

Comparative Antimicrobial Susceptibility Profiles of Uropathogenic Extended-Spectrum β -Lactamase Producing Strains of *Klebsiella pneumonia* and *Escherichia coli* by the CLSI and EUCAST Methodologies

Genişletilmiş Spektrumlu β -Laktamaz Üreten Üropatojenik *Klebsiella pneumonia* ve *Escherichia coli* Suşlarının CLSI ve EUCAST Yöntemlerine göre Karşılaştırmalı Antimikrobiyal Duyarlılık Profilleri

Doğan Akdoğan¹, Mustafa Güzel², Yasemin Genç Bahçe³, Altan Aksoy⁴, Orhan Akpınar⁵

¹Pursaklar state hospital Department of Medical Microbiology, Ankara, Turkey

²Maltepe Medical Center Department of Medical Microbiology, Istanbul, Turkey

³Siirt state hospital Department of Medical Microbiology, Siirt, Turkey

⁴Department of Microbiology Ankara training and research hospital, University of health sciences, Ankara, Turkey

⁵Department of microbiology, Health Sciences Institute, University of Süleyman Demirel, Isparta, Turkey

ABSTRACT

Objective: This study aimed to compare the CLSI and EUCAST interpretations of the antimicrobial susceptibility test results of the ESBL-producing uropathogenic *Escherichia coli* and *Klebsiella pneumonia* strains.

Methods: After obtaining ESBL-producing *E. coli* and *K. pneumonia* isolates from the urine specimens of the patients, Kirby-Bauer's disc diffusion method was used for conducting antimicrobial susceptibility test. The test procedures and the interpretation of the results were carried out according to both of the two guidelines. For the statistical comparison of concordance between the two guidelines, the Kappa coefficients and the concordance rates were calculated.

Results: The results were graded in the range from perfect to poor agreement. For *E. coli*, interpretations of the AST results revealed a moderate to perfect agreement between both methods. Weighted Kappa agreement scores in the range from 0.42 to 1. The agreement for AMC, TPZ30/6, ceftazidime 10, meropenem, and aztreonam was poor without any inconsistencies. For *Klebsiella*, the kappa agreement score was in the range from 0.25 to 1. It was incompatible with AMC, TPZ 30/6, ceftazidime 10, aztreonam; there was poor agreement for cefepime, amikacin and ertapenem.

Conclusions: Our results showed agreement between the two guidelines for *Escherichia coli* and *Klebsiella pneumoniae* but also showed inconsistencies between two guidelines. Therefore, it contribute to the comparison of these guidelines for interpreting antibiotic susceptibilities.

Key Words: CLSI, EUCAST, ESBL Urinary tract infections, *Escherichia coli*, *Klebsiella pneumoniae*, Kappa coefficient

Received: 09.10.2020

Accepted: 11.10.2020

ÖZET

Amaç: Bu çalışma, ESBL üreten üropatojenik *Escherichia coli* ve *Klebsiella pneumonia* suşlarının antimikrobiyal duyarlılık testi sonuçlarının CLSI ve EUCAST yorumlarını karşılaştırmayı amaçlamaktadır.

Yöntem: Hastaların idrar örneklerinden ESBL üreten üropatojenik *Escherichia coli* ve *Klebsiella pneumonia* izolatları elde edildikten sonra antimikrobiyal duyarlılık testi yapmak için Kirby-Bauer disk difüzyon yöntemi kullanıldı. Test prosedürleri ve sonuçların yorumlanması her iki kılavuza göre gerçekleştirildi. İki kılavuz arasındaki uyumun istatistiksel karşılaştırması için Kappa katsayıları ve uyum oranları hesaplandı.

Bulgular: Uyum skoru mükemmelden zayıf uyuma kadar derecelendirildi. *E. coli* için antimikrobiyal duyarlılık testleri sonuçlarının yorumlandığında, her iki yöntem arasında orta ile mükemmel arasında bir uyum vardı. Ağırlıklı Kappa uyum skorları 0.42 ile 1 aralığında bulundu. AMC, TPZ30 / 6, seftazidim 10, meropenem ve aztreonam için zayıf uyum vardı. *Klebsiella* için kappa uyum skoru 0.25 ile 1 aralığındaydı. AMC, TPZ 30/6, seftazidim 10, aztreonam ile uyumsuzluk, sefepim, amikasin ve ertapenem için zayıf bir uyum vardı.

Sonuç: Sonuçlarımız, *Escherichia Coli* ve *Klebsiella Pneumoniae* için iki kılavuz arasında uyumu gösterdiği gibi, aynı zamanda iki kılavuz arasında tutarsızlıkları da göstermiştir. Bu nedenle, antibiyotik duyarlılıklarını yorumlamak için bu kılavuzların karşılaştırılmasına katkıda bulunmaktadır.

Anahtar Sözcükler: CLSI, EUCAST, ESBL, Üriner sistem enfeksiyonları, *Escherichia coli*, *Klebsiella pneumoniae*, Kappa katsayısı

Geliş Tarihi: 10.09.2020

Kabul Tarihi: 10.11.2020

ORCID IDs: D.A.0000-0002-0970-1597, M.G.0000-0001-5408-0905, Y.G.B.0000-0001-8103-7072, A.A.0000-0001-7939-3481, O.A.0000-0001-8397-8247

Address for Correspondence / Yazışma Adresi: Orhan Akpınar, MD Department of microbiology, Faculty of Dentistry, Süleyman Demirel University, East campus 32260 Çünür, Isparta, Turkey E-mail: orhanakpnr@hotmail.com

©Telif Hakkı 2021 Gazi Üniversitesi Tıp Fakültesi - Makale metnine <http://medicaljournal.gazi.edu.tr/> web adresinden ulaşılabilir.

©Copyright 2021 by Gazi University Medical Faculty - Available on-line at web site <http://medicaljournal.gazi.edu.tr/>

doi:<http://dx.doi.org/10.12996/gmj.2021.16>



INTRODUCTION

Urinary tract infections (UTIs) are the most common form of bacterial infection worldwide, and they are associated with high costs and morbidity. The treatment of UTIs has become problematic, and the treatment options are limited due to the common use of antibiotic medications leading to increased antibiotic resistance rates (1, 2). The extended-spectrum beta-lactamase (ESBL) synthesis was first published in 1983 in the members of the Enterobacteriaceae family, and it is currently one of the most critical health problems worldwide (2). Following the identification of ESBL strains of *Klebsiella pneumoniae*, those strains of *E. coli* were demonstrated. Both of these pathogens are mainly involved in UTIs (2, 3). The synthesis of ESBL leads to resistance development to all types of beta-lactam antibiotics, excluding cefamandols and carbapenems. The ESBL synthesis is most commonly seen in *Klebsiella pneumoniae* and *Escherichia coli* strains. Since ESBL-encoding plasmids often carry other resistance genes too, resistance to sulfonamides, aminoglycosides, and fluoroquinolones is common (1,3). The most crucial problem here is the worldwide geographical variability in the frequency of ESBL-producing strains, which are usually resistant to a wide variety of antibiotics. High resistance rates lead to several unfavorable consequences, including treatment failures, recurrent or chronic infections, increased treatment costs, prolonged hospital stay, development of permanent complications, and high morbidity and mortality rates (4). To avoid treatment failures associated with antibiotic resistance, standardization should be ensured in conducting, interpreting, and reporting antimicrobial susceptibility tests (AST). Standardization is essential for determining the resistance profiles at the national level, comparing resistance profiles on international platforms, and taking part in global surveillance systems. For this purpose, two widely-known standards for AST have been developed, and they are used globally. One of these standards is the "Clinical Laboratory Standards Institute" (CLSI), which has been used in our country for many years. The other is the "European Committee on Antimicrobial Susceptibility Testing" (EUCAST) recommendations, which have been used in many European Union member countries since 2015 (5,6). Our laboratory has been using the CLSI guidelines for conducting AST and interpreting the results for many years. However, the increasing number of countries adopting the EUCAST guidelines led us to use the EUCAST methodology in our laboratory frequently. Therefore, our study aimed to investigate the extent of agreement between the AST results obtained by CLSI and EUCAST methodology by examining the antibiotic susceptibility of uropathogenic ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*. It is aimed to evaluate the zone diameters to be yielded, compare the interpretations of test results made according to both of the CLSI and EUCAST guidelines, and to determine possible differences.

MATERIALS and METHODS

Study Setting, Design and Population

This cross-sectional study was conducted prospectively in Ankara Numune Research and Training Hospital. ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* strains obtained from the urine samples of patients were included in the study to evaluate zone diameters according to the standards of both CLSI and EUCAST to determine possible differences between the two methods.

The ESBL-producing strains were excluded from the study when colonization was identified or when the ESBL producing strains were isolated from the same site of infection in the same patient. The uropathogenic strains of ESBL-producing *K. pneumoniae* and *E. coli*, isolated from the hospital- or community-acquired infections were included in the study.

Ethical approval

Before commencing the study, approval of the ethics committee of Ankara Numune Research and Training Hospital (Ref 2017/001) was obtained. Ankara/Turkey

Bacterial Isolate Collection

MALDI-TOF mass spectrometry (BD, Sparks, USA) and the combined disc method were used for identifying the isolates and their ESBL characteristics, respectively. The quality control of the media used in the study, the bacteria identification tests, and AST were performed according to both of the CLSI and EUCAST recommendations about the *E.coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 strains.

Confirmation of ESBL-Producing Strains

The phenotypic confirmation of ESBL synthesis in the isolates was performed using the combined disc method. The Mueller Hinton agar medium (Oxoid LTD, Hampshire, England) was used for the cultivation of the bacterial suspension prepared according to a 0.5 McFarland standard. After placing the discs (BD BBL) 25 mm apart from center to center and incubating the plates at 37°C for 24 hours, the zone inhibitions of the ceftazidime (30µg) and cefotaxime (30µg) discs were compared to the zone inhibitions observed with the clavulanic acid (10µg) containing discs of ceftazidime (30µg) and cefotaxime (30µg). A difference of ≥5 mm between the zone diameters around either of the clavulanic acid-containing discs compared to those of the only antibiotic discs was accepted to indicate the ESBL synthesis in that specific bacterial isolate (7).

Antimicrobial Susceptibility Testing

After inoculating the Muller-Hinton Agar (Oxoid LTD, Basingstoke, Hampshire, United Kingdom) plates with 0.5 Mc Farland turbidity inoculums, the antimicrobial discs (Abtek Biologicals, Liverpool, United Kingdom) were applied to the plates. Then, they were incubated at 37°C for 24 hours. Discs of ampicillin, amoxicillin-clavulanic acid (AMC), ampicillin/sulbactam (SAM), piperacillin/tazobactam 30/6 (TPZ), cefuroxime, cefepime, ceftazidime 10, ceftazidime 30, cefotaxime 5, ceftriaxone, gentamicin, tobramycin, amikacin, imipenem, meropenem, ertapenem, trimethoprim/sulfamethoxazole (TMP/SMX), chloramphenicol, aztreonam, ofloxacin, ciprofloxacin, norfloxacin, levofloxacin, and nitrofurantoin were used in the study. The Kirby-Bauer disc diffusion method was used for conducting AST. The zone diameters formed after the incubation period were measured and recorded. Finally, the inhibition zone diameters were interpreted according to the 2017 guidelines of CLSI and EUCAST. The interpreted results were assigned to one of the susceptible, intermediate, or resistant categories (8,9). The AST results of the *Escherichia coli* and *Klebsiella pneumoniae* isolates were evaluated according to both of the CLSI and EUCAST standards. The susceptibility rates of the isolates and the results of the comparative statistical analyses are shown in Table 1 and Table 2.

Table 1. Susceptibility of uropathogenic extended-spectrum beta-lactamase-producing *Escherichia coli* to various antibiotics; respective concordance rates and kapa statistics comparing the CLSI 2017 and EUCAST 2017 guidelines

	CLSI(%) n=264			EUCAST %(%) n=264			Concordance (%)	Kappa, κ (95 % CI)*
	S	I	R	S	I	R		
Ampicilline	0	2	98	1	0	99	98.5	0.42 (0.29-0.56)
AMC	15.9	20.5	63.6	10.2	0.4	89.4	74.6	0.37(0.27-0.47)
SAM	56.8	17.8	25.4	64	0	36	82.2	0.67(0.60-0.74)
TPZ 30/6	79.5	11.4	9.1	61	11	28	70.8	0.39(0.30-0.47)
Cefuroxime	0	0.4	99.6	0	0	100	99.6	1
Cefepime	8	37.1	54.9	9.5	8.3	82.2	71.2	0.44(0.34-0.53)
Ceftazidime 10	31.1	20.1	48.9	11	8.7	80.3	60.6	0.29(0.22-0.37)
Cefoxitin	92.4	3.8	3.8	90.2	0	9.8	93.9	0.63(0.50-0.76)
Cefotaxime 5	0	0.4	99.6	0	0	100	99.6	1
Ceftriaxone	0	0.8	99.2	0	0	100	99.2	1
Gentamicin	69.3	3.4	27.3	54.9	15.2	29.9	83.0	0.68(0.60,0.76)
Tobramycin	45.1	14.8	40.2	31.4	16.7	51.9	75.4	0.60(0.53,0.67)
Amikacin	95.5	4.5	0	89.8	8.3	1.9	92.4	0.46(0.29-0.62)
Imipenem	98.5	1.1	0.4	98.5	0.8	0.8	99.2	0.75(0.41-1.00)
Meropenem	98.5	0.8	0.8	99.2	0.8	0	98.5	0.33(0.11-0.55)
Ertapenem	75.8	11.4	12.9	58.0	15.2	26.9	70.8	0.43(0.35-0.51)
TMP/SMX	38.6	0.4	61	38.6	0.4	61	100	1
Chloramphenicol	91.7	1.5	6.8	91.7	0	8.3	98.5	0.90(0.82-0.99)
Aztreonam	30.7	32.2	36.4	19.7	8.3	71.2	56.4	0.33(0.26-0.41)
Ofloxacin	34.5	6.1	59.5	31.4	1.9	66.7	90.9	0.82(0.75-0.88)
Ciprofloxacin	31.4	6.1	62.5	31.1	1.9	67	95.1	0.90(0.85-0.95)
Norfloxacin	32.2	2.7	65.2	30.3	1.5	68.2	95.5	0.90(0.85-0.95)
Levofloxacin	33.3	4.9	61.4	30.7	1.5	67.4	92.1	0.84(0.78-0.90)
Nitrofurantoin	92.0	3.0	4.9	95.5	0	4.5	95.8	0.65(0.46-0.84)

EUCAST = European committee for antimicrobial susceptibility testing, CLSI = Clinical and Laboratory Standards Institute,

IS =Intermediate susceptibility, S = Susceptible, R = Resistant, * =Weighted Kappa agreement score, AMC=amoxicillin-clavulanic acid SAM= ampicillin/sulbactam, TPZ= piperacillin/tazobactam 30/6, TMP/SMX= trimeth

Table 2. Susceptibility of uropathogenic extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* to various antibiotics, concordance rates and kapa statistics, comparing the CLSI 2017 and EUCAST 2017 guidelines

	CLSI(%) n=264			EUCAST (%) n=264			Concordance (%)	Kappa, κ (95 % CI)
	S	I	R	S	I	R		
Ampicilline	0	0	100	0	0	100	100	1
AMC	5.1	24.4	70.5	3.8	0	96.2	74.36	0.20 (0.01-0.39)
SAM	35.9	32.1	32.1	44.9	0	55.1	67.95	0.52 (0.40-0.63)
TPZ 30/6	69.2	20.5	10.3	32.1	26.9	41	42.31	0.15 (0.04-0.27)
Cefuroxime	1.3	0	98.7	1.3	0	98.7	100	1
Cefepime	2.6	24.4	73.1	3.8	2.6	93.6	78.21	0.29 (0.09-0.50)
Ceftazidime 10	3.8	10.3	85.9	0	1.3	98.7	85.9	0.06 (-0.05-0.18)
Cefoxitin	85.9	3.8	10.3	83.3	0	16.7	91.03	0.66 (0.46-0.86)
Cefotaxime 5	0	0	100	0	1.3	98.7	98.72	1
Ceftriaxone	0	0	100	0	0	100	100	1
Gentamicin	59	5.1	35.9	43.6	15.4	43.6	76.92	0.61 (0.47-0.74)
Tobramycin	30.8	20.5	48.7	21.8	15.4	62.8	76.92	0.61 (0.47-0.75)
Amikacin	94.9	5.1	0	88.5	7.7	3.8	89.74	0.35 (0.11-0.58)
Imipenem	96.2	2.6	1.3	97.4	1.3	1.3	98.72	0.80 (0.40-1)
Meropenem	96.2	1.3	2.6	97.4	0	2.6	98.72	0.79 (0.40-1)
Ertapenem	64.1	19.2	16.7	37.2	19.2	43.6	51.28	0.25 (0.13-0.38)
TMP/SMX	21.8	1.3	76.9	21.8	0	78.2	98.72	0.96 (0.89-1)
Chloramphenicol	76.9	5.1	17.9	76.9	0	23.1	94.87	0.86 (0.74-0.98)
Aztreonam	12.8	25.6	61.5	5.1	2.6	92.3	66.67	0.20 (0.05-0.36)
Ofloxacin	50	6.4	43.6	35.9	12.8	51.3	79.49	0.65 (0.52-0.78)
Ciprofloxacin	33.3	16.7	50	33.3	3.8	62.8	87.18	0.77 (0.65-0.90)
Norfloxacin	46.2	3.8	50	29.5	6.4	64.1	79.49	0.62 (0.48-0.76)
Levofloxacin	48.7	7.7	43.6	33.3	11.5	55.1	76.92	0.61 (0.47-0.74)
Nitrofurantoin	35.9	21.8	42.3	53.8	0	46.2	73.08	0.56 (0.43-0.69)

EUCAST = European committee for antimicrobial susceptibility testing, CLSI = Clinical and Laboratory Standards Institute, IS = Intermediate susceptibility, S = Susceptible, R = Resistant * = Weighted Kappa agreement score, AMC= amoxicillin-clavulanic acid SAM= ampicillin/sulbactam, TPZ= piperacillin/tazobactam 30/6, TMP/SMX= trimethoprim/sulfamethoxazole.

Statistical analysis

Statistical analyses were carried out by using R (Psych package of R software /cohen.kappa function). The Kappa (κ) coefficient was calculated to compare the study parameters. The concordance rates between the CLSI and EUCAST guidelines were calculated and presented in percentages. The susceptibility to the antimicrobial agents was calculated in percentages for both of the *Escherichia coli* and *Klebsiella pneumoniae*. Weighted kappa values were calculated to find the level of absolute agreement between the two guidelines. Cohen's kappa statistics were used for determining the level of agreement between the AST results found according to both of the CLSI 2017 and EUCAST 2017 guidelines. The results were categorized in the range from a perfect to poor agreement. In practice; to determine the level of agreement with Cohen's Kappa statistics, two independent observations are made. Then, the agreement level above chance is found out. The level of agreement can numerically be in the range from -1 to 1, and a p-value of less than 0.05 but not equal to zero indicates a significant difference occurring not by chance. The calculated Kappa coefficient values in the study were interpreted as follows (10,11), No agreement: $0 \leq \kappa < 0.20$, Poor agreement: $0.20 \leq \kappa < 0.40$, Moderate agreement: $0.40 \leq \kappa < 0.60$, Good agreement: $0.60 \leq \kappa < 0.80$, Perfect agreement: $0.80 \leq \kappa < 1.00$, A p-value of ≤ 0.05 was accepted to indicate a statistically significant difference for all inferential statistics.

RESULTS

The study included 157 ESBL-producing *E. coli* and 95 ESBL-producing *K. pneumoniae* strains that were collected consecutively in Ankara Numune Research and Training Hospital in the period from April 2014 to November 2018.

Table 1 summarizes the AST results obtained for *E. coli* and the respective concordance rates and kappa statistics results comparing the CLSI and EUCAST methods. The concordance between these two methods ranged from 56.4% to 100%. The comparisons revealed that AST results of *E. coli*, found by both methods, were in moderate to perfect agreement for most of the antibiotics tested. The weighted Kappa agreement scores for the AST results of *E. coli* for these antibiotics ranged from 0.42 to 1. However, the level of agreement between these two methods was poor for the following antibiotics: AMC: $\kappa = 0.37$ [95% CI: 0.27-0.47], $p < 0.000$, TPZ 30/6: $\kappa = 0.39$ [95% CI: 0.30-0.47], $p < 0.000$, Ceftazidime 10: $\kappa = 0.29$ [95% CI: 0.22-0.37], $p < 0.000$, Meropenem: $\kappa = 0.33$ [95% CI: 0.11-0.55], $p < 0.000$, Aztreonam: $\kappa = 0.33$ [95% CI: 0.26-0.41], $p < 0.000$ (Table 3). The comparative evaluation of the AST results obtained by using the two guidelines was presented as the kappa agreements in Table 2. The comparisons of the CLSI and EUCAST interpretations made for the AST results for *K. pneumoniae* revealed moderate to perfect agreement for most of the antibiotics. The Kappa agreement scores for these AST results ranged from 0.15 to 0.96 for these antibiotics, and their concordance rates ranged from 42.3% to 100 %. The antibiotic susceptibility patterns interpreted according to both guidelines were found out to be similar. However, the kappa analysis showed that the agreement was hardly present for the following antibiotics: AMC: $\kappa = 0.20$ [95% CI: 0.01-0.39], $p < 0.000$, TPZ 30/6: $\kappa = 0.15$ [95% CI: 0.04-0.27], $p < 0.000$, Ceftazidime 10: $\kappa = 0.06$ [95% CI: -0.05-0.18], $p < 0.000$, Aztreonam: $\kappa = 0.20$ [95% CI: 0.05-0.36], $p < 0.000$. Also, the agreement was poor for the following antibiotics: Cefepime: $\kappa = 0.29$ [95% CI: 0.09-0.50], $p < 0.000$, Amikacin: $\kappa = 0.35$ [95% CI: 0.11-0.58], $p < 0.000$, Ertapenem: $\kappa = 0.25$ [95% CI: 0.13-0.38], $p < 0.000$. The comparative evaluation of the interpretations of the AST results according to both of the guidelines is presented in Table 2. The comparative evaluation of the Kappa agreement scores between the two guidelines is shown in Table 4.

Table-3. Interpretation of the comparative evaluations of the Kappa agreement scores by the two guidelines for uropathogenic extended-spectrum β -lactamase producing *Escherichia coli*

Agreement (Kappa) No agreement (0.01–0.20)	Fair (0.21–0.40)	Moderate (0.41–0.60)	Substantial (0.61–0.80)	Perfect (0.81–1)
-	AMC	Ampicilline	SAM	Cefuroxime
-	TPZ 30/6	Cefepime	Cefoxitin	Cefotaxime 5
-	Ceftazidime 10	Tobramycin	Gentamicin	Ceftriaxone
-	Meropenem	Amikacin	Imipenem	TMP/SMX
-	Aztreonam	Ertapenem	Nitrofurantoin	Chloramphenicol
				Ofloxacin
				Ciprofloxacin
				Norfloxacin
				Levofloxacin

Table-4. Interpretation of the comparative evaluation of the Kappa agreement scores by the two guidelines for uropathogenic extended-spectrum β -lactamase producing *K. pneumoniae*

Agreement (Kappa) No Agreement (0.01–0.20)	Fair (0.21–0.40)	Moderate (0.41–0.60)	Substantial (0.61–0.80)	Perfect (0.81–1)
AMC	Cefepime	SAM	Cefoxitin	Ampicilline
TPZ 30/6	Amikacin	Nitrofurantoin	Gentamicin	Cefuroxime
Ceftazidime 10	Ertapenem		Tobramycin	Cefotaxime 5
Aztreonam			Imipenem	Ceftriaxone
			Meropenem	TMP/SMX
			Ofloxacin	Chloramphenicol
			Ciprofloxacin	
			Norfloxacin	
			Levofloxacin	

DISCUSSION

Extended-spectrum β -lactamases (ESBLs) are responsible for resistance development against β -lactam-antibiotics. This group of antibiotics includes penicillins, cephalosporins, and aztreonam. The ESBL enzymes are usually inhibited by beta-lactamase inhibitors, including clavulanic acid, sulbactam, and tazobactam (12,13). ESBLs are most commonly produced by *Escherichia coli* and *Klebsiella pneumoniae*; which are Gram-negative bacteria of the family Enterobacteriaceae (13,14). Resistance to more than one type of antibiotics is common with ESBL-producing *Klebsiella pneumoniae* and *E.coli* strains, limiting the available treatment options and leading the researchers to look for new therapeutic alternatives (15). β -lactam antibiotics are the main antimicrobial agents used for the treatment of UTI; however, the rates of resistance to β -lactams are on the rise, affecting the treatment effectiveness unfavorably. The most commonly identified microorganisms in UTI are *E. coli* and *Klebsiella* species, which are currently resistant to more than one antibiotic recommended for use in the treatment. There has been an observed increase in the incidences of ESB-producing *E. coli* and *Klebsiella spp* in recent years (12,15). In a multicenter study conducted in Spain, antibiotic susceptibility test results of ESBL-producing *E.coli* blood isolates were compared according to the 2009-2010 CLSI, and 2011 EUCAST guidelines and the study reported a significant difference only between the AMC sensitivity test interpretations (16). Pölsfuss et al. reported no significant differences in the sensitivity of EUCAST 2011 and CLSI 2011 guidelines in detecting ESBL-producing Enterobacteriaceae (17). However, in a different study conducted by Hombach et al., significant differences were reported in the susceptibility rates to cephalosporins for the ESBL-producing Enterobacteriaceae isolates when the test results were obtained according to both of the CLSI 2013 and EUCAST 2013 criteria (18). In a study conducted in Turkey, antibiotic susceptibility test zone diameters for uropathogenic *Escherichia coli* isolates were evaluated according to both CLS 2014 and EUCAST 2014 standards. The results obtained separately by each method showed that amikacin and trimethoprim-sulfamethoxazole susceptibility rates were the same. However, the susceptibility rates were significantly different for gentamicin, cefuroxime axetil, and levofloxacin (19). Kassim et al. reported that the AST patterns of *E. coli* obtained according to the EUCAST 2015 and CLSI 2015 guidelines were similar, excluding AMC, nitrofurantoin, and amikacin. A moderate agreement was noted with AMC; a fair agreement was reported with nitrofurantoin, and a poor agreement was noted with amikacin (6). In a similar study comparing the 2017 guidelines of CLSI and EUCAST, the agreement between these two methods in the AST results of uropathogenic *E. coli* isolates was the highest with trimethoprim and cephalixin with rates of 100% agreement. However, the same study reported that the agreement levels for AMC and ciprofloxacin were the lowest (20). Batista et al. evaluated the clinical isolates of *E. coli* and *Klebsiella* according to both guidelines. The authors reported that the kappa match for amikacin indicated a poor agreement between the guidelines for both microorganisms. Kappa statistics for other antibiotics were found to be consistent (21). In our study, the susceptibility patterns for ESBL-producing *E.coli* were found similar between both EUCAST 2017 and CLSI 2017 guidelines excluding AMC, TPZ, ceftazidime 10, meropenem, and aztreonam. The weighted Kappa agreement scores for these antibiotics indicated a poor agreement between EUCAST 2017 and CLSI 2017 guidelines. For the ESBL-producing *K. pneumoniae* isolates in our study; when the interpretations of the AST results made by both CLSI and EUCAST guidelines were compared, the kappa analysis revealed almost no agreement for AMC, TPZ, ceftazidime 10, and aztreonam, and a poor agreement with cefepime, amikacin, and ertapenem. The guidelines recommend that the ESBL-producing isolates can be treated with cephalosporins based on the categorization of the AST results. Compared to the CLSI 2009 guidelines and partly to the EUCAST 2010 guidelines, the 2013 versions of EUCAST and CLSI classify an increased number of isolates as resistant and recommend higher zone diameter breakpoints, intending to ensure that correct treatment practices are implemented. These recommendations particularly aim for the treatment with cephalosporins (22-25). Recommending cephalosporin therapy for infections caused by ESBL-producing bacteria provides an additional treatment option alternative to the reserve medications, including carbapenems, relieving the pressure felt by treatment providers. However, the data about antibiotic susceptibility patterns of specific ESBL-producing isolates are limited, and there is a scarcity of information in the EUCAST and CLSI guidelines (26).

The role of antibiotics becomes critical, especially when the susceptibility of the bacteria is high and when the treatment is given timely. Therefore, selecting the most appropriate antibiotic for the treatment is of major importance based on the interpretation of the phenotypic AST in treatment-resistant infections (27). AST results play a critical role in guiding critical treatment decisions. Two leading organizations setting standards for AST to be used by clinical microbiology laboratories have used different strategies to overcome these challenges. With our study, we have demonstrated that an acceptable level of agreement exists between the EUCAST 2017 and CLSI 2017 guidelines in the interpretation of AST results of *Escherichia coli* and *Klebsiella pneumoniae*. Our findings indicate that a comparison of susceptibility rates can only be considered in the treatment provider if the discordance generated by the use of different guidelines is established. We would like to see consistency between the recommendations of CLSI and EUCAST to bring standardization to the international reports. The free provision of EUCAST guidelines provides a significant advantage in maintaining the current standards for interpreting antibiotic susceptibility test results.

We compared the AST result interpretations of the guidelines using only two different bacteria species. Therefore, the results may not be generalized and may not represent the comparison of two guidelines for the whole spectrum of clinically relevant gram-positive and negative bacteria. However, the two ESBL-producing bacteria species used in our study represent a significant population of uropathogenic bacteria. Adopting the updated limits in the current recommendations is vital for consistency in reporting the AST results. However, we are still concerned about the inconsistencies between the two guidelines in resistance screening. We recommend the use of the more conservative breakpoints for antibiotics because of the inconsistencies obtained with the kappa analysis results regarding the ESBL-producing uropathogenic strains of *E. Coli* and *K. pneumoniae*.

Conflict of interest

No conflict of interest was declared by the authors.

REFERENCES

- Shakya P, Shrestha D, Maharjan E, Sharma VK, Paudyal R. ESBL production among *E. coli* and *Klebsiella spp.* causing urinary tract infection: a hospital based study. *Open Microbiol J* 2017;28:23-30.
- Ben-Ami R, Rodríguez-Baño J, Arslan H et al A multinational survey of risk factors for infection with extended spectrum beta-lactamase-producing Enterobacteriaceae in nonhospitalized patients. *Clin Infect Dis* 2009;49:82-90.
- Paterson DL, Bonomo RA. Extended-spectrum betalactamases: a clinical update. *Clin Microbiol Rev* 2005;18:657-86.
- Wang Y, Zhang Q, Jin Y, Jin X, Yu J, Wang K. Epidemiology and antimicrobial susceptibility profiles of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* and *Escherichia coli* in China. *Braz J Microbiol* 2019;50:669-75.
- Kahlmeter G, Giske CG, Kirn TJ, Sharp SE. Point-Counterpoint: Differences between the European Committee on Antimicrobial Susceptibility Testing and Clinical and Laboratory Standards Institute recommendations for reporting antimicrobial susceptibility results. *J Clin Microbiol* 2019;26:57-9.
- Kassim A, Omuse G, Premji Z, Revathi G. Comparison of Clinical Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing guidelines for the interpretation of antibiotic susceptibility at a University teaching hospital in Nairobi, Kenya: a cross-sectional study. *Ann Clin Microbiol Antimicrob* 2016;11:15-21.
- Thomson KS, Sanders CC. Detection of extended spectrum beta-lactamases in members of family Enterobacteriaceae: comparison of the double disk and three-dimensional tests. *Antimicrob Agents Chemother* 1992;36:1877-82.
- CLSI. M100-S26. 2017 performance standards for antimicrobial susceptibility testing; Twenty-fifth informational supplement.
- EUCAST 2017. European Committee on Antimicrobial Susceptibility Testing breakpoint tables for interpretation of MICs and zone diameters.
- Revelle W, Revelle MW. Package 'psych'. The Comprehensive R Archive Network 2015.
- Viera AJ, Garrett JM. Understanding interobserver agreement: the kappa statistic. *Fam Med* 2005;37:360-3.

12. Kuster SP, Hasse B, Huebner V, Bansal V, Zbinden R, Ruef C et al. Risk factors for infections with extended-spectrum betalactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* at a tertiary care university hospital in Switzerland. *Infection* 2010;38:33-40.

13. Meier S, Weber R, Zbinden R, Ruef C, Hasse B. Extended spectrum β -lactamase-producing Gram-negative pathogens in community-acquired urinary tract infections: an increasing challenge for antimicrobial therapy. *Infection* 2011;39:333-40.

14. Hyle EP, Lipworth AD, Zaoutis TE, Nachamkin I, Fishman NO, Bilker WB et al. Risk factors for increasing multidrug resistance among extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella* species. *Clin Infect Dis* 2005;40:1317-24.

15. Rodríguez-Baño J, Navarro MD, Romero L, Martínez-Martínez L, Muniain MA, Perea EJ et al. Epidemiology and clinical features of infections caused by extended-spectrum beta-lactamase-producing *Escherichia coli* in nonhospitalized patients. *J Clin Microbiol* 2004; 42:1089-94.

16. Rodríguez-Bano J, Picon E, Navarro MD, Lopez-Cerero L, Pascual A. ESBL-REIPI Group. Impact of changes in CLSI and EUCAST breakpoints for susceptibility in bloodstream infections due to extended-spectrum β -lactamase producing *Escherichia coli*. *Clin Microbiol Infect* 2012;18:894-900.

17. Polsfuss S, Bloemberg GV, Giger J, Meyer V, Hombach M. Comparison of European Committee on Antimicrobial Susceptibility Testing (EUCAST) and CLSI screening parameters for the detection of extended-spectrum β -lactamase production in clinical enterobacteriaceae isolates. *J Antimicrob Chemother* 2012;67:159-66.

18. Hombach M, Mouttet B, Bloemberg GV. Consequences of revised CLSI and EUCAST guidelines for antibiotic susceptibility patterns of ESBL- and AmpC β -lactamase-producing clinical Enterobacteriaceae isolates. *J Antimicrob Chemother* 2013;68:2092-98.

19. Süzük S, Kaşkatepe B, Avcıküçük H, Aksaray S, Başustaoğlu A. The comparison of antibiotic susceptibilities of uropathogenic *Escherichia coli* isolates in transition from CLSI to EUCAST. *Mikrobiyol Bul* 2015;49:494-501.

20. O'Halloran C, Walsh N, O'Grady MC, Barry L, Hooton C, Corcoran GD, Lucey B. Assessment of the comparability of CLSI, EUCAST and Stokes antimicrobial susceptibility profiles for *Escherichia coli* uropathogenic isolates. *Br J Biomed Sci* 2018;75:24-9.

21. Sánchez-Bautista A, Coy J, García-Shimizu P, Rodríguez JC. From CLSI to EUCAST guidelines in the interpretation of antimicrobial susceptibility: what is the effect in our setting? *Enferm Infecc Microbiol Clin* 2018;36:229-32.

22. EUCAST 2013. Breakpoint Tables for Interpretation of MICs and Zone Diameters. Version 3.1

23. Wayne PA. Clinical Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twenty-third Informational Supplement 2013; M100-S23

24. Wayne PA. Clinical Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Nineteenth Informational Supplement 2009; M100-S19

25. EUCAST 2010. Breakpoint Tables for Interpretation of MICs and Zone Diameters. Version 1.0

26. Wang P, Hu F, Xiong Z, Ye X, Zhu D, Wang YF et al. Susceptibility of extended-spectrum beta-lactamase-producing Enterobacteriaceae according to the new CLSI breakpoints. *J Clin Microbiol* 2011;49:3127-31.

27. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. *Clin Infect Dis* 2009;49:1749-55.