



AKADÉMIAI KIADÓ

Acta Microbiologica et
Immunologica Hungarica

68 (2021) 3, 183–188

DOI:

10.1556/030.2021.01458

© 2021 Akadémiai Kiadó, Budapest

ORIGINAL RESEARCH PAPER



*Corresponding author.

E-mail: serapsuzuk@gmail.com



Discrepancy between colistin and polymyxin B susceptibility results among *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates

SERAP SÜZÜK YILDIZ^{1*} , CAN HÜSEYİN HEKİMOĞLU¹,
ZEKIYE BAKKALOĞLU¹ and EMINE ALP²

¹ Department of Microbiology Reference Laboratory and Biological Products, Ministry of Health
General Directorate of Public Health, Ankara, Turkey

² Ministry of Health Ankara, Turkey

Received: March 31, 2021 • Accepted: April 21, 2021

Published online: May 17, 2021

ABSTRACT

The selection of therapeutic agent to be used for the treatment of multidrug-resistant bacteria is a major concern. Polymyxin B use has been commenced in Turkey, although its clinical breakpoint is not listed in the EUCAST. This study aimed to determine the correlation between the MIC values of polymyxin B and colistin. A total of 505 isolates, including 122 isolates of *Escherichia coli* and 383 isolates of *Klebsiella pneumoniae* were included in the present study. All the isolates were assessed for colistin and polymyxin B using the broth microdilution method. The categorical agreement in the *E. coli* isolates was 98.4%, and the rate of very major error was 33.3%. The categorical agreement in the *K. pneumoniae* isolates was 99.5%, the rate of major error was 0.36%, and the rate of very major error was 0.98%. In the evaluation of the essential agreement, 1.6% error in *E. coli* and 2.3% error in *K. pneumoniae* were observed. It was concluded that polymyxin B should never be used in the treatment of the isolates reported as colistin-resistant, and if the MIC values are above 4 mg/L in *E. coli* and *K. pneumoniae*. Our results indicate importance of reporting both polymyxin B and colistin susceptibility results of clinical isolates.

KEYWORDS

colistin, polymyxin b, breakpoint, *E. coli*, *K. pneumoniae*, carbapenem resistant

INTRODUCTION

Antibiotic resistance is among the most worrisome public health concerns worldwide, and this issue is becoming increasingly complex with the accelerating spread of multidrug-resistant microorganisms. The organisms exhibiting multi-drug resistance may be eliminated using a limited number of antibiotics, one among them is the polymyxin group of antibiotics. The polymyxin antibiotics had been discontinued years ago due to their significant side effects and have now emerged at the forefront for the treatment of infections caused by multidrug-resistant microorganisms. The effectiveness of polymyxins in working against *Acinetobacter* spp., *Pseudomonas* spp. and carbapenem-resistant Enterobacterales, which are all multi-drug resistant microorganism groups, confers them an advantageous edge over the other antibiotics [1]. In addition, owing to the sluggish development and introduction of novel antibiotics into the treatment protocols, the use of polymyxins is being continued throughout the world despite their side effects. Although polymyxins have demonstrated limiting properties in pharmacokinetic, pharmacodynamic and toxicodynamic studies, their clinical application is widespread [2].

The antibiotic susceptibility test results and the clinical breakpoints of polymyxins in the microbiology laboratory have changed over time. The MIC values and the zone diameter for

polymyxins were first reported by NCCLS in 1976 for all gram-negative bacteria. In 1980, with the advent of better-tolerated antibiotics such as cephalosporins, polymyxins were removed from the NCCLS list due to their serious side effects. In 2003, CLSI announced the clinical breakpoints for both colistin and polymyxin B, particularly when using these drugs against multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter* species. In 2010, EUCAST published the clinical breakpoints for colistin against *Acinetobacter*, *Pseudomonas*, and *Enterobacterales*. In 2014, both EUCAST and CLSI removed the clinical breakpoints for the disc diffusion of colistin reportedly due to the lack of reliable information, and only the clinical breakpoints for the MIC values of colistin were published. In 2020, while CLSI published the clinical breakpoints for both colistin and polymyxin B, EUCAST published only the breakpoints for colistin against *Enterobacterales*, *Pseudomonas* and *Acinetobacter* species [3].

The antibiotic resistance data for Turkey are abundant, with the multidrug-resistant isolates observed particularly in healthcare-associated infections. Carbapenem resistance is observed in particularly high rates in *Enterobacterales*, *Pseudomonas*, and *Acinetobacter* [4, 5]. Colistin is an antibiotic used for the treatment of multidrug-resistant bacteria, despite its side effects and resistance issues [6]. The EUCAST standards are applied widely in the microbiology laboratories in Turkey [4]. The use of polymyxin B manufactured by a local company in Turkey against multidrug-resistant infection has commenced in 2020. The present study was aimed to evaluate the correlation between the MIC values of polymyxin B and colistin among *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates.

MATERIAL AND METHODS

Collection, identification and antibiotic susceptibility test of the isolates

The isolates that had been previously accepted by the reference laboratory for the determination of the epidemiology of carbapenemase were included in the present study [5]. The isolates were collected from the selected 26 hospitals located in Turkey. A total of 505 isolates, which included 122 isolates of *E. coli* and 383 isolates of *Klebsiella pneumoniae*, were included in the present study. The bacteria were identified at the species level using the MALDI Biotyper (Bruker Daltonics, Germany). The susceptibility test for imipenem (Sigma-Aldrich, St. Louis, MO), meropenem (Sigma-Aldrich, St. Louis, MO), colistin (Sigma-Aldrich, St. Louis, MO), and polymyxin B (Serva, Germany) were performed using the broth microdilution method and the corresponding test results were evaluated according to the standards of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [7]. CLSI was used for evaluation of polymyxin breakpoints [8]. The MIC values 0,0625 between 128 mg/L were used for colistin and polymyxin B in broth micro dilution. *E. coli* ATCC 25922 and *E. coli* NCTC 13846 were used for quality

control in the broth microdilution tests for both colistin and polymyxin B.

Detection of carbapenemase and *mcr* genes

The PCR method developed in-house was employed to identify the carbapenemase genes responsible for the resistance to carbapenems. Eight carbapenemase genes (*bla*_{OXA-23}, *bla*_{OXA-48}, *bla*_{OXA-51}, *bla*_{OXA-58}, *bla*_{NDM}, *bla*_{IMP}, *bla*_{VIM} and *bla*_{KPC}) common to the Turkey region were investigated in the present study. The primers F(5'-GATCGGATTGGA-GAACCAGA-3') and R (5'-ATTTCTGACCGCATTTCCAT-3') for *bla*_{OXA-23}, F (5'-TTGGTGGCATCGATTATCGG-3') and R (5'-GAGCACTTCTTTTGTGATGGC-3') for *bla*_{OXA-48}, F (5'-TAATGCTTTGATCGGCCTTG-3') and R (5'-TGGATTGCACTTCATCTTGG-3') for *bla*_{OXA-51}, F (5'-AAGTATTGGGGCTTGTGCTG-3') and R (5'-CCCCTCTGCGCTCTACATAC-3') for *bla*_{OXA-58}, F (5'-GTAGTGCTCAGTGTCCGCAT-3') and R (5'-GGGCAGTCGCTTCCAACGGT-3') for *bla*_{NDM}, F (5'-GGAATAGAGTGCTTAATTCTC-3') and R (5'-CCAAACCACTACGTATATC-3') for *bla*_{IMP}, F (5'-GTGTTTGGTCGCATATCGC-3') and R (5'-CGCAGCACCAGGATAGAAG-3') for *bla*_{VIM}, and F (5'-ATGTCAGTGTATCGCCGTC-3') and R (5'-TTTTCA-GAGCCTTACTGCCC-3') for *bla*_{KPC} were used [5]. The *mcr*-1, 2, 3, 4, 5, 6, 7 and 8 genes were investigated in the colistin-resistant isolates using the Real-Time PCR Detection Kit (Bio-Speedy, Turkey) [5].

RESULTS

Epidemiological Properties

A total of 505 isolates, including the *E. coli* (122) and *K. pneumoniae* (383) isolates, were included in the present study, among which 25.5% of the isolates were from the outpatients, and 74.5% of the isolates were from the inpatients. According to the epidemiological data, 31% of the isolates were community-acquired, and 69% of isolates were from healthcare-related infection agents. Moreover, 6.1% of the isolates were evaluated as colonization agents, while 93.9% were evaluated as infectious agents. The bacteria were isolated from internal care units (44.1%), intensive care units (32.8%), and surgical clinics (23.1%). The distribution of the clinical samples from which the included isolates were collected was as follows: 57.9% urine, 22.7% blood, 12.4% lower respiratory tract, 3.9% aspirate and 3.1% wound samples.

Antibiotic susceptibility results and detection of resistance genes

The results of the broth microdilution tests conducted for carbapenems, colistin, and polymyxin B are provided in Table 1. The correlation between the MICs of colistin and polymyxin B is presented in Fig. 1. The types of carbapenemases identified in the carbapenemase-producing isolates were OXA-48 (52.2%), KPC (16.1%), NDM-1 (15%), OXA-

*Carbapenems: Ertapenem, Imipenem, Meropenem.

Table 3. The categorical agreement between polymyxin B and colistin for *K. pneumoniae*

Bacteria	Polymyxin B	Results	Colistin		Total
			Susceptible	Resistant	
<i>K. pneumoniae</i>	Polymyxin B	Susceptible	280 (99.6%)	1 (0.4%)	281
		Resistant	1 (1%)	101 (99%)	102
		Total	281 (73.4%)	102 (26.6%)	383

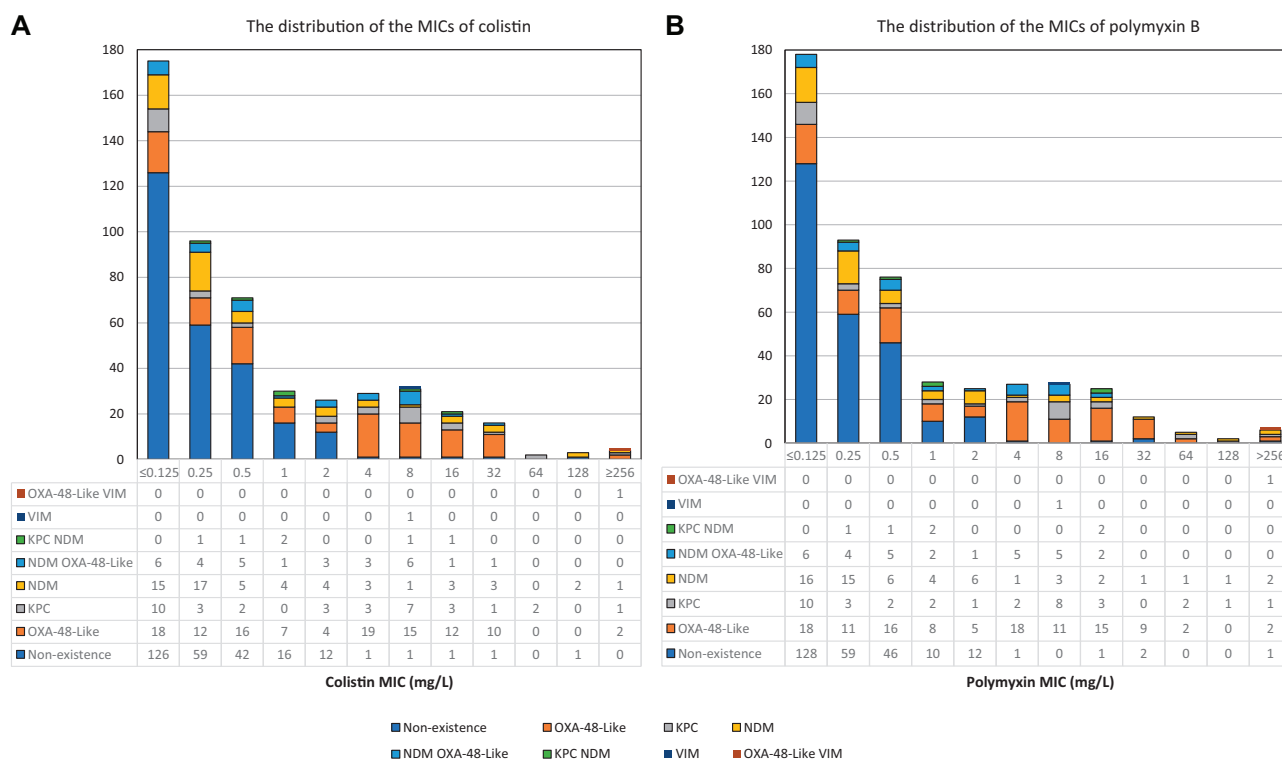


Fig. 2. The distribution of the MICs of colistin (2A) and polymyxin B (2B) according to the carbapenemase type

MIC measurements, the use of colistin sulfate, which is an inactive prodrug of colistin, is recommended [12–14].

Most of the CMS is excreted through the kidneys, and only 20–25% of CMS is converted to colistin. Therefore, approximately five times the required amount of CMS has to be administered to patients to reach adequate plasma concentration. In addition, the rate of conversion of CMS to colistin depends on the relative inter-individual variability. The gradual conversion of CMS accounts for the delay in the killing of bacteria by the active form of colistin. Since the conversion of CMS occurs in the urinary tract, the urinary concentrations of colistin could be relatively higher than those of polymyxin B. Therefore, colistin is preferable over PMB for the treatment of urinary tract infections [9]. Moreover, PMB is associated with a higher incidence of local airway irritation compared to CMS, rendering CMS a better choice for inhalation [15].

The early achievement of adequate serum concentration of polymyxin is critical for its bactericidal activity. Therefore, a loading dose should be administered to all patients [16]. Studies have reported that polymyxin B and colistin present similar nephrotoxicity rates [17, 18].

In Turkey, the use of the EUCAST standards has accelerated gradually since 2014, and nowadays, most of the clinical microbiology laboratories are using EUCAST standards [4]. Since the carbapenemase-carrying *E. coli* and *K. pneumoniae* isolates are endemic to Turkey, the use of colistin is quite common, and the resistance rates against these strains among the citizens are increasing with time [19]. However, with the introduction of polymyxin B by a local pharmaceutical company in Turkey, the use of polymyxin B rather than colistin is expected to become widespread. Since the EUCAST standards are used throughout the country, these were utilized in the present study to determine the sensitivity of polymyxin B in comparison to colistin as a laboratory reference to explore the consequences of using polymyxin B for therapeutic purposes.

In the present study, the MICs of polymyxin B against both carbapenem-sensitive and carbapenem-resistant isolates were studied using the broth microdilution method, which revealed a correlation with the colistin MICs. The CLSI suggests that the clinical breakpoints of colistin could be used for polymyxin B as well [20]. When evaluated microbiologically, the epidemiological threshold value

(wildtype MICs) of colistin against *E. coli* and *K. pneumoniae* was 2 mg/L [21]. Therefore, it was inferred, similar to the findings of previous studies, that the results obtained for colistin could be applied in the case of polymyxin B as well [22]. Since polymyxins do not exhibit good diffusion in agar, it is recommended to determine their antibiotic sensitivity using the broth microdilution test [23]. The results obtained for colistin and polymyxin B in the broth microdilution test conducted using sulfate compounds were compared.

The use of polymyxins has certain limitations. First, the clinical PK/PD data for polymyxins are inconsistent, indicating that these drugs are not reliable [3]. Second, it is mostly recommended to use polymyxins in combination with other active antibiotics [24], although it is reported that the synergy may vary according to each isolate and the corresponding MIC values [25]. Third, it is noteworthy that the efficacy of polymyxins could be low in the case of intravenous administration and the current clinical threshold values for the inhaler form during the treatment are invalid. Therefore, CLSI emphasizes that if the MIC value of colistin and polymyxin B is <4 mg/L, this information should be provided in the microbiology report for the clinician [3]. EUCAST does not report a clinical breakpoint for polymyxin B due to lack of sufficient data, although it does not recommend the use of polymyxin B in the treatment for the isolates resistant to colistin [3].

It was revealed that the carbapenemase type was not an influencing factor for the polymyxins evaluated in the present study. Therefore, it could be inferred that polymyxins are preferable as a treatment choice regardless of their carbapenemase type.

The main limitation of the present study is that the MIC values of polymyxin against the *P. aeruginosa* and *Acinetobacter* spp. isolates were not compared with the MIC values of colistin against these isolates, although these studies are planned for future research. Furthermore, it is considered better to interpret the MIC values together with clinical data, and, therefore, the data of MIC values in the present study would be supported with clinical data in our future studies.

We believe that the colistin-resistant isolates should be accepted as resistant to polymyxin B as well, and the antibiotic susceptibility test (AST) report should also include a clinical microbiology interpretation even if the isolate is detected to be susceptible to colistin. The explanations for the following should be presented to the clinician in the colistin AST with broth microdilution report:

- Polymyxin B should never be used for the treatment of isolates reported with colistin resistance.
- The results may not be reliable due to the lack of sufficient data on the isolates with MIC < 4 mg/L.
- There are insufficient data on PK/PD values for both colistin and polymyxin B, particularly in terms of nephrotoxicity.
- Combination therapies can be preferred when using polymyxins.

- If the use of active agents is reported in the antibiotic susceptibility test, these agents should be preferred for treatment.
- The detected MIC values should not be applied to the use of polymyxin in the inhaler form.

Cooperation between the clinic and laboratory is crucial in the management of multidrug-resistant microorganisms and would be enhanced when the clinical microbiologists provide evidence-based data and related explanations in the antibiotic susceptibility test reports.

REFERENCES

- [1] Quintanilha JCF, Duarte NC, Lloret GR, Visacri MB, Mattos KPH, Dragosavac D, et al. Colistin and polymyxin B for treatment of nosocomial infections in intensive care unit patients: pharmaco-economic analysis. *Int J Clin Pharm* 2019; 41: 74–80.
- [2] Li J, Nation RL, Turnidge JD, Milne RW, Coulthard K, Rayner CR, et al. Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. *Lancet Infect Dis* 2006; 6(9): 589–601.
- [3] Satlin MJ, Lewis JS, Weinstein MP, Patel J, Humphries RM, Kahlmeter G, et al. Clinical and laboratory standards institute and European committee on antimicrobial susceptibility testing position statements on polymyxin B and colistin clinical breakpoints. *Clin Infect Dis* 2020; 71(9): e523–9.
- [4] *Central Asian and European Surveillance of antimicrobial resistance*. Annual report 2020.
- [5] Süzük YS, Şimşek H, Bakkaçoğlu Z, Numanoglu ÇY, Hekimoğlu CH, Kılıç S, et al. National carbapenemase study group: the epidemiology of carbapenemases in *Escherichia coli* and *Klebsiella pneumoniae* isolated in 2019 in Turkey. *Mikrobiyol Bul* 2021; 55(1): 1–16.
- [6] Süzük YS, Kaşkatepe B, Şimşek H, Sarıgüzel FM. High rate of colistin and fosfomycin resistance among carbapenemase-producing Enterobacteriaceae in Turkey. *Acta Microbiol Immunol Hung* 2019; 66(1): 103–12.
- [7] European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters. Version 10.1, 2020.
- [8] Clinical and Laboratory Standard Institute (CLSI). M100-S25, Performance standards for antimicrobial susceptibility testing, 30th Edition, 2020b.
- [9] Bergen PJ, Li J, Rayner CR, Nation RL. Colistin methanesulfonate is an inactive prodrug of colistin against *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 2006; 50(6): 1953–8.
- [10] Nation RL, Velkov T, Li J. Colistin and polymyxin B: peas in a pod, or chalk and cheese? *Clin Infect Dis* 2014; 59(1): 88–94.
- [11] Deris ZZ, Akter J, Sivanesan S, Roberts KD, Thompson PE, Nation RL, et al. A secondary mode of action of polymyxins against Gram-negative bacteria involves the inhibition of NADH-quinone oxidoreductase activity. *J Antibiot* 2014; 67(2): 147–51.
- [12] Kunin CM, Craig WA, Kornguth M, Monson R. Influence of binding on the pharmacologic activity of antibiotics. *Ann N Y Acad Sci* 1973; 226(1): 214–24.

- [13] Craig WA, Kunin CM. Significance of serum protein and tissue binding of antimicrobial agents. *Annu Rev Med* 1976; 27(1): 287–300.
- [14] Nation RL, Li J, Cars O, Couet W, Dudley MN, Kaye KS, et al. Framework for optimization of the clinical use of colistin and polymyxin B: the Prato polymyxin consensus. *Lancet Infect Dis* 2015; 15: 225–34.
- [15] Ziaka M, Markantonis SL, Fousteri M, Zygoulis P, Panidis D, Karvouniaris M, et al. Combined intravenous and intraventricular administration of colistin methanesulfonate in critically ill patients with central nervous system infection. *Antimicrob Agents Chemother* 2013; 57: 1938–40.
- [16] Cheah SE, Li J, Tsuji BT, Forrest A, Bulitta JB, Nation RL. Colistin and polymyxin B dosage regimens against *Acinetobacter baumannii*: differences in activity and the emergence of resistance. *Antimicrob Agents Chemother* 2016; 60(7): 3921–33.
- [17] Rigatto MH, Behle TF, Falci DR, Freitas T, Lopes NT, Nunes M, et al. Risk factors for acute kidney injury (AKI) in patients treated with polymyxin B and influence of AKI on mortality: a multicentre prospective cohort study. *J Antimicrob Chemother* 2015; 70: 1552–7.
- [18] Abdul Rahim N, Cheah SE, Johnson MD, Yu H, Sidjabat HE, Boyce J, et al. Synergistic killing of NDM-producing MDR *Klebsiella pneumoniae* by two ‘old’ antibiotics-polymyxin B and chloramphenicol. *J Antimicrob Chemother* 2015; 70: 2589–97.
- [19] Brolund A, Lagerqvist N, Byfors S, Struelens MJ, Monnet DL, Albiger B, et al. European antimicrobial resistance genes surveillance network EURGen-net capacity survey group.: worsening epidemiological situation of carbapenemase-producing Enterobacteriaceae in Europe, assessment by national experts from 37 countries, July 2018. *Euro Surveill* 2019; 24(9): 1900123.
- [20] *CLSI performance Standards for antimicrobial susceptibility testing M 100*. Wayne, PA: Clinical and Laboratory Standards Institute, 2020.
- [21] Humphries R. *Colistin breakpoints for Pseudomonas aeruginosa and Acinetobacter spp. CLSI rationale document MR01*. Wayne, PA: Clinical and Laboratory Standards Institute, 2018.
- [22] Sader HS, Rhomberg PR, Farrell DJ, Jones RN. Differences in potency and categorical agreement between colistin and polymyxin B when testing 15,377 clinical strains collected worldwide. *Diagn Microbiol Infect Dis* 2015; 83: 379–81.
- [23] Humphries RM. Susceptibility testing of the polymyxins: where are we now? *Pharmacotherapy* 2015; 35: 22–7.
- [24] Lee HJ, Bergen PJ, Bulitta JB, Tsuji B, Forrest A, Nation RL, et al. Synergistic activity of colistin and rifampin combination against multidrug-resistant *Acinetobacter baumannii* in an in vitro pharmacokinetic/pharmacodynamic model. *Antimicrob Agents Chemother* 2013; 57: 3738–45.
- [25] Fan B, Guan J, Wang X, Cong Y. Activity of colistin in combination with meropenem, tigecycline, fosfomycin, fusidic acid, rifampin or sulbactam against extensively drug-resistant *Acinetobacter baumannii* in a murine thigh-infection model. *PLoS One* 2016; 11: e0157757.