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Can colistin susceptibility results determined in multidrug resistant Gram- negative bacteria be used for polymyxin B?

Original Study

Rıza Adaleti[1]*, Nilgün Kansak[2], Şeyma Çalık[3], Neslihan Arıcı[2], Seniha Şenbayrak[4], Sebahat Aksaray[1]

[1] University of Health Sciences, Hamidiye Medical Faculty, Department of Medical Microbiology, Istanbul, Turkey
[2] University of Health Sciences, Haydarpaşa Numune Training and Research Hospital, Laboratory of Medical Microbiology, Istanbul, Turkey
[3] Ministry of Health, Directorate of Public Health, Amasya, Turkey
[4] University of Health Sciences, Hamidiye Medical Faculty, Department of Infectious Diseases and Clinical Microbiology, Istanbul, Turkey

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Abstract

Introduction. The present study aimed to compare the MIC values of colistin and polymyxin B in fermentative and non- fermentative Gram- negative bacteria and to examine whether the susceptibility results determined with colistin can be used for polymyxin B.
Materials and Methods. A total of 268 strains of *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Acinetobacter baumannii* (*A. baumannii*) isolates were included in the study. The reference broth microdilution and the results were evaluated according to CLSI recommendations.
Results. Of the 268 strains, 40.7% were found to be colistin resistant and 34% polymyxin B resistant. Essential agreement (EA) and categorical agreement (CA) ratios for all isolates were found to be 81.3% and 85.1%, respectively. Identical MIC values were found for colistin and polymyxin B in 104 isolates (38.8%), and this rate was 31.1% and 48.7% in Enterobacterales and non-fermentative bacteria, respectively. Colistin resistance rates were 25.4% for *E. coli*, 67.4% for *K. pneumoniae*, 23.8% for *P. aeruginosa*, and 31.5% for *A. baumannii*. Polymyxin B resistance rates were 18.6% for *E. coli*, 64.1% for *K. pneumoniae*, 19% for *P. aeruginosa*, and 16.7% for *A. baumannii*.
Conclusion. According to the results of the study, it was concluded that the MIC values of colistin and polymyxin B antibiotics in all bacteria were not compatible with each other. Colistin susceptibility results cannot be used for treatment with polymyxin B, despite CLSI's recommendation.

Keywords

colistin, polymyxin B • categorical agreement • broth microdilution

1. Introduction

Polymyxins cause the death of bacteria by disrupting the cell membrane of many Gram- negative bacilli [1]. After being discovered in the late 1940s, polymyxins were used for treatment for many years, but their use was discontinued in the 1970s due to their side effects [2]. Since the 1970s and 1980s, more effective antibiotics with fewer side effects have been used for treatment. For a long time, carbapenem antibiotics have been the most important drug in the treatment of multidrug-resistant Gram-negative bacilli (MDRGNB). In recent years, polymyxin group antibiotics have been included in the treatment protocol again, due to the development of resistance to the carbapenem group of antibiotics. With the reintroduction of polymyxin group antibiotics into the treatment protocol, an increasing resistance has developed to this group of antibiotics.

For colistin susceptibility tests, broth microdilution (BMD), broth disk elution, and colistin agar tests are acceptable by the Clinical and Laboratory Standards Institute (CLSI), but for polymyxin B, BMD is the only approved method. Other testing methods, such as disk diffusion and gradient diffusion, are not currently recommended [3]. The current method accepted by both the CLSI and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [4] is the reference BMD method. In the polymyxin group, only polymyxin B and colistin are in clinical use [5]. In the minimal inhibitory concentration (MIC) measurements, the use of colistin sulfate and polymyxin sulfate is recommended. The CLSI [3] has published the clinical breakpoints for colistin and polymyxin B against intermediate and resistant *Enterobacterales* species, *P. aeruginosa* and *Acinetobacter spp*., but EUCAST [4] announced the clinical breakpoint for colistin and published cut-off values for detection of phenotypic resistance to polymyxin B. According to CLSI, colistin and polymyxin B are considered equivalent agents, so MICs obtained from testing of colistin predict MICs for polymyxin B and vice versa, but with EUCAST, there is no interpretation for predicting MICs of polymyxin B according to colistin's MIC value. In recent years, EUCAST standards have been widely used for susceptibility testing in microbiology laboratories in our country. Despite the extensive use of colistin in antimicrobial susceptibility tests in our country, both colistin and polymyxin B are used in the

* *E-mail: rizaadaleti@gmail.com*

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*Table 1. Colistin and polymyxin B evaluation criteria for Enterobacterales, **P. aeruginosa** and **A. baumannii** according to CLSI and EUCAST criteria*

CLSI		Susceptible (μg/mL)	Intermediate(μg/mL)	Resistant(μg/mL)	
	Colistin		≤ 2	≥ 4	
	Polymyxin B		≤ 2	≥ 4	
EUCAST		Susceptible (mg/L)	Intermediate (mg/L)	Resistant (mg/L)	ECOFF (mg/L)
	Colistin*	≤ 2		≥ 4	
	Colistin**	≤ 4		≥ 8	
	Polymyxin B*				2
	Polymyxin B**				4

*ECOFF: epidemiological cut-off value *E. coli, K. pneumoniae, A. baumannii ** P.aeruginosa*

treatment protocol. So, "Can colistin susceptibility test results of MDRGNB be used for polymyxin B?" is the question for the clinical microbiologists and specialists in infectious diseases currently.

In our study, we aimed to investigate the MIC value of both colistin and polymyxin B against MDRGNB strains; ESBL positive *Escherichia coli* (*E.coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Pseudomonas aeuginosa* (*P.aeruginosa*) and *Acinetobacter baumannii* (*A.baumannii*) and evaluated the essential agreement (EA) and categorical agreement (CA) between two antibiotics. We investigated whether the colistin susceptibility results could be used for polymyxin B.

2. Materials and methods

2.1. Bacteria

A total of 268 MDRGNB nonduplicate isolates of ESBL positive *E. coli* (n= 59), multidrug- resistant (including carbapenem-resistant) *K. pneumoniae* (n = 92), *P. aeruginosa* (n = 63), and *A. baumannii* (n = 54) were used in the present study. The bacteria were isolated from blood and tracheal aspirate specimens of patients in Istanbul, Haydarpaşa Numune Training and Research Hospital, from January 2020 to May 2022. Identification up to species level was done by using the VITEK®MS (bioMérieux,Marcy-l'Étoile, France). The routine susceptibility tests were done by VITEK®2 (bioMérieux) according to EUCAST standards. Pure cultures of these isolates were stored at – 80°C. The use of colistin-resistant strains from our stock strains has been prioritized.

2.2. Study design

The reference BMD test was performed using a polystyrene microplate according to CLSI recommendations. The minimal inhibitory concentration of colistin sulfate salt (Sigma-AldrichC4461,USA) and polymyxyn B sulfate (Koçak, Turkey) were determined by BMD using cation- adjusted Mueller Hinton Broth (CAMHB) according to CLSI guidelines [6]. Each well of the microplate was filled with 100 µl CAMHB containing 0.5 to 512 µg/mL concentrations of colistin or polymyxin B and stocked at - 80°C until the working day (1- 4 days). Stock strains were inoculated to sheep blood agar and incubated overnight at 37°C. Strains were prepared according to 0.5 McFarland turbidity on the working day, and they were adjusted to 10^6 CFU/mL by dilution. Then, 100 µl of bacteria were inoculated into the 1–11 wells, which were previously inoculated with 100 µl CAMHB containing 0.5 to 512 µg /mL concentrations of colistin or polymyxin B. The final concentration of antibiotics was 0.25-256 µg/mL. The twelfth well contained only bacteria and was used as positive control.

The MIC study for both antibiotics (colistin and polymyxin B) was performed at the same time using the same bacterial concentration. Since EUCAST does not have break points for the interpretation of polymyxin B, the interpretation of results for colistin and polymyxin B for *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* was done with CLSI guidelines [3, 4] (Table 1).

The broth microdilution test was repeated when the MICs could not be read, the quality control results were not within the expected range, and the results were inconsistent (n = 63). The EA (MIC results within ± 1 doubling dilution) and CA (number of overlapping intermediate (I) and resistant (R) results between polymyxin B and colistin) were evaluated according to ISO criteria (EA and CA > 90%)[7].

E. coli ATCC 25922, *P. aeruginosa* ATCC 27853, and *E.coli* NCTC 13846 (mcr-1positive) were used as control strains.

3. Results

Of the 268 strains, 109 (40.7%) were found to be colistin resistant and 91 (34.0%) polymyxin B resistant. According to the evaluation made with reference to colistin MIC results, EA, CA ratios for all isolates were found to be 81.3%, 85.1%, respectively.

The same MIC values were found for colistin and polymyxin B in 104 isolates (38.8%), and this rate was 31.1% (n=47) and 48.7% (n= 57) in *Enterobacterales* and nonfermentative bacteria, respectively. The essential agreement for *E. coli*, *K. pneumoniae*, *P. aeruginosa,* and *A. baumannnii* were found to be 79.7%, 81.5%, 90.5%, and 72.2%, respectively (Table 2). Colistin and polymyxin B MIC values of isolates with ± one, two, and three double dilution differences are

Table 2. The essential agreement, categorical agreement rates of polymyxin B and resistance rates of colistin and polymyxin B in studied bacteria

			Colistin		Polymyxin B	
Bacteria	EA n (%)	CA n (%)	I n (%)	R n (%)	I n (%)	R n (%)
E. coli	47 (79.7)	39 (66.1)	44 (74.6)	15 (25.4)	48 (81.4)	11 (18.6)
K. pneumoniae	75 (81.5)	89 (96.7)	30 (32.6)	62 (67.4)	33 (35.9)	59 (64.1)
P. aeruginosa	57 (90.5)	54 (85.7)	48 (76.2)	15 (23.8)	51 (81.0)	12(19.0)
A. baumannii	39 (72.2)	46 (85.2)	37 (68.5)	17 (31.5)	45 (83.3)	9 (16.7)
Total	218 (81.3)	228 (85.1)	159 (59.3)	109 (40.7)	177 (66.0)	91 (34.0)

EA: Essential agreement, CA: Categorical agreement, I: Intermediate, R: Resistant
n: Number of isolates,

Table 3. Differences in MIC values between colistin and polymyxin B among tested isolates

Organisms (n)	Polymyxin B more potent by n (%) of strains			Same MIC	Colistin more potent by n (%) of strains		
	3 dilution	2 dilution	1 dilution	n (%)	1 dilution	2 dilution	3 dilution
E.coli (59)	0	4 (6.8)	19 (32.2)	17 (28.8)	11 (18.6)	7 (11.9)	1 (1.7)
K. pneumonia (92)	2 (2.2)	13 (14.1)	39 (42.4)	30 (32.6)	6 (6.5)	2 (2.2)	0
P. aeruginosa (63)	1 (1.6)	4 (6.3)	15 (23.8)	38 (60.3)	4 (6.3)	1 (1.6)	0
A. baumannii (54)	6 (11.1)	9 (16.7)	18 (33.3)	19 (35.2)	2 (3.7)	0	0
Total 268	9 (3.4)	30 (11.2)	91 (34.0)	104 (38.8)	23 (8.6)	10 (3.7)	1 (0.4)

Table 4. Categorical agreement rates changing according to susceptibility status for colistin-polymyxin B intermediate–resistant isolates

Strains	Colistin	Polymyxin B	% of Categorical agreement
Escherichia coli (I)	44	36	81.8
Escherichia coli(R)	15	3	20
Klebseilla pneumoniae(I)	30	30	100
Klebseilla pneumoniae(R)	62	59	95.2
Escherichia coli+ Klebseilla pneumoniae(I)	74	66	89.2
Escherichia coli+ Klebseilla pneumoniae(R)	77	62	80.5
Pseudomonas aeruginoa(I)	48	45	93.8
Pseudomonas aeruginoa(R)	15	9	60
Acinetobacter baumannii(I)	37	37	100
Acinetobacter baumannii (R)	17	9	52.9
Pseudomonas aeruginoa + Acinetobacter baumannii(I)	85	82	96.5
Pseudomonas aeruginoa + Acinetobacter baumannii(R)	32	18	56.3

I: Intermediate, R: Resistant

given in Table 3. The categorical agreement for *E. coli*, *K. pneumoniae*, *P. aeruginosa,* and *A. baumannnii* were found to be 66.1%, 96.7%, 85.7%, and 85.2%, respectively (Table 2). For colistin-polymyxin B intermediate-resistant isolates, CA rates varying according to susceptibility status are given in Table 4.

Colistin and polymyxin B resistance rates of *E. coli* strains were 25.4% (n = 15) and 18.6% (n = 11), respectively. Colistin and polymyxin B resistance rates of *K. pneumoniae* strains were 67.4% (n = 62) and 64.1% (n= 59), respectively (Tables 2, 5). Colistin and polymyxin B resistance rates for *P. aeruginosa* and *A. baumannii* were 23.8% (n =15), 19% (n= 12), and 31.5% (n = 17), 16.7% (n =9), respectively (Tables 2, 6).

In our study, the MIC value of polymyxin B was found to be lower than colistin in 39.0% (n = 23) of *E. coli* strains and higher in 32.2% (n = 19). While the MIC value of polymyxin B was lower than colistin in 58.7% (n = 54) of *K. pneumoniae* strains, it was found to be higher in 8.7% (n = 8) (Table 3). The MIC value of polymyxin B in *P. aeruginosa* and *A. baumannnii* strains was 31.7% (n = 20) and 61.1% (n = 33) lower than colistin, respectively, while 7.9% (n = 5) and 3.7% (n = 2) of strains were found to be higher (Table 3).

MIC values and interpretation of colistin and polymyxin B against all strains studied are given in Table 7.

4. Discussion

The use of colistin and polymyxin B in the treatment of MDRGND infections, including carbapenem resistance, has gained importance again. The chemical structure of colistin and polymyxin B are very similar to each other (differing in one amino acid and the fatty acyl moiety). Despite the different forms in clinical use, it is recommended to use sulfate salt for both drugs in susceptibility tests [8].

Table 5. MIC values (µg/ml) for colistin and polymyxin B in E. coli and K. pneumoniae bacteria (n = 151)
Grey font numbers indicate the number of strains with equivalent MICs for colistin and polymyxin B

Colistin (mg/L)												
	>=256										1K	
	128								1K	2K	1K	
	64							4K	9K	5K		
	32						3K	12K	8K	1K		
	16						4K	4K				
	8				1K+1E	1K	1K					
	4			1K+3E	1K+8E	1K+3E	1K					
	2	2k	2K	5K+9E	2K+7E	2E						
	1	1k	2K+2E	2K+4E	1K+9E	5E						
	0.5	2k	3K+2E	1K	2K+2E	1E						
	<=0.25	3k+1E	2K									
		0.25	0.5	1	2	4	8	16	32	64	128	>=256
		Polymyxin B (mg/L)										

K: Klebsiella pneumoniae, E: Escherichia coli, n: Numbers
NOTE: Vertical column for colistin MIC values and horizontal column for polymyxin B MIC values.

*Table 6. MIC values (µg/ml) for colistin and polymyxin B in **P.aeruginosa** and **A. baumannii** strains (n = 117)*
Grey font numbers indicate the number of strains with equivalent MICs for colistin and polymyxin B.

Colistin (mg/L)												
	>=256										2A	
	128									1A		
	64									1P+1A		
	32								1A			
	16					1A		1P+1A				
	8			1P+2A	1P	2P						
	4		3A	2P+2A	2P+1A	5P+1A	1A					
	2	1A	1P+5A	10P+8A	16P+9A	3P						
	1	1A	4A	12P+4A	1P+1A							
	0.5	1P+2A	1P+2A									
	0.25	2P		1P								
		0.25	0.5	1	2	4	8	16	32	64	128	>=256
		Polymyxin B (mg/L)										

*A: **Acinetobacter baumannii**, P: **Pseudomonas aeruginosa***
NOTE: Vertical column for colistin MIC values and horizontal column for polymyxin B MIC values.

In our country, the interpretation of antibiotic susceptibility tests is based on EUCAST standards. However, since there is no breakpoint value for polymyxin B in EUCAST, MIC values recommended by CLSI were used in our study. Questions that cause disagreement among our guides are whether *in vivo* efficacy can be predicted with different forms used in *in vitro* tests and whether the susceptibility result determined by colistin can be used for polymyxin B is still up to date. To clarify this issue, this study was planned to evaluate the validity of the colistin MIC result for the polymyxin B MIC value in 268 MDRGNB strains using the BMD method.

When we compared the MIC values of both antibiotics, it was found that 109 (40.7%) strains were resistant to colistin, while polymyxin B resistance was 91 (34.0%). It is noteworthy that the

Table 7. MIC values and interpretations of colistin and polymyxin B against ***Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,*** *and* ***Acinetobacter baumannii*** *strains*

Bac.	Col. MIC	Interp.	Poly. MIC	Int.	Bac.	Col. MIC	Interp.	Poly. MIC	Interp.	Bac.	Col. MIC	Int.	Poly. MIC	Int.	Bac.	Col. MIC	Int.	Poly. MIC	Int.	Bac.	Col. MIC	Int.	Poly. MIC	Int.
E1	1	I	2	I	K1	1	I	1	I	K64	64	R	64	R	P1	2	I	1	I	A1	4	R	0.5	I
E2	1	I	4	R	K2	1	I	1	I	K65	64	R	32	R	P2	4	R	1	I	A2	4	R	2	I
E3	4	R	1	I	K3	1	I	2	I	K66	64	R	32	R	P3	1	I	1	I	A3	8	R	1	I
E4	2	I	1	I	K4	1	I	0.5	I	K67	64	R	32	R	P4	1	I	1	I	A4	256	R	128	R
E5	1	I	2	I	K5	1	I	0.25	I	K68	64	R	16	R	P5	2	I	1	I	A5	2	I	0.5	I
E6	2	I	2	I	K6	1	I	0.5	I	K69	64	R	32	R	P6	4	R	2	I	A6	4	R	4	R
E7	1	I	4	R	K7	2	I	1	I	K70	64	R	16	R	P7	2	I	4	R	A7	2	I	1	I
E8	1	I	2	I	K8	2	I	1	I	K71	64	R	32	R	P8	1	I	2	I	A8	0.5	I	0.5	I
E9	1	I	2	I	K9	2	I	1	I	K72	128	R	64	R	P9	1	I	1	I	A9	2	I	0.25	I
E10	4	R	2	I	K10	2	I	0.5	I	K73	128	R	64	R	P10	1	I	1	I	A10	2	I	2	I
E11	1	I	1	I	K11	2	I	1	I	K74	128	R	32	R	P11	1	I	1	I	A11	2	I	1	I
E12	1	I	2	I	K12	2	I	0.25	I	K75	128	R	128	R	P12	2	I	1	I	A12	16	R	4	R
E13	2	I	2	I	K13	2	I	0.25	I	K76	32	R	16	R	P13	2	I	1	I	A13	2	I	2	I
E14	1	I	2	I	K14	2	I	0.5	I	K77	0.25	I	0.5	I	P14	1	I	1	I	A14	0.5	I	0.5	I
E15	0.5	I	2	I	K15	2	I	1	I	K78	0.25	I	0.5	I	P15	4	R	1	I	A15	2	I	1	I
E16	0.5	I	2	I	K16	2	I	2	I	K79	0.25	I	0.25	I	P16	2	I	1	I	A16	2	I	1	I
E17	1	I	1	I	K17	2	I	2	I	K80	0.25	I	0.25	I	P17	16	R	16	R	A17	1	I	0.5	I
E18	1	I	0.5	I	K18	4	R	8	R	K81	0.25	I	0.25	I	P18	2	I	1	I	A18	2	I	2	I
E19	2	I	1	I	K19	4	R	1	I	K82	0.5	I	0.25	I	P19	2	I	2	I	A19	1	I	2	I
E20	4	R	2	I	K20	4	R	4	R	K83	0.5	I	0.5	I	P20	1	I	1	I	A20	2	I	2	I
E21	2	I	2	I	K21	4	R	2	I	K84	0.5	I	0.5	I	P21	1	I	1	I	A21	0.5	I	0.25	I
E22	2	I	1	I	K22	8	R	2	I	K85	0.5	I	1	I	P22	1	I	1	I	A22	2	I	2	I
E23	1	I	2	I	K23	8	R	8	R	K86	0.5	I	0.5	I	P23	1	I	1	I	A23	2	I	1	I
E24	1	I	4	R	K24	8	R	4	R	K87	0.5	I	0.25	I	P24	8	R	2	I	A24	1	I	1	I
E25	4	R	2	I	K25	16	R	8	R	K88	0.5	I	2	I	P25	8	R	4	R	A25	1	I	1	I
E26	2	I	4	R	K26	16	R	16	R	K89	0.5	I	2	I	P26	8	R	4	R	A26	2	I	2	I
E27	1	I	4	R	K27	16	R	16	R	K90	32	R	32	R	P27	2	I	0.5	I	A27	4	R	1	I
E28	2	I	1	I	K28	16	R	16	R	K91	256	R	128	R	P28	2	I	2	I	A28	256	R	128	R
E29	8	R	2	I	K29	16	R	8	R	K92	16	R	8	R	P29	8	R	1	I	A29	16	R	16	R
E30	4	R	2	I	K30	16	R	16	R						P30	2	I	1	I	A30	2	I	1	I
E31	1	I	4	R	K31	16	R	8	R						P31	2	I	1	I	A31	1	I	1	I
E32	1	I	2	I	K32	32	R	8	R						P32	1	I	1	I	A32	128	R	64	R
E33	2	I	2	I	K33	32	R	16	R						P33	64	R	64	R	A33	1	I	0.5	I
E34	0.5	I	4	R	K34	32	R	16	R						P34	1	I	1	I	A34	32	R	32	R
E35	4	R	4	R	K35	32	R	64	R						P35	0.25	I	1	I	A35	2	I	1	I
E36	1	I	1	I	K36	32	R	32	R						P36	2	I	1	I	A36	64	R	64	R
E37	4	R	2	I	K37	32	R	32	R						P37	2	I	2	I	A37	1	I	1	I
E38	2	I	1	I	K38	32	R	16	R						P38	2	I	1	I	A38	0.5	I	0.25	I
E39	1	I	2	I	K39	32	R	32	R						P39	0.5	I	0.5	I	A39	1	I	0.5	I
E40	4	R	2	I	K40	32	R	16	R						P40	2	I	2	I	A40	1	I	0.25	I
E41	4	R	2	I	K41	32	R	32	R						P41	0.25	I	0.25	I	A41	4	R	8	R
E42	2	I	2	I	K42	32	R	8	R						P42	0.25	I	0.25	I	A42	2	I	0.5	I
E43	4	R	4	R	K43	32	R	16	R						P43	0.5	I	0.25	I	A43	2	I	0.5	I
E44	2	I	2	I	K44	32	R	8	R						P44	4	R	2	I	A44	4	R	1	I
E45	4	R	4	R	K45	32	R	16	R						P45	4	R	4	R	A45	4	R	0.5	I
E46	2	I	4	R	K46	32	R	32	R						P46	2	I	4	R	A46	2	I	0.5	I
E47	1	I	0.5	I	K47	32	R	16	R						P47	2	I	2	I	A47	4	R	0.5	S
E48	2	I	1	I	K48	32	R	16	R						P48	2	I	2	I	A48	2	I	0.5	I
E49	1	I	1	I	K49	32	R	16	R						P49	2	I	2	I	A49	8	R	1	I
E50	4	R	2	I	K50	32	R	16	R						P50	2	I	2	I	A50	2	I	2	I
E51	0.25	I	0.25	I	K51	32	R	32	R						P51	2	I	2	I	A51	2	I	2	I
E52	0.5	I	0.5	I	K52	32	R	32	R						P52	4	R	4	R	A52	1	I	0.5	I
E53	2	I	2	I	K53	32	R	16	R						P53	4	R	4	R	A53	2	I	2	I
E54	4	R	1	I	K54	64	R	32	R						P54	2	I	2	I	A54	2	I	1	I

Continued Table 7. MIC values and interpretations of colistin and polymyxin B against **Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,** and **Acinetobacter baumannii** strains

Bac.	Col. MIC	Interp.	Poly. MIC	Int.	Bac.	Col. MIC	Interp.	Poly. MIC	Interp.	Bac.	Col. MIC	Int.	Poly. MIC	Int.	Bac.	Col. MIC	Int.	Poly. MIC	Int.	Bac.	Col. MIC	Int.	Poly. MIC	Int.
E55	0.5	I	0.5	I	K55	64	R	32	R						P55	4	R	4	R					
E56	2	I	1	I	K56	64	R	16	R						P56	2	I	2	I					
E57	2	I	1	I	K57	64	R	32	R						P57	2	I	2	I					
E58	2	I	1	I	K58	64	R	16	R						P58	2	I	2	I					
E59	4	R	1	I	K59	64	R	64	R						P59	2	I	2	I					
					K60	64	R	64	R						P60	2	I	2	I					
					K61	64	R	64	R						P61	2	I	2	I					
					K62	64	R	32	R						P62	2	I	4	R					
					K63	64	R	64	R						P63	4	R	4	R					

E: Escherichia coli, K: Klebsiella pneumoniae, P: Pseudomonas aeruginosa, A: Acinetobacter baumannii
Bac: Bacteria, Poly B: Polymyxin B, Col: Colistin, I: Intermediate, R: Resistant

resistance rates of colistin were higher in all bacterial groups than polymyxin B.

Gales et al. [9] found that polymyxin B and colistin were resistant at rates of 0.1% and 0.2% in *E. coli* (n = 17035) and 1.4% and 1.5% in *Klebsiella spp*. (n = 9774) strains, respectively. The same researchers found that polymyxin B and colistin were resistant at rates of < 0.1% and 0.4% in *P. aeruginosa* (n = 9130) strains and 0.8% and 0.9% *A. baumannii* (n = 4686)strains, respectively. The results of the present study were found to be compatible with our data.

In the study conducted by Chew et al. [10] 67.1% of 76 carbapenem resistant *Enterobacteriaceae* isolates were found to be susceptible to colistin, while 64.5% of the isolates were found to be susceptible to polymyxin B by the BMD test. Contrary to our results, researchers found colistin more potent.

In all bacterial groups (*K. pneumoniae*, *P. aeruginosa, A. baumannii, and polymyxin-resistant E. coli)* we studied, except for the intermediate (2 µg/mL) *E. coli* isolates, polymyxin B was found to be more potent and its MIC values were lower than colistin. Contrary to our results, Sader et al. [11] found a lower colistin MIC value in *K. pneumoniae, P. aeruginosa*, *A. baumannii,* and resistant *E.coli* strains in their study, with a total of 15,377 isolates.

In our study, although colistin and polymyxin B were found to have the same efficacy in *E. coli* isolates with a MIC value of ≤ 2 µg/mL, Sader et al. [11] found that the MIC value of polymyxin B was lower than that of colistin in the same isolates (MIC value ≤ 2 µg/mL). It was thought that the inconsistent results between the two studies might have resulted from the comparison of studies with different methods.

Similar to our findings, Doymaz et al. [12] found polymyxin B to be more effective in their study, with 89 isolates.

When colistin and polymyxin B susceptibility were analyzed according to bacterial species, equal MIC values of both antibiotics were seen, with the highest in *P. aeruginosa* at 60.3% and the lowest in *E. coli* at 28.8%. In the other two bacterial species we studied, equal MIC values for colistin and polymyxin B were found in 35.2% of *A. baumannii* and 32.6% of *K. pneumoniae*.

When all bacteria were evaluated in our study, 228 (85.1%) of 268 isolates did not meet the ISO criteria, although they were categorically compatible. When the results were evaluated separately on the basis of bacteria, 89 (96.7%) of 92 *K. pneumoniae* isolates were found to be categorically compatible (CA ≥ 90%). When colistin intermediate-resistant isolates were evaluated separately on the basis of bacteria, categorical agreement was found to be acceptable (93.8%, 100%, respectively) in *P. aeruginosa* and *A. baumannii* intermediate isolates. Categorical agreement was found to be low in resistant isolates in the same bacterial group (60% and 52.9%, respectively). This was also observed in colistin-resistant *E. coli* and *K. pneumoniae* isolates. As a result, it was thought that the low categorical agreement rate in resistant isolates may be due to the MIC of the isolates close to the limit value.

The study of Süzük et al. [13] found the categorical agreement to be acceptable (98.4%, 99.5%, respectively) and higher than our study in their comparison study with 505 *E. coli* and *K. pneumoniae* isolates. Although the rates of CA, EA, and major error (ME) were acceptable, it is recommended that a separate susceptibility test be performed for polymyxin B and colistin since the rate of very major error (VME) (33.3%) was found to be very high. Since the susceptibility criteria to polymyxins were not given according to the current CLSI criteria, ME and VME evaluation were excluded from our study.

In a review by Humphries [14], it was reported that susceptibility test results for colistin and polymyxin B were compatible in some studies, and it was emphasized that the resistance rate to polymyxins was low in the strains used. These results support our interpretation that a comparison should be made with a close number of susceptible and resistant isolates.

In our study, essential agreement (90.5%) was found to be acceptable only for *P. aeruginosa* isolates, while essential agreement rates were found to be low for other bacteria. In the study of Süzük et al. [13], the essential agreement rates were found to be high for *E. coli* and *K. pneumoniae* bacteria.

5. Conclusions

In our study, with a relatively limited number of samples, we found that colistin and polymyxin B are not compatible with each other for clinically important Gram-negative bacteria.

Colistin and polymyxin B resistance rates were different in the same isolates.

Polymyxin B is more potent than colistin and has lower MIC values.

Categorical agreement rates differ in resistant or intermediate strains of bacteria.

Although categorical agreement was higher in intermediate isolates than in resistant strains, the rate of CA was below acceptable limits in other bacterial groups, except for *K. pneumoniae* isolates.

We concluded that the colistin and polymyxin B susceptibility results cannot be used interchangeably.

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Authors contribution

R.A: Research concept and design, Supervising the project, Carrying out the experiments, Acquisition of data, Data analysis and interpretation, Writing - Original Draft Preparation, Writing - Review and Editing, Visualization, Literature review, Final proofreading and approval of the version for publication, Funding Acquisition

N.K: Research concept and design, Data analysis and interpretation, Writing - Original Draft Preparation, Writing - Review and Editing, Visualization, Literature review, Final proofreading and approval of the version for publication, Funding Acquisition

Ş. Ç: Carrying out the experiments, Acquisition of data, Data analysis and interpretation, Writing - Original Draft Preparation, Writing - Review and Editing, Visualization, Literature review, Final proofreading and approval of the version for publication, Funding Acquisition

N.A: Carrying out the experiments, Acquisition of data, Data analysis and interpretation, Writing - Original Draft Preparation, Writing - Review and Editing, Visualization, Literature review, Final proofreading and approval of the version for publication, Funding Acquisition

S. Ş: Acquisition of data, Writing - Original Draft Preparation, Writing - Review and Editing, Literature review, Final proofreading and approval of the version for publication, Funding Acquisition

S. A: Acquisition of data, Writing - Original Draft Preparation, Writing - Review and Editing, Literature review, Final proofreading and approval of the version for publication, Funding Acquisition

ORCID

Rıza Adaleti https://orcid.org/0000-0001-9576-6794
Nilgün Kansak https://orcid.org/0000-0002-1117-3906
Şeyma Çalık https://orcid.org/0000- 0002- 1281-0073
Neslihan Arıcı https://orcid.org/0000-0003-4788-0044
Seniha Şenbayrak https://orcid.org/0000-0002-4983-6613
Sebahat Aksaray https://orcid.org/0000-0002-0552-1337

Conflict of interest

The authors have no potential conflicts of interest to declare.

Ethics approval

All procedures performed in studies were in accordance with the ethical standards of the institution and/or national research.

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