EFFECTS OF CLINICAL BREAKPOINT CHANGES IN TRANSITION FROM CLSI TO EUCAST FOR ANTIBIOTIC SUSCEPTIBILITY TEST REPORTING OF PSEUDOMONAS AERUGINOSA ISOLATES: A LOCAL STUDY IN TURKEY

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ABSTRACT

***Introduction**: The European Committee on Antimicrobial Susceptibility Testing (EUCAST) is a standard used for the implementation and evaluation of antibiotic susceptibility tests in many European countries. The EUCAST standard has been used in Turkey since 2015. The aim of this study is to evaluate the differences in antibiotic susceptibilities of Pseudomonas aeruginosa (P. aeruginosa) isolates using local data during the transition process from the Clinical Laboratory Standards Institute (CLSI) standards to EUCAST.*

***Materials and methods**: In total, 105 non-duplicate clinical isolates of P. aeruginosa were analyzed. Conventional methods and the Vitek-2 automated system (bioMérieux, France) were used to identify the isolates. Antibiotic susceptibility tests were performed with the Kirby-Bauer disc diffusion method. In addition to the routinely used discs, ceftazidime 10 µg and piperacillin/tazobactam 30/6 µg were added to create different contents for both of the standards. The antibiotic susceptibility zone diameters of the isolates were evaluated in accordance with the CLSI 2014 and EUCAST 2014 standards, and a difference in the ratio of resistance levels was found.*

***Results**: It was observed that antibiotics with different disc contents had different resistance ratios according to the evaluation made with each of the standards. The resistance ratio of ceftazidime (10µg to 30µg) and piperacillin/tazobactam (100/10 µg to 30/6 µg) in P.aeruginosa increased from 1.9% and 0.95% to 4.8% and 15.2%, respectively, when we compared the CLSI 2014 with the EUCAST 2014 standards. There were no significant differences in the susceptibility results for imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, levofloxacin, and cefepime.*

***Conclusion**: Antimicrobial susceptibility testing is one of the most important tasks in clinical microbiology laboratories. Upon the implementation of the EUCAST guidelines, laboratories should be aware of the implications of modified drug susceptibility testing reports on antibiotic prescription policies. Additionally, the continuity of local surveillance activities should be ensured.*

***Keywords**: Pseudomonas aeruginosa, antimicrobial susceptibility tests, Clinical Laboratory Standards Institute (CLSI), European Committee on Antimicrobial Susceptibility Testing (EUCAST).*

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Introduction

Pseudomonas aeruginosa is one of the most common pathogens in nosocomial and ventilator-associated pneumonia, cystic fibrosis (CF), meningitis, abscesses, soft tissue infections, urinary tract infections, catheter-associated infections, corneal infections, and conjunctival erythema[1]. One of the most important problems in the treatment of *P. aeruginosa* infections is the increasing resistance caused by inappropriate use of antibacterial agents[2]. Antibiotic resistance in *P. aeruginosa* strains increases the duration of hospitalization, and has negative impacts on the cost of treatment and mortality[3, 4]. Resistance develops over time to the antibiotics used to treat *P. aeruginosa* infections, and in some cases, the susceptibility status may change during treatment.

Resistance develops especially after the use of specific antibiotics, and resistant strains may be passed on to other patients[5]. When a number of resistances that have developed against various antibiotics are combined, and when cross-resistance develops, the problem of multiple-antibiotic resistance occurs[5]. Knowing the antibiotic resistance of strains is important for selecting the appropriate antibiotic for empirical treatment. Thus, one of the most important and critical tasks of clinical microbiology laboratories in monitoring and directing infectious disease treatments is to report the antimicrobial susceptibility test (AST) results for the isolated pathogen in time and with the correct interpretation.

In order to control resistance-based treatment failures, standardization must be ensured when conducting, interpreting, and reporting AST results. This is not only a valuable guide for antibiotic therapy, but also an important epidemiological tool to examine resistant organisms. Therefore, ASTs should be performed in accordance with the generally accepted and clinically relevant guidelines. The Clinical Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) are the two most common standards used through out the world as guidance for microbiology specialists[6,7].

Turkey adopts the same behavioral patterns as the European Union with regard to various issues, and therefore it is quite important to use the same standards within geographically close European countries, at least for evaluating resistance maps. CLSI standard guidelines were used in Turkey for many years; however, many European Union member states had begun using EUCAST by 2015, and Turkey has now also adopted this standard. Because of the difference in working principles of CLSI and EUCAST standards, some changes are expected in resistance profiles[7].

However, during this transition process, in studies in which we conducted ASTs while reporting P.aeruginosa as an infection pathogen, it was not known to what extent the changes in breakpoints and certain disc contents (ceftazidime 10 μg and piperacillin/tazobactam 30/6 μg) could affect the results. The aim of this study is to evaluate the antibiotic susceptibility zone diameters of various infectious *P. aeruginosa* isolates in ASTs in accordance with the two standards, and to detect the possible differences.

Materials and methods

Clinical isolates

A total of 105 non-duplicate clinical isolates of *P. aeruginosa*, identified during a 12-month period from March 2015 to February 2016 in the Ahi Evran University Training and Research Hospital Microbiology Laboratory, were included in the study. Identification to the species level was performed by routine laboratory methods, including the Vitek 2 system (bioMérieux, Marcy l'Etoile, France). Forty-eight isolates were recovered from urine, 25 from wounds, 24 from respiratory samples, six from blood, and two from ears.

Susceptibility testing

For susceptibility testing, the Kirby-Bauer disc diffusion method was used. Amikacin (30 μg), gentamicin (10 μg), imipenem (10 μg), meropenem (10 μg), ciprofloxacin (5μg), levofloxacin (5μg), cefepime (30μg), ceftazidime (10 μg), ceftazidime (30 μg), piperacillin/tazobactam (30/6 μg), and piperacillin/tazobactam (100/10 μg) antibiotic discs (Bioanalyse, Turkey) were tested during the study period. Susceptibility testing was performed with Mueller-Hinton agar using McFarland 0.5 from overnight cultures, followed by incubation at 36°C for 18-20 h. Inhibition zone diameters were determined with a millimetric ruler and recorded by two experienced microbiologists. Inhibition zone diameters were interpreted according to the EUCAST 2014 and CLSI 2014 M100-S24 guidelines, respectively (see Table 1). Antibiotic susceptibility testing was performed with the quality-control E. coli strain ATCC 25922, according to CLSI and EUCAST protocols.

Comparison of CLSI 2014 and EUCAST 2014 breakpoints

Inhibition zone diameters were used to compare the CLSI 2014 recommended diameter breakpoints with the EUCAST 2014 clinical breakpoints for *P. aeruginosa*[8, 9]. Additionally, a comparison was made between antibiotics with different disc contents (ceftazidime at 10 μg/disc versus 30 μg/disc and piperacillin/tazobactam at 30/6 μg/disc versus 100/10 μg/disc for EUCAST and CLSI, respectively).

Statistical analyses

All statistical analyses were done using Statistical Package for the Social Sciences (SPSS)

version 17 software (SPSS Inc., Chicago, IL, USA). Categorical variables were presented as frequencies and percentages, and continuous variables were expressed as means and SD. The statistical comparison of clinical data between the two groups consisted of unpaired t-tests for parametric data. Correlations were assessed with the Pearson correlation coefficient, and the chi-square test was used for categorical variables. P<0.05 was considered significant.

Discussion

Since *P. aeruginosa* shows resistance to multiple antibiotics, this microorganism can cause problems in treatment. Therefore, it is necessary to monitor its susceptibility to the antibiotics being used. Making use of epidemiological studies in determining the antibiotic susceptibility profiles during the selection process of empirical treatment methods is a rational policy to pursue for antibiotic use. Empirical treatment is a method planned in accor-

Clinical breakpoint (zone diameter [mm])								
Antibiotic	CLSI and EUCAST disk contents (µg)	CLSI disk contents (µg)	EUCAST disk contents (µg)	CLSI 2014			EUCAST2014	
				S	I	R	S	R
Cefepime	30			>=18	15-17	<=14	>=19	<19
Amikacin	30			>=17	15-16	<=14	>=18	<15
Gentamicin	10			>=15	13-14	<=12	>=15	<15
Ciprofloxacin	5			>=21	16-20	<=15	>=25	<22
Levofloxacin	5			>=17	16-14	<=13	<=20	<17
Imipenem	10			>=19	16-18	<=15	>=20	<17
Meropenem	10			>=19	16-18	<=15	>=24	<18
Ceftazidime		30		>=18	15-17	<=14	-	-
Ceftazidime			10	-	-	-	>=16	<16
Piperacillin/tazobactam		100/10		>=21	15-20	<=14	-	-
Piperacillin/tazobactam			30/6	-	-	-	>=18	<18

Table 1: Clinical breakpoint values for inhibition zone diameters of CLSI 2014 and EUCAST 2014 guidelines for antibiotic susceptibility testing.

Results

Antibiotic susceptibility test results of 105 Pseudomonas isolates in this study were evaluated according to both the CLSI and the EUCAST standards. A comparison of the susceptibility ratios obtained with each standard is shown in Table 2, together with statistical evaluation results.

In the evaluation made in accordance with both standards, a significant difference was detected in the results with ceftazidime (10 µg/disk versus 30 µg/disk) and piperacillin/tazobactam (30/6 µg/disc versus 100/10 µg/disc). There was either no difference among the other antibiotics (gentamicin and levofloxacin) or no significant difference (imipenem, meropenem, ciprofloxacin, amikacin, and cefepime). The resistance rates to ceftazidime and piperacillin/tazobactam in P. aeruginosa increased from 1.9% and0.95% to 4.8% and 15.2%, respectively, comparing the CLSI 2014 standards with EUCAST 2014 standards.

Antibiotic	Susceptible isolates (%)		P value
	CLSI	EUCAST	
Cefepime	102 (97.14)	101(96.19)	0.317
Amikacin	101(96.19)	100(95.23)	0.317
Gentamicin	96(91.42)	96(91.42)	
Ciprofloxacin	96(91.42)	95(90.47)	0.317
Levofloxacin	95(90.47)	95(90.47)	
Imipenem	100 (95.23)	98(93.33)	0.317
Meropenem	98(93.33)	97(92.38)	0.317
Ceftazidime*	102 (97.14)	97 (92.38)	0.025
Piperacillin/tazobactam*	103 (98.09)	88 (83.80)	0.012

Table 2: Number and percentage of susceptible isolates according to CLSI and EUCAST standards.
**Ceftazidime at 10 µg/disk versus 30 µg/disk and piperacillin/tazobactam at 30/6 µg/disk versus 100/10 µg/disk for EUCAST and CLSI, respectively.*

dance with local susceptibility ratios based on epidemiological data, together with the individual characteristics of the patient[10]. Epidemiological data is as valuable as the standards in determining treatment protocols. Therefore, both clinicians and microbiology specialists should be aware of their own local epidemiological data, and should evaluate the standards together with epidemiological data when determining treatment protocols[11].

Antimicrobial resistance profiles may change from one hospital to another, and may even change between clinics in the same hospital. The frequency of antimicrobial resistance may vary depending on hospital structure, patient characteristics, the frequency of invasive procedures, and most importantly, the hospital's antibiotic-use policy. Thus, each hospital should keep a regular record of its own isolates, determine the antibiotic resistance ratio of the antibiotics used in antimicrobial treatment, and regulate its own treatment protocols in accordance with these results[12]. The resistance ratio of *P. aeruginosa* isolates that we identified in our hospital was quite low. It is thought that this low ratio may be a result of regional differences, differences in patient populations, and differences in resistance patterns of bacteria in hospital flora. However, generally speaking, the resistance rates of *P. aeruginosa* infections are constantly increasing. Therefore, each hospital should determine its antibiotic susceptibility ratio and adopt appropriate treatment protocols in accordance with the results.

In Turkey, antibiotics in group A (ceftazidime, piperacillin, and gentamicin or tobramycin) are frequently used in empirical treatment, based upon the results of limited and interpreted antibiogram test results using CLSI data. It was decided by the end of 2015 that EUCAST standards were to be used instead of CLSI for AST in Turkey. It is possible to encounter differences between local and national epidemiological data. In this study, the aim was to compare the susceptibility results of antibiotics with the same and different disc contents in accordance with CLSI and EUCAST standards.

EUCAST was established by bringing together local committees from certain European states. The aim of the EUCAST standards is to interpret and compare susceptibility test results and to produce shared wisdom accordingly. With these standards, in addition to clinical cut-off values, another value is called the epidemiological cut-off (ECOFF). ECOFF determines the minimal inhibitory concentration distribution of wild-type roots of a microorganism, and it also determines the one with the highest MIC level among these wild-type microorganisms showing no resistance phenotypically. Thus, it allows the separation of microorganisms with the phenotypic resistance mechanism from those without it. ECOFF is especially useful in estimating the effectiveness of a relevant antibiotic in surveillance studies conducted without clinical cut-off values. With the Kirby-Bauer disc diffusion method, the CLSI method is different from that of EUCAST in determining the clinical cut-off value. The cut-off values used to interpret susceptibility tests in CLSI are merely clinical values that provide information about whether the causative microorganisms can be activated or deactivated according to the blood concentration reached by the routine dose of the relevant antibiotic[7, 11]. CLSI sometimes requires a buffer zone evaluation and interprets that as "moderately susceptible" in AST results. However, EUCAST does not use an intermediate category. Another difference between EUCAST and CLSI is that certain antibiotic disc contents are lower in EUCAST; for example, 10 μg/disc and 30/6 μg/disc with EUCAST versus 30 μg/disc and 100/10 μg/disc with CLSI for ceftazidime and piperacillin/tazobactam, respectively (8, 9). Ceftazidime and piperacillin/tazobactam are the primary preferred agents for the combined treatment of P.aeruginosa infections, and are also used in empirical treatments.

Ceftazidime is an antipseudomonal third-generation cephalosporin that can be produced by gram-positive and gram-negative bacteria, and is highly resistant to beta-lactamases[13]. Piperacillin/tazobactam is a combination of different proportions of piperacillin, which is an ureidopenicillin, and tazobactam, which is a beta-lactamase inhibitor. Like ceftazidime, piperacillin/tazobactam is a highly resistant antipseudomonal agent to beta-lactamases, which are clinically important[14]. In an examination of the literature, in studies in which CLSI and EUCAST were compared, only the antibiotic results with the same disc contents were examined; this point was emphasized as a limitation of these studies[15, 16].

Hombach et al. compared CLSI 2009-11 standards with EUCAST 2011 standards, and found that resistance rates to cefepime, imipenem, and meropenem in *P. aeruginosa* increased from 12.2%, 25.5%, and 20.6% to 19.8%, 30.4%, and 27.7%, respectively. The resistance rates of gentamicin increased from 18.6% to 25.2% in the CLSI 2009-

11 standards compared with the EUCAST 2011 standards due to the elimination of the intermediate category by EUCAST. The resistance rates of ciprofloxacin and levofloxacin increased, respectively, from 15.9% and 21.3% to 29.7% and 30.8% in CLSI 2009-11 standards compared with EUCAST 2011 standards[16].

Another study showed an increase in the rate of resistant strains between the CLSI 2009 and EUCAST 2011 standards based on ASTs of multidrug resistant gram-negative bacteria[16].

In these studies, all isolates were tested with both disc contents in parallel, and were interpreted accordingly. Our study found that the resistance rates of ceftazidime and piperacillin/tazobactam in *P. aeruginosa* increased from 1.9% and 0.95% to 4.8% and 15.2%, respectively, when we compared the CLSI 2014 standards with EUCAST 2014 standards. This was a statistically significant difference. There was either no difference among other antibiotics (gentamicin and levofloxacin) or no significant difference (imipenem, meropenem, ciprofloxacin, amikacin, and cefepime).

A limitation of the present study was the local origin of the clinical strains, the low number of strains, and the fact that the strains were quite susceptible to antibiotics. Since the disc zone diameters of the isolates were quite wide, it was not possible to detect increased resistance of antibiotics with the same disc contents. There is a need for further studies using antibiotics with different disc contents between the two standards.

In some studies that followed CLSI and EUCAST guidelines, discrepancies related to piperacillin/tazobactam were found[17, 18, 19]. Taking into account the importance of piperacillin/tazobactam in clinical practice, further studies are needed to analyze the impact of the new AST guidelines[15].

Changes made in the standards have important effects on the reporting of AST results, and microbiology specialists should be aware of this. The implications of these effects for the clinicians' prescriptions should also be investigated. This issue has been highlighted in several studies[15, 17, 20, 21].

In one study, cumulative antibiogram results were evaluated in the transition process from CLSI to EUCAST, and it was found that the resistance rates to the meropenem and ciprofloxacin groups were reduced in *P. aeruginosa* types based on AST results. It was stated that these changes were important; however, local epidemiological data should also be taken into account in the evaluation of such changes[11].

In conclusion, CLSI 2014 and EUCAST 2014 guidelines displayed significant differences in disc diffusion susceptibility rates for the contents of different drugs, such as ceftazidime and piperacillin/tazobactam. During implementation of the EUCAST system, laboratories should be aware of the implications of modified AST reports on antibiotic prescription policy. The epidemiological data obtained via the follow-up of AST results for locally used antibiotics, in accordance with the latest standards in use, may be the most important factor to guide empirical treatment. The clinical laboratory adoption of current CLSI or EUCAST interpretive criteria for these antimicrobial agents could influence the decision-making of physicians managing patients with *P. aeruginosa* infections, and could determine the treatment implications. More clinical data are necessary to support the present CLSI and EUCAST criteria for *P. aeruginosa* infections.

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