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Re-Evaluation of Enterobacteriaceae Regression Scattergrams using an ESBL-Enriched Population Tested Against Nine ß-Lactams

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AMENDED ABSTRACT

Background: Many ß-lactams are available for therapy of infections caused by Enterobacteriaceae (EBT), but evolving ESBLs require accurate detection and susceptibility tests (ST). Current ß-lactam breakpoints (BP) are generally satisfactory, but need qualifying reports to physicians and confirmatory tests for screen-positive ESBL strains. As part of a NCCLS working group, recent EBTs with a high proportion of ESBL-producing strains were used to re-evaluate BPs by disk diffusion (DD) and broth microdilution methods.
Methods: 350 recent (2003) clinical isolates from bloodstream infections were studied: *E. coli* (74), *Klebsiella* spp. (79), *Enterobacter* spp. (43), *Citrobacter* spp. (29), *P. mirabilis* (30), *Serratia* spp. (32); and 11 other species (63); 20% were ESBL-producers. Isolates were tested against ceftazidime (TZ), ceftriaxone (TX), cefotaxime (CT), ceftizoxime (CZ), cefotetan (CN), cefoxitin (FX), cefuroxime (XM), cefepime (PM) and aztreonam (AT) using reference MIC ST and Etests. Analysis compared the accuracy for current and proposed (generally lower) MIC BP based on PK/PD considerations and Monte Carlo simulations.
Results: Regression plot analyses (current BP) showed (r) values ranging from 0.87 (CN) to 0.97 (AT, TZ, CT) and errors of (Mi=minor %, Ma=major %, Vm=very major %): AT (Mi=4.6, Ma=0.3); TZ (Mi=2.0), CT (Mi=9.4); TX (Mi=6.0, Ma=0.3); CZ (Mi=8.3), CN (Mi=0.9, Ma=0.3, Vm=1.1); FX (Mi=8.9); XM (Mi=6.3, Vm=0.9); and PM (Mi=4.0); all acceptable. BPs adjusted to lower MICs to accurately detect ESBLs and predict clinical outcomes produces satisfactory DD zone correlates of: FX and XM due to no BP change (4.3 - 6.0% error); AT, TZ and PM at ≤ 4 µg/ml (0.0 - 4.3% error); CN and CZ at ≤ 2 µg/ml (6.6 - 9.4% error); and CT and TX at ≤ 1 µg/ml (1.1 - 1.4% error). No intermediate category was assigned to FX and XM increasing serious intermethod errors, but absolute agreement remained high at 90.6 - 93.4%.
Conclusions: Current NCCLS EBT BPs remain acceptable based on error rate analysis for contemporary isolates. Lower PK/PD-directed BPs would eliminate the routine need for separate ESBL ST and DD zone correlates can be selected to maximize intermethod accuracy pending final approval by the NCCLS.

INTRODUCTION

Cephalosporins and monobactam form the backbone of Gram-negative antimicrobial therapy, specifically for treatment of infections caused by the Enterobacteriaceae. However, recent emergence and evolution of extended-spectrum ß-lactamase (ESBL) enzymes compromise therapy by these agents. Isolates with susceptible or intermediate MICs to one or more cephalosporins may harbor ESBLs and lead to treatment failure in clinical practice. Although higher, more prolonged serum levels are required for efficacy against organisms with higher MICs, the presence of an ESBL did not necessarily affect the target PK-PD parameters (i.e., percent of time the drug was above the MIC (% T > MIC)) even at higher challenge inocula (Craig et. Al, 2003). These data suggest that decisions regarding treatment and susceptibility should focus on the absolute MIC value, and not on the specific mechanism, type of enzyme or inoculum.

The NCCLS has set guidelines for screening of ESBL-producers when MICs to at least one of the index drugs (ceftazidime or ceftriaxone or aztreonam) have MIC at ≥ 2 µg/ml. Such isolates require additional tests to confirm inhibition by clavulanic acid. According to a survey, many clinical laboratories in the US do not routinely perform ESBL tests. This lack of information can lead to ineffective therapy or the withholding of usable cephalosporins because of possibility (but non-confirmed) of ESBLs. On the other hand, ESBLs may go undetected because the clavulanic acid inhibition effect could be masked by other inhibitor- resistant ß-lactamases such as Class C enzymes.

Consequently, there is a need for re-evaluation of susceptibility breakpoints for MIC and corresponding disk zone diameters to better predict presence of ESBLs while minimizing the need (epidemiology) for separate screening tests. As part of the deliberations of the NCCLS Working Group, we evaluated cephalosporin and monobactam breakpoints for Enterobacteriaceae by MIC and disk diffusion methods with a collection of ESBL-enriched Enterobacteriaceae clinical isolates. Intermethod correlations were also studied by regression and error-rate bounded analysis.

MATERIALS AND METHODS

Bacterial isolates. A total of 350 recent clinical isolates of Enterobacteriaceae (2003) from bloodstream infections were tested which included *E. coli* (74), *K. pneumoniae* (79), *Enterobacter* spp. (43), *Citrobacter* spp. (29), *P. mirabilis* (30), *Serratia* spp. (32) and 11 other species. Twenty percent of these isolates (n= 70) were ESBL producers, belonging to following species: *E. coli* (22), *Klebsiella* spp. (44), *P. mirabilis* (one), indole positive *Proteae* (two) and *Serratia* spp. (one).

Reagents and susceptibility testing. Standardized broth microdilution susceptibility tests were performed. ß-lactam disks (30-µg) were purchased from Remel (Lenexa, Kansas, USA). Disk diffusion and Etest assays (AB BIODISK, Solna, Sweden) were performed simultaneously on Mueller-Hinton agar plates (Remel) as described by the NCCLS [2003a and b] and the manufacturer. Isolates meeting the MIC criteria for possible ESBL-producers were confirmed by ceftazidime ± clavulanic acid (CLAV), ceftriaxone ± CLAV and cefepime ± CLAV Etest and interpreted as ESBL producers per NCCLS guidelines (M7-A6). The following ATCC control strains were included in the testing for quality assurance: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213 and 25923.

Data analysis. Regression curves were obtained by plotting number of isolates at a given MIC versus their corresponding disk diffusion inhibitory zone diameter. Error rates were calculated based on the current and proposed (lower) breakpoint criteria [NCCLS, M100-S14] using all tests (350) as the denominator for percentage calculations.

RESULTS

- Table 1 illustrates in vitro activity of cephalosporins and aztreonam against species of Enterobacteriaceae. The ESBL-producing population of *E. coli*, *K. pneumoniae*, *P. mirabilis* and indole-positive *Proteae* are reflected as lowered percent of susceptible isolates to ESBL index compounds (aztreonam, cefotaxime, ceftriaxone, ceftazidime).
- Regression plot analyses showed correlation coefficients ranging from 0.87 - 0.97. Most drugs showed linear regression curves with excellent intermethod correlation (Figures 1 - 9).
- Considering the current NCCLS (2004) breakpoints, the error rates for each drug were calculated, which are presented in Table 2. All the error rates remain within acceptable limits.
- Only cefotetan and cefuroxime showed 0.9 and 1.1% very major (false-susceptible) intermethod error, respectively. Only 0.3% major (false-resistant) errors were recorded for aztreonam, ceftriaxone and cefotetan, which accounts for only one isolate among the 350 tested strains. Minor error rates varied from 0.9% for cefotetan to 9.4% for cefotaxime.
- Proposed/modified breakpoints for all drugs continue to have acceptable levels of intermethod error (Table 3; broken lines in figures).
- Adjustments of the breakpoints requires the use of several parameters including PK/PD, MIC population distributions (cefoxitin and cefuroxime) and clinical outcomes data, where available. Some ß-lactams appear not to require modification at this time, but an intermediate category would not be justified by target attainment results (Figures 5 and 9).
- Suggested disk diffusion criteria were also consistently larger zones to detect ESBLs and better predict clinical therapeutic success.
- These changes produced relatively minor changes in the error rates of the compounds. These error rates are shown in Table 3. Increases in the very major error rates for only cefoxitin (0.0 to 4.3%), cefuroxime (0.9 to 2.9%) and cefotetan (1.1 to 1.4%) were observed.
- ESBL index (screening) drugs and all but one other drug showed a decline in error rates at lower breakpoints (except cefotetan).

Table 2. Error rates of Enterobacteriaceae based on current NCCLS criteria [2004].

Antimicrobial agent	Method	Current BP S	Current BP I	Current BP R	Error rates Very major	Error rates Major	Error rates Minor	Intermethod agreement
Aztreonam	MIC	≤8	16	≥32	0.0	0.3	4.6	95.1
	Disk	≥22	16-21	≤15				
Cefepime	MIC	≤8	16	≥32	0.0	0.0	4.0	96.0
	Disk	≥18	15-17	≤14				
Cefotaxime	MIC	≤8	16-32	≥64	0.0	0.0	9.4	90.6
	Disk	≥23	15-22	≤14				
Cefotetan	MIC	≤16	32	≥64	1.1	0.3	0.9	97.7
	Disk	≥16	13-15	≤12				
Cefoxitin	MIC	≤8	16	≥32	0.0	0.0	8.9	91.1
	Disk	≥18	15-17	≤14				
Ceftazidime	MIC	≤8	16	≥32	0.0	0.0	2.0	98.0
	Disk	≥18	15-17	≤14				
Ceftriaxone	MIC	≤8	16-32	≥64	0.0	0.3	6.0	93.7
	Disk	≥21	14-20	≤13				
Ceftizoxime	MIC	≤8	16	≥32	0.0	0.0	8.3	91.7
	Disk	≥20	15-19	≤14				
Cefuroxime	MIC	≤8	16	≥32	0.9	0.0	6.0	93.1
	Disk	≥18	15-17	≤14				

Table 1. Susceptibility profiles of Enterobacteriaceae[a] (n=350) species tested in the experiment.

% susceptible of antimicrobial tested (MIC range µg/ml):

Organism (no. tested)	Aztreonam	Cefepime	Cefotaxime	Cefotetan	Cefoxitin	Ceftazidime	Ceftriaxone	Ceftizoxime	Cefuroxime
Citrobacter spp. (29)	93.1 (0.03-128)	100.0 (≤0.016-8)	89.7 (0.06->256)	89.7 (0.06->256)	41.4 (2->256)	89.7 (0.12->256)	89.7 (0.06->256)	89.7 (0.03->256)	55.2 (2->256)
Enterobacter spp. (43)	79.1 (0.03->256)	97.7 (≤0.016-16)	76.7 (0.03->256)	55.8 (0.12->256)	9.3 (2->256)	79.1 (0.12->256)	76.7 (0.06->256)	79.1 (≤0.016->256)	27.9 (2->256)
E. coli (74; 22 ESBLs)	81.1 (≤0.016->256)	86.5 (≤0.016-64)	78.4 (≤0.016->256)	97.3 (0.06-64)	77.0 (1->256)	82.4 (0.06->256)	75.7 (≤0.016->256	90.5 (≤0.016-32)	48.6 (2->256)
Klebsiella spp. (79; 44 ESBLs)	53.2 (≤0.016->256)	77.2 (0.03-128)	67.1 (≤0.016->256)	98.7 (0.06-128)	78.5 (1->256)	51.9 (0.06->256)	58.2 (≤0.016->256)	92.4 (≤0.016-256)	38.0 (≤0.016->256)
Proteus mirabilis (30; 1 ESBL)	100.0 (≤0.016-2)	96.7 (0.06->256)	90.0 (≤0.016->256)	100.0 (0.12-4)	93.3 (1-64)	93.3 (≤0.016-32)	93.3 (≤0.016->256)	93.3 (≤0.016-64)	90.0 (0.5->256)
Indole positive *Proteae* (24; 2 ESBLs)	100.0 (≤0.016-2)	95.8 (0.03->256)	83.3 (≤0.016->256)	100.0 (0.12-16)	58.3 (2-128)	95.8 (0.06-32)	87.5 (≤0.016->256)	91.7 (≤0.016-64)	33.3 (0.12->256)
Salmonella spp. (20)	100.0 (≤0.016-8)	100.0 (0.03-0.5)	95.0 (≤0.016-16)	100.0 (0.06-8)	90.0 (1-128)	95.0 (0.06->16)	95.0 (0.03-32)	95.0 (≤0.016-32)	55.0 (1-64)
Serratia spp. (32; 1 ESBL)	90.6 (≤0.016->256)	100.0 (0.03-4)	87.5 (0.06-32)	96.9 (0.125-32)	6.3 (8->256)	90.6 (0.03-64)	87.5 (≤0.016-32)	96.9 (0.03-16)	0.0 (8->256)
Shigella spp. (15)	100.0 (0.03-0.25)	100.0 (0.03-0.5)	100.0 (0.03-0.12)	100.0 (0.12-2)	100.0 (2-8)	100.0 (0.12-0.5)	100.0 (0.03-0.12)	100.0 (0.03-0.12)	86.7 (2-8)

a. Only species with > 10 isolates are listed.

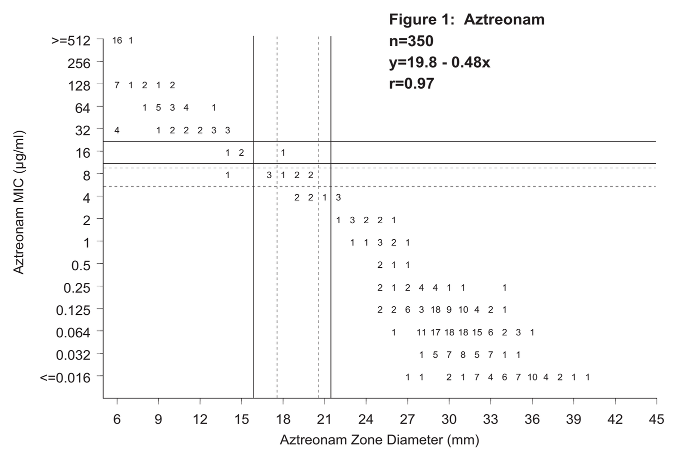
Figure 1: Aztreonam n=350 y=19.8 - 0.48x r=0.97

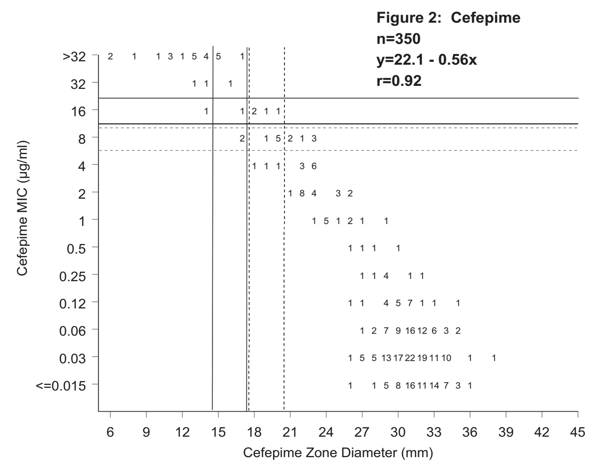
Figure 2: Cefepime n=350 y=22.1 - 0.56x r=0.92

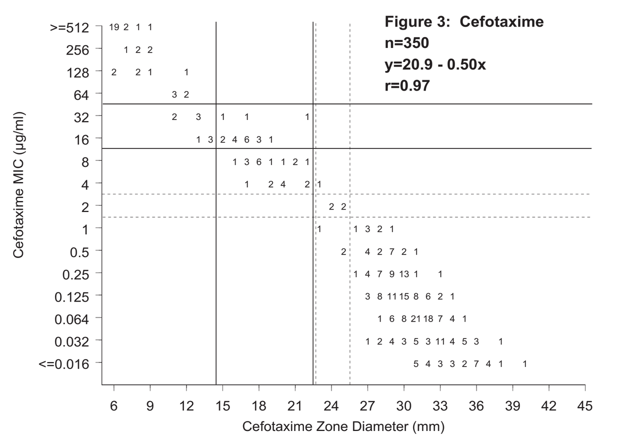
Figure 3: Cefotaxime n=350 y=20.9 - 0.50x r=0.97

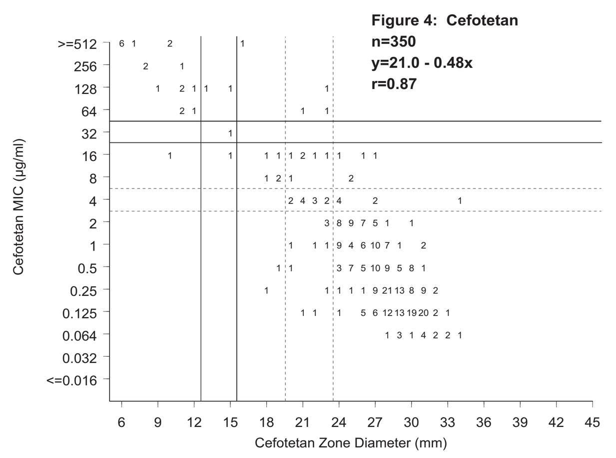
Figure 4: Cefotetan n=350 y=21.0 - 0.48x r=0.87

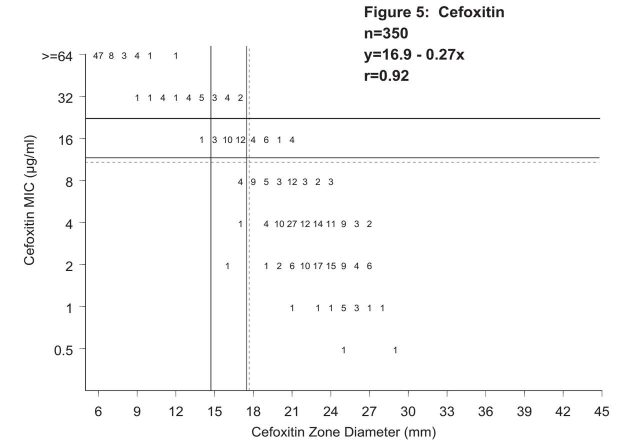
Figure 5: Cefoxitin n=350 y=16.9 - 0.27x r=0.92

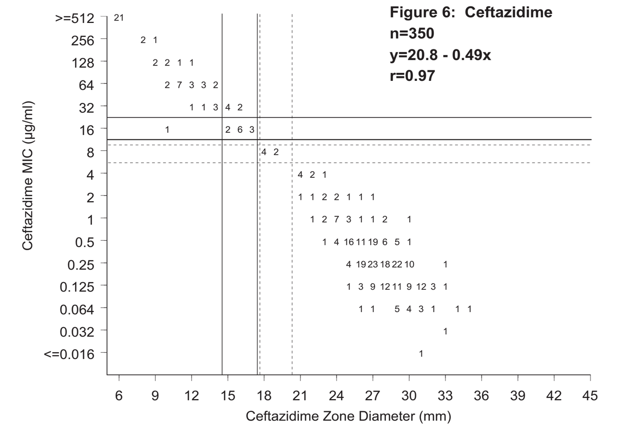
Figure 6: Ceftazidime n=350 y=20.8 - 0.49x r=0.97

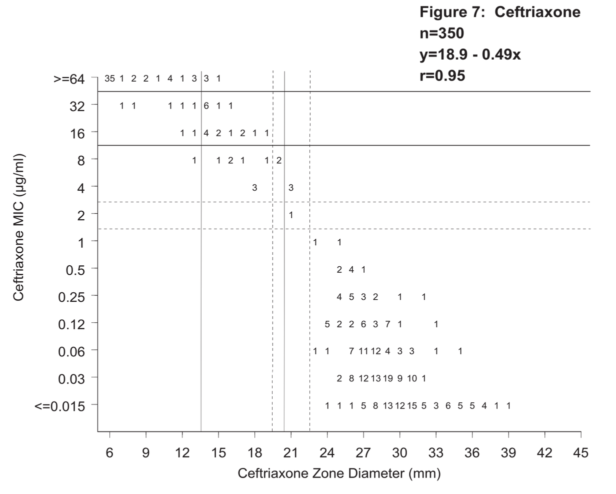
Figure 7: Ceftriaxone n=350 y=18.9 - 0.49x r=0.95

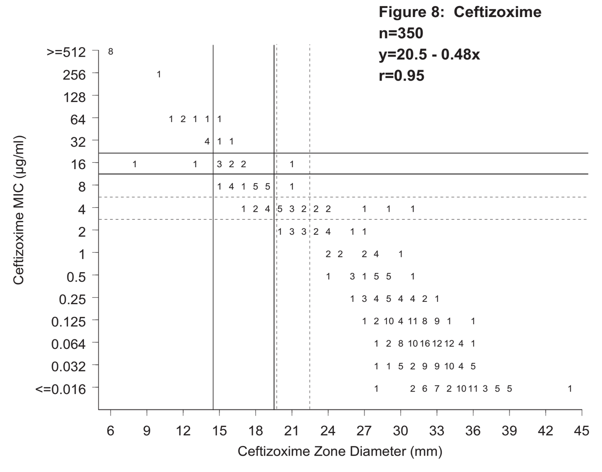
Figure 8: Ceftizoxime n=350 y=20.5 - 0.48x r=0.95

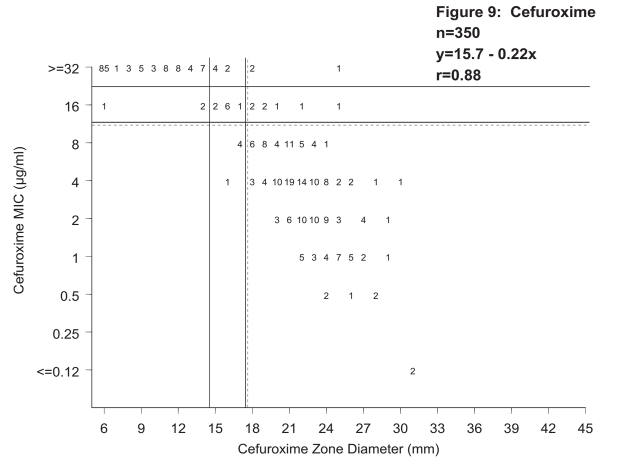
Figure 9: Cefuroxime n=350 y=15.7 - 0.22x r=0.88

Table 3. Error rates and MIC to disk diffusion intermethod agreement among Enterobacteriaceae with proposed lower breakpoints to accommodate ESBL detection and optimal prediction of clinical success.

Antimicrobial agent	Method	Suggested BP S	Suggested BP I	Suggested BP R	Error rates Very major	Error rates Major	Error rates Minor	Intermethod agreement
Aztreonam	MIC	≤4	8	≥16	0.0	0.0	2.6	97.4
	Disk	≥21	18-20	≤17				
Cefepime	MIC	≤4	8	≥16	0.0	0.0	4.3	95.7
	Disk	≥21	18-20	≤17				
Cefotaxime	MIC	≤1	2	≥4	0.0	0.0	1.1	98.9
	Disk	≥26	23-25	≤22				
Cefotetan	MIC	≤2	4	≥8	1.4	0.6	7.4	90.6
	Disk	≥24	20-23	≤19				
Cefoxitin	MIC	≤8	-	≥16	4.3	1.7	NA[a]	94.0
	Disk	≥18	-	≤17				
Ceftazidime	MIC	≤4	8	≥16	0.0	0.0	0.0	100.0
	Disk	≥21	18-20	≤17				
Ceftriaxone	MIC	≤1	2	≥4	0.0	0.0	1.4	98.6
	Disk	≥23	20-22	≤19				
Ceftizoxime	MIC	≤2	4	≥8	0.0	0.0	6.6	93.4
	Disk	≥23	20-22	≤19				
Cefuroxime	MIC	≤8	-	≥16	2.9	1.4	NA[a]	95.7
	Disk	≥18	-	≤17				

a. NA = not applicable.

CONCLUSIONS

- Current MIC and disk diffusion breakpoint criteria for Enterobacteriaceae remained acceptable related to intermethod agreement (90.6 to 98.0%), even with an ESBL enriched population of isolates.
- Selecting lower breakpoints generally decreased the intermethod error rates for the ESBL index and other ß-lactam drugs to predict clinical therapeutic outcomes via combining PK/PD, microbiology and clinical success parameters.

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