Emergence of New Strains of Methicillin-Resistant *Staphylococcus aureus* in a Neonatal Intensive Care Unit

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Background. Genetically distinct strains of methicillin-resistant *Staphylococcus aureus* (MRSA) of community rather than hospital origin have emerged in many areas of the United States. We determined if MRSA strains causing bacteremia in infants treated from birth in a neonatal intensive care unit (NICU) demonstrated the genetic traits of community-associated MRSA.

Methods. A retrospective cohort study was conducted among NICU infants with bacteremia due to MRSA during 2003 in a large tertiary care center NICU in Houston. MRSA isolates were characterized by antimicrobial susceptibility testing and staphylococcal cassette chromosome mec (SCCmec) typing by polymerase chain reaction. All MRSA cases were reviewed for clinical severity of infection and outcome.

Results. During 2003, a total of 8 (47%) of 17 infants with bacteremia due to *S. aureus* had MRSA infection. Isolates from 6 (75%) of these 8 infants carried the SCCmec genes (class B mec and ccr2) that are characteristic of community MRSA; 4 isolates were type IVa. All 6 isolates were resistant to β-lactam antibiotics and erythromycin; 1 was also resistant to clindamycin. One isolate was nontypeable, and another carried the SCCmec type II gene (typical of hospital-associated strains) and was susceptible only to vancomycin. Seven (88%) of 8 infants presented in septic shock. Despite initial treatment with vancomycin, 3 (38%) died, and 3 survivors had complications requiring prolonged antimicrobial therapy; these 6 infants had MRSA isolates with genetic characteristics of isolates of community origin.

Conclusions. Community-associated MRSA strains have emerged as a significant cause of sepsis in neonates hospitalized in NICU since birth and have caused disseminated infection with substantial morbidity and mortality.
Table 1. Antimicrobial susceptibility and molecular genetic characteristics of methicillin-resistant Staphylococcus aureus isolates

<table>
<thead>
<tr>
<th>Infant</th>
<th>Penicillin</th>
<th>Oxacillin</th>
<th>Vancomycin</th>
<th>Gentamicin</th>
<th>TMP-SMZ</th>
<th>Erythromycin</th>
<th>Clindamycin&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Molecular genetics analysis: SCCmec type</th>
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<tbody>
<tr>
<td>1</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
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<td>R</td>
<td>R</td>
<td>IV&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>R</td>
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<td>R</td>
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<td>IV</td>
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<tr>
<td>6</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<td>S</td>
<td>IV</td>
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<tr>
<td>7</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>Nontypeable</td>
</tr>
<tr>
<td>8</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>Nontypeable</td>
</tr>
</tbody>
</table>

<sup>a</sup> Excluded inducible macrolide-lincosamide-streptogramin B resistance. Resistance was defined by a positive result of a D-zone test for the isolate from infant 2 and by disk diffusion zone size for the isolate from infant 3.

<sup>b</sup> Did not amplify the IV<sub>a</sub> region.

NOTE. R, resistant; S, susceptible; SCC, staphylococcal cassette chromosome; TMP-SMZ, trimethoprim-sulfamethoxazole.

Figure 1. Repetitive-element PCR showing the genetic patterns of methicillin-resistant Staphylococcus aureus isolated from 8 infants in our neonatal intensive care unit. Results of this analysis grouped isolates from infants 1–3 together, indicating that these isolates are closely related despite differences in the staphylococcal cassette chromosome (SCC) mec type (1 and 2 were SCCmec IV, 3 was SCCmec II; see table 1). Isolates from patients 4–7 were grouped together and shared the repetitive element pattern associated with the predominant community-associated MRSA clone from our hospital [27]. RFLP, restriction fragment–length polymorphism.

genetic traits of community MRSA and to review the clinical presentation, course, and outcome of these neonatal MRSA infections. We report the first documentation that MRSA with genetic characteristics attributable to strains circulating in the community can be the principal cause of MRSA infections in neonates who have never left the NICU.

METHODS

Patient population. Texas Children’s Hospital is a 737-bed tertiary care hospital located in Houston. It has a 140-bed NICU (76 beds of level III and 64 of level II) and had 3027 admissions in 2003. The Infectious Disease Laboratory at Texas Children’s Hospital maintains a prospective database of patients from whom S. aureus is isolated and collects all S. aureus strains isolated by the hospital clinical microbiology laboratory. Using this database as our information source, we performed a retrospective cohort study of NICU infants who had bacteremia due to MRSA during the 12-month period from 1 January 2003 through 31 December 2003. Data gathered from each infant’s medical record determined demographic characteristics, antenatal and delivery history, complications of prematurity, use of parenteral nutrition or central intravascular catheters, clinical presentation, laboratory and diagnostic imaging studies, antimicrobial susceptibility pattern of MRSA isolates, therapeutic interventions, and outcome.

Laboratory methods. Isolates were identified as S. aureus according to standard methods [19]. The disk diffusion method was used to screen for methicillin resistance and test for antimicrobial susceptibility by use of NCCLS methodology [20, 21]. Antibiotics tested included penicillin, oxacillin, vancomycin, gentamicin, trimethoprim-sulfamethoxazole, erythromycin, and clindamycin. Results were determined after 24 h of incubation at 35°C according to NCCLS breakpoints [21]. When an isolate was erythromycin resistant and clindamycin susceptible, inducible macrolide-lincosamide-streptogramin B (MLS<sub>B</sub>) resistance was excluded by disk diffusion and the D-zone test [22–25].

All MRSA strains from NICU patients with bacteremia were cultured onto tryptic soy agar plates containing 5% sheep blood (BBL Beckton Dickinson) for DNA isolation by use of the UltraClean Microbial DNA Kit as recommended by the manufacturer (MO Bio Laboratories). The SCCmec type was determined by the method described by Okuma et al. [16]. Positive controls (NCTC 10492 [SCCmec type I], N315 [SCCmec type II], 85/2082 [SCCmec type III], and CA 05 [SCCmec type IVa]) were provided by Keiichi Hiramatsu and Teruyo Ito (De-
Table 2. Clinical characteristics of infants with bloodstream infection due to methicillin-resistant *Staphylococcus aureus* (MRSA).

<table>
<thead>
<tr>
<th>Infant</th>
<th>Sex/ethnicity</th>
<th>Gestational age, weeks</th>
<th>Birth weight, g</th>
<th>Underlying condition(s)</th>
<th>Age in days at infection onset</th>
<th>Risk factor(s)</th>
</tr>
</thead>
</table>
| 1      | M/Hispanic    | 28                     | 1205            | RDS, patent ductus arteriosis
          |               |                       |                 |                          | 13                          | IVC in situ    |
| 2      | M/Hispanic    | 31                     | 1418            | Gastrochisis repaired, RDS |
          |               |                       |                 |                          | 34                          | IVC in situ, TPN |
| 3      | F/Hispanic    | 23                     | 543             | RDS, patent ductus arteriosis,* intraventricular hemorrhage, hypothyroidism, microcolon, *Candida albicans* endocarditis |
          |               |                       |                 |                          | 53                          | IVC in situ, TPN |
| 4      | M/white       | 35                     | 2800            | None                     |
          |               |                       |                 |                          | 11                          | None           |
| 5      | F/Hispanic    | 24                     | 665             | RDS, jaundice            |
          |               |                       |                 |                          | 14                          | IVC in situ, TPN |
| 6      | First isolate M/African American | 27                 | 1160            | PHACE syndrome, congenital heart disease, cholestatic liver disease, necrotizing enterocolitis |
          | Second isolate |                       |                 |                          | 54                          | IVC in situ, colonized with MRSA |
| 7      | First isolate M/Hispanic | 28                 | 701             | Small for gestational age, chronic lung disease, blood dyscrasias, short gut syndrome, cholestatic liver disease, recurrent infections |
          | Second isolate |                       |                 |                          | 394                         | IVC in situ, TPN |
| 8      | F/Hispanic    | 39                     | 1715            | Congenital anomalies (Dandy-Walker syndrome) |
          |               |                       |                 |                          | 18                          | IVC in situ, TPN |

**NOTE.** IVC, intravascular catheter; PHACE, posterior fossa malformations, hemangiomas, arterial anomalies, coarctation of the aorta and cardiac defects, and eye abnormalities; RDS, respiratory distress syndrome; TPN, total parenteral nutrition.

* Successfully treated with indomethacin in the first week of life.
* Trimethoprim-sulfamethoxazole was used to treat *Stenotrophomonas maltophilia* that was isolated, in addition to MRSA, from empyema fluid.
* Clindamycin was used to treat suspected necrotizing enterocolitis.

department of Bacteriology, Juntendo University, Tokyo). A few PCR amplicons were purified by use of the PCR Min-elute kit (Qiagen) and subsequently sequenced and compared by BLAST analysis (http://www.ncbi.nlm.nih.gov/BLAST/), with the SCC\textit{mec} sequences deposited into GenBank.

All isolates were typed by repetitive-element PCR by use of a commercial repetitive-element PCR typing kit according to the manufacturer’s instructions (Bacterial Barcodes). Repetitive-element PCR is designed to amplify the regions between the noncoding repetitive elements that are dispersed throughout the bacterial genomes and results in several amplified fragments of different sizes. The position of many of these elements is not conserved between bacterial clones, and the banding patterns will differ between microorganisms at a subspecies level. A PTC-200 Peltier thermocycler PCR System (MJ Research) was used for PCR analysis, and the amplicons were separated by electrophoresis on a 1.5% agarose gel in 1× TAE (0.04 mol/L Tris-HCl, and 0.001 mol/L EDTA). Genetic patterns were visualized by UV analysis after ethidium bromide staining and were compared digitally by means of Pearson correlation analysis with the GelComparII computer software (Applied Maths).

**RESULTS**

Eight (47%) of 17 NICU infants with bacteremia due to *S. aureus* during 2003 were infected with MRSA. Antimicrobial susceptibility patterns and genetic characteristics of MRSA isolates from these 8 infants are listed in table 1. Six (75%) of 8 MRSA isolates contained the class B \textit{mec} and \textit{cr}2 genes as-
associated with SCCmec type IV [16, 26]. Four of these 6 isolates rendered a PCR product with the IVa primers, classifying these as SCCmec type IVa [16]. One strain contained SCCmec type II, and 1 was nontypeable. All SCCmec type IVa isolates had genetic patterns that were identical to those of the predominant community-associated MRSA strain found in our hospital [27]; the remaining isolates differed from this clone by >60% (figure 1). Isolates from infants 1 and 2 closely resembled a less common community-associated MRSA strain found in our hospital.

The demographic and clinical characteristics of the 8 infants with invasive MRSA infections are summarized in table 2. All infants were singleton births. All infections were late onset (occurring at >72 h of age) and presented at a median age of 26 days (range, 11–394 days). Seven infants were hospitalized from birth. Infant 4 was hospitalized in the level II NICU from birth and discharged home on day of life 9. Thirty-six hours later, he was readmitted to the NICU in septic shock. In retrospect, signs of illness (mild irritability and decreased feeding) were present at the time of discharge. The mean gestational age of the 8 infants was 29.4 weeks (range, 23–39 weeks), and the mean birth weight was 1206 g (range, 543–2800 g); 6 had a very low birth weight (<1500 g).

Each infant received vancomycin administered intravenously at the onset of clinical signs of sepsis. Vancomycin was continued (with or without other antimicrobial agents [table 2]) for the duration of therapy, which ranged from 10 days after documentation of sterile blood cultures (or until death occurred, whichever occurred first) to 42 days for infants with endocarditis or osteomyelitis.

Seven infants had severe sepsis, best exemplified in infant 1. The initial neonatal course was complicated by mild respiratory distress syndrome and patent ductus arteriosus. The latter was successfully treated with indomethacin given for 2 days. At the time MRSA bacteremia developed (day of life 13), infant 1 was clinically much improved, having been weaned from mechanical ventilation on day of life 2 and given oral feeding. The acute clinical presentation indicated respiratory failure, refractory hypotension, and upper gastrointestinal bleeding. Despite
prompt initiation of antimicrobial therapy and aggressive supportive care, septic emboli, seizures, disseminated intravascular coagulation, and renal insufficiency rapidly developed. Cranial ultrasonography, the results of which were previously normal, showed cystic changes in the midbrain and ventriculomegaly, with multiple septations visible within the ventricles. Intravenous rifampin (20 mg/kg per day) was added to the antimicrobial regimen when cultures of blood were reported to yield MRSA. The peripheral venous central catheter was removed. No other sites of disseminated infection were detected clinically or after extensive diagnostic imaging studies. Bacteremia cleared after antimicrobial therapy for 7 days. Death occurred 3 days later. Necrotizing pneumonia and encephalomalacia with extensive brain hemorrhage and cystic necrosis were found at autopsy; autopsy cultures were sterile.

Infants 6 and 7 developed recurrent septicemia. Infant 6 developed his second episode on day of life 75 and had evidence of disseminated infection despite having received vancomycin intravenously for 10 days after documented sterile blood cultures during the initial episode of MRSA bacteremia. Infant 7 developed MRSA bacteremia on day of life 394. Despite prompt removal of his central venous catheter and treatment with vancomycin for 10 days after documentation of sterile blood cultures, MRSA bacteremia recurred on day of life 411. He had an excellent clinical response to the reinstitution of vancomycin therapy, but cultures of blood continued to yield MRSA until he unexpectedly died from cardiorespiratory arrest 3 days later. At autopsy, MRSA was isolated from a previously unrecognized lung abscess.

DISCUSSION

We have documented that bacteremia due to MRSA among infants treated in our NICU since birth is predominately caused by isolates genetically identical to strains circulating in the community rather than to those that are health care associated [16, 26]. To our knowledge, this is the first report of this occurrence. Two neonates with bacteremia due to a community-associated MRSA isolate have been described [18, 28], but in one report, concurrent maternal-fetal infection was obvious at birth [18].

We believe that the introduction of community strains of MRSA into our NICU reflects the high prevalence of these strains in the Houston community [10], but their transmission in the nursery setting deserves further study. We do not believe that our cohort represents a single outbreak of infections, because several different genetic patterns were found by repetitive-element-polymorphism PCR, affected infants were in different areas of the NICU and were treated by multiple different health care professionals, and infections occurred sporadically. Each infant with invasive infection due to a community strain of MRSA developed infection after the immediate neonatal period (>72 h of age), making maternal-infant transmission less likely [18, 29]. There were no documented staphylococcal-like illnesses in family contacts of any infant, and our NICU adheres to standard infection-control guidelines [30–32], with all infants being placed in isolation with contact precautions once MRSA is isolated from a culture of a specimen obtained from any site. However, horizontal transmission of S. aureus from colonized visitors or health care workers to infants in the NICU previously has been documented [4, 18, 28] and could have been a mode of transmission in some of our patients. Our findings have important implications for health care–associated infection trends in other hospitals, especially NICUs, as the prevalence of community MRSA colonization and infection increases in other geographic regions of the United States.

S. aureus is the second most common pathogen causing late-onset septicemia in NICU infants with very low birth weights [33]. Poorly developed host defense mechanisms, the necessity for central venous catheters, endotracheal and upper gastrointestinal tract tube placement, procedures causing interruption in skin integrity, prolonged total parenteral nutrition, and use of steroids or antimicrobial agents all increase the risk of staphylococcal infection in premature infants. Six (75%) of our patients were of very low birth weight; 5 had ≥1 factor predisposing to invasive infection. S. aureus bacteremia in the neonate historically is associated with septic shock, which can be rapidly fatal [33–36]. Seven infants (88%) in our cohort of 8 had this clinical presentation. Our case-fatality ratio was 38%, and 60% of surviving infants had complications requiring prolonged antimicrobial therapy. These high fatality and morbidity rates, which occurred despite the prompt initiation of appropriate antimicrobial agents and intensive care support, highlight 2 points. First, despite epidemiological studies of adults and older children linking community-associated MRSA more with superficial (i.e., skin and soft tissue) infections than with deep-seated infections [8, 10, 11, 14], this pathogen is highly virulent in certain hosts. Future case-comparison studies of genetically characterized community versus health care–associated MRSA strains involving NICU patients and other vulnerable populations would be useful in further defining this variation in virulence. Second, on the basis of our experience, in geographic regions where community MRSA is prevalent, eliminating vancomycin from the empirical therapy for presumed late-onset neonatal septicemia potentially could be harmful. Community MRSA strains demonstrate less in vitro antimicrobial resistance than do traditional health care–associated isolates. However, many agents with in vitro activity, such as clindamycin or trimethoprim-sulfamethoxazole, are inappropriate for the treatment of invasive infections in NICU patients, either because of their bacteriostatic rather than bactericidal activity (clindamycin) or the lack of data regarding their pharmacokinetics, safety, and efficacy (trimethoprim-sulfamethoxazole).

Molecular studies have shown an association between certain
genetic determinants and community MRSA [15, 16, 26]. Most important, the SCCmec cassette in community strains is smaller than is that of the health care–associated equivalents, and its genetic composition likely enhances mobility and ability to be transferred between strains [15]. The genetic differences have made it possible, by means of molecular methods, to distinguish health care–associated isolates from strains derived from the community [16]. Therefore, although "community-associated" is a definition based primarily on the patient’s history regarding underlying illnesses, presence of percutaneous medical devices, previous hospitalization or residence in a long term care facility, and timing of MRSA isolation on arrival to the hospital [8], it has been possible to use molecular methods to distinguish health care–associated isolates from strains derived from the community [16].

Our finding that community strains of MRSA have become a significant cause of late-onset neonatal sepsis in a cohort of patients hospitalized in a NICU from birth may herald the necessity for new definitions and nomenclature if our observations are confirmed in other hospitalized patient groups. The genetic traits that thus far have been attributed only to MRSA circulating in the community may in the future also characterize health care–associated strains, if community strains of MRSA become widely distributed in health care facilities. Our findings also have implications for the treatment of health care–associated infections, especially for suspected late-onset sepsis in NICU patients. MRSA causes a significant proportion of S. aureus infections in the NICU, both in our NICU and in other centers [33], resulting in substantial morbidity and mortality, whether the infecting strain is health care associated or has the genetic characteristics of community strains. Although we support efforts to restrict use of vancomycin to prevent the emergence of drug-resistant staphylococci [37], we believe that eliminating vancomycin from the initial therapy for late-onset neonatal sepsis is imprudent in areas where MRSA is prevalent in the community.

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References


