Washington University School of Medicine
Digital Commons@Becker

Open Access Publications

2006

Blinded comparison of repetitive-sequence PCR and multilocus sequence typing for genotyping methicillin-resistant Staphylococcus aureus isolates from a children's hospital in St. Louis, Missouri

Robert S. Liao
Washington University School of Medicine in St. Louis

Gregory A. Storch
Washington University School of Medicine in St. Louis

Richard S. Buller
Washington University School of Medicine in St. Louis

Rachel C. Orscheln
Washington University School of Medicine in St. Louis

Elaine R. Mardis
Washington University School of Medicine in St. Louis

See next page for additional authors

Follow this and additional works at: http://digitalcommons.wustl.edu/open_access_pubs

Authors

Robert S. Liao, Gregory A. Storch, Richard S. Buller, Rachel C. Orscheln, Elaine R. Mardis, Jon R. Armstrong, and W. Michael Dunne Jr.

Journal of Clinical Microbiology

Blinded Comparison of Repetitive-Sequence PCR and Multilocus Sequence Typing for Genotyping Methicillin-Resistant *Staphylococcus aureus* Isolates from a Children's Hospital in St. Louis, Missouri

Robert S. Liao, Gregory A. Storch, Richard S. Buller, Rachel C. Orscheln, Elaine R. Mardis, Jon R. Armstrong and W. Michael Dunne Jr.

Updated information and services can be found at:
http://jcm.asm.org/content/44/6/2254

These include:

REFERENCES

This article cites 24 articles, 16 of which can be accessed free at: http://jcm.asm.org/content/44/6/2254#ref-list-1

CONTENT ALERTS

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), more»

Information about commercial reprint orders: http://journals.asm.org/site/misc/reprints.xhtml
To subscribe to to another ASM Journal go to: http://journals.asm.org/site/subscriptions/

Journals.ASM.org

Vol. 44, No. 6

Blinded Comparison of Repetitive-Sequence PCR and Multilocus Sequence Typing for Genotyping Methicillin-Resistant *Staphylococcus aureus* Isolates from a Children's Hospital in St. Louis, Missouri

Robert S. Liao,[1]† Gregory A. Storch,[2] Richard S. Buller,[2] Rachel C. Orscheln,[2]
Elaine R. Mardis,[3] Jon R. Armstrong,[3] and W. Michael Dunne, Jr.[1]*

*Departments of Pathology and Immunology[1] and Pediatrics[2] and the Genome Sequencing Center,[3]
Washington University School of Medicine, St. Louis, Missouri 63110*

We performed a blinded study to compare repetitive-sequence PCR and multilocus sequence typing for genotyping hospital- and community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA). The MRSA strains that were sequence type 8 (ST8), staphylococcal cassette chromosome *mec* (SCC*mec*) type IV, and Panton-Valentine leukocidin-positive clustered separately from those that were ST5 and SCC*mec* type II.

Until recently, *Staphylococcus aureus* infections in the community were caused mostly by methicillin-susceptible *S. aureus* (MSSA) strains, and the dissemination of hospital-acquired methicillin-resistant *S. aureus* (HA-MRSA) into the community was infrequent (21). However, since the mid-1990s, there have been increased reports of community-acquired MRSA (CA-MRSA) infections in otherwise healthy community-dwelling adult and pediatric patients who do not share the known risk factors for MRSA colonization (13, 22). CA-MRSA isolates have been shown to contain a unique genetic element called staphylococcal cassette chromosome *mec* (SCC*mec*) type IV and V (17). These isolates are frequently clindamycin susceptible and have a narrower antibiotic resistance pattern than HA-MRSA (21). In addition, CA-MRSA isolates are isolated commonly from skin and soft-tissue infections (13) and possess genes encoding the Panton-Valentine leukocidin (PVL) toxin (25).

Twenty-eight clinical isolates of *S. aureus* were obtained from individual pediatric patients between January 2002 and January 2004. These isolates were identified as *S. aureus* by the clinical laboratory with standard methods, using Gram's stain, catalase, and Staphaurex latex agglutination (Remel, Lenexa, KS) (1). Susceptibility testing was performed by the clinical laboratory using the CLSI (formerly NCCLS) guidelines for disk diffusion susceptibility testing (15). For the purpose of this study, two definitions of CA and HA were used. One definition was based on the susceptibility phenotype, in which clindamycin-susceptible strains, including those shown to have inducible clindamycin resistance, were considered to be CA. Clindamycin-resistant strains were assumed to be HA. The second def-

inition was based on day of acquisition. For the latter, MRSA strains were considered to be CA if the cultures were positive in an outpatient setting or within 48 h of hospitalization. Strains that were recovered from patients after 48 h of hospitalization were defined as HA. The study set was chosen to contain representative CA and HA strains based on either definition.

Prior to PCR genotyping, individual clinical isolates were grown overnight in trypticase soy broth. The cells were centrifuged, and the DNA was purified from the pellets using the extraction method described by Kalia et al. (9) in combination with a QIAamp genomic DNA extraction kit (QIAGEN, Inc., Chatsworth, CA). Repetitive-sequence PCR (rep-PCR) typing using the RW3A primer sequences was performed according to the procedure of Del Vecchio et al. (4) and with Ready-To-Go randomly amplified polymorphic DNA analysis beads (Amersham Biosciences, Piscataway, NJ). To investigate the prevalence of resistance determinants, PCR amplification for known resistance genes was performed using Ready-To-Go randomly amplified polymorphic DNA analysis beads (Amersham Biosciences). PCR detections of the *ermA* (10), *ermC* (10), *msrA* (12), *aacA-aphD* (24), *mecA* (24), and *lukS-PV-lukF-PV* (8) genes were performed using the primers and thermal cycling profile previously described. Typing of the staphylococcal chromosome *mec* element was done using a multiplex real-time PCR assay described by Francois et al. (7) using an Applied Biosystems 7300 real-time PCR system (Applied Biosystems, Foster City, CA). For multilocus sequence typing (MLST), PCRs using *S. aureus* genomic DNA isolates were performed as described previously (6). PCR fragments of the seven housekeeping genes, *arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*, were obtained from chromosomal DNA and were directly sequenced. The sequencing reactions were cycled in a PTC-225 (MJ Research, Waltham, MA) and loaded onto a 3730xl DNA analyzer (Applied Biosystems, Foster City, CA).

Rep-PCR genotyping was performed blinded to the source of the study isolates or their susceptibility profiles, MLST sequence types (STs), or SCC*mec* types. Each strain was classi-

* Corresponding author. Mailing address: Division of Laboratory Medicine, Department of Pathology and Immunology, Washington University School of Medicine, 660 South Euclid Avenue, Campus Box 8118, St. Louis, MO 63110. Phone: (314) 362-2998. Fax: (314) 362-1461. E-mail: dunne@wustl.edu.
† Present address: Pathology and Molecular Medicine, Kingston General Hospital, Douglas 3, Room 315, 76 Stuart Street, Kingston, Ontario K7L 2V7, Canada.
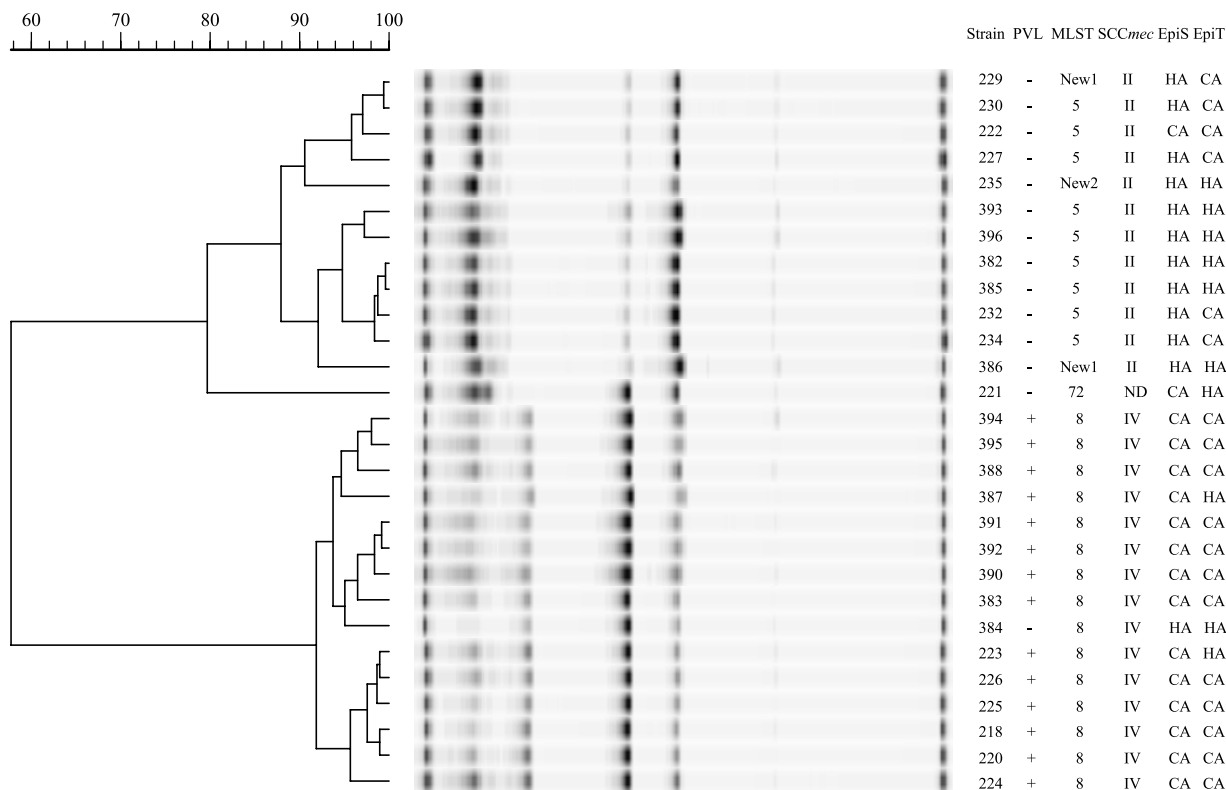
FIG. 1. Cluster analysis of PCR products from all 28 strains of MRSA using repetitive-sequence PCR with RW3A primers. The presence of the PVL gene is indicated by (+) and the absence by (−). MLST results are indicated by allelic profile, and newly identified genotypes are indicated by "New." SSC*mec* typing results that are not determined are indicated with ND. SSC*mec* typing results II and IV are indicated by II and IV. The two columns labeled EpiS and EpiT differentiate strains that were determined to be CA or HA based on susceptibility phenotype and day of acquisition, respectively.

fied using the nomenclature put forward by Enright et al. of naming MRSA clones according to their MLST-resistance phenotype-SCC*mec* types (5). The majority of the 28 strains of MRSA belonged to two epidemic clones that were identified as ST5 (1-4-1-4-12-1-10)-MRSA-II (9 strains) and ST8 (3-3-1-1-4-4-3)-MRSA-IV (15 strains). The two MSSA strains that were included in this study were also ST8. Historical designations previously given to ST8-MRSA-IV and ST5-MRSA-II are New York/Japan and Clone V (or New York clone V), respectively. Three additional SCC*mec* type II strains of MRSA (Sa229, Sa235, and Sa386) had allelic profiles that represented two novel ST entries in the database. These novel profiles, 1-36-1-4-12-1-10 and 1-4-1-4-12-1-80, most closely resembled the allelic profile for ST5 after a comparison utilizing the MLST website (http://saureus.mlst.net). A single MRSA strain, Sa221, was shown to be ST72 (1-4-1-8-4-4-3) using MLST. Although this strain was positive for the *mec* gene, it could not be SCC*mec* typed. RW3A rep-PCR of the 28 MRSA strains produced two distinct clusters of strains that correlated strongly with MLST-SCC*mec* type (Fig. 1) and the presence or absence of the *msrA* and *erm* resistance genes. Quantitative analyses of the RW3A rep-PCR products show that the clusters shared less than 60% similarity.

One of the two clusters contained all 15 of the ST8-MRSA-IV strains. The strains of this ST8-MRSA-IV cluster also included all of the PVL-positive strains with the exception

of a single strain, Sa384. These ST8-MRSA-IV strains have an overall similarity of 92% based on the comparison of amplified RW3A rep-PCR products (Fig. 1). Ninety percent of the ST8-MRSA-IV strains were determined to be from abscesses from the lower extremities.

The second cluster was composed of the single ST72 strain and the 12 SCC*mec* type II strains that included the 9 ST5-SCCmec-II strains plus the 3 strains with closely related but novel ST types (Sa229, Sa235, and Sa386). The strains of this SCC*mec* type II cluster were uniformly PVL negative and shared 88% similarity (Fig. 1). Ten of the 13 strains in the SCC*mec* type II cluster were from respiratory specimens. Strain Sa221 (ST72-SCC*mec* nontypeable) shared only 80% similarity with the other strains in the PVL-negative SCC*mec* type II cluster.

The 28 strains that compose the SCC*mec* type II and ST8-MRSA-IV clusters also correlated strongly with the presence or absence of the *msrA* and *erm* resistance genes. The *msrA* gene was present in 13 of the 15 ST8-MRSA-IV strains, compared to 0 of the 13 SCC*mec* type II strains. In contrast, the *erm* gene was present in all 13 strains of the SCC*mec* type II cluster, although 4 of the ST8-MRSA-IV strains also possessed *erm*.

MLST is a powerful genotyping method that is usually applied to the evaluation of evolutionary relationships because the variation that is quantified accumulates more slowly as a

result of the neutral mutations. As a consequence, isolates with the same ST may be identified as members of the same clone but have very different pulsed-field gel electrophoresis (PFGE) patterns (6). PCR-based genotyping methods, such as RW3A rep-PCR, and PFGE detect genetic events in MRSA that occur on a shorter time scale (23). While rep-PCR has been shown to have high discriminatory power as well as reproducibility for genotyping MRSA, the method is still regarded as having less discriminatory power than PFGE (23). As a consequence, there have been very few direct comparisons between rep-PCR and MLST for genotyping bacteria. This blinded study compared a collection of 28 HA- and CA-MRSA strains using rep-PCR and MLST. The RW3A rep-PCR genotyping method was reproducible and generated highly discriminatory fragment patterns. The success obtained with rep-PCR in separating HA and CA-MRSA strains was due in large part to the fact that the ST8-MRSA-IV (Clone V) and ST5-MRSA-II (New York/Japan) clones have been shown to have very different chromosomal backgrounds (20).

Nine of the 13 strains that composed the SCC*mec* type II cluster, as determined by RW3A rep-PCR, were ST5-MRSA-II. ST5-MRSA-II is a well described pandemic clone of HA-MRSA and is the dominant clone in hospitals in Miami, New York, New Jersey, Pennsylvania, and Connecticut (19). The 13 strains in the SCC*mec* type II cluster shared several traits that are commonly attributed to HA-MRSA clones (22): having clindamycin resistance and causing serious invasive infections. All 13 of the strains in the SCC*mec* type II cluster were clindamycin resistant; 4 of these strains were also resistant to gentamicin. Additionally, 10 of these strains were isolated from respiratory specimens, which is a common body site for hospital-acquired infections. Of note, half of the strains belonging to the SCC*mec* type II cluster could not be distinguished as possible HA-MRSA strains from a determination based on day of acquisition. Thus, the epidemiology associated with these 28 strains of MRSA proved to be less reliable than the information obtained by PCR and genotyping for making a determination of CA- versus HA-MRSA infections.

Strain Sa221 was determined to be ST72 by using MLST and shared only 80% similarity to the ST5-MRSA-II strains. Unfortunately, the SCC*mec* element for Sa221 could not be determined; however, ST72-MRSA-IV has been described as a cause of CA-MRSA infections in Miami (3), San Francisco (2), and Mississippi (18). ST72-MRSA-IV belongs to CC8 and has also been designated pulsed-field type USA700 (14).

RW3A rep-PCR grouped all 15 MRSA strains determined to be ST8 into the ST8-MRSA-IV cluster. The ST8-MRSA-IV strains shared several traits known to be common to CA-MRSA clones (25). Fourteen of the 15 ST8-MRSA-IV strains were isolated from skin and soft-tissue infections and possessed the PVL toxin. In addition, 13 of these 15 ST8-MRSA-IV strains, 12 of which were susceptible to clindamycin, possessed the *msrA* gene. Interestingly, two strains of PVL-positive ST8 MSSA were also genotyped using RW3A rep-PCR, and these strains shared a greater-than-90% similarity with the ST8-MRSA-IV cluster. ST8-MRSA-IV is an epidemic clone of CA-MRSA that exists in the United States and has been designated pulsed-field type USA300 (14). USA300 is present in diverse regions of the United States, where it has been identified in community onset MRSA outbreaks associated with skin and soft-tissue infections in athletic teams, correctional facilities, and nurseries (14).

The presence or absence of the PVL determinant consistently distinguished the SCC*mec* type II cluster from the ST8-MRSA-IV cluster as determined by RW3A rep-PCR, with the exception of a single PVL-negative, ST8-MRSA-IV strain, Sa384. Despite the fact that the PVL gene has been described as a stable genetic marker of CA-MRSA strains (25), the current understanding of SCC*mec* type IV CA-MRSA dictates that its detection worldwide must consider that not all community-acquired MRSA strains will possess the PVL gene (11). Interestingly, the Sa384 strain shared the same MLST allelic profile as the other ST8-MRSA-IV strains in the SCC*mec* type IV cluster, indicating that these strains share a common evolutionary pathway. Strain Sa384 differed from the CA-MRSA ST8-MRSA-IV strains in being obtained from a lower-respiratory specimen (bronchoalveolar) and having multidrug resistance to clindamycin, gentamicin, and trimethoprim-sulfamethoxazole (18, 25). To date, most SCC*mec* IV CA-MRSA isolates have had MLST allelic profiles that are not found in studies of HA-MRSA (18). However, CA-MRSA has the potential to move into the hospital setting and cause outbreaks (16). This study reiterates that the detection of SSC*mec* type IV CA-MRSA does not guarantee PVL production or predictable resistance profiles.

Other investigators have reported that the relative frequencies of *S. aureus erm* genes are not the same worldwide or static over time and that different *erm* genes have been found in different *S. aureus* clones (26). Among our MRSA isolates, there was a strong predominance of the *ermA* over the *ermC* determinant. In our study, the only 2 MRSA strains that possessed the *ermC* gene, Sa221 and Sa384, were remarkably different from the other strains in their respective clusters, and *ermC* could be used as a marker for genetic variation in this group of CA-MRSA isolates.

The results of MLST and SCC*mec* typing and the detection of genes that encode PVL and macrolide/lincosamide resistance were very encouraging in suggesting the strength of rep-PCR for separating this collection of strains into HA- and CA-MRSA. RW3A rep-PCR should be investigated further to determine whether this inexpensive and rapid method of genotyping can be used by individual laboratories to follow the dissemination of CA-MRSA SCC*mec* type IV isolates.

REFERENCES

1. **Bannerman, T. L.** 2003. *Staphylococcus, Micrococcus,* and other catalase-positive cocci that grow aerobically, p. 384–404. *In* P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.), Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
2. **Carleton, H. A., B. A. Diep, E. D. Charlebois, G. F. Sensabaugh, and F. Perdreau-Remington.** 2004. Community-adapted methicillin-resistant *Staphylococcus aureus* (MRSA): population dynamics of an expanding community reservoir of MRSA. J. Infect. Dis. **190:**1730–1738.
3. **Chung, M., G. Dickinson, H. de Lencastre, and A. Tomasz.** 2004. International clones of methicillin-resistant *Staphylococcus aureus* in two hospitals in Miami, Florida. J. Clin. Microbiol. **42:**542–547.
4. **Del Vecchio, V. G., J. M. Petroziello, M. J. Gress, F. K. McCleskey, G. P. Melcher, H. K. Crouch, and J. R. Lupski.** 1995. Molecular genotyping of methicillin-resistant *Staphylococcus aureus* via fluorophore-enhanced repetitive-sequence PCR. J. Clin. Microbiol. **33:**2141–2144.
5. **Enright, M. C., D. A. Robinson, G. Randle, E. J. Feil, H. Grundmann, and B. G. Spratt.** 2002. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc. Natl. Acad. Sci. USA **99:**7687–7692.
6. **Enright, M. C., N. P. Day, C. E. Davies, S. J. Peacock, and B. G. Spratt.** 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J. Clin. Microbiol. **38:**1008–1015.

7. **Francois, P., G. Renzi, D. Pittet, M. Bento, D. Lew, S. Harbarth, P. Vaudaux, and J. Schrenzel.** 2003. Rapid detection of methicillin-resistant *Staphylococcus aureus* directly from sterile or nonsterile clinical samples by a new molecular assay. J. Clin. Microbiol. **41:**254–260.

8. **Jarraud, S., C. Mougel, J. Thioulouse, G. Lina, H. Meugnier, F. Forey, X. Nesme, J. Etienne, and F. Vandenesch.** 2002. Relationships between *Staphylococcus aureus* genetic background, virulence factors, *agr* groups (alleles), and human disease. Infect. Immun. **70:**631–641.

9. **Kalia, A., A. Rattan, and P. Chopra.** 1999. A method for extraction of high-quality and high-quantity genomic DNA generally applicable to pathogenic bacteria. Anal. Biochem. **275:**1–5.

10. **Khan, S. A., M. S. Nawaz, A. A. Khan, and C. E. Cerniglia.** 1999. Simultaneous detection of erythromycin-resistant methylase genes ermA and ermC from *Staphylococcus* spp. by multiplex-PCR. Mol. Cell. Probes **13:**381–387.

11. **Liassine, N., R. Auckenthaler, M. C. Descombes, M. Bes, F. Vandenesch, and J. Etienne.** 2004. Community-acquired methicillin-resistant *Staphylococcus aureus* isolated in Switzerland contains the Panton-Valentine leukocidin or exfoliative toxin genes. J. Clin. Microbiol. **42:**825–828.

12. **Lina, G., A. Quaglia, M. E. Reverdy, R. Leclercq, F. Vandenesch, and J. Etienne.** 1999. Distribution of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. Antimicrob. Agents Chemother. **43:**1062–1066.

13. **Marcinak, J. F., and A. L. Frank.** 2003. Clindamycin treatment of invasive infections caused by community-acquired, methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* in children. Pediatr. Infect. Dis. J. **22:**593–598.

14. **McDougal, L. K., C. D. Steward, G. E. Killgore, J. M. Chaitram, S. K. McAllister, and F. C. Tenover.** 2003. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. J. Clin. Microbiol. **41:**5113–5120.

15. **National Committee for Clinical Laboratory Standards.** 2004. Performance standards for antimicrobial susceptibility testing, 14th informational supplement M100-S14. NCCLS, Wayne, Pa.

16. **O'Brien, F. G., J. W. Pearman, M. Gracey, T. V. Riley, and W. B. Grubb.** 1999. Community strain of methicillin-resistant *Staphylococcus aureus* involved in a hospital outbreak. J. Clin. Microbiol. **37:**2858–2862.

17. **O'Brien, F. G., G. W. Coombs, J. C. Pearson, K. J. Christiansen, and W. B. Grubb.** 2005. Type V staphylococcal cassette chromosome *mec* in community staphylococci from Australia. J. Clin. Microbiol. **49:**5129–5132.

18. **Okuma, K., K. Iwakawa, J. D. Turnidge, W. B. Grubb, J. M. Bell, F. G. O'Brien, G. W. Coombs, J. W. Pearman, F. C. Tenover, M. Kapi, C. Tiensasitom, T. Ito, and K. Hiramatsu.** 2002. Dissemination of new methicillin-resistant *Staphylococcus aureus* clones in the community. J. Clin. Microbiol. **40:**4289–4294.

19. **Roberts, R. B., M. Chung, H. de Lencastre, J. Hargrave, A. Tomasz, D. P. Nicolau, J. F. John, Jr., O. Korzeniowski, et al.** 2000. Distribution of methicillin-resistant *Staphylococcus aureus* clones among health care facilities in Connecticut, New Jersey, and Pennsylvania. Microb. Drug Resist. **6:**245–251.

20. **Robinson, D. A., and M. C. Enright.** 2003. Evolutionary models of the emergence of methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother. **47:**3926–3934.

21. **Saïd-Salim, B., B. Mathema, and B. N. Kreiswirth.** 2003. Community-acquired methicillin-resistant *Staphylococcus aureus*: an emerging pathogen. Infect. Control Hosp. Epidemiol. **24:**451–455.

22. **Sattler, C. A., E. O. Mason, Jr., and S. L. Kaplan.** 2002. 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. **21:**910–917.

23. **Stranden, A., R. Frei, and A. F. Widmer.** 2003. Molecular typing of methicillin-resistant *Staphylococcus aureus*: can PCR replace pulsed-field gel electrophoresis? J. Clin. Microbiol. **41:**3181–3186.

24. **Strommenger, B., C. Kettlitz, G. Werner, and W. Witte.** 2003. Multiplex PCR assay for simultaneous detection of nine clinically relevant antibiotic resistance genes in *Staphylococcus aureus*. J. Clin. Microbiol. **41:**4089–4094.

25. **Vandenesch, F., T. Naimi, M. C. Enright, G. Lina, G. R. Nimmo, H. Heffernan, N. Liassine, M. Bes, T. Greenland, M. E. Reverdy, and J. Etienne.** 2003. Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: worldwide emergence. Emerg. Infect. Dis. **9:**978–984.

26. **Westh, H., D. M. Hougaard, J. Vuust, and V. T. Rosdahl.** 1995. Prevalence of erm gene classes in erythromycin-resistant *Staphylococcus aureus* strains isolated between 1959 and 1988. Antimicrob. Agents Chemother. **39:**369–373.