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Assessing Colistin Resistance Among Clinical Isolates: A Comparative Study of BD Phoenix System versus Broth Microdilution Method

Klinik İzolatlarda Kolistin Direncinin Değerlendirilmesi: BD Phoenix Sistemi ile Sıvı Mikrodilüsyon Yönteminin Karşılaştırmalı Çalışması

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Abstract

Background: This study aims to compare the colistin susceptibility detected by the BD Phoenix automated system among multidrug-resistant bacteria with the broth microdilution (BMD) method results.

Materials and Methods: The study included 236 isolates (92 *A. baumannii, 68 K. pneumoniae, 55 P. aeruginosa, and 21 E. coli*) isolated from clinical samples submitted to the bacteriology laboratory of Dicle University Hospital. The BD Phoenix 100 automated system was evaluated using BMD as a reference method. Categorical agreement (CA, the number of concordant results), major errors (ME, results falsely categorized as resistant by the automated system despite being sensitive by the reference method), and very major errors (VME, results falsely categorized as sensitive by the automated system despite being resistant by the reference method) were calculated.

Results: Of the 236 isolates, colistin resistance was found in 34 (14.4%) isolates, with *A. baumannii, K. pneumoniae, P. aeruginosa, and E. coli* exhibiting resistance rates of 15.2%, 20.6%, 1.8%, and 23.8%, respectively. *A. baumannii* showed 89.1% CA between the two methods, with 10.8% VME. *K. pneumoniae* had an 83.8% CA with a 14.7% VME rate. *P. aeruginosa* exhibited a 98.1% CA with a 1.8% VME rate, while E. coli showed an 80.9% CA with a 19% VME rate. The BD Phoenix 100 automated systems had a 10.5% VME rate compared to BMD. The automated system identified 9 out of 34 isolates as susceptible, contrary to the reference method which reported them as resistant

Conclusions: Categorical agreement rates of the automated system varied according to bacterial isolates.The high VME rate of the BD Phoenix 100 system demonstrated the need to confirm colistin results reported as susceptible to SMD.

Keywords: Colistin, Antibiotic resistance, *Acinetobacter baumannii, Klebsiella pneumoniae*

ÖZ

Amaç: Amaç: Bu çalışma, çoklu ilaca dirençli bakterilerde BD Phoenix otomatize sistem tarafından tespit edilen kolistin duyarlılığını, sıvı mikrodilüsyon (SMD) yönteminin sonuçları ile karşılaştırmayı amaçlamaktadır.

Gereç ve Yöntem: Çalışma, Dicle Üniversitesi Hastanesi bakteriyoloji laboratuvarına gönderilen klinik örneklerden izole edilen 236 izolatı (*A. baumannii-92 izolat, K. pneumoniae-68 izolat, P. aeruginosa-55 izolat ve E. coli-21 izolat*) içermektedir. Çalışmada BD Phoenix otomatize sistem sonuçları, referans yöntem olarak kabul edilen SMD ile karşılaştırılmıştır. Otomatize sistem sonuçlarının kategorik uyum (KU, uyumlu sonuçların oranı), büyük hata (BH, referans yöntem ile duyarlı olup otomatize sistem tarafından dirençli bulunan sonuçlar) ve çok büyük hata (ÇBH, referans yöntem ile dirençli olup otomatize sistem tarafından duyarlı bulunan sonuçlar) oranları hesaplanmıştır.

Bulgular: Toplam 236 izolatın 34'ünde (%14,4) kolistin direnci saptanmış olup, *A. baumannii, K. pneumoniae, P. aeruginosa* ve *E. coli* izolatları sırasıyla %15,2, %20,6, %1,8 ve %23,8 direnç oranlarına sahiptir. *A. baumannii* izolatlarında iki yöntem arasında %89,1 kategorik uyum (KU) ve %10,8 ÇBH saptanırken *K. pneumoniae* izolatlarında %83,8 KU, %14,7 ÇBH saptanmıştır. *P. aeruginosa ve E. coli* izolatlarında ise KU oranları sırasıyla %98,1 ve %80,9, ÇBH oranları sırasıyla %1,8 ve %19 olarak bulunmuştur. BD Phoenix 100 otomatik sistem, SMD ile karşılaştırıldığında %10,5 ÇBH oranına sahiptir; referans yöntemin dirençli bulduğu 34 izolattan 9'unu duyarlı olarak raporlamıştır.

Sonuç: Otomatize sistemin kategorik uyum oranları bakteri izolatlarına göre değişkenlik göstermektedir. BD Phoenix 100 sisteminin yüksek ÇBH oranı, otomatize sistemde duyarlı olarak raporlanan kolistin sonuçlarının SMD ile doğrulanması gerekliliğini ortaya koymaktadır.

Anahtar kelimeler: Kolistin, antibiyotik direnci, *Acinetobacter baumannii, Klebsiella pneumoniae*

Highlights

- Categorical agreement between the BD Phoenix 100 system and BMD ranged from 80.9% to 98.1%.
- The BD Phoenix 100 system misidentified 9 out of 34 resistant isolates as susceptible.
- The high VME rate highlights the need to confirm colistin susceptibility results.

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Introduction

Colistin is a polymyxin group antimicrobial agent which was synthesized in the 1940s and remained in use till the late 1970s. Among approximately thirty different polymyxin compounds, polymyxin B and polymyxin E (colistin) share a similar structure and are used in clinical application (1). Since the 1970s, the intravenous administration of colistin has decreased gradually because of its considerable harmful effects on the kidneys and nerves. However, the emergence of resistance against aminoglycosides followed by carbapenem groups in Gram-negative bacteria has led to the resurgence of parenteral colistin use in the 2000s (2,3). The unnecessary use of antibiotics and incorrect antibiotic use policies, including clinicians' preference for broad-spectrum antibiotics over narrow-spectrum ones, have contributed to the rise in carbapenem resistance, prominent antibiotics in recent decades. Consequently, the necessity of employing colistin in treating carbapenem-resistant bacteria or incorporating it into combination therapies has become unavoidable. With the rising demand for colistin, particularly in combating multidrug-resistant (MDR) strains of *Acinetobacter baumannii (A. baumannii)*, carbapenem-resistant *Pseudomonas aeruginosa*, and various Enterobacterales, the significance of colistin sensitivity testing has become increasingly apparent. Various methods have been employed to compare the effectiveness of colistin against resistant bacterial strains, including *A. baumannii,* such as diffusion (Kirby Bauer disc diffusion and gradient diffusion) tests and dilution (agar dilution, broth microdilution) tests (4-6). Studies have shown that methods based on diffusion (disc and gradient diffusion tests) which are commonly used in microbiology labs due to their simplicity, are not dependable for testing colistin susceptibility. Therefore, the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) do not suggest the use of diffusion tests for detecting colistin sensitivity (5,7,8). The same publications also indicate that semi-automated systems lack validation for colistin testing and advocate for the broth microdilution method as the sole reliable approach for determining colistin sensitivity (7-9).

This study aims to compare colistin susceptibilities detected by BD Phoenix automated system among drug resistant bacterial isolates (such as *K. pneumoniae, A. baumannii, E. coli* and *P. aeruginosa)* with the broth microdilution (BMD) method's results.

Material and Methods

Study design

A total of 236 clinical isolates were included in the study, comprising 92 *A. baumannii* isolates, 68 *K. pneumonia*e isolates, 55 *P. aeruginosa* isolates, and 21 *E. coli* isolates. These were obtained from clinical samples that were sent to the Central Laboratory Bacteriology unit of xxxxxx Hospital. Sterile body fluids, including peripheral and catheter blood samples, cerebrospinal fluid (CSF), joint fluid, and pleural fluid, were collected into Bactec Plus aerobic/F or BD Bactec Peds Plus/F bottles and incubated in the BACTEC FX system (Becton Dickinson, U.S.A.). Subcultures were then prepared from the bottles onto solid media, including 5% Sheep Blood Agar, Eosine Methylene Blue Agar, and Sabouraud Dextrose Agar (all from RTA, Turkey). Urine, nephrostomy, tracheal aspirate, and bronchoalveolar lavage samples were inoculated into the media using the quantitative method. Other samples were inoculated using the streak plate method. Gram staining was performed initially for tracheal aspirate and sputum samples. Samples with a Bartlett score greater than zero were evaluated using the Bartlett scoring system (10). Wound culture samples were evaluated using the Quality (Q) scoring system. Samples with a Q score greater than zero were considered (11). For catheter-related bloodstream infections, a blood culture was taken simultaneously from the catheter tip and/or catheter blood culture and a peripheral vein. In case of growth in both the catheter tip and/or catheter blood culture and the blood culture sample taken from the peripheral vein, catheter-related bacteremia was considered (12). The infectious agents that grew in culture were identified using the mass spectrometry method (Matrix-Assisted Laser Desorption/Ionization-Time Of Flight-Mass Spectrometry: MALDI-TOF-MS) with the Maldi Biotyper 3 (Bruker Daltonics, USA) system. Antimicrobial susceptibility tests (AST) tests were conducted using the BD Phoenix 100 (Becton Dickinson, U.S.A.) automated microbiology system. The Phoenix UNMIC-401/ID Panel and NMIC-400/ID panels were used for urine and other samples, respectively. EUCAST v.13.0 criteria and expert guidelines were used to evaluate the AST results (13).

The results of the colistin sensitivity tests conducted by the automated system were recorded. Both the broth microdilution (BMD) method and the automated system were used to test colistin sensitivity in multidrug-resistant bacteria. The BMD studies were conducted following the American Clinical Standards Institute M7 guidelines (14). To prepare for the study, the following steps were taken:

First, 100 µl of cation-adjusted Mueller Hinton Broth (CAMHB) was added to all the wells. Then, 100 µl of the

antibiotic stock solution (at a concentration of 256 mg/L) was added to the wells in the eleventh column.
Next, 100 µl was taken from the wells in the 11th column and transferred to the wells in the 10th column, creating a serial dilution from the 11th column to the first column.
The solutions taken from the wells in the first column (100 µl each) were discarded. As a result, the final antibiotic concentrations in the wells were 0.125, 0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, and 128 mg/L, respectively.
A bacterial suspension was prepared to achieve a turbidity of 0.5 McFarland, followed by a 1/100 dilution. Subsequently, 10 µl of the bacterial suspension was added to each well, resulting in a bacterial concentration of 10^5 colony-forming units (CFU)/ml in each well. In each study, bacterial suspensions of colistin-sensitive (*E. coli ATCC 25922*) and colistin-resistant (mcr-1-carrying *E.coli NCTC 13846*) were used as quality control isolates. Following the addition of bacterial suspensions, the plates were covered and then incubated for 16-18 hours at 35-37 °C. Subsequently, the wells were examined for turbidity, with the MIC value recorded as the concentration at the first well showing no turbidity. In each study, one well was designated as a growth control (bacteria present, no antibiotics) and another as a medium sterility control (only medium present, no bacteria or antibiotics). During the plate evaluation phase, the tests were deemed valid if there was no bacterial growth in the medium's sterility control well, if growth was observed in the growth control well, and if the MIC values of the quality control isolates fell within the expected ranges.
In the current study, the MIC limit values were adopted as established by EUCAST for Enterobacterales, *P. aeruginosa* and *A. baumannii*. Accordingly, for *A. baumannii* and Enterobacterales (*K. pneumoniae and E. coli*) isolates, those with MIC values of 2 mg/L or lower were classified as susceptible, while those with values above 2 mg/L were deemed resistant. Similarly, for *P. aeruginosa*, isolates with MIC values of 4 mg/L or lower were classified as susceptible, whereas those with values exceeding 4 mg/L were considered resistant.

Analysis

The BD Phoenix 100 automated system was evaluated using BMD as a reference method. Categorical agreement (CA; the rate of concordant results), major errors (ME; results falsely categorized as resistant by the automated system despite being sensitive by the reference method), and very major errors (VME; results falsely categorized as sensitive by the automated system despite being resistant by the reference method) were calculated. Results were assessed according to International Organization for Standardization (ISO) criteria (CA higher than 90%, ME lower than 3%, VME lower than 3%) (15). Percentages were calculated to express the distribution of categorical variables

Results

The study encompassed a total of 236 multidrug resistant isolates isolated from various clinical samples **(Table 1).**
Of 92 *A. baumannii* strains, 78 (84.7%) were classified as sensitive by the broth microdilution (BMD) method, whereas 88 (95.6%) were classified as sensitive by the automated system. Among the *A. baumannii* isolates, 14 were deemed resistant by BMD, of which 10 were classified as susceptible by the automated system (MIC ≤-less than or equal to 1 mg/L in 9 isolates, MIC equal to 2 mg/L in one isolate). The CA rate for *A. baumannii* isolates was 89.1%, with a VME rate of 10.8%; no major errors were observed. Four *A. baumannii* isolates identified as resistant by the automated system were also classified as resistant by BMD. **Table 2** summarized the MIC values of *A. baumannii* isolates determined by the BD Phoenix 100 system and the BMD method.

Table 1. Number of clinical samples from which the isolates included in the study

Variables	Tracheal Aspirate	Urine	Blood	Wound	Abscess	Drain	Pleura	Total
A. baumannii	46	11	18	13	0	2	2	92
K. pneumoniae	6	37	19	2	2	0	2	68
P. aeruginosa	11	9	9	26	0	0	0	55
E. coli	2	7	5	3	2	2	0	21
Total	65	64	51	44	4	4	4	236

Table 2. The number of *Acinetobacter baumannii* isolates with colistin minimal inhibitory concentration (MIC) values determined by BD Phoenix automated system and broth microdilution.

		Broth Microdilution MIC levels (mg/L)											Total n
		≤0.125(n)	0.25(n)	0.5(n)	1(n)	2(n)	4(n)	8(n)	16(n)	32(n)	64(n)	≥128(n)	
BD Phoenix MIC (mg/L) levels	≤1 n	1		11	53	13	4	2	1	1		1	87
	2 n											1	1
	≥4 n										3	1	4
Total n		1		11	53	13	4	2	1	1	3	3	92

Abbreviations: MIC: Minimum inhibitory concentration, n: number of isolates, ≤: less than or equal to, ≥: more than or equal to

Upon examining *K. pneumoniae* isolates, it was found that 54 out of 68 strains (79.4%) were sensitive with the BMD method, while 63 (92.6%) were found to be sensitive with the BD Phoenix 100 automated system. Among the *K. pneumoniae* isolates, 14 were deemed resistant by BMD, of which 10 were classified as susceptible by the BD Phoenix 100 automated system. Conversely, one isolate classified as susceptible by BMD was deemed resistant by the automated system. The CA rate for *K. pneumoniae* isolates was 83.8%, with a VME rate of 14.7% and ME rate of 1.4%. Four of the five *K. pneumoniae* strains classified as resistant by the automated system were also classified as resistant by BMD, while one strain was classified as sensitive. **Table 3** provided a summary of the MIC values of *K. pneumoniae* isolates determined by the BD Phoenix automated system and the BMD method.

Table 3. The number of *Klebsiella pneumoniae* isolates with colistin minimal inhibitory concentration (MIC) values determined by BD Phoenix automated system and broth microdilution.

		Broth Microdilution MIC levels (mg/L)											Total n
		≤0.125(n)	0.25(n)	0.5(n)	1(n)	2(n)	4(n)	8(n)	16(n)	32(n)	64(n)	≥128(n)	
BD Phoenix MIC (mg/L) levels	≤1 n			24	21	7	5	2		1		2	62
	2 n				1								1
	≥4 n			1				1	1		1	1	5
Total n				25	22	7	5	3	1	1	1	3	68

Abbreviations: MIC: Minimum inhibitory concentration, n: number of isolates, ≤: less than or equal to, ≥: more than or equal to

Out of the *P.aeruginosa* isolates that were examined, 54 (98.1%) were found to be sensitive when tested with BMD, whereas 55 (100%) were found to be sensitive when performed with the automated system. Only one *P. aeruginosa* isolate was deemed resistant by BMD, but this isolate was classified as susceptible by the Phoenix 100 automated system. For *P. aeruginosa* isolates, CA rate was 98.1%, with a VME rate of 1.8%, and no ME were detected. The MIC values of *P. aeruginosa* isolates determined by the BD Phoenix system and the BMD method were summarized in **Table 4.**

Table 4. The number of *Pseudomonas aeruginosa* isolates with colistin minimal inhibitory concentration (MIC) values determined by BD Phoenix automated system and broth microdilution.

		Broth Microdilution MIC levels (mg/L)											Total n
		≤0.125(n)	0.25(n)	0.5(n)	1(n)	2(n)	4(n)	8(n)	16(n)	32(n)	64(n)	≥128(n)	
BD Phoenix MIC (mg/L) levels	≤1 n			6	39	7	2					1	55
	2 n												
	≥4 n												
Total n				6	39	7	2					1	55

Abbreviations: MIC: Minimum inhibitory concentration, n: number of isolates, ≤: less than or equal to, ≥: more than or equal to

When evaluating *E. coli* isolates, 16 (76.1%) were classified as sensitive by BMD method, whereas 20 (95.2%) were classified as susceptible by automated system. Among 5 *E. coli* isolates which were deemed resistant by BMD, four

were classified as susceptible by the Phoenix 100 automated system while 1 isolate classified as resistant by both systems. For *E. coli* isolates, CA rate was 80.9%, with a VME rate of 19%, and no ME were detected. The MIC values of E. coli isolates determined by the BD Phoenix system and the BMD method were summarized in **Table 5.**

Table 5. The number of *Escherichia coli* isolates with colistin minimal inhibitory concentration (MIC) values determined by BD Phoenix automated system and broth microdilution.

		Broth Microdilution MIC levels (mg/L)											Total n
		≤0.125(n)	0.25(n)	0.5(n)	1(n)	2(n)	4(n)	8(n)	16(n)	32(n)	64(n)	≥128(n)	
BD Phoenix MIC (mg/L) levels	≤1 n			11	4	1	1		1	1		1	20
	2 n												
	≥4 n											1	1
Total n				11	4	1	1		1	1		2	21

Abbreviations: MIC: Minimum inhibitory concentration, n: number of isolates, ≤: less than or equal to, ≥: more than or equal to

Resistance to colistin by BMD was detected in 34 (14.4%) of the total 236 isolates. Colistin resistance among multidrug-resistant isolates of *E. coli, K. pneumoniae, A. baumannii* and *P. aeruginosa* included in the study was 23.8% (5 out of 21 isolates), 20.6% (14 out of 68 isolates), 15.2% (14 out of 92 isolates) and 1.8% (1 out of 55 isolates) respectively. The bar graph illustrates the MIC values of isolates from four bacterial species as determined by the broth microdilution method **(Figure 1).** Upon evaluating all isolates in the study, it was observed that 9 out of the 34 isolates identified as resistant by BMD also appeared resistant in the automated system, while 25 were found as susceptible by the automated microbiology system. Therefore, VME rate of the BD Phoenix 100 automated system was determined as 10.5%. Out of the 10 isolates identified as resistant by the automated system, 9 were found to be resistant and 1 was found to be sensitive by BMD.

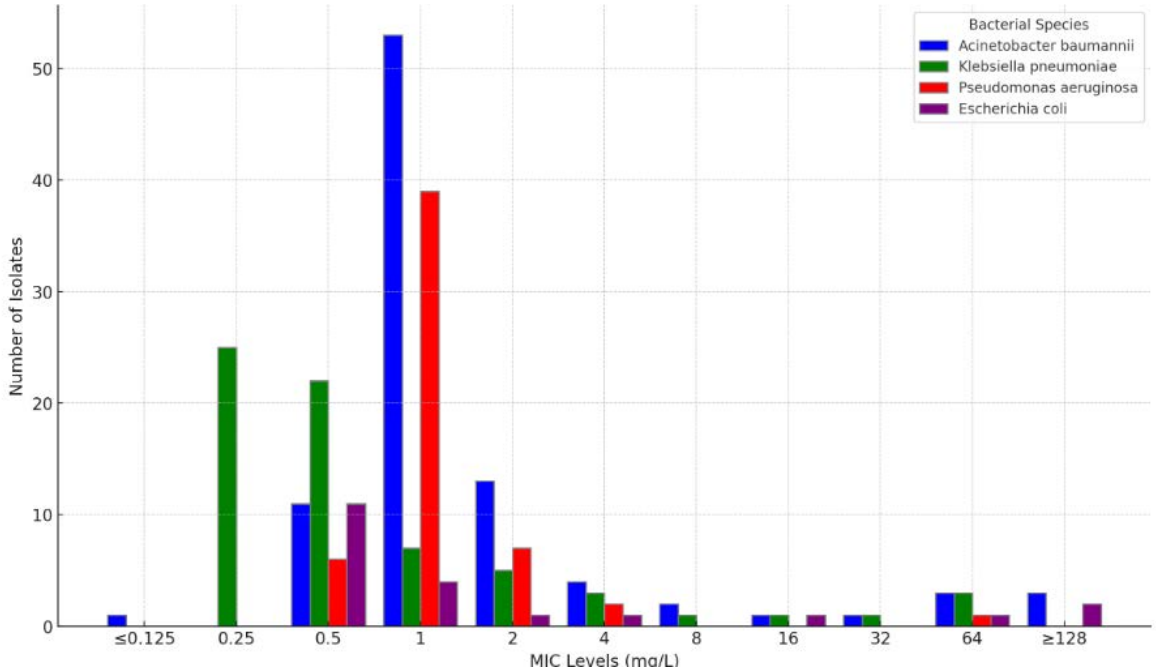

Figure 1. Bar chart showing the minimal inhibitory concentration (MIC) values for the four bacterial species using the broth microdilution method

Discussion

There is an increasing incidence of infections caused by drug resistant Gram-negative bacteria (16). This has led to the reconsideration of using colistin for treating infections caused by multidrug-resistant *P.aeruginosa, A.baumannii* and Enterobacterales members (3,17,18). Colistin, which was avoided before the 2000s due to its side effects on kidneys, is now being used for treating infections of resistant microorganisms, either alone or in combination with other treatments, locally or systemically (2,19,20).

In a joint statement issued in 2016, EUCAST and CLSI declared that BMD was the sole reliable method for testing colistin susceptibility. However, the practical application of BMD poses challenges, prompting the development of alternative methods. Among these alternatives, automated systems have emerged as viable options (21). Widely adopted in clinical microbiology laboratories, automated systems alleviate workload burdens, ensure high repeatability, facilitate expert system analysis for data management, and expedite result generation (22).

Jayol et al. carried on a study about colistin sensitivity of 123 clinical isolates of Enterobacterales. Out of these, 40 isolates were sensitive to colistin while 83 were resistant. They used BMD, BD Phoenix 100, and Rapid Polymyxin NP tests for the study. Based on the results, the BD Phoenix 100 system missed detecting 10 colistin-resistant isolates when compared to the BMD method used as the standard reference. Seven of these isolates were *Enterobacter spp,* and one isolate each was *Salmonella enterica, K. pneumoniae* and *E. coli.* Researchers reported that the Rapid Polymyxin NP test effectively detected all colistin-resistant isolates, with the exception of a single *E. coli* isolate. Furthermore, they noted that the test provided rapid results within a time-frame of 2 hours. The VME rate of the BD Phoenix 100 automated microbiology system was found to be 12% (10 out of 83 isolates) and as a result, it was emphasized that isolates detected as susceptible by the Phoenix 100 automated system should be confirmed with BMD method (6).

In a 2017 study by Vourli et al. in Greece, the susceptibility of 117 *A.baumannii* isolates to colistin was tested using various methods. The study utilized the BMD method as the standard reference, alongside evaluations of the BD Phoenix 100 and Vitek-2 systems—commonly employed in microbiology labs—and the agar dilution (AD) method. While the BMD method yielded a resistance rate of 24.8%, alternative methods displayed different rates: 35.9% with the AD method, 16.2% with the Vitek 2 system, and 15.4% with the Phoenix 100 system. Notably, both automated systems exhibited high rates of VME, recording 41.4% with Phoenix 100 and 37.9% with Vitek 2, while ME rates were exceptionally low at 1.1% for both systems. In contrast, the AD method displayed a considerably higher ME rate of 15.9%. These findings underscore the potential limitations of relying solely on automated systems for detecting colistin-resistant *A. baumannii* isolates, particularly those with a MIC value of 2 mg/L. The study suggests the necessity of confirming isolates using standardized BMD methods to ensure accurate detection (9).

In 2015, a study was conducted by Dafopoulou et al. to test the reliability of gradient tests, which is one of the methods used to detect MIC. The study tested 61 carbapenem-resistant isolates, including 40 *K. pneumoniae* and 21 *A. baumannii* isolates. The study performed gradient test, AD test, Vitek-2 automated system, and MIC test strips (MTS), with the BMD method used as a reference. The study found that the VME rate was 39.3% and 31.1% in the gradient test and MTS, respectively. However, the AD method showed a low VME rate of only 3.3%, and there was no VME detected in the Vitek-2 automated system. The ME rate was also very low in all methods. Based on these results, Dafopoulou et al. concluded that gradient diffusion methods (strip test and MTS) could lead to inappropriate colistin treatments due to falsely sensitive results (23).

In their study, Tuzemen et al. investigated the efficacy of the BMD and gradient test methods, utilizing 36 *K. pneumoniae*, 9 *A. baumanni*i, and 5 *P. aeruginosa* isolates previously identified as colistin-resistant by the BD Phoenix 100 system. In comparison to the BMD method, acknowledged as the gold standard, the CA rate of the Phoenix™ 100 automated system was determined to be 92% across all isolates tested. Specifically, the CA rate was 100% for *K. pneumoniae*, 77.8% for *A. baumannii*, and 60% for *P. aeruginosa*. Notably, the VME rate was recorded at 0% for all isolates, while the ME rate was 8%. Specifically the ME rate was 0% for *K. pneumoniae*, 22.2% for *A. baumanni*i, and 40% for *P. aeruginosa*. In the study, the gradient test diffusion method exhibited a VME rate of 80% across all isolates tested, with specific rates of 83.3%, 77.8%, and 60% observed in *K. pneumoniae, A. baumannii,* and *P. aeruginosa* isolates, respectively. It was noteworthy that the isolates analyzed in this study were pre-identified as resistant by the Phoenix 100 automated system, which accounts for the absence of VME detection within the Phoenix 100 system. While the study indicated that the gradient test diffusion method was inadequate for detecting colistin resistance, it was essential to note that the exclusion of isolates identified as susceptible by the Phoenix 100 system limited the study's ability to provide comprehensive insights into the automated system's detection rate for resistant isolates (24).

Aytac et al. conducted a study to assess the colistin sensitivity of 100 multidrug-resistant *A. baumannii* isolates using Sensititre™ microdilution methods, BD Phoenix™ 100, and MicroScan WalkAway™ automatic systems, with the BMD method as the reference. The study revealed that the categorical agreement of the BD Phoenix 100 automated system was 79%, with a ME rate of 10% and a VME rate of 11%. Based on their findings, Aytaç et al. concluded that optimizing and standardizing commercial methods for colistin sensitivity assessment was imperative (25).

Koyuncu Ozyurt et al. conducted a study to investigate colistin sensitivity using 163 *A. baumannii*, 199 *K. pneumoniae*,

and 34 *E. coli* isolates. They employed the Colistin Disc Elution (CDE) method and the BD Phoenix 100 automated system, with the BMD reference method. In their study, the CA rate for *A. baumannii* isolates was found to be 96.3%, with a VME rate of 3.7%. For *K. pneumoniae* and *E. coli*, the CA rate was 94.1%, and VME rate was 5.9%. Their study revealed that the very large error rate associated with the BD Phoenix 100 system exceeded acceptable limits. Consequently, they concluded that isolates identified as sensitive to colistin should undergo confirmation (26).

In a thesis study conducted with 41 *A. baumannii* isolates in our country, colistin sensitivity was assessed using the BD Phoenix™ 100 automated system in comparison with the reference BMD method. According to the data from the study, the CA between the BD Phoenix™ 100 automated system BMD method was found to be 100%. Furthermore, no discordant results, such as discordant ME or VME were observed (27).

Ceylan et al. analyzed 782 drug-resistant Gram- negative bacterial isolates, including *K. pneumoniae* (n=175), *P. aeruginosa* (n=99), and *A. baumannii* (n=508). The categorical agreement rates were 90.3%, 93.9%, and 94.5%, respectively. The Phoenix M50 system showed a VME rate of 40.4% totally. When examining the VME for each species individually, the rates were 17.7% for *K. pneumoniae*, 75.0% for *A. baumannii*, and 100.0% for *P. aeruginosa* (28). The VME rates for *A. baumannii* and *P. aeruginosa* were higher than in our findings, while the VME rates for *K. pneumoniae* isolates were similar to ours.

Study limitations

A limitation of this study was the small sample size, particularly the limited number of colistin-resistant isolates. Nonetheless, this research can serve as a pioneering study, guiding future investigations that involve larger isolate numbers and employ various testing methods

Conclusion

It was concluded that isolates identified as sensitive to colistin should undergo confirmation using the reference method. This recommendation stems from the observed low categorical agreement and high error rates associated with the BD Phoenix 100 automated microbiology systems when compared to the reference method. Based on the results obtained from our study, further investigations using diverse strains are warranted to ascertain the resistance profiles accurately using the automated system.

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