D-1692

# Susceptibility Testing Accuracy of an Enterobacteriaceae Population Enriched for CTX-M-type ESBL -producing Isolates (China): Intermethod Analysis for Nine Important Parenteral ß-lactams


S POTTUMARTHY, Y YU, HS SADER, M CHEN, RN JONES

JMI Laboratories, North Liberty, IA; Zhejiang Medical College Hangzhou; and Peking Union Medical College Hospital, Beijing, China



ICAAC 2005



JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, fax 319.665.3371
ronald-jones@jmilabs.com


## ABSTRACT


**Background:** To assess the wide geographical applicability of the current (C) or proposed (P) susceptibility (S) breakpoint (BP) criteria for 9 ß-lactams, a collection of Enterobacteriaceae (ENT) enriched with CTX-M-type ESBL-producing isolates from China were tested.

**Methods:** 354 recent clinical isolates (blood cultures, 68.6%) of ENT were analyzed: *E. coli* (100), *Klebsiella* spp. (60), *Proteus* spp. (52), *Enterobacter* spp. (43), *Serratia* spp. (31) and others (68). One-in-five (21.5%) produced an ESBL with predominance of CTX-M-type enzymes (94.7%; endemic types 3, 14, 24 and 28). Isolates were tested by CLSI broth microdilution and disk diffusion (DD) methods. Categorical agreement (CA) using MIC C-BP and those based on PK/PD target attainment (90% at MIC, T > 50%) & Monte-Carlo simulations (P-BP) were analyzed. The intermediate category was removed for cefoxitin and cefuroxime with no change in S-BP because of MIC distributions of wild-type S populations.

**Results:** The error rates and CA results are summarized in the Table.

| | S P-BP | False-S | False-R | CA |
|---|---|---|---|---|
| Antimicrobial agent | MIC (µg/ml/disk (mm) | C-BP/P-BP | C-BP/P-BP | C-BP/P-BP |
| Aztreonam | ≤4/≥21 | 0.0/0.0 | 0.0/0.0 | 93.2/97.5 |
| Cefepime | ≤4/≥21 | 0.0/0.3 | 0.0/0.0 | 95.2/96.3 |
| Cefotaxime (CT) | ≤1/≥26 | 0.0/0.0 | 0.3/0.0 | 92.6/99.2 |
| Cefoxitin | ≤8/≥18 | 0.8/7.3 | 0.0/1.1 | 85.1/91.6 |
| Ceftazidime | ≤4/≥21 | 0.0/0.0 | 0.0/0.0 | 97.2/98.0 |
| Ceftriaxone (TX) | ≤1/≥23 | 0.0/0.0 | 0.3/0.0 | 94.6/99.2 |
| Cefuroxime (XM) | ≤8/≥18 | 0.0/1.7 | 0.0/1.1 | 96.0/97.2 |

Regression analysis showed (r) values ranging from 0.84 (cefotetan [CN]) to 0.98 (CT, XM, TX). The MIC P-BP of ≤ 2 µg/ml for CN and ceftizoxime with corresponding DD correlates, ≥ 24 and ≥ 23 mm, resulted in only 7.6 and 5.1% error rates.

**Conclusions:** Our results confirm that C and P MIC BP criteria with appropriately selected DD correlates have acceptable, generally improved CA when tested against ENT enriched for CTX-M-type ESBL-producing strains endemic in numerous locations outside of the USA.


## INTRODUCTION

Extended-spectrum ß-lactamases (ESBLs) are potent plasmid-mediated enzymes which can hydrolyze oxyimino-cephalosporins and monobactams. These Bush group 2be ESBL enzymes, among others, constitute a heterogeneous molecular cluster comprised of ß-lactamases sharing 20 to > 99% identity. Based on their substrate preferences, these enzymes are broadly classified as ceftazidimases (preferential hydrolysis of ceftazidime > cefotaxime) and cefotaximases (preferential hydrolysis of cefotaxime > ceftazidime). The well known TEM and SHV ESBL groups (> 130 members) described worldwide are predominantly ceftazidimases. Recently, non-TEM and non-SHV plasmid-mediated ESBL types have been reported both in the ceftazidimases and cefotaximases groups. The cefotaximases include SFO-1, BES-1 and CTX-M enzymes. The diversity of molecular structure and hydrolytic spectra of the ESBL enzymes, in addition to the differences of the geographical occurrence of the individual ESBL-types contribute to the challenges in designing appropriate laboratory tests to detect the presence of these enzymes or to guide accurate therapeutic decisions and infection control interventions.

The current Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards [NCCLS]) recommendation for ESBL screening and confirmatory tests of *E. coli* and *Klebsiella* spp. was established in response to the reports of therapeutic failures due to ESBL-producing strains treated with cephalosporins which were tested as susceptible by CLSI methods. However, these recommendations do not address the entire issue for detection of the presence of ESBLs in all species of the Enterobacteriaceae and also require qualifying reports to the physicians. To ensure recommendations that predict favorable clinical response when testing contemporary isolates of Enterobacteriaceae, the standardizing authorities (NCCLS and others) have recognized the need to re-evaluate the existing breakpoints and consider alternative susceptible criteria for all clinically relevant cephalosporins and monobactams.

Studies using Monte-Carlo simulations and pharmacokinetic/pharmacodynamic (PK/PD) target attainment (≥ 90.0%) data support the adjustment of susceptibility MIC breakpoints that are two- to 16-fold lower for aztreonam, cefepime, cefotaxime, cefotetan, ceftazidime, ceftriaxone and ceftizoxime. For cefuroxime and cefoxitin based on the MIC population distributions and target attainment results, removal of the intermediate category accompanied by no change in the susceptible breakpoints has been proposed. Earlier reports have clearly demonstrated that both the current and the proposed alternative MIC breakpoints and corresponding disk diffusion criteria have an acceptable intermethod error rate when testing an ESBL-enriched collection of Enterobacteriaceae from the Americas against cephalosporin and monobactam antimicrobials. However, ESBLs differ in their substrate specificity and there are well recognized differences in the geographical occurrence of the various ESBL-types. To assess the global applicability of these breakpoint criteria, particularly outside of the United States (USA), we have reassessed the intermethod error rates of current and the proposed breakpoint criteria for a collection of Enterobacteriaceae selected for a predominantly CTX-M-type ESBL-producing population, endemic in China and the Far East, but becoming worldwide in range.

## MATERIALS AND METHODS

Strain collection. A collection of 354 strains of Enterobacteriaceae isolated from clinical specimens in 2003 - 2004 were analyzed. The collection was comprised of the following species (strains): *Escherichia coli* (100), *Klebsiella* spp. (60), *Enterobacter* spp. (43), *Serratia* spp.(31), *Citrobacter* spp. (29), *Proteus mirabilis* (28), indole-positive Proteae (24), *Shigella* spp.(15), *Salmonella* spp. (10) and others (14). Specimen type data was available for 83.9% (297) of the isolates. The vast majority of strains were from bloodstream infections (243; 81.8%), with the remaining organisms isolated from urine (24; 8.1%), gastrointestinal tract (22; 7.4%), documented skin and soft tissue infections (five; 1.7%) and respiratory specimens from patients with nosocomial pneumonia (three; 1.0%). More than one in five isolates (76; 21.5%) were confirmed ESBL-producing strains comprised of *E. coli* (48), *K. pneumoniae* (22), *K. oxytoca* (three), and one strain each of *P. mirabilis*, *P. vulgaris* and *Providencia stuartii*. The type of ESBL produced was identified by PCR amplification and gene sequencing procedures for 75.0% (57 of 76) of strains. These isolates were obtained from China and the CTX-M group was the dominant (94.7%; 54 of 57) ESBL-type; containing CTX-M-14 (29), CTX-M-3 (10), CTX-M-24 (five), CTX-M-3 + CTX-M-14 (five), CTX-M-28 (three), CTX-M-14 + SHV-12 (two), with SHV-type ESBLs constituting the remainder of isolates (SHV-12 [two] and SHV-5 [one]).

Susceptibility testing methods. Minimal inhibitory concentrations (MICs) of nine ß-lactams were determined by reference broth microdilution method, and interpreted according to CLSI criteria. MIC results were also determined using the Etest strips in accordance with the manufacturer's recommendations (AB BIODISK, Solna, Sweden). Simultaneous disk diffusion testing was performed for all ß-lactams analyzed and interpreted in accordance with the CLSI guidelines. The ß-lactams analyzed included: aztreonam, cefepime, cefotaxime, cefotetan, cefoxitin, ceftazidime, ceftriaxone, ceftizoxime and cefuroxime.

Selection of proposed MIC breakpoints: Using published mean PK parameter estimates and dispersion measures from volunteer studies, and PK-PD targets derived from a murine infection model (% free-drug time > $MIC_{90}$), Monte-Carlo simulation (10,000 subjects) was used to evaluate the probability of PK/PD target attainment (≥ 90.0%) for standard ß-lactam dosing regimens. Simulation results supported the reduction of some susceptibility MIC breakpoints two- to eight-fold for aztreonam, cefepime, cefotaxime, cefotetan, ceftazidime, ceftizoxime and ceftriaxone. Current susceptibility breakpoints were noted to bisect the wild-type MIC population distribution for cefoxitin. It was also evident that susceptibility breakpoints bisecting MIC population distributions could be avoided assuming the use of higher labeled doses for serious infections for cefuroxime. For both cefuroxime and cefoxitin, proposed criteria include removal of the intermediate category with no modification in the susceptible breakpoint. Correlate disk diffusion criteria were recommended corresponding to the proposed changes in the MIC breakpoints. Current MIC and disk diffusion breakpoint criteria were applied as published by the CLSI document M100-S15.

Analysis of the results: Results of the disk diffusion tests were compared with those of the reference broth microdilution supported by the Etest (AB BIODISK, Solna, Sweden) method. The data was plotted as scattergrams and the least squares method was used to calculate the regression equation. Intermethod-error rates were calculated, and the results were compared to an earlier experiment for cefepime, used as an internal control. The criteria used to calculate the intermethod agreement for MIC and disk diffusion testing included the current CLSI criteria for the nine ß-lactams, and the proposed (generally lower) interpretive criteria.

## RESULTS

**Table 1.** Susceptibility profiles of nine ß-lactams tested against 354 Enterobacteriaceae by reference/standardized NCCLS methods.

| | $MIC_{50/90}$ (% susceptible)[b] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Organism (no. tested)[a] | Aztreonam | Cefepime | Cefotaxime | Cefotetan | Cefoxitin | Ceftazidime | Ceftizoxime | Ceftriaxone | Cefuroxime |
| *E. coli* (100) | 0.5/64(81) | 0.5/32(74) | 1/>256(54) | 0.5/2(98) | 8/32(70) | 0.5/8(90) | 0.5/8(92) | 0.25/>256(52) | 32/>256(45) |
| *Klebsiella* spp. (60) | 0.12/128(75) | 0.12/16(80) | 0.12/256(72) | 0.25/1(100) | 4/16(87) | 0.5/32(87) | 0.06/4(93) | 0.12/>256(57) | 8/>256(53) |
| *Enterobacter* spp. (43) | 0.06/32(79) | 0.06/4(98) | 0.25/>256(77) | 16/>256(56) | >256/>256(9) | 0.5/>256(79) | 0.12/>256(79) | 0.25/>256(77) | 8/>256(61) |
| *Serratia* spp. (31) | 0.12/4(94) | 0.12/1(100) | 0.25/8(90) | 1/16(97) | 32/>256(7) | 0.25/2(94) | 0.12/4(100) | 0.25/8(90) | >256/>256(3) |
| *Citrobacter* spp. (29) | 0.06/16(89) | 0.06/0.5(100) | 0.12/16(89) | 1/128(89) | 64/>256(41) | 0.5/64(89) | 0.25/64(89) | 0.12/64(89) | 4/256(83) |
| *P. mirabilis* (28) | ≤0.016/≤0.016(100) | 0.06/0.12(96) | ≤0.016/0.03(96) | 0.25/1(100) | 4/8(100) | 0.12/0.12(100) | ≤0.016/0.03(100) | ≤0.016/≤0.016(100) | 2/4(96) |
| Indole-positive Proteae (24) | ≤0.016/1(100) | 0.06/4(98) | 0.06/16(83) | 0.5/8(100) | 8/32(58) | 0.25/2(96) | 0.06/4(92) | 0.03/32(88) | 128/>256(33) |
| *Shigella* spp. (15) | 0.06/0.12(100) | 0.03/0.25(100) | 0.03/0.12(100) | 0.25/1(100) | 2/8(100) | 0.25/0.5(100) | 0.06/0.12(100) | 0.03/0.06(100) | 4/8(100) |
| *Salmonella* spp. (10) | 0.06/0.12(100) | 0.06/0.12(100) | 0.12/0.12(95) | 1/1(100) | 2/8(90) | 0.5/1(95) | 0.06/0.12(95) | 0.06/0.12(95) | 4/8(90) |

a. Only the species or genus groups with ≥ 10 strains were tabulated.
b. Susceptibility as defined by CLSI excluding ESBL screening criteria.

- *P. mirabilis*, *Shigella* spp. and *Salmonella* spp. had the highest susceptibility rates (ranging from 90.0 to 100.0%) compared to the other species of Enterobacteriaceae when tested against the ß-lactams (Table 1). For *E. coli* and *Klebsiella* spp. containing the majority of ESBL- producing strains (96.1%; 73 of 76 strains), the susceptibility rates for the ESBL screening compounds (cefotaxime, ceftriaxone, ceftazidime and aztreonam) ranged from only 52.0 to 90.0%. Noteworthy for these two species, was the lower susceptibility rates to ceftriaxone (≤ 57.0%) and cefotaxime (≤ 72.0%) compared to the susceptibility rates to ceftazidime (≥ 87.0%); characteristics of CTX-M-type enzymes.
- The scattergrams for the entire collection of 354 Enterobacteriaceae including the 76 ESBL-producing strains tested against the nine ß-lactams are plotted in Figures 1 through 9. Regression statistics showed excellent correlation between the two methods for most of the antimicrobials analyzed, with the regression coefficient ranging from r = 0.84 (cefotetan) to r = 0.98 (cefotaxime, cefuroxime and ceftriaxone).
- The intermethod categorical concordance for the ß-lactams analyzed remained acceptable for the CTX-M-type ESBL enriched Enterobacteriaceae isolates analyzed in the present study when applying the CLSI interpretive criteria for eight of the nine ß-lactams analyzed (except cefoxitin), with an absolute intermethod agreement ranging from 92.6 (cefotaxime) to 97.8% (ceftazidime), see Table 2 and Figures 1-9.
- Very major errors (false-susceptible) were nil (0.0%) for seven of the nine ß-lactams. Major errors (false-resistant) were absent for all ß-lactams, except cefotaxime, cefotetan and ceftriaxone (0.3%). Minor error rates ranged from 0.8% for cefotetan to the highest rate of 14.1% noted for cefoxitin, resulting in a low intermethod concordance rate of 85.1% overall.

**Table 2.** Intermethod error rates for 354 Enterobacteriaceae isolates based on current CLSI interpretive criteria.

| | | Current criteria[a] | | | Error rates[b] | | | |
|---|---|---|---|---|---|---|---|---|
| Antimicrobial agent | Method | S | I | R | Very major | Major | Minor | Intermethod agreement |
| Aztreonam | MIC | ≤8 | 16 | ≥32 | 0.0 | 0.0 | 6.8 | 93.2 |
| | Disk | ≥22 | 16-21 | ≤15 | | | | |
| Cefepime | MIC | ≤8 | 16 | ≥32 | 0.0 | 0.0 | 4.8 | 95.2 |
| | Disk | ≥18 | 15-17 | ≤14 | | | | |
| Cefotaxime | MIC | ≤8 | 16-32 | ≥64 | 0.0 | 0.3 | 7.1 | 92.6 |
| | Disk | ≥23 | 15-22 | ≤14 | | | | |
| Cefotetan | MIC | ≤16 | 32 | ≥64 | 1.1 | 0.3 | 0.8 | 97.8 |
| | Disk | ≥16 | 13-15 | ≤12 | | | | |
| Cefoxitin | MIC | ≤8 | 16 | ≥32 | 0.8 | 0.0 | 14.1 | 85.1 |
| | Disk | ≥18 | 15-17 | ≤14 | | | | |
| Ceftazidime | MIC | ≤8 | 16 | ≥32 | 0.0 | 0.0 | 2.8 | 97.2 |
| | Disk | ≥18 | 15-17 | ≤14 | | | | |
| Ceftriaxone | MIC | ≤8 | 16-32 | ≥64 | 0.0 | 0.3 | 5.1 | 94.6 |
| | Disk | ≥21 | 14-20 | ≤13 | | | | |
| Ceftizoxime | MIC | ≤8 | 16 | ≥32 | 0.0 | 0.0 | 3.4 | 96.6 |
| | Disk | ≥20 | 15-19 | ≤14 | | | | |
| Cefuroxime | MIC | ≤8 | 16 | ≥32 | 0.0 | 0.0 | 4.0 | 96.0 |
| | Disk | ≥18 | 15-17 | ≤14 | | | | |

a. S = susceptible, I = intermediate, and R = resistant.
b. Very major = false-susceptible, major = false-resistant, and minor = intermediate result by one method and a resistant or susceptible result with the other test.

- The intermethod concordance rates for the modified breakpoint criteria analyzed using an Enterobacteriaceae collection enriched with CTX-M-type ESBL-producing strains (Table 3 and Figures 1-9) was very acceptable, with intermethod agreement rates ranging from 91.6 (cefoxitin) to 99.2% (ceftriaxone and cefotaxime). The intermethod agreement improved for seven of the nine ß-lactams analyzed; aztreonam (+ 4.3%), cefepime (+ 1.1%), cefotaxime (+ 6.6%), cefoxitin (+ 6.5%), ceftazidime (+ 0.8%), ceftriaxone (+ 4.6%) and cefuroxime (+1.2%).
- While the proposed interpretive criteria for cefoxitin and cefuroxime (no change in the susceptible breakpoints and elimination of the intermediate category) resulted in an increase in significant errors (very-major [+ 1.7% cefuroxime; + 6.5% cefoxitin] and major [+ 1.1%] error) for both antimicrobials) the intermethod categorical agreement remained at acceptable levels of 91.6% (cefoxitin) and 97.2% (cefuroxime).
- Noteworthy was the absence of both very major (false-susceptible) and major (false-resistant) errors for the ESBL index and screening compounds (aztreonam, cefotaxime, ceftazidime and ceftriaxone).

**Table 3.** Error rates and MIC/disk diffusion zone intermethod interpretive agreement for 354 Enterobacteriaceae strains using proposed, lower PK/PD-based MIC breakpoints to accommodate ESBL detection and optimize prediction of clinical outcomes.

| | | Proposed criteria[a] | | | Error rates[b] | | | |
|---|---|---|---|---|---|---|---|---|
| Antimicrobial agent | Method | S | I | R | Very major | Major | Minor | Intermethod agreement |
| Aztreonam | MIC | ≤4 | 8 | ≥16 | 0.0 | 0.0 | 2.5 | 97.5 |
| | Disk | ≥21 | 18-20 | ≤17 | | | | |
| Cefepime | MIC | ≤4 | 8 | ≥16 | 0.3 | 0.0 | 3.4 | 96.3 |
| | Disk | ≥21 | 18-20 | ≤17 | | | | |
| Cefotaxime | MIC | ≤1 | 2 | ≥4 | 0.0 | 0.0 | 0.8 | 99.2 |
| | Disk | ≥26 | 23-25 | ≤22 | | | | |
| Cefotetan | MIC | ≤2 | 4 | ≥8 | 1.4 | 0.0 | 6.2 | 92.2 |
| | Disk | ≥24 | 20-23 | ≤19 | | | | |
| Cefoxitin | MIC | ≤8 | - | ≥16 | 7.3 | 1.1 | NA[c] | 91.6 |
| | Disk | ≥18 | - | ≤17 | | | | |
| Ceftazidime | MIC | ≤4 | 8 | ≥16 | 0.0 | 0.0 | 2.0 | 98.0 |
| | Disk | ≥21 | 18-20 | ≤17 | | | | |
| Ceftriaxone | MIC | ≤1 | 2 | ≥4 | 0.0 | 0.0 | 0.8 | 99.2 |
| | Disk | ≥23 | 20-22 | ≤19 | | | | |
| Ceftizoxime | MIC | ≤2 | 4 | ≥8 | 0.3 | 0.0 | 4.8 | 94.9 |
| | Disk | ≥23 | 20-22 | ≤19 | | | | |
| Cefuroxime | MIC | ≤8 | - | ≥16 | 1.7 | 1.1 | NA[c] | 97.2 |
| | Disk | ≥18 | - | ≤17 | | | | |

a. S = susceptible, I = intermediate, and R = resistant.
b. Very major = false-susceptible, major = false-resistant, and minor = intermediate result by one method and a resistant or susceptible result with the other test.
c. NA = not applicable, no intermediate category was assigned.

**Figure 1.** Scattergram comparing the results of aztreonam broth microdilution MIC to the inhibition zone diameters around a 30 µg aztreonam disk for the 354 isolates of Enterobacteriaceae. The solid lines represent the current CLSI [2005] criteria; susceptible = ≤ 8 µg/ml / ≥ 22 mm, intermediate = 16 µg/ml / 16-21 mm, and resistant = ≥ 32 µg/ml / ≤ 15 mm. The interrupted lines represent the proposed criteria; susceptible = ≤ 4 µg/ml / ≥ 21 mm, intermediate = 8 µg/ml / 18-20 mm, and resistant = ≥ 16 µg/ml / ≤ 17 mm.

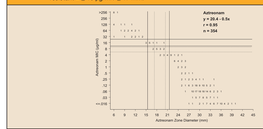



**Figure 4.** Scattergram comparing the results of cefotetan broth microdilution MIC to the inhibition zone diameters around a 30 µg cefotetan disk for the 354 isolates of Enterobacteriaceae. The solid lines represent the current CLSI criteria; susceptible = ≤ 16 µg/ml / ≥ 16 mm, intermediate = 32 µg/ml / 13-15 mm, and resistant = ≥ 64 µg/ml / ≤ 12 mm. The interrupted lines represent the proposed criteria; susceptible = ≤ 2 µg/ml / ≥ 24 mm, intermediate = 4 µg/ml / 20-23 mm, and resistant = ≥ 8 µg/ml / ≤ 19 mm.

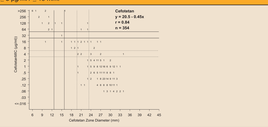



**Figure 7.** Scattergram comparing the results of ceftizoxime broth microdilution MIC to the inhibition zone diameters around a 30 µg ceftizoxime disk for the 354 isolates of Enterobacteriaceae. The solid lines represent the current CLSI [2005] criteria; susceptible = ≤ 8 µg/ml / ≥ 20 mm, intermediate = 16 µg/ml / 15-19 mm, and resistant = ≥ 32 µg/ml / ≤ 14 mm. The interrupted lines represent the proposed criteria; susceptible = ≤ 2 µg/ml / ≥ 23 mm, intermediate = 4 µg/ml / 20-22 mm, and resistant = ≥ 8 µg/ml / ≤ 19 mm.

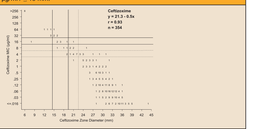



**Figure 2.** Scattergram comparing the results of cefepime broth microdilution MIC to the inhibition zone diameters around a 30 µg cefepime disk for the 354 isolates of Enterobacteriaceae. The solid lines represent the current CLSI [2005] criteria; susceptible = ≤ 8 µg/ml / ≥ 18 mm, intermediate = 16 µg/ml / 15-17 mm, and resistant = ≥ 32 µg/ml / ≤ 14 mm. The interrupted lines represent the proposed criteria; susceptible = ≤ 4 µg/ml / ≥ 21 mm, intermediate = 8 µg/ml / 18-20 mm, and resistant = ≥ 16 µg/ml / ≤ 17 mm.

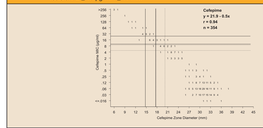



**Figure 5.** Scattergram comparing the results of cefoxitin broth microdilution MIC to the inhibition zone diameters around a 30 µg cefoxitin disk for the 354 isolates of Enterobacteriaceae. The solid lines represent the current CLSI [2005] criteria; susceptible = ≤ 8 µg/ml / ≥ 18 mm, intermediate = 16 µg/ml / 15-17 mm, and resistant = ≥ 32 µg/ml / ≤ 14 mm. The interrupted lines represent the proposed criteria; susceptible = ≤ 8 µg/ml / ≥ 18 mm, and resistant = ≥ 16 µg/ml / ≤ 17 mm.

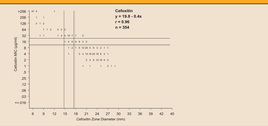



**Figure 8.** Scattergram comparing the results of ceftriaxone broth microdilution MIC to the inhibition zone diameters around a 30 µg ceftriaxone disk for the 354 isolates of Enterobacteriaceae. The solid lines represent the current CLSI [2005] criteria; susceptible = ≤ 8 µg/ml / ≥ 21 mm, intermediate = 16-32 µg/ml / 14-20 mm, and resistant = ≥ 64 µg/ml / ≤ 13 mm. The interrupted lines represent the proposed criteria; susceptible = ≤ 1 µg/ml / ≥ 23 mm, intermediate = 2 µg/ml / 20-22 mm, and resistant = ≥ 4 µg/ml / ≤ 19 mm.

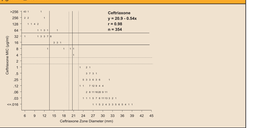



**Figure 3.** Scattergram comparing the results of cefotaxime broth microdilution MIC to the inhibition zone diameters around a 30 µg cefotaxime disk for the 354 isolates of Enterobacteriaceae. The solid lines represent the current CLSI [2005] criteria; susceptible = ≤ 8 µg/ml / ≥ 23 mm, intermediate = 16 - 32 µg/ml / 15-22 mm, and resistant = ≥ 64 µg/ml / ≤ 14 mm. The interrupted lines represent the proposed criteria; susceptible = ≤ 1 µg/ml / ≥ 26 mm, intermediate = 2 µg/ml / 23-25 mm, and resistant = ≥ 4 µg/ml / ≤ 22 mm.

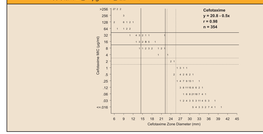
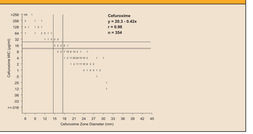



**Figure 6.** Scattergram comparing the results of ceftazidime broth microdilution MIC to the inhibition zone diameters around a 30 µg ceftazidime disk for the 354 isolates of Enterobacteriaceae. The solid lines represent the current CLSI [2005] criteria; susceptible = ≤ 8 µg/ml / ≥ 18 mm, intermediate = 16 µg/ml / 15-17 mm, and resistant = ≥ 32 µg/ml / ≤ 14 mm. The interrupted lines represent the proposed criteria; susceptible = ≤ 4 µg/ml / ≥ 21 mm, intermediate = 8 µg/ml / 18-20 mm, and resistant = ≥ 16 µg/ml / ≤ 17 mm.

**Figure 9.** Scattergram comparing the results of cefuroxime broth microdilution MIC to the inhibition zone diameters around a 30 µg cefuroxime disk for the 354 isolates of Enterobacteriaceae. The solid lines represent the current CLSI [2005] criteria; susceptible = ≤ 8 µg/ml / ≥ 18 mm, intermediate = 16 µg/ml / 15-17 mm, and resistant = ≥ 32 µg/ml / ≤ 14 mm. The interrupted lines represent the proposed criteria; susceptible = ≤ 8 µg/ml / ≥ 18 mm and resistant = ≥ 16 µg/ml / ≤ 17 mm.




## CONCLUSIONS

- CTX-M type enzymes are cefotaximases which have rapidly disseminated in the last decade to constitute the most widespread group of ESBL enzymes worldwide. The distinctive features of these potent enzymes compared to the other ESBL types include: 1.) emergence not limited to nosocomial strains of *E. coli* and *Klebsiella* spp. with detection in community bacterial strains of *Vibrio cholerae*, non-typhoidal *Salmonella* spp. and *Shigella* spp.; 2.) identification of potential reservoirs of the resistant determinants in commensal *E. coli* isolates from healthy children and animals; 3.) rapid dissemination resulting in small or countrywide or international outbreaks; and 4.) emergence linked to the widespread use of cefotaxime and ceftriaxone.
- Our results with CTX-M type strains indicates that using the current CLSI interpretive criteria, the intermethod (MIC versus zone diameter) error rates continue to be acceptable (92.6 to 97.8%) for eight of the nine antimicrobials analyzed. The new susceptible breakpoints also had acceptable intermethod error rates for all ß-lactams (91.6 to 99.2%), with significant improvements (+ 1.1% for cefepime to + 6.6% for cefotaxime) in the categorical agreement for nearly of the antimicrobials analyzed.
- The proposed (generally lower) MIC breakpoints were selected using a rigorous scientific process, taking into consideration the PK/PD data and Monte-Carlo simulations, as well as the use of MIC population analyses. These modified breakpoints will assist clinicians in the choice of an appropriate antimicrobial agent based on the in-vitro activity against the pathogen in question, and not by extrapolation of the putative resistance mechanism to unaffected peer drugs.

## SELECTED REFERENCES


Ambrose PG, Bhavnani SM, Jones RN, Craig WA, Dudley MN. (2004).Use of pharmacokinetic-pharmacodynamic and Monte-Carlo simulation as decision support for the re-evaluation of NCCLS cephem susceptibility breakpoints for Enterobacteriaceae. abstr. #A-138. In: *Programs and Abstracts of the 44th Interscience Conference on Antimicrobial Agents and Chemotherapy.* Washington D.C., October 30- November 2, 2004.

Bonnet R. (2004). Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes. *Antimicrobial Agents and Chemotherapy* 48:1-14.

Clinical and Laboratory Standards Institute. (2005) *Performance standard for antimicrobial susceptibility testing. Document M100-S15.* Wayne, PA:CLSI.

Craig WA, Kiem S, Andes D, Ambrose P, Jones R. (2003). Impact of ESBLs on in vivo activity of four cephalosporins in the neutropenic mouse-thigh infection model, abstr. #A-1318. In: *Programs and Abstracts of the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy.* Chicago, IL, September 14-17, 2003.

Jones RN, Bhavnani SM, Ambrose PG, Mutnick AH, Sader HS. (2003). Experience with ESBL-producing Enterobacteriaceae treated with carbapenems compared to other ß-lactams: Outcome report from SENTRY Antimicrobial Surveillance Program. abstr. #K-706. In: *Programs and Abstracts of the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy.* Chicago, IL, September 14-17, 2003.

Jones RN, Craig WA, Ambrose PG, Dudley MN, Pottumarthy S. (2005). Re-Evaluation of Enterobacteriaceae MIC/disk diffusion zone regression scattergrams for nine ß-lactams: Adjustments of breakpoints for strains producing extended spectrum ß-lactamases. *Diagnostic Microbiology and Infectious Diseases* 52:235-246.

Kahlmeter G, Brown DF, Goldstein FW, MacGowan AP, Mouton JW, Osterlund A, Rodloff A, Steinbakk M, Urbaskokva P, Vatopoulos A. (2003). European harmonization of MIC breakpoints for antimicrobial susceptibility testing of bacteria. *Journal of Antimicrobial Chemotherapy* 52:145-148.

National Committee for Clinical Laboratory Standards. (2001). *Development of in vitro susceptibility testing criteria and quality control parameters, 2nd ed. Approved standard M23-A2.* Wayne, PA:NCCLS.

National Committee for Clinical Laboratory Standards. (2003). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, 6th ed. M7-A6.* Wayne, PA:NCCLS.

National Committee for Clinical Laboratory Standards. (2003). *Methods for antimicrobial disk susceptibility tests; Approved standard, 8th ed. Document M2-A8.* Wayne, PA: NCCLS.

Pottumarthy S, Deshpande LM, Sader HS, Jones RN. (2005). Re-evaluation of the cefepime MIC and disk diffusion test zone diameter relationship for a worldwide collection of Enterobacteriaceae enriched for extended-spectrum-ß-lactamase (ESBL)-producing organisms. *Diagnostic Microbiology and Infectious Diseases* 52:95-99.