EDUCATIONAL COMMENTARY - Methicillin-Resistant *Staphylococcus aureus*: An Update

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**LEARNING OUTCOMES**

Upon completion of this exercise, the participant should be able to

- discuss the recent epidemiologic trends of methicillin-resistant *Staphylococcus aureus* (MRSA).
- list and explain two mechanisms of oxacillin resistance in *S aureus*.
- explain current (2011) CLSI standards for detecting antibiotic resistance in *S aureus*.

**Infection Rates**

The emergence of antibiotic resistance in *Staphylococcus aureus* began in the 1940s with the appearance of penicillin-resistant strains which, for many years remained susceptible to the β-lactamase-stable penicillins. Then, in the mid-1980s, *S aureus* strains that were resistant to the β-lactamase-stable penicillins appeared. These strains were termed “methicillin-resistant *Staphylococcus aureus*” (MRSA) because methicillin was first used to detect resistance to the β-lactamase-stable penicillins. The term MRSA, or methicillin-resistant *S aureus*, is still used today even though methicillin is no longer used to detect resistance.

Initially, MRSA appeared almost exclusively in the healthcare setting and quickly became a major cause of nosocomial infections. Today, however, MRSA strains increasingly appear outside the healthcare setting; these strains are termed “community-associated MRSA.” Persons at higher risk for acquiring community-associated MRSA include: athletes, children in day care centers, students living in dormitories, soldiers living in military barracks, inmates in correctional facilities, and men who have sex with men. Cases have also been reported in people with none of these risk factors.

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**What are “β-lactamase-resistant,” β-lactamase-stable,” and “β-lactamase-sensitive” antibiotics?**

The β-lactam antibiotics, which include penicillins, cephalosporins, carbapenems, penems, monobactams, and β-lactam inhibitors, all contain a core molecular structure known as a β-lactam ring. The β-lactam ring can be broken by enzymes known as β-lactamases. This inactivates the antibiotic and thus confers resistance to the bacteria. Penicillinase is an example of a β-lactamase enzyme.

Some β-lactam antibiotics are impervious to β-lactamases. These drugs are termed “β-lactamase-resistant” (because they resist the action of β-lactamases) or “β-lactamase-stable” (because the β-lactam ring remains intact in the presence of β-lactamases). Antibiotics which are inactivated by β-lactamases, on the other hand, are termed “β-lactamase-sensitive.” Sometimes the terms “penicillinase-resistant,” “penicillinase-stable,” and “penicillinase-sensitive” are also used to describe these antibiotics.
A recent report from the Centers for Disease Control and Prevention (CDC) found that the incidence of healthcare-associated MRSA infections is declining. During the years from 2005 to 2008, incidences of hospital-acquired invasive MRSA infections declined 28%. Similarly, the incidence of healthcare-associated MRSA among persons who were not hospitalized (such as patients receiving dialysis) declined 17%. These statistics are encouraging because they imply that although MRSA is still a significant nosocomial pathogen, efforts to reduce infection rates in healthcare facilities have been effective.

In contrast to the trend of declining rates of infection in the healthcare setting, the rates of community-associated MRSA infection have increased during the past decade. Although community-associated MRSA typically manifests as a skin infection and is usually successfully treated, it is nevertheless an important pathogen with the potential to cause serious invasive disease.

Finally, Waters and colleagues report that contamination of food could be a potential source of antibiotic-resistant *S. aureus*. These investigators found that *S. aureus* contaminated 47% of retail meat and poultry products. Further, 52% of the *S. aureus* isolates were multidrug-resistant, including one isolate with intermediate resistance to vancomycin and one isolate resistant to daptomycin. Further investigations are needed both to identify sources of contamination and to determine whether this contamination presents a substantial threat to public health.

**Antibiotic Resistance**

Today, about 90% of *S. aureus* strains are resistant to penicillin, but many penicillin-resistant strains are still susceptible to β-lactamase-stable penicillins such as oxacillin and methicillin. However, methicillin-resistant strains are resistant to all β-lactam agents, including cephalosporins and carbapenems. Moreover, healthcare-associated MRSA strains and community-associated MRSA strains differ in their susceptibility patterns. Many healthcare-associated MRSA strains are also resistant to other antibiotics such as clindamycin, erythromycin, and tetracycline. Since 1996, MRSA strains that are partially or completely resistant to vancomycin have been detected, a development that concerns public health officials because vancomycin is often used to treat severe MRSA infections. Unlike healthcare-associated MRSA strains, most community-associated MRSA strains are resistant only to β-lactam drugs and erythromycin.

The most common mechanism of resistance to the β-lactamase-stable penicillins is the *mecA* gene. This gene encodes for the presence of penicillin-binding protein 2a or 2’ (PBP 2a or PBP 2’) on the cell surface, and it is this protein that confers the classic resistance to β-lactamase-stable penicillins in MRSA. Although all cells in a population of *S. aureus* may carry the *mecA* gene, often only a few cells will express
the gene. As a result, an isolate of *S aureus* can contain both resistant and nonresistant bacteria, a phenomenon known as heteroresistance.

Other mechanisms of resistance to penicillins, not related to the presence of the *mecA* gene, are the production of excessive amounts of β-lactamase or the presence of altered penicillin-binding proteins other than PBP 2a and PBP 2'. When susceptibility tests are performed on strains with these mechanisms of resistance, the minimum inhibitory concentrations or zones of inhibition are often very close to the breakpoint for susceptibility, which makes them difficult to differentiate from classic MRSA.

**Susceptibility Testing**

The phenomenon of heteroresistance, the existence of multiple mechanisms of resistance to penicillins, and the emergence of multiple-drug–resistant strains of *S aureus* have impacted susceptibility testing standards for this pathogen. For example, resistant bacteria in a heteroresistant isolate often grow more slowly than the susceptible bacteria, thus necessitating a longer (24-hour) incubation time to allow the resistant bacteria to grow. Screening tests can detect the production of β-lactamase or the presence of the *mecA* gene, and so identify the mechanism of resistance. The Clinical Laboratory Standards Institute (CLSI) annually updates standards that detail which antibiotics to test, what incubation conditions to use, how to detect resistance phenotypes or mechanisms, and how to interpret and report the results.

The 2011 CLSI susceptibility testing standards suggest that *Staphylococcus* isolates should be routinely tested with the following antibiotics:

- Azithromycin or clarithromycin or erythromycin
- Clindamycin
- Oxacillin or cefoxitin
- Penicillin
- Trimethoprim-sulfamethoxazole

Susceptibility or resistance to a wide range of antibiotics can be inferred from the results for penicillin and oxacillin. Isolates that are susceptible to penicillin are also susceptible to other penicillins, β-lactam/β-lactamase inhibitor combinations, anti-staphylococcal cephems, and carbapenems. Similarly, isolates that are susceptible to oxacillin are also susceptible to other penicillinase-stable penicillins, β-lactam/β-lactamase inhibitor combinations, anti-staphylococcal cephems, and carbapenems. Isolates that are resistant to penicillin or oxacillin are likewise resistant to other antibiotics in these classes.

The CLSI standards also list other antibiotics that may be tested against *S aureus* isolates. Along with the first-line drugs, isolates should also be tested with vancomycin, daptomycin, linezolid, telithromycin, rifampin, and either doxycycline, minocycline, or tetracycline. However, the results for these antibiotics
should be reported selectively, that is, only if the isolate is resistant to the first-line drugs or the clinical situation warrants.

To ensure reliable results, the testing conditions specified in the CLSI standards for medium, inoculum, and incubation must be followed. The approved media are Mueller-Hinton agar (MHA) and cation-adjusted Mueller-Hinton broth for disk diffusion and minimum inhibitory concentration testing, respectively. For both disk diffusion and minimum inhibitory concentration testing, the inoculums should be a direct colony suspension prepared to an equivalent 0.5 McFarland standard. To detect MRSA, plates or tubes should be incubated in ambient air at no more than 35°C. All methods require a 24-hour incubation time for oxacillin and vancomycin; a 24-hour incubation period is also required for cefoxitin if disk diffusion is used.

In addition to conventional susceptibility testing, resistance or susceptibility to certain drugs can be inferred or confirmed from the results of several screening tests. Nitrocefin-based tests detect β-lactamase production, which is a mechanism of penicillin resistance. Oxacillin resistance can be detected by agar dilution using Mueller-Hinton agar with 4% NaCl infused with 6 μg/mL oxacillin. Alternatively, cefoxitin can be used as a surrogate for oxacillin in both disk diffusion and broth microdilution tests to detect meca-mediated oxacillin resistance. Resistance to vancomycin can be detected by agar dilution using brain heart infusion agar infused with 6 μg/mL vancomycin. Inducible clindamycin resistance in isolates that are resistant to erythromycin and susceptible or intermediate to clindamycin can be detected by placing erythromycin and clindamycin in the same well (for broth microdilution testing) or disks adjacent to one another (disk diffusion testing). High-level mupirocin resistance can also be detected by disk diffusion or broth microdilution using a 200-μg mupirocin disk or 256 μg/mL mupirocin well. The CLSI document *Performance Standards for Antimicrobial Susceptibility Testing* specifies conditions for these tests, including proper media, incubation conditions, interpretive standards, and whether confirmatory tests are needed.

**Conclusion**

The antibiotic susceptibility patterns and epidemiology of *S aureus* continue to evolve. So too do the susceptibility testing standards as new antibiotics and new testing methodologies are developed. Each year, the CLSI standards are revised to reflect the current expert consensus on best testing practices. Laboratories should keep abreast of new developments concerning MRSA and always use the most recent CLSI standards to design susceptibility testing protocols.
REFERENCES AND SUGGESTED READING


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