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Impact of Different EUCAST and CLSI Interpretive Breakpoints on Antimicrobial Susceptibility of *Pseudomonas aeruginosa* – SMART 2007

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Revised Abstract

Objectives: CLSI guidelines are used in many countries; however, in Europe EUCAST breakpoints (BPs) are primarily used. Since EUCAST BPs often differ from CLSI's, we evaluated the impact on reported susceptibility of *P. aeruginosa* of using EUCAST vs. CLSI BPs for selected anti-pseudomonal agents.
Methods: Investigators in 93 hospitals in 33 countries in Europe, North and South America, Asia/Pacific, Middle East, and Africa collected up to 100 consecutive gram-negative isolates from intra-abdominal infections in 2007; 654 of the organisms were *P. aeruginosa*. Minimum inhibitory concentrations (MICs) were determined by broth microdilution following CLSI methods, and results for drugs commonly used to treat *P. aeruginosa* were interpreted using both CLSI and EUCAST BPs.
Results:

Drug	CLSI S/I/R* Breakpoint	EUCAST S/I/R	CLSI %S/I/R	EUCAST %S/I/R
Amikacin	16/32/64	8/16/1932	87/4/9	74/12/14
Cefepime	8/16/1932	8/-/16	75/10/15	75/-/25
Ceftazidime	8/16/1932	8/-/16	74/6/20	74/-/26
Ciprofloxacin	1/2/2004	0.5/1/2	72/4/24	69/3/28
Imipenem	4/8/2016	4/8/2016	73/8/19	73/8/19
Levofloxacin	2/4/2008	1/2/2004	72/4/24	65/6/29
Piperacillin-tazobactam	64/-/128	16/-/32	85/-/15	76/-/24

*S=susceptible, I=intermediate, R=resistant
Conclusions: The tendency of EUCAST BPs to be one doubling dilution lower than CLSI's caused reductions in %S with 4 drugs (amikacin, ciprofloxacin, levofloxacin, and pip-tazo), the other 3 drugs evaluated (cefepime, ceftazidime, and imipenem) had equivalent %S since the S BP was the same in both guidelines.
Absence of an "I" category in EUCAST for 3 drugs (amikacin, cefepime, and pip-tazo) resulted in reported resistance rates 6-10% higher than in countries using CLSI BPs.
The tendency of European *P. aeruginosa* isolates to have lower MICs than is seen in other regions is partially "masked" by lower EUCAST breakpoints.
Using EUCAST guidelines, none of the study drugs achieved %S higher than 76%; even using CLSI only 2 drugs (amikacin and pip-tazo) were above 80%. If 90% or even 80% susceptible is considered to be a minimum indication of a drug's utility for empiric therapy, the list of agents remaining active *in vitro* against *P. aeruginosa* is limited.

Introduction

Laboratories around the world use established guidelines to interpret results from antimicrobic susceptibility testing (AST), whatever the testing methodology used (agar diffusion, agar dilution, broth dilution), and whether using commercially- or in-house-prepared AST products. Clinical and Laboratory Standards Institute (CLSI) guidelines [1,2] are used in most countries outside of the European Union, in which European Committee for Antimicrobial Susceptibility Testing (EUCAST) guidelines [3] are increasingly followed. EUCAST interpretive breakpoints tend to be 1-2 doubling dilutions lower than those of CLSI, which may be due to the fact that EUCAST guidelines for many drugs (especially those that have been marketed for many years already) were developed more recently than many of those appearing in CLSI's guidelines, and therefore may tend to be more reflective of relatively recently identified resistance mechanisms.

Additionally, much more pharmacokinetic/pharmacodynamic (PK/PD) data were available to consider during EUCAST's breakpoint deliberations than were available when many CLSI guidelines were established years ago, further contributing to some of the differences. In fact, when CLSI revisits and alters interpretations for a drug the outcome is almost always lowering of the breakpoints, ultimately aligning more closely with those of EUCAST. The Study for Monitoring Antimicrobial Resistance Trends (SMART) is a global longitudinal surveillance study focused on aerobic gram-negative intra-abdominal infections (IAI). As such, it can be used to help determine regional susceptibility and resistance rates of drugs commonly used to treat IAI. This report evaluates the impact of differing CLSI and EUCAST interpretive breakpoints on reported susceptibility and resistance levels for seven anti-pseudomonal antimicrobics.

Materials & Methods

- All isolates were non-repeat isolates derived from IAIs. Only one isolate per species per patient was accepted into the study. 93 laboratories in 33 countries in Europe, North America, South America, Asia/Pacific, Middle East, and Africa each collected up to 100 consecutive non-selected gram-negative pathogens in 2007. Isolates were identified to the species level and tested for susceptibility at each site. *P. aeruginosa* represented 8.6% of all isolates.
- Minimum inhibitory concentrations (MICs) were determined using MicroScan dehydrated broth microdilution panels manufactured by Siemens Medical Solutions Diagnostics (West Sacramento, California, USA), following CLSI guidelines [1]. All antimicrobial agents were supplied by the panel manufacturer. The following antimicrobial agents with activity against *P. aeruginosa* were included on the panels with their dilution ranges (expressed in mcg/ml): imipenem 0.06-8, cefepime 0.5-32, ceftazidime 0.5-128, ciprofloxacin 0.25-2, amikacin 4-32, levofloxacin 0.5-4, and piperacillin/tazobactam 2/4-64/4.
- MIC results were interpreted following two sets of guidelines: those published by the Clinical and Laboratory Standards Institute (CLSI) [2] and those published by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), www.eucast.org [3].
- Quality control testing (QC) was done by each testing site on each day of testing using the CLSI-recommended QC strains: *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. [2].
- Development of a centralized database of study results was managed by International Health Management Associates, Inc. located in Schaumburg, IL, USA.

References

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2. CLSI, *Performance Standards for Antimicrobial Susceptibility Testing*, *Document M100-S19*. 2009: Clinical and Laboratory Standards Institute (CLSI), 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
3. European Committee on Antimicrobial Susceptibility Testing (EUCAST) website, http://www.eucast.org, 2008.

Acknowledgements

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Results

Tables 1a-1g show the cumulative percentages of 654 *P. aeruginosa* isolates inhibited at each concentration tested in this study. Susceptible, intermediate, and resistant concentrations from CLSI [2] and EUCAST [3] guidelines are highlighted in green, yellow, and red, respectively. There is no EUCAST intermediate category for cefepime or ceftazidime, and none from either standard for pip-tazo. Figure 1 is a bar graph depicting relative percents susceptible and resistant by both CLSI and EUCAST.

Table 1a.

Amikacin	≤4	8	16	32	>32
CLSI	40.7	74	86.5	90.8	100
EUCAST	40.7	74	86.5	90.8	100

Table 1b.

Pip-Tazo	≤2	4	8	16	32	64	>64
CLSI	28	56.7	69.1	76	81	85.3	100
EUCAST	28	56.7	69.1	76	81	85.3	100

Table 1c.

Cefepime	≤0.5	1	2	4	8	16	32	>32
CLSI	3.5	10.7	37.9	58.7	74.9	84.6	90.1	100
EUCAST	3.5	10.7	37.9	58.7	74.9	84.6	90.1	100

Table 1d.

Ceftazidime	≤0.5	1	2	4	8	16	32	64	128	>128
CLSI	3.5	18.8	53.4	67	73.9	80.1	86.4	91.1	93.1	100
EUCAST	3.5	18.8	53.4	67	73.9	80.1	86.4	91.1	93.1	100

Table 1e.

Imipenem	≤0.06	0.12	0.25	0.5	1	2	4	8	>8
CLSI	1.2	2.4	5.8	32.9	62.4	70.2	73.4	80.9	100
EUCAST	1.2	2.4	5.8	32.9	62.4	70.2	73.4	80.9	100

Table 1f.

Ciprofloxacin	≤0.25	0.5	1	2	>2
CLSI	58.6	68.5	71.9	75.8	100
EUCAST	58.6	68.5	71.9	75.8	100

Table 1g.

Levofloxacin	≤0.5	1	2	4	>4
CLSI	52.4	65.3	71.6	75.8	100
EUCAST	52.4	65.3	71.6	75.8	100

Figure 1. Comparison of percents susceptible and resistant of 654 *P. aeruginosa* isolates by CLSI and EUCAST interpretive guidelines.

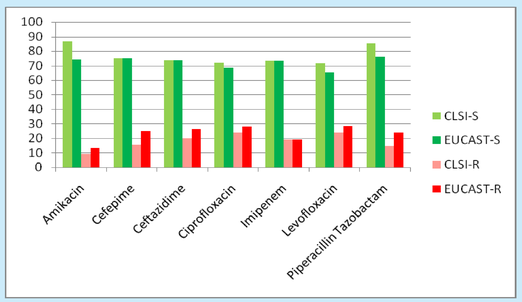

Table 2 compares susceptibility levels of European vs. Rest of World (ROW) isolates (which includes the SMART isolates from North and South America, Asia/Pacific, and Middle East/Africa), using both CLSI and EUCAST breakpoints. When European data were evaluated using CLSI guidelines, three drugs (cefepime, ceftazidime, and pip-tazo) had statistically significant higher percents susceptible than ROW.

Table 2. Comparison of susceptibility of European vs. Rest of World (ROW) *P. aeruginosa* isolates in 2007, using both CLSI and EUCAST breakpoints.

	ROW-CLSI n=431	EU-CLSI n=223	p*	EU-EUCAST n=223
Amikacin	85.2	89.2	0.1833	77.1
Cefepime	72.2	80.3	0.0284	80.3
Ceftazidime	70.8	79.8	0.0145	79.8
Ciprofloxacin	70.1	75.3	0.1693	69.5
Imipenem	72.4	75.3	0.4557	75.3
Levofloxacin	71.9	70.9	0.7844	63.2
Pip-Tazo	82.8	90.1	0.0141	78.5

Conclusions

- The tendency of EUCAST breakpoints to be one doubling dilution lower than CLSI's would, if applied to the global data, cause reductions in percent susceptible and increases in percent resistance. More specifically, amikacin, ciprofloxacin, levofloxacin, and pip-tazo susceptibility would diminish, while cefepime, ceftazidime, and imipenem would remain unchanged since the susceptible breakpoint was the same in both guidelines. The largest decreases in percent susceptible would be seen with amikacin and piperacillin-tazobactam: 13 and 9%, respectively.
- Absence of an "intermediate" category in EUCAST for 3 drugs (amikacin, cefepime, and pip-tazo) would result in reported resistance rates 6-10% higher than with CLSI breakpoints.
- When comparing *P. aeruginosa* susceptibility rates in Europe to those in other regions of the world, it is important to note that application of EUCAST breakpoints usually decreases reported percents susceptible. Using CLSI breakpoints for European data, results from the SMART study indicate that European susceptibility levels for these seven drugs are actually somewhat higher than in other regions, with three (cefepime, ceftazidime, and pip-tazo) being significantly so ($p<0.05$).
- Using EUCAST guidelines, none of the study drugs achieved percent susceptible higher than 76%; even using CLSI only 2 drugs (amikacin and pip-tazo) were above 80%. If 90% or even 80% susceptible is considered to be a minimum indication of a drug's utility for empiric therapy, the list of agents retaining *in vitro* activity against *P. aeruginosa* is limited.