What's New in the 2006 Standards for Antimicrobial Susceptibility Testing (AST)?

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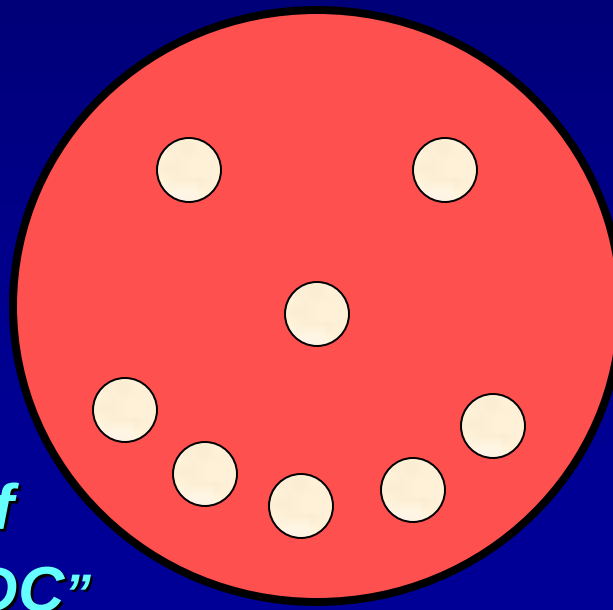

"working as a consultant with the Association of Public Health Laboratories with support from CDC"

At the conclusion of this talk, you will be able to……

- Outline the major changes found in the new CLSI tables (M100-S16) and standards for disk diffusion (M2-A9) and MIC testing (M7-A7).
- Discuss how to optimally use the new Disk Diffusion and MIC QC Troubleshooting Guides.
- Describe a strategy for implementing the new practice guidelines in your laboratory, as appropriate.

CLSI Standards - 2006

- **M100-S16 Tables (2006)***

New!

…..to be used with text documents explaining how to perform the tests….

M2-A9 Disk Diffusion (2006)**

M7-A7 MIC (2006)**

*** M100 updated yearly**
****M2, M7 updated every 3 years**

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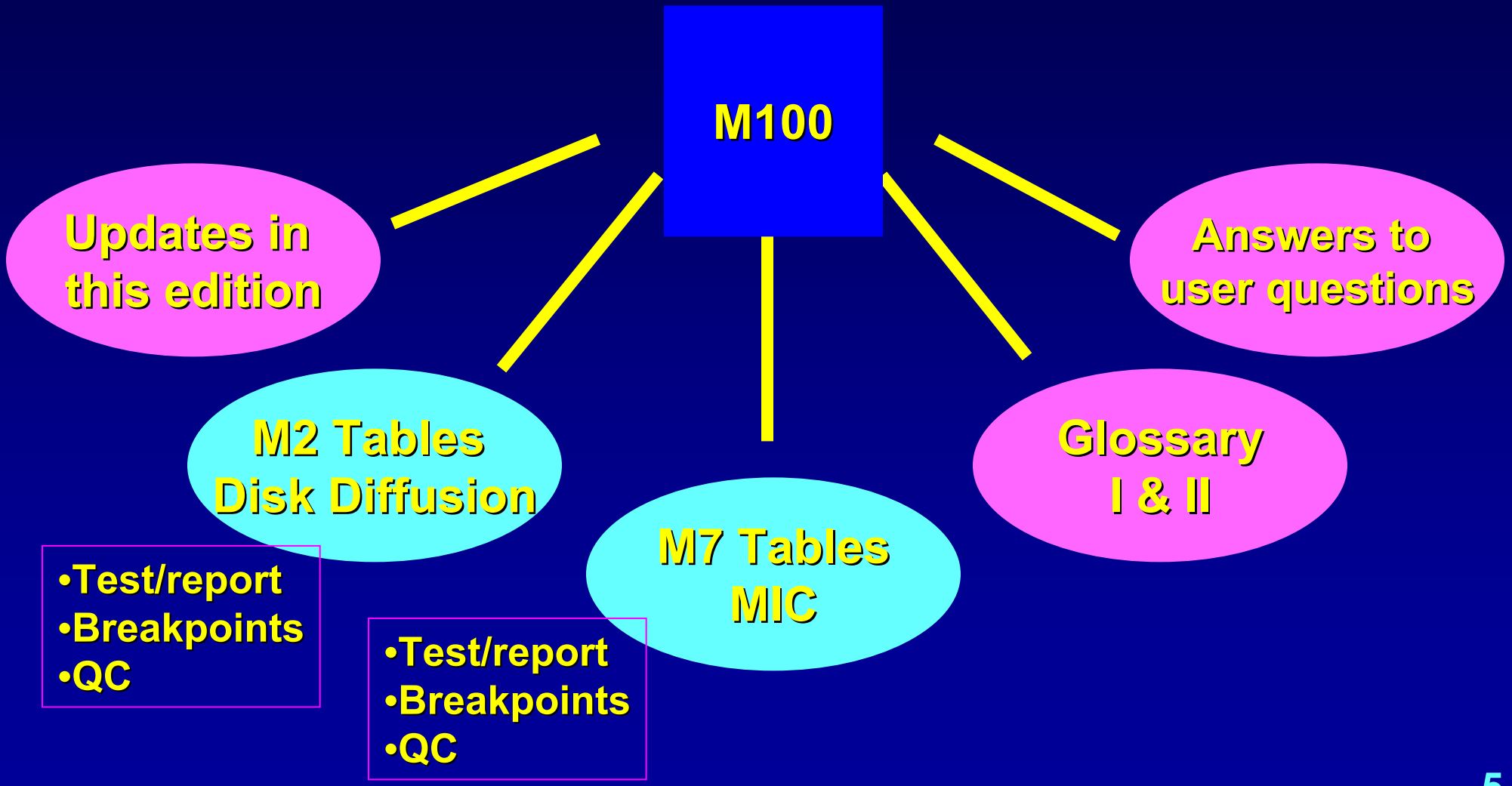
Reference Terminology

….when I refer to….

- M100 -- this means the new tables M100-S16
- M2 -- this means the new disk diffusion document M2-A9
- M7 -- this means the new MIC document M7-A7
- M45 -- this means the new fastidious organism testing document M45-P
- CLSI = formerly NCCLS

CLSI M100 contains…..



Updated information in M100-S16

Updated Information in This Edition

This document includes all of the tables from the Clinical and Laboratory Standards Institute Disk Diffusion (M2) susceptibility testing and Aerobic Dilution (M7) susceptibility testing documents. There are several important changes to the tables that have resulted from meetings of the Subcommittee on Antimicrobial Susceptibility Testing during 2005. Included below is a summary of the changes in this document, which supersede the tables published in 2005 and in earlier years.

Summary of Major Changes in This Document

The list includes the "major" changes in this document. Other minor or editorial changes have been made to the general formatting and to some of the table footnotes. Boldface type is used to highlight the changes in each table.

Additions/Changes/Deletions

The following are additions or changes unless otherwise noted as a "***deletion***."

All Tables Throughout (M2, Tables 2A-2J; M7, Tables 2A-2L)

Clarification of incubation temperature (M2 and M7)

Introduction to Tables:
Expanded rationale for listing drugs in Tables 1 and 1A (M2, p. 17 and M7, p. 91)

Changes 2006
CLSI M100-S16

CLSI Standard Reference Method and Breakpoints (M100-S16, page 14)

It is important for users of M2-A9, M7-A7, and the M100 Informational Supplement to recognize that the standard methods described in CLSI documents are reference methods. These methods may be used for routine antimicrobial susceptibility testing of clinical isolates, for evaluation of commercial devices that will be used in clinical laboratories, or by drug or device manufacturers for testing of new agents or systems. Results generated by reference methods, such as those contained in CLSI documents, may be used by regulatory authorities to evaluate the performance of commercial susceptibility testing devices as part of the approval process. Clearance by a regulatory authority indicates that the commercial susceptibility testing device provides susceptibility results that are substantially equivalent to results generated using reference methods for the organisms and antimicrobial agents described in the device manufacturer's approved package insert.

CLSI breakpoints may differ from those approved by various regulatory authorities for many reasons, including the following: different data bases, differences in interpretation of data, differences in doses utilized in different parts of the world and public health policies. Differences also exist because the CLSI proactively evaluates the need for changing breakpoints. The reasons why breakpoints may change and the manner in which the CLSI evaluates data and determines breakpoints are outlined in CLSI document M23—*Development of* In Vitro *Susceptibility Testing Criteria and Quality Control Parameters*.

Following a decision by CLSI to change an existing breakpoint, regulatory authorities may also

CLSI M2, M7, M100

- Describe standard consensus "reference methods"
- U.S. clinical labs can use:
  - CLSI test method as written
  - Method that performs comparably to CLSI "reference method" (e.g. FDA-cleared diagnostic AST device)

Diagnostic AST device = commercial instrument or test used to determine antimicrobial susceptibility in vitro

New Antimicrobial Agent Pathway

Pharmaceutical Company

Submits "New Drug Approval" (NDA) request packet to FDA
- -clinical outcome data
- -microbiological data
  - -breakpoints
  - -QC ranges
- -other

If FDA approved

Therapeutic details provided in pharmaceutical product labeling; includes FDA breakpoints

Submits condensed version of NDA request packet to CLSI
- -clinical outcome data
- -microbiological data
  - -breakpoints
  - -QC ranges
- -other

If CLSI approved

Testing details and CLSI breakpoints provided in laboratory testing "reference standards"….CLSI M2, M7 and M100

Testing to establish FDA breakpoints done using CLSI "standard reference methods"

Diagnostic Manufacturer

Submits diagnostic AST device performance data to FDA

If FDA approved

Testing details provided in diagnostic AST device product labeling; includes FDA breakpoints

Diagnostic AST device performance data is based on manufacturer demonstrating that their device produces results comparable to results produced with CLSI "standard reference methods"

FDA vs. CLSI Breakpoints

- Nearly always agree!
- Sometimes disagree
- Sometimes only FDA breakpoints (e.g. tigecycline)
- Sometimes only CLSI breakpoints (before drug is FDA cleared or if drug used in other countries)
- Sometimes modified by CLSI (e.g., 2006, vancomycin – *S. aureus*)

2006 Vancomycin MIC (µg/ml) Breakpoints – *S. aureus*

CLSI		
≤2	4-8	≥16

FDA		
≤4	8-16	≥32

Presently, diagnostic manufacturers must use FDA breakpoints

Clinical laboratories can use CLSI or FDA breakpoints
Caveat: if using commercial AST instrument, system uses FDA breakpoints

CLSI Introduction to Tables 1-1B; 2A-2L

Introduction to Tables 1 Through 1B and 2A Through 2L for Use With M7-A7—MIC Testing

On the following pages, you will find:

1. Tables 1 and 1A—Suggested groupings of antimicrobial agents that should be considered for routine testing and reporting by clinical microbiology laboratories. **These guidelines are based on drugs with clinical indications approved by the Food and Drug Administration (FDA) in the United States. In other countries, placement in Table 1 or 1A of antimicrobial agents should be based on available drugs approved for clinical use by relevant regulatory agencies.**
2. For each organism group, an additional table (Tables 2A through 2L) that contains:
   a. Recommended testing conditions.
   b. Minimal QC recommendations. (See also the M7-A7 text document, Section 16.)
   c. General comments for testing the organism group and specific comments for testing particular drug/organism combinations.
   d. Suggested agents that should be considered for routine testing and reporting by clinical microbiology laboratories as specified in Tables 1 and 1A (test/report groups A, B, C, U; the latter for "urine").
   e. Additional drugs that have an approved indication for the respective organism group, but would generally not warrant routine testing by a clinical microbiology laboratory in the United States (test/report group O for "other"; test/report group Inv. for "investigational" [not yet FDA approved]).
   f. Minimal inhibitory concentration (MIC) interpretive standards.

CLSI Introduction to Tables "Clinical Indications"

"These guidelines are based on drugs with clinical indications approved by the Food & Drug Administration (FDA) in the United States. In other countries, placement in Tables 1 and 1A of antimicrobial agents should be based on available drugs approved for clinical use by relevant regulatory agencies."

CLSI M100-S16; Introduction (M2, M7)

Example of Drug Labeling

Once-A-Day

CUBICIN®

(daptomycin for injection)

Rx only

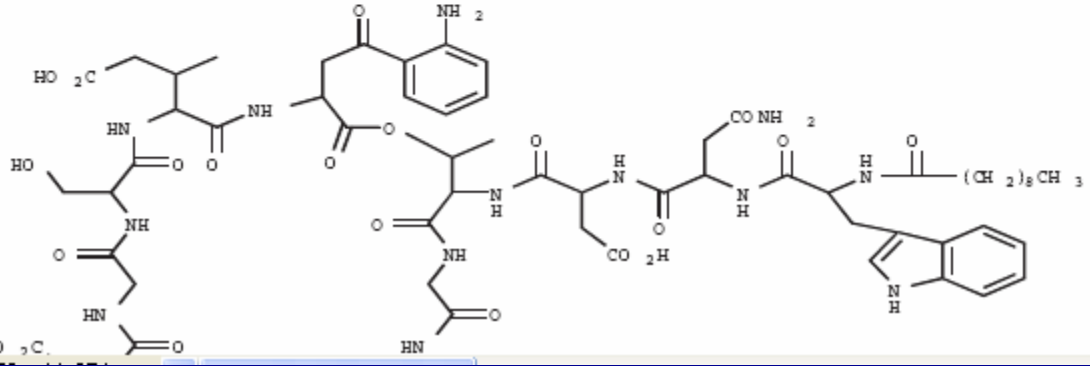
To reduce the development of drug-resistant bacteria and maintain the effectiveness of Cubicin and other antibacterial drugs,

infections caused by bacteria.

le antibacterial agent derived from the fermentation of *Streptomyces*

-L-tryptophyl-L-asparaginyl-L-aspartyl-L-threonylglycyl-L-ornithyl-L-aspartyl-

l-L-glutamyl-3-anthraniloyl-L-alanine ε_1-lactone. The chemical structure is:



removed by 4 hours of hemodialysis and 48 hours of CAP

every 24 hours for patients with $CL_{CR} \geq 30$ mL/min and

those on hemodialysis and CAPD. Daptomycin should b

hemodialysis days (see **DOSAGE AND ADMINISTRATIO**

Table 2. Mean (SD) Daptomycin Population Pharmacokin
Intravenous Infusion of 4 mg/kg to Infected Patients and N
Renal Function

Renal Function	$AUC_{0-\infty}$ (µg*h/mL
Normal (CL_{CR}>80mL/min) (N=165)	417 (15
Mild Renal Impairment (CL_{CR} 50-80 mL/min) (N=64)	466 (17
Moderate Renal Impairment (CL_{CR} 30-<50 mL/min) (N=24)	560 (25
Severe Renal Impairment (CL_{CR}<30 mL/min) (N=8)	925 (46
Hemodialysis and CAPD (N=21)	1244 (37

Note: CL_{CR}= Creatinine clearance estimated using the

Hepatic Insufficiency

The pharmacokinetics of daptomycin were evaluated in 10
and compared with healthy volunteers (n=9) matched for
not altered in subjects with moderate hepatic impairment.
mycin to patients with mild to moderate hepatic impairme
hepatic insufficiency have not been evaluated.

Gender

No clinically significant gender-related differences in dapt

Daptomycin product labeling…
http://www.cubicin.com/PDF/1004-2_MARKETING_gt.pdf.

Drug product labeling….

- Has many sections, including…
  - (Clinical) indications and usage
    - Based on demonstrated clinical efficacy
    - Examines in vitro susceptibility test and clinical outcome data
  - Microbiological activity (in vitro)
    - Does not mean clinical efficacy
- Provides information for
  - Clinicians
  - Pharmacists
  - Diagnostic AST manufacturers
  - Patients
  - Others

"Clinical Indication and Usage"

- **Clinical indication = a disease entity that has a specific set of signs, symptoms and laboratory findings that can be described to clinicians in product labeling.**
- **Example:**
  Daptomycin has clinical indication for complicated skin and skin structure infections

  This means…
  - **Daptomycin can be used to treat *S. aureus* (including MRSA) wound infections as noted in package insert**
- **Clinical indication is linked to specific pathogens**

INDICATIONS AND USAGE

Cubicin (daptomycin for injection) is indicated for the treatment of complicated skin and skin structure infections caused by susceptible strains of the following Gram-positive microorganisms (see also **DOSAGE AND ADMINISTRATION**): *Staphylococcus aureus* (including methicillin-resistant strains), *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae* subsp. *equisimilis* and *Enterococcus faecalis* (vancomycin-susceptible strains only). Combination therapy may be clinically indicated if the documented or presumed pathogens include Gram-negative or anaerobic organisms. (see **CLINICAL STUDIES**).

Daptomycin is not indicated for the treatment of pneumonia.

Appropriate specimens for microbiological examination should be obtained in order to isolate and identify the causative pathogens and to determine their susceptibility to daptomycin. Empiric therapy may be initiated while awaiting test results. Antimicrobial therapy should be adjusted as needed based upon test results.

To reduce the development of drug-resistant bacteria and maintain the effectiveness of Cubicin and other antibacterial drugs, Cubicin should be used only to treat or prevent infections that are proven or strongly suspected to be caused by susceptible bacteria. When culture and susceptibility information are available, they should be considered in selecting or modifying antibacterial therapy. In the absence of such data, local epidemiology and susceptibility patterns may contribute to the empiric selection of therapy.

CONTRAINDICATIONS

Cubicin is contraindicated in patients with known hypersensitivity to daptomycin.

WARNINGS

Pseudomembranous colitis has been reported with nearly all antibacterial agents, including daptomycin, and may range in

Daptomycin product labeling…
http://www.cubicin.com/PDF/1004-2_MARKETING_gt.pdf.

is co-administered with probenecid.

MICROBIOLOGY

Daptomycin is an antibacterial agent of a new class of antibiotics, the cyclic lipopeptides. Daptomycin is a natural product which has clinical utility in the treatment of infections caused by aerobic Gram-positive bacteria. The *in vitro* spectrum of activity of daptomycin encompasses most clinically relevant Gram-positive pathogenic bacteria. Daptomycin retains potency

Daptomycin has been shown to be active against most isolates of the following microorganisms both *in vitro* and in clinical infections, as described in the **INDICATIONS AND USAGE** section.

Aerobic and facultative Gram-positive microorganisms:

Enterococcus faecalis (vancomycin-susceptible strains only)
Staphylococcus aureus (including methicillin-resistant
Streptococcus agalactiae
Streptococcus dysgalactiae subsp. *equisimilis*
Streptococcus pyogenes

both in vitro and in clinical infections

The following *in vitro* data are available, but their clinical significance is unknown. Greater than 90% of the following microorganisms demonstrate an *in vitro* MIC less than or equal to the susceptible breakpoint for daptomycin versus the bacterial genus. The efficacy of daptomycin in treating clinical infections due to these microorganisms has not been established in adequate and well-controlled clinical trials.

Aerobic and facultative Gram-positive microorganisms:

in vitro

Corynebacterium jeikeium
Enterococcus faecalis (vancomycin-resistant strains)
Enterococcus faecium (including vancomycin-resistant strains)
Staphylococcus epidermidis (including methicillin-resistant strains)
Staphylococcus haemolyticus

Daptomycin product labeling…
http://www.cubicin.com/PDF/1004-2_MARKETING_gt.pdf.

Daptomycin FDA MIC Breakpoints (same as CLSI) 2006 - CLSI eliminated disk diffusion breakpoints

Table 3. Susceptibility Interpretive Criteria for Daptomycin

Pathogen	Minimal inhibitory concentration (μg/mL)[a]			Disk diffusion zone Diameter (mm)[b]		
	S	I	R	S	I	R
Staphylococcus aureus (methicillin-susceptible and methicillin-resistant)	≤1	(c)	(c)	≥16	(c)	(c)
Streptococcus pyogenes, *Streptococcus agalactiae*, and *Streptococcus dysgalactiae* subsp. *equisimilis*	≤1	(c)	(c)	≥16	(c)	(c)
Enterococcus faecalis (vancomycin–susceptible only)	≤4	(c)	(c)	≥11	(c)	(c)

a. The MIC interpretive criteria for *S. aureus* and *E. faecalis* are applicable only to tests performed by broth microdilution using Mueller-Hinton broth adjusted to a calcium content of 50 mg/L; the MIC interpretive criteria for *Streptococcus* spp. other than *S. pneumoniae* are applicable only to tests performed by broth microdilution using Mueller-Hinton broth adjusted to a calcium content of 50 mg/L, supplemented with 2 to 5% lysed horse blood, inoculated with a direct colony suspension and incubated in ambient air at 35°C for 20 to 24 hours.

b. The zone diameter interpretive criteria for *Streptococcus* spp. other than *S. pneumoniae* are applicable only to tests

Daptomycin product labeling…
http://www.cubicin.com/PDF/1004-2_MARKETING_gt.pdf

Table 1. Suggested Groupings of Antimicrobial Agents With FDA Clinical Indications That Should Be Considered for Routine Testing and Reporting on Nonfastidious Organisms by Clinical Microbiology Laboratories in the U.S.

	Enterobacteriaceae[a]	*Pseudomonas aeruginosa* and Other Non-Enterobacteriaceae[j]	*Staphylococcus* spp.	*Enterococcus* spp.[n]
GROUP A PRIMARY TEST AND REPORT	Ampicillin [b]	Ceftazidime	Oxacillin[l]	Penicillin[o] or ampicillin
	Cefazolin[a] Cephalothin[a]	Gentamicin	Penicillin[l]	
	Gentamicin	Mezlocillin or ticarcillin Piperacillin		
GROUP B[e] PRIMARY TEST SELECTIVELY	Amikacin	Amikacin	Azithromycin[d] or clarithromycin[d] or erythromycin[d]	Daptomycin
				Linezolid
				Quinupristin-dalfopristin[q]
	Amoxicillin-clavulanic acid or ampicillin-sulbactam Piperacillin-tazobactam Ticarcillin-clavulanic acid	Cefepime		Vancomycin
	Cefamandole or cefonicid or cefuroxime	Aztreonam Cefoperazone	Clindamycin[q]	
			Daptomycin	
		Ciprofloxacin Levofloxacin	Linezolid	
			Telithromycin[q]	
	Cefepime	Imipenem Meropenem		
	Cefmetazole Cefoperazone[q]	Ticarcillin-clavulanic acid[k]		

CLSI Table 1 (M7) Drugs to Test/Report

Why are some drugs listed in Table 2 but not in Table 1?

CLSI Table 1 (M7)
Drugs to Test/Report

Table 1. Suggested Gr… …n FDA Clinical Indications That Should Be Considered for R… …Nonfastidious Organisms by Clinical Microbiology Laboratories in the U.S.

…UP A …RY TEST …REPORT	Enterobacteriaceae[a]	*Pseudomonas aeruginosa* and Other Non-Enterobacteriaceae[j]	*Staphylococcus* spp.	*Enterococcus* spp.[n]
	Ampicillin[g]	Ceftazidime	Oxacillin[l]	Penicillin[o] or ampicillin
	Cefazolin[g]	Gentamicin	Penicillin[l]	

CLSI Table 2B-1 (M7)
Non-Enterobacteriaceae Breakpoints

Test/Report Group	Antimicrobial Agent	MIC (µg/mL) Interpretive Standard S	I	R	Comments
PENICILLINS					
A	Mezlocillin or	≤ 64	-	≥ 128	For *P. aeruginosa* only
		≤ 16	32-64	≥ 128	For other non-Enterobacteriaceae
		≤ 64	-	≥ 128	*For P. aeruginosa* only
		≤ 16	32-64	≥ 128	For other non-Enterobacteriaceae
		≤ 64	-	≥ 128	*For P. aeruginosa* only
		≤ 16	32-64	≥ 128	For other non-Enterobacteriaceae
		≤ 128	256	≥ 512	For *P. aeruginosa* only
		≤ 16	32	≥ 64	For other non-Enterobacteriaceae
		≤ 64	-	≥ 128	For *P. aeruginosa* only
…IATIONS					
B	Ticarcillin-clavulanic acid	≤ 16/2	32/2-64/2	≥ 128/2	For other non-Enterobacteriaceae (4) May be indicated for testing of some *Pseudomonas* spp. (other than *P. aeruginosa*)
O	Ticarcillin-clavulanic acid	≤ 64/2	-	≥ 128/2	For *P. aeruginosa* only
O	Piperacillin-tazobactam	≤ 64/4	-	≥ 128/4	For *P. aeruginosa* only

Possible Reasons why drug may be in Table 2 but not Table 1…

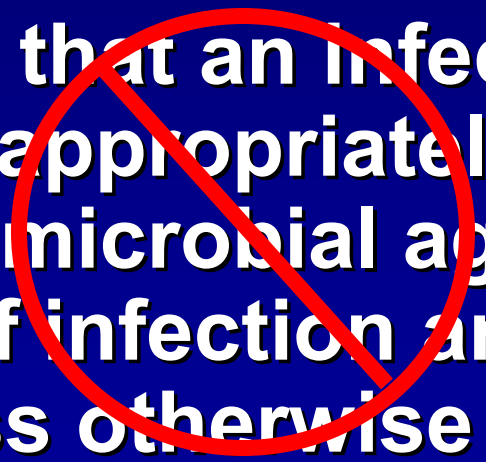
- Drug does not have an FDA clinical indication for organism
- Drug may not be used in the USA
- Drug is not a first-choice or alternative drug suggested for routine testing for organism

  Example:

  – piperacillin-tazobactam and *P. aeruginosa*

Susceptible ("S") Reworded

- **New** -…implies that isolates are inhibited by the usually achievable concentrations of antimicrobial agent when the recommended dosage is used for the site of infection.
- **Old** -…implies that an infection due to the strain may be appropriately treated with the dosage of antimicrobial agent recommended for that type of infection and infecting species, unless otherwise contraindicated.



Intermediate ("I")

- The "intermediate" category includes isolates with antimicrobial agent MICs that approach usually attainable blood and tissue levels and for which response rates may be lower than for susceptible isolates. The intermediate category implies clinical efficacy in body sites where the drugs are physiologically concentrated (e.g., quinolones and β-lactams in urine) or when a higher than normal* dosage of a drug can be used (e.g., β-lactams). This category also includes a buffer zone, which should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations, especially for drugs with narrow pharmacotoxicity margins.

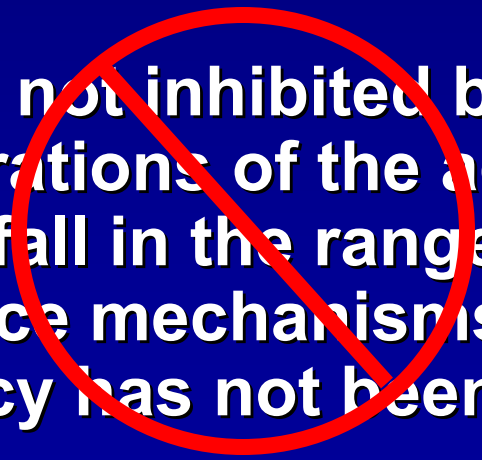
*previously stated "high dosage"

CLSI M100-S16; Introduction (M2, M7)

Resistant ("R") Reworded

- **New –…implies that isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage schedules and/or that demonstrate MICs that fall in the range where specific microbial resistance mechanisms are likely (e.g., β-lactamases), and clinical efficacy of that agent against the isolate has not been reliably shown in treatment studies.**

- **Old - …strains are not inhibited by the usually achievable systemic concentrations of the agent with normal dosage schedules and/or fall in the range where specific microbial resistance mechanisms (e.g., β-lactamases) and clinical efficacy has not been reliable in treatment studies.**



CLSI M100-S16; Introduction (M2, M7)

"S" only Definition

rare occurrence

If only "S" criteria are specified:

For some organism/antimicrobial combinations, the absence or rare occurrence of resistant strains precludes defining any results categories other than "susceptible." For strains yielding results suggestive of a "nonsusceptible" category, organism identification and antimicrobial susceptibility test results should be confirmed. Subsequently, the isolates should be saved and submitted to a reference laboratory that will confirm results using a CLSI reference dilution method.

CLSI M100-S16; Introduction (M2, M7)

"Absence" vs. "rare occurrence"….
How do we know if there has EVER been a "NS" isolate?

- Check CLSI M100-S16 "Suggestions for Verification of AST Results and Confirmation of Organism Identification" [Table 4 (M2) or Table 8 (M7)]

NS, not susceptible

Excerpt from: "Suggestions for Verification of AST Results and Confirmation of Organism Identification"

Organism or Group	Category I Verify at all labs	Category II Verify – institution specific
Staphylococcus aureus	daptomycin - NS linezolid – NS quin-dalfo – I or R vancomycin – I or R	oxacillin – R
Streptococcus pneumoniae	fluoroquinolone - NS linezolid[c] – NS vancomycin[c] – NS	penicillin - R 3rd-gen cephalosporin- R

NS, not susceptible
Note: superscript "c" means "never reported"

CLSI M100-S16; Tables 4 (M2) and 8 (M7)

[c]NS Example
NS, not susceptible

S. pneumoniae - Vancomycin[c]

MIC (µg/ml)

	Susc	Int	Res
vancomycin	≤ 1.0	-	-

*investigate any NS isolate
..Repeat ID and AST
..Save isolate
..Send to reference lab (test by CLSI MIC method)
Note: vancomycin-NS *S. pneumoniae* have NEVER been reported

CLSI M100-S16; Table 2G (M7)

NS Example
NS, not susceptible

S. aureus - Linezolid MIC (µg/ml)

	Susc	Int	Res
linezolid	≤ 4.0	-	-

*investigate any NS isolate
..Repeat ID and AST
..Save isolate
..Send to reference lab (CLSI MIC method)
Note: linezolid-NS *S. aureus* have been reported on rare occasions

CLSI M100-S16; Table 2C (M7)

CLSI Introduction to Tables
Added ESBLs to "Warning" List

V. Warning

Some of the comments in the tables relate to dangerously misleading results that can occur when certain antimicrobial agents are tested and reported as susceptible against specific organisms. These are denoted with the word "**Warning.**"

"Warning": The following antimicrobial agent/organism combinations may appear active *in vitro* but are not effective clinically and should not be reported as susceptible.

Location	Organism	Antimicrobial Agents That Must Not be Reported as Susceptible
Table 2A	**ESBL-producing *K. pneumoniae*, *K. oxytoca*, *E. coli*, and *P. mirabilis***	**penicillins, cephalosporins, and aztreonam**
Table 2A	*Salmonella* spp., *Shigella* spp.	1st- and 2nd-generation cephalosporins, **cephamycins,** and aminoglycosides
Table 2C	oxacillin-resistant *Staphylococcus* spp.	penicillins, β-lactam/β-lactamase inhibitor combinations, cephems, and carbapenems
Table 2D	*Enterococcus* spp.	aminoglycosides (except high concentrations), cephalosporins, clindamycin, and trimethoprim-sulfamethoxazole
Table 2K (Table 2A)	*Yersinia pestis*	β-lactam antimicrobial agents
Table 7	*Listeria* spp.	cephalosporins

Clarification of Incubation Temperature Range

- **35°C +/- 2°C (except staphylococci as shown below and *Neisseria gonorrhoeae*)**

Table 2C. MIC Interpretive Standards (µg/mL) for *Staphylococcus* spp.

Testing Conditions	
Medium:	Broth dilution: Cation-adjusted Mueller-Hinton broth (CAMHB) CAMHB plus 2% NaCl for oxacillin, methicillin, and nafcillin; CAMHB supplemented to 50 µg/mL calcium for daptomycin Agar dilution: Mueller-Hinton agar (MHA); Agar dilution **has not been validated** for daptomycin. MHA plus 2% NaCl for oxacillin, methicillin, and nafcillin
Inoculum:	Direct colony suspension, equivalent to a 0.5 McFarland standard
Incubation:	35 ± 2 °C; ambient air; 16 to 20 hours; 24 hours for oxacillin, methicillin, nafcillin, and vancomycin. **Testing at temperatures above 35 °C may not detect MRS.**

Minimal
ranges.)

Staphyl
Escheri
combina

Staphyl
ATCC® E
test)

CLSI M45-P Guideline

"Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently-Isolated or Fastidious Bacteria"

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CLSI M45-P Guideline

- "Guideline" vs. "Standard"
- M45 is….
  - Based upon data in published literature
  - Based on MIC distributions and resistance mechanisms of organisms

  Limited clinical data available to support decisions
- M100 is a "standard" and is based on substantial clinical data in addition to in vitro data (see CLSI M23)
- "P" = proposed; will likely become M45-"A" (A= approved)

On inside cover of each CLSI document is description of Standard, Guideline, Proposed, Approved and more….

Clinical and Laboratory Standards Institute

Providing NCCLS standards and guidelines, ISO/TC 212 standards, and ISO/TC 76 standards

The Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) is an international, interdisciplinary, nonprofit, standards-developing, and educational organization that promotes the development and use of voluntary consensus standards and guidelines within the healthcare community. It is recognized worldwide for the application of its unique consensus process in the development of standards and guidelines for patient testing and related healthcare issues. Our process is based on the principle that consensus is an effective and cost-effective way to improve patient testing and healthcare services.

In addition to developing and promoting the use of voluntary consensus standards and guidelines, we provide an open and unbiased forum to address critical issues affecting the quality of patient testing and health care.

PUBLICATIONS

A document is published as a standard, guideline, or committee report.

Standard A document developed through the consensus process that clearly identifies specific, essential

Most documents are subject to two levels of consensus—"proposed" and "approved." Depending on the need fo field evaluation or data collection, documents may also b made available for review at an intermediate consensu level.

Proposed A consensus document undergoes the first stag of review by the healthcare community as a propose standard or guideline. The document should receive a wid and thorough technical review, including an overall review of its scope, approach, and utility, and a line-by-line review of its technical and editorial content.

Approved An approved standard or guideline has achieve consensus within the healthcare community. It should b reviewed to assess the utility of the final document, t ensure attainment of consensus (i.e., that comments o earlier versions have been satisfactorily addressed), and t identify the need for additional consensus documents.

Our standards and guidelines represent a consensus opinio on good practices and reflect the substantial agreement b materially affected, competent, and interested partie obtained by following CLSI's established consensu procedures. Provisions in CLSI standards and guideline may be more or less stringent than applicable regulations

CLSI M45-P Guideline

Abiotrophia / Granulicatella	Lactobacillus
*Aeromonas / Plesiomonas	Leuconostoc
Bacillus spp. (not anthrax)	Listeria monocytogenes
Campylobacter jejuni / coli	Moraxella catarrhalis
Corynebacterium	*Pasteurella
Erysipelothrix	Pediococcus
HACEK Group	*Vibrio spp. (not cholera)

*disk diffusion method described in addition to MIC method

CLSI M45-P Guideline

- "Testing should only be undertaken in consultation with infectious diseases or other expert clinicians that can assist in determining if susceptibility testing is needed in the management of a specific patient."

CLSI M45-P; Indications

Table 5. *Corynebacterium* spp. (Including *C. diphtheriae*)—Information and Interpretive Criteria for Broth Microdilution Susceptibility Testing

Testing Conditions	
Medium	Cation adjusted Mueller-Hinton broth with lysed horse blood (2.5-5%)
Inoculum	Direct colony suspension, equivalent to a 0.5 McFarland standard
Incubation	35 °C; ambient air for 24-48 hours

Minimal QC Recommendations
Streptococcus pneumoniae ATCC® 49619

Agents to Consider for Primary Testing
Penicillin
Vancomycin
Erythromycin
Gentamicin

General Comments

(1) Growth characteristics on routine media:
Nonfastidious; grows well on BAP; ambient air; 20-24 hours

(2) For some organism/antimicrobial combinations, the absence or rare occurrence of resistant strains precludes defining any results categories other than "susceptible." For strains yielding results suggestive of a "nonsusceptible" category, organism identification and antimicrobial susceptibility test results should be confirmed. Subsequently, the isolates should be saved and submitted to a reference laboratory for confirmation.

NOTE: Information in boldface type is considered tentative for one year.

Antimicrobial Class	Antimicrobial Agent	MIC (µg/mL) Interpretive Criteria			Comments
		S	I	R	
PENICILLINS					(3) Interpretive criteria may not apply to meningitis
	Penicillin	≤1	2	≥4	
CEPHEMS					(3) Interpretive criteria may not apply to meningitis
	Cefepime	≤1	2	≥4	
	Cefotaxime	≤1	2	≥4	
	Ceftriaxone	≤1	2	≥4	
CARBAPENEMS					(3) Interpretive criteria may not apply to meningitis
	Imipenem	≤4	8	≥16	
	Meropenem	≤4	8	≥16	
GLYCOPEPTIDES					
	Vancomycin	≤4	-	-	
	Teicoplanin	≤8	16	≥32	

CLSI M45-P Table 5
***Corynebacterium* spp.**

CLSI M45-P Table 5 (con't) *Corynebacterium* spp.

Supplemental Information

Resistance:
Some species of *Corynebacterium* may exhibit resistance to multiple drug classes.

Reasons for Testing/Not Testing:
Testing of isolates from normally sterile sources (blood cultures, deep tissue, implanted prosthetic devices) may be warranted, especially in immunodeficient patients.

Derivation of Interpretive Criteria:
Interpretive criteria for penicillin and erythromycin are based primarily on MIC distributions following testing of a large number of isolates. Cephalosporin interpretive criteria are adapted from those for *Streptococcus* spp.; linezolid interpretive criteria are adapted from those for *Enterococcus* spp.; remaining interpretive criteria are adapted from those for *Staphylococcus* spp. as published in the current edition of CLSI document M100.

Testing Notes:
Resistant results can be reported at 24 hours. Isolates demonstrating susceptible results for beta-lactams should be reincubated and results reported at 48 hours.

Changes 2006
CLSI M100-S16
Gram Negatives

GNR

Enterobacteriaceae

CLARIFICATIONS…

- **ESBL screening breakpoints for *Proteus mirabilis***
- **Warning comment for *Salmonella/Shigella***
- **AST of *Salmonella* spp. from feces**

Proteus mirabilis ESBL Screen Test

Drug	Disk Screen (mm)	MIC Screen (µg/ml)
Cefpodoxime	≤17*	>1*
Ceftazidime	≤22	>1
Aztreonam	NA	NA
Cefotaxime	≤27	>1
Ceftriaxone	NA	NA

*unique for *P. mirabilis* (as compared to ≤22 mm and >4 µg/ml for *E. coli* and *Klebsiella* spp.); NA, not applicable

CLSI M100-S16; Table 2A (M2, M7)

"Warning" Comment *Salmonella/Shigella*

- "*Warning*: For *Salmonella* and *Shigella* spp., aminoglycosides, 1st - and 2nd -generation cephalosporins and cephamycins may appear active in vitro but are not effective clinically and should not be reported as "S".

CLSI M100-S16; Table 2A (M2, M7)

CLSI Glossary I (Part 1) Cephems

		ticarcillin-clavulanic acid
cephems (parenteral)	cephalosporin I[c,b]	cefazolin cephalothin cephapirin cephradine
	cephalosporin II[c,b]	cefamandole cefonicid cefuroxime (sodium)
	cephalosporin III[c,b]	cefoperazone cefotaxime ceftazidime ceftizoxime ceftriaxone
	cephalosporin IV[c,b]	cefepime
	cephamycin[d]	cefmetazole cefotetan cefoxitin
	oxacephem	moxalactam
cephems (oral)	cephalosporin[b]	cefaclor cefadroxil cefdinir cefditoren cefetamet cefixime cefpodoxime cefprozil ceftibuten cefuroxime (axetil) cephalexin cephradine
	carbacephem	loracarbef

Salmonella Reporting

- When fecal isolates of *Salmonella* and *Shigella* spp. are tested only ampicillin, a quinolone, and trimeth-sulfa should be tested and reported routinely. In addition, chloramphenicol and a 3rd-generation cephalosporin should be tested and reported for extraintestinal isolates of *Salmonella* spp.

CLSI M100-S16; Table 2A (M2, M7)

Salmonella spp. (feces)

ampicillin	S
ciprofloxacin	S
trimeth-sulfa	S

Notes:

- Add chloramphenicol (if your MDs want this) and 3rd-generation cephalosporin for extraintestinal isolates
- May not need to do AST on *Salmonella* spp. isolated from patients with mild diarrhea as disease is self-limiting

Non-Enterobacteriaceae

- **Added separate breakpoint tables:**
  - ***Pseudomonas aeruginosa* (disk diffusion)**
  - ***Pseudomonas aeruginosa* and other non-Enterobacteriaceae (MIC)**
  - ***Acinetobacter* spp.**
  - ***Burkholderia cepacia***
  - ***Stenotrophomonas maltophilia***
- **Delete *P. aeruginosa "Rx"* comment**
- **Colistin / polymyxin B**
  - **Deleted disk diffusion QC ranges**
  - **Added colistin MIC breakpoints for *Acinetobacter* spp.**

CLSI Table 1 (M7) Drugs to Test/Report

Table 1. Suggested Groupings of Antimicrobial Agents With FDA Clinical Indications That Should Be Considered for Routine Testing and Reporting on Nonfastidious Organisms by Clinical Microbiology Laboratories in the U.S.

	Enterobacteriaceae[d]	*Pseudomonas aeruginosa* and Other Non-Enterobacteriaceae[i]	*Staphylococcus* spp.	*Enterococcus* spp.[n]
GROUP A PRIMARY TEST AND REPORT	Ampicillin [g]	Ceftazidime	Oxacillin[l]	Penicillin[o] or ampicillin
	Cefazolin[a] Cephalothin[a]	Gentamicin	Penicillin[l]	
	Gentamicin	Mezlocillin or ticarcillin Piperacillin		

Table 1. (Continued)

	Acinetobacter spp.[j]	*Burkholderia cepacia*[j]	*Stenotrophomonas maltophilia*[j]
GROUP A PRIMARY TEST AND REPORT	Ceftazidime	Trimethoprim-sulfamethoxazole	Trimethoprim-sulfamethoxazole
	Imipenem Meropenem		
	Amikacin	Ceftazidime	Ceftazidime

Table 2B-4 (M7) MIC Breakpoints for *Stenotrophomonas maltophilia*

Table 2B-4. MIC Interpretive Standards (µg/mL) for Breakpoints for *Stenotrophomonas maltophilia*

Testing Conditions	
Medium:	Broth dilution: Cation-adjusted Mueller-Hinton broth (CAMHB) Agar dilution: Mueller-Hinton agar (MHA)
Inoculum:	Growth method or direct colony suspension, equivalent to a 0.5 McFarland standard
Incubation:	35 ± 2 °C; ambient air; 20 to 24 hours

Minimal QC Recommendations (See Table 3 for Acceptable QC Ranges.)

Pseudomonas aeruginosa ATCC® 27853
Escherichia coli ATCC® 25922
Escherichia coli ATCC® 35218 (for β-lactam/β-lactamase inhibitor combinations)

General Comments

(1) Some of these agents may require testing in those institutions that harbor endemic or epidemic strains resistant to other drugs or for reporting to infection control as an epidemiologic aid.

NOTE: Information in boldface type is considered tentative for one year.

Test/Report Group	Antimicrobial Agent	MIC (µg/mL) Interpretive Standard			Comments
		S	I	R	
β-LACTAM/β-LACTAMASE INHIBITOR COMBINATIONS					
B	Ticarcillin-clavulanic acid	≤ 16/2	32/2-64/2	≥ 128/2	
CEPHEMS (PARENTERAL) (Including cephalosporins I, II, III, and IV. Please refer to Glossary I.)					
B	Ceftazidime	≤ 8	16	≥ 32	
TETRACYCLINES					
B	Minocycline	≤ 4	8	≥ 16	
FLUOROQUINOLONES					
B	Levofloxacin	≤ 2	4	≥ 8	
FOLATE PATHWAY INHIBITORS					
A	Trimethoprim-sulfamethoxazole	≤ 2/38	-	≥ 4/76	

S. maltophilia (blood)

	MIC (μg/ml)	
ceftazidime	32	R
levofloxacin	2	S
minocycline	1	S
ticarcillin-clav	16	S
trimeth-sulfa	0.5/9.5	S

These are the only drugs for which there are MIC breakpoints in M100-S16 Table 2B-4 (M7)

What if MD asks for results for other drugs on S. maltophilia?

Option to consider…..

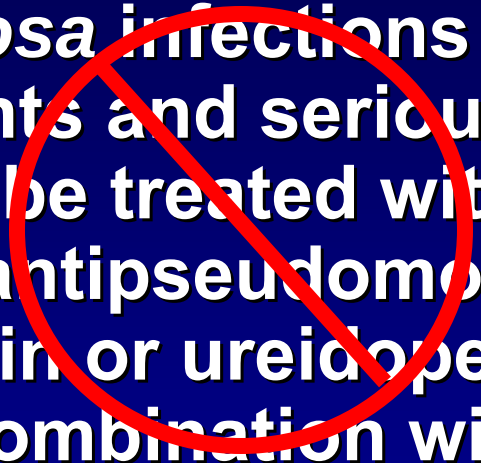
- **Get request in writing, preferably from Infectious Diseases clinician**
- **Test by MIC only**
- **Report results without interpretation**
- **Qualify results**

S. maltophilia (blood)

Report Option		MIC (μg/ml)	
	*aztreonam	8	
	ceftazidime	32	R
	levofloxacin	2	S
	minocycline	1	S
	ticarcillin-clav	16	S
	trimeth-sulfa	≤0.5/9.5	S

*No MIC interpretive criteria available; reported per Dr. Jones request; Infectious Diseases consult suggested

Rx: Comment Deleted *Pseudomonas aeruginosa*



"*Rx: P. aeruginosa* infections in granulocytopenic patients and serious infections in other patients should be treated with maximum doses of the selected antipseudomonal penicillin (carboxypenicillin or ureidopenicilllin) or ceftazidime in combination with an aminoglycoside."

Rationale for deletion – there are currently other options for treatment

CLSI M100-S16; Table 2B (M2, M7)

Colistin / Polymyxin B

- Currently, NO disk diffusion recommendations
- *Acinetobacter* spp.

Antimicrobial Agent	MIC (µg/ml)		
	S	I	R
Polymyxin B or colistin	≤2	-	≥4

- Breakpoints for other bugs forthcoming
- No FDA-cleared test

CLSI M100-S16; Table 2B (M7)

Haemophilus spp.
Neisseria meningitidis

- Procedures in M2, M7 and M100 are for *H. influenzae* and *H. parainfluenzae*
  - CLSI M45-P for other *Haemophilus* species
- *Neisseria meningitidis*
  - Added disk diffusion procedure

Standard Disk Diffusion Method for *Neisseria meningitidis*

New!

Table 2J. Zone Diameter Interpretive Standards and Equivalent Minimal Inhibitory Concentration (MIC) Breakpoints for *Neisseria meningitidis*

Testing Conditions	
Medium:	Mueller-Hinton agar with 5% sheep blood
Inoculum:	Direct colony suspension, equivalent to a 0.5 McFarland standard
Incubation:	35 ± 2 °C; 5% CO_2; 20 to 24 hours

Minimal QC Recommendations (See Tables 3 and 3A for acceptable QC ranges.)

Streptococcus pneumoniae ATCC® 49619 in 5% CO_2. *E. coli* ATCC® 25922 (incubated either in ambient air or 5% CO_2) should be used for ciprofloxacin, nalidixic acid, and minocycline.

General Comments

(1) Recommended precautions: Biosafety Level 2 (BSL2) practices are recommended for this organism. Whenever possible, procedures likely to generat aerosols should be performed within a biological safety cabinet.

(2) For some organism/antimicrobial agent combinations, the absence or rare occurrence of resistant strains precludes defining any results categories other than "susceptible." For strains yielding results suggestive of a "nonsusceptible" category, organism identification and antimicrobial susceptibility test results should be confirmed. Subsequently, the isolates should be saved and submitted to a reference laboratory that will confirm results using a CLSI reference dilution method.

NOTE: Information in boldface type is considered tentative for one year.

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter, Nearest Whole mm			Equivalent MIC Breakpoints (µg/mL)		Comments
			R	I	S	R	S	

CLSI M100-S16; Table 2J (M2)

Neisseria meningitidis
Disk Diffusion Testing

Medium: MHA with 5% sheep blood

Inoculum: direct colony suspension equivalent to 0.5 McFarland standard

Incubation: 35°C +/- 2°C; 5% CO_2; 20-24h

QC: *S. pneumoniae* ATCC 49619

E. coli ATCC 25922 (select drugs)

Biosafety Level 2 (BSL 2) safety practices; use biosafety cabinet

Neisseria meningitidis Disk Diffusion Testing

- Therapeutic agents*
  - *Penicillin
  - *Ampicillin
  - Cefotaxime
  - Ceftriaxone
  - Meropenem
  - Chloramphenicol

All drugs listed are in Test/Report Group C "Supplemental, Report Selectively"

*No disk diffusion breakpoints; must do MIC test

Neisseria meningitidis Disk Diffusion Testing

- "Breakpoints may be appropriate only for prophylaxis of meningococcal case contacts"
  - Azithromycin
  - Ciprofloxacin
  - Minocycline
  - Nalidixic acid (for surveillance only; may detect diminished fluoroquinolone susceptibility)
  - Rifampin
  - Trimethoprim-sulfamethoxazole (predicts susceptibility to sulfonamides also)

All drugs listed are in Test/Report Group C "Supplemental, Report Selectively"

Changes 2006
CLSI M100-S16
Gram Positives

GPC

Daptomycin

For…..

- Staphylococcus
- Enterococcus
- Streptococcus

- Deleted daptomycin disk diffusion breakpoints
  - Rationale for deletion – inability of disk diffusion method to consistently detect those few isolates that are non-susceptible to daptomycin
- Specify agar dilution testing has not been validated for daptomycin
- FDA-cleared for several commercial systems

Staphylococcus spp.

Clarifications…

- For disk diffusion testing, cefoxitin disk is..
  - Preferred over oxacillin disk for detection of *mec*A-mediated resistance
  - A "surrogate" for oxacillin (report oxacillin NOT cefoxitin)
  - Should always be used for *S. lugdunensis* (do not use oxacillin disk)
- Fluoroquinolones – breakpoints for gatifloxacin, levofloxacin, moxifloxacin, and ofloxacin still tentative (for another year)

Cefoxitin Disk for *mec*A-mediated Resistance in Staphylococci

	Cefoxitin zone (mm)	
	Res	Susc
S. aureus	≤19*	≥20**
S. lugdunensis	≤19*	≥20**
CoNS	≤24*	≥25**

* Report as oxacillin resistant

** Report as oxacillin susceptible

CoNS, coagulase-negative staphylococci

CLSI M100-S16; Table 2C (M2)

MIC Breakpoints (μg/ml) *Staphylococcus* and Fluoroquinolones

	Old (M100-S14)*			M100-S15, M100-S16 **		
	S	I	R	S	I	R
Gatifloxacin	≤2	4	≥8	≤0.5	1	≥2
Levofloxacin	≤2	4	≥8	≤1	2	≥4
Moxifloxacin	none			≤0.5	1	≥2

*Same as current FDA breakpoints
**Tentative for another year
Check M100-S16 for corresponding disk diffusion breakpoints

Staphylococcus spp.

Additions/changes…

- Modified vancomycin MIC breakpoints for *S. aureus*
- Table highlighting use of BHI-vancomycin agar for *S. aureus*

CLSI Vancomycin MIC (µg/ml) Breakpoints – *S. aureus*

2006		
≤2	4-8	≥16

2005		
≤4	8-16	≥32

Notes:

2005 same as current FDA breakpoints

No change in disk diffusion breakpoints

No change for coagulase-negative staphylococci

Why did CLSI modify vancomycin breakpoints for S. aureus?

- To detect emerging vancomycin resistance
  - Data shows patients fail vancomycin therapy when infected with *S. aureus* with vancomycin MICs of ≥4 µg/ml
  - Clinical labs have been advised by CDC to investigate *S. aureus* with vancomycin MICs of ≥4 µg/ml as potential VISA or VRSA
  - Some VISA test 4 µg/ml by some methods

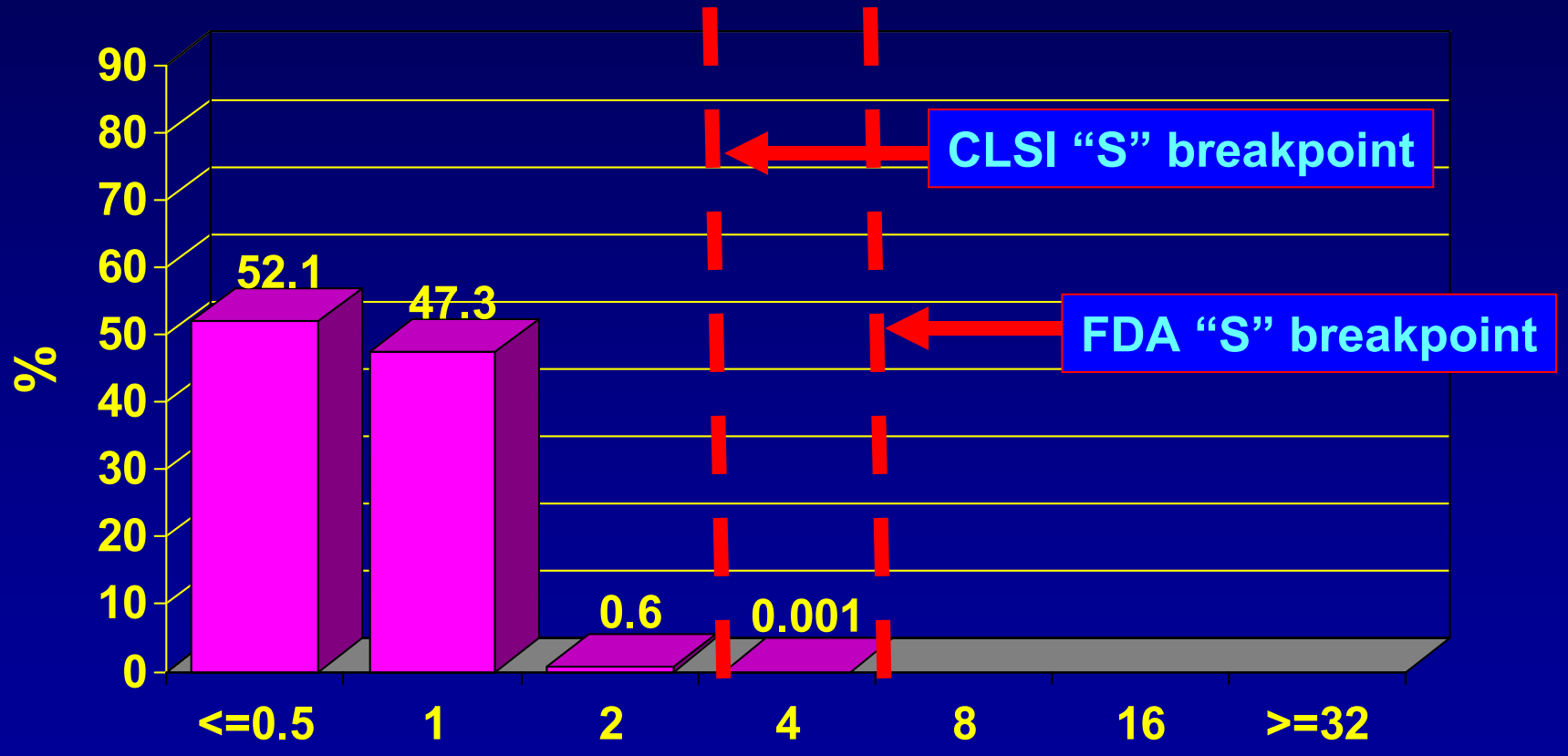
Modifying CLSI Breakpoints

CLSI has mechanism for reevaluation of breakpoints when needed as defined in **CLSI M23-A2** "Development of In Vitro Susceptibility Testing Criteria and QC Parameters"

Will it matter what vancomycin breakpoints we use for S. aureus?

- "Either FDA or CLSI susceptibility interpretive breakpoints are acceptable to clinical laboratory accrediting bodies" **(CLSI M100-S16, page 14)**
- …and we should all pursue *S. aureus* with vancomycin MICs ≥4 µg/ml since these could be VISA (or VRSA if ≥16 µg/ml)
  http://www.cdc.gov/ncidod/dhqp/ar_visavrsa_algo.html
- So, if we ALL diligently pursue vancomycin MICs of ≥4 µg/ml in *S. aureus*, it really won't matter

S. aureus - Vancomycin MICs* UCLA 1/00-10/05 (n=13981)



*CLSI broth microdilution reference method

Table 2C. (Continued)

Screening Tests for Oxacillin Resistance and Reduced Susceptibility to Vancomycin in *Staphylococcus aureus*

Screen Test	Oxacillin Resistance	Reduced Susceptibility to Vancomycin
Medium	MHA with NaCl (4% w/v; 0.68 mol/L)	BHI agar
Antimicrobial concentration	6 µg/mL oxacillin	6 µg/mL vancomycin
Inoculum	Direct colony suspension to obtain 0.5 McFarland turbidity Using a 1 µL loop that was dipped in the in ipped a nt.	Direct colony suspension to obtain 0.5 McFarland turbidity. Preferably, using a micropipette, spot a 10 µL drop onto agar surface. Alternatively, using a swab dipped in the suspension and the excess liquid expressed, spot an area 10 to 15 mm in diameter or streak a portion of the plate.
		35 °C; ambient air 24 hours
Results	>1 colony = resistant Examine carefully with transmitted light for >1 colony or light film of growth.	>1 colony = presumptive reduced susceptibility Examine carefully with transmitted light for >1 colony or light film of growth. Perform vancomycin MIC using a validated MIC method to confirm reduced susceptibility
QC Recommendations	*Staphylococcus aureus* ATCC® 29213 - Susceptible *Staphylococcus aureus* ATCC® 43300 - Resistant	*Enterococcus faecalis* ATCC® 29212 - Susceptible *Enterococcus faecalis* ATCC® 51299 - Resistant

BHI-Vancomycin (6 µg/ml) Screen

- **Add BHI-V screen if using automated system (unless fixed, check with manufacturer) or use CLSI MIC reference method or Etest to detect reduced susceptibility to vancomycin in *S. aureus***
- **For workup of VISA and VRSA, see http://www.cdc.gov/ncidod/dhqp/ar_visavrsa_algo.html**

VRSA (as of 12/05)

	MIC (ug/ml) [1]	Source	Date	Location
1	1024	foot ulcer	4/02	Michigan
2	32	foot ulcer	9/02	Pennsylvania
3	64	nephrostomy tube	3/04	New York
4	256	foot ulcer	2/05	Michigan
5	512	wound	10/05	Michigan

[1] Reference broth microdilution MIC

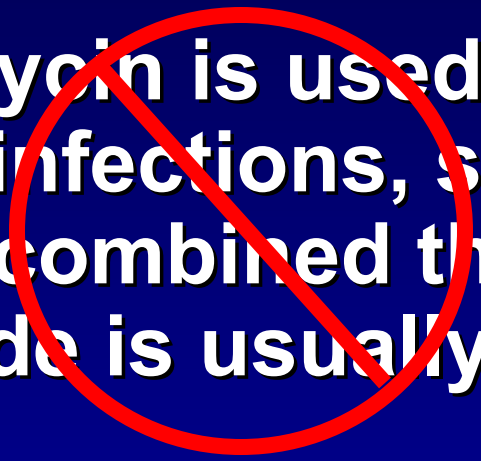
Enterococcus spp.

Additions/changes…

- Deleted vancomycin-synergy *Rx* comment
- Added definitions for high-level aminoglycoside resistance (HLAR) testing for disk diffusion (similar to those for MIC testing)

Enterococcus *Rx* Comment Deleted

- ***Rx:*** **If vancomycin is used for serious enterococcal infections, such as endocarditis, combined therapy with an aminoglycoside is usually indicated.**



- **Rationale for deletion – use of vancomycin should not be encouraged; ampicillin or penicillin are the preferred agents to use in combination therapy for enterococci**

CLSI M100-S16; Table 2D (M2, M7)

Enterococcus spp.
Disk diffusion screen for HLAR

Table 2D. (Continued)

Disk Diffusion Screening Tests for High-Level Aminoglycoside Resistance (HLAR)[a]

Test/Report Group	Antimicrobial Agent	Disk Content	Zone Diameter, Nearest Whole mm			Equivalent MIC Breakpoints (µg/mL)		Comments
			R[b]	I[c]	S[d]	R	S	
C	Gentamicin (HLAR)	120 µg	≤ 6	7-9	≥ 10	> 500	≤ 500	(15) If the zone is 7 to 9 mm, the test is inconclusive, and an agar dilution or broth microdilution screen test should be performed to confirm resistance. See comments (2) and (7).
C	Streptomycin (HLAR)	300 µg	≤ 6	7-9	≥ 10	-	-	(15) MIC correlates for streptomycin broth microdilution are resistant >1000 µg/mL and for agar dilution >2000 µg/mL. See comments (2), (7), and (15).

Footnotes

a. For QC of HLAR screen tests, use *Enterococcus faecalis* ATCC® 29212 (see Table 3, Footnote f [Disk Testing] for acceptable QC ranges).

b. Resistant, will not be synergistic with cell-wall-active agent (e.g., ampicillin, penicillin, vancomycin).

c. Inconclusive, perform an agar dilution or broth microdilution test to confirm.

d. Susceptible, will be synergistic with cell-wall-active agent (e.g., ampicillin, penicillin, vancomycin) that is also susceptible.

See M7, Table 2D (MIC Testing) which summarizes additional screening tests for vancomycin, high-level aminoglycoside resistance, and supplemental tests for identification that may be helpful for vancomycin-resistant enterococci.

Enterococcus spp.
Disk diffusion Screen for HLAR

- **Resistant** -- will not be synergistic with cell-wall-active agent (e.g., ampicillin, penicillin, vancomycin)
- **Inconclusive** -- perform an agar dilution or broth microdilution test to confirm
- **Susceptible** -- will be synergistic with cell-wall-active agent (e.g., ampicillin, penicillin, vancomycin) that is also susceptible

CLSI M100-S16; Table 2D (M2)

Streptococcus pneumoniae
Modified reporting recommendations for meropenem…

- "Penicillin **and** cefotaxime or ceftriaxone **or** meropenem should be tested by a reliable MIC method and reported routinely with **CSF isolates** of ***S. pneumoniae.***"

CLSI M100-S16; Table 2G (M2, M7)

New Antimicrobial Agents in M100-S16*

Agent	Drug class	Route of administration	FDA approved
ceftobiprole	cephem	IV	No
faropenem	penem	PO	No

*In Glossary and QC tables only

Ceftobiprole

- **Manufacturer:**
  - Johnson & Johnson
- **Possible clinical use:**
  - Nosocomial pneumonia
  - Complicated skin and skin structure infections

Ceftobiprole (con't)

- **Microbiological activity:**
  - *Staphylococci* (including MRSA)
  - *Streptococci* (including penicillin-R *S. pneumoniae*)
  - *Enterococcus faecalis*
  - Most *Enterobacteriaceae*
  - *Haemophilus influenzae* (including BLNAR)
  - Many *Pseudomonas aeruginosa* and *Acinetobacter baumanii*
- **Limited activity:**
  - Many non-*Enterobacteriaceae* [Gram-negatives]
  - *Enterococcus faecium*
  - *ESBL*-producers
  - Metallo-beta-lactamase producers
  - Beta-lactamase-producing anaerobes

Faropenem

- **Manufacturer:**
  - Replidyne
- **Possible clinical use:**
  - Bacterial sinusitis
  - Acute exacerbations of chronic bronchitis
  - Community-acquired pneumonia
  - Uncomplicated skin and skin structure infections

Faropenem (con't)

- **Microbiological activity:**
  - Respiratory pathogens *(S. pneumoniae, H. influenzae, M. catarrhalis)*
  - *S. pyogenes*
  - MSSA
  - Some *Enterobacteriaceae*
- **Limited activity:**
  - *Non-Enterobacteriaceae*
  - *Enterobacter* spp.
  - *Enterococcus faecium*
  - MRSA

Changes 2006
CLSI M100-S16
Quality Assessment / Quality Control

QA/QC

Quality Control

ADDITIONS / Changes…

- **Added QC ranges:**
  - **Ceftobiprole**
  - **Faropenem**
  - **Drugs for testing *Campylobacter jejuni* ATCC 33560 using broth dilution method**

QC Tables

Table	M2 (disk diffusion)	M7 (MIC testing)
3	Nonfastidious	Nonfastidious
3A	Fastidious	Fastidious (broth dilution)
3B	DD QC Testing Frequency	Fastidious (agar dilution) New!
3C	DD Troubleshooting Guide New!	Fastidious (broth dilution + supplement)*
3D	NA	Fastidious (Brucella broth dilution)* New!

DD, disk diffusion; NA, not applicable;
*when testing potential agents of bioterrorism

QC Tables (con't)

Table	M2	M7
3E	NA	MIC QC Testing Frequency
3F	NA	MIC Troubleshooting Guide New!

*NA, not applicable

Table 3C. Disk Diffusion QC Troubleshooting Guide

New!

Table 3C. Disk Diffusion QC Troubleshooting Guide

This table provides guidance for troubleshooting and corrective action for out-of-range quality control, primarily using antimicrobial susceptibility tests with Mueller Hinton Agar. Refer to M2-A9, Disk Diffusion, Section 15, Quality Control Procedures and Appendix A, Quality Control Protocol Flow Charts for additional information. Out-of-range quality control tests should first be repeated. If the issue is unresolved, this troubleshooting guide provides additional suggestions for troubleshooting out-of-range quality control results. In addition, if unresolved, manufacturers should be notified of potential product problems.

General Comments

(1) QC organism maintenance: avoid repeated subcultures. Retrieve new QC strain from stock. If using lyophilized strains, follow the maintenance recommendations of the manufacturer. Store *E. coli* ATCC® 35218 and *K. pneumoniae* ATCC® 700603 stock cultures at -60 °C or below and prepare working stock cultures weekly.

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments/Action
Aminoglycosides	Any	Zone too small	pH of media too low	Acceptable pH range = 7.2-7.4 Avoid CO_2 incubation which lowers pH.
Aminoglycosides	Any	Zone too large	pH of media too high	Acceptable pH range = 7.2-7.4
Aminoglycosides	*P. aeruginosa* ATCC® 27853	Zone too small	Ca++ and/or Mg ++ content too high	Use alternative lot of media.
Aminoglycosides	*P. aeruginosa* ATCC® 27853	Zone too large	Ca++ and/or Mg ++ content too low	Use alternative lot of media.
Amoxicillin-clavulanic acid	*E. coli* ATCC® 35218	Zone too small	Clavulanic acid is labile Disk has lost potency	Use alternative lot of disks. Check storage conditions and

Disk Diffusion QC Troubleshooting Guide Table 3C (M2) *Examples:*

Antimicrobial Agent	QC Strain	Observation	Probable Cause	Comments / Action
Beta-lactam group	Any	Zone initially acceptable but decreases and possibly out-of-range over time	Disk has lost potency	Use alternative lot of disks. Check storage conditions and package integrity. Imipenem, cefaclor, and clavulanic acid are especially labile.
Quinolones	Any	Zone too large	pH of media too high	Acceptable pH range =7.2=7.4

CLSI M100-S16; Table 3C (M2)

CLSI Standards - 2006

- **M100-S16 Tables (2006)***

  …..to be used with text documents explaining how to perform the tests….

  M2-A9 Disk Diffusion (2006)**

  M7-A7 MIC (2006)**

New!

* M100 updated yearly

**M2, M7 updated every 3 years

CLINICAL AND LABORATORY STANDARDS INSTITUTE™

M2-A9, M7-A7
Primary Changes

- Primarily expanded discussions and detailed recommendations for test procedures in M100-S16 including those for:
  - Oxacillin-resistant staphylococci
  - *Streptococcus pneumoniae*
  - *Streptococcus* spp.
  - *Neisseria meningitidis*

M2-A9, M7-A7
Primary Changes (con't)

- Additions to antimicrobial agent descriptions
- Additional tips for media/reagent preparation
- Supplemental QC suggestions

Last pages of M2 and M7

Summary of Comments and Subcommittee Responses

Clinical and Laboratory Standards Institute consensus procedures include an appeals process that is described in detail in Section 8 of the Administrative Procedures. For further information, contact CLSI or visit our website at www.clsi.org.

Summary of Comments and Subcommittee Responses

M2-A8: *Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eighth Edition*

General

1. Could you please clarify a point for me about cefuroxime testing? Your standard gives different zone size criteria for the parenteral and oral forms of the drug and specifies that different discs should be used. Is the implication that you could get an isolate testing sensitive with the one disk and resistant with the other? If this is true, could you use the parenteral (cefuroxime sodium) disk to predict sensitivity for both forms, accepting that you may miscall some strains resistant that would respond to oral, or is it the other way around? The standard gives no details about these particular recommendations.

- **Although there are two formulations for cefuroxime, one for parenteral and one for oral administration, there is only one disk for laboratory testing. Different interpretive criteria were developed based on the different pharmacodynamic/pharmacokinetic data and clinical indications for the two formulations. Tables 2A through 2J should be used to guide interpretation for individual organisms.**

Q&A Example (paraphrased)

- Q – Why is D zone test not recommended for *Streptococcus pneumoniae*?
- A – Isolates of *Streptococcus pneumoniae* can have *erm*-mediated resistance to erythromycin. However, the vast majority of these are also resistant to clindamycin (constitutive phenotype). Rare isolates of pneumococci may have inducible resistance; however the clinical significance of this has not been established. Therefore, routine testing for inducible clindamycin resistance is not recommended for this species.

Issues Under Discussion by CLSI

- Additional recommendations for testing colistin / polymyxin B
- Disk diffusion test for *Campylobacter* spp.
- Detection of ESBLs
- Review recommendations for drugs to Test / Report (Table 1)
- Improved communication of CLSI AST Subcommittee decisions

Material on your CD-ROM…

1. PowerPoint presentation
2. PDF of “New Antimicrobial Agent Pathway” slide (slide #10)
3. M100-S16 checklist
4. References
5. CLSI information flier
6. CLSI catalogue

To Ask a Question….

- Please send to neoffice@nltn.org
- Questions will be compiled and answers will be published on http://www.phppo.cdc.gov/nltn/nphtcs/ast012506.aspx

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http://www.phppo.cdc.gov/nltn/default.aspx

Thank you!