JPPT

REVIEW ARTICLE

Clinical and Laboratory Issues in Community-acquired MRSA

Toni Beavers-May, BSMT(ASCP)[1,2] and Richard F. Jacobs, MD[2,3]

[1]Microbiology Section, Clinical Laboratory, [2]Arkansas Children's Hospital and the [3]University of Arkansas for Medical Sciences, Little Rock, Arkansas

The prevalence of Methicillin-resistant *Staphylococcus aureus* (MRSA) has steadily increased over the past 40 years. Today, intravenous clindamycin or vancomycin is recommended for anti-staphylococcal coverage. Failure to identify resistant strains could result in the overuse of vancomycin and subsequent resistance to that antibiotic. Concerns over the emergence of this pathogen has caused many hospital laboratories to reassess their ability to identify antibiogram patterns and epidemiological shifts, determine appropriate laboratory testing, and review empiric therapy guidelines. Reliance on automated instrumentation to detect these isolates can result in major errors that cause false susceptible interpretations. The emergence of methicillin/oxacillin resistant strains has required additional laboratory analysis. This paper will review these tests and will focus on the role of the "D-test" in directing antibiotic therapy.

KEYWORDS: antibiotic resistance, children, clindamycin, Community-acquired MRSA, D-test, MRSA

J Pediatr Pharmacol Ther 2004;9:82-88

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first recognized as a significant nosocomial pathogen in the 1960s, and the prevalence has steadily increased since that time.[1-3] According to the National Nosocomial Infections Surveillance (NNIS) system, *S. aureus* resistant to methicillin, oxacillin, or nafcillin increased to > 55% in 2000 for hospital-acquired infections in adult and pediatric intensive care units. This is a 29% increase in resistance in comparison to the data collected during 1995–1999.[4,5] In the 1980s and early 1990s, community-acquired MRSA (CA-MRSA) was seen in adult and pediatric patients with clearly defined risk factors such as drug abuse or underlying medical conditions.[6,7] In the 1990s, MRSA strains began to be isolated from patients without traditional risk factors. Four such pediatric cases resulted in death.[8] Predisposing factors such as prior antibiotic usage, colonization of a family member, and indirect contact with the health care system were documented.[9-13]

Due to recent dramatic increases in CA-MRSA, several institutions are reevaluating their incidence to identify epidemiological shifts, determine appropriate laboratory testing, and review empiric therapy guidelines. One pediatric facility has shown no difference between risk factors in patients with nosocomially-acquired strains and those with CA-MRSA.[9] Other investigators have reported the incidence of CA-MRSA as higher than nosocomial isolates. These reports have included significant numbers of cases with no risk factors[10] and have identified colonization in healthy children.[11] In contrast, one adult health care facility has reported no significant increase in CA-MRSA during the same time period.[14] We have observed a recent dramatic increase in CA-MRSA at our institution. The emergence of this

ABBREVIATIONS: BORSA, Borderline oxacillin-resistant *S. aureus*; CA-MRSA, community-acquired Methicillin-resistant *Staphylococcus aureus*; MRSA, Methicillin-resistant *Staphylococcus aureus*; NNIS, National Nosocomial Infections Surveillance; NCCLS, National Committee for Clinical Laboratory Standards (NCCLS); PBP2a, penicillin-binding protein 2a; PCR, polymerase chain reaction; SCC, staphylococcal cassette chromosome (SCC)

Address reprint requests to: Toni Beavers-May, Clinical Laboratory, Arkansas Children's Hospital, 800 Marshall Street, Little Rock, AR 72202, e-mail: maytk@archildrens.org

community-acquired pathogen susceptible to non-beta-lactam antibiotics resulted in a reassessment of our laboratory testing and reporting.

NOSOCOMIAL MRSA

The molecular mechanism responsible for methicillin resistance is the presence of the staphylococcal cassette chromosome (SCC) *mec* mobile element in the genome of a susceptible strain. This element contains the *mec*A gene, which encodes the penicillin-binding protein 2a (PBP2a). Nosocomial isolates have been extensively studied and have been found to predominantly carry the SCC*mec* types I-III.[15,16] These types, especially II and III, are associated with the "capturing" of other resistance mechanisms due to their large size. This would explain the resistance to other antibiotics such as tetracycline, clindamycin, and erythromycin in these isolates.[17]

Laboratory testing of *S. aureus* isolates to determine methicillin/oxacillin resisitance can be performed by several methods. The "gold standard" method of determination used by some laboratories is the detection of the *mec*A gene by polymerase chain reaction (PCR).[18,19] However, this method is costly, time consuming, and not feasible by most hospital-based clinical laboratories. Automated instrumentation such as the Vitek® (bioMérieux Vitek®, Hazelnut, MO) and Microscan® (Dade Behring, West Sacramento, CA) systems offer a more streamlined approach in addition to providing multiple drug testing (Figure 1). This methodology determines the minimum inhibitory concentration (MIC) in μg/mL. The National Committee for Clinical Laboratory Standards (NCCLS) established interpretative break points for resistance at ≥ 4 μg/mL and as susceptible at ≤ 2 μg/mL.[20]

The Kirby Bauer disk diffusion method utilizes an oxacillin disk on a Mueller Hinton agar plate in which the diameter of growth around the disk determines resistance or susceptibility (Figure 2). This method is inexpensive and a good choice for the smaller clinical laboratory. The Etest® (AB BIODISK, Piscatway, NJ) system is another MIC method which incorporates use of a Mueller Hinton plate with an oxacillin Etest® strip with increasing concentrations of the antibiotic (Figure 3). The oxacillin screen agar plate incorporates oxacillin into the agar: presence of growth indicates resistance, and lack of growth represents susceptibility (Figure 4). These methods of antimicrobial susceptibility testing (AMS) can take up to 24 hours.

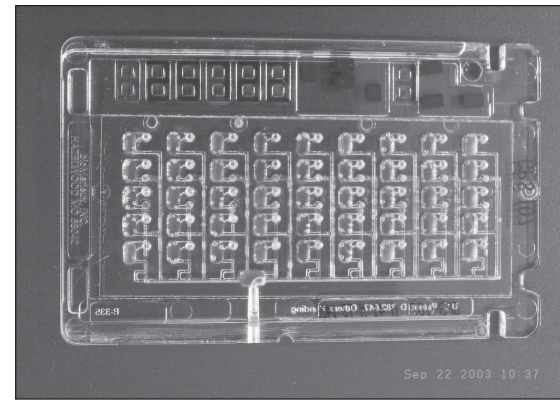

Figure 1. Vitek® GPS-107 card (bioMérieux Vitek®, Hazelnut, MO). Methicillin-resistant *S. aureus* isolate tested in a gram positive susceptibility card. Absence of growth in microwells depict minimum inhibitory concentration. Several antibiotics are tested utilizing one card.

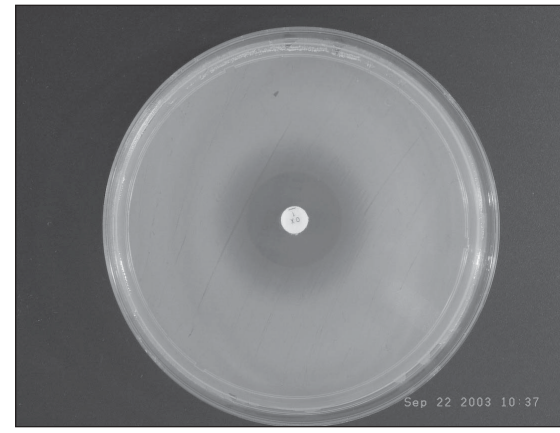

Figure 2. Kirby Bauer Disk Diffusion test. Oxacillin disk on Mueller Hinton agar with a lawn of susceptible *S. aureus*.

During the late 1990s, rapid methods based on detecting the presence of PBP2a in clinical isolates were made available.[18,19,21] These slide latex agglutination tests produce results in approximately 20 minutes. However, this method is also expensive and can disrupt the workflow of the laboratory. Some studies have shown false negatives. One study showed that retesting *mec*A positive, slide agglutination negative isolates with a larger inoculum resulted in positive results.[21] Another possibility for false negatives could be suppression of *mec*A gene transcription. This can be remedied by overnight incubation with methicillin, but with an increase in turn around time.[18] Regardless of the methodology utilized, *mec*A-positive, PBP2a-positive, or oxacillin-resistant strains infer resistance to all penicillins, cephalosporins,

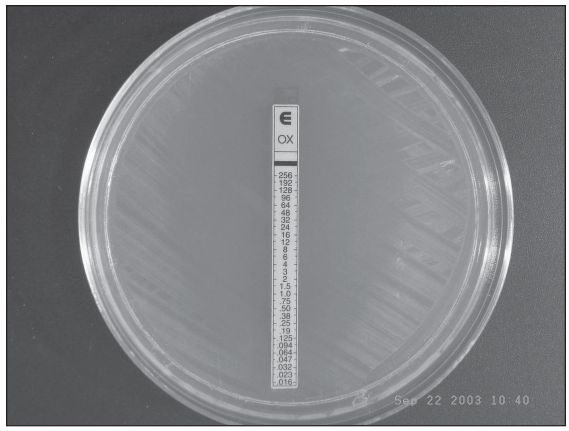

Figure 3. Etest® (AB BIODISK, Piscatway, NJ). Oxacillin gradient strip on Mueller Hinton agar with a lawn of susceptible *S. aureus*.

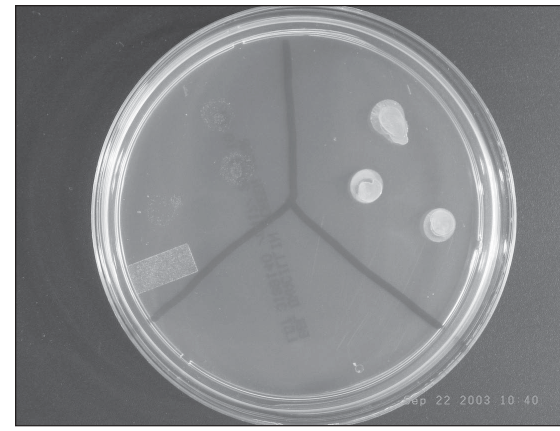

Figure 4. Oxacillin screen agar plate. Mueller Hinton agar with oxacillin incorporated in media. Upper right quadrant depicts growth of methicillin-resistant *S. aureus*, upper left shows a haze of growth by a oxacillin intermediate strain, and the lower quadrant contains a susceptible strain showing no growth.

and other beta-lactams such as the carbapenems. These testing methods and interpretations have been approved and addressed by the NCCLS.[21]

Borderline oxacillin-resistant *S. aureus* (BORSA) are *mec*A-negative strains, which have a MIC of 2 to 8 μg/mL. Growth will occur on the oxacillin screen agar plate, and any MIC method will falsely show resistance if the MIC is > 6 μg/mL. Failure to identify these strains could result in the overuse of vancomycin. Beta-lactam combination drugs may be effective in these strains.[11,22,23]

Predictable antibiogram patterns show that nosocomial MRSA is resistant to multiple drugs. Thus, prohibitive costs associated with additional AMS testing is ruled out for some institutions. Limiting additional testing on nosocomial isolates can be a significant cost. These same concerns do not apply to the new era of community-acquired MRSA isolates in the microbiology laboratory.

COMMUNITY-ACQUIRED MRSA

CA-MRSA strains have similar genetic characteristics, but the majority have been found to carry the SCC*mec* type IV. This cassette is smaller than the previously mentioned nosocomial isolates, and additional resistance mechanisms are lacking due to the smaller size.[15] These strains can remain susceptible to certain antibiotics other than the beta-lactams. However, superantigens and other toxins present in some strains increase virulence.[16] Laboratory testing for these organisms, as per NCCLS current guidelines, are the same as for the nosocomial strains. However, with the emergence of CA-MRSA, reports of *mec*A- positive isolates testing oxacillin negative with automated instrumentation have been reported. These strains were classified as oxacillin resistant by Kirby Bauer and the oxacillin agar screen methods.[24]

With the emergence of CA-MRSA, additional laboratory analysis has become necessary. Treatment options for pediatric patients require clindamycin and trimethoprim-sulfamethoxazole testing and reporting. Treatment failures have been documented utilizing clindamycin even though the organism was tested as susceptible by the MIC method.[25] This is due to the presence and expression of the *erm* gene in some clinical isolates of *S. aureus*. When expressed, enzymes are encoded which lead to ribosomal methylation, causing a conformational change that results in an alteration in binding sites. Since macrolide, lincosamide, and streptogramin B target sites overlap, resistance by this mechanism can be inferred to all antibiotics in these groups.[26,27]

Erythromycin acts *in vitro* as an inducer of clindamycin resistance.[26-28] When an erythromycin disk and a clindamycin disk, using the Kirby Bauer disk diffusion method, are placed 15–22 mm apart, a growth pattern in a "D" shape can be detected around the clindamycin after overnight incubation (Figure 5). This is referred to as a "D test". Studies involving treatment failures with clindamycin have positive "D tests" in retrospect.[25] Our institution performs a "D test" on every MRSA isolate in addition to MIC testing. If the "D test" is positive, the following comment is attached to the AMS report: "The method of erythromycin

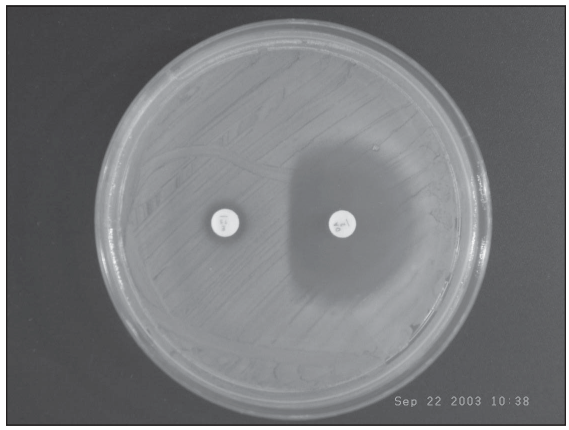

Figure 5. D-test. Mueller Hinton agar with an erythromycin disk on left with a clindamycin disk placed 18 mm apart. This positive D-test represents the presence of the *erm* gene in this MRSA isolate.

resistance indicates possible inducible resistance to clindamycin during therapy." Erythromycin-resistant, clindamycin-susceptible strains that do not show inducible resistance are reported as clindamycin susceptible as supported by previous literature.[27,28] Current NCCLS guidelines do not address inducible clindamycin resistance. However, the 2004 guidelines will address inducible *erm* resistance criteria (Dr. Paul Schreckenberger, personal communication).[30] Trimethoprim-sulfamethoxazole and tetracycline are tested and reported by the MIC method.

CLINICAL ASPECTS

The beta-lactam antibiotics have historically been the gold standard for treatment of a community-acquired infection due to *S. aureus*. These antibiotics are bactericidal and are recommended for their quick and efficient killing.[35] Since comprehensive antibiotic susceptibility testing isn't typically available until 48 hours after culture collection, empiric therapy choices can be critical.

Several studies have addressed the changing epidemiology of MRSA, especially in the pediatric setting. Increasing cases of CA-MRSA without traditional risk factors have been documented. The broadened risk factors for CA-MRSA include IV drug abuse, prior surgery or hospitalization, exposure to self-administered antibiotics, prolonged or current antibiotic usage, household contact of a colonized individual, day care attendance, and underlying medical conditions such as cystic fibrosis.[6-13,31-33] Recently, cases have

Table 1. *S. aureus* in outpatients at Arkansas Children's Hospital. Increased prevalence and decreased susceptibility of outpatient *S. aureus*.

Year	No. of isolates	% susceptible to oxacillin
2000	190	81.6
2001	197	70.6
2002	338	64.8
2003 (6 months)	221	54.8

emerged among competitive sports participants.[34] Most commonly to date, these patients present with skin and soft tissue infections in contrast to the nosocomial strains which are frequently isolated from blood, indwelling devices, and the respiratory tract.[6,9,12]

Since 2000, we have documented a dramatic increase in outpatient CA-MRSA infections which were susceptible to non-beta-lactam antibiotics. Data collected in the first six months of 2003 have been compared to the previous three years (Table 1). The number of *S. aureus* isolates increased with a decreased susceptibility to oxacillin. Clindamycin was added to the testing and reporting protocol during mid-2002. Thirty-one outpatient MRSA isolates were tested, and 96.8% were susceptible. During the first six months of 2003, this decreased to 80.2% among 101 MRSA outpatient strains, suggesting another emerging problem.

TREATMENT

Prior to 2000, empiric treatment of an outpatient who presented with a staphylococcal infection typically was an oral beta-lactam antibiotic. Due to the emerging epidemiology and growing resistance of staphylococcal infections, institutions are reevaluating treatment options. For serious infections and the critically ill, intravenous clindamycin or vancomycin is now recommended for anti-staphylococcal coverage.[12,25,36] Although expensive, another option in these patients is linezolid.[37] For mild to moderate infections, utilization of clindamycin as empiric therapy in the pediatric outpatient setting has been recommended by some experts.[10,12,36,38] Some institutions are using a beta-lactam antibiotic initially, and if no improvement is seen and laboratory data are unavailable, a switch to clindamycin or trimethoprim-sulfamethoxazole for skin or soft tissue infections is considered.[31] A patient that presented in our outpatient setting with a *S. aureus* infection in 2000 had a $< 20\%$ probability of be-

ing resistant to the beta-lactam antibiotics. During the first half of 2003, the resistance has more than doubled.

CONCLUSION

Community-acquired MRSA is an emerging pathogen and increasing in frequency at an astounding rate. Even with the broadened definition of risk factors, the dramatic increase in incidence cannot be explained. This phenomenon has been most apparent in the pediatric setting. Frequent review of the rate of isolation and the antibiogram against *S. aureus* is imperative at each institution. Effectively detecting MRSA in the clinical microbiology laboratory has become problematic due to the evolving nature of this organism. Reliance on automated instrumentation with these isolates can result in major errors resulting in false susceptible interpretations. Each laboratory must recognize the limitations of each testing methodology and assess the specific needs while providing the most accurate test available. Once MRSA is detected, laboratory reports must include accurate interpretations stating resistance to all beta-lactams regardless of MIC results.

Additional susceptibility testing should be performed due to the absence of other resistance mechanisms in CA-MRSA isolates. However, laboratories testing for clindamycin should perform the "D test" in addition to MIC testing in order to detect the presence of the *erm* gene. The omission of this test could result in treatment failures. Other antibiotic choices for mild to moderate infections such as trimethoprim-sulfamethoxazole are being used with success.[32] Each institution must determine the empiric therapy options based on a frequent assessment of their antibiogram and case presentations.

DISCLOSURE: The authors declare no conflicts or financial interest in any product or service mentioned in the manuscript, including grants, equipment, medications, employment, gifts, and honoraria.

ADDENDUM: The new NCCLS guidelines for laboratory testing of community-acquired MRSA with inducible clindamycin resistance have been published since the original submission of this review and support the approach outlined above.[39]

ACKNOWLEDGEMENTS: This study was supported, in part, by a grant from the Horace C. Cabe Foundation. Special thanks to Dr. Dale Ellison, Director of Surgical Pathology, University of Arkansas for Medical Sciences, Arkansas Children's Hospital for photographs and to Holly Maples, Pharm. D., Assistant Professor, Dept. of Pharmacy Practice, Assistant Professor, Dept. of Pediatrics, University of Arkansas for Medical Sciences for review of our manuscript.

REFERENCES

1. Thompson RL, Cabezudo I, Wenzel RP. Epidemiology of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus*. Ann Intern Med 1982;97:309-17.
2. Boyce JM, Causey WA. Increasing occurrence of methicillin-resistant *S. aureus* in the United States. Infection Control 1982;3:377-83.
3. Saravolatz LD, Markowitz N, Arking L, Phohlod D, Fisher E. Methicillin-resistant *Staphylococcus aureus*. Epidemiologic observations during a community-acquired outbreak. Ann Intern Med 1982;96:11-6.
4. Centers for Disease Control NNIS System. National nosocomial infections surveillance (NNIS) system report, data summary from January 1992 to April 2001, issued August 2001. Am J Infect Control 2001;29:400-21.
5. Centers for Disease Control NNIS System. National nosocomial infections surveillance (NNIS) system report, data summary from January 1992 to June 2002, issued August 2002. Am J Infect Control 2002;30:458-75.
6. Frank AL, Marcinak JF, Mangat PD, Schreckenberger PC. Community-acquired and clindamycin-susceptible methicillin-resistant *Staphylococcus aureus* in children. Pediatr Infect Dis J 1999;18:993-1000.
7. Layton MC, Hierholzer WJ, Patterson JE. The evolving epidemiology of methicillin-resistant *Staphylococcus aureus* at a university hospital. Infect Control Hosp Epidemiol 1995;16:12-7.
8. Centers for Disease Control. Four pediatric deaths from community-acquired methicillin-resistant *S. aureus*: Minnesota and North Dakota, 1997-1999. MMWR 1999;48:707-10.
9. Campbell AL, Bryant, KA, Stover, B, Marshall, GS. Epidemiology of methicillin-resistant *Staphylococcus aureus* at a children's hospital. Infect Control Hosp Epidemiol 2003;24:427-30.
10. Hussain FM, Boyle-Vavra S, Bethel CD, Daum RS. Current trends in community-acquired methicillin-resistant *Staphylococcus aureus* at a tertiary care pediatric facility. Pediatr Infect Dis J 2000;19:1163-6.

11. Hussain FM, Boyle-Vavra S, Daum, RS. Community-acquired methicillin-resistant *Staphylococcus aureus* colonization in healthy children attending an outpatient pediatric clinic. Pediatr Infect Dis J 2001;20:763-7.
12. Sattler CA, Mason EO, Kaplan SL. Prospective comparison of risk factors and demographic and clinical characteristics of community-acquired, methicillin-resistant versus methicillin-susceptible *Staphylococcus aureus* infection in children. Pediatr Infect Dis J 2002;21:910-6.
13. Johnson, LB, Bhan, A, Pawlak J, Manzor O, Saravolatz LD. Changing epidemiology of community-onset methiciliin-resistant *Staphylococcus aureus* bacteremia. Infect Control Hosp Epidemiol 2003;24:431-5.
14. Suntharam N, Hacek D, Peterson L. Low prevalence of community-acquired methicillin-resistant *Staphylococcus aureus* in adults at a university hospital in the central United States. J Clin Microbiol 2001;39:1669-71.
15. Säid-Salim B, Mathema B, Kreiswirth BN. Community-acquired methicillin-resistant *Staphylococcus aureus*: An emerging pathogen. Infect Control Hosp Epidemiol 2003;24:451-5.
16. Fey PD, Säid-Salim B, Rupp ME, Hinrichs SH, Boxrud DJ, Davis CC, et al. Comparative molecular analysis of community-or hospital-acquired methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2003;47:196-203.
17. Lowry FD. Antimicrobial resistance: the example of *Staphylococcus aureus*. J Clin Invest 2003;111:1265-73.
18. Van Leeuwen WB, Van Pelt C, Luijendijk A, Verbrugh HA, Goessens WH. Rapid Detection of methicillin resistance in *Staphylococcus aureus* isolates by the MRSA-screen latex agglutination test. J Clin Microbiol 1999;37:3029-30.
19. Van Griethuysen A, Pouw M, Van Leeuwen N, Heck M, Willemse P, Buiting A, Kluytmans J. Rapid slide latex agglutination test for detection of methicillin resistance in *Staphylococcus aureus*. J Clin Microbiol 1999;37:2789-92.
20. National Committee on Clinial Laboratory Standards. MIC testing: supplemental tables, M100-S13. Villanova, PA: NCCLS 2003.
21. Louie L, Matsumura SO, Choi E, Louie M, Simor AE. Evaluation of three rapid methods for detection of methicillin resistance in *Staphylococcus aureus*. J Clin Microbiol 2000;38:2170-3.
22. Backo M, Gaenger E, Burkart A, Chai YL, Bayer AS. Treatment of experimental Staphylococcal endocarditis due to a strain with reduced susceptibility in vitro to vancomycin: efficacy of ampicillin-sulbactam. Antimicrob Agent Chemother 1999:43:2565-8.
23. Lee NL, Yuen KY, Kumana C. Beta-lactam antibiotic and beta-lactamase inhibitor combinations. JAMA 2001;285:386-8.
24. Issued by Biomerieux Vitek®: Emerging Resistance Alert; unusual methicillin resistant *Staphylococcus aureus*. Hazelwood (MO): October 25 2002.
25. Frank AL, Marcinak JF, Mangat PD, Tjhio JT, Kelkar S, Schreckenberger PC, Quinn JP. Clindamycin treatment of methicillin-resistant *Staphylococcus aureus* infections in children. Pediatr Infect Dis J 2002;21:530-4.
26. Weisblum B, Demohn V. Erythromycin-inducible resistance in *Staphylococcus aureus*: Survey of antibiotic classes involved. J Bacteriol 1969;98:447452.
27. Westh H, Hougaard DM, Vuust J, Rosdahl VT. Prevalence of *erm* gene classes in erythromycin-resistant *Staphylococcus aureus* strains isolated between 1959 and 1988. Antimicrob Agents Chemother 1995;39:369-73.
28. Weisblum B. Insights into erythromycin action from studies of its activity as inducer of resistance. Minireview. Antimicrob Agents Chemother 1995;39:797-805.
29. Weisblum B. Erythromycin resistance by ribosome modification. Antimicrob Agents Chemother 1995;39:577-85.
30. Hindler JF. Everything you wanted to know about antimicrobial testing of *Staphylococcus aureus*. [teleconference]. Sept 2003.
31. Fergie JE, Purcell K. Community-acquired methicillin-resistant *Staphylococcus aureus* infection in south Texas children Pediatri Infect Dis J 2001;20:860-3.
32. Faden H, Ferguson S. Community-acquired methicillin-resistant *Staphylococcus aureus* and intra family spread of pustular disease. [letter] Pediatr Infect Dis J 2001;20:554-5.

33. Miller MB, Gilligan PH. Minireview: Laboratory aspects of management of chronic pulmonary infections in patients with cystic fibrosis. J of Clin Microbiol 2003;41:4009-15.
34. Centers for Disease Control. Methicillin-resistant *Staphylococcus aureus* infections among competitive sports participants: Colorado, Indiana, Pennsylvania, and LosAngeles County, 2000-2003.MMWR 2003;52:793-5.
35. Chesney PJ. *Staphylococcus aureus.* In: Long SS, Pickering LK, Prober CG, eds. Principles and Practice of Pediatric Infectious Diseases, 2nd ed. New York, NY: ChurchillLivingstone; 2003:694-707.
36. Martinez-Aguilar G, Hammerman WA, Mason EO, Kaplan SL. Clindamycin treatment of invasive infections caused by community-acquired, methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* in children. Pediatr Infect Dis J 2003;22:593-9.
37. Kaplan SL, Deville JG, Yogev R, Morfin MR, Wue E, Adler S, et al. and the Linezolid Pediatric Study Group. Linezolid versus vancomycin for the treatment of resistant gram-positive infections in children. Pediatr Infect Dis J 2003;22:677-85.
38. Panagea S, Perry, JD, Gould FK. Should Clindamycin be used as treatment of patients with infections caused by erythromycin-resistant Staphylococci? J Anti Micro Chemo 1999;44:581-2.
39. National Committee on Clinical Laboratory Standards. Performance Standards for Antimicrobial Susceptibility Testing: Fourteenth Informational Supplement, M100-S14. Wayne, Pennsylvania: NCCLS 2004.