CLSI M100-S20 (2010) Cephalosporin and Aztreonam Breakpoint Revisions
Fact Sheet

I. Terminology / Processes

A. It seems that CLSI and others use the term "breakpoints" and "interpretive criteria" interchangeably. Are there any differences in these two terms?
- No, breakpoints and interpretive criteria refer to the same values.

B. Where can I find explanations of how CLSI establishes breakpoints?
- There is a very brief explanation of how breakpoints are established in CLSI M100-S20 on page 17. This paragraph references the comprehensive CLSI guideline for developing breakpoints, CLSI document M23—*Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters.*

C. What is the CLSI process for revising breakpoints?
- Briefly, revising breakpoints involves systematic review of microbiological, pharmacologic, and clinical data. Recognized experts, sponsors (pharmaceutical industry), and regulators participate in the process which includes discussions at public meetings of the CLSI Subcommittee on Antimicrobial Susceptibility Testing that take place twice a year. When establishing original breakpoints for new agents, controlled clinical trial data are required. Although controlled clinical trial data are desirable when revising breakpoints, they are often not feasible when addressing rapid changes in bacterial resistance mechanisms and "older" drugs. Thus, the Subcommittee must rely on best practices supported by evidence in the published literature, expert opinion, and a consensus process. Epidemiological, clinical practice, and regulatory implications of any breakpoint revision must be considered.

  Minutes of CLSI Subcommittee meetings can be found at http://www.clsi.org/Content/NavigationMenu/Committees/Microbiology/AST/AST.htm

  In the USA both the FDA and CLSI establish breakpoints. Sometimes there are differences between the breakpoints set by these two organizations.

II. Specific Breakpoint Changes and Rationale

A. Why do breakpoints sometimes need to be revised?
- Breakpoints need to be revised due to changing resistance mechanisms and bacterial population distributions, changing science leading to a better understanding of the pharmacologic determinants of clinical response, and adoption of "best practices" by clinicians. Breakpoints for many drugs routinely used in clinical practice were derived from data generated over 25 years ago under practices and standards that would no longer be considered acceptable according to today's regulatory and quality assurance standards. The need for ongoing review and update of breakpoints has been recognized by microbiologists, clinicians, and regulators in both the USA and Europe. For example, the Food and Drug Administration Amendments Act (FDAAA) passed in 2007 includes a charge to the FDA to update breakpoints and the USA FDA has responded in 2009 through issuance of a guidance document for industry for updating labeling of susceptibility test information in systemic antibacterial drug products and antimicrobial susceptibility testing devices.

  FDAAA 2007
  http://www.fda.gov/RegulatoryInformation/Legislation/FederalFoodDrugandCosmeticActFDCAct/SignificantAmendmentstotheFDCAct/FoodandDrugAdministrationAmendmentsActof2007/default.htm

  Guidance document 2009
  http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM169359.pdf

B. What breakpoints were revised in 2010?

- Select cephalosporin and aztreonam breakpoints for Enterobacteriaceae were revised as noted below (for comparison, the old breakpoints are included):

MIC breakpoints (µg/ml):

Agent	Old (M100-S19)			Revised (M100-S20)		
	Susc	Int	Res	Susc	Int	Res
Cefazolin	≤8	16	≥32	≤1	2	≥4
Cefotaxime	≤8	16-32	≥64	≤1	2	≥4
Ceftizoxime	≤8	16-32	≥64	≤1	2	≥4
Ceftriaxone	≤8	16-32	≥64	≤1	2	≥4
Ceftazidime	≤8	16	≥32	≤4	8	≥16
Aztreonam	≤8	16	≥32	≤4	8	≥16

Disk diffusion breakpoints (mm):

Agent	Old (M100-S19)			Revised (M100-S20)		
	Susc	Int	Res	Susc	Int	Res
Cefazolin	≥18	15-17	≤14	NA	NA	NA
Cefotaxime	≥23	15-22	≤14	≥26	23-25	≤22
Ceftizoxime	≥20	15-19	≤14	≥25	22-24	≤21
Ceftriaxone	≥21	14-20	≤13	≥23	20-22	≤19
Ceftazidime	≥18	15-17	≤14	≥21	18-20	≤17
Aztreonam	≥22	16-21	≤15	≥21	18-20	≤17

NA = not available

- In addition, the following MIC breakpoints were reevaluated for Enterobacteriaceae but were not revised (similarly, the corresponding disk diffusion breakpoints for these were not revised; see CLSI M100-S20):

MIC breakpoints (µg/ml)

Agent	M100-S19			M100-S20		
	Susc	Int	Res	Susc	Int	Res
Cefuroxime (parenteral)	≤8	16	≥32	≤8	16	≥32
Cefepime	≤8	16	≥32	≤8	16	≥32
Cefotetan	≤16	32	≥64	≤16	32	≥64
Cefoxitin	≤8	16	≥32	≤8	16	≥32

C. Why were cefazolin, cefotaxime, ceftizoxime, ceftriaxone, ceftazidime and aztreonam breakpoints for the *Enterobacteriaceae* revised?

- The breakpoints were revised in order to better represent the effect these agents might have when they are used to treat infections caused by contemporary isolates with currently recommended dosage regimens. Knowledge gained from ESBL-producing organisms played a major role. The initial CLSI recommendations to perform ESBL screening and confirmatory tests and to change penicillin, cephalosporin, and aztreonam results from susceptible to resistant for isolates with a positive ESBL confirmatory test were based upon: 1) the observations that some ESBL producing isolates demonstrate elevated but susceptible MICs (using former breakpoints) to these drugs and 2) limited clinical observations of poor outcomes in patients with infections due to isolates harboring ESBLs. The ESBL testing recommendations were to be a short term solution to address a new mechanism of resistance. Subsequently, additional mechanisms of resistance have been identified (e.g., new types of ESBLs and AmpC-like enzymes) and with increased frequency multiple enzymes are identified in a single isolate which can complicate ESBL testing (1). These issues coupled with improved understanding of the PK-PD determinants of efficacy with cephalosporins and monobactams resulted in the decision to revise the breakpoints.

The revised breakpoints eliminate the need to perform ESBL screen and confirmatory tests for making treatment decisions. Phenotypic tests for ESBL detection and confirmation are less accurate when multiple enzymes are present (e.g., false-negative results occur when isolates express both ESBLs and AmpC-type enzymes) (13) and the presence of multiple enzymes are more common in contemporary isolates (4, 8). The MIC of an isolate correlates better with clinical outcome than knowledge of resistance mechanisms (e.g., ESBLs) (16).

CLSI believes that the new breakpoints will provide improved information for directing patient care and result in less uncertainty and work for the clinical laboratory.

D. Were all cephems reevaluated during the cephem and aztreonam breakpoint revision process?
   - No, breakpoints for cephems generally not used or available in the USA including cefamandole, cefonicid, cefoperazone and moxalactam were not reevaluated. Consequently, ESBL screening and confirmatory testing should be performed if these drugs are tested and reported on *E. coli, Klebsiella spp.* and *Proteus mirabilis.* A susceptible result for any of these agents should be reported as resistant on isolates for which the ESBL confirmatory test is positive.

E. Why were the breakpoints for the cephamycins, cefoxitin and cefotetan not revised?
   - Breakpoints for cefoxitin were not revised as review of current data supported the current breakpoints. The PK-PD evaluation showed that exposures were within target ranges for the indicated doses. No changes were made for cefotetan because of insufficient data to suggest that revision was warranted. Cephamycins are not susceptible to hydrolysis by ESBLs (2) and susceptible results for cephamycins are not changed to resistant for isolates for which the ESBL confirmatory test is positive.

F. Why were the breakpoints for cefepime and cefuroxime (parenteral) not revised?
   - The cefepime breakpoints were not revised based upon clinical trial data and PK-PD evaluations. The clinical trial data showed cefepime efficacy for patients infected with isolates that tested cefepime susceptible (MIC ≤8 µg/ml), but produced an ESBL. PK-PD evaluations showed that daily doses of cefepime exceeding 3 g per day (i.e., 1g every 8h or 2 g every 12 hrs) would result in levels of cefepime that meet target exposure criteria used in the breakpoint revision decisions.

     Review of data suggested it was not necessary to revise the current cefuroxime (parenteral) breakpoints and these only apply to dosage regimens of 1.5 g every 8h dosage or higher.

G. Why are there no disk diffusion breakpoints for cefazolin?
   - Studies have not yet been completed to identify the zone diameter breakpoints that correlate with the revised MIC breakpoints for cefazolin. Initial studies did not reveal clear zone diameter breakpoints and disk diffusion testing of cefazolin may require a new disk with alternate disk content.

H. Why did cephalothin get moved to Test/Report Group U for Enterobacteriaceae?
   - Cephalothin for injection is no longer available in the USA. As related to Enterobacteriaceae, orally administered cephalothin is primarily used for treatment of urinary tract infections. Results from testing cephalothin can be used to represent activities of several other oral agents FDA-approved for treatment of urinary tract infections which include cefadroxil, cefpodoxime, cephalexin, and loracarbef.

I. Are cephalosporin and aztreonam breakpoints changing for non-Enterobacteriaceae?
   - No, however, cephalosporin and aztreonam breakpoints are currently being reevaluated and will likely be revised in the future.

III. Laboratory Testing and Reporting - General

A. Is it necessary to inform the medical staff, pharmacy, infectious diseases physicians, infection control practitioners, and/or the P&T committee about the cephalosporin and aztreonam breakpoint revisions?

- Yes, the new breakpoints should be discussed with stakeholders and users of laboratory reports in each institution. Infectious disease clinicians, pharmacists and others knowledgeable in pharmacotherapy will need to educate other clinicians about the revisions and how the new breakpoints may allow use of drugs previously excluded by the change in interpretation (from susceptible to resistant) dictated by ESBL confirmatory tests.

  It is important that those using antimicrobial susceptibility test results for guiding therapy decisions are aware that the new breakpoints apply to FDA-approved dosage regimens listed in Table 2A in M100-S20. These reflect standard adult dosing regimens as extracted from the pharmaceutical company's Prescribing Information or Product Label for the respective agent. Institutional policies concerning drug dose and dosage adjustment will have to be reviewed to insure they are consistent with the CLSI-recommended breakpoints. It is unlikely that the medical staff would request that the laboratory list the dosing regimens on the routine patient reports.

IV. Laboratory Testing and Reporting – Detecting Resistance Mechanisms

A. Will the revised cephalosporin and aztreonam breakpoints detect any specific beta-lactam resistance mechanisms?
   - No, the revised breakpoints are not intended to identify any specific beta-lactam resistance mechanisms. They will enhance detection of resistance among Enterobacteriaceae with a variety of resistance mechanisms.

Revised cephalosporin and aztreonam breakpoints and ESBLs

B. Will all ESBL producers test resistant with the revised cephalosporin and aztreonam breakpoints?
   - No. The revised cephalosporin and aztreonam breakpoints are focused on the MICs and drug pharmacokinetics and NOT the resistance mechanism. As has been shown, some ESBLs hydrolyze some cephalosporins more efficiently than others, thus the MIC for one agent (e.g., cefotaxime) may be considerably higher than the MIC for another (e.g., ceftazidime) in an ESBL-producing strain. Also, organisms may vary in the amount of β-lactamase produced, so that MICs will be higher if more β-lactamase activity is present (1, 2, 11).

C. When using the revised breakpoints, should we continue to perform ESBL screen and confirmatory tests for patient management? for infection control?
   - When using the revised breakpoints, it is not necessary to perform ESBL screen and confirmatory tests when reporting results to guide management of patients' therapy.

     Deciding whether or not to perform ESBL confirmatory testing for infection control or epidemiological purposes should be made following consultation with infectious disease practitioners, the pharmacy and the pharmacy and therapeutics and infection control committees of your medical staff.

D. If we use the revised breakpoints but continue to perform ESBL testing for infection control purposes, how can we explain to our clinicians that an ESBL-producing isolate of Enterobacteriaceae may now be reported as susceptible to some cephalosporins in subclass III (see Glossary I in M100-S20, page 144) and resistant to others?
   - There are differences in the activity of resistance mechanisms, such as ESBLs, to cephalosporins and aztreonam. These differences can result in different MICs. For example, with the revised breakpoints some ESBL-producing isolates may test susceptible to ceftazidime but resistant to ceftriaxone. Similarly, another ESBL-producing isolate may test resistant to ceftriaxone, but susceptible to ceftazidime. It now recommended that these results be reported without changing the cephalosporin susceptible result to resistant because studies indicate that MIC is the best predictor of treatment outcome of infections caused by β-lactamase-producing Enterobacteriaceae (3).

E. The CLSI ESBL rules indicate that all penicillin susceptible results should be edited to resistant for *E. coli, Klebsiella spp.,* and *Proteus mirabilis* that are positive with the ESBL confirmatory test. Are there some ESBL producers that might test susceptible to ticarcillin or piperacillin?
   - Since the ESBL reporting rules were initially published many years ago, several studies have shown that MICs for piperacillin and ticarcillin will fall in the resistant range (≥128ug/ml) for ESBL producers (6, 9, 10, 12).

Revised cephalosporin and aztreonam breakpoints and AmpC beta-lactamases

F. Will all AmpC producers test resistant with the revised cephalosporin and aztreonam breakpoints?
   - No. As with ESBL producers, not all AmpC producers will test resistant based on the revised breakpoints. This is because most Enterobacteriaceae, with the exception of the *Klebsiella* spp. and some *E. coli*, produce a chromosomal AmpC beta-lactamase that is produced at a low (basal) level, and MICs tend to be low and in the susceptible range even with the revised breakpoints (5). However, the most common type of AmpC-mediated resistance is due to the selection of a mutant strain that produces a high level of AmpC ("derepressed mutant"), providing sufficient amounts of the cephalosporinase to inactivate all cephalosporins and produce resistant results. An exception is possibly cefepime which is less vulnerable to inactivation by AmpC enzymes (14). In all cases, it is recommended that results be reported as they test. If sufficient AmpC is produced by strains that also have a porin defect, resistance to carbapenems such as imipenem, as well as to penicillins and cephalosporins, may also occur.

G. When using the revised breakpoints, should we do any additional tests to identify AmpC producers for patient management? for infection control?
   - Currently CLSI does not recommend any specific tests to detect AmpC production in Enterobacteriaceae. Several phenotypic tests for detection of these enzymes have been described but at this time no assay has been sufficiently evaluated for CLSI to make a recommendation to test for AmpC beta-lactamase. Like ESBL detection assays, AmpC detection assays are not recommended by CLSI for making treatment decisions.

     Deciding whether or not to do additional tests to identify AmpC producers for infection control should be made following consultation with infectious disease practitioners, the pharmacy and the pharmacy and therapeutics and infection control committees of the medical staff. Since there are no standardized phenotypic methods for detecting AmpC enzymes, the limitations of these methods must be communicated to those using results from such testing.

V. Laboratory Testing and Reporting - Implementing the Revised Breakpoints

A. How should a laboratory go about implementing the revised breakpoints?
   - Each laboratory should develop a plan for implementing the revised breakpoints. This plan should include the following steps:
     a) Determine if the antimicrobial susceptibility test system (AST) used can accommodate the revised breakpoints now. If using disk diffusion testing or a reference MIC method (e.g., in-house prepared broth microdilution panels or agar dilution plates), the revised breakpoints can be implemented now. For other commercial AST systems, see below.
     b) Discuss the revised breakpoints with infectious disease practitioners, the pharmacy and the pharmacy and therapeutics and infection control committees of the medical staff.
     c) Validate the revised breakpoints (see below)

   Note: verify (not validate) is the official term used by CLIA when establishing performance specifications of a test system in a user's laboratory, however, the more commonly accepted term, "validate" will be used here.

B. Will accrediting agencies and proficiency survey providers mandate that we adopt the revised breakpoints when the new CLSI tables (CLSI M100-S20) are released?

- No. As it states on page 18 in M100-S20, "Laboratories that use FDA-approved susceptibility testing devices are allowed to utilize existing FDA interpretive breakpoints. Either FDA or CLSI susceptibility interpretive breakpoints are acceptable to clinical laboratory accrediting bodies."

C. Is it acceptable to use CLSI M100-S19 until we can implement the revised breakpoints?
   - Yes. The breakpoints published in CLSI M100-S19 should be used until the revised breakpoints listed in CLSI M100-S20 can be implemented. If using the old breakpoints, laboratories should continue to follow ESBL testing and reporting rules.

D. Why can't manufacturers of commercial AST systems adopt the revised breakpoints now?
   - Performance of commercial AST devices is regulated in the United States by the FDA. Part of the FDA clearance requirements for an AST device involves evaluating interpretive category agreement [susceptible (S), intermediate (I), resistant (R)] of results obtained from the test (commercial) system to those obtained from a reference method. Currently, for this comparison manufacturers of AST systems must by law use the FDA breakpoints listed in the drugs "Prescribing Information" or "drug label".

     When CLSI revises an existing breakpoint, the FDA may also review data in order to determine how that change may affect the safety and effectiveness of the antimicrobial agent for the approved indications of use. If the FDA changes a breakpoint, AST device manufacturers may then have to initiate additional studies, submit the data to the FDA, and await review and regulatory clearance. In addition, AST system software changes, including LIS interface system changes, must also be coded and validated. Thus, formal implementation of revised breakpoints by device manufacturers is a regulated process that may take several months to years to complete.

     At this time, FDA has not made revisions to the cephalosporin or aztreonam breakpoints, however, they are reviewing the revisions made by CLSI. Consequently, manufacturers of commercial AST systems cannot proceed with any modification to their systems until FDA revises breakpoints.

     (see also CLSI M100-S20 page 17)

E. Despite the limitations noted above, is it possible for laboratories to use the revised breakpoints on commercial AST systems now?
   - Yes. This is a decision that must be made by each laboratory director. The revised breakpoints could be used providing: 1) the AST system contains the lower antimicrobial concentrations needed to accommodate the revised breakpoints; 2) there is a mechanism to interpret MIC results using the revised breakpoints (e.g., ability to modify breakpoints in the system software or interpreting results manually); and 3) an in-house validation is performed. This strategy is highlighted in CLSI M100-S20 page 18 where it is states: "Following discussions with appropriate stakeholders such as infectious disease practitioners and the pharmacy department, as well as the Pharmacy and Therapeutics and Infection Control committees of the medical staff, newly approved or revised breakpoints may be implemented by clinical laboratories. CLSI disk diffusion test breakpoints may be implemented as soon as they are published in M100. If a device includes antimicrobial test concentrations sufficient to allow interpretation of susceptibility to an agent using the CLSI breakpoints, a laboratory could, after appropriate validation, choose to interpret and report results using CLSI breakpoints."

F. How can a laboratory validate the revised breakpoints for FDA-cleared panels that contain the lower concentrations of cefazolin, cefotaxime, ceftriaxone, ceftizoxime, ceftazidime, and/or aztreonam? Note: this pertains to panels that are FDA cleared using the old breakpoints and contain the lower drug concentrations that accommodate the revised breakpoints.
   - There are no standard recommendations for performing this type of validation and each laboratory's director must determine what is appropriate for his/her laboratory. The validation is done to ensure there is acceptable "categoric agreement". This means that S, I, and R

results obtained using the revised breakpoints with the test system are comparable to S, I, and R results that are obtained from a CLSI reference disk diffusion, broth dilution or agar dilution method. The most conservative approach for obtaining the reference S, I, and R results would be to select from options 1 and 2 below. However, as for any laboratory test the laboratory director is ultimately responsible for the validation and can determine if an alternative procedure is appropriate. There are no commercial tests currently FDA cleared using the revised breakpoints.

Option	Reference Method	Comment
1	Disk diffusion	During recent reevaluation of breakpoints, the revised disk diffusion S, I, R results correlated within acceptable limits as described in CLSI M23-A2 with S, I, and R results obtained using the revised breakpoints with a reference broth microdilution MIC method
2	CLSI broth or agar dilution reference methods	For most, this would involve sending isolates to a reference lab that uses a CLSI M07-A8 broth or agar dilution reference methods
3	Other	An individual lab director might decide that an FDA-cleared commercial MIC test could be used as the reference method providing it contains the drug concentrations needed

Any discrepancies obtained between the test system and disk diffusion testing should be arbitrated using a CLSI broth or agar dilution reference method.

Note: Although CLIA regulations do not address verification of AST systems specifically, laboratories should be aware of CLIA requirements for verification of diagnostic tests.

CLIA regulations (CLIA 493.1253):
(http://wwwn.cdc.gov/clia/regs/toc.aspx and
http://www.cms.hhs.gov/CLIA/downloads/apcsubk1.pdf)

G. Why isn't it necessary to validate the MIC results when using the revised breakpoints?
   - If an FDA cleared panel that contains the lower concentrations of drug is used, the manufacturer has already demonstrated that MIC results obtained from the test system are comparable to those obtained from a CLSI broth or agar dilution reference method.

H. How many isolates and what types of isolates might be tested during the validation?
   - There are no standard recommendations for this and each laboratory's director must determine what is appropriate for that laboratory. The sample might contain 30 isolates which might include:
     5 isolates that are ESBL confirmatory test positive (these will likely be *Klebsiella pneumoniae* or *E. coli*)
     5 isolates that are ESBL screen positive but ESBL confirmatory test negative (these will likely be *E. coli*)
     20 isolates selected from *Citrobacter*, *Enterobacter, E. coli*, *Klebsiella*, *Proteus*/*Providencia/Morganella*, *Serratia*, other Enterobacteriaceae (select isolates that have MICs within the susceptible range using the old breakpoints; no more than 3 from a given genus)

I. What would be an acceptable level of performance for the validation studies as described above?
   - There are no standard recommendations and any of the acceptable limits described in various publications generally involve a larger sample size than what a clinical laboratory might use here. For FDA clearance, manufacturers are required to show categoric agreement of >90% with less than 1.5% very major errors (false susceptible) and less than 3% major errors (false resistance). Jorgensen suggested a very major error rate of ≤3% and a combined major and minor error rate of ≤7% for unselected isolates (7). A recent Cumitech

indicates for a small sample size, 0% for very major, less than 5% for major and less than 10% for combined major and minor are acceptable error rates (15). For calculating very major and major errors, the denominators are the numbers of resistant and susceptible isolates, respectively.

CLSI M23-A3 describes an alternative for calculating acceptable error rates which is particularly useful when many of the isolates used in the validation have MICs near the breakpoints. Less than 10% very major and less than 10% major errors are acceptable. These error rates are calculated using a denominator that includes the number of isolates with MICs within +/- 1 dilution of the intermediate MIC or intermediate MIC range. See CLSI M23-A3 for more details.

If using only 30 isolates and using strict selection criteria as suggested here, it may be difficult to attain the performance specifications referenced. The laboratory director should decide what would be acceptable performance prior to commencing the validation process.

FDA clearance document for manufacturers:
(http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm080564.htm)

J. Is there any special quality control that must be performed when using the revised breakpoints?
   - No. No changes in quality control procedures are needed when using the revised breakpoints.

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Thank you to CLSI Enterobacteriaceae Working Group for assisting in compiling these Q&As.