

2009 CLSI M100-S19 Update

Nebraska Public Health Laboratory

Discussion points

- Changes most important to routine antimicrobial susceptibility testing.
- Documents available
 - Janet Hindler discussion slide handout
 - Janet Hindler M100-S19, M02-A10, and M07-A8 checklist
 - If you have budgetary issues purchasing M100-S19, please call NPHL or me (402) 559-2122.

#1-format of tables has changed

- Disk diffusion and MIC tables are on the same page. Very nice addition.
- Go to a quiet place and familiarize yourself with format and tables so you can easily move to appropriate place in manual. Changes are noted in bold.
- Pages 26-35. Read over these pages and re-familiarize yourself with antibiotics to test and report. Have changed some rules in regards to whether one can predict susceptibility based on a report from a similar antibiotic.

#2-non-susceptible category

- Newer antibiotics (e.g. daptomycin) where there have not been enough resistant isolates obtained to determine resistant breakpoints.
- Report as NS with a comment (after confirming with another methodology)

#3-Penicillin susceptible Staphylococci

- If staphylococcal isolate is susceptible to penicillin, confirm with other methodology (nitrocephin) after induction with oxacillin (on agar plate)

#4-Detection of oxacillin-resistance in the staphylococci

- Cefoxitin detects oxacillin-resistance better than oxacillin. Has to do with induction of *mecA* through regulatory loci.
- Still zone diameter breakpoints for oxacillin against *S. aureus* and *S. lugdunensis*, but not coagulase-negative staphylococci.

#5-Detection of VISA using disk diffusion

- Do not use disk diffusion test to detect vancomycin resistance in the staphylococci. Use alternate methodology if disk diffusion is method of choice.
- If you have a suspect VISA strain, please call NPHL and we can advise on best ways to confirm.
- New discussion on Hetero-resistance (VISA) against vancomycin.

#6 High level mupirocin resistance in *S. aureus*

- Page 122-Isolates with a mupirocin MIC ≥ 512 are difficult to eradicate in the nares.
- Important for those institutions that are screening patients for MRSA colonization.

#7-Carbapenemases within the *Enterobacteriaceae*

- KPC carbapenemases
- Difficult to detect using current MIC breakpoints.
- Isolates that have an MIC of 2 $\mu\text{g/ml}$ to ertapenem or an MIC of 2-4 $\mu\text{g/ml}$ to meropenem or imipenem.
- Modified Hodge test is confirmatory. Discussed in manual. PCR is gold standard.

#7-Carbapenemases within the *Enterobacteriaceae*

- Remember that other mechanisms of carbapenem-resistance are known (e.g. porin mutation in addition to ESBL or AmpC production).
- KPC plasmid mediated-resistance may be a more difficult infection control issue due to plasmid transfer.
- KPC carbapenemases hydrolyze expanded spectrum cephalosporins in addition to carbapenems

Mechanisms of Carbapenem Resistance

- Carbapenemase hydrolyzing enzymes
- Porin loss "OprD"
- ESBL or AmpC + porin loss

Carbapenemases

- The most versatile family of β -lactamases
- Two major groups based on the hydrolytic mechanism at the active site
 - Serine at the active site: class A and D
 - Zinc at the active site: class B
- All carbapenemases hydrolyze penicillins, extended spectrum cephalosporins, and carbapenems

Carbapenemase Classification

Molecular Class	A	B	D
Functional Group	2f	3	2d
Aztreonam Hydrolysis	+	-	-
EDTA Inhibition	-	+	-
Clavulanate Inhibition	+	-	±

Klebsiella pneumoniae

- Ampicillin R
- Piperacillin R
- Cephalothin R
- Cefoxitin S
- Cefotaxime R
- Ceftazidime I
- Ceftriaxone R
- Aztreonam I
- Cefepime S
- Pip/Tazo R
- Imipenem I
- Might need to screen for carbapenemase

Carbapenemases Class A

- First identified 1982 in UK
- Four major families
- Chromosomally encoded
 - *Serratia marcescens* enzyme (SME)
 - Not metalloenzyme carbapenemases (NMC)
 - Imipenem-hydrolyzing β -lactamases (IMI)
- Plasmid encoded
 - *Klebsiella pneumoniae* carbapenemases (KPC)
 - Guiana Extended-Spectrum (GES)

KPC

- Molecular class A and functional group 2f
- Inhibited by clavulanic acid but not by EDTA
- Confers resistance to ALL β -LACTAM antibiotics
- Plasmid-encoded
 - Associated with other resistant genes (aminoglycosides, fluoroquinolones)
 - Transferable

KPC Epidemiology

- Predominantly in *K. pneumoniae* (KP)
- Reported in *Enterobacter* spp., *Salmonella* spp., *E. coli*, *P. aeruginosa*, and *Citrobacter* spp.
- First identified in KP clinical isolate from North Carolina in 1996 (KPC-1)
- KPC-2, -3, and -4 have been reported.
- Mostly identified on the East coast

KPC Epidemiology

- KPC producers have been identified outside USA
 - France
 - Brazil
 - Columbia
 - China
- Not detected at the University of Nebraska Medical Center
 - 45 ESBL-like isolates collected-6 had elevated carbapenem MICs-none contained KPC

When to Suspect a KPC Producer

- *Enterobacteriaceae*
- Resistance to extended spectrum cephalosporins (cefotaxime, ceftazidime, and ceftriaxone)
- Variable susceptibility to cephamycins (cefoxitin, cefotetan)
- Carbapenem MICs $\geq 2 \mu\text{g/ml}$

How to Detect a KPC Producer

- Antimicrobial susceptibility tests (ASTs)
 - MIC
 - Carbapenem MIC $\geq 2 \mu\text{g/ml}$
 - Disk diffusion
 - Carbapenem: "I" or "R"
 - Among carbapenems, ertapenem:
 - Most sensitive
 - less specific

Anderson et al. 2007. JCM 45 (8): 2723

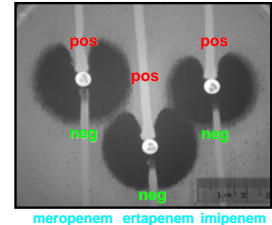
How to Detect a KPC Producer

- Commercial systems
 - Inconsistent detection of KPC-producing isolates
 - » Tenover et al. 2006. EID. 12:1209-1213
 - Breakpoints do not match CLSI recommendations

Definitive ID of a KPC Producer

- Modified Hodge test
 - 100% sensitivity to detect KPC

1. Swab *E. coli* ATCC 25922 onto plate to create lawn. Place imipenem disk in center.
2. Streak test isolates from edge of disk to end of plate.
3. Incubate overnight.
4. Look for growth of *E. coli* around test isolate streak - indicates carbapenem-hydrolyzing enzymes.



Janet Hindler, What's New in the 2008 CLSI Standards for (AST)?

Definitive ID of a KPC Producer

- PCR
 - The method of choice to confirm KPC

#8-Purchase QC strains

- ATCC BAA-1708- *mupA* *S. aureus* isolate
- ATCC BAA-1705 and BAA-1706. Positive and Negative modified Hodge test isolates, respectively.
- New ampicillin, piperacillin and ticarcillin QC tests for *E. coli* ATCC35218. Pages 92 and 100

**#9-*Enterobacteriaceae*-New
cephalosporin breakpoints**

- Probably next year, susceptible breakpoints will go from ≤ 8 $\mu\text{g/ml}$ to ≤ 1 $\mu\text{g/ml}$.
- Expanded-spectrum cephalosporins, cefazolin, and aztreonam.