 gene in 16 (48.48%) isolates. While the mean hospitalization was 20.3 days in 13 patients with a colistin minimum inhibitory concentration (MIC) of 2 µg/ml, it was 33.9 days in 20 patients with a colistin MIC of > 2 µg/ml. The average length of stay in the hospital was 21.8 days in mcr-1 negative patients and 35.7 days in mcr-1 positive patients. Carbapenemase and mcr-1 positivities were found at dramatically high rates in Diyarbakır, Turkey. It was indicated that plasmid-mediated antimicrobial resistance in Kp isolates was problematic. Each hospital should monitor the colistin and carbapenem resistance mechanisms by molecular methods. Colistin resistance should be confirmed by the broth microdilution method (BMD).

Key Words: *Klebsiella pneumoniae, carbapenemase, bla OXA-48, mcr-1, multiplex PCR, broth microdilution.*

Klebsiella Pneumoniae Kan İzolatlarında Karbapenem ve Kolistin Direnç Mekanizmalarının Moleküler Olarak İncelenmesi

ÖZ

Karbapenem Dirençli Klebsiella pneumoniae (KDKp) enfeksiyonları azalan tedavi seçenekleri nedeniyle endişe verici sağlık sorunları oluşturmaktadır. Bu çalışmada, KDKp izolatlarının karbapenemaz (OXA-23,24, 48, 51, 55, 58, KPC, NDM-1, VIM, IMP) ve mcr-1 genleri araştırılmaktadır. Dicle Üniversitesi Tıp Fakültesi Hastanesi yoğun bakım ünitelerinden (YBÜ) Şubat 2020 ile Haziran 2020 tarihleri arasında alınan hasta kan örneklerinden izole edilen toplam 33 KDKp izolatı çalışmaya dahil edildi. Tüm KDKp izolatları, bla OXA-23, 24, 48, 58, bla KPC, blaNDM-1, bla VIM, bla IMP dahil olmak üzere karbapenemaz kodlayan genlerin varlığı multipleks Polimeraz Zincir Reaksiyonu (PZR) ile araştırıldı. KDKp izolatları monoplex PZR ile mcr-1 geni ve bla OXA-51, bla OXA-55 genleri için test edildi. Kirby Bauer Disk Difüzyon Test (DDT) (%100) ile çalışılan tüm KDKp izolatları ertapenem'e dirençliydi; 9'u (%2.27) imipeneme, 23'ü (%69.70) meropeneme dirençliydi. İzolatların 20'si (%60,61) kolistine dirençli bulundu. bla OXA-48, bla NDM-1 ve bla OXA-24 genleri sırasıyla %75.76 (n=25), %6.06 (n=2) ve %3.03 (n=1) izolatında bulundu. İki (%6.06) izolatta hem bla OXA-48 hem de bla NDM-1 genleri, 16 (%48,48) izolatta mcr-1 geni saptandı. Kolistin minimum inhibitör konsantrasyonu (MİK) değeri 2 µg/ml olan 13 hastada ortalama yatış süresi 20.3 gün iken, kolistin MİK değeri > 2 µg/ml olan 20 hastada 33.9 gündü. Hastanede ortalama kalış süresi mcr-1 negatif hastalarda 21.8 gün, mcr-1 pozitif hastalarda 35.7 gündü. Karbapenemaz ve mcr-1 pozitiflikleri Diyarbakır, Türkiye'de çarpıcı biçimde yüksek oranlarda bulundu. Kp izolatlarında plazmit aracılı antimikrobiyal direncin sorunlu olduğu belirtildi. Her hastane moleküler yöntemlerle kolistin ve karbapenem direnç mekanizmalarını izlemelidir. Kolistin direnci sıvı mikrodilüsyon yöntemi ile doğrulanmalıdır.

Anahtar Kelimeler: *Klebsiella pneumoniae, karbapenemaz, bla OXA-48, mcr-1, multipleks PZR, sıvı mikrodilüsyon.*

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INTRODUCTION

Antibiotic resistance of Gram-negative bacteria has become a concern for public health. Patients with Multi-Drug Resistant (MDR) bacterial infections have to undergo intravenous treatment in the hospital, as there are no effective oral medications. Resistance in empirical antibiotic therapy results in increased mortality rates, prolonged hospital stay, difficulty in treatment, and higher costs.

K. pneumoniae is a Gram-negative bacteria belonging to the Enterobacterales order. As being a part of the healthy human microbiome, this microorganism is colonized in the gut and other parts of the human body. It can cause bloodstream, urinary tract infections, and severe pneumoniae, especially in critically ill or immune-compromised patients, neonates, or patients with risk factors in hospital settings. The frequency of Extended-spectrum β-lactamase (ESBL)-producing *K. pneumoniae* (Kp) has raised substantially (Knother, 1983; Pitout, 2006). ESBL-producing *Kp has* been reported as resistant to cephalosporins and various antibiotics, including quinolones (Mugnaioli, 2006). Therefore, carbapenems have been proposed for the treatment of ESBL-producing Kp infections. Recently, CRKp increased globally over the years. CRKp infections pose a significant threat to human health, as the appropriate treatment options are limited and the mortality rate is relatively high. Due to the increase in CRKp outbreaks in hospitals worldwide, many studies are carried out to reveal the resistance mechanisms. Two main mechanisms mediate carbapenem resistance. First, CRKp isolates may produce β-lactamase enzymes along with reduced cell wall membrane permeability (Leavitt, 2009). The second mechanism is the synthesis of beta-lactamases which can hydrolyze most beta-lactams, including carbapenems. According to the Ambler classification, these carbapenemases are divided into three groups; class A (*K. pneumoniae* carbapenemase, KPC), class B (Metallo-β-lactamases, NDM), and class D (oxacilinases, OXA-48-like) (Pitout, 2015). Under these circumstances, colistin has become the antibiotic of last resort for CRKp infections (Perez, 2013).

Colistin (polymyxin E) is a polypeptide antimicrobial among polymyxin agents (polymyxin B and E) (Bergen, 2006). It has been kept as a backup agent for a long time due to severe nephrotoxicity and neurotoxicity problems, and less toxic antibiotics were preferred (Beringer, 2001). The alarming increase in the prevalence of MDR Gram-negative bacteria has led to the reassessment of colistin as a viable treatment option, especially in critically ill patients. Colistin resistance has been reported worldwide secondary to the increase in the use of colistin in the treatment of MDR bacterial infections (Landman, 2008). Previously, it was known that colistin resistance was coded by chromosomal genes. However, plasmid-mediated mobilized colistin resistance (mcr) genes (mcr-1 and its variants) have been reported to lead to colistin resistance. The original variant mcr-1 can make horizontal transitions among various strains of a bacterial species. The potential for mcr genes to spread rapidly across strains raises concerns regarding the use of colistin as a last-resort therapeutic option. Infections of MDR isolates are frequently encountered in hospitalized patients of ICUs. Infections caused by MDR isolates are frequently encountered, especially in patients hospitalized in intensive care units. If these isolates become resistant to colistin, treatment of serious infections such as bacteremia in intensive care patients will be difficult, mortality will increase, and outbreaks will occur in the hospital (Ling, 2020).

In this study, we aimed to investigate carbapenemase genes responsible for carbapenem resistance and *mcr-1* gene among CRKp isolates. It was also aimed to determine the MIC values of colistin with the BMD method, to compare it with the automated system for ensuring the selection of the appropriate antimicrobial treatment.

MATERIAL AND METHODS

A total of 33 non-duplicated CRKp isolates were included from hospitalized ICU patients in Dicle University Medical Faculty Hospital between February 2020, and June 2020 were included in the study. The Non-Interventional Clinical Research Ethics Committee of Dicle University Faculty of Medicine approved the study (no:56/2020).

The identification of bacteria grown in media was performed using mass spectrometry by Maldi Biotyper version 1.3 (Bruker Daltonics, Germany). Antimicrobial susceptibilities of the identified strains were performed by BD Phoenix 100 (Becton Dickinson, USA) automated microbiology system with Phoenix 100 NMIC panel.

Isolates resistant to at least one of the carbapenems (meropenem, imipenem, ertapenem) were confirmed with DDM. Imipenem (Oxoid, England), meropenem (Oxoid, England), and ertapenem (Oxoid, UK) discs with 10 µg content each were used for DDM. The minimum inhibitory concentration (MIC) values and the zone diameters were evaluated according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (EUCAST, 2019).

The antimicrobial susceptibility of colistin was performed with the broth microdilution (BMD) method according to EUCAST. The lowest antimicrobial concentration preventing growth was taken as the MIC value. Accordingly, those with MIC values > 2 mg / L were considered resistant to colistin, while those 2 mg / L were deemed to be sensitive to colistin. *E. coli* ATCC 25922, mcr-1 positive *E. coli* NCTC 13846, and *P. aeruginosa* ATCC 27853 were used as quality control strains according to the recommendations of EUCAST (EUCAST, 2019).

Isolates were stored in Tryptic Soy Broth with 16% glycerol (Merck, Germany) medium at -20 °C until molecular study. DNAs of CRKp isolates were extracted by boiling method. Subsequently, two separate multiplex PCR were performed for *bla* OXA-23, 24, 48,58 genes and for *bla* KPC, *bla* NDM-1, *bla* VIM, *bla* NDM-1, *bla* IMP genes. Monoplex PCR was performed for *bla* OXA-51, *bla* OXA-55, and *mcr-1* gene (12). Table 1 demonstrates the primers used in this study (Table 1.) (Arabaci, 2019).

Table1. The properties of primers used in the study

Oligoname	Base pairs 5'-3'	Amplicon length (bp)
CLR5	F: CGGTCAGTCCGTTTGTTC R: CTTGGTCGGTCTGTAGGG	305
OXA-23	F:GATCGGATTGGAGAACCAGA R: ATTTCTGACCGCATTTCCAT	501
OXA-24	F: GGTTAGTTGGCCCCCTTAAA R:AGTTGAGCGAAAAGGGGATT	246
OXA-48	F: TTGGTGGCATCGATTATCGG R: GAGCACTTCTTTTGTGATGGC	743
OXA-51	F: TAATGCTTTGATCGGCCTTG R: TGGATTGCACTTCATCTTGG	353
OXA-55	F: CATCTACCTTTAAAATTCCC R: AGCTGTTCCTGCTTGAGCAC	975
OXA-58	F: AAGTAT TGGGGCTTGTGCTG R: CCCCTCTGCGCTCTACATAC	599
IMP	F: CATGGTTTGGTGGTTCTTGT R: ATAATTTGGCGGACTTTGGC	488
VIM	F: ATTGGTCTATTTGACCGCGTC R: TGCTACTCAACGACTGAGCG	780
NDM-1	F: GAGATTGCCGAGCGACTTG R: CGAATGTCTGGCAGCACACTT	497
KPC	F: ATGTCACTGTATCGCCGTCT R: TTTTCAGAGCCTTACTGCCC	893

PCR conditions were as follows; final volume was 25 µl, initial denaturation at 94° C for 4 minutes, a total of 40 cycles including 94° C for 30 seconds, a final extension step of 40 seconds at 52° C and 50 seconds at 72°C, 10 minutes at 72 C. Gel DocTM XR + (Bio Rad, USA) device was used for imaging. Colistin resistant *mcr-1* positive *E. coli NTCC 13846, K. pneumoniae* NCTC 13443 (NDM-1), *E. coli* NCTC 13476 (IMP), *A. baumannii* NCTC 13301 (OXA-23) and *A. baumannii* ATCC 19606 (OXA-51), *A. baumannii* NCTC 13302 (OXA-58), *K. pneumoniae* NCTC 13442 (OXA-48), *K. pneumoniae* NCTC 13439 (VIM-1) strains were used.

RESULTS AND DISCUSSION

Results of the Patients' Data

Of the 33 CRKp isolates included in the study, 19 (58%) were isolated from females and 14 (42%) from male patients. The average age of the female patients was 55.4, while of the male patients was 47.4. All of the patients were hospitalized in ICUs of the hospital including 8 (24.24%) chest diseases, 7 (21.21%) internal medicine, 5 (15.15%) pediatrics, 4 (12.12%) neurology, 3 (9.09%) hematology, 2 (0.06%) cardiology, 2 (0.06%) anesthesia and reanimation, 1 (0.03%) emergency and 1 (0.03%) gastroenterology departments.

According to the BMD, the mean hospitalization duration in 13 patients with 2 µg / ml colistin MIC value was 20.3 days, in 20 patients with > 2 µg / ml colistin MIC was 33.9 days. The mean hospitalization period of 8 patients with 32 µg / ml MIC values was 37.63 days; 6 patients with a MIC value of 64 µg / ml was 34.2 days. The average length of stay in the hospital was 21.8 days in *mcr-1* negative patients; 35.7 days in *mcr-1* positive patients. Nine (27.3%) patients died while they were hospitalized, and all of the isolates of these patients were resistant to colistin by BMD. In addition, 5 of the deceased patients had a colistin MIC of 64 µg/ml, 2 of them 32 µg/ml, 1 of them 16 µg/ml and 1 of them 4 µg/ml. Of these 9 patients, 7 were mcr-1 positive. The mortality rate was 43.75% (n=7) in mcr-1 positives (n=16) and 11.8% (n=2) in mcr-1 negatives (n=17). While the mortality rate of colistin-resistant patients (>2 µg/ml) according to BMD was 45% (n=9), no death was detected in susceptible patients.

Results of Antimicrobial Susceptibility Methods

The antibiotic susceptibility testing results by automated system and disk diffusion test:

piperacillin-tazobactam 93.94% (n=31) R (resistant), 6.06% (n=2) S (sensitive); amikacin % 69.70 (n = 23) R, 30.30% (n = 10) S; gentamicin 66.67% (n = 22) R, 33.33% (n = 11) S; ciprofloxacin 84.85% (n = 28) R, 15.15% (n = 5) S; ceftazidime 90.91% R (n = 30), 9.09% (n = 3) S; 87.88% R (n = 29), 9.09% (n = 3) S and 3.03% (n = 1) I for cefepime (moderately sensitive); 87.88% (n = 29) R, 12.12% (n = 4) S for trimethoprim-sulfamethaxazole; 48.48% (n = 16) R for colistin, 51.52% (n = 17) S; and 96.97% (n = 32) R for ertapenem, 3.03% (n = 1); 60.61% (n =20) R, 3.03% (n = 1) S and 36.36% (n = 12) I for imipenem; for meropenem, 84.85% (n = 28) R, 9.09 % (n = 3) S and 6.06 % (n = 2).

Distribution of the numbers of isolates according to colistin MIC values is given in (Figure 1.). The MIC values of colistin by BMD method were 0.25 µg/ml (n=6), 1.00 µg/ml (n=4), 2.00 µg/ml (n=3), 4.00 µg/ml (n=2), 8.00 µg/ml (n=2), 16.00 µg/ml (n=2), 32 µg/ml (n=8), and 64 µg/ml (n=6) (Figure 1).

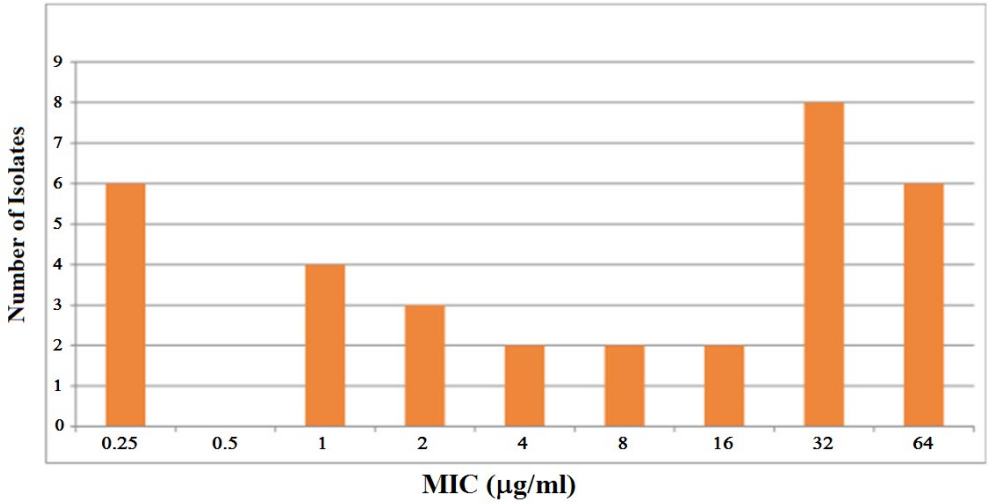

Figure 1. Distribution of isolate numbers according to Colistin MIC values determined by BMD method.

Results of Molecular Methods

PCR Findings of Carbapenemase Genes: The carbapenemase genes detected by PCR method of 33 KDKp isolates studied in total are shown in Figure 2. These rates were 75.76% (n = 25) for *bla* OXA-48, 6.06% for *bla* NDM-1 (n=2), and 3.03% (n=1) for *bla* OXA-24. Isolates from *bla* OXA-23, *bla* OXA-51, *bla* OXA-55, *bla* OXA-58, *bla* KPC, *bla* VIM, and *bla* IMP was detected. Two (6.06%) isolates and *bla* OXA-48 both *bla* NDM-1 genes, were carrying each other (Figure 2.).

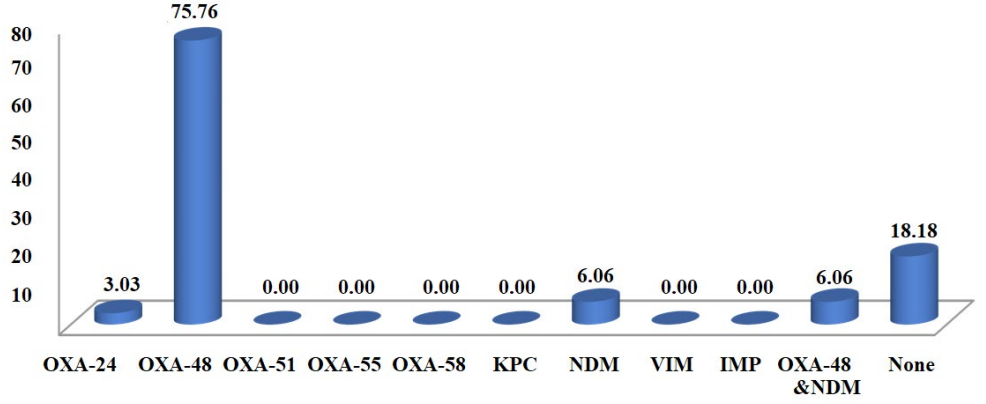

Figure 2. Percentage distribution of carbapenemase genes detected by PCR methods of CRKp 33 isolates.

Of the 33 CRKp isolates, 16 (48.48%) were found to be *mcr-1* positive, and 17 (51.52%) were found to be *mcr-1* negative. The numbers of isolates with mcr-1 positivity according to the carbapenemase positivity are shown in (Table 2.).

Table 2. The numbers of isolates with mcr-1 positivity according to carbapenemase positivity

Carbapenemase	*mcr-1* (+)	*mcr-1* (-)
OXA-48	15	10
OXA-24	0	1
NDM-1	0	2
Negative	1	6

Comparison of Phenotypic and Molecular Methods

Among the 25 *bla* OXA-48 gene positive isolates all (100%) were ertapenem, and 11 (44%) were imipenem; 21 (84%) were meropenem, 23 (93.54%) piperacillin-tazobactam, 22 (70.97 %) amikacine, 16 (64%) gentamicin, 23 (92%) ciprofloxacin, 24 (96%) ceftazidime, 24 (96%) cefepime and 14 (56%) colistin resistant. Among the *bla* OXA-48 gene positive isolates, 15 (48.39%) were found to carry the *mcr-1* gene, simultaneously.

Twenty-two (66.67%) of 33 CRKp isolates were susceptible to amikacin and/or gentamicin. Out of the colistin-resistant isolates, 55% (n:11) were susceptible to amikacin and/or gentamicin. Nine (56.35%) of mcr-1 positive isolates (n = 16) were found sensitive to amikacin and/or gentamicin.

In the study, the number of colistin-resistant isolates by the automated system was 16 and by BMD method was 20. Four isolates that were susceptible to colistin by the automated system were resistant to BMD.

Among 16 *mcr-1* gene-positive isolates, colistin resistance was detected in 14 (87.50%) isolates by BMD. Of 17 the *mcr-1 gene* negative isolates, 6 (35.29%) isolates were resistant against colistin by BMD.

Discussion

The *mcr-1* positive results (48.48%) obtained were found to be well above the reported results from other studies (Arabaci, 2019; Yildiz, 2021; Karki, 2021). PCR results may also be false-positive and must be confirmed by sequencing. Besides, this condition can originate from regional differences in the patients treated in Dicle University Hospitals. In the study, the majority of the patients receiving treatment at Dicle University Hospitals were from Diyarbakir and its surrounding provinces and immigrants from Syria.

Colistin-resistant *Klebsiella pneumoniae* isolates are an important health problem worldwide. In our study, 9 (27.3%) patients died, and all of the isolates of these patients were resistant to colistin. Additionally, eight of the isolates belonging to these patients' colistin MIC was ≥16 μg/ml, and seven were *mcr-1* positive. In our study, the mortality rate was 45% in colistin-resistant patients and 43.75% in mcr-1 positives; the average length of stay in the hospital was 33.9 days in patients with MIC> 2 μg / ml Kp isolates and 37.63 days in patients with MIC values are> 32 μg / ml Kp. The average hospital stay was 21.8 days in mcr-1 negatives; 35.7 days in *mcr-1* positives. Giacobbe et al. reported that in patients with risk factors such as the presence of colistin resistance and hospitalization duration of more than 30 days in ICUs, the mortality rate was 51% (Giacobbe, 2015). Our data and literature data indicated that the length of hospitalization and mortality rate was higher in colistin-resistant Kp and/or *mcr-1* positive Kp isolated patients.

All isolates in our study were ESBL positive, and cefepime resistance was 87.88%. Amikacin and/or gentamicin are antibiotics that can be used as a combined therapy in colistin/carbapenem-resistant Kp infection due to adverse effects, including nephrotoxicity (Vardakas, 2012). Our study showed that the significant majority of the isolates were resistant against amikacin and/or gentamicin, but approximately %30

of the isolates were susceptible. These results indicated that amikacin and/or gentamicin could be an alternative drug in combined therapy in antimicrobial-resistant infections.

Colistin resistance in Kp isolates has been reported worldwide. Colistin resistance rates in Kp isolates were reported as 10.5-20% in Greece, 6.8% in South Korea, 6.3% in Singapore (Ah, 2014). In the last ten years, colistin resistance in Kp isolates from different hospitals has increased tremendously (from 6% to 75.6%) in Turkey (Aris, 2020). In our study, the colistin resistance rate was 60.61% by BMD, and colistin resistance was an important problem in our hospital.

BMD method was recommended for testing the colistin resistance by EUCAST due to false results can be obtained by gradient tests and automated processes (Jayol, 2015). The study of Poirel et al. reported that the rate of false-sensitive results was 15% with the automated system (Poirel, 2017). In our study, four of 16 isolates that were susceptible to colistin by the automated system were found to be resistant to BMD. The false sensitivity finding of 18.75% in our study was compatible with the literature, and the automated system was found to be inadequate in detecting some colistin-resistant strains. Our data clearly indicated that colistin susceptibility should be confirmed by BMD.

In our study, 16 isolates were *mcr-1* positive 17 isolates were *mcr-1* negative. In addition to this, 20 isolates were colistin-resistant by BMD. Of 20 colistin-resistant isolates, 14 isolates were *mcr-1* positive, and 6 were *mcr-1* negative. However, among the 16 *mcr-1* positive isolates, 14 isolates were colistin-resistant, and two isolates were colistin susceptible by BMD. Our data showed that chromosomal resistance mechanisms and other mcr variants should be investigated in Kp isolates in further studies (Hadjadj, 2019). Genome analysis studies showed that in strains with *mcr-8* gene, different antibiotic resistance genes (the beta-lactams, aminoglycosides, sulfonamides, fluoroquinolones) could be seen together (Hadjadj, 2019). *mcr-1* positivity in colistin susceptible Kp isolates showed that *mcr-1* and *mcr* gene variants screening of colistin susceptible Kp isolates could be recommended for controlling of infection and spreading of *mcr-1* gene by the plasmid.

OXA-48-producing *K. pneumoni*ae infections are common in Turkey, Europe, Middle East, and Africa with the rate of %10-72 (Aktaş, 2008; Carrër, 2010; Davarcı, 2019). In our study OXA-48 positivity rate was 75.76%. The OXA-48 beta-lactamase enzyme can successfully hydrolyze penicillins but shows weak or no activity against broad-spectrum cephalosporins (such as ceftazidime, cefotaxime, cefepime), carbapenems. In this study, among 25 (93.94%) isolates whose OXA-48 gene was detected, all were resistant to ertapenem, 44% to imipenem, 84% to meropenem, 56% of to colistin, and antibiotic resistance rates were more than 55 % in OXA-48 positive Kp isolates. *Neuner et al.* In their study, the colistin, amikacin, and gentamicin sensitivities of OXA-48 positive isolated from blood samples of patients hospitalized in ICUs were found to be 86%, 45%, and 22%, respectively (Neuner, 2011).

In two multicenter studies in Turkey NDM-1 ratio was 7.25% and 6.5% (Grundmann, 2017; Çakar, 2016). In the study of Çakar et al., the rate of NDM + OXA-48 coexistence was found to be 2.4% (Çakar, 2016). In our study, this rate was 6.06% and is within similar limits. In EUSCAP study (European Survey on Carbapenemas of Producing Enterobacteriaceae) of Turkey centers in a different region, in 124 Kp isolates, two isolates (1.6%) were IMP positive (Çakar, 2016). KPC-producing isolates have been found mainly in Greece, the USA, Israel, Turkey (24,26, current study). VIM, IMP and KPC were not detected in our study, but this plasmid-mediated enzyme poses a problem for our country.

CONCLUSION

Consequently, *K. pneumoniae* with colistin and carbapenem resistance is a significant public health problem in ICUs and hospitals in Turkey. OXA-48 beta-lactamase is the most commonly reported carbapenemase enzyme type in our country. Colistin resistance and mcr-1 positivities were the risk factors for extended hospitalization and high mortality. Automated systems may be insufficient to detect colistin sensitivity, and these results should be confirmed by BMD. *mcr-1* gene can also be detected in colistin susceptible isolates. For the controlling of plasmid-mediated spread, screening of the *mcr-1* and *mcr* variant genes is helpful in both colistin-resistant and susceptible isolates.

CONFLICT OF INTEREST

All the authors of this article declared no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT

Developing hypothesis and experimenting (Genişel, N., Dal, T., Özcan, N.), preparing the study text and literature research (Genişel, N., Gül, K.), analysis and interpretation of the data (Genişel, N., Dal, T.), designed the concept and drafted the manuscript (Genişel, N., Özcan, N., Dal, T.), prepared the figures (Genişel, N., Kenar, L.), held ethical approval and collected relevant samples and clinical data (Özcan, N., Gül, K., Akpolat, N., and Atmaca, S.,), carried out the laboratory applications of this study (Genişel, N., Dal, T.), reviewed the existing journal policy (Altanlar, N., Kenar, L.), contributed to the writing of the final version of the manuscript (Dal, T., Kenar, L.).

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