



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Everything You Wanted to Know About Antimicrobial Susceptibility Testing (AST) of *Staphylococcus aureus*!

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**currently working with CDC's Division of Laboratory Systems through an Interagency Personnel Agreement*

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

- ◆ Describe current NCCLS recommendations for disk diffusion and MIC testing of *S. aureus*.
- ◆ Discuss current strategies for reporting AST results on *S. aureus*.
- ◆ List steps for verifying and reporting VISA and VRSA.



At the conclusion of this presentation, you will be able to..... (con't)

- ◆ List supplemental drugs that might be reported on MRSA, VISA, and VRSA and discuss testing of these agents.
- ◆ Describe a method for assessing competency of staff in detecting and reporting MRSA, VISA and VRSA.



Acronyms

MRSA – methicillin-resistant *S. aureus*

ORSA – oxacillin-resistant *S. aureus*

(MRSA = ORSA)

BORSA – borderline ORSA

CA-MRSA – community-associated MRSA

VISA – vancomycin-intermediate *S. aureus*

VRSA – vancomycin-resistant *S. aureus*

PBP2' (PBP2a) – penicillin-binding protein 2' (2a)

Staphylococcus aureus - Rx

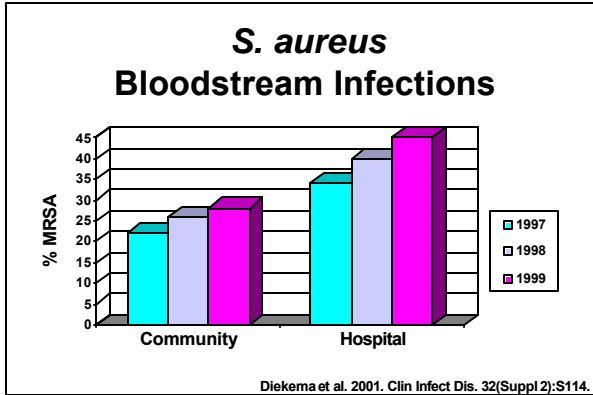
Organism	1st Choice Drugs	Alternative Drugs
	oxacillin, nafcillin	a cephalosporin, vancomycin, β-lac/β-lac inhibitor combo, carbapenem, macrolide, clindamycin, linezolid, quin- dalfo, fluoroquinolone
	vancomycin	linezolid, quin-dalfo, fluoroquinolone, trim-sulfa

Sanford Guide, 2003

MRSA Epidemiology

Methicillin-resistant *Staphylococcus aureus*

MRSA



- ### **MRSA in Non-Healthcare Settings Community Associated MRSA (CA-MRSA)**
-
- ◆ **Competitive sports**
 - ◆ **School children**
 - ◆ **Correctional facilities**
 - ◆ **Men who have sex with men (MSM)**
- Salgado et al. 2003. Clin Infect Dis. 36(Suppl. 2):S131.
MMWR. 2003; 52:88.

- ### **CA-MRSA (con't)**
-
- ◆ **May possess Panton Valentine leucocidin (PVL)**
 - **Facilitates MRSA crossing intact skin barrier**
 - **Can cause septicemia in immunocompetent patients**
 - **Associated with lethal necrotizing pneumonia**

CA-MRSA (con't)

- ◆ MRSA carry staphylococcal cassette chromosome *mec* (SCC*mec*) as methicillin resistant determinant
- ◆ Novel SCC*mec* (Type IV) in some CA-MRSA
- ◆ CA-MRSA may have distinct origin of derivation

CA-MRSA (con't)

- ◆ Often susceptible to:

clindamycin	rifampin
erythromycin	tetracyclines
fluoroquinolones	trimeth-sulfa
linezolid	vancomycin

S. aureus Testing / Reporting

Test/Report

NCCLS Antimicrobial Susceptibility Testing (AST) Standards

◆ Instructions for performing test (2003)

- M2-A8 Disk Diffusion
- M7-A6 MIC
- (Updated every 3 years)

◆ M100-S13 (2003) "The Tables"

- Drugs to test/report
- Interpretive Criteria (breakpoints)
- Quality Control ranges
- (Updated annually)



NCCLS AST Standards

◆ Describe "reference methods"

◆ Clinical labs can use:

- NCCLS methods as written OR
- Method that performs comparably to NCCLS "reference method" (e.g. FDA-cleared diagnostic device)

Vitek	Etest
MicroScan	Other

S. aureus - Oxacillin Special Testing Concerns

Inoculum: McFarland 0.5 suspension from fresh colonies (direct colony suspension method)

Incubation: 35°C; 24h

Disk diffusion (DD): MHA*
oxacillin (1 µg)
transmitted light

MIC testing: CAMHB** + 2% NaCl

Agar screen: MHA + 4% NaCl + 6 mg/ml oxacillin

*Mueller-Hinton agar

**Cation-adjusted Mueller-Hinton broth

NCCLS M100-S13

Penicillinase-Labile Penicillins

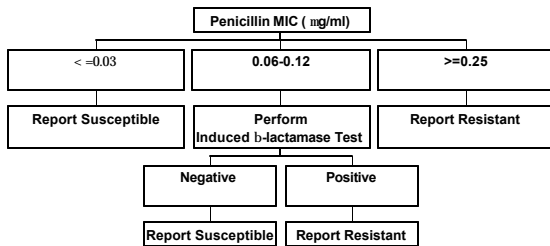
◆ Include:

amoxicillin penicillin
ampicillin piperacillin
carbenicillin ticarcillin
mezlocillin

◆ Inactivated by staphylococcal β -lactamase (penicillinase)

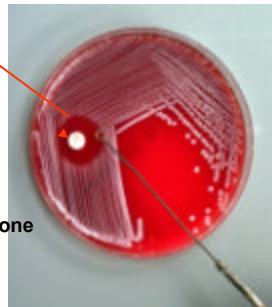
◆ β -lactamase positive = resistant to agents listed

Staphylococcus spp. Penicillin "MIC" Testing/Reporting



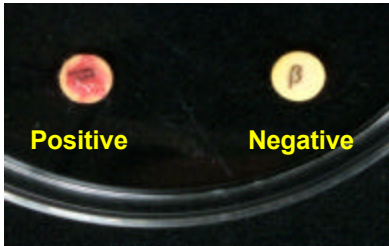
Induced β -lactamase Test

Oxacillin
(inducer)



- Sub isolate to BAP
- Test cells from periphery of zone
- Other methods

β-lactamase Test (Nitrocefin)

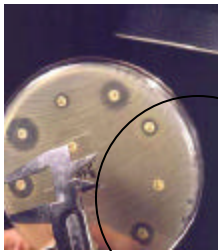


Penicillinase-Stable Penicillins

- ◆ Oxacillin = group representative
- ◆ Oxacillin performs best in in vitro test systems
- ◆ Oxacillin results used to predict results of other penicillinase-stable penicillins:
 - Nafcillin
 - Dicloxacillin
 - Methicillin

Measuring Oxacillin and Vancomycin Zones for *Staphylococcus* spp.

USE Transmitted
Light



NOT Reflected
Light

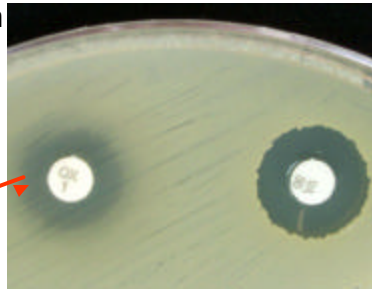


Classic MRSA - Expression

- ◆ *mecA* = genetic determinant of MRSA
- ◆ Heterogeneous expression - in MRSA (*mecA* +) population, some cells appear "S" and others appear "R" under standard test conditions
- ◆ Homogeneous expression - in MRSA (*mecA* +) population, all cells appear "R"

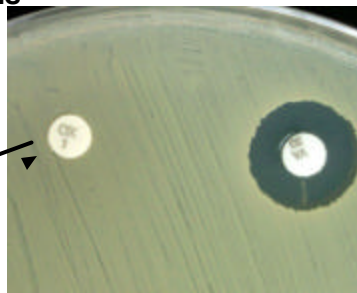
Heterogeneous expression

Haze within zone due to oxacillin-R cells



Homogeneous expression

Confluent growth up to disk



Oxacillin-salt agar
 screen for *S. aureus*
 (MHA + 4% NaCl + 6 ug/ml
 oxacillin)

+ control – *S. aureus* ATCC 43300
 (heteroresistant)

- control – *S. aureus* ATCC 29213

need > 1 colony for R

***Staphylococcus* spp.**

“Isolates of staphylococci that are shown to carry the *mecA* gene, or that produce PBP2a, the gene product, should be reported as oxacillin resistant.”

NCCLS M100-S13; Table 2C

PBP2a rapid latex agglutination assay

- Perform on isolated colonies
- Extract PBP2a
- React with latex antibody to PBP2a

Utility of *mecA* or PBP2a Tests

- ◆ **Rapid result**
 - Communicate to physician rapidly to realize value of test
- ◆ **If *mecA* or PBP2a negative, physician may discontinue vancomycin**
 - Empiric therapy for *S. aureus* often vancomycin if high incidence of MRSA

Utility of *mecA* or PBP2a Tests (con't)

- ◆ **Usually not sufficient as a “stand alone” susceptibility test**
 - Other drug results needed
 - Possible exception - MRSA surveillance cultures
- ◆ **Value in previous MRSA patients?**
- ◆ **Good for arbitrating equivocal results from DD, MIC, or agar screen tests**

Report Example (following PBP2a assay): Leg Wound Culture

GS (day 1):
Many GPC clusters
Many WBCs

Preliminary Culture Report (day 2):
Many:
Staphylococcus aureus, oxacillin-resistant (MRSA)

“Additional susceptibility results to follow”

Staphylococcus aureus

clindamycin	R
erythromycin	R
oxacillin	R
penicillin	R
vancomycin	S

"Oxacillin-R staphylococci are resistant to all β -lactams. MRSA isolated, please check Infection Control policies."

Staphylococcus aureus

cefazolin	S R*
clindamycin	R
erythromycin	R
oxacillin	R
penicillin	R
vancomycin	S

If any b-lactam is tested and tests "S", do not report or change to "R" for MRSA

Staphylococcus aureus

clindamycin	S
erythromycin	S
oxacillin	R
penicillin	R
vancomycin	S

Historically, MRSA has been multiply resistant to other anti-staphylococcal agents. However, some MRSA, particularly community-associated strains are not multiply resistant.

“Borderline” MRSA

BORSA

MRSA

Resistance *mecA*? Mechanism Multiply R?

Classic yes PBP2a yes*

Borderline no β -lactamase no
(*bla_c*)

Borderline no mod PBPs 1,2,4 no
(MOD-SA)

*CA-MRSA often not multiply R

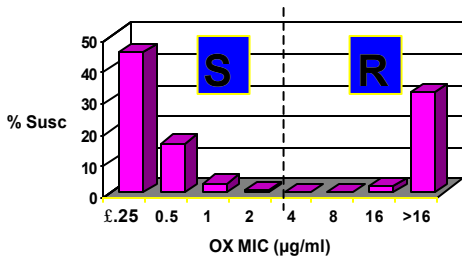
Staphylococcus aureus Oxacillin Breakpoints

MIC (mg/ml)

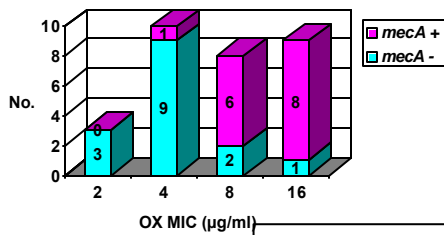
	S	I	R
<i>S. aureus</i>	£ 2	-	³ 4

NCCLS M100-S13 (M7); Table 2C

**Oxacillin MIC ($\mu\text{g/ml}$) vs. *S. aureus*
UCLA (n=4192)**



***mecA* PCR vs. Oxacillin MIC ($\mu\text{g/ml}$)
S. aureus (n=30)**



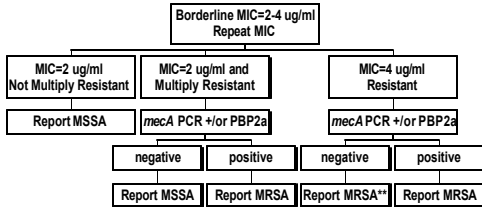
UCLA unpublished data
Isolates collected 1995-2004

**“Borderline” MRSA
(*mecA* neg) Therapy**

- ◆ Can oxacillin be used?
- ◆ Literature suggests β -lactamase inhibitor combination or other β -lactam might be effective.

Pefanis, et al. 1993. AAC. 37:507.

“Borderline” MRSA Workup



*Add report comment: “*mecA* (or PBP2a) negative” Discuss with physician.

Staphylococcus aureus (*mecA* neg)

	MIC (ug/ml)
cefazolin	1 S R
clindamycin	0.5 S
erythromycin	0.5 S
oxacillin	4 R*
penicillin	R
vancomycin	0.5 S

***Atypical oxacillin resistance (*mecA* negative);
Infectious Diseases consult suggested.”

S. aureus Clindamycin

Clindamycin

**Staphylococcus spp.
Erythromycin/Clindamycin**

Mechanism	Determinant	Erythro	Clinda
Efflux	<i>msrA</i>	R	S
Ribosome alteration	<i>erm</i>	R	(R)*

msrA = macrolide streptogramin resistance
erm = erythromycin ribosome methylase

*may test resistant or may test susceptible and require induction to show resistance)

**Staphylococcus spp.
erm-mediated Resistance**

- ◆ *erm* confers resistance to macrolide, lincosamide, streptogramin B (MLS)
- ◆ MLS resistance occurs via methylation of the 23S rRNA and reduces binding of MLS agents to the ribosome
 - MLS_Bⁱ = inducible resistance to lincosamide (clindamycin); “D test” required
 - MLS_B^c = constitutive resistance to lincosamide; shows clindamycin resistance in routine ASTs

Staphylococcus aureus

clindamycin S
 erythromycin R
 oxacillin R
 penicillin R
 vancomycin S

Is this phenotype due to:
msrA and truly clindamycin S?
erm with inducible clindamycin R?

Staphylococcus aureus

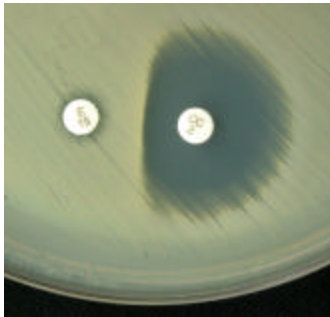
erythromycin R
oxacillin R
penicillin R
vancomycin S

“Contact laboratory if clindamycin results needed”

If clindamycin-S, erythromycin-R, do not report as clindamycin-S without performance of “D Test”

“D Test”

Inducible Clindamycin Resistance (*erm*-mediated)



“D Test” positive

Staphylococcus aureus

erythromycin R
oxacillin R
penicillin R
vancomycin S

“This *S. aureus* demonstrates inducible clindamycin resistance in vitro and isolate may develop clindamycin resistance during therapy”

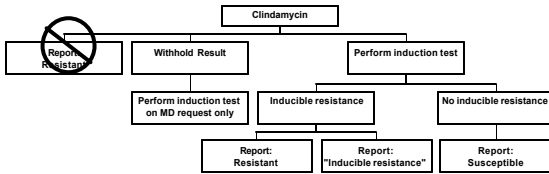
"D Test" negative

Staphylococcus aureus

clindamycin **S**
erythromycin **R**
oxacillin **R**
penicillin **R**
vancomycin **S**

"This *S. aureus* DOES NOT demonstrate inducible clindamycin resistance in vitro"

**Clindamycin Test/Report Options
Staphylococcus spp.
Erythromycin-R & Clindamycin-S**



Staphylococcus aureus

clindamycin **S**
erythromycin **R**
oxacillin **R**
penicillin **R**
vancomycin **S**

Many community-associated MRSA are clindamycin-S (msrA type) and clindamycin is a therapy option.

VISA / VRSA

Vancomycin-intermediate *Staphylococcus aureus*
Vancomycin-resistant *Staphylococcus aureus*

VISA VRSA

Staphylococcus spp. Vancomycin

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

Susceptible \leq 4
Intermediate 8 - 16
Resistant \geq 32

VISA = (4) - 16 $\mu\text{g/ml}$
VRSA = \geq 32 $\mu\text{g/ml}$

NCCLS M100-S13; Table 2C

Detection of VISA / VRSA

- ◆ Disk diffusion
 - VISA - not detected; MIC of 8 $\mu\text{g/ml}$ are "S" by DD (even at 24 h)
 - VRSA - "R"; use transmitted light
- ◆ MIC
 - VISA, VRSA - generally, overnight broth microdilution systems detect both
 - VISA or VRSA - Vitek, MicroScan may not detect (missed 2nd VRSA)
 - Check *S. aureus* with vanco MIC \geq 4 $\mu\text{g/ml}$

Detection of VISA / VRSA (con't)

- ◆ Brain heart infusion (BHI) agar with 6 µg/ml vancomycin*
 - VISA and VRSA grow on this
 - Incubate 24 h
 - QC
 - *S. aureus* ATCC 29213
 - *E. faecalis* ATCC 51299
 - Consider use if routine test method has been shown to miss VISA / VRSA

*medium used for VRE screening

Detection of VISA

	MIC (µg/ml)
◆ MicroScan ON	8
◆ Etest	6-8
◆ Sensititre	4,8
◆ Vitek	4
◆ BHI-Van (6 µg/ml)*	growth
◆ MicroScan rapid	£2, ³ 16
◆ Disk diffusion	inadequate

*medium used for VRE screening

Tenover, et al. 1998. JCM. 36:1020

VISA

- ◆ 8 cases to date in USA
- ◆ Pts. previously had MRSA
- ◆ Pt. previously treated with vancomycin
- ◆ Most are MRSA

Fridkin et. al. 2001. Clin. Infect. Dis. 32:108.
Fridkin et. al. 2003. Clin. Infect. Dis. 36:429.

Case Study VISA - Pt. JB (UCLA)

- ◆ 27 y.o. referral patient
- ◆ Cholecystitis
- ◆ Bile drainage (liver abscesses)
- ◆ Culture results
 - 2 strains VISA
 - MRSA (vancomycin-S)
 - *S. maltophilia*

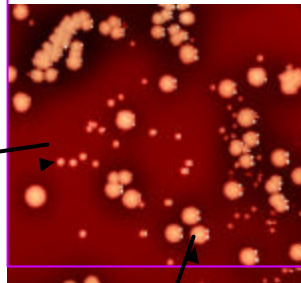


Case Study - VISA Pt. JB

Strain	<i>mecA</i>	MIC (mg/ml)	
		OX	Van
1	-	0.5	8
2	+	>16	8

VISA colonies may be smaller and slower growing than typical *S. aureus* as shown on this 48h BAP

VISA



MRSA (not VISA)

Marlowe, et al. 2001. JCM. 39:2637.

VRSA – 1st Case Michigan June 2002

- ◆ 40 y.o. female, diabetic, hemodialysis
- ◆ Pt. previously had MRSA and VRE (*vanA*)
- ◆ Pt. previously treated with vancomycin
- ◆ VRSA isolated from catheter exit site and foot wound
- ◆ Wounds healed at 3 months
- ◆ VRSA had *mecA* and *vanA*

MMWR. 2002; 51:565-7.
Chang et al. 2003. NEJM 348:1342

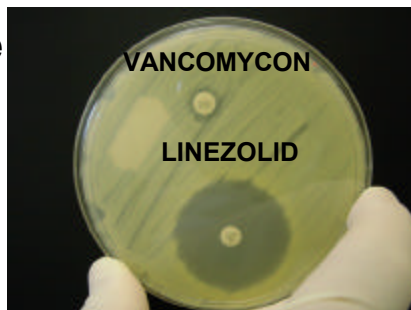
VRSA – 1st Case Michigan June 2002 (con't)

- ◆ Routine vancomycin tests (NCCLS methods)
 - DD = no zone (or heavy haze within zone)
 - Screen plate (BHI + 6 µg/ml vancomycin) = growth
 - MIC = 1024 µg/ml
- ◆ Resistance detected by automated systems (MicroScan, Vitek)
- ◆ “S” to chloramphenicol, linezolid, minocycline, quin-dalfo, TMP-SMZ

MMWR 2002; 51:565-7.

VRSA 1st Case

From Michigan
Health Dept. Lab

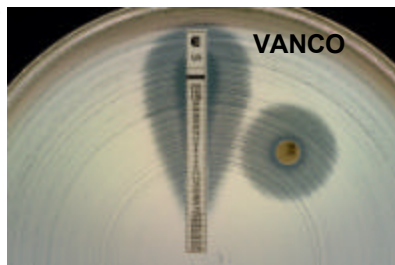


VRSA – 2nd Case Pennsylvania September 2002

- ◆ 70 y.o. male, morbid obesity
- ◆ Chronic foot ulcer grew VRSA
- ◆ Routine vancomycin tests (NCCLS methods)
 - MIC = 32 µg/ml
 - DD = 12 mm
 - Screen plate (BHI + 6 µg/ml vancomycin) = growth
- ◆ Not detected by automated systems (MicroScan, Vitek)
- ◆ “S” to chloramphenicol, linezolid, minocycline, quin-dalfo, rifampin, TMP-SMZ

MMWR 2002; 51:902.

VRSA 2nd Case



From CDC Lab

Confirmation of VISA / VRSA

If you suspect a VISA / VRSA:

1. Repeat ID and susceptibility tests
2. Contact your institution's infection control department
3. Contact CDC at SEARCH@cdc.gov
4. Contact your local health department
5. SAVE ISOLATE!!

***S. aureus* AST QA/QC/Competency**

QA/QC

QC of AST for *S. aureus* NCCLS Recommendations

- ◆ **Disk diffusion and MIC tests**
 - *S. aureus* ATCC 25923 (DD); *S. aureus* ATCC 29213 (MICs)
 - *E. coli* ATCC 35218 (b-lactam/ b-lactamase inhibitor combos)
 - QC daily; weekly (if meet acceptable daily criteria)
- ◆ **Oxacillin-salt agar screen**
 - *S. aureus* ATCC 43300
 - *S. aureus* ATCC 29213
 - QC each day of use

NCCLS M100-S13

Supplemental QA/QC of AST for *S. aureus*

- ◆ **Verify AST results on patient's isolates**
 - NCCLS M100-S13 (2003) - Verification Tables (Table 4 - disk diffusion, Table 8 - MIC)
- ◆ **Assess competency of staff**
- ◆ **Proficiency surveys**
- ◆ **Antibiogram review**
- ◆ **Other**

Verifying Results *S. aureus*

- ◆ All labs should verify the following results for *S. aureus*:
 - Quinupristin-dalfopristin – I or R
 - Vancomycin – I or R
 - Linezolid – non-susceptible
- ◆ Confirm with NCCLS dilution reference method

NCCLS M100-S13

Staphylococcus spp. Linezolid

	<u>Susc</u>	<u>Int</u>	<u>Res</u>
DD (mm)	≥ 21	-	-
MIC (µg/ml)	£ 4	-	-

- *investigate any non-“S” isolate
- ..Repeat ID and AST
- ..Save isolate
- ..Send to reference lab

“Verify” Results ~~Patient’s Isolates – HOW?~~

- ◆ Check transcription
 - ◆ Reexamine plate/tray, purity plate, etc.
 - ◆ Check previous isolates on patient
- THEN.....**
- ◆ Confirm ID and/or
 - ◆ Repeat AST (alternate method?)
 - ◆ Get assistance from reference lab

NCCLS M100-S13

Verifying MRSA

- ◆ NCCLS suggests verification if you feel this is appropriate for your institution
- ◆ ..from JH..consider verifying MRSA when isolated from new patient (first time MRSA for that patient)

Why Verify MRSA?

- ◆ Clinical consequences of reporting MRSA are significant
 - Isolation of patient
 - Broad spectrum (e.g. vancomycin) therapy
 - "MRSA" label
- ◆ *S. aureus* is often present in culture with oxacillin-resistant organisms (e.g. enterococcus, coagulase-negative staphylococci) – increases risk for mixed susceptibility tests

Verify?*

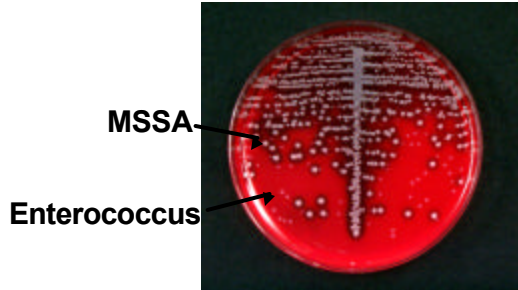
Staphylococcus aureus

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

clindamycin	>8 R
erythromycin	>8 R
oxacillin	>16 R
penicillin	R
vancomycin	1 S

*Institution Specific (NCCLS M100-S13)

Examine test closely -
MIC purity plate on patient's *S. aureus*



FINAL Report*

Staphylococcus aureus

	MIC ($\mu\text{g/ml}$)
clindamycin	£0.5 S
erythromycin	£0.5 S
oxacillin	£0.5 S
penicillin	R
vancomycin	1 S

After repeat MIC test without enterococcus
contaminating the inoculum.

S. aureus
Susceptibility Statistics
(Annual Antibiogram)

STATS

NCCLS M39-A Guideline

“Analysis and Presentation of Cumulative Antimicrobial Susceptibility Test Data”

May 2002



M-39A Recommendations

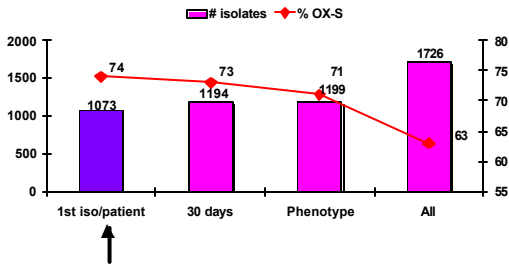
- ◆ Analyze/present data annually
 - Sufficient to GUIDE EMPIRIC THERAPY
- ◆ Calculate %S (do not include %I)
- ◆ Include diagnostic (not surveillance) isolates
- ◆ Include the 1st isolate/patient, irrespective of
 - body site
 - antimicrobial pattern
- ◆ For *S. aureus* present all and MRSA subset

Handling Repeat Isolates Options

- ◆ Count 1st isolate/patient/year
 - Irrespective of body site or overall susceptibility profile
 - Count each patient once
 - recommended in NCCLS M39-A
- ◆ Count repeat isolates after 30 days have elapsed since testing the previous isolate from a given patient
- ◆ Count repeat isolates that have different phenotypes or different results for one (or more drugs (e.g., S to R, R to S)

Example of %S for oxacillin when analyzing a single data set (N=1726) and eliminating duplicates 3 ways.

S. aureus - Oxacillin



Example: US Hospital Antibigram 2002

	No.	% Susceptible					
		clin	ery	ox	pen	t-s	van
all SA	1073	80	50	74	9	97	100
MRSA	449	34	4	0	0	94	100

Summary

- ◆ Use the most current NCCLS standards (new tables published every January).
- ◆ Know specific protocols for detecting:
 - Heteroresistant MRSA (with *mecA*)
 - Borderline MRSA (without *mecA*)
 - Inducible clindamycin resistance
 - VISA, VRSA

Summary

- ◆ Make certain your reports are clearly understood by your physicians.
- ◆ Test and report supplemental agents on *S. aureus* based on the needs of your physicians.
- ◆ Without delay, follow up on potential VISA and VRSA with infection control and your public health department.

Summary (con't)

- ◆ Verify all patient results. This may require retesting to confirm ID and AST (e.g., first time MRSA; VISA; VRSA)
- ◆ Develop a comprehensive QA/QC program for testing *S. aureus* in your laboratory
 - Include assessing competency of staff
 - Carefully review annual antibiogram
- ◆ If using a commercial AST system, regularly review the product literature provided by the manufacturer.



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www.phppo.cdc.gov/dls/master/default.asp
