Comparison of Colistin Susceptibility Tests / *Kolistin Duyarlılık Testlerinin Karşılaştırılması*

Nurullah ÇİFTÇİ[1], Uğur ARSLAN[2], Hatice TÜRK DAĞI[3]

1. Selcuk University Faculty of Medicine Medical Microbiology Department, ciftcinurullah72@gmail.com

2. Selcuk University Faculty of Medicine Medical Microbiology Department, drarslanugur@gmail.com

3. Selcuk University Faculty of Medicine Medical Microbiology Department, haticeturkdagi@yahoo.com

Gönderim Tarihi | Received: 31.03.2023, Kabul Tarihi | Accepted: 21.07.2023, Yayım Tarihi | Date of Issue: 31.03.2024,

Atıf | Reference: "ÇİFTÇİ, N., ARSLAN, U. and TÜRK DAĞI, H. (2024). Comparison of colistin susceptibility tests. *Health Academy Kastamonu (HAK), 9(*2), 400-406. https://doi.org/10.25279/sak.1274681"

Abstract

Introduction: Polymyxins are important antimicrobial agents for the treatment of infections caused by Gram-negative bacteria. The susceptibility testing for polymyxins is a challenge for clinical laboratories due to the difficulty of performance, reproducibility, and accuracy of available methods. Aim: To compare the performance of the colistin susceptibility test of an automated system and a gradient test with the gold standard broth microdilution method (BMD). Materials and Methods: Multidrug-resistant isolates of *Acinetobacter baumannii (n=102), Klebsiella pneumoniae (n=40),* and *Pseudomonas aeruginosa(n=11)* were included. The VITEK 2 systems and gradient test were studied according to the manufacturer's instructions. Broth microdilution tests were performed according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Commercial susceptibility testing methods were compared to BMD. Results: Rates of essential agreement of colistin test results between BMD, VITEK 2, and gradient test were 96.1% and 79.7%, respectively. The VITEK 2 and gradient test showed 95.4% and 94.8% of categorial agreement. The very major error rate of VITEK 2 was 3.2%, and the gradient test was 5.2%. The major error rate of VITEK 2 was 1.3%, and there was no major error for the gradient test. Conclusion and Suggestions: The very major error rate was higher in the gradient test (5.2%) than VITEK 2 (3.2%). Even if the very major error rate of VITEK 2 was lower, both resistance and susceptility results of VITEK 2 should be confirmed with the BMD test. Further studies for susceptibility testing are needed with a focus on the correlation of MIC's results of different tests.

Keywords: Antimicrobial drug resistance, Colistin, Microbial sensitivity tests, Minimum inhibitory concentration

Öz

Giriş: Polimiksinler, Gram negatif bakterilerin neden olduğu enfeksiyonların tedavisinde kullanılan önemli bir antimikrobiyal ajandır. Bu antibiyotiklerin çalışıldığı duyarlılık testlerinin performans, tekrar edilebilirlik ve doğru yöntemin uygulanmasındaki zorluklar nedeniyle klinik laboratuvarlar için problem oluşturmaktadır. Otomatize edilmiş antimikrobiyal duyarlılık testlerinin doğruluğu halen belirsizdir. Amaç: Bu çalışmada, kolistin duyarlılık testi çalışılan otomatize sistem ve gradient testin altın standart olan sıvı 2ikrodilüsyon testi ile karşılaştırılması amaçlanmaktadır. Gereç ve yöntem: Çoklu ilaç direncine sahip 102 *A. baumannii*, 40 *K. pneumoniae* ve 11 *P. aeruginosa* suşu çalışmaya dahil edildi. VITEK 2 ve gradient test firma önerileri doğrultusunda çalışıldı. Sıvı mikrodilüsyon testi ise EUCAST kriterlerine göre

değerlendirildi. Bu çalışmada ticari testler ile sıvı mikrodilüsyon testi karşılaştırıldı. Bulgular: Sıvı mikrodilüsyon testi ile VITEK 2 ve gradient test arasındaki temel uyum oranı sırasıyla %96.1 ve %79.7 olarak hesaplandı. VITEK 2 ve gradient test ile sıvı mikrodilüsyon yöntemi arasında %95.4 ve %94.8 kategorik uyum saptandı. Çok büyük hata oranı VITEK 2 ile %3.2, gradient test ile %5.2 olarak tespit edildi. Büyük hata oranı VITEK 2 ile %1.3 olarak hesaplandı ve gradient test ile büyük hata tespit edilmedi. Sonuç ve Öneriler: Çalışmamızda çok büyük hata oranı gradient testte VITEK 2'ye göre daha yüksek oranda saptandı. VITEK 2 yönteminde çok büyük hata oranı düşük olsa bile bu yöntemle elde edilen duyarlılık ve direnç sonuçları sıvı mikrodilüsyon yöntemi ile doğrulanmalıdır. Farklı testler ile elde edilen MIK sonuçları arasında uyumu gösteren daha fazla çalışmaya ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Antimikrobiyal ilaç direnci, Kolistin, Mikrobiyal duyarlılık testleri, En düşük engelleyici yoğunluk

1. Introduction

Polymyxins were first isolated in 1947 from the soil by *Bacillus polymxa* (Storm et al., 1977). Although there are many types of polymyxins (A-E), which are polypeptide antibiotics, only polymyxin B and colistin (polymyxin E) are used clinically. Systemic use of colistin has been limited due to its severe nephrotoxic effect (Li et al., 2006). However, after the emergence of multi-drug resistant strains such as *Acinetobacter baumannii* (*A. baumannii)*, *Klebsiella pneumoniae* (*K. pneumoniae*), and *Pseudomonas aeruginosa* (*P.aeruginosa)*, it is used as a last resort in the treatment of these infections. Recently, resistance to this antibiotic has been observed due to increased use of colistin (Karaiskos and Giamarellou, 2014). Therefore, it is important to use an antibiotic susceptibility test that most accurately detects susceptibility to colistin. Both CLSI and EUCAST suggested that the most reliable antibiotic susceptibility test for colistin is the broth microdilution test (Matuschek et al., 2018). In this study, it was aimed to study the colistin susceptibility of *A. baumannii*, *K. pneumoniae,* and *P. aeruginosa* strains isolated from different clinical specimens with the VITEK 2, gradient test (E test) and to compare them with the reference method, the broth microdilution test.

2. Material and Methods

2.1. Type of Research

This is an original research study.

2.2 Place and Timing of Research

This study was carried out in Selcuk University Faculty of Medicine Medical Microbiology Laboratory between January 2022 and December 2022.

2.3 Population, Sample and Sampling Method of Research

One hundred and two *Acinetobacter baumannii,* forty *Klebsiella pneumoniae*, and eleven *Pseudomonas aeruginosa,* which are multidrug-resistant isolates, were included in this study. Bacteria were identified using conventional methods and the VITEK 2 (bioMérieux, France) automated system. The susceptibility of bacteria to colistin was studied with the VITEK 2 automated system and the gradient test (bioMérieux, France) method and was confirmed by the reference method, the broth microdilution test (BMD). Antibiotic susceptibility of all strains was evaluated according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (EUCAST, 2021).

Colistin gradient and VITEK 2 (AST-N038 susceptibility card) tests were carried out according to the manufacturer's guidelines. The Gradient tests were carried out briefly as follow; bacterial suspension was prepared at 0.5 McFarland value then homogeneously inoculated on MH agar, the antibiotic strips placed on the cultural media and the results were noted after incubation at 37 ºC for 24 hours. The broth microdilution test was performed in accordance with the instructions in the EUCAST guidelines. Colistin (Sigma-Aldrich) was scaled in powder form and diluted to 0.125-64 mg/L and distributed to the sterile 96-well microplates. Then, the bacterial suspension was adjusted to 0.5 McFarland and diluted 1/20, and added to each well. In each assay, the last two wells were set as sterility control and growth control, then the plates were incubated at 35±2º ºC for 18-20 hours. The results were evaluated visually, and the minimum inhibitory concentration (MIC) values were evaluated as sensitive ≤2 mg/L and resistant >2mg/L. In each study, *E. coli* ATCC 13846 strain was used as quality control (EUCAST, 2021).

2.4 Data Collection

In our study, the criteria for acceptance of antibiotic susceptibility tests were evaluated by calculating the essential agreement (EA), categorical agreement (CA), very major error (VME), and major error (ME) values (very mjor error and major error below 3%, categorical agreement above 90%). Essential agreement was defined as a MIC result within a 2-fold dilution of the BMD result. Categorical agreement was defined as agreement in the interpretation of the MICs of the commercial kit and BMD. VME occurred where the tested method's MIC interpretation was susceptible and the BMD's MIC interpretation was resistant. ME occurred where the tested method's MIC interpretation was resistant and the BMD's MIC interpretation was susceptible. The VME rates were calculated using the number of isolates resistant by BMD, while the ME rates were calculated using the number of isolates susceptible to BMD. The acceptance criteria of the tests require that the VME and ME values be below 3%, and the categorical agreement be higher than 90% (ISO 2019).

2.5 Ethical Consideration

This study is carried out with samples in our stocks; therefore, an ethical committee report is not required for this study. We prove that our study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

3. Results

A total of 153 gram-negative bacterial strains, including 102 *A. baumannii*, 40 *K. pneumoniae*, and 11 *P. aeruginosa*, which have multidrug resistance, were included in this study. Eight of the strains were found to be resistant to colistin with the broth microdilution test, and five of the strains were resistant with the VITEK 2 system. All strains were determined to be susceptible by the gradient test method. MIC values determined by different antibiotic susceptibility test methods are shown in Table 1.

Rates of EA of colistin test results between BMD, VITEK 2, and gradient test were 96.1% and 79.7%, respectively. The VITEK 2 and gradient test showed 95.4% and 94.8% of CA respectively. In addition, very major error rate of VITEK 2 were detected 3.2%, gradient test were 5.2%. Major error rate of VITEK 2 was 1.3% and there was not major error for E test.

Table 1. Minimum Inhibitory Concentration of Bacterial Strains (mg/L)

Methods	≤0.5	1	2	4	8	≥16
BMD	124	12	9	2	-	6
VITEK 2	143	2	3	-	-	5
Gradient test	123	27	3	-	-	-

BMD: Broth Microdilution

The essential agreement, categorical agreement, very major error and major error values between the reference method (BMD test) and VITEK 2 and E test are given in Table 2.

Table 2. Comparison of Different Commercial Tests with Reference Method

Bacteria	Method	Susceptible Isolate (n)	Essential Agreement n (%)	Categorical Agreement n (%)	Very Major Error n (%)	Major Error n (%)
A. baumannii	**VITEK 2**	101	98 (%96.1)	98 (%96.1)	3 (%2.9)	1 (%0.9)
	Gradient test	102	80 (%78.4)	99 (%97.1)	3 (%2.9)	0
	BMD	99				
K. pneumoniae	**VITEK 2**	36	38 (%92.7)	37 (%92.5)	2 (%5)	1 (% 2.5)
	Gradient test	40	32 (%78.1)	35 (%87.5)	5 (%12.5)	0
	BMD	36				
P. aeruginosa	**VITEK 2**	11	11 (%100)	11 (%100)	0	0
	Gradient test	11	10 (%90.9)	11 (%100)	0	0
	BMD	11				

BMD: Broth microdilution

In both commercial tests, very major error and major error values were below 3% in *A. baumannii* and *P. aeruginosa* strains. However, in *K. pneumoniae* strains very major error rate was detected over 3%. Categorical agreement was over 90% in all strains with the VITEK 2 method. In the gradient test method, the false susceptibility rate in *K. pneumoniae* strains was found to be below 90%.

4. Discussion

Recently, the number of infections caused by multidrug-resistant gram-negative bacteria has increased. The usage of colistin has rised recently due to increased resistance to many antibiotics. Therefore, the use of reliable antibiotic susceptibility testing for colistin will contribute to treatment (Li et al., 2006; Li et al., 2005). Many test methods are used to detect colistin susceptibility. However, EUCAST recommends using the broth microdilution method as the reference method (EUCAST 2016).

Among commercial methods, the gradient test is used by many clinical laboratories because it is cheap and easy to apply. Studies have reported that the colistin gradient test has a high

rate of wrong susceptible results for resistant strains. Therefore, it is not recommended to use the gradient test as a colistin susceptibility (EUCAST, 2016; Dafopoulou et al., 2015; Maalej et al., 2011). In a study, the essential agreement between the gradient test and the reference method was reported as 52%, and the categorical agreement was reported as 33% (Maalej et al., 2011). In another study, the basic agreement between the gradient test and the reference method was 52.8%, and the categorical agreement was 59% (Dafopoulou et al., 2015). These rates are below the acceptable criteria of the test (90%); therefore the use of the gradient test for colistin susceptibility is not recommended. In another study, unlike other studies, it was suggested that the colistin gradient test had high compatibility with other tests, so it could be used as an antimicrobial test (Akın et al., 2010; Paköz et al., 2018). In a study by Altınkanat Gelmez et al. (2021), it was reported that the categorical agreement between the gradient test and the reference method was high, but the major error rate was found to be above the acceptance criteria (3%). In this study, it was reported that the high major error rate may be related to the low number of isolates, and this rate may decrease if the study is continued with more isolates. In our study, the essential and categorical agreement between BMD and gradient test was 78.4% and 97.1% in *A. baumannii* strains, 78.1% and 87.5% in *K. pneumoniae* strains, and 90.9% and 100% in *P. aeruginosa* strains, respectively. In *A. baumannii* and *P. aeruginosa* strains, very major error and major error rates of the gradient tests were detected under 3%. However, a very major error rate of gradient test was 12.5% in *K. pneumoniae* strains. Therefore, we recommend for centers that detect colistin susceptibility in *K. pneumoniae* strains with the gradient test to confirm their results with the reference method.

The VITEK 2 automated system is frequently used in identification of bacteria and antibiotic susceptibility. Studies have reported that the VITEK 2 system is reliable for detecting colistin susceptibility (Dafopoulou et al., 2015; Paköz et al., 2018, Lee et al., 2013; Lo-Ten-Foe et al., 2007). However, recent studies have reported that very major error rates are high in VITEK 2 results (Chew et al., 2017; Vourli et al., 2017; Girardello et al., 2018). In a study, although between VITEK 2 and the reference method the essential agreement was 93.4% and the categorical agreement was 88.2%, the very major error rate was determined as 36% for colistin (Chew et al., 2017). Vourli et al. (2017) compared colistin susceptibility of Phoenix 100 and VITEK 2 automated systems with the reference method and determined the very major error rates as 41.4% and 37.9%, respectively. Very major errors were generally detected in isolates with a MIC value of 1-2 mg/L. They suggest that the isolates detected as susceptible in the automated system should be confirmed with the reference method. In another study, it was found that the best performance with VITEK 2 was obtained in *K. pneumoniae* and *E. coli* strains with MIC values of ≤0.5 and ≥16 mg/L, and they suggest that all strains with MIC values of 1-8 mg/L should be confirmed by the reference method (Girardello et al., 2018). In our study, the results are reliable level, as categorical agreement was over 90% in all strains with the VITEK 2 method. Very major error and major error rate are acceptable, because it is under 3% for the VITEK 2 test in *A. baumannii* and *P. aeruginosa* strains. The very major error rate in *K. pneumoniae* strains is 5% in the VITEK 2 automated system. Therefore, we suggest that if this test is to be used, the results should be confirmed with the reference method. In addition, we carried out our study with 40 *K. pneumoniae* strains, and very major error rate may have been high. We believe that if the study is continued and more strains are used, the error rate could change. For this reason, it would be beneficial to re-evaluate these rates with further studies and more strains.

5. Conclusion and Suggestions

Considering that many laboratories frequently use these methods in the laboratory, it is highly possible for colistin to give wrong results with these methods. This situation will lead clinicians

to use inappropriate colistin treatments. For this reason, by the CLSI-EUCAST Polymyxin Working Group, only the broth microdilution method is recommended for the determination of colistin susceptibility (EUCAST 2016; Gelmez et al., 2021). Broth microdilution method is not preferred in routine laboratories due to difficulties in solution preparation, long duration, and difficulty in working. Therefore, it is necessary to develop more practical and inexpensive methods to detect colistin resistance in routine microbiology laboratories.

References

Akın, Ö.F.E., Bayram, A., Balcı, İ.(2010). Çoğul dirençli *Acinetobacter baumannii* izolatlarında kolistin, polimiksin B ve tigesiklin direncinin saptanmasında disk difüzyon, E-test ve buyyon mikrodilüsyon yöntemlerinin karşılaştırılması. Mikrobiyol Bul, 44(2), 203-10.

Altınkanat Gelmez, G., Sayın, E., Hasdemir Gökboğa, M., Söyledir, G.(2021). Kolistin Duyarlılık Testi İçin Diagnostics Colistin MIC-Strip Testinin Değerlendirilmesi. Ankem Derg, 35(1), 9-13. doi: 10.5222/ankem.2021.009

Chew, K.L., La, M.V., Lin, R.T., Teo, J.W. (2017) Colistin and polymyxin B susceptibility testing for carbapenem-resistant and mcr-positive *Enterobacteriaceae*: comparison of Sensititre, MicroScan, Vitek 2, and Etest with broth microdilution. J clin microbiol, 55(9), 2609-16. https://doi.org/10.1128/jcm.00268-17

Dafopoulou, K., Zarkotou, O., Dimitroulia, E., Hadjichristodoulou, C., Gennimata, V., Pournaras S, et al.(2015) Comparative evaluation of colistin susceptibility testing methods among carbapenem-nonsusceptible *Klebsiella pneumoniae* and *Acinetobacter baumannii* clinical isolates. Antimicrob agents and chemother, 59(8), 4625-30. https://doi.org/10.1128/aac.00868-15

EUCAST (2016). Recommendations for MIC determination of colistin (polymyxin E) as recommended by the joint CLSI-EUCAST Polymyxin Breakpoints Working Group. EUCAST: Växjö, Sweden. 2016.

EUCAST (2021). European Committee on Antimicrobial Susceptibility Testing 2021 [Availablefrom:https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_11.0_Breakpoint_Tables.pdf.

Girardello, R., Cury, A.P., Franco, M.R.G., Di Gióia, T.R., de Almeida, Jr J.N., de Araújo, M.R.E., et al. (2018). Colistin susceptibility testing and Vitek-2TM: is it really useless? Diagn Microbiol Infect Dis, 91(4), 309-311. https://doi.org/10.1016/j.diagmicrobio.2018.03.019

ISO (2019). Clinical Laboratory Testing and in Vitro Diagnostic Test Systems-Susceptibility Testing of Infectious Agents and Evaluation of Performance of Antimicrobial Susceptibility Test Devices: Reference Method for Testing the in Vitro Activity of Antimicrobial Agents Against Rapidly Growing Aerobic Bacteria Involved in Infectious Diseases: ISO; 2019. https://www.iso.org/standard/70464.html

Karaiskos, I., Giamarellou, H. (2014). Multidrug-resistant and extensively drug-resistant Gram-negative pathogens: current and emerging therapeutic approaches. Expert opin pharmacother, 15(10), 1351-70. https://doi.org/10.1517/14656566.2014.914172

Lee, S.Y., Shin, J.H., Lee, K., Joo, M.Y., Park, K.H., Shin, M.G., et al. (2013). Comparison of the Vitek 2, MicroScan, and Etest methods with the agar dilution method in assessing

colistin susceptibility of bloodstream isolates of *Acinetobacter* species from a Korean university hospital. J clin microbiol, 51(6), 1924-6. https://doi.org/10.1128/jcm.00427-13

Li, J., Nation, R.L., Milne, R.W., Turnidge, J.D., Coulthard, K. (2005). Evaluation of colistin as an agent against multi-resistant Gram-negative bacteria. Int j antimicrob agents, 25(1), 11-25. https://doi.org/10.1016/j.ijantimicag.2004.10.001

Li, J., Nation, R.L., Turnidge, J.D., Milne, R.W., Coulthard, K., Rayner, C.R., et al. (2006). Colistin: the re-emerging antibiotic for multidrug-resistant Gram-negative bacterial infections. The Lancet infect dis, 6(9), 589-601. https://doi.org/10.1016/S1473-3099(06)70580-1

Lo-Ten-Foe, J.R., de Smet, A.M.G., Diederen, B.M., Kluytmans, J.A., van Keulen, P.H. (2007) Comparative evaluation of the VITEK 2, disk diffusion, Etest, broth microdilution, and agar dilution susceptibility testing methods for colistin in clinical isolates, including heteroresistant Enterobacter cloacae and *Acinetobacter baumannii* strains. Antimicrob agents chemother, 51(10), 3726-30. https://doi.org/10.1128/aac.01406-06

Maalej, S., Meziou, M., Rhimi, F., Hammami, A. (2011) Comparison of disc diffusion, E test and agar dilution for susceptibility testing of colistin against *Enterobacteriaceae*. Lett appl microbiol, 53(5), 546-51. https://doi.org/10.1111/j.1472-765X.2011.03145.x

Matuschek, E., Åhman, J., Webster, C., Kahlmeter, G. (2018). Antimicrobial susceptibility testing of colistin–evaluation of seven commercial MIC products against standard broth microdilution for Escherichia coli, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp. Clin Microbiol and Infect,24(8), 865-70. https://doi.org/10.1016/j.cmi.2017.11.020

Paköz, N.İ.E., Kaya, E., Orhan, Z., Kayış, A., Aral, M. (2018). Farklı klinik örneklerden izole edilen çoğul dirençli *Acinetobacter baumannii* izolatlarında tigesiklin, kolistin direncinin disk difüzyon, E-test ve otomatize sistem yöntemleri ile karşılaştırılması. Turk Hij Den Biyol Derg, 75(2), 109-16. https://doi.org/10.5505/TurkHijyen.2018.13334

Storm, D.R, Rosenthal, K.S, Swanson, P.E. (1977) Polymyxin and related peptide antibiotics. Annu rev biochem, 46(1):,723-63.

Vourli, S., Dafopoulou, K., Vrioni, G., Tsakris, A., Pournaras, S. (2017). Evaluation of two automated systems for colistin susceptibility testing of carbapenem-resistant *Acinetobacter baumannii* clinical isolates. J Antimicrob Chemother, 72(9), 2528-30. https://doi.org/10.1093/jac/dkx186

Declarations

We have no conflicts of interest to disclose. All author's evaluated results of antimicrobial studies and read and approved final manuscript. This study was not produced from the thesis. This study was carried out with bacteria in our laboratory stocks therefore ethical committee report is not required for this study. We declare that our study was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. This study was not presented as an oral/poster presentation at any meeting. Author contributions: Idea: HTD, NÇ, UA, Design: HTD, NÇ, UA, Inspection: UA,NÇ, HTD; Materials: NÇ; Data collection and / or processing: NÇ, HTD, UA, Analysis and / or interpretation: NÇ, UA, HTD, Literature review: NÇ, HTD, Resources: NÇ, Writing: NÇ, Critical Review: NÇ, UA, HTD.