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Validity of CLSI Disk Diffusion Zone Correlates for *Enterobacteriaceae* versus Older Cephalosporins

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Revised Abstract

Background: CLSI disk diffusion correlates for older cephalosporins and *Enterobacteriaceae* were established many years ago. As mechanisms of resistance have evolved, it has become necessary to re-evaluate those correlates to determine if they remain valid with contemporary bacterial isolates. **Methods**: 205 *Enterobacteriaceae* were selected from year 2008 isolates to represent relevant wild-type and susceptible or resistant subsets. Broth microdilution and disk diffusion susceptibility testing were done with cephalothin (CF), cefazolin (CFZ), cefaclor (CCL), cefpodoxime (CPD), cefprozil (CPZ), cefoxitin (CFX), cefuroxime (CRM), loracarbef (LOR), and cefdinir (CDN). All tests used CLSI M02-A10 or M07-A8 (2009) methods and results were evaluated for interpretive errors using M100-S19 criteria. **Results**: Minor, major (false resistance), and very major (false susceptibility) error rates of disk tests compared to broth microdilution are summarized below:

Drug	Minor	Major	Very Major
CF	19.5	0	0
CFZ	5.4	0	0
CCL	1.5	0	0
CPD	2.9	0	0
CPZ	4.4	0	0.5
CFX	4.4	0	0
CRM	25.4	0	0
LOR	5.9	0	0.5
CDN	3.9	0	1

Conclusions: Disk diffusion correlates for contemporary *Enterobacteriaceae* appear to be accurate for most of the cephalosporins studied, except for CF and CRM, which had minor error rates of at least 20%. Further study of these two drugs including testing concentrations of up to 64 µg/ml (2 doubling dilutions higher than the Intermediate category of 16 µg/ml) would be necessary to determine if the observed error rates fall within the CLSI allowed rate of 40% for MICs within one doubling dilution of the Intermediate category.

Introduction

The Clinical and Laboratory Standards Institute (CLSI) has developed standardized reference methods for antimicrobial susceptibility testing, including agar dilution, broth microdilution, and disk diffusion (also known as agar diffusion). The interpretation of disk diffusion zone diameters is based on their correlation to broth microdilution minimum inhibitory concentrations (MICs). For many antimicrobial agents, the MIC-zone diameter correlations were established many years ago, prior to the known existence of many of today's mechanisms of resistance. This is particularly true for many of the first- and second-generation oral cephalosporins versus *Enterobacteriaceae*.

During recent deliberations regarding revision of interpretive breakpoints for third-generation cephalosporins, the CLSI Subcommittee for Antimicrobial Susceptibility Testing decided that it would be prudent to revisit the MIC-zone diameter correlation of many of the early-generation oral cephalosporins versus *Enterobacteriaceae*. This study was undertaken to develop at least a preliminary idea of whether or not the breakpoints established many years ago were still acceptably reliable at identifying resistance among a contemporary collection of bacterial isolates.

Materials & Methods

- Bacterial isolates: 205 *Enterobacteriaceae* isolates collected from clinical specimens in 2008 were selected to represent common resistance phenotypes. Organisms included 96 *Escherichia coli* (54 ampicillin-susceptible, 37 ampicillin-resistant, 5 extended-spectrum-beta-lactamase producers), 64 *Klebsiella pneumoniae* (59 wild-type, 5 extended-spectrum-beta-lactamase producers), 33 *Proteus mirabilis* (30 wild type, 3 ampicillin-resistant), 4 *Enterobacter aerogenes* (all wild type), 4 *Enterobacter cloacae* (all wild type), and 4 *Serratia marcescens* (all wild type).
- Broth microdilution MIC panels were manufactured and tested following CLSI guidelines [1]. Drugs included on the panels were cephalothin, cefazolin, cefaclor, loracarbef, cefprozil, cefuroxime, cefoxitin, cefdinir, and cefpodoxime. All drugs were tested at concentrations ranging from 16-0.12 µg/ml in cation-adjusted Mueller-Hinton broth.
- Disk diffusion tests were done following CLSI guidelines [2], using 30 µg disks for all drugs except cefpodoxime (10 µg) and cefdinir (5 µg). Disk tests were done on 150mm Mueller-Hinton agar plates manufactured by Becton-Dickinson (Franklin Lakes, NJ).
- CLSI quality control criteria were applied for all tests, using *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus* aureus ATCC 29213 [3].
- Error rates were calculated following CLSI published guidelines [4].

References

1. CLSI, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Eighth Edition, in Document M07-A8. 2009: Clinical and Laboratory Standards Institute (CLSI), 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
2. CLSI, Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Tenth Edition, in Document M02-A10. 2009: Clinical and Laboratory Standards Institute (CLSI), 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
3. CLSI, Performance Standards for Antimicrobial Susceptibility Testing, in Document M100-S19. 2009: Clinical and Laboratory Standards Institute (CLSI), 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
4. CLSI, Development of *In Vitro* Susceptibility Testing Criteria and Quality Control Parameters; Approved Guideline—Third Edition, in Document M23-A3. 2008: Clinical and Laboratory Standards Institute (CLSI), 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.

Results

Figures 1-9 show the scatterplots for the 9 cephalosporins included in this study. Current CLSI Susceptible/Intermediate/Resistant (S/I/R) breakpoints for MICs and zone diameters are represented by the dark horizontal and vertical lines, respectively; minor errors are yellow-highlighted and very major errors are red-highlighted, where applicable. There were no major errors observed.

Figure 1. Cephalothin MIC-zone diameter scatterplot.

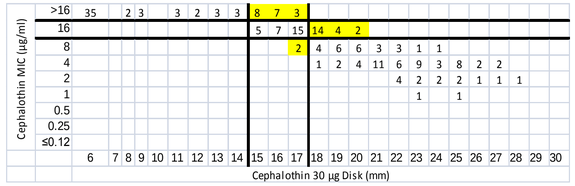

Figure 2. Cefazolin MIC-zone diameter scatterplot.

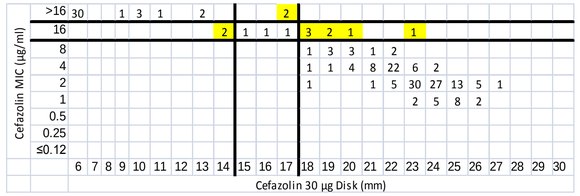

Figure 3. Cefaclor MIC-zone diameter scatterplot.

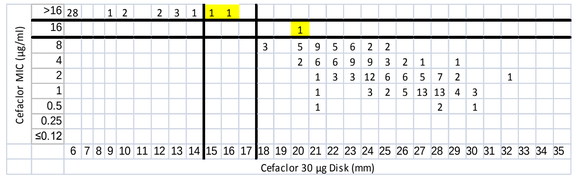

Figure 4. Cefpodoxime MIC-zone diameter scatterplot.

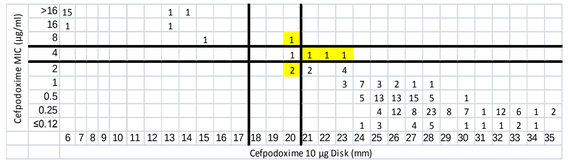

Figure 5. Cefprozil MIC-zone diameter scatterplot.

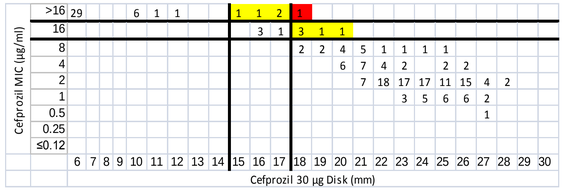

Figure 6. Cefoxitin MIC-zone diameter scatterplot.

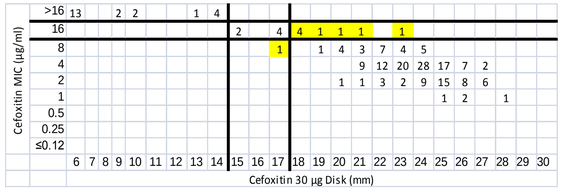

Figure 7. Cefuroxime MIC-zone diameter scatterplot.

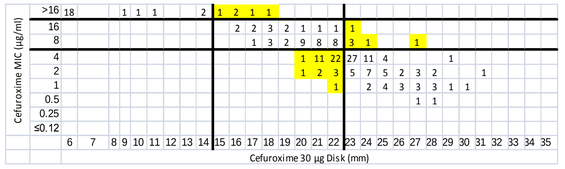

Figure 8. Loracarbef MIC-zone diameter scatterplot.

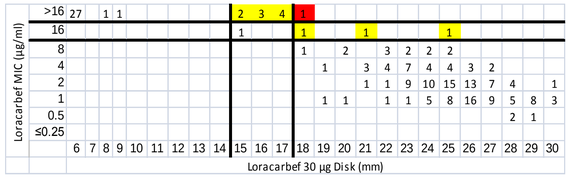

Figure 9. Cefdinir MIC-zone diameter scatterplot.

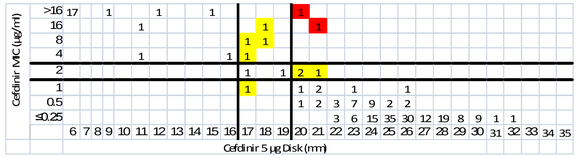

Table 1. Error rates for MIC results versus disk diffusion zone diameters.

Drug	% Error			% Categorical Agreement
	Very Major	Major	Minor	
Cephalothin	0.0	0.0	19.5[a]	80.5
Cefazolin	0.0	0.0	5.4	94.6
Cefaclor	0.0	0.0	1.5	98.5
Cefpodoxime	0.0	0.0	2.9	97.1
Cefprozil	0.5	0.0	4.4	95.1
Cefoxitin	0.0	0.0	4.4	95.6
Cefuroxime (oral)	0.0	0.0	25.4	74.6
Loracarbef	0.5	0.0	5.9	93.6
Cefdinir	1.0	0.0	3.9	95.1

[a]Underlined values are potentially outside limits specified by CLSI as representing acceptable performance [4].

Conclusions

- Disk diffusion correlates for contemporary *Enterobacteriaceae* appear to be accurate for most of the cephalosporins studied, except for cephalothin and cefuroxime axetil, which had minor error rates of 19.5 and 25.4%, respectively.
- Further study of cephalothin and cefuroxime axetil including testing concentrations of up to 64 µg/ml (2 doubling dilutions higher than the Intermediate concentration of 16 µg/ml) would be necessary to determine if the observed error rates fall within the CLSI allowed rate of 40% for MICs within one doubling dilution of the Intermediate category.