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Comparision of Antibiotic Susceptibility Testing As Per CLSI and Eucast Guidelines for Gram Negative Bacilli

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Abstract: *Non-availability of CLSI guidelines and presence of some degree of confusion to clinician in regard to intermediate susceptibility, have led to refer to EUCAST guidelines. The aim of this study was to compare the CLSI and EUCAST inhibitory zone diameters by disk diffusion. Total 100 clinical isolates of Enterobacteriaceae were analysed. Inhibition zone diameters were determined for Cefotaxime, Ceftazidime, Piperacillin Tazobactam and Nitrofurantoin. CLSI and EUCAST guidelines were applied .For Escherichia coli, the resistance rate to cefotaxime as per CLSI and EUCAST was 100% for each, for Ceftazidime 100% for each, for Piperacillin Tazobactam 77.4% for each. For Klebsiella ,resistance rate to cefotaxime as per CLSI and EUCAST was 94.3% and 62.8%,for Ceftazidime 100% for each, for Piperacillin Tazobactam 48.6% and 68.6%.For Proteus,resistance rate to cefotaxime as per CLSI and EUCAST to was 100% for each,for Ceftazidime 100% for each, for Piperacillin Tazobactam 71.4 % and 100%.For Pseudomonas,resistance rate to ceftazidime as per CLSI and EUCAST was 59.3% and 66.7%,for Piperacillin Tazobactam 26% and 40.7%.The resistance rate to Nitrofurantoin as per CLSI and EUCAST for Escherichia coli was 10% and 10%, Klebsiella 0% and 28.6%,Pseudomonas 100% and 100%.We conclude from the observations of our study that antibiotic susceptibility testing by EUCAST guidelines is more feasible than CLSI guidelines to both the microbiologist and clinician.*

Keywords: *Antibiotic Susceptibility testing, CLSI, EUCAST, Inhibitory zone diameter, Intermediate Susceptibility, Resistance rate.*

I. Introduction

Aerobic Gram negative bacilli are common agents of infection in hospitalized patients. They are the cause for community and hospital acquired bacteremia, and the majority of cases of hospital acquired pneumonia, both being severe infections associated with a high mortality. The outcome of severe infections caused by aerobic Gram negative bacilli may depend on rapid and appropriate therapy[1,2].

The Clinical and Laboratory Standards Institute and the European Committee on Antimicrobial Susceptibility testing can be considered the major international contribution to antimicrobial susceptibility testing[3].

The main difference between EUCAST and CLSI is the elimination, or at least a reduction of the intermediate AST category. EUCAST has removed the intermediate zone. Consequently, AST reports are simplified by reporting an isolate as either susceptible or resistant. This strategy will change AST reports, mostly by reporting isolates as resistant that were formerly considered intermediate[4].

This study aimed at comparing AST done according to CLSI and EUCAST guidelines for Gram negative bacilli using Cefotaxime, Ceftazidime, Piperacillin/Tazobactam and Nitrofurantoin antibiotic discs. The results of this study will support clinical microbiological laboratories in correct interpretation and antibiotic therapy recommendations to clinicians during the transition phase from the CLSI system to EUCAST system. Close interaction with and information from clinicians is needed to avoid uncertainities in the interpretation of changes in AST reports[4].

II. Methods

Clinical isolates : A total of 100 clinical isolates of Gram negative bacilli were included in the study. The 100 clinical isolates comprised of 73 Enterobacteriaceae (31 Escherichia coli, 35 Klebsiella species, 7 Proteus species) and 27 Pseudomonas species.

Susceptibility testing : Antibiotic susceptibility testing was performed by the disk diffusion method, with Mueller- Hinton agar, according to CLSI and EUCAST methodology[5]. The plates were inoculated with samples of each strain adjusted to a turbidity of 0.5Mc Farland. The discs were applied to the surface of inoculated plates and the plates were incubated for 20 hours at 37°c[4-7]. The inhibition zone diameters were interpreted according to CLSI and EUCAST guidelines. Testing with control strains (Escherichia coli ATCC 25922 and Pseudomonas

aeruginosa ATCC 27853) was also done and the results were as per the recommended CLSI and EUCAST criteria.
The antibiotic disc loads as per CLSI and EUCAST[4] guidelines are listed in TABLE I

Table I : Antibiotic disc loads as per CLSI and EUCAST guidelines

DRUG	CLSI	EUCAST
Cefotaxime	30 µg	10 µg
Ceftazidime	30 µg	10 µg
Piperacillin/ tazobactam	100/10 µg	30/6 µg
Nitrofurantoin	300 µg	100 µg

The Clinical breakpoint values of CLSI 2011 and EUCAST 2011 for AST of Enterobacteriaceae are listed in TABLE II

Table II- Clinical breakpoint values of CLSI 2011 and EUCAST 2011 for AST of Enterobacteriaceae

DRUG	CLSI 2011	EUCAST
Cefotaxime	S ≥26 I 15-22 R≤22	S≥20 R<17
Ceftazidime	S ≥21 I 18-20 R≤17	S≥22 R<19
Piperacillin tazobactam	S≥21 I 18-20 R≤17	S≥20 R≤17
Nitrofurantoin	S≥17 I 15-16 R≤14	S≥11 R<11

S – susceptible I – Intermediate R- Resistant

The Clinical breakpoint values of CLSI 2011 and EUCAST 2011 for AST of Pseudomonas are listed in TABLE III

Table III- Clinical breakpoint values of CLSI 2011 and EUCAST 2011 for AST of Pseudomonas

DRUG	CLSI 2011	EUCAST
Cefotaxime	Inherently resistant	Inherently resistant
Ceftazidime	S≥18 I 15-17 R≤14	S>16 R<16
Piperacillin tazobactam	S≥21 I 15-20 R≤14	S>18 R<18
Nitrofurantoin	S≥17 I 15-16 R≤14	Not mentioned

S – susceptible I – Intermediate R- Resistant

When the inhibitory zone edge is heaped or colonies are growing within the inhibition zone, the particular drug is reported as resistant. Thus, zone edge quality was also taken into account[8].(fig 1,2)

Fig 1 shows inhibitory zones with heaped edges

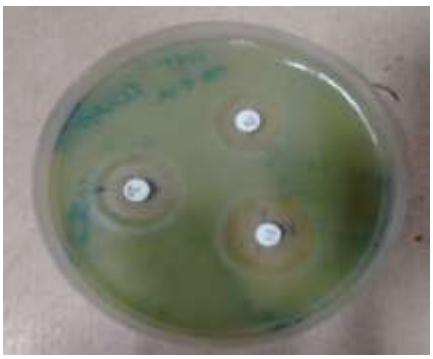

Figure 1

Fig 2 shows colonies within the inhibitory zone

Figure 2

Inhibitory zones as in the above fig 1 and fig 2 indicate resistance.

III. Results

For Escherichia coli, the resistance rate as per CLSI to cefotaxime was 100%, for ceftazidime 100% and for Piperacillin tazabactam 77.4%. By EUCAST to cefotaxime was 100%, for ceftazidime 100% and for Piperacillin tazabactam 77.4% as noted in TABLE IV

Table IV- Assignment of Escherichia coli(%) to antibiotic susceptibility interpretative categories

n =31

DRUG	CLSI (%)			EUCAST (%)		
	S	I	R	S	I	R
Cefotaxime	0	0	100	0	0	100
Ceftazidime	0	0	100	0	0	100
Piperacillin Tazobactam	6.4	16.2	77.4	0	0	100

S – susceptible I – Intermediate R- Resistant

For Klebsiella, resistance rate as per CLSI to cefotaxime was 94.3%, for ceftazidime 100% and for Piperacillin tazobactam 48.6%. By EUCAST to cefotaxime was 62.8%, for ceftazidime 100% and for Piperacillin tazobactam 68.6% as noted in TABLE V

Table V- Assignment of Klebsiella species (%) to antibiotic susceptibility interpretative categories

n=35

DRUG	CLSI (%)			EUCAST (%)		
	S	I	R	S	I	R
Cefotaxime	5.7	0	94.3	25.7	11. 4	62.8
Ceftazidime	0	0	100	0	0	100
Piperacillin Tazobactam	20	31.4	48.6	0	31.4	68.6

S – susceptible I – Intermediate R- Resistant

For Proteus, resistance rate as per CLSI to cefotaxime was 100%, for ceftazidime 100% and for Piperacillin tazabactam 71.4%. By EUCAST to cefotaxime was 100%, for ceftazidime 100% and for Piperacillin tazabactam 100% as noted in TABLE VI

Table VI- Assignment of Proteus species (%) to antibiotic susceptibility interpretative categories

n=7

DRUG	CLSI (%)			EUCAST (%)		
	S	I	R	S	I	R
Cefotaxime	0	0	100	0	0	100
Ceftazidime	0	0	100	0	0	100
Piperacillin Tazobactam	0	28.6	71.4	0	0	100

S – susceptible I – Intermediate R- Resistant

For Pseudomonas,resistance rate as per CLSI to ceftazidime was 59.3% and for Piperacillin tazobactam 26%. By EUCAST to ceftazidime was 66.7% and for Piperacillin tazobactam 40.7% as noted in TABLE VII

Table VII- Assignment of Pseudomonas species (%) to antibiotic susceptibility interpretative categories

n=27

DRUG	CLSI (%)			EUCAST (%)		
	S	I	R	S	I	R
Cefotaxime	Inherently resistant			Inherently resistant		
Ceftazidime	33.3	7.4	59.3	33.3	0	66.7
Piperacillin Tazobactam	33.3	40.7	26	51.9	7.4	40.7

S – susceptible I – Intermediate R- Resistant

The resistance rate to Nitrofurantoin as per CLSI for Escherichia coli was 10%, klebsiella species 0% and Pseudomonas species 100%. By EUCAST, for Escherichia coli was 10%, Klebsiella species 28.6% and Pseudomonas species 100% as noted in TABLE VIII

Table VIII- Assignment of clinical isolates to Nitrofurantoin susceptibility in urine samples n=29

ORGANISM	CLSI (%)			EUCAST (%)		
	S	I	R	S	I	R
Escherichia coli (n=20)	45	45	10	90	0	10
Klebsiella species (n=7)	28.6	71.4	0	71.4	0	28.6
Proteus species (n=0)	–	–	–	–	–	–
Pseudomonas species(n=2)	0	0	100	0	0	100

S – susceptible I – IntermediateR- Resistant

IV. Discussion

AST reports influence prescription policy and antibiotic use[4].Non-availability of CLSI guidelines and presence of some degree of confusion to clinician in regard to intermediate susceptibility, have led to refer to EUCAST guidelines.

Besides the national AST systems (e.g in Germany, France, UK and Sweden), many laboratories, particularly in countries without a rational AST system, have been using CLSI guidelines for many years[4]. Many European laboratories are currently prepared to implement the new EUCAST guidelines for AST.

Limitations of EUCAST:

1. Implementation of EUCAST guidelines will affect antibiotic prescription, in part because of the partial elimination of the intermediate category. Defining isolates as resistant that were formerly considered intermediate will most likely lead clinicians to use other antimicrobial classes[4].

2. The number of useful antimicrobial treatment options for Gram negative bacilli like Enterobacteriaceae and glucose non- fermenting GNB like pseudomonas will probably decrease after implementation of EUCAST guidelines due to higher resistance rates. The choice of drugs available to the clinician is limited[4].

3. More Gram negative bacilli will be reported as multidrug resistant, resulting in higher rates of patients in isolation and concomitantly higher costs. A higher rate of multidrug- resistant Gram negative bacilli will not only result in higher costs for hospitals and hospital hygiene measures, but will also result in more confirmatory testing in the laboratory[4].

Comparision of AST done by CLSI and EUCAST was done by Michael Hombach, Guido V. Bloemberg, Erik C. Bottger et al., by using antibiotics discs with same concentration as per CLSI and EUCAST. Higher resistance rate was reported by following EUCAST guidelines as the intermediate category drugs were reported as resistant. Their study did not include cefotaxime, ceftazidime, piperacillin tazobactam and nitrofurantoin as they differ in antibiotic disc concentration as per CLSI and EUCAST[4]. Though, discs with different concentrations were included in our study, we found that the inhibitory zone diameters were almost similar and the resistance rates were higher as per EUCAST than CLSI. These results correlated with the work done by Michael Hombach, Guido V. Bloemberg, Erik C. Bottger et al.,

CLSI documents are not freely available. There is also some degree of confusion to the clinician[1], as for the drugs reported as intermediate susceptible, the clinician uses higher concentration of drugs. Moreover the success of therapy is not predictable.

Whereas EUCAST documents are freely available on net making them feasible for the microbiologist to implement. Implementation of these guidelines gives a clear picture to the clinician as intermediate category is eliminated and only susceptibility and resistance are reported. Implementation of EUCAST standards for antibiotic susceptibility testing made results in Europe more comparable, incorporating PK-PD studies and clinical data[1].

E. Matuschek, D. F. J. Brown and G. Kahlmeter expressed that EUCAST encourages laboratories with expertise in susceptibility testing to participate in a network of collaborating laboratories interested in contributing to the development and maintainance of the disk diffusion test. With this network, the future of the EUCAST disk diffusion method is secured. Automated susceptibility testing may relieve laboratories of some AST work, but their lack of versatility, the unavailability of some agents and tests for some species, and their long development times, still favour the use of disk diffusion testing for many years to come[5].

V. Conclusion

From the observations of our study, we conclude that antibiotic susceptibility testing by EUCAST guidelines is more feasible than CLSI guidelines to both the microbiologist and the clinician. Advantages though less, outweigh the disadvantages as it helps in specific reporting of sensitivity.

References

[1]. Håkan Hanberger, MD, PhD; José-Angel Garcia-Rodriguez, MD, PhD; Miguel Gobernado, MD, PhD; Herman Goossens, MD, PhD; Lennart E. Nilsson, PhD; Marc J. Struelens, MD, PhD; and the French and Portuguese ICU Study Groups. Antibiotic Susceptibility Among Aerobic Gram-negative Bacilli in Intensive Care Units in 5 European Countries. JAMA. 1999;281(1):67-71. doi:10.1001/jama.281.1.67.

[2]. R. C. B. Slack, Hospital infection, Greenwood Medical Microbiology, 18th edition, 2012, David Greenwood, Mike Barer, Richard Slack, Will Irving, pg no 720-726

[3]. P. Silley. Susceptibility testing methods, resistance and breakpoints: what do these terms really mean? Rev. sci. tech. Off. int. Epiz., 2012, 31 (1), 33-41

[4]. Michael Hombach, Guido V. Bloemberg and Erik C. Bottger. Effects of clinical breakpoint changes in 2010/2011 and EUCAST guidelines 2011 on antibiotic susceptibility test reporting of Gram negative bacilli. Journal of Antimicrobial Chemotherapy 2011.

[5]. E. Matuschek, D. F. J. Brown and G. Kahlmeter. Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories. Clinical Microbiology and Infection, Volume 20 Number 4, April 2014.

[6]. Bailey and Scott, Laboratory methods and strategies for antimicrobial susceptibility testing, Bailey and Scott's diagnostic microbiology, 13th edition, 2007, Andrew Allen, Pg no 174-178.

[7]. R. S. Miles, S. G. B. Amyes, Laboratory control of antimicrobial therapy, Mackie and McCartney practical medical Microbiology, 14th edition, 2012, J. G. Collee, A. G. Fraser, B. P. Marmion, A. Simmons, Pg no 151-166.

[8]. Kristin Hegstad, Christian G. Giske, and Arnfinn Sundsfjord. Performance of the EUCAST Disk Diffusion Method, the CLSI Agar Screen Method, and the Vitek 2 Automated Antimicrobial Susceptibility Testing System for Detection of Clinical Isolates of Enterococci with Low- and Medium-Level VanB-Type Vancomycin Resistance: a Multicenter Study. J clin.microbiol 2014 May;52(5):1582-1589