

MicroFocus

A MACRO LOOK AT MICRO ISSUES

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Introduction

MicroScan, a product line of Dade Behring, continues their commitment to education by sponsoring a series of newsletters and related offerings devoted to emerging issues in microbiology. The consensus from a recent opinion leader forum in which I participated, clearly indicates that laboratorians would benefit from technical articles and bench references addressing some of the complex issues they face every day. The reply card is for you to return with your suggestions for future topics. We hope that you will find MicroFocus a valuable resource for your lab or practice.

The purpose of this first publication is to provide new information as well as practical tools to assist clinical microbiologists in producing more helpful laboratory reports. Enclosed is a "pull-out" table summarizing the new CLSI recommendations. We anticipate this table will be kept at the bench and serve as a frequent reference.

In the first of two technical papers, Susan Munro describes the steps to be taken by laboratorians for accurate detection of oxacillin (methicillin), vancomycin, and clindamycin resistance in staphylococci. Detection of oxacillin-resistant strains carrying the genetic information (*mecA*) for the altered cell wall forming enzyme (penicillin-binding-protein 2a - PBP2a) is essential. Your system or procedure must accurately detect isolates producing PBP2a resulting in oxacillin resistance. Most laboratories are not equipped to perform the PCR procedure for the *mecA* genes but should perform phenotypic testing which best detects PBP2a containing (oxacillin resistant) isolates.

Janet Hindler provides some very practical guidelines for refining testing regimens to improve the clinical utility of laboratory reports. It is a surprise to many technologists that their bench decisions can have a major impact on physician treatment decisions. Appropriate and often essential when dealing with true pathogens, performing antimicrobial testing on all isolates from wounds or other non-sterile sites can be more harmful than helpful. Another key point made by Janet is the follow-up by microbiologists of unusual results. The first clindamycin-resistant *Bacteroides fragilis* and vancomycin-resistant *S. aureus* were found in my laboratory by astute technologists who followed-up unusual results. The take-home message is: do not just accept or reject such findings but verify and refer for confirmation.

William J. Brown, Ph.D., D(ABMM), F(AAM), Editor

Beyond Routine Antimicrobial Susceptibility Testing for *Staphylococcus* spp. – When Is Extra Testing Needed?

Susan D. Munro, MT(ASCP)

Technical Specialist, Antibiotics

Stanford University Medical Center, Stanford, CA

Introduction

Rapid emergence of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) has created a new phenotype of MRSA, and the clindamycin induction test for detection of the *erm* gene has gained added importance. The recently described cefoxitin disk test enhances the laboratory's ability to detect oxacillin resistance in staphylococci. Vancomycin-intermediate and vancomycin-resistant *S. aureus* (VISA and VRSA respectively) are

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Acting Responsibly in the Clinical Microbiology Laboratory to Encourage Prudent Prescribing of Antimicrobial Agents

Janet F. Hindler, MCLS MT(ASCP) F(AAM)

UCLA Medical Center Los Angeles, CA

"Emerging resistance," "multi-drug resistance," and "pan resistance" are terms that have become all too familiar to clinicians, microbiologists, pharmacists, other healthcare practitioners and now, even the public at large. But healthcare

professionals have a particular responsibility to prevent antimicrobial resistant bacteria from becoming more widespread. For the clinician this means prudent prescribing, for the pharmacist this means keeping current on new developments in

Healthcare professionals have a particular responsibility to prevent anti-microbial resistant bacteria from becoming widespread.

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still uncommon, but laboratory detection is critical for patient care.

Oxacillin resistance

The cefoxitin disk is used as a surrogate marker for detection of *mecA*-mediated resistance to oxacillin.² Cefoxitin is a more potent inducer of *mecA* than oxacillin. The cefoxitin disk does not demonstrate the light haze of growth common around the oxacillin disk with heterogeneously resistant strains, and therefore is easier to read. For these reasons, the cefoxitin disk should be substituted for the oxacillin disk for laboratories using

disk diffusion testing as their primary antimicrobial susceptibility testing (AST) method. The cefoxitin disk may also be used as an alternative or supplement to the oxacillin MIC, especially for

The cefoxitin disk is used as a surrogate marker for detection of *mecA*-mediated resistance to oxacillin.²

coagulase-negative staphylococci (CoNS) with oxacillin MICs of 0.5 – 2 µg/ml. CoNS strains which do not possess *mecA* may be interpreted as falsely resistant using CLSI oxacillin MIC interpretations (≥0.5 resistant). Swenson et al studied a group of CoNS with oxacillin MICs 0.5 – 2 µg/ml and found the specificity of oxacillin MIC to be only 17%, whereas the cefoxitin disk specificity was 93%.¹⁰

Figure 1

Comparison of Cefoxitin Disk and Oxacillin Salt Agar Screen for Detection of Oxacillin Resistance in <i>S. aureus</i>			
	Method	Sensitivity %	Specificity %
<ul style="list-style-type: none"> 110 Stanford isolates and 10 UCLA BORSA tested PCR <i>mecA</i> gold standard <small>Abstracts ASM Gen. Mtg. 2005. Comparison of cefoxitin disk diffusion screen test and oxacillin salt agar screen for detection of oxacillin resistance in <i>S. aureus</i>. MH Nourbakhsh, M Mudambi, SD Munro, EJ Baron. Stanford Hosp and Clinics.</small>	Cefoxitin Disk	98	100
	Oxacillin agar screen	98	100
	Oxacillin MIC (MicroScan)	98	100
	PBP2a latex	100	99

How does the oxacillin salt agar screen compare to the cefoxitin disk for *S. aureus*? Our laboratory evaluated the two tests and MicroScan oxacillin MIC using 110 unique

clinical isolates and 10 previously identified borderline oxacillin-resistant *S. aureus* (BORSA) strains.⁹ We found cefoxitin disk, MicroScan oxacillin MIC, and oxacillin salt agar equally able to detect resistance (fig. 1). This raises the question as to whether multiple tests are needed to confirm MRSA. To determine the best testing strategy, the laboratory should evaluate: 1) the reliability of their routine testing system, 2) the known incidence of errors, 3) the source of the specimen, and 4) whether the patient previously had MRSA.

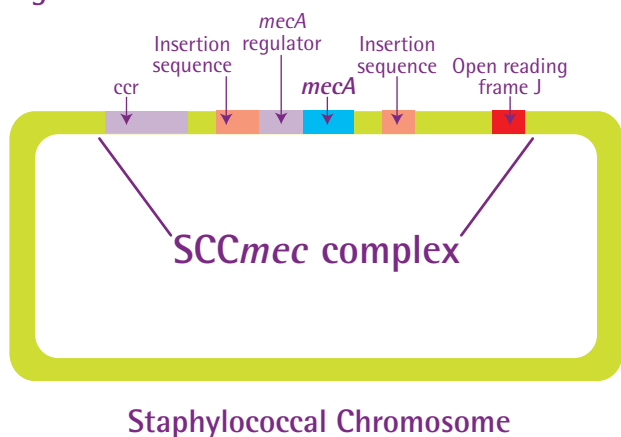
Community-Acquired MRSA (CA-MRSA)

Healthcare-associated MRSA (HA-MRSA) are nosocomial or associated with known behavior risks (e.g. substance abuse). CA-MRSA were first reported in the 1990's in a study done at University of Chicago Children's Hospital.⁶ These infections appeared in the community in children with none of the traditional risk factors for MRSA. Furthermore, CA-MRSA had resistance to fewer classes of antibiotics and the clinical syndromes were similar to infections with methicillin-susceptible isolates from the community.

Subsequent study has revealed a unique staphylococcal cassette chromosome *mec* (SCC*mec*) responsible for CA-MRSA.⁴ SCC*mec* contains the *mecA* gene complex (*mecA* and its regulators) and *ccr* complex (site specific recombinases responsible for mobility of SCC*mec*) (fig. 2). HA-MRSA have SCC*mec* types I, II, or III. CA-MRSA demonstrate novel SCC*mec* types IV and V, which are smaller in size than I – III, making them too small to carry the other transposable elements and genes for resistance to non-beta-lactam antibiotics. This small size provides added mobility. It is theorized that the smaller SCC*mec* elements are disseminated strain to strain by a bacteriophage, plasmid, or yet undiscovered mechanism. They parasitize CA-MSSA strains, converting them to CA-MRSA. CA-MRSA has spread rapidly in diverse geographic areas of the U.S., supplanting HA-MRSA in some hospitals.

Some CA-MRSA are highly invasive, due to the presence of a gene-encoded cytotoxin, Panton-Valentine leukocidin (PVL). PVL causes tissue necrosis and leukocyte destruction,

Figure 2



Courtesy of Dr. Ellen Jo Baron

and may play a role in the severity of skin and soft tissue infections and severe necrotizing pneumonia.⁵ PVL is uncommon in SCCmec types I – III but frequent in type IV.

Skin and skin structure infections are the most common sources of CA-MRSA. The patient may present with what appears to be a bug or spider bite. Specific patient populations are associated with CA-MRSA: children and adolescents, prison inmates, military personnel, competitive athletes, Native Americans, IV drug abusers, and HIV patients.

Clindamycin Induction Test

Since there are more oral therapeutic options for CA-MRSA, (i.e. trimethoprim-sulfamethoxazole, doxycycline, and clindamycin), the clindamycin induction test provides important information to the clinician.

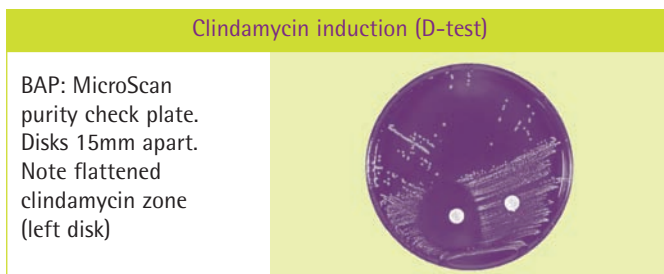
Macrolide-Lincosamide-Streptogramin B (MLS_B) resistance in staphylococci is mediated by two resistance genes, *erm* (methylase inhibition) or *msrA* (efflux) (fig. 3). Erythromycin-resistant, clindamycin-susceptible isolates require performance of the clindamycin induction test (D- test) to determine the presence of the *erm* gene. Bacteria with the inducible phenotype produce inactive mRNA which is unable to encode the methylase. The mRNA becomes active only in the presence of a macrolide inducer. If erythromycin and clindamycin disks are placed 15- 26 mm apart (edge to edge) on Mueller Hinton agar, and a D-shaped inhibition zone is formed around clindamycin, the isolate is positive for clindamycin

induction, and clindamycin should be reported as resistant. The D test may also be performed on the blood agar purity plate used for AST, with the disks placed 15 mm apart (fig.4).⁷ Some laboratories choose to perform the D test on all staphylococci. Other options: 1) if the isolate is erythromycin-resistant and clindamycin-susceptible, suppress the clindamycin result and add a comment to the report, "If clindamycin is considered for therapy, call the lab for further testing," or 2) perform the D test only on MRSA isolates which are erythromycin-R and clindamycin-S. For erythromycin-resistant, clindamycin-susceptible MSSA and CoNS suppress clindamycin, add a comment as described above. Note that both options create incomplete clindamycin data for an annual antibiogram since not all isolates have had the D test performed.

Figure 3

Macrolide-Lincosamide-Streptogramin B (MLS _B) Resistance in Staphylococci			
Mechanism	Determinant	Erythromycin	Clindamycin
Efflux	<i>msrA</i>	R	S
Methylation of ribosomal target	<i>erm</i>	R	R Constitutive
Methylation of ribosomal target	<i>erm</i>	R	S Induction to show resistance

Figure 4



VRSA and VISA

Six VRSA isolates have been confirmed in the U.S. by the CDC. Vancomycin resistance is due to the *vanA* resistance gene, most likely acquired by interspecies transfer from enterococci. Not all of the first three isolates were reliably detected by automated AST methods,¹ but were detected by reference broth microdilution, agar dilution (including vancomycin agar screen), and Etest[®] methods.

In contrast, vancomycin-intermediate *Staphylococcus aureus* (VISA) isolates demonstrate reduced susceptibility to vancomycin due to thickened cell walls and altered metabolic pathways.³ VISA appear to derive from MRSA. VISA colonies may be smaller and slower growing than typical *S. aureus*, requiring 48 hours incubation of the original plates.⁸

The disk diffusion test fails to identify VISA, and VRSA may be difficult to detect due to a light haze of growth. CLSI and CDC have recommended the use of the vancomycin agar screen to enhance detection.² Not all automated AST methods reliably detect VRSA or VISA. MicroScan overnight panels have recently received FDA clearance for a reformulation of vancomycin allowing detection of these organisms, therefore a backup vancomycin agar screen for VRSA is no longer required.

In 2006 CLSI revised vancomycin interpretive criteria for *S. aureus* ($\leq 2 \mu\text{g/ml}$ = susceptible).² These interpretations differ from existing FDA vancomycin breakpoints ($\leq 4 \mu\text{g/ml}$ = susceptible). All automated AST systems were FDA-cleared using the older FDA vancomycin breakpoints. Regardless of which vancomycin breakpoints are implemented, laboratories should check any *S. aureus* that has a vancomycin MIC $\geq 4 \mu\text{g/ml}$ or grows on vancomycin agar screen. If the result is the same on repeat testing, refer to a public health lab or CDC who will verify the vancomycin MIC. In the meantime, report as, "Vancomycin non-susceptible; Possible VISA/VRSA," and use proper notification procedures as shown in the CDC algorithm available online at: www.cdc.gov/ncidod/dhqp/ar_visavrsa_algo.html

References 1. CDC. Brief report: vancomycin-resistant *Staphylococcus aureus* – New York, 2004. *MMWR* 2004;53:322-323. 2. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Sixteenth informational supplement M100-S16. 2006. CLSI, Wayne, PA. 3. Cui L, Murakami H, Kuwahara-Arai K, Hanaki H, Hiramatsu K. Contribution of a thickened cell wall and its glutamine nonamidated component to the vancomycin resistance expressed by *Staphylococcus aureus* Mu50. *Antimicrob Agents Chemother*. 2000;44:2276-2285. 4. Daum RS, Ito T, Hiramatsu K, Hussain F, Mongkolrattanothai K, Jamklang M, Boyle-Vavra, S. A novel methicillin-resistance cassette in community-acquired methicillin-resistant *Staphylococcus aureus* isolates of diverse genetic backgrounds. *Jour Infect Dis*. 2002;186:1344-7. 5. Etienne J. Pantone-Valentine leukocidin: a marker of severity of *Staphylococcus aureus* infection? *Clin Infect Dis*. 2005; 41:591-3. 6. Herhold BC, Immergluck LC, Maranan MC, Lauderdale DS, Gaskin RE, Boyle-Vavra, S, Leitch CD, Daum RS. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. *JAMA*. 1998;279:593-598. 7. Hindler JF, Bruckner DA. Performance of the D zone test on *Staphylococcus aureus* using the MicroScan Prompt inoculation system. Proceedings of the American Society for Microbiology General Meeting. 2005. Atlanta, GA. Abstract C-325. 8. Marlowe EM, Cohen MD, Hindler JF, Ward KV, Bruckner DA. Practical strategies for detecting and confirming vancomycin-intermediate *Staphylococcus aureus*: a tertiary-care hospital laboratory's experience. *Jour Clin Microbiol*. 2001;39: 2367-2639. 9. Nourbakhsh MH, Mudambi M, Munro SD, Baron EJ. Comparison of ceftioxin disk diffusion screen test and oxacillin salt agar screen for detection of oxacillin resistance in *Staphylococcus aureus*. Proceedings of the American Society for Microbiology General Meeting. 2005. Atlanta, GA. Abstract A-088. 10. Swenson JM, Tenover FC, and the Ceftioxin Study Group. Results of disk diffusion testing with ceftioxin correlate with presence of *mecA* in *Staphylococcus* spp. *Jour Clin Microbiol*. 2005;43:3818-3823.

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Acting Responsibly in the Clinical Microbiology Laboratory to Encourage Prudent Prescribing of Antimicrobial Agents

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order to guide clinician prescribing, and for every health-care practitioner, this means maintaining the highest standards for infection control.

The responsibility of the clinical microbiologist is to generate laboratory reports that are meaningful. This includes providing antimicrobial susceptibility test results only when the isolate is likely to be contributing to an infectious process. For example, it is unlikely that the gram-negative rods isolated in the example in Figure 1 are causing an infection, particularly since neither gram-negative rods nor WBCs are noted on gram stain and the specimen contains large numbers of bacteria typically associated with skin flora. If complete identification and susceptibility test results are provided for the gram-negative rods, the physician may conclude that these bacteria are contributing to an infection and antimicrobial therapy is warranted. Such reporting can lead to imprudent prescribing and possible selection of resistant bacteria. This may also discourage the physician from seeking the true cause of the patient's problem.

Figure 1

Specimen:	Groin lesion
Diagnosis:	Hernia repair
Gram stain:	<ul style="list-style-type: none"> ■ Many gram-positive cocci in clusters ■ Moderate pleomorphic gram-positive rods ■ No WBCs
Culture:	<ul style="list-style-type: none"> ■ Many coagulase-negative staphylococci ■ Many diphtheroids ■ Few lactose-positive gram-negative rods (two types)

Acting responsibly also involves reporting those antimicrobial agents appropriate for the species and infection source. For example, daptomycin, a newer antimicrobial agent, is active against many gram-positive pathogens but has not been shown to be effective in treating pneumonia and should not be reported on isolates from respiratory sources. This information may not be widely available to clinical microbiologists and emphasizes the need to develop testing and reporting protocols in consultation with infectious diseases, pharmacy, infection control and possibly other services within one's facility.

Table 1. Examples of AST results requiring verification in all laboratories and the suggested follow-up action.

Organism	Results	Comments/Action
<i>E. coli</i> / peritoneal fluid	Gentamicin-R and S to other drugs on panel	Verify gentamicin-R; very unusual for gentamicin-R in strain S to all other agents, including other aminoglycosides, on routine gram-negative panel. <ul style="list-style-type: none"> ■ Repeat AST using same method ■ If still gentamicin-R, repeat using alternate approved method ■ If both methods agree, report ■ If methods disagree, send to reference lab for confirmation with CLSI reference method ■ Confirm identification
<i>S. aureus</i> / abscess	MRSA that is Linezolid-R	Linezolid-R <i>S. aureus</i> are rare; sometimes linezolid disk diffusion and MIC tests show hazes which result in false R calls. <ul style="list-style-type: none"> ■ Repeat AST using same method; take caution to avoid reading hazes as R ■ If still linezolid-R, repeat using alternate approved method ■ If both methods agree, report ■ If methods disagree, send to reference lab for confirmation with CLSI reference method ■ Confirm identification
<i>Enterococcus faecalis</i> / blood	VRE that is quinupristin-dalfopristin-S	<i>E. faecalis</i> by definition are quinupristin-dalfopristin-R, whereas <i>E. faecium</i> are typically quinupristin-dalfopristin-S. <ul style="list-style-type: none"> ■ Repeat using same method ■ Confirm identification ■ If still quinupristin-dalfopristin-S and <i>E. faecalis</i>, repeat using alternate approved methods for both ID and AST ■ If methods agree, report ■ If methods disagree, send to reference lab for confirmation with CLSI reference method and confirmation of ID

Although many laboratories practice selective or cascade reporting, unexpected resistance should always be reported even if it contradicts a laboratory's selective reporting rules. Linezolid resistance is very uncommon among *Enterococcus* species and most physicians assume all enterococci are linezolid-susceptible. Use of artificial intelligence is invaluable in catching such unexpected observations. If a laboratory includes linezolid on an enterococcal panel and notes resistance, it should be reported following verification, particularly if linezolid is on that facility's formulary. Recent information suggests that linezolid-resistant enterococci may be more likely in

patients who have undergone prior treatment.² Providing linezolid results only when resistant or non-susceptible, may become problematic in some facilities. The assumption of susceptible may lead to increased utilization, therefore each institution may want to address reporting protocols to ensure they are appropriate for their formulary and organism population.

Every clinical microbiologist appreciates the need for a comprehensive quality assurance program for AST. This includes testing the routine QC strains and verifying each result on a patient's isolate before reporting. Even if a result for a specific agent is not reported, it should be verified as it is possible that the result may subsequently be reported upon specific request. In addition, all data are likely to be considered when preparing a cumulative antibiogram and it is assumed that all these are accurate. Table 1 lists several examples.

When isolates are encountered that are resistant to all appropriate agents routinely tested, the microbiologist has a responsibility to take action. Although each laboratory must devise their own strategy, the following are steps to consider:

1. Verify the results if they have not been previously verified.
2. Contact the patient's physician to:
 - a. Report the isolate
 - b. Establish the significance of the isolate; in some cases further testing will not be essential to therapeutic management of the patient
 - c. Discuss suggestions for additional testing, if necessary. If the physician is not an infectious diseases specialist, it may be appropriate to mention other resources available to assist in any subsequent antimicrobial testing decisions. These would include infectious diseases, infection control, pharmacy and/or other services. In some facilities, however, such advice from the laboratory may not be appropriate.
3. Contact infection control to report the isolate. Infection control is likely to have a protocol for responding to reports of highly resistant bacteria.
4. Review recommendations in Tables 1 and 2 of CLSI M100-S16¹ to determine which, if any, additional agents might be tested. If interpretive criteria are listed for the specific organism, testing of that agent is acceptable. However, testing narrower spectrum drugs against isolates resistant to broader and expanded spectrum agents within an antimicrobial class will likely be futile.
5. If additional advice is still needed, interact with infectious diseases, infection control, and/or the pharmacy services who are likely to have the most current information on treating infections with multiply resistant bacteria. In some cases it may be necessary to check the latest literature and/or contact experts outside of one's own facility.

Examples of supplemental agents that might be tested on specific multiply resistant bacteria are shown in Table 2. This discussion has highlighted several areas where the clinical

microbiologist, acting responsibly, can impact prudent prescribing. In this era of increasing resistance, every effort is essential to preserve our antimicrobial agent armamentarium.

Table 2. Examples of resistant bacteria, antimicrobial agents often routinely reported and supplemental agents to consider for testing and reporting.

Community-associated MRSA / abscess		
Routine results	Clindamycin - S (S results reported only after D zone testing) Erythromycin - R Oxacillin - R Penicillin - R Vancomycin - S	
Supplemental (all have PO forms which make them attractive for treating infections in outpatients; CA-MRSA often susceptible these agents)	Tetracycline or doxycycline or minocycline	Tetracycline-S isolates are "S" to doxycycline and minocycline. Tetracycline-I or -R isolates may be "S" to doxycycline or minocycline.
	Rifampin	Add note "Rifampin should not be used alone for chemotherapy"
	Trimethoprim-Sulfamethoxazole	
	Linezolid	On request only
VRE / blood		
Routine	Ampicillin - R Vancomycin - R Gentamicin (high-level resistance screens for synergy) - R Streptomycin (high-level resistance screens for synergy) - R	VRE are typically resistant to ampicillin, penicillin and synergy screens
Supplemental	Chloramphenicol	On request only
	Linezolid	On request only
	Quinupristin-Dalfopristin	For <i>E. faecium</i> only (not <i>E. faecalis</i>)
	Rifampin	Add note "Rifampin should not be used alone for chemotherapy"
	Tetracycline or doxycycline or minocycline	Tetracycline-S isolates are "S" to doxycycline and minocycline. Tetracycline-I or -R isolates may be "S" to doxycycline or minocycline.
Pan-resistant <i>Acinetobacter baumannii</i> / pleural fluid		
Routine (agents that might be on one's routine gram-negative panel)	Amikacin - R Gentamicin - R	Other aminoglycosides likely R*
	Ceftazidime - R Ceftriaxone - R Imipenem - R Piperacillin-Tazobactam - R	Other beta-lactams likely R*
	Ciprofloxacin - R	Other fluoroquinolones likely R*
	Tetracycline - R	Tetracycline-S isolates are "S" to doxycycline and minocycline. Tetracycline-I or -R isolates may be "S" to doxycycline or minocycline
	Trimethoprim-Sulfamethoxazole - R	
	Supplemental	Ampicillin-Sulbactam
Colistin or polymyxin		New MIC breakpoints for these agents in CLSI M100-S16
Tigecycline		Newer agent: currently no CLSI breakpoints; breakpoints in FDA labeling could be used. Protocol for reporting with FDA breakpoints must be decided in each laboratory.

*It is unlikely that any other members of the drug class would be active against the *Acinetobacter baumannii* presented here. Nevertheless, each laboratory must decide if any other agents within the class warrant testing following input from clinical staff.

Explanation of terms used in table: S, susceptible; I, intermediate; R, resistant; PO, per os or oral; MRSA, methicillin (oxacillin) resistant *Staphylococcus aureus*; VRE, vancomycin-resistant *Enterococcus*; On request only, report only when specifically requested to help preserve spectrum of activity by limiting widespread use

References 1. CLSI. 2006. Performance Standards for Antimicrobial Susceptibility Testing; Sixteenth Informational Supplement. Document M100-S16, Vol.26, No.3. CLSI, Wayne, PA. 2. Devasia, R. et al. 2005. The first reported hospital outbreak of linezolid-resistant enterococcus: An infection control problem has emerged. Infectious Disease Society of America Meeting 2005, Abstract 1079.

Commentary



William J. Brown, Ph.D., D(ABMM), F(AAM)

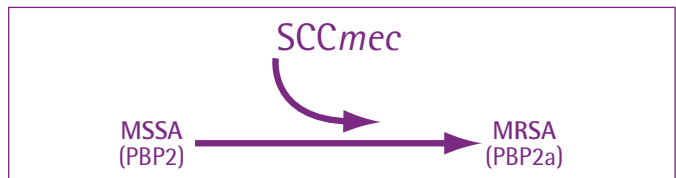
The increasing knowledge of antimicrobial resistance mechanisms places greater demands on the microbiologists to understand mechanisms of antimicrobial action and how bacteria are developing resistance. With this knowledge, the micro-

biologist can best implement new guidance to adjust testing regimens and better support physicians in their therapeutic decisions.

S. aureus has demonstrated ability to develop resistance to virtually all classes of antibiotics and as such has become one of the most challenging pathogens to manage from both a diagnostic and therapeutic perspective. Many of the pathogenic and resistance mechanisms are acquired from exogenous nucleic acid particles received via a number of mobile genetic elements.

One of the mobile genetic units is named pathogenicity islands (PI) and varies in size from about 15 to 70 kilobases. The base sequences of the PIs have a different nucleic acid base ratio than the native bases in the *S. aureus* chromosome. The heterogeneous DNA of the PIs has different base pair ratios and make up a significant portion of virulent *S. aureus* chromosome. For example, strains of *S. aureus* which contain the PI with genes for TSST-1 toxin are the etiological strains causing *Staphylococcal* toxic shock syndrome. Virulent *S. aureus* contain other PIs which provide the genetic information for other toxins. Dr. Hiramatsu has described a special type of PI in *S. aureus* which contains genes for antimicrobial resistance rather than the usual toxin genes.¹⁴ These PI-like structures are named "*Staphylococcal* Cassette Chromosome *mec*" (SCC*mec*), are the mobile genetic elements which spread from strain to strain and provide the genes for oxacillin resistance (*mec* genes). Also essential for mobility of the SCC elements are the genes for recombinase (*ccrA* or *ccrB*) which catalyze the removal of the SCC*mec* from one chromosome and insertion into another. The remaining region of the SCC*mec* is referred to as "J" section (J for junkyard).³ Of the five SCC*mec* types that have been described so far, types I, II, and III have large J regions and carry resistance determinants for antibiotics other than beta-lactams. Types IV and V are small and only carry the *mec* resistance genes.

MRSA produce an altered cell wall forming enzyme (PBP2a) to which all currently available beta-lactam antibiotics have a low affinity and are clinically resistant (regardless of susceptibility testing results). For example, oxacillin or ceftazidime resistant staphylococci are also resistant to ceftazidime regardless of ceftazidime testing results. Therefore, it is critical for the microbiology laboratory to accurately detect *mec* positive strains. This becomes more of a challenge when only a small percentage of the



organisms in the population are expressing the production of PBP2a (heterogeneous resistance). It is on these strains that ceftazidime appears better at detection of resistance since it appears to be a better inducer of PBP2a production.

The PVL cytotoxin that Susan Munro describes appears to have a major role in the pandemic of skin and soft tissue community acquired MRSA infections. Because of the importance of these organisms, laboratories should be sure they are identifying and susceptibility testing all *S. aureus* from these specimens, regardless of their quantity or presence of other organisms.

For *S. aureus*, many hospitals are reporting up to 60% of their ICU isolates as oxacillin-resistant (MRSA) which makes the organism clinically resistant to all current beta-lactam drugs (penicillins and cephalosporins). This markedly restricts therapeutic options for physicians and has led to increased use of vancomycin. Furthermore, positive blood cultures with Gram positive cocci in clusters frequently initiates vancomycin therapy until organisms are proven to be oxacillin-susceptible. These events and the frequent use of third generation cephalosporins has resulted in marked increases in vancomycin-resistant *Enterococcus* species and even rare isolates of vancomycin-resistant *S. aureus* (VRSA) which acquire the resistance genes from enterococci. A second form of reduced susceptibility to vancomycin (VISA) has been observed in strains of MRSA with thicker than normal cell walls. This mechanism has been further clarified in a recent article by Cui et al. Attachment of the large vancomycin molecules to the excess binding sites forms a barrier. Additional molecules of vancomycin are blocked from reaching the intended targets at the cell membrane and result in VISA strains.²

It is clear that appropriate patient care management is a multi-disciplinary process, guided in part by informed laboratory testing practices and prudent reporting by the clinical microbiology laboratory. When unexpected resistance is determined, as Janet Hindler indicates, it is our responsibility to ensure that these results are validated and then communicated in a timely manner to the clinician, pharmacist and infection control practitioner. Only through an informed and combined effort can the escalation of multi-resistant bacterial be minimized.

References 1. Chongtrakool, P., T. Ito, X.X. Ma, et al. 2006. Staphylococcal cassette chromosome *mec* (SCC*mec*) typing of methicillin-resistant *Staphylococcus aureus* strains isolated in 11 Asian countries: a proposal for a new nomenclature for SCC*mec* elements. *Antimicrob. Agents Chemother.* 50: 1001-1012. 2. Cui, L., A. Iwamoto, J.Q. Lian, et al. 2006. Novel mechanism of antibiotic resistance originating in vancomycin-intermediate *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 50: 428-438. 3. Ito, T., K. Ouma, X.X. Ma, et al. 2003. Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. *Drug Resist. Updat.* 6: 41-52. 4. Katayama, Y., T. Ito, and K. Hiramatsu. 2000. A new class of genetic element, staphylococcal cassette chromosome *mec*, encodes methicillin resistance in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 44: 1549-1555.



Zero in on Emerging Resistance

MicroScan
Resistance has met its match