Impact of Different FDA and EU Breakpoints on Bacterial Susceptibility Patterns - Analysis of Data from the T.E.S.T Program

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M. Hackel[1], R. Badal[1], S. Bouchillon[1], B. Johnson[1], J. Johnson[1], D. Hoban[1], M. Dowzicky[2]

[1]International Health Management Associates, Schaumburg, IL, USA
[2]Wyeth Pharmaceuticals, Collegeville, PA, USA

IHMA, Inc.
2122 Palmer Dr.
Schaumburg, IL
60173
Tel: (847) 303-5003
Fax: (847) 303-5601
www.ihmainc.com

REVISED ABSTRACT

Background: The MIC testing methodology recommended by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee for Antimicrobial Susceptibility Testing (EUCAST) is nearly identical, but interpretive MIC breakpoints established by the US FDA, based on CLSI methods, often differ from those set by EUCAST, sometimes leading to significantly different interpretations of the same MIC. It is important to understand the impact of these differences when interpreting susceptibility data reported in the literature. This study evaluated the impact of discrepant S/I/R breakpoints on susceptibility data from the global Tigecycline Evaluation Surveillance Trial (T.E.S.T.). **Methods:** A total of 57,704 pathogens from 320 sites in 42 countries were identified at each site and confirmed at a reference laboratory. MICs were determined at each site utilizing supplied broth microdilution panels and interpreted according to FDA and EUCAST guidelines. **Results:** There were discrepancies for gram-pos and -neg organisms with several drugs, but the most significant (FDA %S >90% S, EUCAST <90%, or vice versa) were seen only with the gram-neg organisms summarized below:

Organism	Tigecycline	Cefepime	Ceftriaxone	Meropenem
Enterobacter spp.	94.6 / 89.2	93.7 / 79.1	-	-
E. coli	-	-	91.5 / 88.8	-
Klebsiella spp.	95.6 / 89.8	91.6 / 83.6	-	-
K. oxytoca	-	-	93.1 / 84.1	-
Serratia spp.	96.8 / 85.6	-	91.3 / 80.9	-
S. pneumoniae	-	-	-	75.9 / 91.7

Conclusion: Differences between FDA vs. EUCAST MIC interpretive breakpoints can lead to significantly different assessments of an antimicrobial's potency vs. various bacterial species. Although the quantitative differences in %S were usually relatively small, there were several drug/bug combinations in this analysis for which use of EUCAST breakpoints caused the %S to fall below 90%. Since >90% susceptibility is often viewed as the minimum for a drug to be considered useful vs. a given species of bacteria, it is essential when evaluating reports of a drug's activity to be aware of which interpretive breakpoints were used in the analysis, and to bear in mind that there can be significant differences when using EUCAST instead of FDA breakpoints.

INTRODUCTION

Different regulatory and/or advisory organizations around the world establish and publish guidelines for antimicrobial susceptibility testing. Although differences in testing methods used to create most of these guidelines have narrowed over the past few years, discrepancies in interpretive criteria (MIC values corresponding to susceptible, intermediate, or resistant) still exist. Such discrepancies can cause the susceptibility of a given strain to some drugs to appear completely different (e.g., resistant by one set of standards but susceptible by another).

Many factors are considered during the deliberations of breakpoint-setting groups, and often there is not a clear-cut or unanimously-accepted breakpoint value-even within a given organization setting the breakpoint. While it is therefore not altogether surprising that sometimes different breakpoints for the same drug are published by different groups of experts working independently of each other, it can nevertheless be problematic for those trying to interpret and compare susceptibility data generated from different parts of the world.

The Tigecycline Evaluation Surveillance Trial (T.E.S.T.) is a global study comparing the in vitro susceptibility of commonly-isolated bacterial pathogens to a variety of antimicrobials. The data generated can be used to help guide therapy by identifying regional differences in susceptibility levels of pathogens to locally-used antimicrobials; however, the interpretation of those data should be done using locally-acceptable guidelines. In light of the fact that the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [1] breakpoints are becoming more widely accepted and applied within Europe, it is advisable when evaluating antibiograms of European isolates to apply EUCAST interpretive guidelines before reaching conclusions about a given drug's in vitro efficacy in Europe. Conversely, countries choosing to follow other guidelines (FDA, CLSI, etc.) should apply those used in their respective regions to the quantitative data available. For example, an antimicrobic that has acceptable activity against extended spectrum beta-lactamase producing *Enterobacteriaceae* using one set of interpretive guidelines may not be perceived as such if evaluated using alternative guidelines. On the other hand, MIC_{90} values (assuming common testing methodology was used to determine the MICs) are more universal and may be used more readily used to compare regional antibiograms.

The Tigecycline Evaluation and Surveillance Trial (TEST) is a longitudinal global antimicrobial susceptibility surveillance study designed to determine and track susceptibility of commonly-isolated bacterial pathogens to several antimicrobics, including the new glycyclcycline, tigecycline. Since different interpretive breakpoints are utilized in Europe and other parts of the world, this analysis was undertaken to determine if the perceived activity of tigecycline and other antimicrobics in the TEST program is altered significantly when using EUCAST breakpoints as opposed to those accepted by the United States Food and Drug Administration (FDA) [2].

MATERIALS & METHODS

- For the T.E.S.T program all isolates were derived from blood, respiratory tract, urine (no more than 25% of all isolates), skin, wound, fluids, and other defined sources. Only one isolate per patient was accepted.
- For this study 57,704 clinical isolates were collected from 2004 to 2006 from 320 sites in 42 countries around the world.
- Minimum inhibitory concentrations (MICs) were determined by the CLSI recommended broth microdilution testing method [3]. Tigecycline was supplied by Wyeth Pharmaceuticals (Collegeville, PA, USA). All other agents were supplied by the panel manufacturer, MicroScan (Dade Behring Inc., Sacramento, CA, USA). The following antimicrobial agents were included on the panels with their dilution ranges (expressed in mcg/ml): amikacin (0.5-64); amoxicillin/clavulanic acid (0.12/0.06-32/16); ampicillin (0.5-32, gram-negative panel, and 0.06-16, gram-positive panel); cefepime (0.5-32); ceftriaxone (0.06-64); imipenem (0.06-16); linezolid (0.5-8); levofloxacin (0.008-8); minocycline (0.5-16); tigecycline (0.008-16); penicillin (0.06-8); piperacillin/tazobactam (0.06/4-128/4) and vancomycin (0.12-32).
- MICs were interpreted following published guidelines established by EUCAST and the United States FDA. If either set of guidelines had no interpretive standard for a given drug/organism combination tested in this study, that combination was excluded from this analysis.
- Isolates were identified to genus and species by the local laboratory. Each site tested the isolates using broth microdilution.
- *Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* were screened for ESBL activity when MIC results for ceftriaxone were >1 mcg/ml using broth microdilution panels. ESBL activity was confirmed using the CLSI (2006) phenotypic confirmatory disk test (Oxoid, Ogdensburg, NY, USA) on Mueller-Hinton agar (Remel Inc., Lenexa, KS, USA) according to CLSI (2006) guidelines. ESBL presence was confirmed by testing the following antibiotic disks: cefotaxime (30-mcg), cefotaxime/clavulanic acid (30/10-mcg), ceftazidime (30-mcg), and ceftazidime/clavulanic acid (30/10-mcg). Antimicrobial disks were manufactured by Oxoid, Inc. (Ogdensburg, NY, USA). Mueller-Hinton agar used in testing was manufactured by Remel, Inc. (Lenexa, KS, USA). An organism was interpreted as containing an ESBL if there was an increase of >5 mm in the inhibition zone of the combination disk when compared to that of the cephalosporin alone.
- Quality control of broth microdilution panels followed manufacturer's and CLSI guidelines using the following ATCC strains: *Enterococcus faecalis* ATCC 29212; *Escherichia coli* ATCC 25922 and ATCC 35218; *K. pneumoniae* ATCC 700603; *Haemophilus influenzae* ATCC 49247 and ATCC 49766; *Staphylococcus aureus* ATCC 29213; *Streptococcus pneumoniae* ATCC 49619; and *Pseudomonas aeruginosa* ATCC 27853. *K. pneumoniae* ATCC 700603 was used for ESBL confirmation by the reference lab.
- The collection and transportation of organisms, confirmation of identification, and construction and management of a centralized database were conducted and coordinated by Laboratories International for Microbiology Studies (LIMS), a subsidiary of International Health Management Associates, Inc. (IHMA, Schaumburg, IL, USA).

REFERENCES

1 European Committee on Antimicrobial Susceptibility Testing (EUCAST) website, http://www.eucast.org, 11 August 2006.

2. *Physicians' Desk Reference, 2006*, 59th Edition, Thompson PDR, Montvale, NJ, USA.

3. Clinical and Laboratory Standards Institute (CLSI), Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard-Seventh Edition, Document M7-A7. 2006: Clinical Laboratory Standards Institute (CLSI), 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

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RESULTS

Table 1. Comparison of susceptible (S), intermediate (I), and resistant (R) percentages using EUCAST and FDA interpretive breakpoints.

		EUCAST			FDA		
Organism (n)	**Drug**	**%S**	**%I**	**%R**	**%S**	**%I**	**%R**
Acinetobacter spp. (4010)	Amikacin	71.6	5.9	22.5	77.5	7.9	21.1
	Imipenem	78	8.6	13.4	84.5	2.2	13.4
	Levofloxacin	50.1	3.2	46.7	53.3	8.7	38
	Meropenem	68.1	12.2	19.7	75.9	4.4	19.7
P. aeruginosa (6259)	Amikacin	90.1	3.9	6	94	2.5	3.5
	Cefepime	76	n/a	24	76	12.7	11.3
	Imipenem	83	7.8	9.2	83	7.8	9.2
	Levofloxacin	55.2	8.6	36.2	63.8	7.1	29.1
	Meropenem	73.8	13.9	12.3	80.4	7.3	12.3
E. coli (7983)	Amikacin	98.1	1	0.9	99.1	0.5	0.4
	Cefepime	90.3	4.5	5.2	94.8	1.1	4
	Ceftriaxone	88.81	0.8	10.4	91.5	1.9	6.6
	Imipenem	99.7	0.2	0.1	99.9	0	0.1
	Levofloxacin	74.1	0.8	25.1	74.9	2.5	22.6
	Meropenem	99.6	0.4	0	99.6	0.4	0
	Tigecycline	99.7	0.3	0	100	0	0
E. coli ESBL+ (431)	Amikacin	86.3	7.2	6.5	93.5	3.5	3
	Cefepime	8.6	25.1	66.4	33.6	9.7	56.6
	Ceftriaxone	4.4	2.1	93.5	13	7.7	79.4
	Imipenem	99.2	0.8	0	99.7	0.3	0
	Levofloxacin	16.9	0.9	82.1	17.9	5.3	76.8
	Meropenem	100	0	0	100	0	0
	Tigecycline	98.4	1.6	0	100	0	0
Klebsiella spp. (7536)	Amikacin	93.9	3.2	2.9	97.1	1.3	1.6
	Cefepime	83.6	8	8.4	91.6	2.2	6.3
	Ceftriaxone	80.5	1.6	17.9	86.1	4.3	9.7
	Imipenem	98.7	1	0.3	99.5	0.2	0.3
	Levofloxacin	85.5	1.9	12.6	87.4	2.3	10.3
	Meropenem	96.1	2	1.9	97	1.1	1.9
	Tigecycline	89.8	5.8	4.4	95.6	3.5	0.9
K. pneumo ESBL+ (781)	Amikacin	63.5	20.1	16.4	83.6	8.5	7.9
	Cefepime	11.3	38.2	50.6	49.4	10.4	40.2
	Ceftriaxone	1.5	3.5	95	18.7	23.4	57.9
	Imipenem	93.8	4.9	1.3	97.5	1.3	1.3
	Levofloxacin	32.9	5.4	61.7	38.3	8.2	53.5
	Meropenem	84.9	6.8	8.2	86.3	5.5	8.2
	Tigecycline	78.2	13.7	8.1	91.9	5.9	2.2
Enterobacter spp. (6980)	Amikacin	96.7	1.6	1.7	98.3	0.8	1
	Cefepime	79.1	14.6	6.3	93.7	2.2	4.1
	Ceftriaxone	65.9	3.4	30.7	76.2	9.9	13.9
	Imipenem	99.2	0.7	0.1	99.8	0.1	0.1
	Levofloxacin	86.7	2.4	10.9	89.1	2.5	8.4
	Meropenem	99.3	0.6	0.1	99.6	0.3	0.1
	Tigecycline	89.2	5.4	5.4	94.6	3.8	1.5
Serratia spp. (2926)	Amikacin	96.3	1.5	2.2	97.8	1.2	1
	Cefepime	90.2	6.6	3.2	96.8	0.8	2.4
	Ceftriaxone	80.9	4	15.1	91.3	4.6	4.1
	Imipenem	98.8	1.1	0.1	99.8	0.1	0.1
	Levofloxacin	90.8	3.9	5.3	94.7	2.4	2.9
	Meropenem	98.6	0.3	1	99	0	1
	Tigecycline	85.6	11.1	3.2	96.8	2.6	0.6
S. aureus (meth-R) (3181)	Levofloxacin	20.8	3.3	75.9	24.1	19.9	56
	Linezolid	100	0	0	100	0	0
	Vancomycin	100	0	0	100	0	0
	Tigecycline	100	0	0	100	0	0
S. aureus (meth-S) (3923)	Levofloxacin	96.8	1.8	1.4	98.6	0.4	1
	Linezolid	100	0	0	100	0	0
	Vancomycin	100	0	0	100	0	0
	Tigecycline	100	0	0	100	0	0
Enterococcus spp. (4642)	Imipenem	73.1	2.8	24.2	73.1	2.8	24.2
	Linezolid	100	0	0	97.6	2.4	0
	Tigecycline	100	0	0	100	0	0
	Vancomycin	83.8	0.4	15.8	83.8	0.4	15.8
Enterococcus spp. (VRE) (697)	Linezolid	100	0	0	97.1	2.9	0
	Tigecycline	100	0	0	100	0	0
	Vancomycin	0	0	100	0	0	100
Streptococcus agalactiae (2791)	Ceftriaxone	99.7	n/a	0.3	100	0	0
	Levofloxacin	98.4	1.3	0.3	99.7	0.3	0
	Linezolid	100	0	0	100	0	0
	Meropenem	100	0	0	100	0	0
	Tigecycline	100	0	0	100	0	0
	Vancomycin	100	0	0	100	0	0
Streptococcus pneumoniae (3903)	Ceftriaxone	88.9	10.1	1	88.9	8.8	2.3
	Imipenem	99.5	n/a	0.5	99.6	0.1	0.2
	Levofloxacin	99.8	n/a	0.2	99.8	0.2	0
	Linezolid	100	0	0	100	0	0
	Meropenem	84.72	4.62	10.72	80.4	0	19.6
	Vancomycin	100	0	0	100	0	0
S. pneumoniae (Pen-I) (1042)	Ceftriaxone	92.7	6.9	0.4	92.7	5.9	1.4
	Imipenem	99	n/a	1	99.4	0.2	0.3
	Levofloxacin	99.7	n/a	0.3	99.7	0.3	0
	Linezolid	100	0	0	100	0	0
	Meropenem	91.7[2,3]	4.92	3.42	75.9	0	24.1
	Vancomycin	100	0	0	100	0	0
S. pneumoniae (Pen-R) (424)	Ceftriaxone	16.3	75.5	8.3	16.3	66.3	17.5
	Imipenem	97.7	n/a	2.3	98	0.6	1.4
	Levofloxacin	99.3	n/a	0.7	99.3	0.7	0
	Linezolid	100	0	0	100	0	0
	Meropenem	5.1	24	70.9	2.5	0	97.5
	Vancomycin	100	n/a	0	100	0	0
Haemophilus influenzae (3570)	Ceftriaxone	97.8	n/a	2.2	99.9	0	0.1
	Imipenem	99.2	n/a	0.8	100	0	0
	Levofloxacin	99.8	n/a	0.2	100	0	0
	Meropenem	100	n/a	0	100	0	0

[1] Yellow highlighting indicates FDA %S > 90%S, EUCAST %S < 90%.
[2] Blue highlighting indicates EUCAST %S > 90%, FDA %S <90%.
[3] EUCAST meningitis breakpoints were used.

CONCLUSIONS

- Although EUCAST interpretive breakpoints tend to be 1-2 doubling dilutions lower than those approved by FDA, the impact of the differences on the percent susceptible is almost always less than 10 points.
- Among the species and antimicrobics evaluated in this study, only 10 times did discordant breakpoints between EUCAST and FDA cause the percent susceptible of one guideline to be below 90% while the other was above 90%. 9/10 times EUCAST yielded lower percent susceptible, and in only 1/10 cases did FDA yield a lower percent susceptible (meropenem vs. penicillin-intermediate *S. pneumoniae*).
- When evaluating the in vitro efficacy of antimicrobics, it is important to be aware of which interpretive standards were used to determine percent susceptible, intermediate, and resistant. This is especially critical when looking at %S, where >90% is usually used as a cut-off for considering a drug to be adequately active against a given organism type.