

## Original Article

**Detection of colistin resistance via four methods in multidrug-resistant Gram-negative rods isolated from blood cultures**Rama Alhamwi<sup>1,2</sup>, Lütfiye Öksüz<sup>1</sup><sup>1</sup> Department of Medical Microbiology, Istanbul University, Istanbul Faculty of Medicine, Capa-Istanbul, Türkiye<sup>2</sup> Istanbul University Institute of Graduate Studies in Health Sciences, Istanbul, Türkiye**Abstract**

**Introduction:** The broth microdilution (BMD) method recommended for the detection of colistin resistance is labor-intensive, time-consuming, and difficult to apply in routine laboratories. Thus, various methods, such as disk elution, commercial microdilution, and rapid polymyxin-NP tests have been developed for the detection of colistin resistance. In this study, a total of 102 multi-resistant Gram-negative bacteria isolated from blood cultures were evaluated by four different methods for the detection of colistin resistance, and compared with the reference method. **Methodology:** For the detection of the compatibility of these methods with the reference method, categorical and essential agreements, very major, major, and minor error rates were determined. Colistin-tigecycline and colistin-meropenem combinations were investigated in colistin-resistant isolates.

**Results:** Of the isolates, 15 (15%) [*K. pneumoniae* (n = 12), *A. baumannii* (n = 2), *E. coli* (n = 1)] were resistant to colistin with reference BMD method. MIC<sub>50</sub> and MIC<sub>90</sub> values of all isolates were ≤ 0.25 µg/mL and 16 µg/mL, respectively. The categorical agreement rates were 100% for commercial microdilution, disk elution, and RPNP test. The essential agreement rates of commercial microdilution, disk elution, and broth microdilution were 78.4%, 86.3%, and 100%, respectively. Although there were no major errors in these methods, the macrodilution (12%) and commercial microdilution (20.6%) methods showed the most minor errors. Colistin-meropenem combination showed a 100% synergistic effect, but the colistin-tigecycline combination showed an 80% synergistic effect and 20% indifference effect.

**Conclusions:** Disk elution and RPNP tests are suitable for routine use because they are the most efficient, easiest, low-cost, and good performance tests in detecting colistin resistance.

**Key words:** Colistin; multi-drug resistance; disk elution; synergy; *mcr-1*.

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**Introduction**

Bacteremia caused by multi-drug resistant Gram-negative bacteria (MDR-GNB) is increasing and has serious consequences. Treatment options against MDR-GNB have become limited over the years, often resulting in treatment failure in bacteremia [1]. Therefore, the use of polymyxins, as the last antibiotic choice, against multi-drug resistant bacteria has restarted, even though they had been abandoned due to their neurotoxic and nephrotoxic side effects [2]. Unfortunately, in recent years, bacteremia treatment has become increasingly difficult due to the increase in the number of colistin-resistant Gram-negative bacteria (GNB) [3,4]. The use of disk diffusion test in detecting the susceptibility of colistin is problematic due to its large molecular structure that cannot diffuse into agar. This makes disk diffusion test results unreliable [3,5]. Furthermore, the colistin molecule readily binds to plastic surfaces, so broth microdilution method remains

the only reliable test to achieve minimal inhibitory concentrations (MIC) [6,7].

Colistin-resistant GNB has become a serious threat due to the spread of the plasmid-mediated colistin-resistant (*mcr*) gene family. Recently, nine genes (*mcr-1-9*) of *mcr* family have been isolated from different countries in the world [8,9]. It is important to be mindful of the use of colistin to prevent the spread of colistin-resistant GNB. For this purpose, empirical use of the drug must be avoided, proven antibiotic susceptibility test methods should be used, and reliable and practical methods that can be used in routine laboratories should be suggested [10]. Thus, it may be possible to prevent the overuse of colistin in therapy and limit the spread of resistance. In this study, the susceptibility to colistin of MDR-GNB isolated from blood cultures was detected by macro dilution, disk elution, commercial microdilution, and rapid polymyxin NP tests, and was compared to broth

microdilution. Then the combination of colistin with tigecycline and with meropenem was tested by the checkerboard method. Additionally, the presence of *mcr-1* gene was investigated by PCR in colistin-resistant isolates.

## Methodology

This study was carried out following the ethical values specified in the Declaration of Helsinki and was approved by the Istanbul Faculty of Medicine Clinical Research Ethics Committee (2019/1279).

### Bacterial isolates

Bacteria were isolated from blood cultures sent to the medical microbiology laboratory from various clinics of our hospital during the 18 months-period covering 2019-2020. The blood culture bottles were incubated in a blood culture system (BACTEC FX, Beckton Dickinson, USA) for five days at 35 °C, after receiving the positive signal, Gram staining was performed and they were incubated in blood agar and chocolate agar medium for 24-48 hours.

### Bacterial identification

Isolated bacteria were identified using conventional biochemical methods and - upon request - an automatic identification system (Phoenix 100, Beckton Dickinson, USA). A total of 102 MDR-GNB were isolated from blood cultures during the study period. The bacterial isolates were stored in brain-heart infusion broth supplemented with 20% glycerol. All isolates were stored at -20 °C until further analysis.

### Antibiotic susceptibility tests

Routine antibiotic susceptibility tests were performed by Kirby Bauer disk diffusion method and evaluated by the recommendations of Clinical Laboratory Standards Institute (CLSI) guideline [11]. These antibiotics were tested: Ampicillin (10 µg), ampicillin + sulbactam (10/10 µg), amoxicillin + clavulanic acid (20/10 µg), piperacillin + tazobactam (10/10 µg), sefazolin (30 µg), cefuroxime (30 µg), cefoxitine (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefepime (30 µg), imipenem (10 µg), meropenem (10 µg), ertapenem (10 µg), gentamisin (10 µg), tobramycin (10 µg), amikacin (30 µg), trimethoprim + sulfamethoxazole (1.25/23.75 µg), ciprofloxacin (5 µg). Isolated bacteria were categorized according to the susceptibilities of third-generation cephalosporins, carbapenems, and fluoroquinolones, and divided into three groups.

### Broth microdilution tests (BMD)

Minimal inhibitory concentrations (MIC) for colistin were detected by the broth microdilution method [12,18]. BMD panels were prepared in-house using cation-adjusted Mueller-Hinton broth, colistin sulphate powder, and round-bottom polystyrene 96-well microplate. The range of concentrations of colistin (Carbosynth, UK) was between 0.25 and 128 mg/L. *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 13846 (*mcr-1* harboring *E. coli*), and *Pseudomonas aeruginosa* ATCC 27853 were used as standard control strains.

### Broth macro-dilution tests

MIC for colistin were detected by the broth macrodilution method [18]. This test was performed to detect whether the glass tube affects antibiotic susceptibility testing [7]. The range of concentrations of colistin (Carbosynth) was between 0.25 and 128 mg/L.

### Commercial microdilution tests (Diagnostics, Slovakia)

The range of concentration for colistin was 0.25-16 µg/mL. Ready-to-use microplates were supplied by the manufacturer. This test was applied according to manufacturer recommendations.

### Colistin broth disk elution method

This test was performed using four cation-adjusted Mueller-Hinton broth containing a different number (0, 1, 2, and 4) colistin disks resulting final concentration of 0, 1, 2, and 4 µg/mL. After 30 minutes of incubation at room temperature, A suspension of bacteria at 0.5 McFarland turbidity was added to each tube. MIC values were read visually after 16 to 20 hours of incubation at 35 °C [13].

### Rapid Polymyxin NP test

This test was performed for fermentative bacteria (*E. coli*, *K. pneumoniae*) which testing was based on glucose metabolism in presence of a colistin concentration of 3.75 µg/mL. Red phenol was used as a pH indicator. The result of the test (susceptible/resistant) could be read visually after 4 hours of incubation at 35 °C [14,15].

### Resazurin-related Rapid Polymyxin NP test

This test was performed for non-fermentative bacteria (*A. baumannii*, *P. aeruginosa*). A colistin concentration of 3.75 µg/mL was prepared in cation-adjusted Mueller-Hinton broth. After three hours of incubation at 35 °C, resazurin stains were added to each well and read visually after one hour of incubation [16].

**Checkerboard method**

This test was used to compare the effect of antibiotic combinations (colistin-tigecycline-CT-TIG and colistin-meropenem-CT-MEM) on colistin-resistant isolates with their activities alone. Fractional inhibitory concentration (FIC) index values were then calculated. According to the FIC index, interactions of antibiotics were categorized as synergy, antagonism, and indifference [12,17,18].

**DNA extraction**

To extract bacterial DNA for use in PCR assays, a few colonies were taken from the 18–24 hours pure culture in Tryptic Soy agar medium and suspended in 1 mL of filtered sterile distilled water. The suspension was boiled in a water bath at 95 °C for 10 minutes and then centrifuged at 13000 g. After centrifugation, the supernatant on the top of the pellet was separated and transferred to another sterile Eppendorf tube. The obtained DNA extract was stored at -20 °C [19,20].

**Detection of mcr gene**

The presence of *mcr-1* gene in colistin-resistant isolates was investigated using PCR method [20].

**Statistical analysis**

To evaluate the compatibility of colistin MIC values determined by four different methods with the gold standard method, essential agreement (EA) and categorical agreement (CA), minor error, major error, and very major error (VME) rates were determined [21].

**Results**

Most of the strains were isolated from adult patients (79%) while other strains were isolated from children (21%). In this group, 51% of the patients were male and 49% were female. The age range of the patients was between 0-96 years and the mean age was 50 ± 27 years.

**Table 1.** Underlying diseases or infections of patients with bacteria isolated from blood culture.

Underlying diseases	N (%)
Cancer	14 (13)
COVID-19	9 (9)
Urinary system infections	9 (9)
Diabetes mellitus	8 (7.5)
Liver disease	7 (6.5)
Pneumonia	6 (6)
Solid organ transplantation	6 (6)
Digestive system disease	6 (6)
Kidney failure	6 (6)
Leukemia	5 (5)
Gallbladder cirrhosis	3 (3)
Hepatitis B	3 (3)
West Nile fever	3 (3)
Febrile neutropenia	2 (2)
Soft tissue infections	1 (1)
Unknown	14 (14)
<b>Total</b>	<b>102</b>

The underlying diseases of the patients were given in Table 1.

Multi-resistant Gram-negative rods obtained from blood cultures sent to the laboratory during the 18 months were included in the study. Of the bacteria isolated, 46 (45%) were *E. coli*, 43 (42%) were *K. pneumoniae*, eight (8%) were *A. baumannii*, and five (5%) were *P. aeruginosa*. The patients from whom the bacteria were isolated were mostly sent from the Department of Internal Diseases (51%), followed by the Departments of Pediatrics (19%), Department of General Surgery (9%), Pandemic Clinic (9%), Department of Gynecology (6%), Intensive Care Unit (2%), and the other wards (4%).

The isolated bacteria were divided into three different groups according to disk diffusion method results: Group 1 (ESBL positive isolates), Group 2 (isolates resistant to carbapenems), and Group 3 (isolates resistant to all tested antibiotic groups, except colistin and tigecycline). Group 1 contains 63% of the isolates, while each Group 2 and Group 3 contain 18% and 5% of all isolates, respectively. Of the *K. pneumoniae* isolates, 44% of the bacteria were in Group

**Table 2.** MIC<sub>50</sub> and MIC<sub>90</sub> values of all isolates by four methods.

	BMD (µg/ml)		BMaD (µg/mL)		Commercial BMD (µg/mL)		Disk Elution (µg/mL)	
	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>
<i>K. pneumoniae</i>	≤ 0.25	16	0.5	16	0.5	16	< 1	> 4
<i>E. coli</i>	≤ 0.25	≤ 0.25	≤ 0.25	0.5	0.5	1	< 1	< 1
<i>A. baumannii</i>	0.5	8	≤ 0.25	8	1	8	< 1	> 4
<i>P. aeruginosa</i>	0.5	1	0.5	2	2	2	< 1	< 1
Total	≤ 0.25	16	≤ 0.25	8	0.5	8	< 1	> 4
Group 1	≤ 0.25	≤ 0.25	≤ 0.25	0.5	0.5	1	< 1	< 1
Group 2	0.5	16	0.5	8	1	16	< 1	> 4
Group 3	4	32	4	16	8	16	4	> 4

BMD: Broth microdilution; BMaD: Broth microdilution; MIC: Minimal Inhibitor Concentration.

1, 19% in Group 2, and 37% in Group 3. Among the *E. coli* isolates, 98% of the bacteria were in Group 1, and only 2% were in Group 2. According to disk diffusion susceptibility results, 7% of the isolates were susceptible to cefepime, 40% to gentamicin, 51% to amikacin, 14% to cotrimoxazole, 35% to piperacillin-tazobactam, and 16% to ciprofloxacin in *K. pneumoniae* isolates. Among the *E. coli* isolates, 13% were susceptible to cefepime, 48% to gentamicin, 96% to amikacin, 39% to cotrimoxazole, 87% to piperacillin tazobactam and 24% to ciprofloxacin. In our study, imipenem and meropenem resistance rates in *K. pneumoniae* isolates were 35% and 42%, respectively. Imipenem and meropenem resistance in *P. aeruginosa* isolates were found to be 40% and 80%, respectively; while the resistance in *A. baumannii* isolates was 87% and 100%, respectively. Although no resistance to imipenem and meropenem was detected in *E. coli* isolates, 2% resistance was found to ertapenem.

The minimal inhibitory concentrations for colistin in the MDR-GNB isolates were evaluated by different methods, and the results were presented in Table 2. The comparison of the categorical and essential agreements for each method were presented in Table 3. No very major error was found in any of the methods. Disk elution showed 100% of categorical and essential agreement with no errors at all.

According to all tested methods, 87 isolates were susceptible to colistin but 15 isolates [*K. pneumoniae* (n = 12), *A. baumannii* (n = 2), and *E. coli* (n = 1)] were resistant to colistin (Figure 1). Of the patients whom isolated colistin-resistant strains, 40% were cancer, 20% were transplant patients, 13.3% had COVID-19 infection, and 6.6% had urinary tract infection.

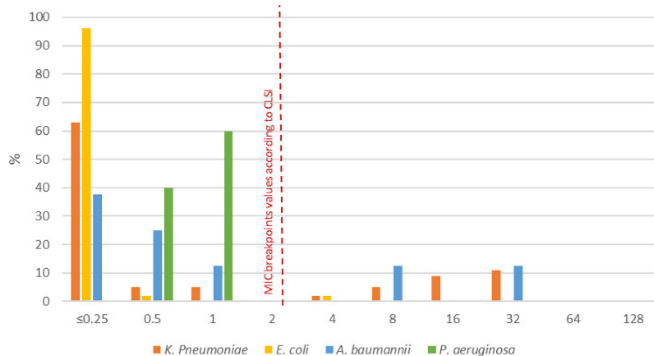
In colistin-meropenem combination, 100% synergism was found, while in colistin-tigecycline combination 80% synergism and 20% indifference effects were found.

No *mcr-1* gene was detected in colistin-resistant isolates.

**Discussion**

In recent years, the number of MDR-GNB isolated from blood cultures has been increasing gradually,

**Figure 1.** Distribution of MIC values for colistin by BMD method.



which causes both difficulties in treatment and increased morbidity and mortality. Colistin is an important treatment option as a "last-choice drug" for MDR-GNB. Unfortunately, today, colistin resistance exists in many *Enterobacterales* family members, such as *E. coli*, *Klebsiella* spp., and *Enterobacter* spp. [1,4,22].

When automated systems are used to detect the MIC of colistin, it comes into contact with the plastic at every stage of the test, and concentration losses occur due to the adhesion of colistin to the plastic and causes resistant strains to appear susceptible. For this reason, most studies reported that various automated systems such as Phoenix 100, Microscan, and VITEK-2, are not reliable [23-25]. Although there are various colistin susceptibility tests, there is still no practical, easy-to-use, and low-cost method that can be used in routine laboratories instead of BMD. The reason for the planning of this study is to investigate the sensitivity of the disk elution method found by Simner *et al.* and its applicability in the laboratory [13].

At the time of this study, the rate of positivity in blood cultures was 14.2% in our laboratory. In this process, resistance to cefotaxime was 46.5%, 17% to amikacin, 30% to ciprofloxacin, and 5.6% to imipenem in *E. coli* isolated from blood cultures. In our laboratory, among *K. pneumoniae* isolates, 64% were resistant to cefotaxime, 32% to amikacin, 45% to ciprofloxacin, and 35% to imipenem [26].

According to the data of the National Antimicrobial Resistance Surveillance System (NARSS) covering 69

**Table 3.** Categorical and essential agreements of the studied methods, and major–minor error rates.

Methods	Categorical agreement (%)	Essential agreement (%)	Very-major error (%)	Major error (%)	Minor error (%)
BMaD	98	86.3	0	2.3	12
Commercial BMD	100	78.4	0	0	20.6
Disk Elution	100	100	0	0	0
RPNP Test	100	-	-	-	-

BMD: Broth microdilution; BMaD: Broth microdilution; RPNP: Rapid Polymyxin NP.

centers in our country, resistance to cefotaxime, gentamicin, carbapenem, and ciprofloxacin in *E. coli* isolates was 51%, 29%, 5%, and 55%, respectively. Among *K. pneumoniae* isolates, resistance rates against cefotaxime, gentamicin, carbapenem, and ciprofloxacin were 68%, 49%, 40%, and 63%, respectively. Rates of resistance to carbapenems and fluoroquinolones in *P. aeruginosa* isolates were 46% and 38%, and 92% and 91% in *A. baumannii* isolates, respectively [27]. Additionally, Central Asian and Eastern European Surveillance of Antimicrobial Resistance (CAESAR) results supported the results of NARSS [28]. Although resistance rates in the present study were similar to NARSS results, resistance rates to fluoroquinolones were found to be higher (76% for *E. coli*, 84% for *K. pneumoniae*) because this study included multiple resistant isolates.

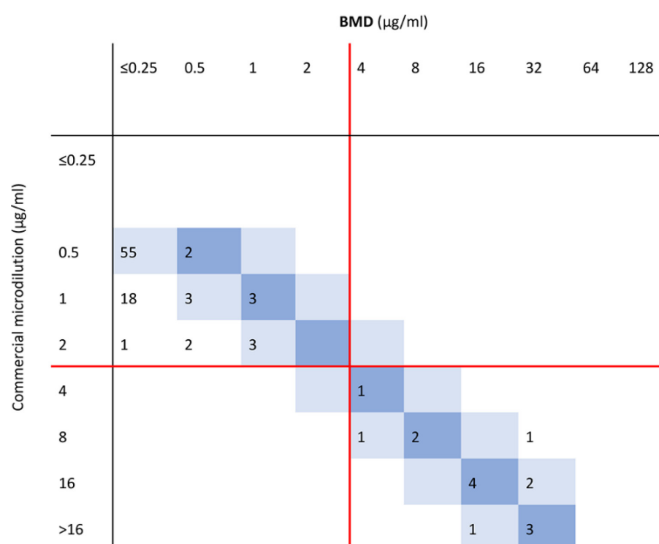
Resistance to colistin may develop due to excessive use of colistin in agriculture and livestock, bacterial mutations, and mistakes in empirical therapy applications [2,29]. Although MDR-GNB were previously susceptible to colistin, they started to develop resistance mechanisms in the 1990s, such as mutations that cause loss or change in the structure of LPSs, efflux pumps, loss of porin, enzymes that inactivate colistin, or more intense production of the polysaccharide capsule [9,20,25,30,31]. Studies testing colistin susceptibility using BMD in Greece, Italy, India, Egypt, Germany, and Poland found resistance rates of 95.1%, 13.5%, 32.4%, 45.1%, and 17%, respectively [32-37]. Colistin resistance has been detected in many studies conducted in Turkey, and resistance rates vary between 27% and 60% [24,38,39].

The prevalence of MDR-GNB has been observed at varying rates all over the world in recent years, but the incidence continues to increase steadily. It has been reported that mortality rates increased by 57% in bloodstream infections due to ESBL-producing bacteria. ESBL-producing bacteria tend to develop resistance to antibiotics such as carbapenems and colistin and transfer their resistance mechanisms to other bacteria via plasmids [40]. Consistent with this information, in our study, 13% of Group 1 (ESBL positive) isolates, 16% of Group 2 (carbapenem-resistant bacteria), and 53% of Group 3 (bacteria resistant to all antibiotics except colistin and tigecycline) isolates were found to be resistant to colistin. Of the patients infected with colistin-resistant Gram-negative rods, 50% had undergone solid organ transplantation, 36% had cancer, 16% had kidney failure, and 22% had COVID-19.

The difficulties of detecting colistin resistance have encouraged many researchers to investigate new practical methods. Some researchers evaluated the potency of the macro-dilution method and found CA greater than 90% and good EA [34,41]. In our study, when the data obtained by the macro-dilution method were compared with the results of the reference method BMD, 12 (12%) minor errors and two (2%) major errors were found, and no very major error was detected; the CA was 100% and the EA 86.3%. However, to determine the accuracy of some results during the study, the macro-dilution test was repeated several times and the reproducibility of the test was found to be low. Thus, this cost-effective method is labor-intensive, time-consuming, and has low reproducibility; therefore, it can cause minor and major errors. In this context, it is considered unsuitable for routine laboratory use.

Recently, many laboratories tend to develop commercial microdilution methods that are easier and more practical as well as reliable and can replace conventional microdilution. [10,42]. Some researchers evaluated these commercial products such as Sensitive UMIC Colistin, SensiTest, Microscan, and Micronut-MIC, and all products were reported to show acceptable CA and EA with varying amounts of very major errors. Yusuf et al. tested four different commercial products for colistin susceptibility, found high CA and EA in all, and reported that these products were acceptable. [43]. Similarly, Altınkanat- Gezmez et al. tested the commercial product tested in our study and found 98% CA, and 84% EA with 3.8% major errors [44]. This study presented a CA of 100% but a low EA rate of 78.4%, which was not suitable for commercial microdilution kits although it was practical and easy to

**Figure 2.** Categorical and essential agreements graphics of commercial microdilution method compared with BMD method.



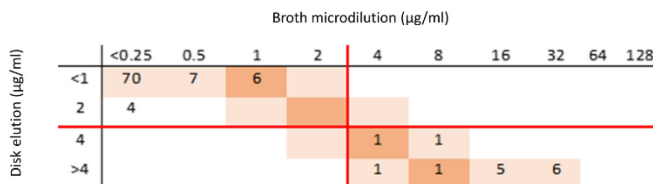
perform (Figure 2). Therefore, this commercial method is relatively expensive, easy, and fast to be applied and the results can be easily read with the naked eye. Although the exact MIC value could not be obtained, it gave accurate results about susceptibility and resistance.

Although there are various colistin susceptibility tests, Simmer *et al.* developed an easy-to-apply disk elution method, since there is still no practical, easy, and low-cost method that can be used in routine laboratories instead of BMD [13]. The disk elution method is a rather new method used in Slovakia to detect colistin resistance and because it is performed in glass tubes, it was thought that it could be a reliable alternative to the BMD method in routine microbiology laboratories. When the disk elution method was evaluated by Simmer *et al.*, they found 98% CA and 99% EA, with no errors at all [13]. Koyuncu *et al.* also evaluated this method compared to BMD and found 99% CA with 0.5% very major errors [24]. In the present study, the disk elution method had 100% CA and 100% EA, with no errors at all (Figure 3). Disk elution was very easy to perform and interpret and it is also relatively affordable as compared to the other four methods [45,46].

Although RPNP tests did not detect MIC values and only detected resistance, they were affordable, practical, and very easy to interpret, due to color change. It has been reported that RPNP testing may be a rapid and reliable option for detecting colistin resistance in *Enterobacteriaceae* strains isolated from blood cultures in routine laboratories [14]. Conceição-Neto *et al.* reported that the RPNP test showed 91% CA, 6.4% major error, and 9.7% very major error in 170 *K. pneumoniae* isolated from different clinical specimens [47]. When Shoaib *et al.* evaluated the RPNP test, they found that it showed 97% sensitivity and 100% specificity [48]. In this study, the results of the RPNP test were found to show very good (100%) categorical agreement when compared to the MIC results of the BMD method.

Based on the RPNP test, the resazurin-dependent rapid polymyxin NP (R-RPNP) test, which can detect colistin resistance in non-fermentative bacteria *A. baumannii* and *P. aeruginosa* strains, has also been developed [49]. Jia *et al.*, in their study in 2020, compared the R-RPNP test with the BMD method in *A. baumannii* and *P. aeruginosa* isolates and investigated its accuracy. They reported that the test showed only two major errors, 100% sensitivity and 96% specificity for *A. baumannii* strains and 100% sensitivity and 100% specificity for *P. aeruginosa* strains [50]. In this

**Figure 3.** Categorical and essential agreements graphics of disk elution method compared with BMD method.



study, when the data obtained with R-RPNP were compared with the data of the reference method BMD, no minor or major errors were observed, and very good (100%) categorical agreement, but a slower color change was observed in *P. aeruginosa* isolates compared to *A. baumannii* strains. It is considered that this test is easy to apply in a routine laboratory, has a low cost, can give fast results in four hours, and is very easy to read due to color change. In RPNP and R-RPNP tests, a categorical agreement was found to be high because colistin concentrations were 3.75 µg/mL and the MIC breakpoint for colistin was > 2 µg/mL.

Colistin combinations have been used in the treatment of colistin-resistant Gram-negative bacterial infections. The combination of colistin provides more effective treatment as well as help to prevent toxic side effects and the development of resistance. The most commonly preferred agents in combinations are carbapenems and protein synthesis inhibitors [51-53]. In addition, it is recommended to use tigecycline in combination with colistin, which can have side effects when used alone and is the last option for treatment against colistin resistance [54]. In our study, colistin-meropenem and colistin-tigecycline combinations were studied in 15 colistin-resistant isolates [*K. pneumoniae* (n = 12), *A. baumannii* (n = 2) and *E. coli* (n = 1)]. Colistin-meropenem combination showed a 100% synergistic effect, while the colistin-tigecycline combination showed an 80% synergistic effect and 20% indifference effect. The results of the some studies were consistent with our study, which found a synergistic and/or indifferent effect between colistin-meropenem and colistin-tigecycline using the checkerboard method in colistin-resistant strains [52,53,55,56], but a study from USA reported low synergistic effect and even antagonism in the same combinations [57].

The first *mcr-1* gene was isolated in China in 2015 followig in Belgium in 2016, and nine different plasmid-mediated colistin resistance genes have been isolated from different places until today [8,9,31]. In Turkey, the *mcr-1* gene was found for the first time in *E. coli* isolates isolated from chicken meat by Kurekci

*et al.* in 2018 [58]. In a study conducted by Arabacı *et al.* in 2019, they detected 5.25% (n = 3) *mcr-1* gene in 57 carbapenem-resistant *K. pneumoniae* isolated from 56 patients [38]. Özkaya *et al.* found 0.13% (n = 2) *mcr-1* gene in 14657 *Enterobacteriaceae* isolates [59]. In this study, none of colistin-resistant isolates harbored *mcr-1* gene. The resistance to colistin in isolates of our study may be due to different mechanisms such as the presence of the *mcr* genes other than *mcr-1*, the presence of efflux pumps, and the absence of cell wall lipopolysaccharides. To understand these reasons, investigation of the other *mcr* genes and efflux pumps in multiple resistant strains was planned in future studies.

### Conclusions

The results of this study may guide microbiologists as to whether the commonly used tests to detect colistin susceptibility can be used reliably in routine laboratories. Disk elution and RPNP tests are suitable for routine use because they are the most effective, easy-to-use, cost-effective, and have good performance. Although the commercial microdilution method was relatively expensive, it was fast, easy-to-use and the results could be read with the naked eye, but the MIC value cannot give. The tests showing a relatively high minor error were the macrodilution and commercial microdilution tests. The repeatability of the macrodilution method was low. Colistin-meropenem combination may be a good treatment option in colistin-resistant bloodstream infections. Therefore, monitoring of colistin resistance in infections caused by multi-drug resistant bacteria is substantial to prevent the spread of resistance, because infections caused by colistin-resistant strains are relatively common in the world.

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