



Evaluation of Colistin Performance of Phoenix M50 with Sensititre FRCOL in Clinical Isolates

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What is known on this subject?

The Clinical Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing, Polymyxin Breakpoints Working Group published a report in 2016 indicating that the colistin susceptibility test should only be performed with the broth microdilution method, in accordance with the rules set in International Organization for Standardization. Users of semi-automated devices should apply rigorous quality control and check with the manufacturer whether or not they are confident that their method for colistin antibiotic susceptibility test gives correct results.

What this study adds?

In this study, we compared the colistin susceptibility results of gram-negative bacteria isolated between June 2021 and June 2022, studied with Sensititre FRCOL and Phoenix M50. Thus, we aimed to determine the reliability of Phoenix M50 for reporting colistin results.

ABSTRACT

Objective: In the report published by the Clinical Laboratory Standards Institute-European Committee on Antimicrobial Susceptibility Testing (EUCAST), Polymyxin Breakpoints Working Group, they recommended that laboratories using semi-automatic devices take into account the manufacturer's recommendations and implement strict quality control (QC) studies when reporting the colistin result. In this study, we compared the one-year colistin susceptibility results with those of Sensititre FRCOL and Phoenix M50. Thus, we aimed to determine the reliability of Phoenix M50 for reporting colistin results.

Material and Methods: Extensively drug-resistant Gram-negative bacteria grown from clinical samples that arrived at the laboratory between June 2021 and June 2022 were included. MALDI-TOF Microflex LT/SH Smart MS was used for bacterial identification, and Phoenix M50 and Sensititre FRCOL were used for colistin antibiotic susceptibility testing, according to the manufacturer's recommendations. The results obtained were evaluated in line with the EUCAST criteria. QC was performed using *Escherichia coli* ATCC 25922 and NCTC 13846 strains in accordance with EUCAST recommendations.

Results: We studied 782 strains of *K. pneumoniae* (n=175), *P. aeruginosa* (n=99), and *A. baumannii* (n=508). Categorical agreements were 90.3%, 93.9%, and 94.5%. The very major error rate (VME) of Phoenix M50 was found to be 40.4%. Considering the VME for *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa*, 17.7%, 75.0%, and 100.0% were found, respectively. The ME rates of *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* were 5.3%, 0.8%, and 1.1%, respectively.

Conclusion: The susceptible colistin results of these bacteria by Phoenix M50 should be confirmed by broth microdilution as the VME is above acceptable values. While the results of colistin detection resistant by Phoenix M50 could be reported for *P. aeruginosa* and *A. baumannii*, it needs to be confirmed with broth microdilution for *K. pneumoniae*.

Keywords: Colistin susceptibility test, broth microdilution, Phoenix M50, *K. pneumoniae*, *A. baumannii*

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Introduction

Colistin, initially isolated from the soil bacterium *Paenibacillus polymyxa* subsp. *colistinsi* in 1947, is a polypeptide antibiotic effective against Gram-negative bacteria (1). Although colistin was used for years after its discovery, because of its high toxicity, it was replaced with other less toxic antibiotic groups in the 1970s. The rapid increase in multidrug-resistant Gram-negative bacteria recently has again led colistin to come into question as a treatment option (2).

While determining the harm-benefit balance of this highly toxic drug, the sensitivity result from the laboratory is critical in terms of guiding clinicians. In addition, the Clinical Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) Polymyxin Breakpoints Working Group published a report in 2016 indicating that the colistin susceptibility test should only be performed with broth microdilution method, in accordance with the rules set in International Organization for Standardization (ISO) standards, and other methods, including agar dilution, disc diffusion, and gradient strip test, should not be used (3). Although the recommendations favor using the microdilution method, its use is limited as it is time-consuming and expensive, and the results are dependent on the experience of the laboratory staff.

Finally, this report was updated in May 2020 and includes the following statement: "we could not systematically evaluate semi-automated colistin methods, but by sending isolates with minimum inhibitory concentration (MIC) values in the non-susceptible range to colleagues around the world, we have disclosed the frequent occurrence of very major errors." Users of semi-automated devices should apply rigorous quality control (QC) and check with the manufacturer whether or not they are confident that their method for colistin AST gives correct results. QC of colistin must be performed with both a susceptible QC strain (*Escherichia coli* ATCC 25922 or *Pseudomonas aeruginosa* ATCC 27853) and the colistin-resistant *E. coli* NCTC 13846 (*mcr-1* positive). For *E. coli* NCTC 13846, the colistin MIC target value is 4 mg/L and should only occasionally be 2 or 8 mg/L (4). When the 2023 guidelines of these two organizations are examined, EUCAST states that the only method that can be used for colistin is the broth microdilution method, and CLSI states that broth microdilution, agar dilution, and disk elution methods can be used for colistin (5,6).

Our institution is a large hospital with a capacity of 2,700 beds, serving national and international patients. Extensive drug-resistant Gram-negative bacteria grow, especially in

samples taken from hospitalized patients, and the use of colistin for treating these microorganisms is inevitable. Phoenix M50 (semi-automated system) is used for antibiotic susceptibility tests in our laboratory. Colistin susceptibility tests for extensively drug-resistant Gram-negative bacteria are reported with the results of Sensititre FRCOL. In this study, we compared the susceptibility results of colistin in Gram-negative bacteria isolated between June 2021 and June 2022, which were studied with Sensititre FRCOL (commercial broth microdilution system) and Phoenix M50. Thus, we aimed to determine the reliability of Phoenix M50 in reporting colistin susceptibility test results.

Material and Methods

In this study, extensively drug-resistant *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* isolates grown from clinical samples that arrived at the laboratory between June 2021 and June 2022 were included. MALDI-TOF Microflex LT/SH Smart MS (Bruker Daltonics, Germany) was used for bacterial identification, and Phoenix M50 (BD Diagnostics, USA) and Sensititre FRCOL (Thermo Scientific, West Sussex, UK) were used for colistin antibiotic susceptibility testing, according to the manufacturer's recommendations. The results obtained were evaluated in line with the EUCAST criteria (7,8,9). The study was approved by the University of Health Sciences Turkey, Başakşehir Çam and Sakura City Hospital Clinical Research Ethics Committee (decision no: 2022-138, date: 27.04.2022).

According to EUCAST version 12.0 recommendations, MIC breakpoints of colistin ≤ 2 mg/L for *Klebsiella pneumoniae* and *Acinetobacter baumannii*; ≤ 4 mg/L for *P. aeruginosa* considered susceptible and > 2 mg/L for *K. pneumoniae* and *A. baumannii*; > 4 mg/L *P. aeruginosa* considered resistant. QC studies for Phoenix M50 and Sensititre FRCOL were regularly performed with *E. coli* ATCC 25922 and *E. coli* NCTC 13846 strains (4).

The sensitivity, specificity, positive predictive values (PPV), and negative predictive values (NPV) of the Phoenix M50 were evaluated based on the sensitivity FRCOL. Results were evaluated according to the ISO criteria. Categorical agreement (agreement of sensitive and resistant results of the two systems), major error (ME) (susceptible by Sensititre FRCOL, but resistant by the Phoenix M50), and very major error (VME) (resistant by Sensititre FRCOL, but susceptible by the Phoenix M50) of the Phoenix M50 were calculated according to the Sensititre FRCOL [categorical agreement (CA) $> 90\%$; ME and VME $< 3\%$] (10).

Statistical Analysis

Statistical analyses were conducted with using “IBM SPSS Statistics” (version 26.0, Chicago) statistical software. The agreements between the Phoenix M50 and sensitivity were evaluated using Cohen’s Kappa (κ) analysis. A κ value above 0.80 was interpreted as excellent, between 0.60 and ≥ 0.80 as good, between 0.40 and ≥ 0.60 as moderate, and between 0.20 and ≥ 0.40 as low moderate agreement.

Results

The susceptibility results of all isolates studied with Phoenix M50 and sensitivity FRCOL are shown in Tables 1 and 2.

Susceptibility test results in *K. pneumoniae* isolates revealed a significant correlation between Sensititre and Phoenix M50 (κ : 0.784, $p < 0.001$; Figure 1A). Moreover, susceptibility results in *P. aeruginosa* revealed a low correlation between Sensititre and Phoenix M50 (κ : 0.004, $p = 0.883$; Figure 1B). The susceptibility results in *A. baumannii* revealed a low-significant correlation between Sensititre and Phoenix M50 (κ : 0.149, $p < 0.001$; Figure 1C).

The sensitivity of Phoenix M50 was 94.38% [confidence interval (CI): 92.43%-95.96%], specificity was 84.29% (CI: 73.62%-91.89%), PPV was 98.39% (CI: 97.26%-99.06%), and NPV was 59.60% (CI: 51.78%-66.96%) in all strains (Table 3).

For *K. pneumoniae*, the sensitivity of Phoenix M50 was 90.68% (CI: 83.93%-95.25%), specificity was 89.47% (CI: 78.48%-96.04%), PPV was 94.69% (CI: 89.30%-97.44%), and NPV was 82.26% (CI: 72.40%-89.13%) (Table 4).

For *P. aeruginosa*, the sensitivity of Phoenix M50 was 94.90% (CI: 88.49%-98.32%), specificity was 0.00% (CI: 0.0%-97.50%), PPV was 98.94% (CI: 98.89%-98.98%), and NPV was 0% (CI: -) (Table 4).

For *A. baumannii*, the sensitivity of Phoenix M50 was 99.16% (CI: 97.86%-99.77%), specificity was 25.0% (CI: 11.46%-43.40%), PPV was 95.16% (CI: 94.15%-96.00%), and NPV was 66.67% (CI: 38.88%-86.28%) (Table 4).

When the susceptibility test results of 782 isolates were analyzed, the CA was 93.5%. Among 782 isolates, *A. baumannii* (508), *K. pneumoniae* (175), and *P. aeruginosa* (99) were analyzed separately and CA was 94.5%, 90.3%, and 93.9%, respectively. Essential agreement could not be calculated because the Phoenix M50 device had few colistin wells.

The percentages of VME for *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* were 17.7%, 100.0% and 75.0%, respectively. In contrast, the percentages of ME for *K. pneumoniae*, *P. aeruginosa* and *A. baumannii* were 5.3%, 1.1% and 0.8%, respectively.

Table 1. MIC values of all isolates by sensitivity Sensititre FRCOL

Organism	0.25	0.5	1	2	4	8	16	32	64	≥ 128	Total	Susceptibility
<i>Acinetobacter baumannii</i>	1	108	316	51	11	7	8	4	0	2	508	93.7%
<i>Klebsiella pneumoniae</i>	5	66	19	23	15	7	16	15	6	3	175	64.5%
<i>Pseudomonas aeruginosa</i>	1	5	23	61	4	3	1	0	1	0	99	94.9%

MIC: Minimum inhibitory concentration

Table 2. MIC values of all isolates by Phoenix M50

Organism	≤ 1	2	4	> 4	Total	Susceptibility
<i>Acinetobacter baumannii</i>	494	2	2	10	508	97.6%
<i>Klebsiella pneumoniae</i>	118	0	0	57	175	67.4%
<i>Pseudomonas aeruginosa</i>	98	0	0	1	99	98.9%

MIC: Minimum inhibitory concentration

Table 3. Comparison of antibiotic sensitivity results of Phoenix M50 with sensitivity FRCOL

		Phoenix M50		Total (n)
		Susceptible (n)	Resistant (n)	
Sensititre	Susceptible	672	11	683
	Resistant	40	59	99
	Total	712	70	782

MIC results of colistin-susceptible *E. coli* ATCC 25922 and colistin-resistant *E. coli* NCTC 13846 were <1 mg/L and 4 mg/L, respectively.

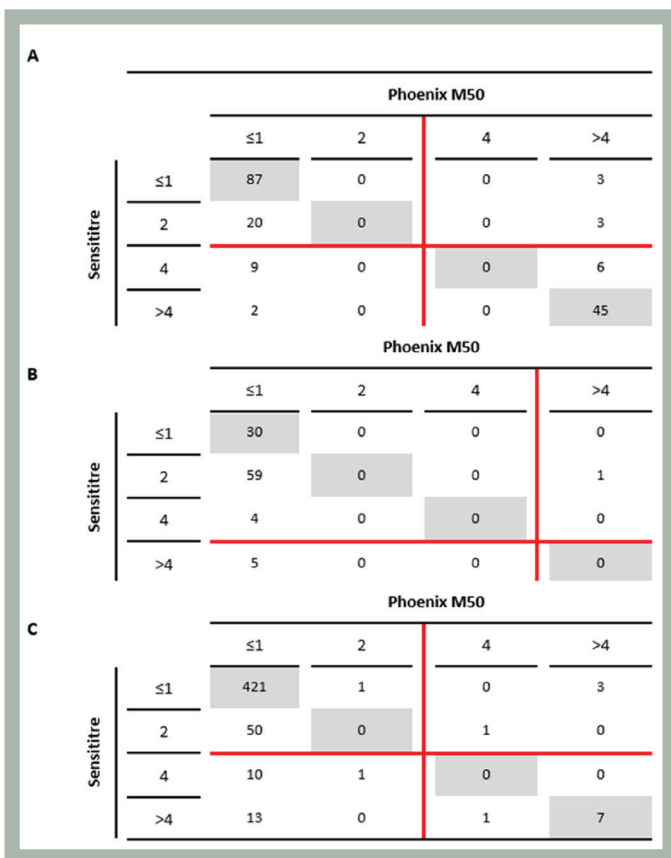


Figure 1. Distribution of MIC values for *K. pneumoniae* (A), *P. aeruginosa* (B), and *A. baumannii* (C) determined using Sensititre FRCOL and Phoenix M50

MIC: Minimum inhibitory concentration

Table 4. Comparison of antibiotic sensitivity results of Phoenix M50 with sensitivity FRCOL for *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii*

<i>K. pneumoniae</i>	Sensititre	Phoenix M50		
		Susceptible (n)	Resistant (n)	Total (n)
	Susceptible (n)	107	6	113
	Resistant (n)	11	51	62
	Total (n)	118	57	175
<i>P. aeruginosa</i>	Sensititre	Phoenix M50		
		Susceptible (n)	Resistant (n)	Total (n)
	Susceptible (n)	93	1	94
	Resistant (n)	5	0	5
	Total (n)	98	1	99
<i>A. baumannii</i>	Sensititre	Phoenix M50		
		Susceptible (n)	Resistant (n)	Total (n)
	Susceptible (n)	472	4	476
	Resistant (n)	24	8	32
	Total (n)	496	12	508

Discussion

Colistin is one of the last-choice drugs that is preferred for treating extensively drug-resistant and pan-drug-resistant Gram-negative bacteria, including *P. aeruginosa*, *A. baumannii*, and *K. pneumoniae* infections (7). Therefore, determining colistin sensitivity with accurate and reliable tests is very important considering the benefit it will provide for the patient's treatment. In 2017, the CLSI and EUCAST working group recommended (9) applying the reference BMD test using a polystyrene microplate and colistin sulfate salt according to the recommendations of the ISO-20776-1 to determine colistin susceptibility (10). The EUCAST development laboratory has published these results by comparing various commercial broth microdilution systems, including the sensititre, with the reference method. Sensititre stated that the commercial broth microdilution system can be used to test the susceptibility of colistin (7). According to this study, essential agreement for sensitivity was 96% and CA was 95%. No VME was found among the 75 isolates; only 4 isolates had ME. In different studies, commercial broth microdilution systems and reference methods were compared, and it was determined that colistin sensitivity could be studied with commercial broth microdilution systems (7,11,12). The CLSI and EUCAST working group reported, which was updated in 2020; "Users of semi-automated devices should apply rigorous QC and check with the manufacturer whether or not they are confident that their method for colistin AST gives correct results. QC of colistin must be performed with both a susceptible QC strain (*E. coli* ATCC 25922 or *P. aeruginosa*

ATCC 27853) and colistin-resistant *E. coli* NCTC 13846 (*mcr-1* positive). For *E. coli* NCTC 13846, the colistin MIC target value is 4 mg/L and should only occasionally be 2 or 8 mg/L.” as proposal (4). In our laboratory, Phoenix M50 is routinely used for antibiotic susceptibility testing, and a Sensititre™ FRCOL (commercial BMD) is used for colistin susceptibility testing in line with the recommendations of EUCAST. For both systems, QC strains (*E. coli* ATCC 25922 and *E. coli* NCTC 13846) were tested weekly. Since the QC results for colistin with Phoenix M50 were within the recommended mean limits without exception, we thought that we could report the colistin susceptibility with Phoenix M50, instead of the more expensive and difficult to implement BMD. Based on this thought, we aimed to retrospectively evaluate our one-year data and compare the results of colistin using Phoenix M50 and Sensititre FRCOL, according to the ISO criteria (10).

In a study conducted in Greece in 2017, the colistin susceptibility of 117 *A. baumannii* strains was compared with the semi-automatic systems and reference method, according to ISO criteria (10). The CA of Vitek 2 and Phoenix 100 was found to be 89.7% and 88.9%, respectively, and VME rates of 37.9% and 41.4%, respectively (13). In another study, CA for Vitek 2 was 88.2%, and the ME was 36.0% (12). According to the study by Carretto et al. (14), comparing the reference method and the Phoenix 100, the CA was found to be 96.8%, while the ME was 10%. In another study (15), the performance of Phoenix M50 was evaluated using 533 Gram-negative clinical isolates, and BMD was used as the reference method for colistin performance. In the same study, CA was found for 131 Gram-negative bacteria, 96 of which were colistin-resistant, with a VME of 0% and a ME of 1.5% (15). A study conducted in India in 2021 included 25 clinical isolates (14 *E. coli* and 11 *K. pneumoniae*) and compared the colistin susceptibility performance of the Phoenix M50 system with the Mikrolatest MIC colistin susceptibility testing kit as a reference method. The ratio of VME and ME for colistin in the Phoenix M50 system was 0% (16). We compared Phoenix M50 and Sensititre FRCOL, a commercial BMD test that EUCAST indicated can be used for colistin sensitivity. When we examined the susceptibility results of 782 isolates, the CA was found to be 93.5%. *A. baumannii* (n=508), *K. pneumoniae* (n=175) and *P. aeruginosa* (n=99) were analyzed separately, and the CA was calculated as 94.5%, 90.3%, and 93.9%, respectively.

Although the CA rates seem high (>90%), when the incompatible results are examined in detail, the percentages of VME for *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* were 17.7%, 100.0% and 75.0%, respectively. In contrast, the

percentages of ME for *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* were 5.3%, 1.1%, and 0.8%, respectively.

Based on these percentages, it appears that Phoenix M50 “susceptible” results for colistin should not be reported for these bacteria. The ME rates for *A. baumannii*, *K. pneumoniae*, and *P. aeruginosa* isolates were 0.8%, 5.3%, and 1.1%, respectively. When compared with Sensititre, it is seen that “resistant” results gained from Phoenix M50 could be reported for *A. baumannii* and *P. aeruginosa*, but should not be reported for *K. pneumoniae* (17).

Broth microdilution is one of the most reliable methods for reporting colistin susceptibility. However, in addition to the difficulties and cost in implementation, there are procedures that must be applied in the study to ensure accurate results. As possible contamination may give erroneous results, the test should be studied in a biosafety cabinet and must be performed with a single experienced person to ensure a standardization. In our study, as a final control, we also inoculated the suspension on 5% sheep blood agar after BMD procedures to check whether it is pure or not.

Semi-automated systems are frequently used in clinical microbiology laboratories to study susceptibility testing because they reduce workload, perform data management with expert system analysis software, and provide results in a shorter time than conventional methods (18).

Conclusion

As a result, it has been seen that susceptible colistin results on the Phoenix M50 for *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* should be validated by broth microdilution, since the ME rates are above acceptable values. While the results of colistin detection resistant by Phoenix M50 could be reported for *P. aeruginosa* and *A. baumannii*, it needs to be confirmed with broth microdilution for *K. pneumoniae*. Most of the isolates in our study were susceptible. Studies with larger sample sizes are required, including more colistin-resistant isolates.

Ethics

Ethics Committee Approval: The study was approved by the University of Health Sciences Turkey, Başakşehir Çam and Sakura City Hospital Clinical Research Ethics Committee (decision no: 2022-138, date: 27.04.2022).

Informed Consent: Not necessary.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: A.N.C., S.K., A.G., Design: A.N.C., A.G., Data Collection or Processing: A.N.C., S.K., A.G., B.Ö., Analysis or Interpretation: A.N.C., A.G., Literature Search: A.N.C., S.K., A.G., B.Ö., Writing: A.N.C., S.K., A.G., B.Ö.

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