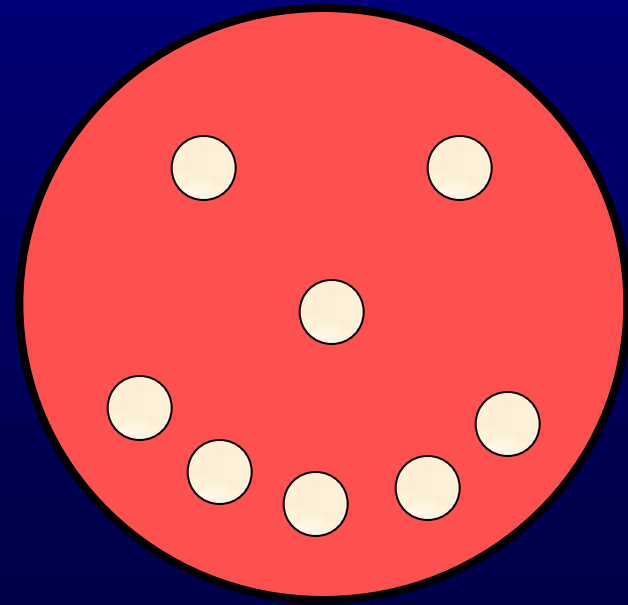


# Antimicrobial Susceptibility Testing (AST) Update

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*...also consultant with the Association  
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# At the conclusion of this talk, you will be able to.....

- ◆ Discuss detecting and reporting of multidrug-resistant (MDR) gram-negative bacteria.
- ◆ Describe current recommendations for reporting vancomycin for *Staphylococcus aureus*.
- ◆ Discuss strategies for coping with continual changes in recommendations for AST and reporting.



OCTOBER 30, 2008

**THE CAMPAIGN TRAIL**  
A remarkable discussion with Jaw Meyer, Ryan Lizza, Heidi Hertzberg, and Dorothy Wisniewski.



**BLOGS**  
James Surowiecki describes what happens when the news doesn't check its facts.  
Heidi Hertzberg on the last-ditch efforts of the Republican Party.  
Steve Coll shares the primary sources for the musical "Dr. Atomic."  
George Parker compares anti-Obama blogging to the political rumors he heard in Iraq.  
Roger Angell wishes Tim McCarver referenced Jean Simmons not Gene Simmons.  
Sasha Freire-Jones on how the Corliss took over YouTube's homepage.  
**The Book Bench:** Cartoonists talk about books. Garfield's mama

MEDICAL DISPATCH

## SUPERBUGS

The new generation of resistant infections is almost impossible to treat

by Jerome Groopman

AUGUST 11, 2008

TEXT SIZE: A | A+ | A+  
PRINT | EMAIL | FEEDS

In August, 2000, Dr. Roger Wetherbee, an infectious-disease expert at New York University's Tisch Hospital, received a disturbing call from the hospital's microbiology laboratory. At the time, Wetherbee was in charge of handling outbreaks of dangerous microbes in the hospital, and the laboratory had isolated a bacterium called *Klebsiella pneumoniae* from a patient in an intensive-care unit. "It was literally resistant to every meaningful antibiotic that we had," Wetherbee recalled recently. The microbe was sensitive only to a drug called colistin, which had been



Doctors fear that dangerous bacteria may become entrenched in hospitals.

KEYWORDS

**Features...**  
**MDR *Klebsiella***  
**Other MDR GNR**

**Features...**  
**ESBLs**  
**MDR**  
***A. baumannii***  
***P. aeruginosa***  
***E. aerogenes***

THE INFORMED PATIENT | OCTOBER 1, 2008

## 'Superbugs' That Strike the Sickest Patients

In hospitals' war against drug-resistant superbugs, a class of bacteria once thought to be fairly benign is emerging as a deadly threat to the sickest and most vulnerable patients. The scourge -- known as gram-negative bacteria -- is throwing a new wrench into efforts to contain the spread of deadly infections.

Amid more than 1.7 million infections annually in hospitals, prevention efforts have been aimed at the most widespread organisms, like the staph infection MRSA and others in the so-called gram-positive category. These can still be thwarted by antibiotics such as vancomycin.

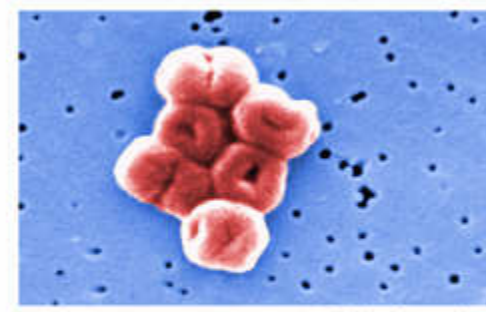


Photo Researchers

Acinetobacter is becoming resistant to all antibiotics.

But some of these bugs' wily cousins -- which don't pick up the purplish dye used in the test to distinguish them from gram-positive bacteria -- are becoming ultra-resistant. The extra outer membrane that rejects the stain also gives them additional armor against antibiotics. Some also produce an enzyme, known as ESBL, that enables them to break down antibiotics and develop even more resistance.

While they don't cause disease in healthy people, infections by gram-negative bacteria

# International effort ongoing to standardize definitions for:

- ◆ **MDR** – multidrug-R  
(e.g., “R” to  $\geq 3$  drug classes)
- ◆ **XDR** – extensively drug-R  
(e.g., “R” to almost all classes but retains “S” to at least one drug class)
- ◆ **PDR** – pandrug-R  
(e.g., “R” to all drug classes)

**Definitions apply to “acquired” (vs. “intrinsic”) resistance and to drugs that might be used to treat an infection caused by the species.**

# Management of Multidrug-Resistant Organisms In Healthcare Settings, 2006

Jane D. Siegel, MD; Emily Rhinehart, RN MPH CIC; Marguerite Jackson, PhD; Linda Chiarello, RN MS; the Healthcare Infection Control Practices Advisory Committee

**Acknowledgement:**

The authors and HICPAC gratefully acknowledge Dr. Larry Strausbaugh for his many contributions and valued guidance in the preparation of this guideline.



**“Individual facilities should seek appropriate guidance and adopt effective measures that fit their circumstances and needs.” (page 31)**

<http://www.cdc.gov/ncidod/dhqp/>

**Specimen: Fluid**

**Diagnosis: Surgical wound infxn**

***Serratia marcescens***

**Case #1**

	<u>MIC (<math>\mu\text{g/ml}</math>)</u>
amikacin	1 S
ampicillin	>32 R
cefazolin	>32 R
ceftriaxone	$\leq 0.5$ S
ciprofloxacin	$\leq 0.25$ S
gentamicin	$\leq 0.5$ S
imipenem	>16 R
piper-tazobactam	$\leq 8$ S
tobramycin	1 S
trimeth-sulfa	$\leq 1/19$ S

***What about imipenem? cephalosporins?***

# ***Results verified with standard disk diffusion test...***



# Appendix G. Screening and Confirmatory Tests for Carbapenemases in Enterobacteriaceae

## Appendix G. Screening and Confirmatory Tests for Suspected Carbapenemase Production in *Enterobacteriaceae*

It is not necessary to test an isolate for a carbapenemase by the modified Hodge test when all of the carbapenems that are reported by a laboratory test either intermediate or resistant (ie, these carbapenem susceptibility results should be reported as tested). However, the modified Hodge test may be useful in this case for infection control and epidemiological purposes.

Test	Initial Screen Test		Phenotypic Confirmatory Test
When to do this test:			Positive screening test and resistance to one or more agents in cephalosporin subclass III (ie, cefoperazone, cefotaxime, ceftazidime, ceftizoxime, and ceftriaxone)
Test Method	Disk diffusion	Broth microdilution	Modified Hodge test
Medium	MHA	CAMHB	MHA
Antimicrobial concentration	Ertapenem 10 µg or Meropenem 10 µg  (NOTE: The imipenem disk test performs poorly as a screen for carbapenemases.)	Ertapenem 1 µg/mL or Imipenem 1 µg/mL or Meropenem 1 µg/mL	Ertapenem disk 10 µg or Meropenem disk 10 µg
Inoculum	Standard disk diffusion recommendations	Standard broth dilution recommendations	(1) Prepare a 0.5 McFarland standard suspension (using either direct colony suspension or growth method) of <i>E. coli</i> ATCC® 25922 (the indicator organism) in broth or saline and dilute 1:10 in saline or

**MHT**

**CLSI  
M100-S19.**

# ***What are the mechanisms of carbapenem resistance in Enterobacteriaceae...***

- ◆ **Carbapenemase** ( $\beta$ -lactamase that hydrolyzes carbapenems)
  - Exmp. KPC (*Klebsiella pneumoniae* carbapenemase)
- ◆ **Cephalosporinase combined with porin loss**
  - Some cephalosporinases (e.g., AmpC  $\beta$ -lactamases or ESBLs) have low-level carbapenemase activity
  - Porin loss limits entry of carbapenem into the cell

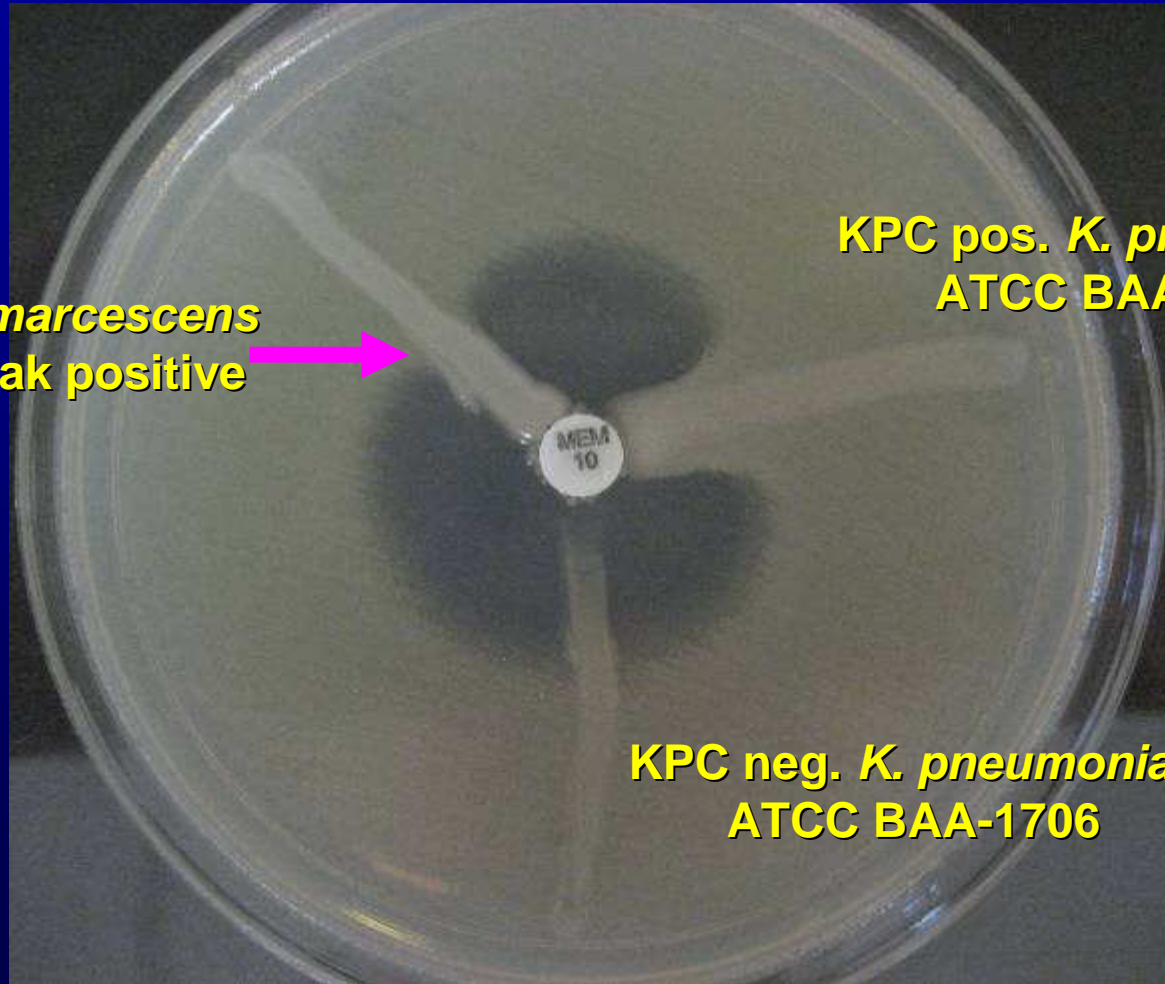
# Modified Hodge Test

*S. marcescens*  
weak positive



KPC pos. *K. pneumoniae*  
ATCC BAA-1705

KPC neg. *K. pneumoniae*  
ATCC BAA-1706



## ***Molecular testing revealed SME-2. Where does SME fit among carbapenemases?***

<b>Molecular Class</b>	<b>Carbapenemase</b>	<b>Found in:</b>
<b>A</b>	<b>KPC</b>	<i>K. pneumoniae</i> and other Enterobacteriaceae
	<b>SME</b>	<i>S. marcescens</i>
	also IMI, NMC, GES	Enterobacteriaceae
<b>B</b>	Metallo beta-lactamases (IMP, VIM, GIM, SPM)	<i>P. aeruginosa</i> , Enterobacteriaceae, <i>Acinetobacter</i> , <i>S. maltophilia</i>
<b>D</b>	<b>OXA</b>	<i>Acinetobacter baumannii</i>

**Adapted from Queenan & Bush. 2007. Clin Microbiol Rev. 20:440.**

# Some features of carbapenemases...

Carbapenemase	Hydrolysis profile		Inhibition profile	
	3 <sup>rd</sup> , 4 <sup>th</sup> gen cefs	Aztreonam	EDTA	Clavulanic acid
KPC	+	+	-	+
SME	+/-	+	-	+
MBL	+	-	+	-

3<sup>rd</sup> gen cefs, ceftriaxone, cefotaxime, ceftazidime

4<sup>th</sup> gen cef, cefepime

MBL, metallo beta-lactamase

Adapted from Queenan & Bush. 2007. Clin Microbiol Rev. 20:440.

# ***What is unique about SME as compared to KPC?***

- ◆ Gene located on **chromosome** (vs. KPC on plasmid)
- ◆ **Less hydrolysis** of ceftriaxone, cefotaxime, ceftazidime, cefepime than KPC (*S. marcescens* “S” to these in vitro; **? activity in vivo**)

**Majiduddin et al. 2005. Antimicrob Agents Chemother. 59:3421.**

**Queenan et al. 1992. Antimicrob Agents Chemother. 44:3035.**

- ◆ **Infrequently encountered:**

- 2000-2005

- 50,881 Enterobacteriaceae isolated from N. America, Latin America, Europe

- 5 SME (*S. marcescens*)!

**Castanheira et al. 2008. Antimicrob Agents Chemother. 52:570.**

**Specimen: Fluid**

Final report

**Diagnosis: Surgical wound infxn**

***Serratia marcescens***

**Case #1**

	<u>MIC (µg/ml)</u>
amikacin	1 S
ampicillin	>32 R
cefazolin	>32 R
ceftriaxone	≤0.5 S
ciprofloxacin	≤0.25 S
gentamicin	≤0.5 S
imipenem	>16 R
piper-tazobactam	≤8 S
tobramycin	1 S
trimeth-sulfa	≤1/19 S

**Report comment:**

**“Imipenem-R is due to carbapenemase production (but not KPC). The effectiveness of β-lactams in treating infections due to carbapenemase-producing *S. marcescens* has not been established. ID consult suggested.”**

# Selective Reporting Option

## *Serratia marcescens*


	<u>MIC (<math>\mu\text{g/ml}</math>)</u>
amikacin	1 S
ampicillin	>32 R
cefazolin	>32 R
ceftriaxone	$\leq 0.5$ S
ciprofloxacin	$\leq 0.25$ S
gentamicin	$\leq 0.5$ S
imipenem	>16 R
piper-tazobactam	$\leq 8$ S
tobramycin	1 S
trimeth-sulfa	$\leq 1/19$ S

Some may suppress carbapenem result if “S” to ceftriaxone and piperacillin-tazobactam

....but, always report “unexpected resistance”. (CLSI M100-S19. Table 1. Note 1. p 33. )

[http://www.cdc.gov/ncidod/dhqp/ar\\_kp\\_lab.html](http://www.cdc.gov/ncidod/dhqp/ar_kp_lab.html)

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## *Klebsiella pneumoniae* (*K. pneumoniae*)

[Overview](#) | [Lab Testing & Practices](#)

### Laboratory Testing and Practices for (*K. pneumoniae*)

#### Procedures

- ↓ [Laboratory Protocol for Detection of Carbapenem-Resistant or Carbapenamase-Producing, Klebsiella spp. and E. coli from Rectal Swabs](#) PDF (110 KB / 6 pages)
- ↓ [Modified Hodge Test for Carpenamase Detection in Enterobacteriaceae](#) PDF (127 KB / 3 pages)

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Multi-Level Antimicrobial  
Susceptibility Testing Education

# MMWR March 20, 2009 (1)

## <http://www.cdc.gov/mmwr/>



## Guidance for Control of Infections with Carbapenem-Resistant or Carbapenemase-Producing *Enterobacteriaceae* in Acute Care Facilities

Infection with carbapenem-resistant *Enterobacteriaceae* (CRE) or carbapenemase-producing *Enterobacteriaceae* is emerging as an important challenge in health-care settings (1). Currently, carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is the species of CRE most commonly encountered in the United States. CRKP is resistant to almost all available antimicrobial agents, and infections with CRKP have been associated with high rates of morbidity and mortality, particularly among persons with prolonged hospitalization and those who are critically ill and exposed to invasive devices (e.g., ventilators or central venous catheters). This report provides updated recommendations from CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC) for the control of CRE or carbapenemase-producing *Enterobacteriaceae* in acute care (inpatient) facilities. For all acute care facilities, CDC and HICPAC recommend an aggressive infection control strategy, including managing all patients with CRE using contact precautions and implementing Clinical and Laboratory Standards Institute (CLSI) guidelines for detection of carbapenemase production. In areas where CRE are not endemic, acute care facilities should 1) review microbiology records for the preceding 6-12 months to determine whether CRE have been recovered at the facility, 2) if the review finds previously unrecognized CRE, perform a point prevalence culture survey in high-risk units to look for other cases of CRE, and 3) perform active surveillance cultures of patients with epidemiologic links to persons from whom CRE have been recovered. In areas where CRE are endemic, an increased likelihood exists for importation of CRE, and facilities should consider additional strategies to reduce rates of CRE (2). Acute care facilities should review these recommendations and implement appropriate strategies to limit the spread of these pathogens.

For CRKP, the most important mechanism of resistance is the production of a carbapenemase enzyme, *bla<sub>KPC</sub>*. The gene that encodes the *bla<sub>KPC</sub>* enzyme is carried on a mobile piece of genetic material (transposon), which increases the risk for dissemination. Since first described in North Carolina in 1999, CRKP has been identified in 24 states and is recovered routinely in certain hospitals in New York and New Jersey (3). Analysis of 2007 data regarding health-care-associated infections reported to CDC indicated that 8% of all *Klebsiella* isolates were CRKP, compared with fewer than 1% in 2000 (CDC, unpublished data, 2008). CRKP poses significant treatment challenges, and CRKP infections have been associated with increased mortality, length of stay, and increased cost (4). The emergence and spread of CRKP and other types of CRE is another in a series of worrisome public health developments regarding antimicrobial resistance among gram-negative bacteria and underscores the immediate need for aggressive detection and control strategies (5).

A difficulty in detecting CRE is the fact that some strains that harbor *bla<sub>KPC</sub>* have minimal inhibitory concentrations (MICs) that are elevated but still within the susceptible range for carbapenems. Because these strains are susceptible to carbapenems, they are not identified as potential clinical or infection control risks using current susceptibility testing guidelines. To address this challenge, in January 2009, CLSI published a recommendation that carbapenem-susceptible *Enterobacteriaceae* with elevated MICs or reduced disk diffusion zone sizes be tested for the presence of carbapenemases using the modified Hodge test (MHT) (6). The MHT is a phenotypic test used to detect carbapenemases in isolates demonstrating elevated but susceptible carbapenem MICs and has demonstrated sensitivity and specificity exceeding 90% in identifying carbapenemase-producing *Enterobacteriaceae* (6). If the MHT reveals the presence of a carbapenemase, CLSI recommends that a comment be added to the microbiology report to inform clinicians and infection preventionists. Because treatment information on MHT-positive, carbapenem-susceptible isolates is limited, CLSI guidelines do not recommend any changes regarding the reporting of susceptibility results themselves. Strains of *Enterobacteriaceae* that test intermediate or resistant to carbapenems should be reported as such and do not need to be subjected to the MHT.

Patients with unrecognized CRKP colonization have served as reservoirs for transmission during health care. In addition to a review of infection control practices, active surveillance cultures were performed on patient colonized patients who were not previously known to harbor CRKP and were not placed in contact precautions. Personal adherence to recommendations for gown and glove use was low (62%) at the hospital, and only 48% of patient encounters. The hospital eventually was able to control the outbreak through enhanced infection control measures. Additional cases were identified.

**CRE = carbapenem-resistant  
*Enterobacteriaceae***

Experience from the outbreak in Puerto Rico and elsewhere (notably Israel) suggests that early detection through use of targeted surveillance and introduction of strict infection control measures (including reinforcement of hand hygiene and contact precautions) can help control the spread of CRKP (7). Other reports have demonstrated that infection control measures for CRKP can be associated with both health care-associated infections that are resistant to multiple antibiotics and also with lower

MMWR March 20, 2009 (2)

Laboratory  
Responsibilities...  
Use reliable test  
methods and report  
CRE promptly!

Surveillance

#### Infection Prevention and Control

- All acute care facilities should implement contact precautions for patients colonized or infected with carbapenem-resistant *Enterobacteriaceae* (CRE) or carbapenemase-producing *Enterobacteriaceae*. No recommendation can be made regarding when to discontinue contact precautions.

#### Laboratory

- Clinical microbiology laboratories should follow Clinical and Laboratory Standards Institute guidelines for susceptibility testing (*I*) and establish a protocol for detection of carbapenemase production (e.g., performance of the modified Hodge test).
- Clinical microbiology laboratories should establish systems to ensure prompt notification of infection prevention staff of all *Enterobacteriaceae* isolates that are nonsusceptible to carbapenems or *Klebsiella* spp. or *Escherichia coli* isolates that test positive for a carbapenemase.

#### Surveillance

- All acute care facilities should review clinical culture results for the preceding 6–12 months to determine whether previously unrecognized CRE have been present in the facility.
  - If this review identifies previously unrecognized CRE, a point prevalence survey (a single round of active surveillance cultures) should be performed to look for CRE in high-risk units (e.g., intensive care units, units where previous cases have been identified, and units where many patients are exposed to broad-spectrum antimicrobials).
  - If this review does not identify previously unrecognized CRE, monitoring for clinical infections should be continued.
- If CRE or carbapenemase-producing *Klebsiella* spp. or *E. coli* are detected from one or more clinical cultures OR if the point prevalence survey reveals unrecognized colonization, the facility should investigate for possible transmission by:
  - Conducting active surveillance testing of patients with epidemiologic links to a patient with CRE infection (e.g., patients in the same unit or who have been cared for by the same health-care personnel).
    - Continue active surveillance periodically (e.g., weekly) until no new cases of colonization or infection suggesting cross-transmission are identified.
    - If transmission of CRE is not identified after repeated active surveillance testing, consider altering the surveillance strategy by performing periodic point prevalence surveys in high-risk units.
  - In areas where CRE are endemic, an increased likelihood exists for importation of CRE, and the procedures outlined might not be sufficient to prevent transmission. Facilities in such areas should monitor clinical cases and consider additional strategies to reduce rates of CRE as described in the 2006 Tier 2 guidelines for management of multidrug-resistant organisms in health-care settings (2). Recommendations for rate calculations have been described previously (3).

#### References

1. Clinical and Laboratory Standards Institute. 2009 performance standards for antimicrobial susceptibility testing. Nineteenth information supplement (M100-S19). Wayne, PA: Clinical and Laboratory Standards Institute; 2009.
2. CDC, Healthcare Infection Control Practices Advisory Committee. Management of multidrug-resistant organisms in healthcare settings, 2006. Atlanta, GA: US Department of Health and Human Services, CDC, Healthcare Infection Control Practices Advisory Committee; 2007. Available at <http://www.cdc.gov/ncidod/dhqp/pdf/ar/mdroguideline2006.pdf>.
3. Cohen AL, Calfee D, Fridkin SK, et al. Recommendations for metrics for multidrug-resistant organisms in healthcare settings: SHEA/HICPA position paper. *Infect Control Hosp Epidemiol* 2008;29:901–13.

**Specimen: Blood**

**Diagnosis: Neutropenic fever**

***Klebsiella pneumoniae***

**Case #2**

	<u>MIC (<math>\mu\text{g/ml}</math>)</u>
amikacin	32 R
ampicillin	>32 R
cefazolin	>32 R
ceftazidime	>32 R
ceftriaxone	>32 R
ciprofloxacin	>2 R
gentamicin	2 S
imipenem	>16 R
piper-tazobactam	128 R
tobramycin	>10 R
trimeth-sulfa	>4/76 R

***What about aminoglycosides?***



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The **Subcommittee on Antimicrobial Susceptibility Testing** is composed of representatives from the professions, government, and industry, including microbiology laboratories, government agencies, healthcare providers and educators, and pharmaceutical and diagnostic microbiology industries. Using the CLSI voluntary consensus process, the subcommittee develops standards that promote accurate antimicrobial susceptibility testing and appropriate reporting.

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[M02 \(Disk Diffusion\) and M07 \(MIC\) Summary of Comments and Subcommittee Responses](#)

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## M100-S17, *Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Informational Supplement*

### General

1. Enterobacteriaceae and non-Enterobacteriaceae, which are resistant to tobramycin and amikacin, but susceptible to gentamicin, most likely produce a 6'-acetyltransferase. In this case, only one of the three gentamicin subcomponents, C<sub>1</sub>, remains active. Since the fraction of C<sub>1</sub> varies between gentamicin formulations and C<sub>1</sub> appears to have different pharmacokinetics than gentamicin as a whole (*Antimicrob Agents Chemother.* 1975;7:328-332), are the gentamicin interpretive breakpoints accurate in these cases? Would it be reasonable to report gentamicin susceptibility as intermediate or provide a comment that gentamicin activity is uncertain?
  - The comment raises an interesting question. We have no data that would support changing the susceptible category to intermediate or resistant. However, when an isolate that is gentamicin susceptible and amikacin and tobramycin resistant is encountered and selective reporting is used by the laboratory, the susceptibility to gentamicin and the resistance to tobramycin and amikacin should all be reported.

## Susceptibility Testing Guide

Based on the Journal of Antimicrobial Chemotherapy Supplement (JAC [2001] 48, Suppl. S1).

Individual chapters are available below:-

Chapter One

[History and development of antimicrobial susceptibility testing methodology](#)

July 2001

Chapter Two

[Determination of minimum inhibitory concentrations](#)

March 2006 (chapter under review)

Chapter Three

[Establishing MIC breakpoints and the interpretation of \*in vitro\* susceptibility tests](#)

January 2005

Chapter Four

[The development of the BSAC standardized method of disc diffusion testing](#)

July 2001

Chapter Five

[BSAC standardized disc susceptibility testing method](#)

Version 6 April 2007

Chapter Six

[Detection of beta-lactamase-mediated resistance](#)

August 2005

Chapter Seven

[Detection of methicillin/oxacillin resistance in staphylococci](#)

July 2001

Chapter Eight

[Quality assurance of antimicrobial susceptibility testing by disc diffusion](#)

July 2001

Chapter Nine

[Recommendations for suscep](#)

July 2001

Chapter Ten

[Instrumentation in antimicrobial susceptibility testing](#)

July 2001

Chapter Eleven

[Interpretative reading: recognizing the unusual and inferring resistance mechanisms from resistance phenotypes](#)

March 2004

[http://www.bsac.org.uk/susceptibility\\_testing/guide\\_to\\_antimicrobial\\_susceptibility\\_testing.cfm](http://www.bsac.org.uk/susceptibility_testing/guide_to_antimicrobial_susceptibility_testing.cfm)

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**Table IX.** Phenotypes; interpretation of mechanism and editing of antibiograms: aminoglycosides versus Gram-negative bacteria

GEN	NET	TOB	AMK	KAN	NEO	Interpretation	Frequency	Edit/action and comments
<i>E. coli</i> and other Enterobacteriaceae <u>not</u> shown separately								
S	S	S	S	S	S	classical	common	
R	S	S	S	S	S	AAC(3)I	Rare	Also R to fortimicin
R	R	R	S	R	S	AAC(3)II	rare	Greater R to GEN than to TOB or NET
R	R	R	S	r	R	AAC(3)IV	rare	Also R to apramycin (used in veterinary practice). Mostly in <i>E. coli</i>
S/r	R	R	R	R	R	AAC(6')	rare	One component of GEN remains active but <i>in vivo</i> use best avoided.
R	S	R	S	R	S	ANT(2')	rare	Equal R to GEN and TOB
S	S	S	S	R	R	APH(3')	common	Usually more R to KAN than NEO. Was common, now rarely tested.
r/R	r/R	r/R	r/R	r/R	r/R	'impermeability'	rare	Low-level R to all aminoglycosides

**AAC(6') "common" in *S. marcescens***

**David Livermore (BSAC website)**

**Specimen: Blood**

**Diagnosis: Neutropenic fever**

***Klebsiella pneumoniae***

Final Report

Case #2

	<u>MIC (µg/ml)</u>
amikacin	32 R
ampicillin	>32 R
cefazolin	>32 R
ceftazidime	>32 R
ceftriaxone	>32 R
ciprofloxacin	>2 R
colistin	≤0.12*
gentamicin	2 S
imipenem	>16 R
piper-tazobactam	128 R
tigecycline	1 S
tobramycin	>10 R
trimeth-sulfa	>4/76 R

*Report comment:*

“\*No interpretive criteria available  
Imipenem resistance NOT due to  
carbapenemase production.  
The effectiveness of gentamicin in  
treating infections due to *K.*  
*pneumoniae* that are gentamicin-S,  
amikacin-R and tobramycin-R has  
not been established. ID consult  
suggested.”

**Specimen: Tracheal Aspirate**

**Diagnosis: Pneumonia**

***Acinetobacter baumannii***

**CASE #3**

	<u>MIC (<math>\mu\text{g/ml}</math>)</u>
amikacin	>32 R
ceftriaxone	>32 R
<b>colistin</b>	<b><math>\leq 0.5</math> S</b>
ciprofloxacin	>4 R
gentamicin	>16 R
imipenem	>16 R
piper-tazobactam	>512 R
<b>tigecycline</b>	<b>2*</b>
tobramycin	>16 R
trimeth-sulfa	$\leq 4/76$ R

\* no interpretive criteria

***What about tigecycline?***

# Tigecycline – FDA Breakpoints (no CLSI breakpoints)

Table 2. Susceptibility Test Result Interpretive Criteria for Tigecycline

Pathogen	Minimum Inhibitory Concentrations (µg/mL)			Disk Diffusion (zone diameters in mm)		
	S	I	R	S	I	R
<i>Staphylococcus aureus</i> (including methicillin-resistant isolates)	≤0.5 <sup>a</sup>	-	-	≥19	-	-
<i>Streptococcus</i> spp. other than <i>S. pneumoniae</i>	≤0.25 <sup>a</sup>	-	-	≥19	-	-
<i>Enterococcus faecalis</i> (vancomycin-susceptible isolates only)	≤0.25 <sup>a</sup>	-	-	≥19	-	-
Enterobacteriaceae <sup>b</sup>	≤2	4	≥8	≥19	15-18	≤14
Anaerobes <sup>c</sup>	≤4	8	≥16	n/a	n/a	n/a

<sup>a</sup> The current absence of resistant isolates precludes defining any results other than “Susceptible”. Isolates yielding MIC results suggestive of “Nonsusceptible” should be referred to a reference laboratory for further testing.

<sup>b</sup> Tigecycline has decreased in vitro activity against *Morganella* and *Providencia* spp.

<sup>c</sup> Agar dilution

**Tigecycline**  
<http://www.wyeth.com>  
 (Prescribing Information)

# ***What should we know about testing/ reporting tigecycline on Acinetobacter baumannii?***

- ◆ Currently, **no FDA approval** for use in treating *Acinetobacter* infections
- ◆ Only breakpoints for *Acinetobacter* spp. available are published by AST standards-type organization are from British Society for Antimicrobial Chemotherapy (BSAC)

[http://www.bsac.org.uk/\\_db/\\_documents/](http://www.bsac.org.uk/_db/_documents/)

- MIC ( $\mu\text{g/ml}$ )  $\leq 1$  S, 2 I,  $>2$  R
- Peleg et al. recommends using these for *A. baumannii* infections other than blood

**Peleg et al. 2008. Clin Microbiol Rev. 21:538.**

## ***Why shouldn't we use the BSAC Acinetobacter spp. breakpoints for blood isolates?***

- ◆ **Maximum blood concentration = 0.63 µg/ml**
  - Prudent NOT to report blood isolates with MICs >0.5 µg/ml as “S”
  - Perform MIC not disk diffusion for blood isolates

**Peleg et al. 2008. Clin Microbiol Rev. 21:538.**

# ***What are the activities tigecycline and tetracyclines against MDR A. baumannii?***

**%Susceptible (N=93)**

<b>Tigecycl*</b>	<b>Mino</b>	<b>Doxy</b>	<b>A-S</b>	<b>Amik</b>	<b>Imip</b>	<b>Cipro</b>	<b>Poly B</b>
<b>95</b>	<b>83</b>	<b>56</b>	<b>20</b>	<b>38</b>	<b>20</b>	<b>0</b>	<b>100</b>

**\* Used MIC breakpoint ( $S \leq 2 \mu\text{g/ml}$ ) for **Enterobacteriaceae** from tigecycline product package insert (Wyeth Pharmaceuticals Inc.)**

**Scheetz et al. 2007. Antimicrob Agents Chemother. 51:1621.**

# What are the activities of tigecycline and minocycline against MDR *A. baumannii*?

## Tested:

Amp-sulb  
Pip-tazo  
Aztre  
Ceftriax  
Ceftaz  
Cefotax  
Cefepime  
Imip  
Mero  
Cipro  
Oflox  
SXT  
Tetra  
Gent  
Tob  
Amik

		Tigecycline		Minocycline		
		MIC ( $\mu\text{g/ml}$ )		MIC ( $\mu\text{g/ml}$ )		
Isolate	N	Range	90%	Range	90%	%S
PDR	46	0.5-8	2	0.25-16	16	8.7
XDR	40	0.25-4	2	0.25-32	16	30
IPM-S	64	0.03-2	2	$\leq 0.06-16$	8	60.9
<b>Total</b>	<b>150</b>	<b>0.03-8</b>	<b>2</b>	<b><math>\leq 0.06-32</math></b>	<b>16</b>	<b>36.6</b>

Excluding tigecycline and minocycline....

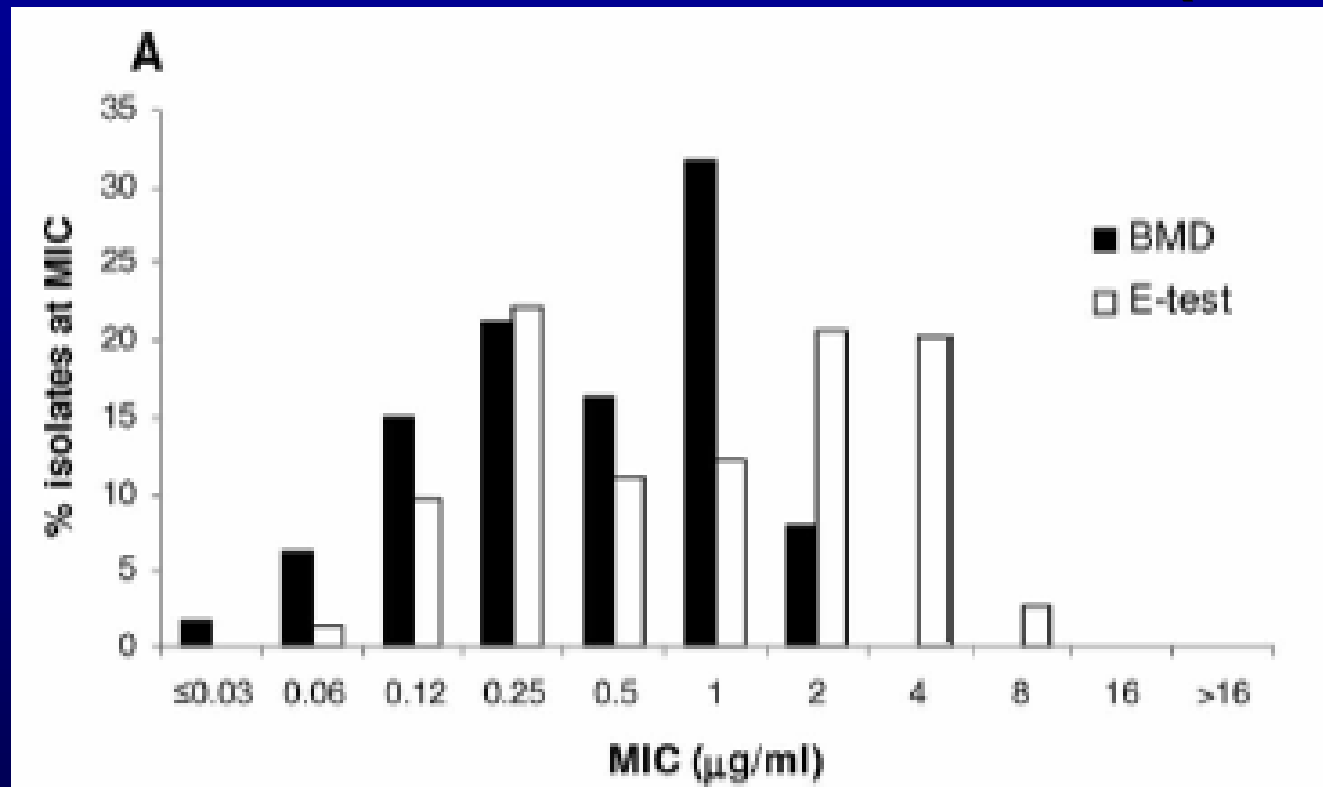
PDR = R to all including colistin

XDR = only S to colistin

IPM-S = variable

Arroyo et al. 2009. Antimicrob Agents Chemother. 53:1295.

# Method-related discordance in tigecycline MICs for *Acinetobacter baumannii* (N=227); Etest vs. broth microdilution (BMD)



**MIC<sub>90</sub>: Etest = 4 µg/ml    BMD = 1 µg/ml**

**Pillar et al. 2008. J Clin Microbiol. 46:2862.**

**See also...Casal et al. 2009. J Antimicrob Chemother. 64:69.**

**Specimen: Tracheal Aspirate**  
**Diagnosis: Pneumonia**  
*Acinetobacter baumannii*

Final Report

**CASE #3**

	<u>MIC (µg/ml)</u>
amikacin	>32 R
ceftriaxone	>32 R
colistin	≤0.5 S
ciprofloxacin	>4 R
gentamicin	>16 R
imipenem	>16 R
piper-tazobactam	>512 R
<b>tigecycline</b>	<b>2*</b>
tobramycin	>16 R
trimeth-sulfa	≤4/76 R

*Report comment:*

**“\*No interpretive criteria;  
Infectious Diseases  
consult suggested.”**

**Specimen: Pleural fluid**  
**Diagnosis: Pneumonia**  
*Staphylococcus aureus*

**Case #4**

	<u>MIC (<math>\mu\text{g/ml}</math>)</u>
clindamycin	>8 R
erythromycin	>8 R
oxacillin	>16 R
penicillin	R
vancomycin	2 S

***Should we do anything about  
vancomycin MIC of 2  $\mu\text{g/ml}$ ?***

# *S. aureus* - Vancomycin MIC Interpretive Criteria ( $\mu\text{g/ml}$ )

Susceptible	Intermediate	Resistant
$\leq 2$	4-8	$\geq 16$

**VSSA**

$\leq 1$  vs  $2 \mu\text{g/ml}$

**hVISA**

**VISA**

**VRSA**

CLSI M100-S19; Table 2C.

hVISA (h=heteroresistant) no CLSI recommendations for detecting hVISA

# Why does it matter if vancomycin MIC is 1 vs. 2 $\mu\text{g}/\text{ml}$ ?

- ◆ Based on PK/PD, there may be different clinical outcomes if vancomycin used for therapy
- ◆ Infectious Diseases Society of America (IDSA) Guidelines; if vancomycin MIC is:
  - $\leq 1 \mu\text{g}/\text{ml}$  – maintain vancomycin trough serum concentration of 15-20 mg/L
  - $\geq 2 \mu\text{g}/\text{ml}$  - use alternative treatment

**Rybak et al. Clin Infect Dis. 2009. 49:325**
- ◆ Increasing numbers of isolates with susceptible MICs of 1 and 2  $\mu\text{g}/\text{ml}$  (as compared to  $\leq 0.5 \mu\text{g}/\text{ml}$ ) - referred to as vancomycin “creep”
- ◆ Some suggest lowering the breakpoint to  $\leq 1 \mu\text{g}/\text{ml}$  for “S”

# Vancomycin MICs are “Creeping up” MRSA Blood Isolates 2001-2005 (N=662)

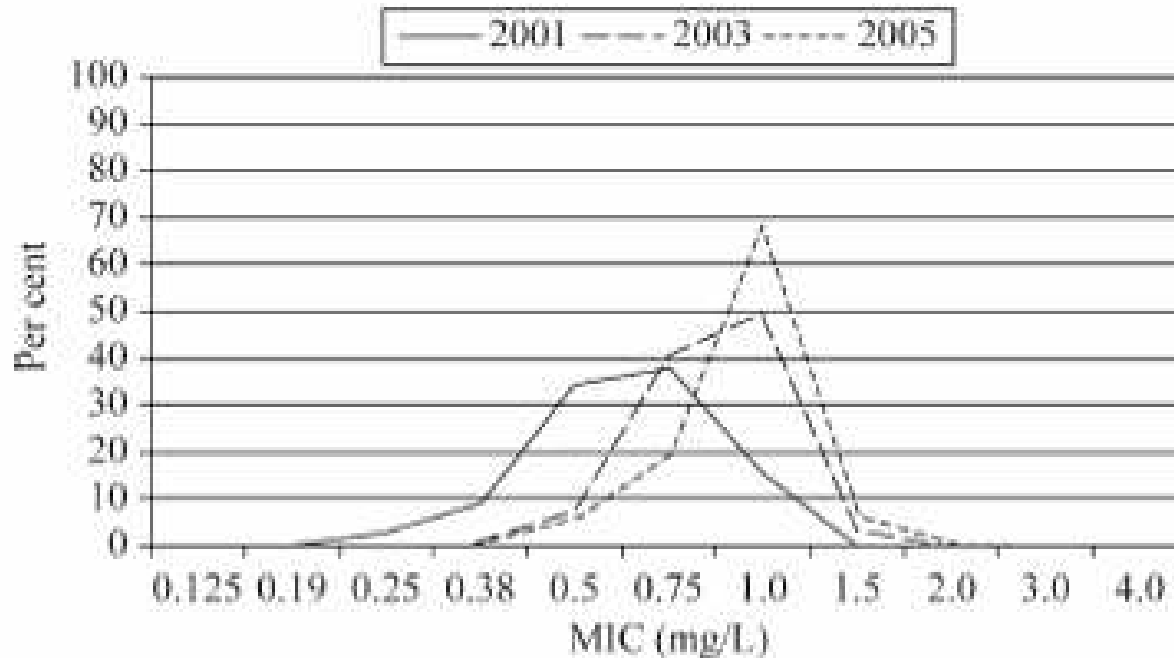


Figure 1. Vancomycin MIC population distribution 2001–05.

**MIC testing performed by Etest method**

**Steinkraus et al. 2007. J Antimicrob Chemother. 60:788.  
See also...Wang et al. 2006. J Clin Microbiol. 44:3883.**

# Outcomes of Vancomycin Therapy in 92 Patients with MRSA Bacteremia (2005-2007)

Outcome	VAN MIC $\geq$ 1.5 (66 patients)	VAN MIC $<$ 1.5 (26 patients)	<i>P</i> value
Overall failure	24 (36.4)*	4 (15.4)	0.049
Hospital length of stay	21 (9.0-43.0)	10.5 (9.0-16.5)	0.02

\* No. (%) of patients

MIC testing performed by Etest

Lodise et al. 2008. Antimicrob Agents Chemother. 52:3315.

See also...

Soriano et al. 2008. Clin Infect Dis. 46:193.

Kollef et. al. 2007. Clin Infect Dis. 45 (Suppl 3): S191.

# *What methods should we use for routine testing of vancomycin and S. aureus?*

- ◆ Is Etest best?
  - More recent outcome data based on Etest
- ◆ Method variability
  - Etest MICs higher than CLSI reference method MICs
- ◆ Reproducibility of MIC tests generally +/- 1 two-fold dilution



**MIC = 1.5 µg/ml**

# Vancomycin MIC (N=101 MRSA)

Etest MICs > Reference Broth Microdilution and Agar Dilution MICs

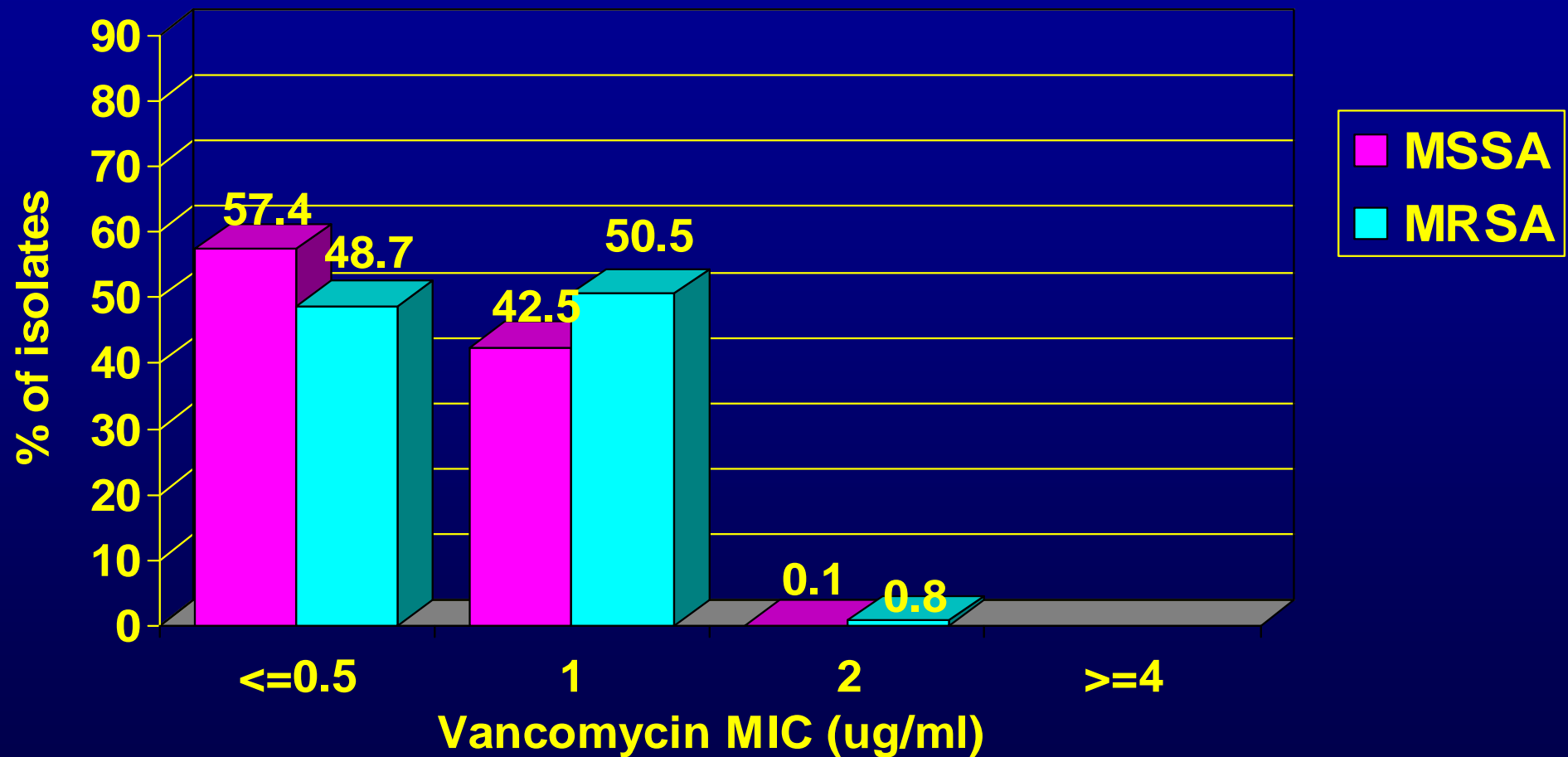
TABLE 1. Comparison of vancomycin MICs determined by broth microdilution, agar dilution, and Etest<sup>a</sup>

Vancomycin MIC (µg/ml)	No. of isolates (%) with MIC (µg/ml) determined by:			
	Broth microdilution	Agar dilution	Etest (Remel agar)	Etest (BBL agar)
0.5	21 (20.8)	1 (1)	0 (0)	0 (0)
0.75			1 (1)	1 (1)
1	77 (76.2)	88 (87)	11 (10.9)	1 (1)
1.5			69 (68.3)	62 (61.4)
2	3 (2.97)	12 (11.9)	20 (19.8)	37 (36.6)
Modal MIC (µg/ml)	1	1	2	2

<sup>a</sup> MICs were determined for 101 MRSA blood isolates obtained between 2002 and 2006.

**Prakash et al. 2008. Antimicrob Agents Chemother. 52:4528.**  
**See also...Hsu et al. 2008. Intl J Antimicrob Agents. 32:378.**

# *S. aureus* - Vancomycin MIC Distribution UCLA 2008 (889 MSSA, 662 MRSA)



CLSI reference broth microdilution method

**Specimen: Pleural fluid**  
**Diagnosis: Pneumonia**  
*Staphylococcus aureus*

Final report

**Case #4**

	<u>MIC (<math>\mu\text{g/ml}</math>)</u>
clindamycin	>8 R
daptomycin	0.5 S
erythromycin	>8 R
linezolid	1 S
oxacillin	>16 R
penicillin	R
vancomycin	2 S*

**“\*Vancomycin MIC determined by Etest method”**

**Specimen: Blood**

**Diagnosis: Endocarditis**

***Staphylococcus aureus***

**Case #5**

MIC ( $\mu\text{g/ml}$ )

clindamycin	>8 R
erythromycin	>8 R
oxacillin	>16 R
penicillin	R
vancomycin	$\leq 0.5$ S

***Could this be hVISA?***

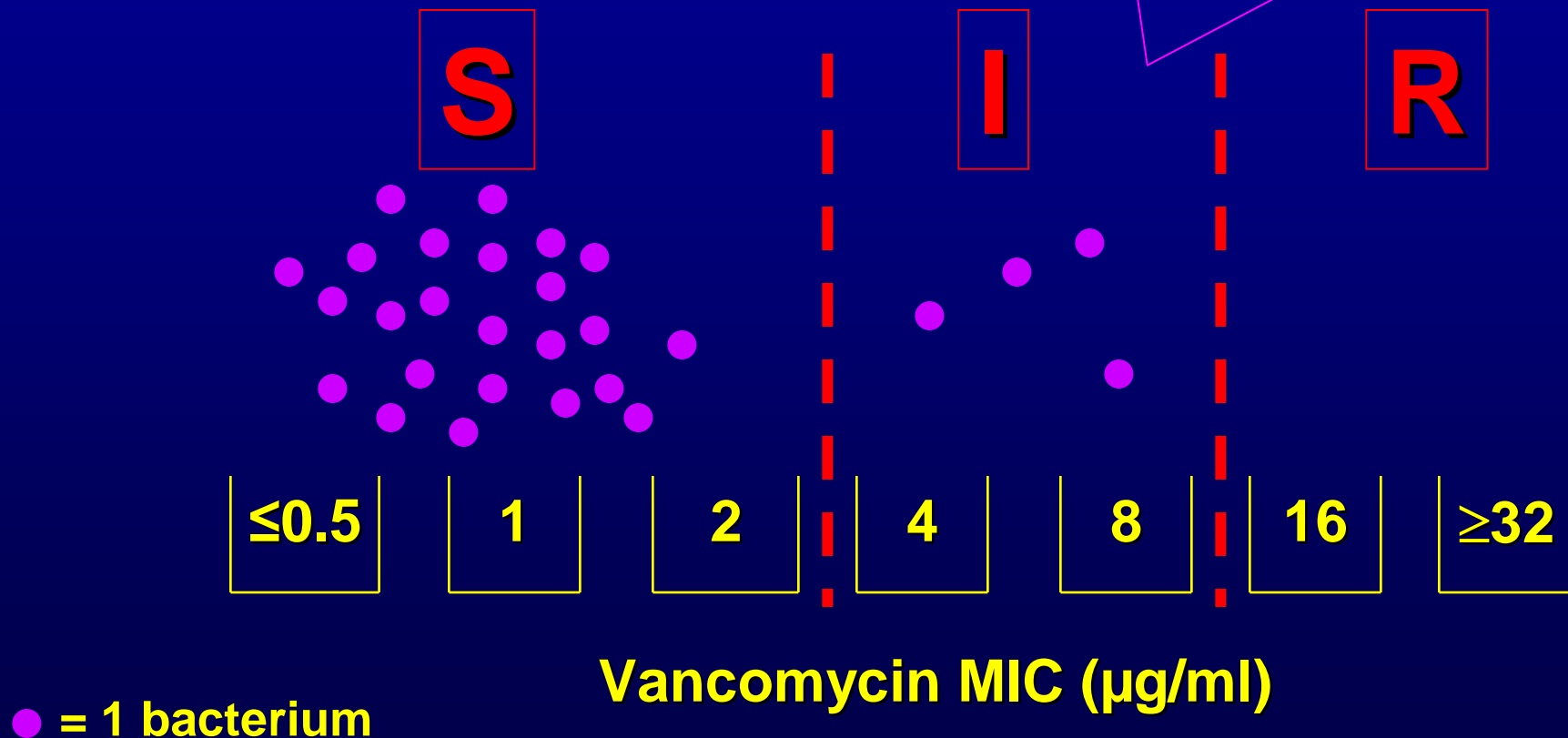
# ***What is hVISA\*?***

- ◆ ***S. aureus* that show....**
  - vancomycin MIC  $\leq 2$   $\mu\text{g/ml}$  (S) by conventional MIC tests
  - subpopulation of cells with MICs 4-8  $\mu\text{g/ml}$
  - associated with increase production of biofilm; more tolerant to “killing”; poor patient outcomes

**\*heteroresistant VISA**

# What might an hVISA inoculum look like?

Too few "NS" cells to detect with standard inoculum size in routine AST (MIC & DD results will be "S");  
often slower growing



Vancomycin MIC (µg/ml)

NS, not susceptible

# What methods have been used to detect hVISA?

## ◆ Population analysis/AUC (PAP/AUC)

- Place high inoculum on agar with varying vancomycin concentrations and divide by area under the population curve (**gold standard**)

## ◆ Etest (test vancomycin and teicoplanin)

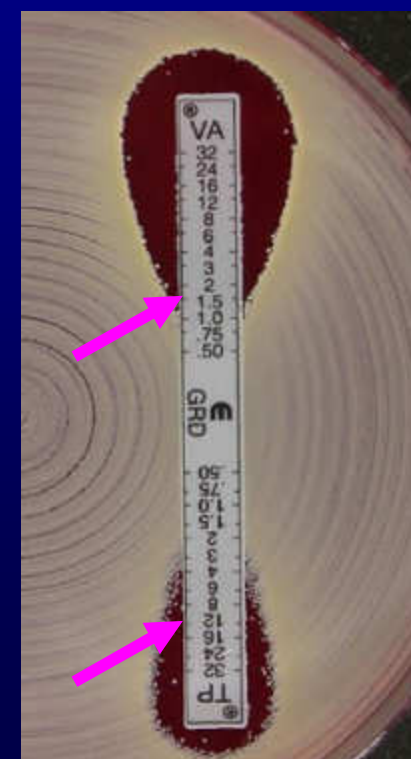
- Macro method - high inoculum (McF #2) on BHI
- GRD (glycopeptide resistance detection)

See Yusof et al. 2008. JCM. 46:3042

## ◆ Screen plates

- MHA + 5 µg/ml teicoplanin
- Other screen plates

**GRD Etest**



# Outcomes in 250 patients with Bacteremia due to hVISA or VS-MRSA

	hVISA (N=27)	VS-MRSA (N=223)	p
Infection-attributable death	12 (44)*	81 (36)	.4
Hospitalization duration, days	12 (0-207)	12.5 (0-184)	.8
Bacteremia, days	12 (1-123)	2 (1-92)	.005
Endocarditis	5 (19)	8 (4)	.007
Osteomyelitis	7 (26)	16 (7)	.006

\*No. (%) of patients or median value (range)  
hVISA identified with Etest macromethod

Maor et al. 2009. J Infect Dis. 199:619.

# Pt. X – 4 MRSA Blood Isolates (same PFGE)

## Endocarditis - failed vancomycin and daptomycin Rx

Isolate Date	MIC ( $\mu\text{g/ml}$ )			
	Dapto	Vanc	Vanc	
	BMD fresh	BMD fresh	Etest* fresh	Etest* frozen
9/16	$\leq 0.5$ S	1 S	2 S	2 S
11/5	$\leq 0.5$ S	2 S	2 S	2 S
12/4	4 NS	2 S	4 I	2 S
12/6	4 NS	4 I	8 I	3 (I)

hVISA Etest		PAP
Macro	GRD	
-	-	-
hVISA	-	hVISA
hVISA	hVISA	hVISA
hVISA	hVISA	hVISA

**BMD, broth microdilution**

**\* Std. method, McFarland 0.5 / MHA**

**Fresh, isolate tested when recovered**

**Frozen, isolate tested after freezing**

**PAP, population analysis**

Tenover et al. 2009.

Intl J Antimicrob Agents. 33:564.

# CLSI Retesting Rule

- ◆ For *S. aureus*, vancomycin "S" isolates may become vancomycin "I" during prolonged therapy.

## Suggestion:

Test subsequent isolates of *S. aureus* from similar body site after 3-4 days to see if isolate is still vancomycin-S.

**Specimen: Blood**

**Diagnosis: Endocarditis**

***Staphylococcus aureus***

Final report

**Case #5**

	<u>MIC (<math>\mu\text{g/ml}</math>)</u>
clindamycin	>8 R
erythromycin	>8 R
oxacillin	>16 R
penicillin	R
vancomycin	$\leq 0.5$ S

**“hVISA identified by Macro Etest method; Macro Etest performed at Dr. Smith’s request”**



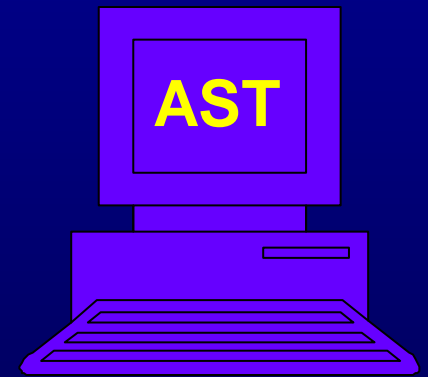
***How can we handle all of this????***

***“Need.....Artificial Intelligence”!***

- ◆ **Flag atypical / inconsistent results**
- ◆ **Suggest confirmatory tests**
- ◆ **Report appropriate drugs**
- ◆ **Edit “S” or “I” results to “R”**
- ◆ **Add comments to report**

**and**

- ◆ ***Informed Clinical Laboratory Scientists!***



# Summary (1)

- ◆ **Carbapenem resistance** in Enterobacteriaceae may be due to carbapenemases or combinations of other  $\beta$ -lactamases and porin changes.
- ◆ **SME carbapenemases** are uncommon, do not readily hydrolyze 3<sup>rd</sup> and 4<sup>th</sup> generation cephalosporins and can be modified Hodge test positive.
- ◆ The effectiveness of gentamicin in **gentamicin-S, amikacin-R and tobramycin-R Enterobacteriaceae** is not known.
- ◆ There are currently no CLSI or FDA breakpoints for interpreting results for **tigecycline** with *Acinetobacter* spp.

# Summary (2)

- ◆ Serious infections caused by MRSA with **vancomycin MICs of  $>1 \mu\text{g/ml}$**  may not respond as well to vancomycin therapy as MRSA with vancomycin MICs of  $\leq 1 \mu\text{g/ml}$ .
- ◆ Different methods may result in **different vancomycin MICs** for vancomycin-S *S. aureus*. Etest produces higher vancomycin MICs than CLSI broth microdilution reference method.
- ◆ There are currently no CLSI or other standard recommendations for testing for **hVISA**.

# Thank you!

***"...there is nothing so satisfying to the spirit, so defining of our character than giving our all to a difficult task!"***  
**President Barack Obama, 1/20/09**

