

# Bacterial resistance: How to detect three types

By Susan M. Shima, MS, MT(ASCP), and Lawrence W. Donahoe, M(ASCP)

Two important — *and opposing* — trends are occurring simultaneously, and they both have a significant influence on the treatment of infectious diseases. One trend is the relentless increase in bacterial resistance to currently used antibiotics. The opposing trend is that fewer new antibiotics are being developed now than in previous decades. There have been only eight antibacterial medications approved since 1998.<sup>1</sup> This means it is crucially important that the microbiology laboratory provide physicians with the most accurate antibiotic susceptibility test data possible. If there are no false resistant antibiotic susceptibility test results, physicians will not be steered away from using an antibiotic that may be effective. If there are no false susceptible results, therapeutic failures due to inappropriate antibiotic use will be avoided. The role of the microbiology laboratory in providing guidance is more urgent than ever in this environment of increasing bacterial resistance and decreasing numbers of new antibiotics.

We have typically thought of antimicrobial susceptibility testing in the microbiology laboratory as providing physicians with data on the susceptibility of microorganisms to a battery of antibiotics. While the determination of relative susceptibility may be very important, we know that a result of *sensitive* to

an antibiotic does not guarantee treatment success. There is, however, a strong correlation between a *resistant* result and treatment failure. It is probably more appropriate to view the microbiology laboratory's work as focusing on bacterial-resistance testing rather than determining antibiotic susceptibility. The failure to recognize antibiotic resistance, when it is present, will certainly make ineffective treatment more likely.

As the focus shifts towards the detection of resistance mechanisms, microbiologists have become increasingly aware of the need to supplement traditional susceptibility testing methods with other tests and software that detect these resistance mechanisms. Microbiologists must understand the limitations of traditional disk-diffusion and agar- or broth-dilution methods in detecting bacterial resistance. They need to know which resistance mechanisms are most difficult to detect. They also need to know if, when, and what types of supplemental testing may be indicated.

The purpose of this review is to discuss three mechanisms of bacterial resistance that are important in terms of their clinical implications, inconsistent detection by microbiology laboratories, and potential need for testing or analysis by methods other than those traditionally employed in antibiotic susceptibility testing.

These three resistance mechanisms are:

1. extended-spectrum beta-lactamase (ESBL) production by Gram-negative bacilli
2. inducible clindamycin resistance in staphylococci
3. community-acquired methicillin resistance in staphylococci

## ESBL production by Gram-negative bacilli

### *The problem*

A rather disturbing finding is that many laboratories are making little or no effort to detect ESBL enzymes.<sup>2</sup> Dr. Paul Schreckenberger of the University of Illinois Medical Center recently conducted a survey of community hospitals in which he found that 61% of survey respondents were incorrectly reporting ESBL-producing organisms, and 47% performing the testing who were incorrectly reporting the cephalosporin susceptibility test results.<sup>3</sup> Empirical reports lead the authors to speculate that the reason for this lack of detection of ESBLs is that microbiologists believe these organisms do not exist or are rare in their institutions — or that their clinical relevance is minimal. It is also possible that there is insufficient knowledge as to how to go about testing for the presence of these enzymes.

In contrast to laboratory practice, clinicians like Dr. David Paterson of the University of Pittsburgh Medical

## CE CONTINUING EDUCATION

To earn CEUs, see test on page 24.

### LEARNING OBJECTIVES

Upon completion of this article the reader will be able to:

1. Discuss the importance of testing for extended-spectrum beta-lactamase production by Gram-negative bacilli.
2. Identify the treatment of choice for infections due to ESBL-producing organisms.
3. List the three phenotypes associated with acquired resistance among the staphylococci.
4. Describe the D test and its importance in the reporting of clindamycin results.
5. Discuss the emergence of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA), and outline the laboratory procedures suggested for its identification.

Center believe that testing for ESBL is crucial for two reasons. He states that:

1. High failure rates have been observed when cephalosporins have been used for the treatment of serious infections due to ESBL producers, even when the organism is apparently “susceptible” to the cephalosporin used in treatment.
2. ESBL-producing organisms can be a serious infection-control problem, but prompt infection-control interventions can curtail outbreaks.<sup>4</sup>

When serious nonESBL-producing *Klebsiella pneumoniae* infections are treated with cephalosporins, a treatment failure rate of approximately 20% has been observed.<sup>5</sup> Contrast this with Dr. Paterson’s report of a >50% treatment failure rate when cephalosporin therapy was used to treat serious infections due to ESBL-producing organisms, even when the organism appears to test susceptible.<sup>4(p35)</sup>

According to Dr. Paterson, the treatment of choice for serious infections due to ESBL-producing organisms is a carbapenem or a quinolone antibiotic. Even though the cephamycin antibiotics — cefotetan, cefmetazole, and ceftioxin — may appear to test susceptible, their usefulness in treating serious infections with ESBL producers is unclear. Dr. Paterson feels that they occupy a place in therapy only if carbapenems or quinolones cannot be used.<sup>4(p35)</sup>

### The background

The third-generation cephalosporins were introduced in the early 1980s. There was great interest in these antibiotics because they were not hydrolyzed by the common  $\beta$ -lactamases found in virtually all species of the Enterobacteriaceae.<sup>6</sup> This broadened the range of organisms that could be treated with cephalosporins. The new antibiotics were called extended-spectrum  $\beta$ -lactams. It was not long after this, however, that the first reports of resistance to the third-generation cephalosporins emanated from Europe.<sup>7</sup> The mechanism of resistance was found to be due to enzymes that were very similar to the known  $\beta$ -lactamases, yet were different enough to inactivate these newer cephalosporins. The newly discovered enzymes became known as extended-spectrum beta-lactamases, or ESBLs.

Most of the ESBLs have now been described. There are more than 100 of them noted in the literature — descendants of two common  $\beta$ -lactamases.<sup>8</sup> The first is the SHV-1  $\beta$ -lactamase that is present in virtually all *Klebsiella pneumoniae* and confers resistance to ampicillin and ticarcillin. The second is the TEM-1  $\beta$ -lactamase found in many *Escherichia coli* and confers resistance primarily to ampicillin.<sup>9</sup> Mutations in the genes encoding for these enzymes cause the enzyme to exhibit very different activity. A mutation that results in a change of only a few amino acids can be quite significant if it occurs near the active site of  $\beta$ -lactamase activity.<sup>10,11</sup> The enzymes created by these mutations became the ESBLs, and they are known to confer resistance to all penicillins, cephalosporins, and aztreonam.<sup>12,13</sup> Interestingly, they are, so far, unable to hydrolyze the cephamycin antibiotics cefotetan, cefmetazole, and ceftioxin, which are close relatives of the cephalosporins. They are also unable to inactivate the imipenem or meropenem (the carbapenem antibiotics).<sup>8,9,14</sup> As mentioned above, imipenem and meropenem remain the drugs of choice for treating infections due to ESBLs.

### Laboratory issues

Surveys and published data document that laboratories are experiencing difficulties in detecting and reporting ESBL-

producing organisms.<sup>2</sup> This is in contrast with the numerous experts who believe it is crucial that clinical microbiology laboratories be able to accurately report ESBL-producing organisms.

The National Committee for Clinical Laboratory Standards (NCCLS) has provided laboratories with guidelines for testing and reporting ESBLs. The NCCLS states in M100-S13 (M7), Table 2A, that: “strains of *Klebsiella* spp and *E coli* that produce extended-spectrum beta-lactamase (ESBLs) may be clinically resistant to therapy with penicillins, cephalosporins, or aztreonam, despite apparent *in vitro* susceptibility to some of these agents. Some of these strains will show minimal inhibitory concentrations (MICs) above the normal susceptible population but below the standard breakpoints for certain extended-spectrum cephalosporins or aztreonam. Other strains may test intermediate or resistant by standard breakpoints to one of more of these agents. Such strains should be screened for potential ESBL production by using the ESBL-screening breakpoints (listed below) before reporting results for penicillins, extended-spectrum cephalosporins, or aztreonam. Other strains may test intermediate or resistant by standard breakpoints to one or more of these agents.

In all strains with ESBLs, the MICs for one or more of the extended-spectrum cephalosporins or aztreonam should decrease in the presence of clavulanic acid as determined in phenotypic confirmatory testing. For all confirmed ESBL-producing strains, the test interpretation should be reported as resistant for all penicillins, cephalosporins, and aztreonam. The decision to perform ESBL-screening tests on all urine isolates should be made on an institutional basis, considering prevalence, therapy, and infection-control issues.”

The reader should consult the NCCLS documents M2-A8 or M7-A6 for specific information on the screening and confirmatory tests. The screening test is based on the MIC or disk-diffusion results of five indicator antimicrobial agents. The confirmatory test involves subsequent testing with ceftazidime and cefotaxime in the presence and absence of clavulanic acid (a beta-lactamase inhibitor).

One possible problem with this two-step screen-and-confirm approach is providing results in a clinically relevant time frame. In many cases, it will be three or more days after specimen collection before results are available. Laboratories may choose to report preliminary results if the screening test is positive, but this strategy can be problematic.

Dr. Fred Tenover, et al, reported on the results of confirmatory testing on 131 isolates that were screen-test positive according to the NCCLS criteria.<sup>15</sup> Only 16% (21 isolates) were actually determined to be ESBL producers by confirmatory testing. The other 84% of these isolates were exhibiting elevated MICs to the screening antibiotics because of resistance mechanisms other than ESBL enzymes.

The poor specificity of the ESBL-screening test can have significant implications. It has not yet been determined whether penicillin, cephalosporin, or aztreonam results should be changed when a mechanism of resistance — other than an ESBL — has been detected. Therefore, if a laboratory were to report the screening tests as suspicious of an ESBL-producing organism, clinicians would most likely prescribe carbapenem therapy, leading to overuse of this class of antibiotics.<sup>4(p44)</sup> Not reporting this finding, however, could result in appropriate therapy being withheld. A laboratory could avoid this dilemma by omitting the screening test and

*Continues on page 14*

immediately performing a confirmatory test, although extra cost may be involved.

Approximately 85% of laboratories participating in the College of American Pathologists' (CAPs) proficiency-testing surveys in 2000 were using either the MicroScan (Dade Behring MicroScan, Sacramento, CA) or the VITEK (bioMérieux Inc., Durham, NC) commercial systems for antibiotic susceptibility testing.<sup>16</sup> With this in mind, the ESβL-detecting capabilities of these systems is quite important.

Standard MicroScan dried MIC panels have antibiotics that provide appropriate concentrations of aztreonam, ceftazidime, cefotaxime, ceftriaxone, cefpodoxime, and aztreonam to determine MIC and ESβL screening. A computer flags identifications of *E coli*, *K pneumoniae* or *K oxytoca* with MICs for any of the five substrates of >2 μg/mL. All MicroScan panels also have wells with cefpodoxime at 4 μg/mL of ceftazidime at 1 μg/mL, and this provides an ad-

*A rather disturbing finding is that many laboratories are making little or no effort to detect ESβL enzymes.*

ditional screen for ESβLs. MicroScan also provides MicroScan ESβL Plus panels that screen and confirm for ESβLs. The confirmatory test is consistent with NCCLS guidelines in testing cefotaxime and ceftazidime with and without clavulanic acid and notes a ≥3 twofold decrease in MICs to either drug in the presence of clavulanic acid. Studies of these panels show sensitivities and specificities between 95% and 100%, respectively.<sup>20</sup>

Most of the routine VITEK Gram-negative susceptibility cards have a confirmatory ESβL test based on the NCCLS principle of demonstrating a reduction in MIC when clavulanic acid is added to cefotaxime and/or ceftazidime. Studies have found the FDA-approved ESβL-confirmatory test to be between 95% and 100% respectively,<sup>17</sup> and test results can be available to clinicians, in many cases, on the day following specimen submission. The VITEK 2's Advanced Expert System compares the obtained results to a database of ESβL phenotypes and, if there are matching patterns, provides a presumptive identification of an ESβL producer. Again, this result can be available within one day of specimen collection. In a study of known ESβL genotypes, it has shown a sensitivity and specificity of 91% and 93%, respectively.<sup>18,19</sup>

Dr. Michael Pfaller of the Department of Pathology at the University of Iowa has stated that "in order to provide the most appropriate and useful information for the care of infected patients, laboratories and the diagnostic-product manufacturers must take pains to define the resistance phenotype of the organism tested."<sup>4(p59)</sup> Accurate detection of ESβL-producing *E coli* and *Klebsiella* spp has been made easier because of developments by MicroScan and VITEK, and benefits patients by preventing the inappropriate use of cephalosporins and optimizing the use of the carbapenems.

**Inducible clindamycin resistance in staphylococci**

*The background*

The macrolides, lincosamides, and streptogramins are three classes of antibiotics that are closely related in function but not structure. There are currently three macrolides in common use: erythromycin (the first macrolide), clarithromycin,

and azithromycin. The lincosamide class includes clindamycin and lincomycin. The streptogramins (quinupristin/dalfopristin), consist of an A component and a B component that act synergistically against bacteria. These agents are often collectively referred to as the MLS group.<sup>21</sup>

As a group, the MLS antibiotics inhibit protein synthesis at the ribosome level in susceptible organisms. Gram-negative organisms are naturally resistant to the MLS antibiotics because entry of the drug into the cell is restricted. The MLS antibiotics are widely used, however, in the treatment of staphylococcal infections. Clindamycin is often a choice for skin and soft tissue staphylococcal infections, as well as an alternative in the penicillin-allergic patient.<sup>22</sup>

Acquired resistance to the macrolides and lincosamides is prevalent among the staphylococci. Two different mechanisms are responsible for most macrolide resistance. Active efflux, encoded by the *msrA* gene, causes resistance to the macrolides and type B streptogramins (but not the lincosamides).<sup>21,22</sup>

**MS phenotype: Erythromycin = R Clindamycin = S**  
Modification of the ribosomal target is encoded by the *erm* genes and causes resistance to the macrolides, lincosamides, and type B streptogramins (MLSb resistance). The *erm* genes cause production of methylase enzymes that decrease binding of the drug to the rRNA target. This resistance can be either constitutive or inducible. If the *erm* genes are consistently expressed, erythromycin, clindamycin, and other members of the MLS group will exhibit resistance.<sup>21,22</sup>

**MLSb constitutive phenotype: Erythromycin = R Clindamycin = R**

In some cases, however, the *erm* genes require an inducing agent to express resistance to clindamycin. Erythromycin can act as such an inducer. These isolates show *in vitro* resistance to erythromycin and susceptibility to clindamycin.<sup>22,23,24</sup>

**MLSb inducible phenotype: Erythromycin = R Clindamycin = S**  
These three phenotypes are summarized below.<sup>25,26</sup>

**Staphylococcus and MLS phenotypes**

Mechanism	Gene	Erythromycin	Clindamycin
Efflux	<i>msrA</i>	R	S
Ribosomal alteration	<i>erm</i>	R	S (inducible)
Ribosomal alteration	<i>erm</i>	R	R (constitutive)

The *in vitro* susceptibility patterns for *msrA* and inducible *erm*-mediated resistance are identical. Infections treated with clindamycin caused by a *Staphylococcus* species possessing the *msrA* gene is likely to be successful. There have been, however, documented cases of treatment failure with clindamycin attributed to the inducible *erm* mechanism.<sup>22</sup> While the incidence of MLSb inducible resistance can vary geographically, some published studies estimate that 45% of staphylococcal isolates have inducible resistance.<sup>22</sup>

If *in vitro* susceptibility results for staphylococci with MLSb inducible resistance is reported as tested, treatment failure with clindamycin is likely if there has been exposure to a macrolide.<sup>22,27</sup> The importance of distinguishing these two phenotypes cannot be overemphasized.

The D test is a relatively simple procedure available to identify inducible clindamycin resistance.<sup>22</sup> Using a standard disk-diffusion procedure, an erythromycin disk is placed 15 mm to 26 mm from a clindamycin disk. Following incu-

*Continues on page 16*

bation, a flattening of the zone in the area between the two disks where both drugs have diffused indicates that the organism has inducible clindamycin resistance. This procedure can now be found in NCCLS 2004 M100-S14 MIC Testing Supplemental Tables.<sup>28</sup>

NCCLS also suggests the following reporting options. If the original susceptibility report shows an erythromycin-resistant, clindamycin-sensitive staphylococci, the clindamycin result should be suppressed. Clindamycin should not be reported unless a D test is performed. If the D test is not routinely done, a comment should be added to contact the laboratory if a clindamycin result is needed. Once a D test is performed, whether by request or as a routine, results can be reported as illustrated below.<sup>25,26,28,29</sup>

**D-test reporting results**

D Test Result	Clindamycin
Negative	Susceptible. A comment may be added: This <i>Staphylococcus</i> does not demonstrate inducible clindamycin resistance <i>in vitro</i> .
Positive	Resistant or suppress clindamycin. Add a comment: This <i>Staphylococcus</i> demonstrates inducible clindamycin resistance <i>in vitro</i> and the isolate may develop clindamycin resistance during therapy.

It is not recommended to simply report clindamycin as resistant (therapeutic correction) whenever erythromycin is resistant. This will only discourage clindamycin use.<sup>22,25,26</sup>

Clindamycin is a useful drug for treating skin, soft tissue, and serious infections caused by staphylococci and anaerobes. It has good oral absorption, which makes it an important treatment option for outpatients.<sup>22</sup> In fact, many community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) are erythromycin-R and clindamycin-S, and clindamycin (often in combination with rifampin) is a valuable treatment option for this difficult organism.<sup>25,28</sup>

**Laboratory issues**

It is the laboratory’s responsibility to report accurate susceptibility results so physicians can make correct therapeutic decisions. In the case of staphylococci and clindamycin, the laboratory must have an awareness and understanding of inducible clindamycin resistance and the impact it has on reporting *in vitro* susceptibility results.

Secondly, there must be a mechanism in place to identify this phenotype and suppress results, pending further evaluation. This can be accomplished by visually checking manual or automated susceptibility reports. Many laboratory information systems (LISs) have software that will screen for specific susceptibility patterns. In addition, most automated susceptibility systems have online validation or “expert” systems that will identify and hold requested phenotypes. Software for creating antibiotic-suppression rules are available on many laboratory information and automated systems.

Third, laboratories must have the capability to perform supplemental testing when necessary and report results appropriately. All procedures must follow the recommendations of scientific committees and accrediting agencies.

Bacteria are rapidly developing resistance to many currently available drugs, while fewer and fewer new drugs are being developed. It is imperative that all possible treatment choices are accurately and reliably reported.

**Community-acquired methicillin-resistant *Staphylococcus aureus***

**The background**

Methicillin, a penicillinase-stable beta-lactam antibiotic, was introduced in 1961 to battle the problem of increasing penicillin resistance in *Staphylococcus aureus*. In 1968, the first infection caused by a methicillin-resistant *Staphylococcus aureus* (MRSA) was reported in the United States. Currently, the incidence of MRSA in the hospital setting is as high as 50%.<sup>30</sup>

Resistance to methicillin (and oxacillin) in both *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) are due to a chromosomal gene of unknown origin. This gene, the *mecA* gene, codes for a variant of the PBP2 penicillin-binding protein.

Penicillin-binding proteins are enzymes that participate in the production of peptidoglycan, a major component of the bacterial cell wall. The altered penicillin-binding protein, designated PBP2a, is able to perform its cell-wall synthesis functions, but has low affinity and does not bind to beta-lactam antibiotics. The presence of the *mecA* gene confers resistance to all beta-lactam antibiotics. In addition, MRSA found in hospitals are typically resistant to multiple antibiotics of various classes.<sup>24,31</sup>

For almost 30 years since its detection, MRSA has been largely confined to the healthcare setting and is considered strictly a nosocomial or hospital-acquired (HA) pathogen. Until a few years ago, MRSA infections found outside the hospital could usually be linked to a recent hospitalization, close contact with a person who was hospitalized, or previous antibiotic therapy.<sup>31</sup>

In 1999, the U.S. Centers for Disease Control and Prevention (CDC) reported four deaths from MRSA in healthy children from Minnesota and North Dakota. Epidemiological studies indicated the children had none of the traditional risk factors for MRSA infection.<sup>32</sup> Phenotypically, the strains were resistant to the beta-lactam antibiotics, but much more susceptible to other antibiotic classes than hospital-acquired MRSA (HA-MRSA).

**MRSA phenotypes**

	Clindamycin	Erythromycin	Oxacillin	Penicillin	Vancomycin
HA-MRSA	R	R	R	R	S
CA-MRSA	S	S	R	R	S

Typing by pulse field gel electrophoresis (PFGE) also indicated that these strains were distinctive.<sup>33</sup> This evidence was strongly suggestive of a new community-acquired MRSA (CA-MRSA). Further reports and studies seem to confirm this.<sup>31,33,34</sup> Comparison of virulence factors between hospital-acquired and community-acquired strains showed CA-MRSA much more likely to be associated with toxic-shock syndrome.<sup>33</sup>

These organisms may also possess the Pantone-Valentine leukocidin (PVL) that facilitates MRSA crossing of the intact skin barrier. This can cause septicemia in immunocompetent patients and has been associated with lethal necrotizing pneumonia.<sup>25</sup> CA-MRSA has gained a foothold in the community and is emerging as an important outpatient pathogen. The exact origin of these CA-MRSA strains is still unclear. At this time, one possible explanation is that the *mec* gene was transferred horizontally from a nosocomial (HA) donor into a susceptible community donor.<sup>30</sup>

*Continues on page 21*

Normally, this might seem like an unlikely occurrence due to the presence of *mec* on the chromosome and its large size. But Hiramatsu and coworkers have identified genes, called *ccrAB* (cassette chromosome recombinase A and B), that code for proteins, which catalyze precise excision and precise integration of *mec* into the *S aureus* chromosome.<sup>35</sup> Recent studies have also identified a unique genetic element called staphylococcal cassette chromosome *mec* (SCC*mec*) type IV in CA-MRSA.<sup>36</sup> Unlike that of HA-MRSA, this element is very small and does not have genetic-resistance determinants for nonbeta-lactam antibiotics.<sup>36</sup>

These studies suggest that *mec* could be spread among *Staphylococcus aureus* isolates and also explain the phenotypic differences.

#### Laboratory issues

Rapid and reliable detection of MRSA is essential for optimal treatment of patients with staphylococcal infections. A variety of testing methods was developed in response to the emergence of HA-MRSA. The oxacillin agar screen test has been used for many years to aid in the identification of MRSA. This method can be used for *S aureus*, but is unreliable for CNS.<sup>28,29,39</sup> Disk-diffusion and broth- or agar-dilution methods can be used for all staphylococci. The accuracy of these procedures, however, can be affected by inoculum size, incubation time, temperature, media, pH, salt concentration, and other factors.<sup>29,37,38</sup>

Despite the standardized recommendations for susceptibility testing of MRSA provided by NCCLS, some MRSA strains fail to be detected. This variation in the phenotypic expression of methicillin resistance is largely due to the heterogeneous nature of the resistance mechanism.<sup>38</sup>

Whether hospital-acquired or community-acquired, MRSA expression can be homogeneous (where all *mecA* cells within a population express resistance) or heterogeneous (where some *mecA*-positive cells appear resistant and others appear sensitive) under standard test conditions.<sup>24</sup> The majority of isolates have heterogeneous drug resistance.<sup>38</sup> It has also been shown that heterogeneous strains exposed to beta-lactams will develop homogeneous oxacillin resistance.<sup>38</sup> For the microbiologist using these methods, determination of oxacillin resistance could be totally dependent on the colonies selected for testing. It is possible that heterogeneous *mecA*-positive cells selected for susceptibility testing could test oxacillin susceptible. Treatment with a beta-lactam would most likely fail, and oxacillin resistance would be further induced.

Subsequent testing of *S aureus* isolates from the patient would then show oxacillin resistance. When this occurs or when results differ between methods, microbiologists tend to first question the accuracy and reliability of test methods rather than taking a closer look at the resistance mechanism involved. Microbiologists with an understanding of *mecA* resistance would likely question an oxacillin-sensitive *S aureus* if it were resistant to multiple other drug classes. On the other hand, an oxacillin-sensitive *S aureus* with no other resistance expressed may not be questioned. This could be a serious mistake, especially if the isolate is from an outpatient source. Heterogeneous CA-MRSA could have just such a phenotypic pattern, and failure to identify it as an MRSA might have severe consequences for the patient.

Other staphylococcal-resistance mechanisms which confer oxacillin resistance (e.g., borderline *S aureus* or BORSA, and modified *S aureus* or MODSA) can be difficult to distin-

guish from MRSA using phenotypic methods.<sup>37,41</sup> With these phenotypes, oxacillin resistance does not imply resistance to all beta-lactam antibiotics. If they are reported, however, as MRSA based strictly on the oxacillin result, vancomycin therapy may be initiated unnecessarily.

It has been shown that isolates displaying a CA-MRSA phenotype do not reliably report resistant to oxacillin with either NCCLS standard microbroth- or agar-dilution methods.<sup>38,40</sup> Strains in one published outbreak consistently reported resistant to oxacillin only when using disk diffusion or the oxacillin salt-agar screening plate.<sup>39</sup> Since many commercial systems use microbroth dilution standardized to broth- or agar-dilution reference methods, oxacillin resistance may have been missed in these cases. It is important to realize that this is not a defect of the commercial system, but rather an apparent characteristic of some CA-MRSA. Laboratories using commercial systems may want to consider periodic screening of their isolates using oxacillin salt-agar or disk-diffusion testing.

This year, NCCLS M100-S14 included a procedure utilizing a 30- $\mu$ g cefoxitin disk and alternate breakpoint to screen for oxacillin resistance in staphylococci.<sup>28</sup> This test can be used in place of the oxacillin disk-diffusion test for *S aureus* and CNS. The test has equal to or greater correlation with the presence of *mecA* as compared to oxacillin disk diffusion and is much easier to read.<sup>26</sup> For CNS, not *Staphylococcus epidermidis*, with oxacillin MIC's 0.5-2.0  $\mu$ g/ml, a cefoxitin disk test may be helpful. If the cefoxitin zone is  $\geq 25$  mm, report oxacillin susceptible. If the cefoxitin zone is  $\leq 24$  mm report "probable oxacillin resistance; contact the laboratory if more definitive testing desired." Then perform a test specific for *mecA*.<sup>26,28</sup>

Since methicillin resistance is almost exclusively caused by PBP2a encoded by the *mecA* gene, tests that detect *mecA* or PBP2a are considered more accurate and reliable than phenotypic tests.<sup>41</sup> Methods based on PCR that target *mecA* are the gold standard by which all new methods are measured.<sup>41</sup> Several DNA-based detection methods, such as probes or PCR assays, have been published. These methods are often too labor intensive or technically demanding for reliable use in the clinical lab.<sup>41</sup> Rapid latex screening methods are now available for detection of *mecA* and PBP2a with high sensitivity and specificity for both *S aureus* and CNS.<sup>24,37,40</sup> These tests are not designed to replace susceptibility tests. They have their greatest value when used in addition to susceptibility tests to arbitrate equivocal results. *MecA*-positive tests should be reported as oxacillin resistant; negative tests are reported as oxacillin susceptible. Rapid communication of these results to the physician will contribute to selection of the most appropriate antibiotic therapy.

Staphylococci are the leading cause of nosocomial and community-acquired infections worldwide.<sup>37</sup> The percent of those infections that are methicillin resistant continues to climb.<sup>30,31,41,42</sup> The increased use of vancomycin to treat these infections has led to vancomycin-resistant enterococcus or VRE, glycopeptide-intermediate *Staphylococcus aureus* (GISA), and now vancomycin-resistant *S aureus* or VRSA.<sup>41</sup> Rapid and reliable detection of MRSA decreases the use of vancomycin and is a valuable tool to help control the spread of MRSA.<sup>40</sup> New methods to detect oxacillin resistance continue to be developed. The emergence of CA-MRSA as a significant outpatient pathogen demands that laboratories evaluate their current procedures and update them as necessary.

*Continues on page 22*

## Summary

Antimicrobial susceptibility testing in the clinical laboratory is becoming more complex. We can no longer be concerned with simply determining accurate susceptibilities. Microbiologists must now possess knowledge of bacterial-resistance mechanisms and implement procedures to reliably detect them. The traditional susceptibility test will most likely need to be supplemented with testing methods and software that allow for phenotypic identification of resistance mechanisms. Resistance mechanisms can be present in apparently susceptible bacterial populations. Accurate identification of these mechanisms will help to control emergence of new resistance by encouraging use of the most appropriate antibiotics. □

Susan M. Shima, MS, MT(ASCP), is group leader, Microbiology Customer Service, and Lawrence W. Donahoe, M(ASCP), U.S. Microbiology marketing manager at bioMérieux Inc, Durham, NC.

## References

- Centers for Disease Control and Prevention. *Infectious Disease News*, December 2003. Vol. 16, No. 12.
- Tenover FC, Mohammed MJ, Gorton TS, Dembek ZF. Detection and reporting of organisms producing extended spectrum beta-lactamases: survey of laboratories in Connecticut. *J Clin Microbiol*. 1999;37:4065-4070.
- Dr. Paul Schreckenberger, Personal communication, results to be presented in an education program in conjunction with the University of Illinois at Chicago and bioMérieux Inc.
- Jorgensen, Sahn, Nicolau, Paterson, Thomson, Tenover, Pfaller. *ESBLs: Pharmacodynamics, Clinical Relevance, Evolution, Prevalence, and Methods for Detection and Reporting*. bioMérieux, Inc. 2003. Monograph, 34-59.
- Korvick JA, Bryan CS, Farber B, et al. Prospective observational study of Klebsiella bacteremia in 230 patients: outcome for antibiotic combinations versus monotherapy. *Antimicrob Agents Chemother*. 1992;36:2639-2644.
- Bush K, Jacoby G. A functional classification scheme for  $\beta$ -LACTAMASES and its correlation with molecular structure. *Antimicrob Agents Chemother*. 1995;37:1637-1644.
- Sirof J, Chanal A, Petit D, Sirof R, Labia R, Gerbaud G. Klebsiella pneumoniae and other Enterobacteriaceae producing novel plasmid-mediated  $\beta$ -lactamases markedly active against third-generation cephalosporins: Epidemiological studies. *Rev Infect Dis*. 10:850-859.
- Bush K. New beta-lactamases in Gram negative bacteria: diversity and impact on the selection of antimicrobial therapy. *Clinical Infectious Diseases*. 2001;32:1085-1089.
- Bradford PA. What's new in  $\beta$ -lactamases? *Curr Infect Dis Repts*. 2001;3:13-19.
- Bush K, Jacoby GA, Madeiros AA. A functional classification scheme for  $\beta$ -lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother*. 1995;39:1211-1233.
- Jacoby GA. Extended spectrum  $\beta$ -lactamases and other enzymes providing resistance to oxymino- $\beta$ -lactams. In: *Infectious Disease Clinics in North America*. Tenover FC and McGowan JE Jr., eds. 1997;Vol. 11:4 W.B. Saunders Co., Philadelphia, PA.
- Bush K, Jacoby GA, Madeiros AA. A functional classification scheme for  $\beta$ -lactamases and its correlation with molecular structure. *Antimicrob Agents Chemother*. 1995;39:1211-1233.
- Jacoby GA. Extended spectrum  $\beta$ -lactamases and other enzymes providing resistance to oxymino- $\beta$ -lactams. In: *Infectious Disease Clinics in North America*. Tenover FC and McGowan JE Jr., eds. Vol. 11:4 W.B. Saunders Co., Philadelphia, PA.
- Jett BD, Ritchie DJ, Reichley R, et al. In vitro activities of various  $\beta$ -lactam antimicrobial agents against clinical isolates of Escherichia coli and Klebsiella spp resistant to oxymino cephalosporins. *Antimicrob Agents Chemother*. 1995;39:1187-1190.
- Tenover FC, et al. Evaluation of the NCCLS Extended Spectrum  $\beta$ -lactamase Confirmation Methods for Escherichia coli with Isolates Collected during Project ICARE. *JCM*. July 2003. 3142-3146.
- Jones RN. Method preferences and test accuracy of antimicrobial susceptibility testing: updates from the College of American Pathologists Microbiology Surveys Program. *Arch Pathol Lab Med*. 125:1285-1289.
- Sanders CC, Barry AL, Washington JA, et al. Detection of extended spectrum  $\beta$ -lactamases producing members of the family Enterobacteriaceae with the VITEK ESBL Test. *J Clin Microbiol*. 1996;34:2997-3001.
- Livermore, DM, Struelens M, Amorim J, et al. Multicentre evaluation of the VITEK 2 Advanced Expert System for interpretive reading of antimicrobial resistance tests. *J Antimicrob Chemother*. 2000;49:289-300.
- Sanders, CC, Peyret M, Moland ES, et al. Ability of the VITEK 2 Advanced Expert System to identify  $\beta$ -lactam phenotypes in isolates of Enterobacteriaceae and Pseudomonas aeruginosa. *J Clin Microbiol*. 2000;38:570-574.
- Paterson, DL, Rihs BL, Ko WC, et al. Evaluation of MicroScan ESBL 98 Confirmation Panel, VITEK ESBL card, Etest  $\beta$  strips, and disk diffusion methodologies in detection of extended spectrum  $\beta$ -lactamases from blood culture isolates of Klebsiella pneumoniae. [Abstract.] 1999;C-252: 155 in Abstracts of the 99th General Meeting of the American Society for Microbiology. American Society for Microbiology, Washington, DC.
- Rice LB, Sahn D, Bonomo RA. Mechanisms of Resistance to Antimicrobial Agents. *Man Clin Microbiol*. 8th ed. 2002:1074-1094.
- Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH. Practical Disk Diffusion Method for Detection of Inducible Clindamycin Resistance in Staphylococcus aureus and Coagulase-Negative Staphylococci. *J Clin Microbiol*. 2003;41:4740-4744.
- Panagea S, Perry JD, Gould FK. Should clindamycin be used as treatment with infections caused by erythromycin-resistant staphylococci? *J Antimicrob Chemother*. 1999;44:581-582.
- Poulter MD, Hindler JF. Challenges in Antimicrobial Susceptibility Testing and Reporting. *Laboratory Medicine*. 2002;11:877-884.
- Hindler JF. Everything you wanted to know about antimicrobial susceptibility testing of Staphylococcus aureus. *National Laboratory Training Network*. 2003.
- Hindler JF. What's New in the 2004 NCCLS Standards for Antimicrobial Susceptibility Testing? *National Laboratory Training Network Teleconference*. 2004.
- Drinkovi D, Fuller ER, Shore KP, Holland DJ, Ellis-Pegler R. Clindamycin treatment of Staphylococcus aureus expressing inducible clindamycin resistance. *J Antimicrob Chemother*. 2001;48:315-316.
- National Committee for Clinical Laboratory Standards. *Performance Standards for Antimicrobial Susceptibility Testing. Fourteenth Informational Supplement*. 2004. Villanova, PA. NCCLS. M100-S14 (M7).
- National Committee for Clinical Laboratory Standards. (2003). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*. 6th Ed. Approved Standard M7-A6. Villanova, PA. NCCLS.
- Estrada B. Methicillin-resistant Staphylococcus aureus in the Community. *Infect Med*. 2001;18(10):452.
- Chambers HF. The Changing Epidemiology of Staphylococcus aureus. *Emerg Infect Dis*. Centers for Disease Control and Prevention. 2001;7:(2).
- Four pediatric deaths from community-acquired methicillin-resistant Staphylococcus aureus – Minnesota and North Dakota, 1997-1999. *MMWR Morb. Mortal. Wkly Rep*. Centers for Disease Control and Prevention. 1999;48:707-710.
- Stratton C. Community-acquired MRSA: A dramatically different strain. *41st Interscience Conference on Antimicrobial Agents and Chemotherapy*. 2001.
- Herold BC, Immergluck LC, Maranan MC, Lauderdale DS, Gaskin RE, Boyle-Vavra S, et al. Community-acquired methicillin-resistant Staphylococcus aureus in children with no identified predisposing risk. *JAMA*. 1998;279:593-598.
- Katayama Y, Ito T, Hiramatsu K. A new class of genetic element, staphylococcus cassette chromosome mec, encodes methicillin resistance in Staphylococcus aureus. *Antimicrob Agents Chemother*. 2000;44:1549-1555.
- Hammerschlag MR. Community-acquired MRSA: A new twist for an old bug. *Infect. Med*. 2003;20(1):8,13.
- Oxoid Ltd. [package insert]. Ogdensburg, NY. Penicillin Binding Protein (PBP2') Latex Agglutination Test. 2003.
- Sakoulas G, Gold HS, Venkataraman L, Degirolami PC, Eliopoulos GM, Qian Q. Methicillin-resistant Staphylococcus aureus: Comparison of susceptibility testing methods and analysis of mecA-positive susceptible strains. *J Clin Microbiol*. 2001;39(11):3946-3951.
- Block J, Orlando MF, McDougal LK, Jevitt L, Dunne WM, Fitzsimmons S, Gerst J. Emerging resistance: Detection of methicillin resistance in unusual strains of Staphylococcus aureus with a community-acquired phenotype and appearing susceptible by standard methods. *ASM 103rd General Meeting*. 2003.
- Swenson JM, Williams PP, Killgore G, Mohr O'Hara C, Tenover F. Performance of eight methods, including two new rapid methods, for detection of oxacillin resistance in a challenge set of Staphylococcus aureus organisms. *J Clin Microbiol*. 2001;39(10):3785-3788.
- Skov RL, Pallesen LV, Poulsen RL, Espersen F. Evaluation of a new 3-h hybridization method for detecting the mecA gene in Staphylococcus aureus and comparison with existing genotypic and phenotypic susceptibility testing methods. *J Antimicrob Chemother*. 1999;43:467-475.
- Tsambras P, Nadler JP, Carter W. Less well-known emerging infections and newer antibiotics. *40th Interscience Conference on Antimicrobial Agents and Chemotherapy*. 2000.