

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

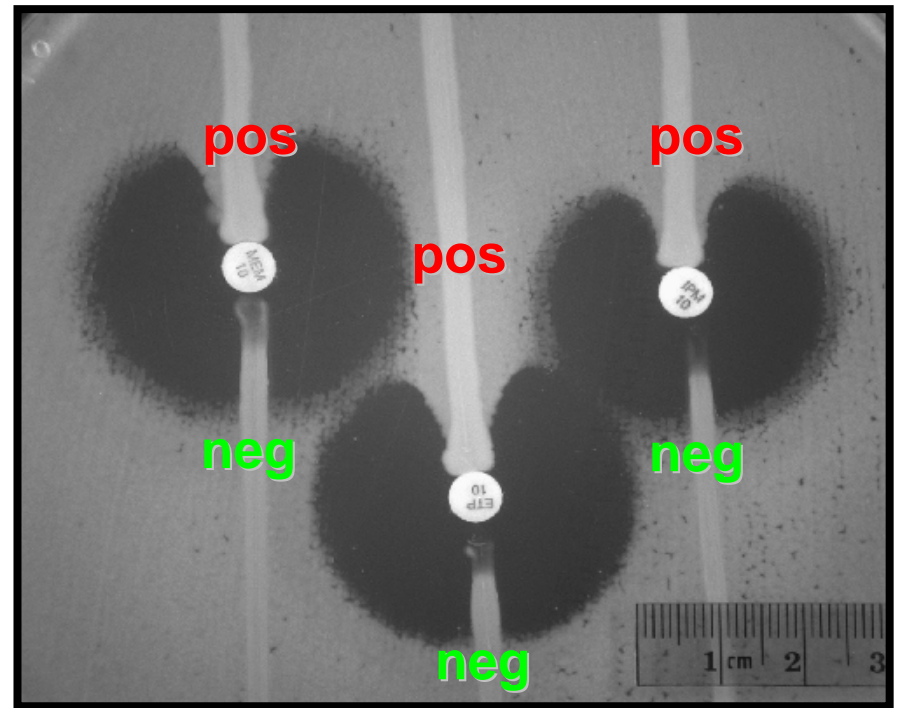
How to Detect a KPC Producer

- 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 enzyme.



meropenem ertapenem imipenem

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 $\leq 8 \mu\text{g/ml}$ to $\leq 1 \mu\text{g/ml}$.
- Expanded-spectrum cephalosporins, cefazolin, and aztreonam.