#P573

Comparison of Ceftriaxone Susceptibility Rates of *Enterobacteriaceae* in the T.E.S.T. Program Using EUCAST vs. 2009 and 2010 CLSI Breakpoints

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Revised Abstract

Objective: EUCAST and CLSI have different breakpoints for many drugs, including cephalosporins, complicating the process of evaluating global data from surveillance studies. In 2010, CLSI lowered susceptibility breakpoints for several cephalosporins and aztreonam vs. *Enterobacteriaceae* by as much as 2 doubling dilutions. The TEST program has been monitoring susceptibility levels of tigecycline and other drugs since 2004. This report evaluates the impact of the CLSI breakpoint change on reported susceptibility of *Enterobacteriaceae* to ceftriaxone (Cax), both in Europe and globally. **Methods:** 74,335 isolates of *Enterobacteriaceae* were tested from 2004-2009 using broth microdilution following CLSI guidelines. Susceptibility to Cax was compared using 2009 and 2010 CLSI (≤8mg/L and ≤1mg/L, respectively) and EUCAST (≤1mg/L) breakpoints.

Isolate Source	BP Used	Enterob.	% Susceptible Ec ESBL+	Ec ESBL-	Kp ESBL+	Kp ESBL-	Ko ESBL+	Ko ESBL-
Europe	EUCAST/CLSI 2010	71	1	92	1	88	2	82
	CLSI 2009	78	8	95	10	92	16	90
Rest of World	EUCAST/CLSI 2010	75	1	91	2	88	5	87
	CLSI 2009	81	8	94	13	92	38	94

ESBL=extended spectrum beta-lactamase
Ec=*E. coli*; Kp=*K. pneumoniae*; Ko=*K. oxytoca*.

Conclusions: The convergence of the EUCAST and CLSI Cax breakpoints in 2010 has eliminated discrepancies in susceptibility levels due to different breakpoints being used in Europe and the rest of the world. Analyses using CLSI will see some large (and appropriate) declines in % susceptible values in some organism types (e.g., ESBL+ *Klebsiella oxytoca*). The new CLSI breakpoint does a good job of classifying most ESBL+ isolates as non-susceptible to Cax, as does the EUCAST breakpoint.

Introduction

The Clinical and Laboratory Standards Institute (CLSI) recently concluded several years of discussion by publishing lower susceptibility breakpoints of *Enterobacteriaceae* for most of the third-generation cephalosporins [1]. Until this change was implemented in January 2010, the cephalosporin interpretive breakpoints specified by the CLSI and the European Committee for Antimicrobial Susceptibility Testing (EUCAST) differed by as much as three doubling dilutions. For example, the previous CLSI susceptibility breakpoint for ceftriaxone for *Enterobacteriaceae* was 8 mg/L, while that of EUCAST was 1 mg/L. The CLSI breakpoint of 8mg/L was lowered to 1mg/L in January 2010, matching that of EUCAST. The change was made to (a) account for pharmacokinetic/pharmacodynamic (PK/PD) data suggesting that the old breakpoint of 8 was likely to lead to treatment failures, and (b) better separate extended spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* from non-ESBL producers.

This report evaluates the susceptibility rates to ceftriaxone of over 74,000 isolates of *Enterobacteriaceae* collected and tested from 2004 through 2009 in the Tigecycline Evaluation and Surveillance Trial (TEST), comparing the 2009 CLSI interpretive criteria to the new 2010 CLSI guidelines (which now are identical to those of EUCAST).

Materials & Methods

- All isolates were derived from blood, respiratory tract, urine (no more than 25% of all isolates), skin, wound, body fluids, and other defined sources. Only one isolate per patient was accepted into the study. There were 74,335 clinical gram-negative *Enterobacteriaceae* collected and tested between 2004 and 2009 from investigative sites in 59 countries in the North America, South America, Europe, Middle East, Africa, and Asia/Pacific regions. Isolates were identified to the species level and tested at each site by the participating laboratory.
- Organism collection, transport, confirmation of organism identification, and development and management of a centralized database, were coordinated by Laboratories International for Microbiology Studies (LIMS), a division of International Health Management Associates, Inc. located in Schaumburg, IL, USA.
- All organisms were deemed clinically significant by local participant criteria. Isolate inclusion was independent of medical history, antimicrobial use, age, or gender. All sites identified each study isolate utilizing local laboratory criteria.
- Minimum inhibitory concentrations (MICs) were determined following the CLSI recommended broth microdilution testing method [2], and interpreted following CLSI 2009 [3], CLSI 2010 [1], and EUCAST [4] guidelines.
- *Escherichia coli*, *Klebsiella pneumoniae,* and *Klebsiella oxytoca* were screened for ESBL activity when MIC results for ceftriaxone were >1 mg/L using the broth microdilution panels. ESBL activity was confirmed using the CLSI phenotypic confirmatory disk test (Oxoid, Ogdensburg, NY, USA) on Mueller-Hinton agar (Remel Inc., Lenexa, KS, USA) according to CLSI guidelines [1]. ESBL presence was confirmed by testing the following antibiotic disks: cefotaxime (30-µg), cefotaxime/clavulanic acid (30/10-µg), ceftazidime (30-µg), and ceftazidime/clavulanic acid (30/10-µg). 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 controls (QC) were performed by each testing site on each day of testing using the following control strains: *E. coli* ATCC 25922; *E. coli* ATCC 35218; and *Pseudomonas aeruginosa* ATCC 27853. Results were included in the analysis only when corresponding QC isolates tested within the acceptable range according to CLSI guidelines [1].

References

1. Clinical and Laboratory Standards Institute, 2010. *Performance Standards for Antimicrobial Susceptibility Testing; Fourteenth Informational Supplement*. CLSI document M100-S20. Clinical and Laboratory Standards Institute (CLSI), Wayne, PA 19087-1898 USA.
2. Clinical and Laboratory Standards Institute, 2008. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Seventh Edition, in Document M7-A7. Clinical and Laboratory Standards Institute (CLSI), Wayne, PA 19087-1898 USA.
3. Clinical and Laboratory Standards Institute, 2009. *Performance Standards for Antimicrobial Susceptibility Testing; Fourteenth Informational Supplement*. CLSI document M100-S19. Clinical and Laboratory Standards Institute (CLSI), Wayne, PA 19087-1898 USA.
4. European Committee on Antimicrobial Susceptibility Testing (EUCAST) website, http://www.eucast.org, 2009-12-22 (version 1.0).

Acknowledgements

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Results

Figure 1. Susceptibility of all *Enterobacteriaceae* and *E. coli, K. pneumoniae,* and *K. oxytoca* ESBL phenotypes to ceftriaxone using CLSI 2009 and CLSI 2010/EUCAST interpretive criteria.

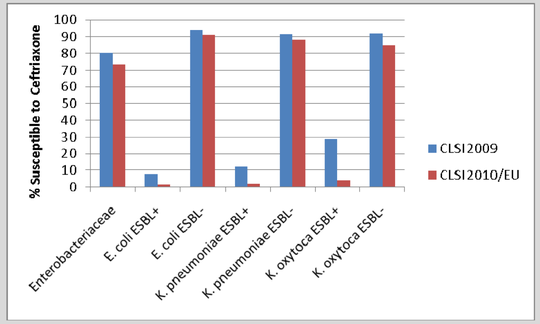


Figure 2. Frequency distribution of ceftriaxone MICs of ESBL+ and ESBL- *Enterobacteriaceae* (*E. coli*, *K. pneumoniae*, and *K. oxytoca*).

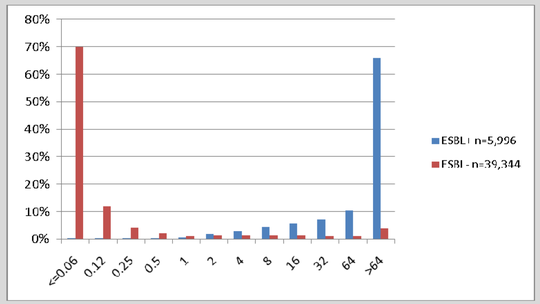


Figure 3. Frequency distribution of ceftriaxone MICs of ESBL+ and ESBL- *E. coli*.

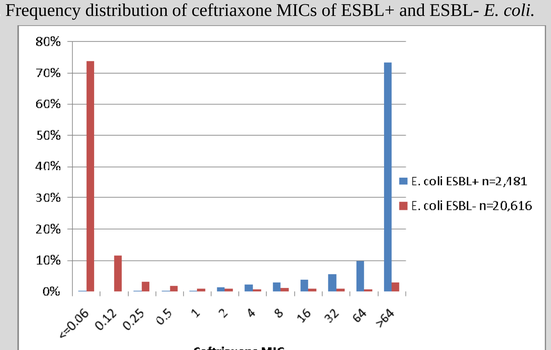


Figure 4. Frequency distribution of ceftriaxone MICs of ESBL+ and ESBL- *K. pneumoniae.*

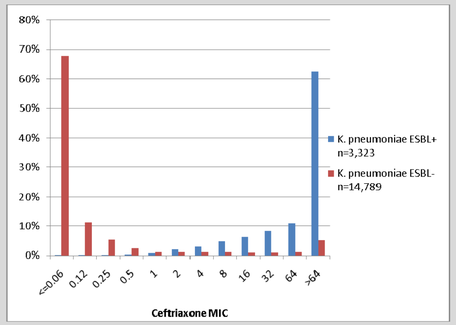
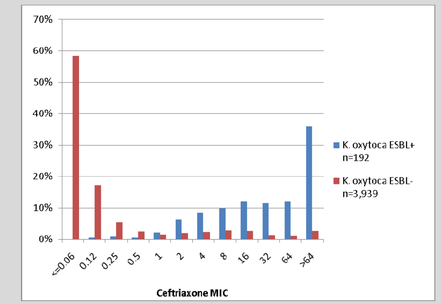


Figure 5. Frequency distribution of ceftriaxone MICs of ESBL+ and ESBL- *K. oxytoca.*



Conclusions

- The convergence of the EUCAST and CLSI ceftriaxone breakpoints in 2010 has eliminated artifactual discrepancies in susceptibility levels due to different breakpoints being used in Europe and the rest of the world.
- Susceptibility analyses using CLSI will see some large (and appropriate) declines in percent susceptible values in some organism types (e.g., ESBL+ *Klebsiella oxytoca*).
- The ceftriaxone susceptibility breakpoint of ≤1 mg/L used by both EUCAST and CLSI (as of January 2010) does a good job of classifying most ESBL+ isolates as non-susceptible to ceftriaxone.