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Comperative Evaluation of Vitek 2 and Etest Methods with the Referance Broth Microdilution for Antimicrobial Susceptibility Testing of Colistin Among Multi-Drug Resistant Gram-Negative Bacteria

Çoklu İlaca Dirençli Gram-Negatif Bakterilerde Kolistin in Vitro Duyarlılığının Belirlenmesinde Referans Sıvı Mikrodilüsyon Metodu ile Vitek 2 ve Etest Yöntemlerinin Karşılaştırmalı Olarak Değerlendirilmesi

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ABSTRACT

Introduction: *Despite the increased need for colistin, especially in serious infections caused by carbapenem resistant gram-negative bacteria, problems and challenges regarding colistin susceptibility testing remain. The aim of this study was to evaluate the performance of Vitek 2, one of the commonly used automated systems, and Etest for colistin susceptibility testing compared with reference broth microdilution method (BMD).*

Materials and Methods: *This study included 657 multi-drug resistance (MDR) Gram negative bacteria obtained from clinical samples; Negative control, Escherichia coli ATCC 25922 and Positive control, Escherichia coli NCTC 13846. The collected MDR isolates were performed colistin BMD according to ISO standard 20776-1, prospectively. Categorical agreement (CA), Very Major Error (VME), and Major Error (ME) rate were calculated. Acceptable performance was evaluated as; CA ≥90%; VME <1.5% and ME <3%.*

Results: *Colistin resistance rates were detected by Vitek 2, Etest and BMD; 40.3%, 48.7%, 53.9%, respectively. CA rates were as follows: Vitek 2 92.4% and Etest 71.9%. While the compatibility of Vitek 2 and BMD was observed (kappa value= 0.85) to be 'excellent agreement'; the agreement of Etest and BMD was found to be 'moderate' (kappa value= 0.45). Although CA varied from 85.7% to 100% for Vitek 2, it ranged 63.6% to 80% for Etest depending on bacterial species. Alarming high rates of VME were determined for Vitek 2 (14.5%) and Etest (36.5%). While MEs were 1.7% by Vitek 2; there was no false resistant isolate with Etest.*

Conclusion: *It may be recommended for laboratories not to rely on Vitek 2 and Etest colistin susceptibility results. Additionally, colistin resistant isolates will be underestimated by reducing colistin susceptibility studies to a specific minimum inhibitory concentration (MIC). In this regard, our suggestion is that laboratories would improve their infrastructure and staff skills to apply BMD routinely.*

Key Words: *Antimicrobial resistance; Colistin; Broth microdilution*

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ÖZ

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Giriş: *Kolistin ihtiyacının artmasına rağmen, özellikle karbapenem dirençli gram-negatif bakterilerin neden olduğu ciddi infeksiyonlarda; kolistin duyarlılık testi ile ilgili sorunlar ve zorluklar devam etmektedir. Bu çalışmanın amacı kolistin duyarlılığının belirlenmesinde yaygın olarak kullanılan otomatize sistemlerden Vitek 2'nin ve Etest yönteminin referans sıvı mikrodilüsyon metodu (BMD) ile karşılaştırılmalı olarak değerlendirilmesidir.*

Materyal ve Metod: *Bu çalışmaya, klinik örneklerden izole edilen çoklu ilaca dirençli (MDR) 657 gram-negatif bakteri ve Negatif kontrol, Escherichia coli ATCC 25922; Pozitif kontrol, Escherichia coli NCTC 13846 suşları dahil edildi. Stoktaki MDR izolatlar ISO standart 29776-1'e uygun olarak kolistin sıvı mikrodilüsyon metodu ile prospektif olarak çalışıldı. Kategorik uyum (KU), Çok Büyük Hata (ÇBH), Büyük Hata (BH) oranları hesaplandı. KU ≥%90; ÇBH <%1.5 ve BH <%3 kabul edilebilir performans kriterleri olarak alındı.*

Bulgular: *Kolistin direnç oranları Vitek 2, Etest ve BMD yöntemleriyle sırasıyla %40.3, %48.7, %53.9 olarak tespit edildi. KU oranları Vitek 2 %92.4; Etest %71.9 olarak bulundu. Vitek 2-BMD uyumu analiz neticesinde mükemmel (kappa değeri: 0.85) iken; Etest-BMD orta derecede uyumlu bulundu (kappa değeri: 0.45). Kategorik uyum Vitek 2 için %85.7 ile %100 arasında değişirken; Etest de bakteri türlerine göre kategorik uyum %63.6 ile %80 aralığında tespit edildi. Endişe veren yüksek çok büyük hata oranları Vitek 2 için %14.5 ve Etest için %36.5 olarak hesaplandı. Vitek 2 için belirlenen büyük hata oranı %1.7 iken; Etest ile hiçbir izolat yanlış dirençli olarak saptanmadı.*

Sonuç: *Laboratuvarlara Vitek 2 ve Etest kolistin duyarlılık testi sonuçlarına güvenmemeleri tavsiye edilebilir. Ek olarak, kolistin duyarlılık çalışmalarını spesifik bir minimum inhibitör konsantrasyon (MİK) değerine göre sınırlamak kolistin dirençli izolatların gözden kaçmasına sebep olabilir. Bu kapsamda, sıvı mikrodilüsyon yönteminin rutin olarak kullanılması amacıyla laboratuvarların altyapı ve becerilerinin geliştirilmesini önermekteyiz.*

Anahtar Kelimeler: *Antimikrobiyal direnç; Kolistin; Sıvı Mikrodilüsyon*

INTRODUCTION

Infections caused by multidrug-resistant Gram-negative bacteria (MDRGN) remain a significant challenge associated with high morbidity and mortality worldwide[1]. The misuse and extensive use of antibiotics have led to widespread resistance to carbapenems, one of the current broad spectrum antibiotics[2]. Due to the limited efficient agent against infections with carbapenem-resistant Enterobacterales (CRE), an older class of antibiotics such as polymyxins have re-emerged[3].

Colistin (Polymyxin E) was synthesized in the 1940s and used by the 1970s[4]. The use of colistin is abandoned due to its serious nephrotoxic and neurotoxic effects. Nevertheless, it is now increasingly being used as a 'last-line' therapeutic option in serious infections caused by MDRGN, particularly carbapenem-resistant (CR) Gram-negative bacteria. Currently, with the increasing use of colistin, a need for reliable and rapid antibiotic susceptibility testing (AST) have became crucial[3]. Because of the its large structure, colistin cannot be sufficiently diffused to agar medium. Therefore, false sensitive results may occur in agar-based antibiotic susceptibility tests (disk diffusion test, gradient test e.g.)[5-7]. Concerning the discrepancy in colistin sensitivity test results, the "European Committee on Antimicrobial Susceptibility Testing" (EUCAST) stated that the minimum inhibitory concentration (MIC)

of the colistin detected in gradient tests underestimate, and the disk diffusion method shall not be used for AST. Both the EUCAST and the "Clinical and Laboratory Standards Institute" (CLSI) joint working group recommended the broth microdilution method (BMD) as the only valid susceptibility testing method for colistin[8,9].

The aim of this prospective study was to investigate the performance of Vitek 2 automated system and Etest compared with reference Broth Microdilution Method for colistin susceptibility test of MDRGN isolates.

MATERIALS and METHODS

Study Design

A total of 657 non-duplicate clinical strains of gram-negative bacteria isolated from various samples sent to Microbiology Laboratory of Necmettin Erbakan University Meram Medical Faculty between January 2019-June 2020 were included. The study was approved by the local ethics committee.

Antimicrobial Susceptibility Testing

The identification and antibiotic susceptibility tests were performed by Vitek 2 (bioMérieux, Marcy l'Etoile, France) for each strain in accordance with EUCAST guidelines[10]. Strains were classified as multi-drug resistance (MDR) if they were resistant to at the least three classes of antimicrobial agents[1]. Of the 203 isolates were performed Etest (bioMйrieux, Marcy l'Etoile, France) simultaneously. Both Vitek 2 and Etest were interpreted according to the manufacturer's recommendations. The possible range of MIC readings for Vitek was <=0.5 mg/L to>=16 mg/L, it was 0.016 to 256 mg/L for Etest.

In-House Broth Microdilution (BMD)

The active ingredient colistin sulphate powder was acquired from Sigma-Aldrich (St Louis, MO, USA). It was dissolved in accordance with the recommendations of the manufacturer and stock solutions were obtained in 128 mg/L concentration. All of the isolates were cultivated from stock cultures for colistin broth microdilution testing. BMD was performed according to International Organization for Standardization (ISO) 20776-1[11] in 96-well untreated polystyrene trays using cation-adjusted BBL Mueller Hinton II broth (Becton Dickinson and Company Sparks, MD 21152, USA). Serial dilutions (0.32-32 mg/L) were prepared in Mueller-Hinton broth on microdilution plates from the stock solution. The last wells served as growth control. After preparing a suspension with 0.5 McFarland standard turbidity from all collected isolates, the final bacteria concentration was added to microdilution plates at $5x10^5$ cfu/ml and the microplates were incubated at 36°C for 18-24 hours. The lowest concentration of colistin without growth was determined as the MIC value. *Escherichia coli* ATCC 25922 (target MIC 0.5 to 1 mg/L, range 0.25 to 2) and mcr-1 positive *E. coli* NCTC 13846 (target MIC 4 mg/L, range 2 to 8) standard bacteria were used for colistin sensitive and colistin resistant controls, respectively.

Analysis of Data

The results were evaluated according to the clinical breakpoints specified in the EUCAST standards (≤2 mg/L colistin sensitive and >2 mg/L colistin resistant)[10]. BMD was accepted as the reference method for determination of colistin sensitivity. MIC50 and MIC90 values were calculated based on BMD results as the MICs at which 50% and 90% of the isolates were inhibited, respectively. Very major errors (VME) were determined as 'susceptible' according to Vitek 2 systems or Etest and 'resistant' according to BMD; major errors (ME) were defined as 'resistant' according to Vitek 2 systems or Etest and 'susceptible' according to BMD. Categorical agreement (CA) was calculated by the rate of isolates with the same susceptibility category using the total number of isolates performed as the denominator. Acceptable performance according to the criteria determined by CLSI; CA ≥90%; VME <1.5% and ME <3%[12,13]. The agreement in categorical outcomes was evaluated by the Cohen's kappa statistics. Analyzes were performed with Jamovi 1.2.22 program.

RESULTS

A total of 657 multi-drug resistant gram-negative bacilli, most of which were isolated from intensive care units (n= 459, 69.9%), included in the study were as follows: *Klebsiella pneumoniae*

n= 538; *Acinetobacter baumannii* complex n=90; *Pseudomonas aeruginosa* n= 14; *Escherichia coli* n= 11; *Enterobacter cloacae* complex n= 4. The sensitivity of colistin for the samples examined is summarized in Table 1, blood (n= 303, 46.1%) and bronchoalveolar lavage (n= 190, 28.9%) samples were constituted the vast majority of the specimens. The distribution of isolates according to colistin MICs values determined by reference broth microdilution method is given in Table 2.

Meropenem resistance was detected in 555 (84.5%) of 657 isolates. Of these 555 isolates with carbapenem resistance, 291 (52.43%) were also found to be resistant to colistin. However, resistance to colistin was detected in only 12 (11.8%) of 102 isolates that were susceptible to meropenem.

The categorical agreement (CA) varied from 85.7% to 100% for the Vitek 2 and from 63.6% to 80% for the Etest (Table 3) CA rate was 92.4% in the colistin susceptibility with the Vitek 2 for all isolates. Despite acceptable CA rate was determined via Vitek 2 generally, significant differences of agreement were observed according to the bacterial specie evaluated (Table 3). In this study, when compared with Enterobacterales; the categorical agreement of Vitek 2 was lower for non-fermentative bacilli. In addition, 14.5% of very major errors (VME) and 1.7% of major errors (ME) were determined by Vitek 2 (Table 3). Although the categorical agreement and major errors were within acceptable limits (>90%; <3%; respectively), high percentage of VME (44 false susceptible, 14.5%) was observed, which was over the criterion of ≤1.5%. The highest number

Table 1. Colistin sensitivity rates according to the source of sampling

Sampling Source	Colistin Resistant (n)	Colistin Sensitive (n)	Total (n)
Cerebrospinal fluid	4	0	4
Pus	0	3	3
Bronchoalveolar lavage	115	75	190
Sputum	6	3	9
Drainage	3	17	20
Urine	28	33	61
Blood	118	185	303
Catheter	6	5	11
Pleura fluid	1	1	2
Wound	22	32	54
Total	303	354	657

Table 2. Distribution of isolates according to colistin MICs values determined by reference broth microdilution method

Type of Bacteria	Distribution of Isolates by MIC Values (mg/L)									
	0.064	0.125	0.25	0.5	1	2	4	8	16	32
K. pneumoniae (n= 538)	5	51	94	76	22	16	15	36	104	119
A. baumannii (n= 90)	-	8	94	17	12	2	-	1	8	17
P. aeruginosa (n= 14)	-	-	4	2	2	3	-	-	2	1
E. coli (n= 11)	-	4	6	1	-	-	-	-	-	-
E. cloacoae complex (n= 4)	-	-	2	2	-	-	-	-	-	-
Total	5	63	131	98	36	21	15	37	114	137

MIC: Minimum inhibitory concentration.

Table 3. Performance of Vitek 2 and Etest on colistin susceptibility test in comparison to referance BMD

Method		Sensitive Number (%)	Resistans Number (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	CA Number (%)	VME Number (%)	ME Number (%)
Broth microdilution	**All isolates (n= 657)**	303 (46.1%)	354 (53.9%)	1	32	Reference	Reference	Reference
	K. pneumoniae (n= 538)	264 (50.9%)	274 (50.9%)	4	32			
	A. baumannii complex (n= 90)	64 (71.1%)	26 (28.9%)	0.5	32			
	P. aeruginosa (n= 14)	11 (78.6%)	3 (21.4%)	1	16			
	E. coli (n= 11)	11 (100%)	0 (0%)	0.25	0.25			
	Enterobacter clocae complex (n= 4)	4 (100%)	0 (0%)	0.25	0.5			
Vitek 2	**All isolates (n= 657)**	392 (59.7%)	265 (40.3%)	≤0.5	≥16	607 (92.4%)	44 (14.5%)	6 (1.7%)
	K. pneumoniae	294 (54.6%)	244 (45.4%)	≤0.5	≥16	502 (93.3%)	33 (12.04%)	3 (1.1%)
	A. baumannii complex	74 (82.2%)	16 (17.8%)	≤0.5	≥16	78 (86.7%)	11 (42.3%)	1 (1.5%)
	P. aeruginosa	9 (64.3%)	5 (35.7%)	≤0.5	≥16	12 (85.7%)	0 (0%)	2 (16.6%)
	E. coli	11 (100%)	0 (0%)	≤0.5	≤0.5	11 (100%)	0 (0%)	0 (0%)
	Enterobacter clocae complex	4 (100%)	0	≤0.5	≤0.5	4 (100%)	0 (0%)	0 (0%)
E test	**All isolates (n= 203)**	104 (51.3%)	99 (48.7%)	2	12	146 (71.9%)	57 (36.5%)	0 (0%)
	K. pneumoniae (n= 187)	96 (51.3%)	91 (48.7%)	2	8	143 (76.5%)	52 (36.04%)	0 (0%)
	A. baumannii complex (n= 11)	5 (45.5%)	6 (54.5%)	4	8	7 (63.6%)	4 (40%)	0 (0%)
	P. aeruginosa (n= 5)	3 (60%)	2 (40%)	1	32	4 (80%)	1 (33.3%)	0 (0%)

MIC: Minimum inhibitory concentration, CA: Categorical agreement, VME: Very major errors (false susceptible), ME: Major errors (false resistant).

Table 4. Colistin MIC (mg/L) distribution by method

		No. of isolates with MIC (mg/L) (n= 657) via BMD									
		0.064	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**
No. of isolates with MIC (mg/L) via VITEK 2 (n= 657)	**<=0,5**	5	63	128	97	34	12	6	2	11	16
	1	-	-	1	1	-	1	2	-	-	1
	2	-	-	-	-	-	6	-	4	-	2
	4	-	-	1	-	-	-	1	6	11	-
	8	-	-	-	-	-	1	0	9	8	4
	>=16	-	-	1	-	2	1	6	16	84	114
	Total	**5**	**63**	**131**	**98**	**36**	**21**	**15**	**37**	**114**	**137**

MIC: Minimum inhibitoryconcentration. The dashed line indicates the EUCAST breakpoint for susceptibility (≤ 2 mg/L). Very major error are represented in bold.

Table 5. Distribution of colistin minimum inhibitory concentration by broth microdilution and E-test for the tested isolates

		No. of isolates with MIC (mg/L) via reference BMD (n= 203)									
		0.064	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**
No. of isolates with MIC (mg/L) via Etest (n= 203)	**0.125**	1	3	1	-	1	-	-	-	-	-
	0.5	-	1	2	1	-	-	-	-	5	2
	0.75	-	1	3	1	1	-	-	1	1	-
	1	1	2	3	1	3	1	2	-	4	5
	1.5	1	2	3	6	-	2	-	2	3	10
	2	-	-	1	1	-	4	1	-	8	13
	4	-	-	-	-	-	-	2	8	23	17
	8	-	-	-	-	-	-	-	2	8	17
	12	-	-	-	-	-	-	-	1	3	3
	16	-	-	-	-	-	-	-	-	-	4
	32	-	-	-	-	-	-	-	1	2	8
Total		3	9	13	10	5	7	5	15	57	79

MIC: Minimum inhibitory concentration. The dashed line indicates the EUCAST breakpoint for susceptibility (≤ 2 mg/L). Very major error are represented in bold.

of VME in relation to the species were observed for isolates of *A. baumannii* complex in both the Vitek 2 (42.3%) and Etest (40%). It is important to emphasize that the majority of VME detected by Etest was at the borderline MIC 1.5-2 mg/L while the MIC majority of VME observed with Vitek 2 was < 0.5 mg/L for *K. pneumoniae* isolates (Table 3). Discrepancies were determined in colisin MIC values reported via the Vitek 2 systems versus the reference in house broth microdilution (Table 4).

The distribution of colistin MIC determined by Etest versus the reference BMD method are given in Table 5. The categorical agreement rate for colistin Etest was observed 71.9% as quite poor. According to BMD, Etest exhibited lower performance than Vitek 2 for all isolates. Although the highest categorical agreement among isolates was detected in *P. aeruginosa*, this CA rate is below the acceptable limit of >90%. Besides, high percentages of VME (57 false susceptible, 36.5%) was detected by Etest for tested isolates.

Although the rate of VME detected for *P. aeruginosa* was high by Etest, only one isolate was given as 'false susceptible'. Therefore; the number of isolates for *P. aeruginosa* tested with the Etest is limited for generalization. Additionally, according to the BMD; no major error (ME) was detected by the Etest. However, Vitek 2 showed marginally higher ME rate (1.7%).

Inter-method agreement was also evaluated using Cohen's kappa statistics. While the compatibility of Vitek 2 and BMD was observed (kappa value= 0.85) to be 'excellent agreement'; The agreement of Etest and BMD was found to be 'moderate' (kappa value= 0.45).

DISCUSSION

The emerging resistance to colistin has become a new threat for global public health. The determination of colistin in vitro antibiotic susceptibility testing (AST) is obviously essential for patient management and the monitoring of colistin resistance[13,14]. However, there is no reliable, reproducible and practical technique. In the report published by EUCAST, it is stated that disc diffusion method cannot be used in determining the sensitivity of colistin; it does not differentiate sensitive-resistant isolates. Another drawback induced by EUCAST; even when quality control results are within range; available gradient stripes underestimate colistin MIC values, undervalued colistin resistance and so, the use of these tests should be avoided. Additionally, the recommended broth microdilution method is ideal; it is impractical, laborious and time consuming method[9,13-15]. Furthermore, the performance of commercial automated systems in detecting colistin sensitivity has not also been evaluated by EUCAST until now[9,15]. However, many studies in the literature have disclosed the frequent occurrence of VMEs in automated systems colistin susceptibility results[6,9,16].

In the present study, we compared the performance of Vitek 2, which are frequently used commercial semi-automated systems and gradient test (Etest) with in house prepared broth microdilution method.

In this study, colistin resistance rate was observed as 53.9% for all isolates via BMD method. These high colistin resistance rates are similar to the recent studies conducted in Turkey[5]. In the studies using the BMD method, colistin resistance rates have been reported as 39.5% by Kocak et al.[17] and 76.2% by Yıldız et al.[18] from Turkey. Colistin resistance was found to be 92.1% (35/38) by Kansak et al.[19].

In this study, among Enterobacterales family members, *K. pneumoniae* was the most common genus associated with colistin resistance (50.9%), followed by *A. baumannii* and *P. aeruginosa* with a resistance rate of 28.9%; 21.4%, respectively. Although colistin resistance rates are reported 'low' percentages among *A. baumannii* and *P. aeruginosa* isolates worldwide[20], the high colistin resistance determined in our study among these groups was considered worrisome. The high proportion of carbapenem resistant (CR) strains among the study isolates may have caused high colistin resistance rates. Besides, a high ratio of colistin resistance among CR Enterobacterales strains has been reported all around the world[20].

Numerous studies have been reported comparing the reference BMD with the available commercial methods. Categorical agreements of colistin AST were mostly within acceptable limits by Vitek 2 in many studies[6,21]. Other automated AST systems such as MicroScan and BD Phoenix also supplied reproducible and accurate categorical results for the testing of colistin in Enterobacterales[5,22]. The main problem in determining the sensitivity of colistin with automated systems is that the results obtained by these systems, with high very major errors (VMEs) rate, do not reliably distinguish colistin susceptible/resistant isolates.

In this study, the Vitek 2 showed rates of 92.4% CA, among all 657 isolates. Furthermore, 14.5% of very major errors (VMEs), 1.7% of major errors (MEs) were observed. In the study by Tanrıverdi et al.[22] evaluating the performance of Vitek 2 according to BMD, the rates of CA, VMEs and MEs are as follows; 84.12%, 55.88% and 1.09%. Chew et al. reported that for colistin testing CA was <90% (67/76 isolates), with high VMEs rate 36%[16].

Although Vitek 2 represented acceptable CA

between referance BMD generally, significant differences of agreement were observed according to the bacterial species evaluated and resistant property of target isolates. Among Enterobacterales isolates, Vitek showed CA (100%) rates for *E. clocae* complex and *E. coli* were higher than those obtained for *K. pneumoniae* (CA 93.3%) (Table 3). While the highest very major error rate among Enterobacterales was detected in *K. pneumoniae* isolates (12.4%); no false susceptible isolates were observed in *E. coli* and *E. cloacae* complex isolates. Vitek 2 showed poor performance for non-fermentative isolates, determined unacceptable CA rates for *A. baumannii* and *P. aeruginosa* 86.7%; 85.7% respectively. While the highest VME rate (42.3%) was found in *A. baumanni*; the highest ME (16.6 %) rate was observed in *P. aeruginosa*. High rates of MEs (16.6%) reported for *P. aeruginosa* in that study could be due to the low number of isolates (n= 14/2.1%), which can magnify ME rates even with few false resistant results. Similar to our results, Vourli et al. have reported unacceptable EA and CA of 88.9% and 89.7% respectively, on a greater number of *A. baumannii* isolates (n= 117), which contained 29 (24.8%) colistin-resistant isolates. In addition, Vitek-2 showed unacceptable rates of VMEs (37.9%) in this study[6]. The principal disadvantage of the Vitek 2 is its' poor performance to detect resistant subpopulations (heteroresistance) [23]. Heteroresistance can be expansively describe as the existence of subpopulations with an MIC higher (variably more than two-fold to eight-fold) than the MIC of the basic population. This resistant subpopulation is clinically important as it may cause treatment failures[24]. Both of *Acinetobacter* spp. and *Enterobacter* spp. have been reported in the literature as well-known producers of heteroresistant populations. Therefore, it could be a limiting factor on performance of automated systems such as Vitek 2[25]. On the account the fact that the number of *E. cloacae* complex isolates in this study is very low to be represented; resistant subpopulation, false susceptible or false resistant isolates may not have been detected at all.

In this study, Vitek 2 showed variable performance between different gram-negative bacteria species for colistin susceptibility testing, with a categorical concordance of 85.7% -100%. In one of the recent studies, it has been emphasized that the isolates with MICs of ≤0.5 and ≥16 mg/L by Vitek 2 was perfectly compatible with broth microdilution[25]. In another study, it was stated that isolates determined by Vitek 2 MICs between 1 to 4 should be repeated with BMD; the rest of the isolates having MICs of <1 mg/ L and >4 mg/L can be released without any testing[26]. Vourli et al. have reported that VMEs were more frequent by both automated (Vitek 2 and Phoneix) isolates with MICs of 2 mg/L rather than <1 mg/L, refering that isolates MIC values close to the susceptibility breakpoint should be favourably re-tested by referance BMD[6]. In contrast, based on the results of our study, the majority of isolates (35/44, 79.5%) determined VME were found MIC ≤0.5 mg/L by Vitek 2. In addition to that, ll of the *A. baumannii* isolates detected as false susceptible (n= 11) were found as MIC <= 0.5 mg/L by Vitek 2.

In spite of high concordance (kappa value= 0.85) with BMD in this study, Vitek 2 was detected to be unreliable due to unacceptable high rates VMEs (14.5%). Moreover, it cannot be recommended for colistin antibiotic susceptibility test (AST).

Polymyxins are cationic large molecules which hardly diffuse into agar. This property causes false sensitive test results in Etest. Among previous studies comparing gradient test with BMD for colistin AST, good categorical and essential agreement have been observed in some[16], while some has shown low performance[21]. Several studies have shown that colistin susceptibility test results obtained by Etest methods have high very major error rates[15,16,21], some of them as high as 41.5%[2]. Etest result was correlated moderate agreement (kappa value=0.45) with in-house reference tests in the present study. The results were poorer for Etests (CA: 63.6%-80% depending on bacterial species).

The lowest categorical agreement (63.6%) and the highest VMEs rate (40%) among isolates was observed in A. baumanni species by Etest. With the gradient tests, a larger number of resistant

isolates were overlooked, resulting in a significant amount of false susceptible results (57 of total 203 isolates) in this study. A previous study has reported that colistin Etest demonstrated good performance, close to 90% CA, moreover, the very major error (VME) rate was found to be high (12% to 36%), similar to our results[16].

The strength of the present study is the included isolates, in particularly, colistin resistant *K. pneumoniae* isolates, are represented adequately in the study. The limiting factors of this study is that the Etest method was not applied to all isolates and colistin resistance genes could not be detected by molecular methods.

CONCLUSION

The importance of reliable colistin susceptibility test method has increased. However, colistin AST still remains a big challenge for many laboratories. Although we detected that the agreement of Vitek 2 with BMD was higher than the Etest-BMD in our study; the VME rates of both methods were determined to be above acceptable limits. Previous studies have reported that it may be useful in clinical settings to propose stringent MIC breakpoints considering heteroresistant isolates and mcr-1 carrying strains (which is MIC close to breakpoint)[16]. On the contrary, colistin resistant isolates will be underestimated by reducing susceptibility studies to a specific MIC since the MICs of VME isolates determined by Vitek 2 were mostly <= 0.5 mg/L in our study.

Based on the results of this study, it may be recommended not to rely on Vitek 2 and colistin gradient test results. It may also cause treatment failure not to prefer performing the broth microdilution method due to the specific MIC value being above or below. Finally, our suggestion to laboratories is to improve their skills and necessary infrastructure to use the broth microdilution method in their routine workflow.

ETHICS COMMITTEE APPROVAL

This study was approved by the Necmettin Erbakan University Research Ethics Committee [protocol number: 2020/2914, permission date: November 20, 2020].

CONFLICT of INTEREST

No conflict of interest was declared by the authors.

AUTHORSHIP CONTRIBUTIONS

Concept and Design: SU, MD

Data Collection or Processing: SU

Analysis/Interpretation: MD, SU

Literature Search: SU

Writing: SU

Final Approval: MD, SU

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