EDUCATIONAL COMMENTARY – ANTIBIOTIC RESISTANT STAPHYLOCOCCUS AUREUS

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Learning Outcomes

Upon completion of this exercise, the participant will be able to:

- Discuss recent epidemiological trends of antibiotic resistant Staphylococcus aureus.
- Describe 2 mechanisms of resistance to penicillins in S. aureus.
- Discuss changes in susceptibility testing protocols for S. aureus in the 2006 CLSI standards.

One of the most significant developments in clinical microbiology since the middle of the last century has been the emergence of antibiotic resistance in common pathogens such as Staphylococcus aureus. Penicillin-resistant strains of S. aureus appeared as early as the 1940s, but for many years these remained susceptible to β-lactamase-stable penicillins. Then, in the mid-1980s, S. aureus strains emerged that were resistant to the β-lactamase-stable penicillins. These strains were termed “methicillin-resistant S. aureus” (MRSA), because methicillin was initially used to detect their resistance to β-lactamase-stable penicillins. Although MRSA strains are often resistant to multiple drug classes, they have been reliably sensitive to vancomycin. Recently, however, reduced susceptibility to vancomycin has been detected in a few cases, a development which has raised fears that an untreatable strain of S. aureus could emerge.

When MRSA strains first appeared, they occurred predominantly in the healthcare setting. They continue to be a major cause of nosocomial infections. Now, however, they increasingly appear outside the healthcare setting. Data collected in the National Health and Nutrition Examination Survey (NHANES) from 2001-2002 indicate that about 2.3 million people in the United States carry MRSA.

Overall, colonization with MRSA is higher in people older than 60 years and in women; however, community-associated MRSA is more prevalent in young children and non-Hispanic black people. Factors associated with higher risk for community-associated MRSA infection include intravenous drug use, recent hospitalization, serious underlying disease, and treatment with antibiotics. However, cases have also been reported in people with none of these risk factors.

The emergence of community-associated MRSA strains and the recent appearance of strains with reduced susceptibility to vancomycin are important factors to consider when developing susceptibility testing protocols for S. aureus. At a minimum, laboratories in geographic regions known to harbor community-associated MRSA should routinely either perform susceptibility testing on S. aureus isolates or send the isolates to a reference laboratory.
Mechanisms of Resistance to Penicillins

In *S. aureus*, resistance to penicillins occurs through 2 mechanisms: the production of the ß-lactamase enzyme and the presence of the *mecA* gene.

Eighty-five to ninety percent of *S. aureus* strains today produce ß-lactamase and are thus resistant to penicillin. Some of these strains produce excessive amounts of ß-lactamase, which makes them appear borderline resistant to oxacillin. These strains are termed borderline oxacillin-resistant *S. aureus* (BORSA), and they can be difficult to differentiate from classic MRSA.

The second mechanism of resistance—the *mecA* gene—encodes for the presence of a penicillin-binding protein (PBP 2a or PBP 2') on the surface of the bacterial cell wall. It is this protein that confers the classic resistance to ß-lactamase-stable penicillins in MRSA. Although all cells in a population of *S. aureus* may carry the *mecA* gene, often only a few of the cells will express the gene. Thus, both resistant and nonresistant bacteria can exist in the same culture, a phenomenon known as heteroresistance.

The presence of both resistant and nonresistant bacteria, along with the fact that the resistant bacteria often grow more slowly, can make it difficult to detect methicillin resistance. Accordingly, the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards [NCCLS]) has developed susceptibility testing protocols for *S. aureus* that encourage the growth of the resistant subpopulation by incorporating 4 conditions:

- Neutral pH,
- Incubation temperature of 33°C-35°C,
- Mueller-Hinton agar or broth infused with 2%-4% NaCl, and
- 24-hour incubation time.

Changes in 2006 CLSI Standards

The 2006 editions of the CLSI standards for susceptibility testing contain 2 significant changes for detecting susceptibility to oxacillin and vancomycin in *S. aureus*.

First, for disk diffusion testing, a 30 µg cefoxitin disk is now preferred over the 1 µg oxacillin disk to detect *mecA*-mediated resistance. This is because the cefoxitin disk test is easier to read, and it is as sensitive and as specific as MIC methods. However, the results should still be reported as "oxacillin resistant" or "oxacillin susceptible."

Second, the MIC breakpoints for vancomycin have changed. The new breakpoints are:

- \( \leq 2 \mu g/mL \)  
  Susceptible
- \( 4 \mu g/mL-8\mu g/mL \)  
  Intermediate
- \( \geq 16 \mu g/mL \)  
  Resistant
Commercial antimicrobial susceptibility testing instruments, however, are required to use breakpoints established by the Food and Drug Administration. Unlike the new CLSI breakpoints, the FDA breakpoints still interpret a minimum inhibitory concentration of \( \leq 4 \mu g \) as susceptible, but data have shown that vancomycin treatment is ineffective if the MIC is \( > 4 \mu g \). To detect these intermediate strains, the Centers for Disease Control and Prevention advises laboratories to include a vancomycin agar screen plate (BHIA with 6 \( \mu g/mL \) of vancomycin) as part of the primary testing methods in both MIC and disk diffusion protocols. The CDC’s Multi-level Antimicrobial Susceptibility Testing Resources (MASTER) web pages contain links to an algorithm for testing \( S. \) aureus with vancomycin, a procedure for the BHI agar with vancomycin screening test, and other useful resources. These are available at [www.phppo.cdc.gov/dls/master/default.aspx](http://www.phppo.cdc.gov/dls/master/default.aspx).

**Direct Detection Methods**

Finally, in addition to conventional susceptibility testing, several methods can directly detect the \( mecA \) gene or the protein expressed by the \( mecA \) gene. These methods, which include DNA amplification and hybridization tests, fluorescence tests, and slide latex agglutination tests, are the most accurate way to predict oxacillin resistance. They have high sensitivity and specificity, and they can detect classic \( mecA \)-mediated resistance much faster than conventional susceptibility testing.

Although direct detection methods can detect classic resistance, they cannot detect resistance by other mechanisms. Consequently, these methods cannot replace conventional susceptibility testing. The CLSI standards include instructions for reporting the results of direct detection methods used in conjunction with conventional MIC and disk diffusion testing.

**Suggested Reading**


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