Methicillin-Resistant *Staphylococcus aureus* (MRSA): Identification and Susceptibility Testing Techniques

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Many traditional techniques are useful for identification of MRSA strains, including techniques for detection of penicillin-resistance, such as the nitrocefin disk. Techniques for assessing methicillin-resistance vary from growth on special media or at a lower temperature, to detection of the *mecA* gene by manual (latex agglutination) and automated (PCR) methods. Technique development is now geared toward making MRSA identification more rapid. Real-time PCR has sped MRSA detection, but can be costly. Resistance to other drugs is also an issue. Clindamycin resistance may need to be induced, so a special disk diffusion test can be performed. Vancomycin resistance is becoming an issue, so alternative drugs need to be identified. Drugs that are currently available for MRSA infections include: daptomycin, linezolid, quinupristin/dalfopristin, and tigecycline. Drugs that are in the development phase include: ceftobiprole, dalbavancin, oritavancin, and telavancin. These drugs provide a promising arsenal against MRSA.

Index Terms: MRSA, identification techniques, susceptibility testing, antimicrobial agents

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LEARNING OBJECTIVES

1. Discuss the standards techniques for identification of *Staphylococcus aureus*.
2. Describe how methicillin-resistance is induced and detected.
3. Compare the different PCR methods for MRSA identification.
4. Describe other drugs that some MRSA strains may be resistant to and how that resistance is detected.
5. Differentiate among the new MRSA drugs and among those drugs in the developmental phase.

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The traditional techniques used to identify a culture isolate as *Staphylococcus aureus* are neither difficult nor time consuming. When a patient specimen is cultured and *S. aureus* is a potential pathogen, the laboratory scientist looks for β-hemolytic white to yellowish colonies on the blood agar plate. A gram stain (which may or may not have also been performed on the direct specimen) of a suspect colony shows gram-positive cocci in clusters. As protocol usually dictates, a catalase test is performed, which should be positive. This confirms that the isolate probably contains staphylococci and not streptococci. Then the isolate is tested for coagulase production; either by the traditional slide (bound - clumping factor) and tube (free coagulase) methods, or the more modern and rapid combined latex agglutination methods, such as Staphaurex, which detects clumping factor and protein A production[1]. The average time frame to get from specimen to coagulase positive is around 24 hours. Because so many *S. aureus* are now penicillin-resistant, a standard practice is to perform a rapid β-lactamase production test using a nitrocefin disk. Nitrocefin is a chromogenic cephalosporin. If the bacteria produce β-lactamase, the β-lactam ring of the nitrocefin is hydrolyzed. This creates an electron shift which results in a color change, to a brown-red, within 10 minutes[2].

With the rise in MRSA isolates, and the ever-increasing threat of VRSA strains, the time required for proper iden-

tification has become a major issue. Hospitals and other health-care facilities want to isolate patients with MRSA, so they need results very rapidly to prevent the spread of MRSA within the facility. Not only do the isolates have to be identified as *S. aureus*, but they also have to be shown to be methicillin-resistant (actually tested for by using an alternative). Traditional methods for susceptibility testing, MIC determination by dilution, or disk diffusion methods, usually require an additional 8–16 hours. Attempting to combine the identification and susceptibility testing steps with MRSA is complicated by the fact that in most clinical isolates methicillin-resistance is expressed in a heterogeneous manner when grown using standard conditions. This means that standard susceptibility testing methods could produce false negative results. Fortunately, the expression of the *mecA* gene, which is responsible for methicillin-resistance, can be induced by varying the composition of the growth media and/or the growth temperature. Addition of NaCl to the growth media and/or growing the cultures at 30° C instead of 37° C induces expression of *mecA* and allows for detection of methicillin-resistance[3]. Since cefoxitin is a better inducer of *mecA* than oxacillin, a cefoxitin disk diffusion test will detect MRSA strains with better accuracy[4]. One study that compared mannitol salt agar (MSA) with MSA-containing oxacillin showed that the plain MSA plate actually detected MRSA as accurately as the MSA plate containing oxacillin, 79% vs. 65%, respectively[5].

Much effort has been put into decreasing the time that it takes to identify an isolate as MRSA. A commonly used rapid test is the PBP2a (PBP2′) latex agglutination assay (from Oxoid), which detects the altered PBP produced by the *mecA* gene. It has to be performed on culture isolates from agar plates (that do not contain β-lactam antibiotics). This means an overnight incubation has already occurred. The test latex particles are sensitized with a monoclonal antibody against PBP2a. This assay takes only about 15 minutes[7].

Another attempt to simplify (and thereby hopefully shorten the time to) the identification of MRSA was the development of chromogenic agars. One that is available in the U.S. is CHROMagar MRSA® (from BBL). This agar contains chromogenic substrates and cefoxitin. MRSA strains are able to grow in the presence of the cefoxitin and produce mauve-colored colonies. Other bacteria may produce blue to blue-green colonies, or if not able to utilize the chromogenic substrates, white or colorless colonies. This allows direct screening of patient specimens, particularly nasal swabs, for detection of MRSA. Since this type of agar can only be used for screening purposes, a definitive identification would require an additional test, such as the PBP2a latex agglutination assay. The culture also has to be incubated for at least 16-24 hours, so while it might be a relatively inexpensive method to use for screening purposes, it doesn't appreciably speed up the identification process[7].

The older automated methods that combine identification and susceptibility testing, such as the Vitek® (bioMérieux) and the MicroScan® (Siemens), don't really save time because a pure culture is necessary for loading a specimen onto these analyzers. The newer identification methods that are truly rapid are also far more expensive than the culture methods. These newer methods include several PCR-based assays. The older PCR assays required the use of pure cultures, and so don't save any time. Some of these, such as nested PCR and multiplex PCR, may also be complex and labor intensive[8].

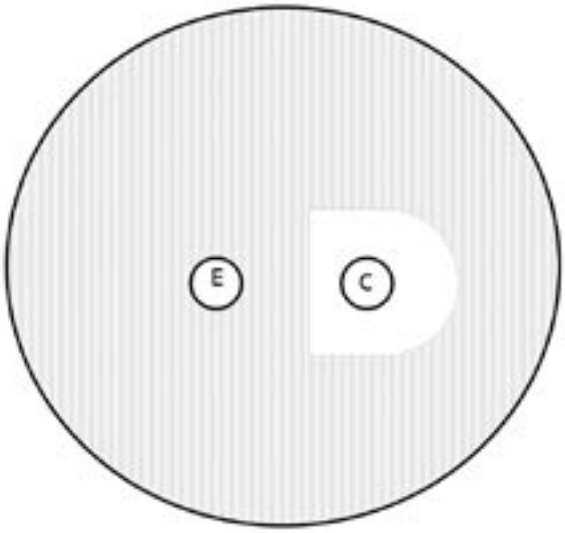
Newer PCR assays, including some real-time PCR methods, allow the direct use of patient specimens (e.g. nasal swabs). These can shorten the identification time dramatically, to a few hours. The downside is the cost. Not all laboratories can afford to do patient admission MRSA screen testing using these faster methods. One real-time method that is available in the U.S. is the BD GeneOhm MRSA® assay (formerly the IDI-MRSA® assay). It is used for the rapid identification of MRSA from nasal swab specimens. It includes primers for most of the SCC*mec* elements (types I-IV), where the *mecA* gene is located, so it can detect MRSA strains from either HA-MRSA or CA-MRSA origins. It also includes a primer for the *orfX* gene, which is *S. aureus* specific. The amplified targets are detected by fluorescent molecular beacons, results are available in two to three hours, and the assay can only be run in batches[8, 9, 10].

The very latest in real-time PCR assays is the GeneXpert MRSA® assay kit, from Cepheid. This assay uses the same PCR target sequences as the GeneOhm MRSA® assay, the various SCC*mec* elements and the *orfX* gene. Currently, it is designed for use with nasal swabs, but is now being evaluated for use with skin and soft tissue specimens. Preparation time is minimal because the specimen is directly inoculated into a disposable cartridge that contains all the necessary reagents. The assays can be performed by random access, and it only takes about 75 minutes for the results. The GeneXpert systems offer the flexibility to purchase units that will hold 1, 4, or 16 cartridges[11].

Unfortunately being able to rapidly identify an isolate as MRSA is not the end of the story. The other big issue is the

possibility of resistance to other drugs. Some CA-MRSA strains may possess inducible clindamycin resistance, many HA-MRSA strains are resistant to multiple drugs, and now we have begun to see developing resistance to vancomycin. Clindamycin is sometimes used to treat MRSA infections of skin and soft tissue (SSTIs). These types of infections are most commonly caused by CA-MRSA strains. In *S. aureus*, macrolide and lincosamide drugs have a common mode of action. Both types of drugs inhibit protein synthesis in bacteria by binding to the 50S ribosomal subunit. Bacterial resistance to these drugs can also be mediated by the same process, methylation of the ribosomal target site. The mechanism of resistance can be constitutive, always present; or inducible, where the target is only methylated when the offending drug is present. Any *S. aureus* strain that tests as resistant to erythromycin (a macrolide) should be tested for inducible resistance to clindamycin (a lincosamide). It is usually more time efficient to just test any suspected MRSA stain for this inducible resistance at the same time as you are running other susceptibility tests. Probably the easiest method to use is the clindamycin disk diffusion (DD) test. Since erythromycin is a much stronger resistance inducer than clindamycin, the test uses a disk of each drug. A positive result shows no growth around the erythromycin disk, and the zone of inhibition around the clindamycin disk is blunted on the side nearest the erythromycin disk (due to the induced resistance), forming the characteristic "D" shaped zone (Figure 1)[12].

Figure 1. Clindamycin disk diffusion (DD) test—positive result for induction of resistance



There is an increasing amount of resistance to multiple drugs in HA-MRSA strains. In a study that analyzed over 14,600 MRSA isolates, these isolates were considered to be multidrug resistant if they were also resistant to three or more of the following drugs (which are possibilities when treating uncomplicated MRSA infections): ciprofloxacin, clindamycin, erythromycin, gentamicin, and trimethoprim/sulfamethoxazole (SXT). The results are summarized in Table 1[13].

Until recently physicians have not been overly concerned about the threat of MRSA. They assumed that they could rely on vancomycin to treat serious MRSA infections, and infections with MRSA that were clindamycin and/or multidrug resistant. Unfortunately, not only is it not wise to abuse vancomycin to the point that VRSA strains become a huge threat, but also, in reality, vancomycin does not have that great a track record for treating MRSA infections. Vancomycin is administered intravenously because it is not absorbed well from the intestinal tract, is expensive, has limited tissue penetration (especially in the lungs), and demonstrates slower bactericidal activity than some other drugs[14, 15]. In addition, there have been studies that showed alarming treatment failure percentages (as high as 40% or more) when using vancomycin to treat MRSA isolates[16, 17, 18, 19, 20].

If vancomycin cannot be relied on to treat MRSA infections, what can be used? Since many of the old favorite drugs are producing resistant strains of MRSA (see Table 1), some attention has focused on the use of another older drug, rifampin. It is potent, and has good tissue penetration; however, if it is used alone, resistance develops fairly rapidly[3]. There is also the issue of adverse effects, especially when used for treating serious infections[21]. It has been shown to be fairly effective in treatment of MRSA colonization when used in combination with drugs such as: clindamycin, doxycycline,

Table 1. Percent of MRSA Isolates Resistant to Other Antimicrobial Agents

Number of Other Agents	Percent Resistant
0	4.5
1	0.2
2	1.0
3	4.2
4	8.0
5	2.1

minocycline, trimethoprim/sulfamethoxazole, or even with vancomycin[22, 23, 24].

In the last few years, several new drugs have become available for treatment of MRSA: daptomycin, linezolid, quinupristin/dalfopristin, and tigecycline. Only one of these, linezolid, can be given orally as well as intravenously. Daptomycin has been shown to be fairly effective in the treatment of MRSA SSTIs, but not very effective against MRSA bacteremia or endocarditis. Due to the fact that drug activity is inhibited by pulmonary surfactant, it should not be used to treat pneumonia. Linezolid is expensive for an oral drug and is only bacteriostatic. It has been shown to be fairly effective against complicated SSTIs (cSSTIs—defined as deep infections that require surgical intervention), but less effective against pneumonia and bacteremia[25, 26]. The combination drug, quinupristin-dalfopristin is only recommended for cSSTIs, and there have been complaints of adverse effects[27]. Tigecycline is also only recommended for cSSTIs, and has been shown to be fairly effective[25].

There are also several drugs in development for MRSA infections. These include: ceftobiprole, dalbavancin, oritavancin, and telavancin, all of which are administered intravenously. Ceftobiprole is a broad spectrum cephalosporin ,-lactam drug that has a high affinity for the PBP2a of MRSA. It has been shown to be effective in cSSTIs, and pneumonia, and is a promising treatment for osteomyelitis and some VISA and VRSA infections[28]. Dalbavancin is a semisynthetic glycopeptide drug which has an extremely long half life (5–7 days) which will allow once weekly dosing. It has been shown to be effective against cSSTIs and catheter-related bloodstream infections (CR-BSIs)[26]. Oritavancin is also a semisynthetic glycopeptide drug that has been shown to be effective against cSSTIs, CR-BSIs, and nosocomial pneumonia. It also has shown activity against VRSA strains[29]. Telavancin is yet another semisynthetic glycopeptide drug and has a dual mechanism of action. It inhibits cell wall synthesis and is also able to insert into the bacterial cell membrane, which increases cell membrane permeability. This allows the drug to have a rapid bactericidal activity. It has been shown to be effective against SSTIs, nosocomial pneumonia, and uncomplicated bacteremia. Synergistic effects have been shown when telavancin was used in combination with imipenem or piperacillin-tazobactam against VISA isolates, and with cefepime, imipenem, or piperacillin-tazobactam against VRSA isolates[30].

With rapid identification methods and the hope of new, more effective drugs, the outlook for the fight against MRSA is beginning to look a lot less grim. The most daunting problem is how labs will find the resources for performing surveillance cultures on all patient admissions. If quick results are required, then the lab will need to have some type of real-time PCR assay available. At least the treatment options seem to be improving. Physicians should approach MRSA treatment with caution, choosing which drug(s) to use based on factors such as: the type and site of infection, the antimicrobial susceptibility profile of the particular MRSA strain, and the potential cost of treatment. If the newer drugs are used wisely, perhaps we can prevent the creation of *S. aureus* strains that are untreatable.

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