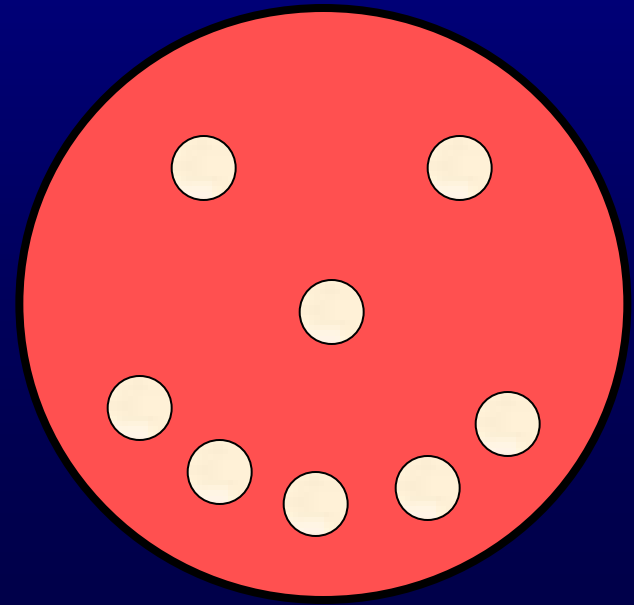


Detection and Reporting of Antimicrobial Resistance: gram positives (MRSA, VISA, VRSA, VRE, Spneu)

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

- ◆ Describe the methods available for detection of **MRSA**.
- ◆ Discuss concerns related to **vancomycin** and ***S. aureus*** including VISA, VRSA and hVISA.
- ◆ List points to consider for AST of **coagulase-negative staphylococci (CoNS)**.
- ◆ Describe the recent changes in **penicillin** breakpoints for ***Streptococcus pneumoniae***



Antimicrobial Agent Options for MSSA Infections

Predictable activity

Need AST

Beta- lactams

dicloxacillin / nafcillin
cephalexin / cefazolin
amoxicillin-clavulanic acid
ampicillin-sulbactam
carbapenem (e.g., imipenem)
vancomycin
daptomycin
linezolid
quinupristin-dalfopristin
tigecycline

clindamycin
trimethoprim-sulfa
macrolide (e.g.
clarithromycin)
tetracycline
fluoroquinolone (e.g.
ciprofloxacin)
(gentamicin)
(rifampin)

Antimicrobial Agent Options for MRSA Infections

Predictable activity

Need AST

**Beta-
lactams**

~~dicloxacillin / nafcillin
cefnalexin / cefazolin
amoxicillin-clavulanic acid
ampicillin-sulbactam
carbapenem (e.g., imipenem)~~
vancomycin
daptomycin
linezolid
quinupristin-dalfopristin
tigecycline

clindamycin
trimethoprim-sulfa
macrolide (e.g.
clarithromycin)
tetracycline
fluoroquinolone (e.g.
ciprofloxacin)
(gentamicin)
(rifampin)

MRSA – methicillin-resistant *S. aureus*

- Oxacillin vs. cefoxitin tests to detect *mecA*-mediated oxacillin resistance

Tests for *mecA*-mediated Oxacillin Resistance in *S. aureus*

◆ Phenotypic tests

– Disk diffusion or MIC

- Cefoxitin as a surrogate

- Report results for **OXACILLIN**, not cefoxitin

- Oxacillin

– Oxacillin-salt agar screen for *S. aureus*

◆ Detection of gene or gene product

- Gene = *mecA*

- Gene product = penicillin binding protein (PBP) 2a

Specimen: Joint fluid
Diagnosis: Septic arthritis

Case #1

Staphylococcus aureus

	<u>MIC ($\mu\text{g/ml}$)</u>
(cefoxitin	≤ 4 S)
clindamycin	> 8 R
erythromycin	> 8 R
oxacillin	4 R
penicillin	R
vancomycin	≤ 0.5 S

What should we do?

(commercial panels often contain both cefoxitin and oxacillin)

Results for 135 “Difficult” Isolates of *S. aureus*

Method	<i>mecA</i> pos (n=79)		
	No. w/ result		SENS
	S	I or R	
CX-DD 18h	2	77	98%
OX DD 24h	7	72	91
*CX-MIC 18h	1	78	99
OX MIC 24h	12	67	85%
OX salt agar 24h	12	67	85%

<i>mecA</i> neg (n=56)		
No. w/ result		SPEC
S	I or R	
56	0	100%
33	23	59
53	3	95
49	7*	88%
28	28	50%



* borderline MRSA

Swenson et al. 2007. Diagn Microbiol Infect Dis. 58:33.

Detection of Oxacillin Resistance in *S. aureus*

<i>mecA</i> or PBP2a	Oxacillin MIC ($\mu\text{g/ml}$)	Report oxacillin
Pos	any	resistant
Neg	≤ 2	susceptible
Neg	≥ 4	resistant ^a

^a Rare occurrence of oxacillin resistance mechanisms other than *mecA* (e.g. “borderline” oxacillin resistance)...
“These isolates may test susceptible to cefoxitin”

CLSI M100-S19. Table 2C.

Cefoxitin Tests for *mecA*-mediated Oxacillin Resistance in *S. aureus*

- ◆ Highly sensitive and specific in detecting *mecA*-mediated resistance; better than oxacillin for “difficult to detect MRSA”
- ◆ Fails to detect oxacillin-R *mecA* negative isolates (e.g., “borderline” isolates)

What about “borderline MRSA”?

- ◆ *mecA* negative and oxacillin MICs slightly above “R” breakpoint
 - ◆ **Mechanisms:**
 - 1) β -lactamase - \uparrow β -lactamase causes \uparrow oxacillin MIC
 - 2) MOD-SA - modified PBPs 1,2,4
 - ◆ Infrequently encountered
 - ◆ Limited **clinical information** in literature re: therapy
 - May be treatable with β -lactam agents
 - Some reports that they do respond to β -lactam
- Chambers. 1997. Clin Microbiol Rev. 10:781.**

Cloxacillin Clinical Failure for Endocarditis due to Borderline Oxacillin Resistant *S. aureus*

- ◆ 43 yo IV drug user
- ◆ Past 16 months, 4 admissions for MSSA endocarditis, osteomyelitis
 - Incomplete Rx – pt. left against medical advice
 - Rx. cloxacillin + rifampin
- ◆ 5th admission blood cultures – *S. aureus* (*mecA* neg)
 - Oxacillin: MIC = 12 µg/ml (Etest); ≥4 µg/ml (Vitek2); no zone (KB)
 - Rifampin – R
 - Pt responded to vancomycin
 - Some *S. aureus* isolates atypical phenotype

Commercial AST Software

S. aureus – OX / CX Testing

OX	CX	Report:
S	S	S
R	R	R
R	S	Expertize R*
S	R	Expertize R

*may be borderline MRSA

Phoenix vs. Cefoxitin Disk Diffusion

S. aureus (N=1,066: 347 MRSA, 719 MSSA)

Discrepancies
Between OX/CX

N	Phoenix		CX-DD	PBP2a
	OX	CX		
1	R	R	S*	Neg
6	S	R	R	Pos
1	R	S	R	Pos

- Isolates collected from 2006-2007 (Italy)
- Variety of sources
- Phoenix: 100% sensitivity; 99.86% specificity

*Broth dilution MIC ($\mu\text{g/ml}$): oxacillin = 1; cefoxitin = 4

Mencacci, et al. 2009. JCM. 47:2288.

Phoenix and Vitek 2 vs. *mecA*

S. aureus (N=620: 448 MRSA; 172 MSSA)

System	Sensitivity		
	OX	CX	Combined
Phoenix	97.8	99.8	99.8
Vitek 2	98.2	99.1	99.8

Specificity		
OX	CX	Combined
100	100	100
99.4	100	99.4

Phoenix:

- 1 *mecA* positive isolate S to both OX and CX
- 9 OX-S/CX-R (*mecA* +) changed to OX R

Vitek 2:

- 1 *mecA* positive isolate S to both OX and CX
- 1 *mecA* negative isolate R to OX and S to CS
- 7 OX-S/CX-R (*mecA* +) changed to OX R
- 3 OX-R/CX-S (3 *mecA* +, 1 *mecA* -) changed to OX R

Junkins et al. 2009.
JCM. Epub 22 July 2009.

Specimen: Joint fluid
Diagnosis: Septic arthritis

Final Report

Staphylococcus aureus

Case #1

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

clindamycin	>8 R
erythromycin	>8 R
oxacillin	4 R
penicillin	R
vancomycin	≤ 0.5 S

If oxacillin or cefoxitin = R
then report oxacillin R

May be a rare “borderline oxacillin-R” isolate.
Currently, no rule to distinguish these from *mecA* strains.

How should we define MRSA?

- ◆ *mecA*?
- ◆ Oxacillin MIC >2 µg/ml?

Definition may be a **hospital-acquired infection (HAI)** issue as well as a clinical issue

Scenario: MRSA Surveillance vs. Diagnostic Testing for MRSA

◆ Patient's results:

- MRSA surveillance: negative
- Wound culture: MRSA (oxacillin MIC 4 µg/ml)

Is this an HAI?

Surveillance tests: target *mecA*

Diagnostic tests: target *mecA* and/or oxacillin MIC >2 µg/ml

Penicillin and *Staphylococcus* spp.
- Induced β -lactamase testing

Specimen: Bone

Diagnosis: Osteomyelitis

Case #2

Staphylococcus aureus

	<u>MIC ($\mu\text{g/ml}$)</u>
clindamycin	≤ 0.5 S
erythromycin	≤ 0.5 S
oxacillin	≤ 0.25 S
penicillin	≤ 0.06 S
vancomycin	≤ 0.5 S

What should we do?

Surveillance

- Surveillance Studies
- Surveillance Data Reports

Gram-Positive Pathogens

Resources

► **Visit Surveillance Data**
to generate reports on Gram-
positive pathogens in your region.

Surveillance

Gram-Positive Cocci Susceptibility Data Quick Report Results

Summary of the antimicrobial activity of linezolid and comparison agents tested against *Staphylococcus aureus* from North America (n = 17,827).

Antimicrobial agent	MIC ₅₀	MIC ₉₀	Range	% susceptible/ resistant ^a
Linezolid	2	2	<=0.25 - >8	>99.9 / -
Oxacillin	>2	>2	<=0.25 - >2	49.8 / 50.2
Ceftriaxone	4	>32	<=0.25 - >32	49.8 / 50.2
Ciprofloxacin	0.5	>4	<=0.03 - >4	54.9 / 43.7
Clindamycin	<=0.5	>2	<=0.5 - >2	71.7 / 27.9
Daptomycin	0.25	0.5	<=0.06 - >1	99.8 / -
Erythromycin	>2	>2	<=0.25 - >2	37.5 / 61.6
Gentamicin	<=2	<=2	<=2 - >8	95.4 / 4.3
Levofloxacin	<=0.5	>4	<=0.5 - >4	55.9 / 43.2
Penicillin	16	>32	<=0.015 - >32	10.1 / 89.9

Penicillin only 10.1% "S"

<http://www.gpcsusceptibilitydata.com/content/home.asp?page=home>

Staphylococcus spp. - Penicillin

- ◆ An induced β -lactamase test should be performed on staphylococcal isolates if penicillin...
 - MIC $\leq 0.12 \mu\text{g/ml}$
 - Zone diameter $\geq 29 \text{ mm}$
-before reporting the isolate as penicillin susceptible

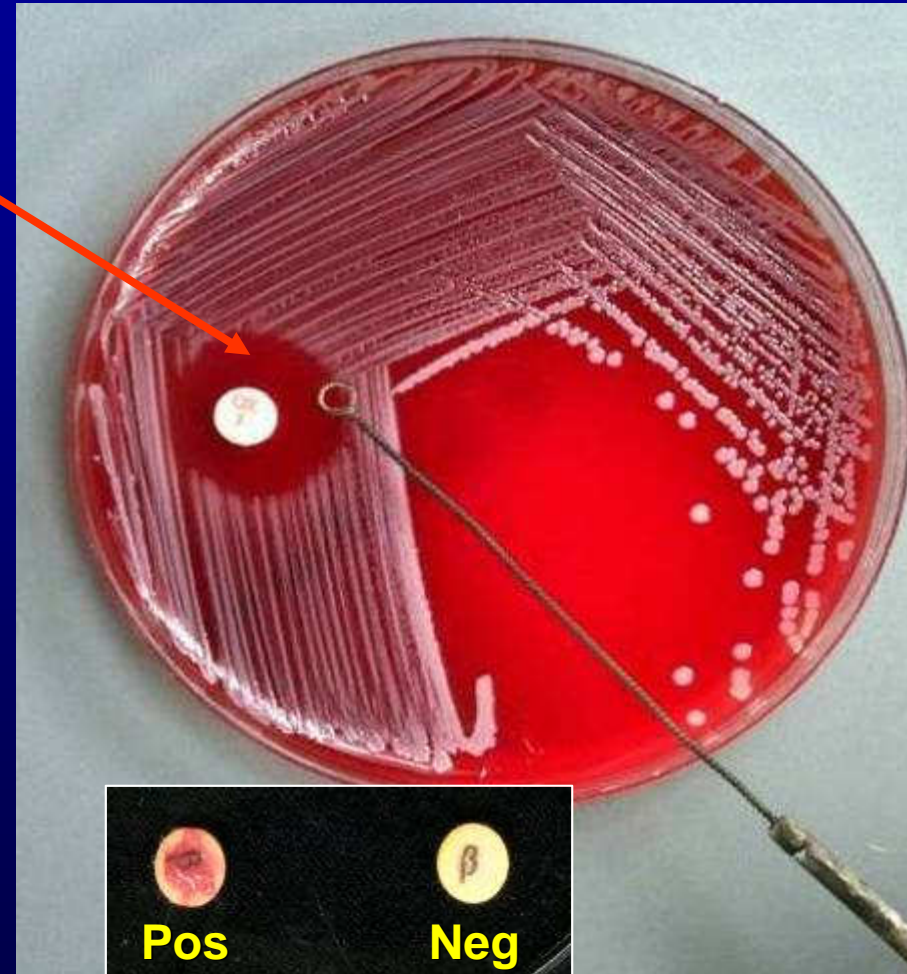
CLSI M100-S19. Table 2C.

Penicillin	10 units	≥ 29	-	≤ 28	≤ 0.12	-	≥ 0.25	(10) Penicillin-resistant strains of staphylococci produce β -lactamase, and the testing of penicillin instead of ampicillin is preferred. Penicillin should be used to test the susceptibility of all staphylococci to all penicillinase-labile penicillins, such as ampicillin, amoxicillin, azlocillin, carbenicillin, mezlocillin, piperacillin, and ticarcillin. An induced β -lactamase test should be performed on staphylococcal isolates with penicillin MICs $\leq 0.12 \mu\text{g/mL}$ or zone diameters $\geq 29 \text{ mm}$ before reporting the isolate as penicillin susceptible. A positive β -lactamase test predicts resistance to penicillin, ampicillin, amoxicillin, carbenicillin, ticarcillin, mezlocillin, and piperacillin. For oxacillin-resistant staphylococci, report penicillin as resistant or do not report. See Appendixes B and C.
		S		R	S		R	

Example of Induced β -lactamase Test

**Oxacillin
(inducer)**

- Sub isolate to agar (e.g., BAP, MHA)
- Drop β -lactam disk (e.g., oxacillin, cefoxitin)
- Incubate overnight
- Test cells from periphery of zone
- If β -lactamase positive (with or without induction), report penicillin R



Staphylococcus aureus

Phenotypic Methods for Penicillinase (1)

197 *S. aureus* with “S” penicillin MICs

Penicillin MIC (µg/ml)*	N	<i>blaZ</i> +
0.03	17	0
0.06	81	5
0.12	99	23
Total	197	28

***Vitek 2**

blaZ* = gene coding for penicillinase production in *S. aureus

Kaase et al. 2008. Clin Microbiol Infect. 14:614.

Staphylococcus aureus

Phenotypic Methods for Penicillinase (2)

28 *blaZ* Positive *S. aureus*

N (%) positive for penicillinase with 3 phenotypic methods

Disk Diffusion (≤ 28 mm zone)	Disk Diffusion (sharp zone edge)	Induced Nitrocefin β-lactamase test (60 min)
16/28 (57.1)	20/28 (71.4)	11/28 (39.3)

***Oxoid nitrocefin stick**

Conclusion: phenotypic methods may not detect staphylococci that have *blaZ* and that might not respond to penicillin therapy

Kaase et al. 2008. Clin Microbiol Infect. 14:614.

MSSA (N=24¹) isolated over 12 days...

Patient JD w/ endocarditis

Day	No. isolates	Source	Penicillin MIC ($\mu\text{g/ml}$)	Nitrocefin β -lactamase ²	blaZ PCR
1, 6	3	Blood	0.25 R	Neg	Pos
3, 4, 5, 6	6	Blood	0.12 S	Neg	Pos
7, 8, 10, 11, 12	10	Blood	>2	Pos	Pos
10	4	Mitral valve	>2	Pos	Pos
10	1	Mitral valve	0.12 S	Neg	Pos

¹ All isolates erythromycin-R, clindamycin-R (inducible), same PFGE type

² Tests performed following induction with cefoxitin, oxacillin, and penicillin; examined following incubation @ RT for 10 and 60 min

Staphylococcus spp. - Penicillin

◆ UCLA protocol

- Report penicillin if “R”
- Suppress penicillin if “S” and add note “Contact laboratory if penicillin results needed”

If penicillin needed, perform:

- induced β -lactamase test
- broth dilution MIC (penicillin 0.015-4 $\mu\text{g/ml}$)
- retest subsequent isolates from the patient
- communicate with physician

Specimen: Bone

Diagnosis: Osteomyelitis

Staphylococcus aureus

Final Report

Case #2

	<u>MIC ($\mu\text{g/ml}$)</u>
clindamycin	≤ 0.5 S
erythromycin	≤ 0.5 S
oxacillin	≤ 0.25 S
vancomycin	≤ 0.5 S

“Contact laboratory if penicillin results needed”

Vancomycin and *S. aureus*

- VSSA** – vancomycin-susceptible *S. aureus*
- VISA** – vancomycin-intermediate *S. aureus*
- VRSA** – vancomycin-resistant *S. aureus*
- hVISA** – heteroresistant VISA

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

Susceptible	Intermediate	Resistant
≤ 2	4-8	≥ 16

VSSA

≤ 1 vs $2 \mu\text{g/ml}$

hVISA

VISA

VRSA

CLSI M100-S19; Table 2C.

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

Ability of Various Methods to Detect Levels of Vancomycin Susceptibility in *S. aureus*

	Vancomycin MIC ($\mu\text{g/ml}$)	MIC Method	Disk Diffusion Method	Vancomycin Agar Screen*
VSSA {	≤ 2 S	yes	no	yes
VISA** {	4 I	yes	no	variable
	8 I	yes	no	yes
VRSA** {	16 R	yes	no	yes
	≥ 32 R	yes	yes	yes

* BHI + 6 $\mu\text{g/ml}$ vancomycin – does not detect all VISA

** Report following reference lab confirmation

CLSI M02-A10.
CLSI M07-A8.

Disk Diffusion (DD) Breakpoints (mm)

Staphylococcus spp. - Vancomycin

CLSI Document	Susceptible	Intermediate	Resistant
M100-S18 2008	≥15	-	-
New! M100-S19 2009*	-	-	-

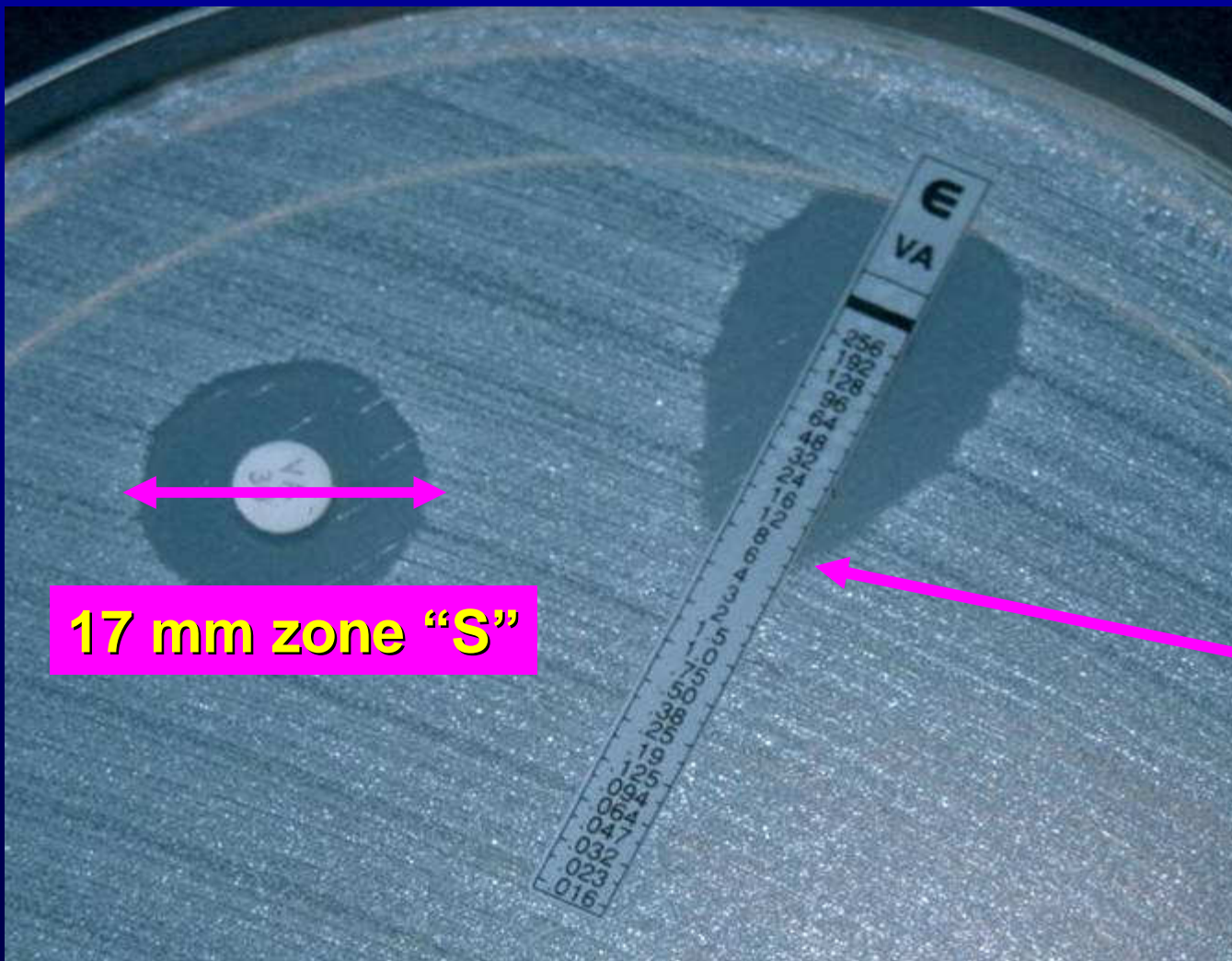
For. *S. aureus*:

*If zone ≥7 mm, perform MIC test if vancomycin needed

- Vancomycin DD is only reliable for detecting *S. aureus* containing *van-A* (VRSA); must confirm no zone (≤6 mm) results
- DD does not separate VSSA from VISA
- DD does not work for CoNS

CLSI M100-S19. Table 2C.

VISA - falsely "S" by previous (CLSI M100-S18) vancomycin disk diffusion breakpoints...



S. aureus

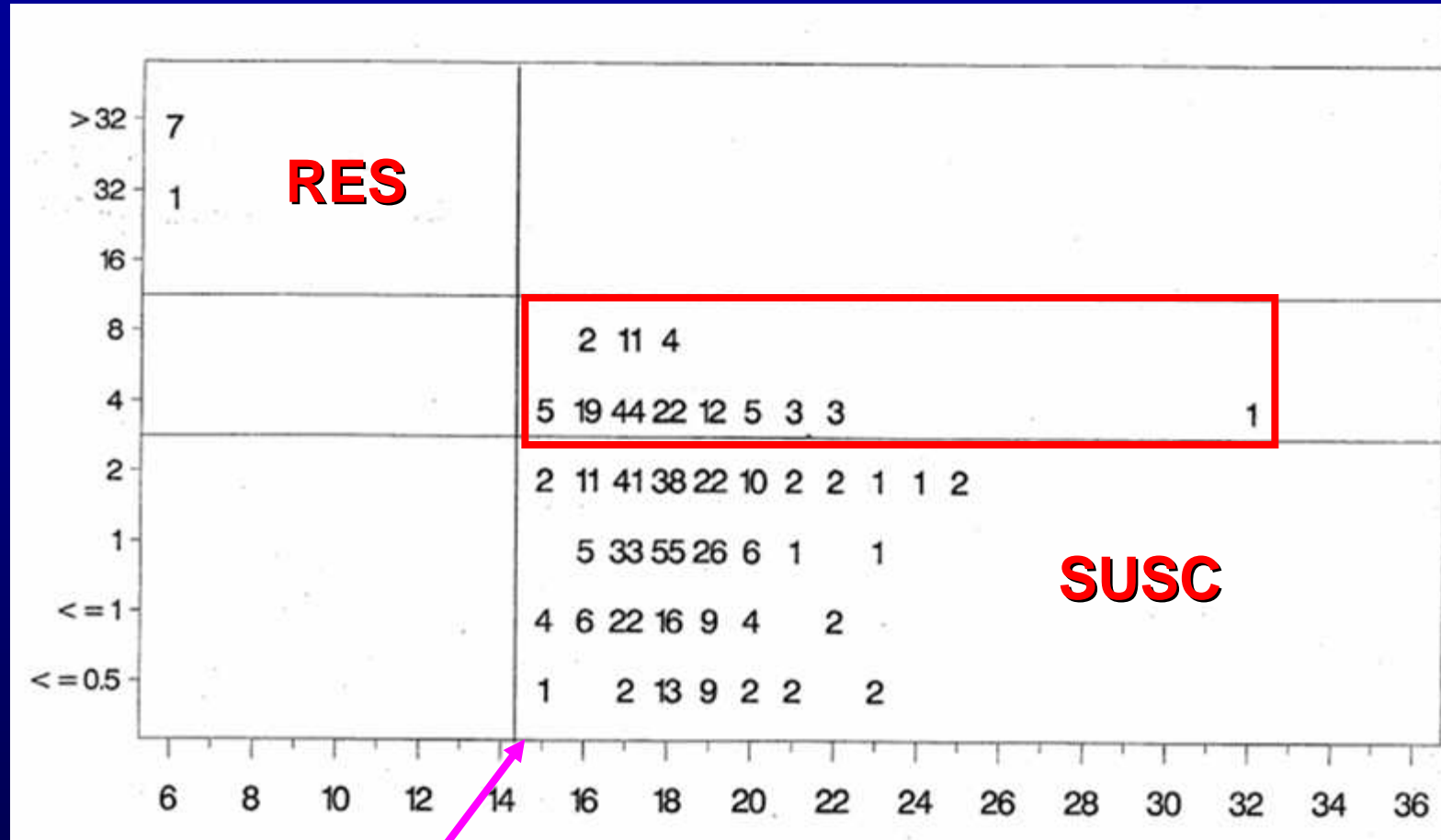
17 mm zone "S"

MIC - 8 µg/ml "I"

Vancomycin Zone Diameter vs. MIC *S. aureus*

VRSA
VISA
VSSA

Vancomycin MIC (µg/ml)



former (2008) 15 mm cutoff

Vancomycin Zone (mm)

Specimen: Pleural fluid
Diagnosis: Pneumonia
Staphylococcus aureus

Case #3

	<u>MIC ($\mu\text{g/ml}$)</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
≤ 2	4-8	≥ 16

VSSA

≤ 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 Rx
- ◆ 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”**

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

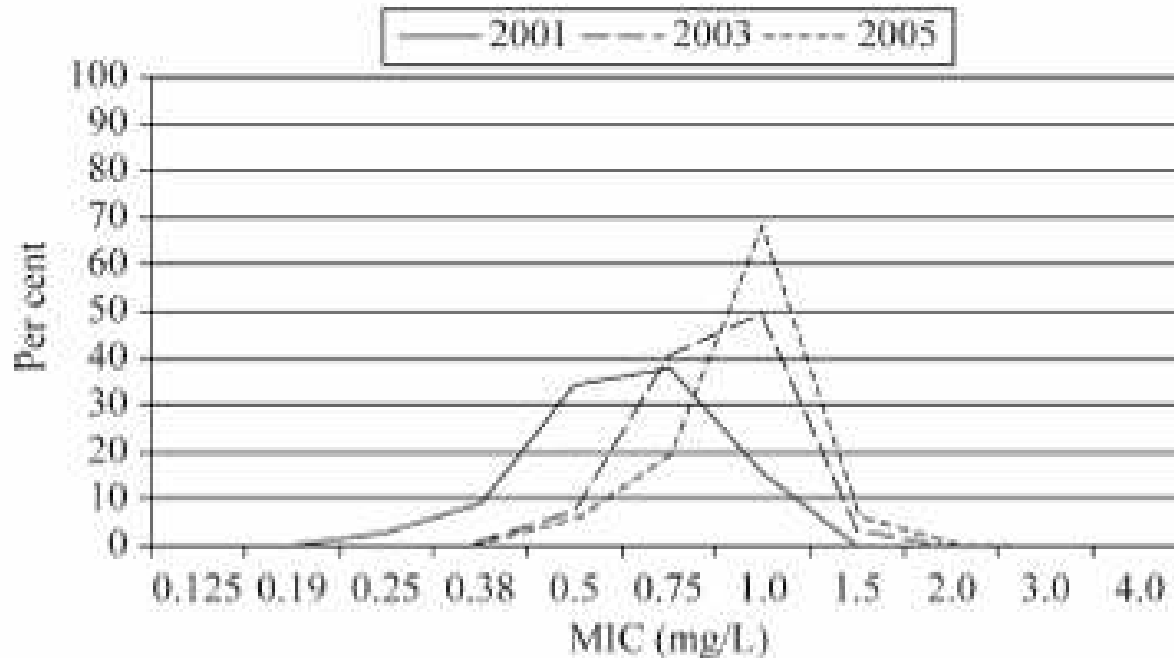


Figure 1. Vancomycin MIC population distribution 2001–05.

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

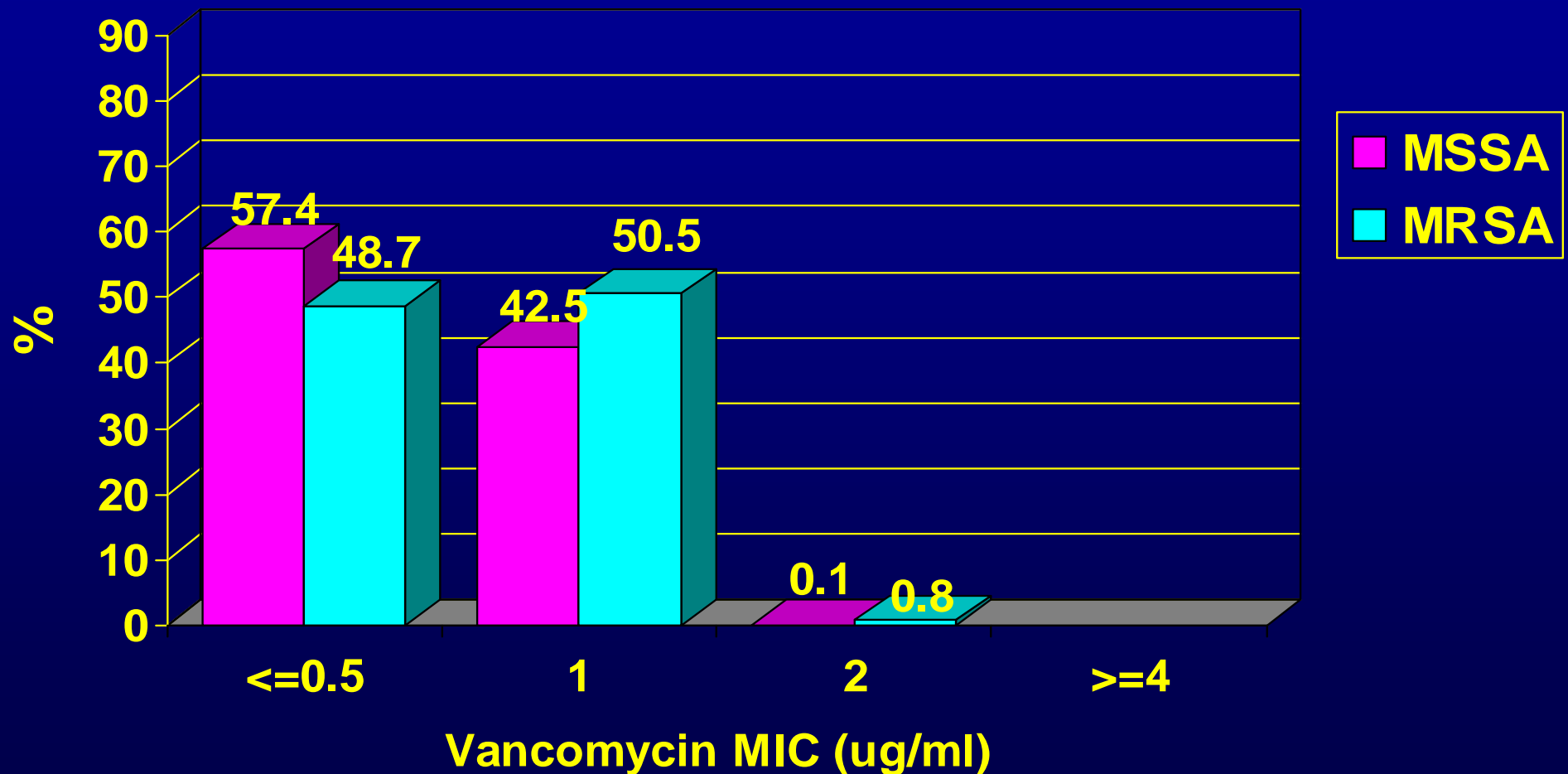
TABLE 1. Comparison of vancomycin MICs determined by broth microdilution, agar dilution, and Etest^a

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

^a 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 #3

	<u>MIC ($\mu\text{g/ml}$)</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 #4

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

Could this be hVISA?

What is hVISA*?

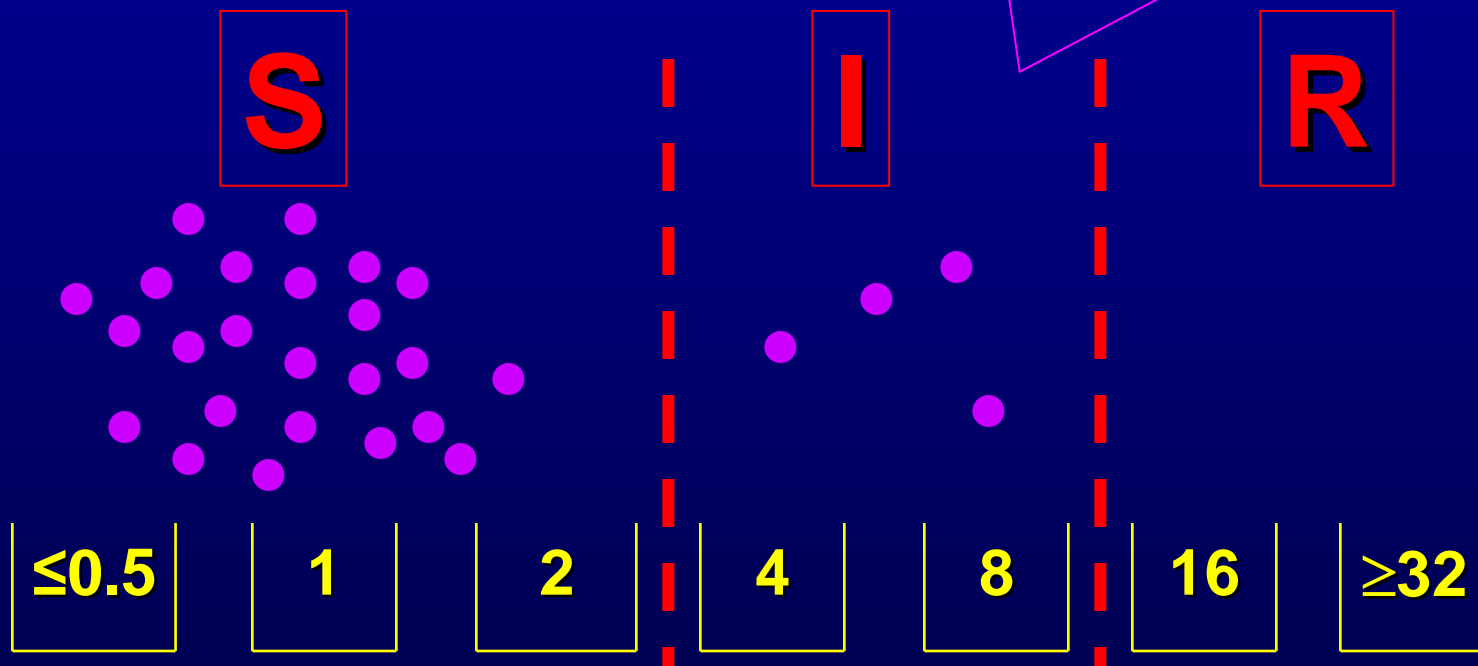
◆ *S. aureus* that show....

- vancomycin MIC ≤ 2 $\mu\text{g/ml}$ (S) by conventional MIC tests**
- subpopulation of cells with MICs 4-8 $\mu\text{g/ml}$**
- 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



● = 1 bacterium

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

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)

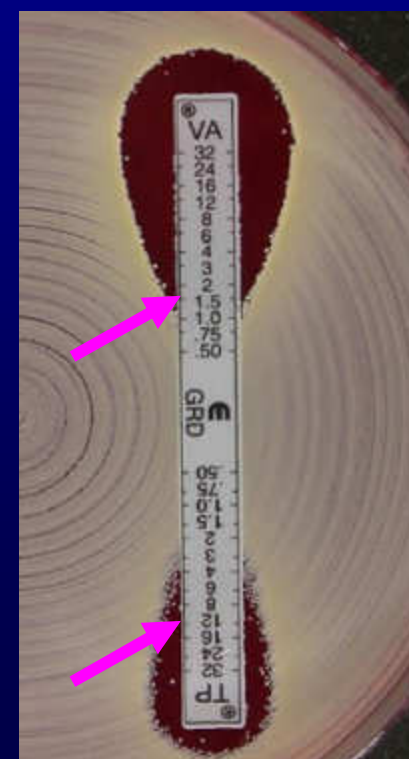
- Macro method - high inoculum (McF #2) on BHI
- GRD (glycopeptide resistance detection)

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 Etest	
	BMD fresh	BMD fresh	Std. fresh	Std. frozen
9/16	≤ 0.5 S	1 S	2 S	2
11/5	≤ 0.5 S	2 S	2 S	2
12/4	4 NS	2 S	4 I	2
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, standard Etest 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 #4

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

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

Specimen: Blood
Diagnosis: Sepsis
Staphylococcus aureus

Case #5

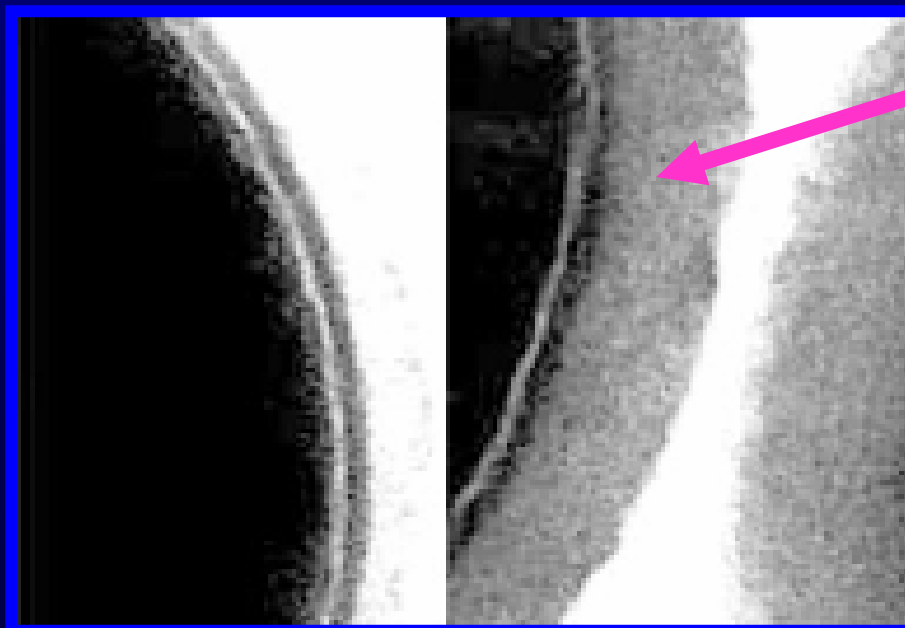
	<u>MIC ($\mu\text{g/ml}$)</u>
clindamycin	>8 R
erythromycin	>8 R
oxacillin	>16 R
penicillin	R
vancomycin	4 I

What about VISA?

VISA

Resistance due to:	Thickened cell wall
# reported in USA:	Less than 100
Detection:	Often difficult; phenotype unstable
Colony appearance:	Atypical for <i>S. aureus</i>

**Vancomycin
susceptible**

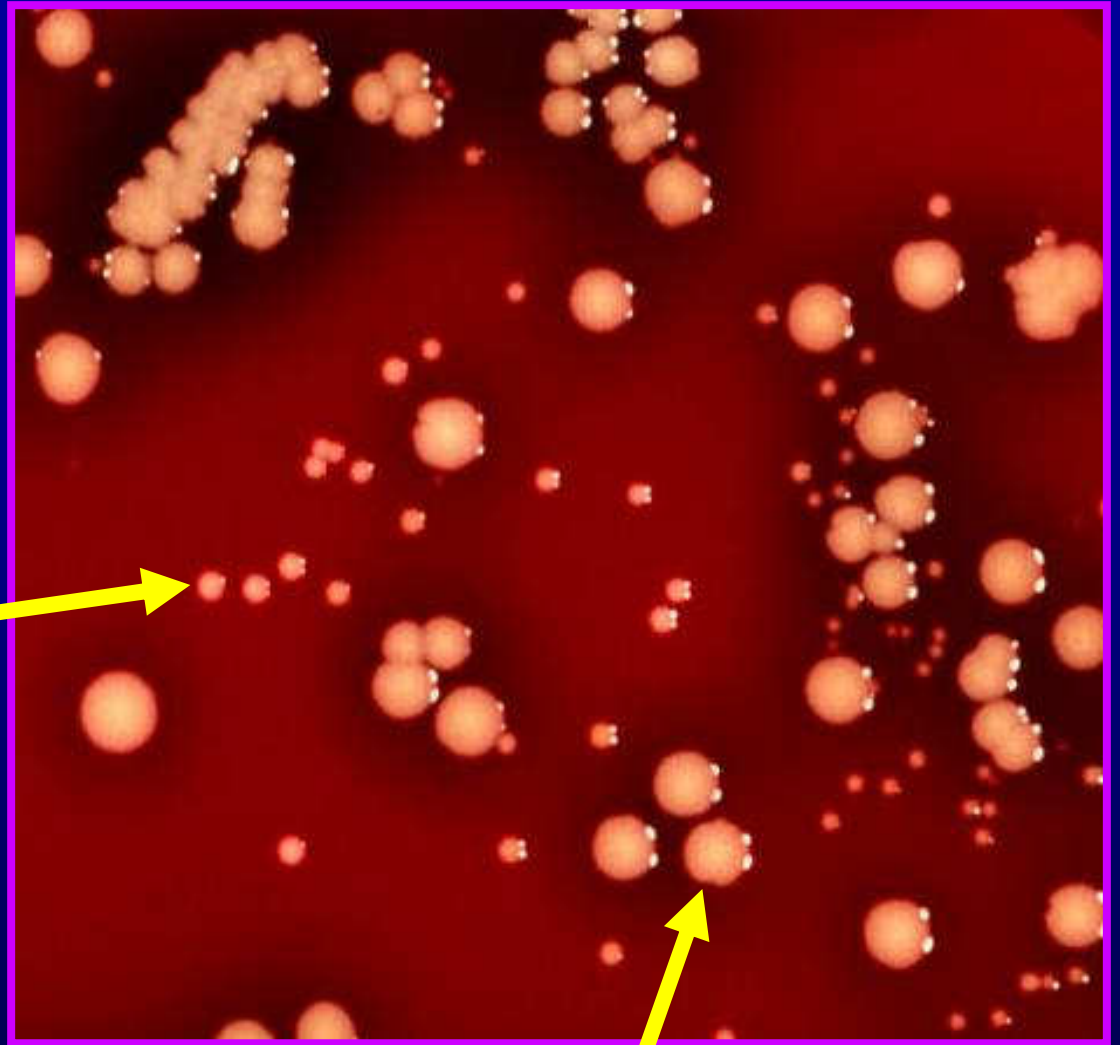


Cell wall

**Vancomycin
intermediate**

**48 hour growth on
blood agar plate**

VISA

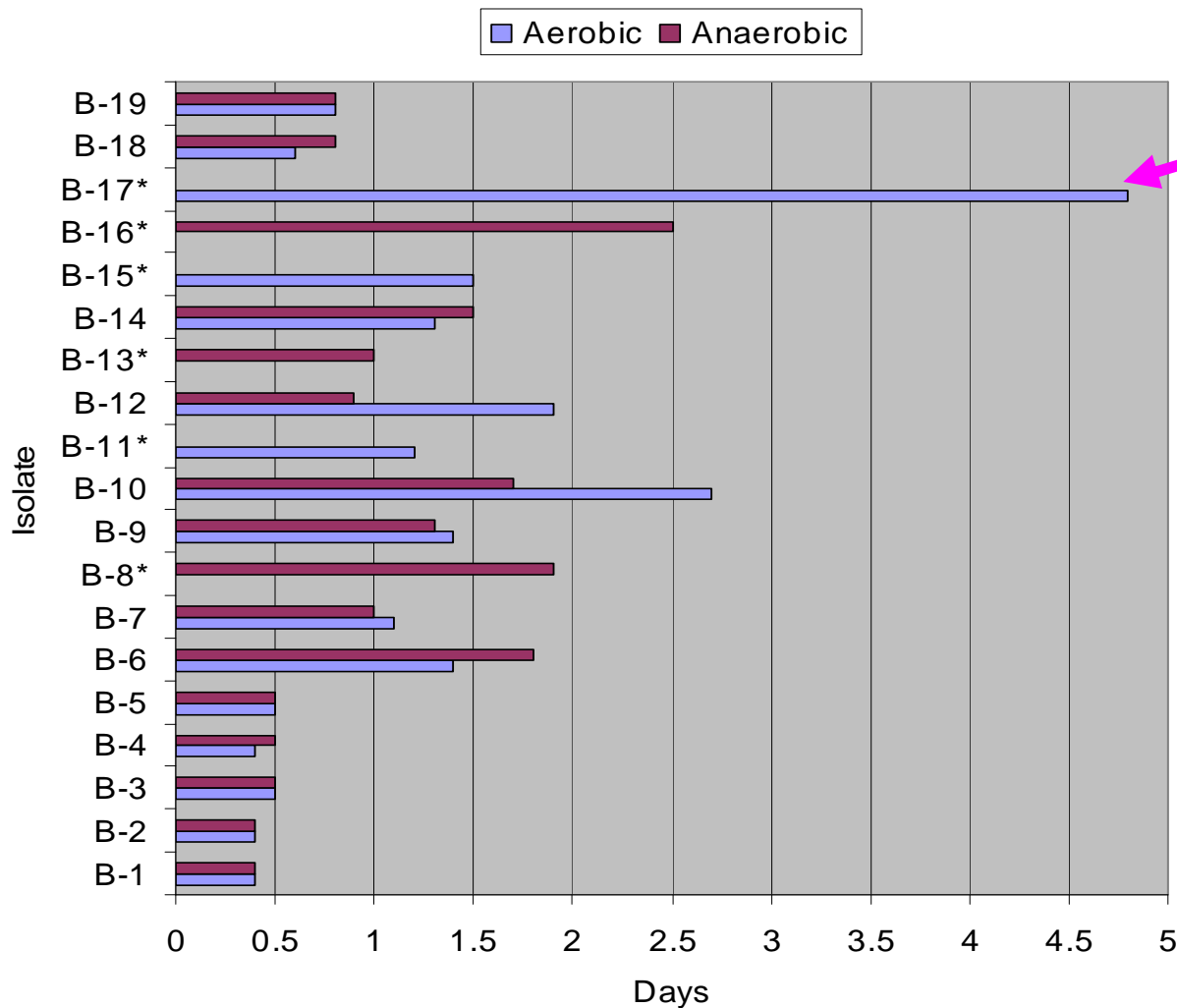


Marlowe, et al. 2001. J Clin Microbiol. 39:2637.

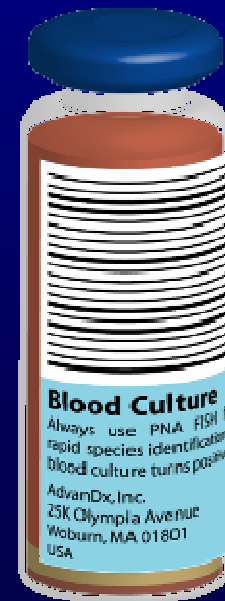
MRSA (not VISA)

UCLA Patient #SS - MRSA (non-VISA) and VISA, Time to Detection of Growth in Blood Cultures

Patient B Blood Culture Time to Positivity
* Paired bottle remained negative



One VISA; all others MRSA (non-VISA)



Characteristics sometimes observed for VISA...

- ◆ Colony morphology may be atypical for *S. aureus* (some pinpoint)
- ◆ Delayed growth in broth (e.g., blood culture)
- ◆ Weak / delayed coagulase reaction
- ◆ After several subcultures:
 - Colony morphology becomes typical for *S. aureus*
 - Vancomycin MIC decreases and isolate becomes vancomycin susceptible
- ◆ MICs for β -lactams decrease
- ◆ MICs for daptomycin increase

Specimen: Blood
Diagnosis: Sepsis
Staphylococcus aureus

Final Report

Case #5

	<u>MIC ($\mu\text{g/ml}$)</u>
clindamycin	>8 R
daptomycin	4 NS
erythromycin	>8 R
linezolid	≤ 0.5 S
oxacillin	>16 R
penicillin	R
vancomycin	4 I

**“Vancomycin-intermediate, daptomycin non-susceptible*
S. aureus; Infectious Diseases consult suggested.”**

What should we do about testing vancomycin and MRSA?

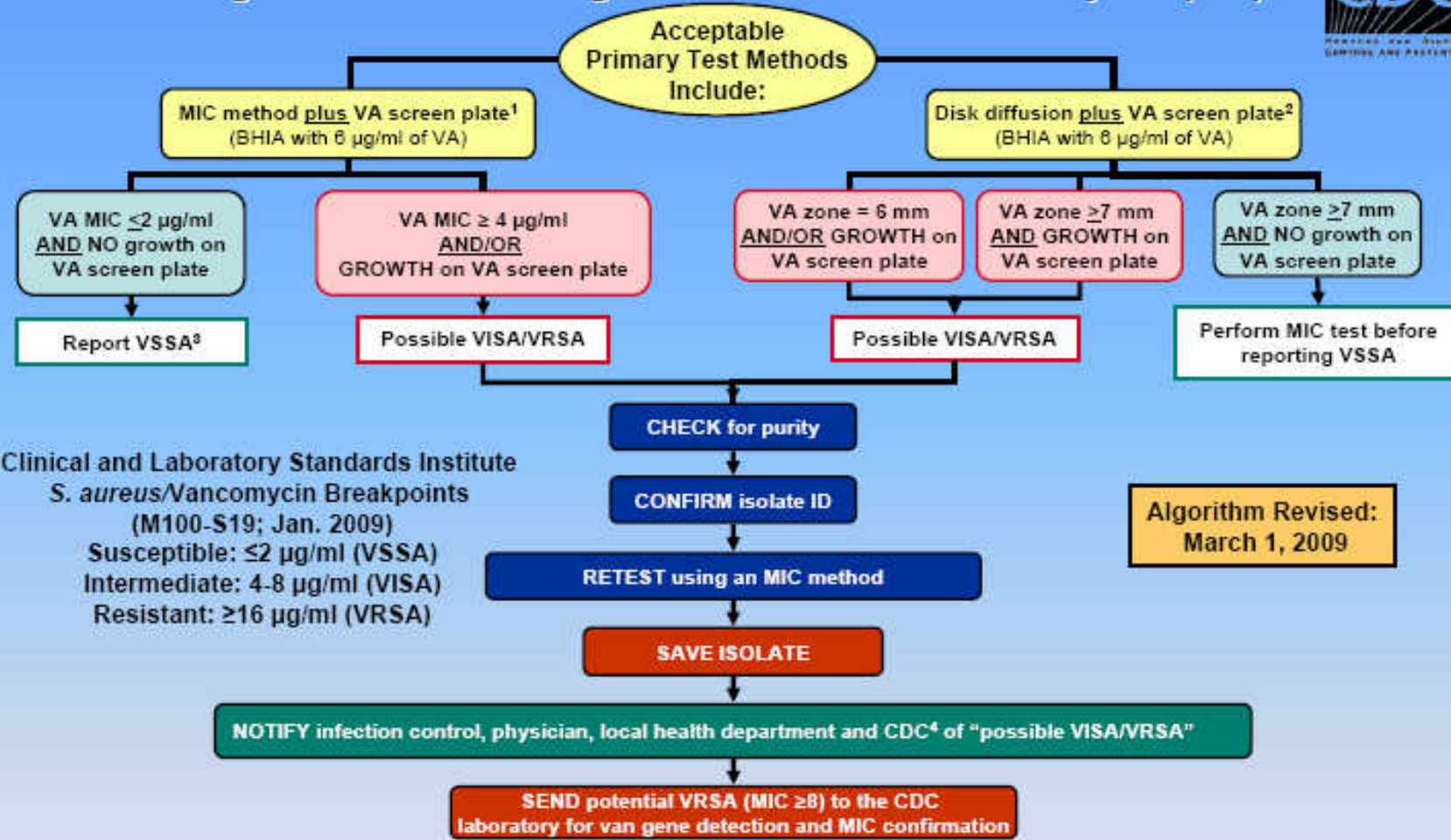
- ◆ Use reliable MIC method for **VSSA, VISA** and **VRSA**
 - Avoid repeated subcultures
- ◆ Be **consistent** with testing/reporting
- ◆ Perform special testing on request
 - There are no standardized recommendations for detecting **hVISA** in the clinical laboratory
 - Some questions remain re: the significance of **hVISA** or isolates with **vancomycin MICs of 2 µg/ml**

**Some refs... Falagas et. al. 2008. Clin Microbiol Infect. 14:101.
Sakoulas & Moellering. 2008. Clin Infect Dis. 46(S):360.
Tenover & Moellering. 2007. Clin Infect Dis. 44:1208.**

...and remember

- ◆ Clinicians must factor in the **clinical status of the patient** as well as laboratory data in decision making

Algorithm for Testing *S. aureus* with Vancomycin (VA)



Clinical and Laboratory Standards Institute
S. aureus/Vancomycin Breakpoints
 (M100-S19; Jan. 2009)
 Susceptible: ≤ 2 µg/ml (VSSA)
 Intermediate: 4-8 µg/ml (VISA)
 Resistant: ≥ 16 µg/ml (VRSA)

Algorithm Revised:
 March 1, 2009

Important Footnotes

- ¹ Laboratories using automated MIC methods that have not been validated for VRSA detection should add a commercial VA agar screen plate (6 µg/ml).
- ² Disk diffusion will not differentiate VISA (MICs 4-8) from susceptible strains (MICs 0.5-2). The vancomycin disk test will detect VRSA isolates containing the *vanA* resistance gene by showing no zone of inhibition around the disk (zone = 6 mm). VA screen plate will not reliably detect strains for which MIC=4.
- ³ If concerned about a result based on a patient's history, send to a reference lab for MIC testing.
- ⁴ Report only isolates with MIC ≥ 8 µg/ml or zone diameter = 6 mm to CDC by email: SEARCH@cdc.gov

More VISA/VRSA info: http://www.cdc.gov/ncidod/dhqp/ar_visavrsa.html

http://www.cdc.gov/ncidod/dhqp/pdf/ar/VRSA_testing_algo09v4.pdf

Re: vancomycin MIC... when should we send staphylococci for further testing?

◆ *S. aureus*

- MIC 4 µg/ml – maybe
- MIC ≥8 µg/ml – yes

◆ CoNS

- MIC ≥32 µg/ml – yes

Check with your public health department!

Staphylococcus spp.

-Inducible clindamycin resistance

Specimen: Pus (L buttock lesion)

Diagnosis: Localized Abscess

CASE #6

Many *Staphylococcus aureus*

clindamycin	S ???
erythromycin	R
oxacillin	R
penicillin	R
vancomycin	S

What should we do about clindamycin results?

S. aureus “D Zone Test”

D zone test is only for
staphylococci that are:
Erythromycin “R” and
Clindamycin “S” or “I”

Photo 1: inducible clindamycin R

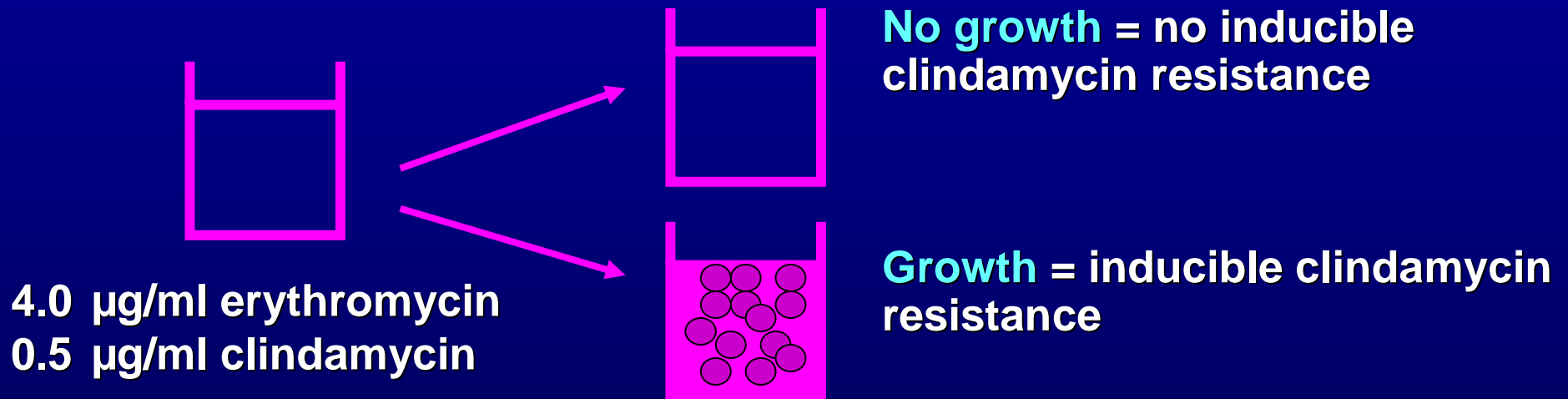
Photos 2 and 3: erythromycin R
and clindamycin R (D zone test
NOT needed)



Staphylococcus spp.

Inducible Clindamycin Resistance

MIC Method



Be careful with inoculum preparation

Anecdotal data:

False "S" if young colonies or underinoculated

Specimen: Pus (L buttock lesion)

Diagnosis: Localized Abscess

Final Report

Many *Staphylococcus aureus*

CASE #6

clindamycin	R
erythromycin	R
oxacillin	R
penicillin	R
vancomycin	S

Erythromycin-R, Clindamycin-S *Staphylococci*

Tests for Inducible Clindamycin Resistance

◆ Notes

- Test applies to *S. aureus* and CoNS (OX-S and OX-R)
- Test not routinely done on CoNS as clindamycin rarely used for CoNS
- Most hospital-associated MRSA are clindamycin-R
- Many community-associated MRSA are clindamycin-S (*msrA* genotype)
- Clindamycin can be give orally

Newer Antimicrobial Agents

S. aureus

Drug	Route			Comments
	PO	IM	IV	
daptomycin			x	FDA indications for complicated skin infxns, bacteremia; NOT for respiratory infxns (lung surfactant inhibits drug); S only breakpoint
linezolid	x		x	FDA indications for nosocomial pneumonia, complicated skin infxns, uncomplicated skin infxns (MSSA); severe VRE infxns; community-acquired pneumonia (MSSA); S only breakpoint
tigecycline			x	FDA indications for complicated skin infxns (MRSA and MSSA); complicated intra-abdominal infections (MSSA only). Usually if tetracycline-S, tigecycline-S; S only breakpoint.

Antimicrobial Agents for *S. aureus* in the Pipeline!

- ◆ **Lipoglycopeptides (IV administration)***
 - Dalbavancin
 - Oritavancin
 - Televancin
- ◆ **Ceftobiprole (IV administration)**
 - Broad-spectrum cephalosporin with MRSA coverage
 - Projected to be widely prescribed

***vancomycin is glycopeptide**

Coagulase-negative *staphylococci*
(CoNS)

- AST rules for various species

Specimen: Shunt fluid

Diagnosis: Spina Bifida

Coagulase-negative Staphylococcus

	<u>MIC ($\mu\text{g/ml}$)</u>
clindamycin	>8 R
erythromycin	>8 R
oxacillin	1 R
penicillin	R
vancomycin	≤ 0.5 S

Case #7

***Should we do more testing?
(isolate is PYR negative)***

What are CLSI rules for detecting MRCoNS?

Species	Rule
<i>S. lugdunensis</i>	Use oxacillin and cefoxitin breakpoints as for <i>S. aureus</i>
<i>S. saprophyticus</i>	Don't test; add comment to report re: appropriate therapy for <i>S. saprophyticus</i> UTI
<i>S. epidermidis</i>	Use CoNS oxacillin (MIC or DD) and/or cefoxitin (DD only) breakpoints - work well
Other CoNS	Use caution for oxacillin MICs 0.5-2.0; cefoxitin testing is better!

Eliminated oxacillin disk diffusion test for CoNS (2009)!

Staphylococcus lugdunensis Identification



	<u>Slide Coag</u>	<u>Latex agglu</u>	<u>Tube Coag</u>	<u>Ornithine Decarbox</u>	Poly B ⁺	PYR
<i>S. aureus</i>	+	+	+	-	R	- or weak
<i>S. intermedius</i> (dogs)	V	V	+	-	S	+
<i>S. lugdunensis</i>	V	V	-	+	R	+
<i>S. schleiferi</i>	+	+	-	-	S	+
<i>S. saprophyticus</i> **	-	V	-	-	S	-
<i>S. epidermidis</i>	-	-	-	V-delayed	R	-
most other CoNS	-	-	-	-	S	V

* 1.0 McFarland inoculum; **Novobiocin resistant;

+ or -, >95% of isolates demonstrate reaction

Refs: Manual of Clin Microbiol 8th ed 2003, p. 392-393; Clin Microbiol Proc Manual 2004, p. 3.18.1.7

Most commercial ID kits acceptable

Courtesy of Mary York

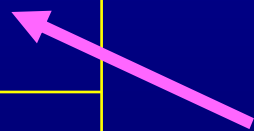
Tests for Oxacillin Resistance in Coagulase-negative Staphylococci (CoNS) (except *S. lugdunensis*)

Method	Susceptible	Intermediate	Resistant
MIC – oxacillin	$\leq 0.25 \mu\text{g/ml}$	-	$\geq 0.5 \mu\text{g/ml}$
Disk Dif – cefoxitin*	$\geq 25 \text{ mm}$	-	$\leq 24 \text{ mm}$

*Detects *mecA*-mediated oxacillin resistance (preferred test)
Cefoxitin is surrogate; report oxacillin

Results for 196* “Routinely Encountered” CoNS

Method	Sensitivity	Specificity
CX-DD 24h	99%	97%
OX DD 24h	99%	89%
OX MIC 24h	98%	91%

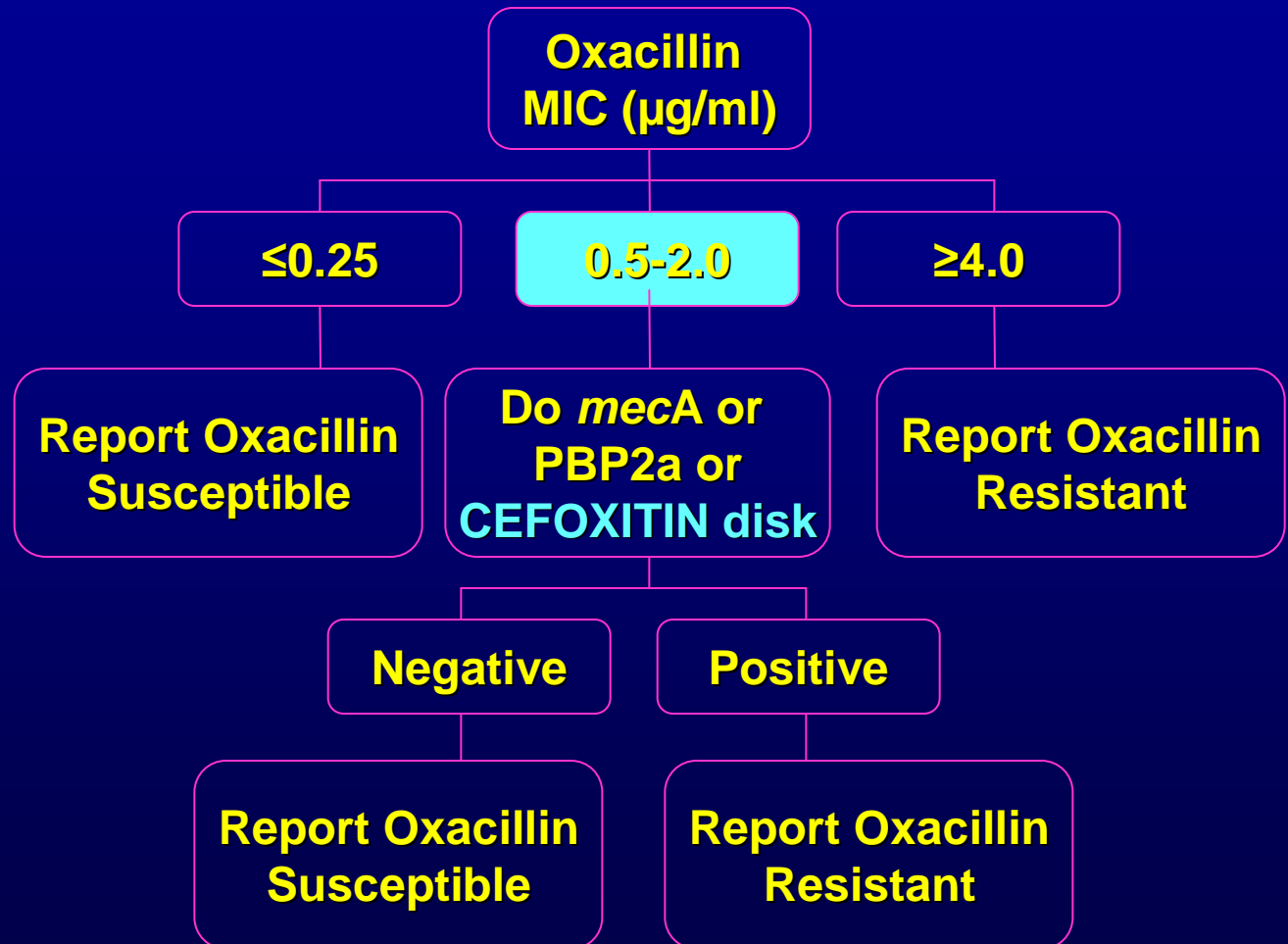


***126 *mecA* positive and 70 *mecA* negative**

Cefoxitin disk diffusion test least likely to call oxacillin falsely resistant!

Reporting Strategy Oxacillin MIC Results for CoNS*

*"For testing non-*S. epidermidis* isolates from sterile sites where CoNS is causing an infection"



Specimen: Shunt fluid
Diagnosis: Spina Bifida

Final Report

Coagulase-negative Staphylococcus

	<u>MIC ($\mu\text{g/ml}$)</u>
clindamycin	>8 R
erythromycin	>8 R
oxacillin	1 R
penicillin	R
vancomycin	≤ 0.5 S

Case #7

Oxacillin may be false resistant. Discuss case with physician and determine if further testing (e.g., cefoxitin disk diffusion, *mecA*, PBP2a) is warranted.

Why is reporting “false resistance” to oxacillin in staphylococcus a concern?

- ◆ For oxacillin-R *staphylococci*, drug most likely to be used is **vancomycin** or maybe a newer agent (e.g., linezolid, daptomycin)
- ◆ **Vancomycin** kills staphylococci slower than **β -lactams** and has **poorer tissue penetration**

Specimen: Blood

Diagnosis: Endocarditis

Coagulase-negative staphylococcus

	<u>MIC ($\mu\text{g/ml}$)</u>
clindamycin	≤ 0.5 S
erythromycin	≤ 0.5 S
oxacillin	0.5 ?
penicillin	≤ 0.06 ?
vancomycin	≤ 0.5 S

Case #8

Should we rule out *S. lugdunensis*?

What are some noteworthy features of S. lugdunensis?

- ◆ **Common skin commensal**, often in inguinal area
- ◆ Has been associated with septicemia, breast abscesses, peritonitis, infections w/ prosthetic devices, osteomyelitis, septic arthritis
- ◆ Can cause “**native valve**” endocarditis; most CoNS “**prosthetic valve**” endocarditis due to *S. epidermidis*
- ◆ **Usually very susceptible to antimicrobial agents...including penicillin-S**
 - Only 5 - 25% penicillin-R, β -lactamase positive

CLSI Interpretive Criteria *S. lugdunensis*

Method	Susceptible	Intermediate	Resistant
MIC – oxacillin*	≤ 2 $\mu\text{g/ml}$	-	≥ 4 $\mu\text{g/ml}$
Disk Dif – cefoxitin*	≥ 22 mm	-	≤ 21

* Oxacillin MIC and cefoxitin disk diffusion breakpoints are the same as those for *S. aureus*

CLSI M100-S19. Table 2C.

Staphylococcus lugdunensis

Typical MICs	MIC ($\mu\text{g/ml}$)	Interpreted w/ oxacillin breakpoints for:	
		<i>S. aureus</i>	CoNS
cefazolin	≤ 0.5	S	R*
clindamycin	≤ 0.5	S	S
erythromycin	≤ 0.5	S	S
oxacillin	0.5	S	R
penicillin	≤ 0.06	S	R*
vancomycin	≤ 0.5	S	S

correct!

incorrect!

* Edited to "R" because of oxacillin-R result

Specimen: Blood

Final Report

Diagnosis: Endocarditis

Staphylococcus lugdunensis

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

clindamycin	≤ 0.5 S
erythromycin	≤ 0.5 S
oxacillin	0.5 S
penicillin	≤ 0.06 S
vancomycin	≤ 0.5 S

Case #8

Can S. lugdunensis be a contaminant in blood cultures?

- ◆ 11/01-1/08 - **29 / 5784** single pos blood culture w/ CoNS = *S. lugdunensis*
- ◆ 14/29 (48%) not acknowledged
- ◆ 6/29 (21%) considered contaminant
- ◆ **9/21 (31%)** true bacteremia

***S. lugdunensis* can be blood culture contaminant**

Specimen: Urine

Diagnosis: Acute cystitis

Case #9

>10⁵ CFU/ml *S. saprophyticus*

Should we perform AST?

Final Report

Specimen: Urine

Diagnosis: Acute cystitis

>10⁵ CFU/ml *S. saprophyticus*

Case #9

“*Staphylococcus saprophyticus* typically responds to urine concentrations of agents commonly used to treat acute, uncomplicated UTIs (e.g. nitrofurantoin, TMP-SMX, or a fluoroquinolone).”

CLSI says no need to test...

CLSI M100-S19. Table 2C.

What about S. saprophyticus vs. other CoNS in urine?

◆ *S. saprophyticus*

- Predominantly from young, sexually active females
- Can cause upper and lower UTI
- Contaminant in urine only 5% of the time

◆ Other CoNS (predominantly *S. epidermidis*)

- Almost exclusively from inpatients with complications of urinary tract (e.g., catheter, urological surgery, transplant, stones)
- When present in urine, significant only 10% of the time

Archer, GL et. al. 2005. Chpt. 193. In Mandell, Douglas, and Bennett's Principles and Practices of Infectious Diseases. 6th ed. Elsevier Churchill Livingstone.

Streptococcus pneumoniae

- **Newer penicillin breakpoints**
- **AST and reporting**

Specimen: Blood
Diagnosis: Pneumonia
Streptococcus pneumoniae

Case #10

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

ceftriaxone (meningitis)	0.5 S
ceftriaxone (non-meningitis)	0.5 S
erythromycin	>1 R
levofloxacin	1 S
meropenem	≤ 0.25 S
penicillin	1 ???
vancomycin	0.5 S

How do we interpret penicillin results?

Streptococcus pneumoniae

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

	Susc	Int	Res
Penicillin parenteral (nonmeningitis)	≤ 2	4	≥ 8
Penicillin parenteral (meningitis)	≤ 0.06	-	≥ 0.12
Penicillin (oral penicillin V)	≤ 0.06	0.12-1	≥ 2

Table 2G has several therapy (Rx) comments for each category

CLSI M100-S19. Table 2G.

Specimen: Blood

Final Report

Diagnosis: Pneumonia

Streptococcus pneumoniae

Case #10

	<u>MIC ($\mu\text{g/ml}$)</u>
ceftriaxone (meningitis)	0.5 S
ceftriaxone (non-meningitis)	0.5 S
erythromycin	>1 R
levofloxacin	1 S
meropenem	≤ 0.25 S
penicillin (meningitis)	1 R
penicillin (nonmeningitis)	1 S
penicillin (oral penicillin V)	1 I
vancomycin	0.5 S

Specimen: CSF

Diagnosis: Meningitis

Streptococcus pneumoniae

	<u>MIC ($\mu\text{g/ml}$)</u>
ceftriaxone (meningitis)	0.5 S
meropenem	≤ 0.25 S
penicillin (meningitis)	≤ 0.06 S
vancomycin	0.5 S

Rx. Use of ceftriaxone in meningitis requires therapy with maximum doses.

Rx. Use of penicillin in meningitis requires therapy with maximum doses of IV penicillin (e.g., at least 3 million units every four hours in adults with normal renal function).

Applying penicillin, cefotaxime, ceftriaxone breakpoints....

◆ CSF specimens

- Report penicillin, cefotaxime, ceftriaxone using meningitis breakpoints ONLY

◆ All other specimens

- Report penicillin, cefotaxime, ceftriaxone using both meningitis and nonmeningitis breakpoints

What about all of the *S. pneumoniae*

Rx comments?

- ◆ Most relate to recommended dosages
- ◆ Some relate to predicting results for other beta-lactams based on penicillin MIC
- ◆ Add to your reports.... if your medical director deems this is appropriate
 - ...or perhaps there are other mechanisms for proper dosing in your facility?

Streptococcus pneumoniae

Disk diffusion method - Use oxacillin disk as surrogate for penicillin susceptibility

	Disk content	Zone (mm)			MIC ($\mu\text{g/ml}$) Equivalent	
		R	I	S	R	S
Penicillin	1 μg oxacillin	-	-	≥ 20	-	$\leq 0.06^*$

*** correlates with meningitis and oral breakpoints**

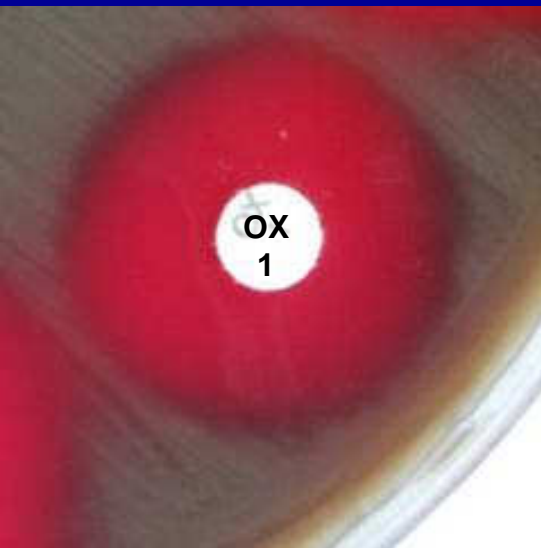
CLSI M100-S19. Table 2G.

Streptococcus pneumoniae

Oxacillin Disk Test for Penicillin Susceptibility

- ◆ **≥20 mm** - Report “S” for:
 - penicillin
 - cefotaxime/ ceftriaxone
 - other β-lactams
- ◆ **≤19 mm** - Perform MICs for:
 - penicillin
- ◆ Oxacillin disk S or **≥20 mm** correlates with meningitis or penicillin oral S breakpoints (MIC **≤0.06 μg/ml**)

Oxacillin Disk as Surrogate for Penicillin Susceptibility in *S. pneumoniae*



≥ 20 mm – report penicillin as S

When penicillin MIC was performed on this isolate, MIC = 0.03 $\mu\text{g/ml}$



≤ 19 mm – perform penicillin MIC

When penicillin MIC was performed on this isolate, MIC = 1 $\mu\text{g/ml}$

“S” for non-meningitis infections (e.g., pneumonia)

How are *S. pneumoniae* AST results used to guide therapy of meningitis?

- ◆ **Empiric therapy** – generally 3rd generation cephalosporin (e.g., cefotaxime or ceftriaxone) + vancomycin
- ◆ **AST results**
 - May **discontinue vancomycin** and use β -lactam alone if:
 - Penicillin MIC ≤ 0.06 $\mu\text{g/ml}$
 - Ceftriaxone / cefotaxime MIC ≤ 0.5 $\mu\text{g/ml}$

Sanford Guide 2009.

How are *S. pneumoniae* AST results used to guide therapy of CAP (community-acquired pneumonia)?

◆ Empiric therapy - resources

- IDSA (Infectious Disease Society of America) and ATC (American Thoracic Society)

Mandell et al. 2007. Clin Infect Dis. 44:227.

- Published surveillance data
- Local cumulative antibiogram data

◆ AST results

- May modify therapy if poor response to empiric therapy

Recommended Empirical Antibiotics for CAP in Outpatients

Clinical Condition	Recommended Antimicrobials
Previously healthy, no antimicrobials within previous 3 mos.	A macrolide Doxycycline
Comorbidities (e.g. chronic heart, lung, liver, or renal disease, diabetes mellitus, alcoholism, malignancies, asplenia; immunosuppressed; use of antimicrobials within previous 3 mos; other risks for DRSP)	Respiratory fluoroquinolone (levofloxacin [750 mg], moxifloxacin, gemifloxacin) β-lactam plus a macrolide
In regions where high incidence of macrolide-R <i>S. pneumoniae</i> (including those w/o comorbidities)	“ “ “

Adapted from Mandell L et al. 2007. Clin Infect Dis. 44(suppl 2):S27.

Summary (1)

- ◆ **Cefoxitin disk diffusion and cefoxitin MIC tests** are more sensitive in detecting *mecA*-mediated resistance in *S. aureus* than tests using oxacillin.
- ◆ **Borderline oxacillin resistance** in *S. aureus* may be missed with cefoxitin tests; borderline oxacillin resistance is rare in *S. aureus* and has not been reported in CoNS.
- ◆ **Borderline oxacillin resistant *S. aureus*** may not be clinically significant.
- ◆ Current phenotypic methods may not detect staphylococci that produce **low levels of beta-lactamase**.

Summary (2)

- ◆ **Vancomycin disk diffusion** test should only be used to rule out VRSA; it cannot distinguish VISA from VSSA.
- ◆ 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**.
- ◆ **VISA** and **hVISA** are often slow growing and may yield a variety of colony morphologies.

Summary (3)

- ◆ Erythromycin-R and clindamycin-S staphylococci should NOT be reported as clindamycin-S without performance of a test for **inducible clindamycin resistance** (e.g., D zone test).
- ◆ **Oxacillin disk diffusion test** was eliminated for CoNS in 2009.
- ◆ Oxacillin MIC tests work well for *S. epidermidis* but can overcall oxacillin-R in other CoNS that lack *mecA*. The cefoxitin disk diffusion test is better.
- ◆ Most *S. lugdunensis* are *mecA* negative and more than 75% are β -lactamase negative.

Summary (4)

- ◆ *S. saprophyticus* are predictably susceptible to drugs commonly used to treat UTIs; susceptibility testing of *S. saprophyticus* from urine is not needed.
- ◆ Species of CoNS other than *S. saprophyticus* rarely cause UTIs.
- ◆ The newer CLSI penicillin breakpoints should be used for *S. pneumoniae* as they better predict potential utility of penicillins in treating pneumococcal pneumonia.
- ◆ Penicillin MIC should be done on *S. pneumoniae* that have oxacillin zones ≤ 19 mm.

Thank you!

