Latex Agglutination-Negative Methicillin-Resistant *Staphylococcus aureus* Recovered from Neonates: Epidemiologic Features and Comparison of Typing Methods

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Received 11 March 1992/Accepted 3 July 1992

An unusual strain of methicillin-resistant *Staphylococcus aureus* (MRSA) was repeatedly isolated from infants in a newborn special care unit (NBSC) and a newborn intensive care unit. Between January 1989 and March 1990, approximately 100 isolates from infected or colonized infants were recovered. Surveillance cultures taken during this time revealed a 20% colonization rate, which was defined as recovery of MRSA from the nares, umbilicus, or groin. Isolates were identified as *S. aureus* by tube coagulase reactivity and heat-stable nuclease production but were unreactive in a latex agglutination assay. Representative isolates that were collected during the outbreak and that were found to share the latex agglutination assay-negative phenotype were compared by antibiogram (12 isolates), bacteriophage typing (20 isolates), capsular polysaccharide typing (30 isolates), and plasmid as well as chromosomal DNA analyses (20 isolates). All isolates known to be associated with the outbreak had nearly identical antibiograms and were notably susceptible to clindamycin. Staphylococcal bacteriophage typing was not useful in determining the relatedness of the isolates, since the majority were nontypeable. Plasmid pattern analysis revealed one large plasmid (approximately 100 kb) of equivalent size among the isolates. Capsular polysaccharide typing revealed that 14 of 30 isolates tested were type 5. Isolates identified in children at two other hospitals in the city which were also unreactive by the latex agglutination assay and clindamycin susceptible had plasmid and antibiogram patterns identical to those of isolates from the NBSC. Pulsed-field gel electrophoresis of restriction enzyme-digested genomic DNAs from the outbreak isolates demonstrated identical patterns which could be clearly differentiated from those of other unrelated MRSA. The strain from the NBSC is, therefore, unique and underscores the need for caution in interpreting the latex agglutination reactivities of MRSA isolates.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a common cause of nosocomial infections in hospitalized adult patients, especially those who remain in critical care units for extended periods of time (23). While *S. aureus* is the most frequently reported nosocomial pathogen on pediatric and newborn services in large hospitals, resistance to methicillin is uncommon (11). The few reported outbreaks of MRSA involving colonization and infection of pediatric patients have been characterized by high rates of morbidity and mortality (6, 22). This has been attributed in part to the rapidity with which the organism spreads in a hospital unit and the propensity for these organisms to be resistant to multiple antibiotics (7, 12).

Control of the spread of MRSA in a hospital relies upon the timely identification of these organisms in the patient population and then the immediate institution of appropriate control methods (18, 25). Rapid and accurate identification of the species of *Staphylococcus* involved is critical, since the control measures implemented will vary depending on the identity of the outbreak organism. A rapid method commonly used by hospital microbiology laboratories to differentiate *S. aureus* from other staphylococcal species is latex agglutination. In this assay, latex particles coated with fibrinogen and immunoglobulin G are used to detect protein A and/or clumping factor on the cell surface of *S. aureus* (19). Questions have been raised as to the utility of this test in identifying MRSA since decreased sensitivity has been reported (20, 24).

This report describes an outbreak of clindamycin-susceptible, latex agglutination-negative MRSA among infants in a newborn special care unit (NBSC) and newborn intensive care unit (TNIC) in Houston, Tex. Isolates obtained from the outbreak were characterized by antibiogram, bacteriophage and capsular polysaccharide typing, and DNA analysis. Comparisons were made between these isolates and MRSA strains recovered from adult patients in other areas of the hospital as well as MRSA strains recovered from pediatric patients in two other hospitals within the city.

**MATERIALS AND METHODS**

**Epidemiological investigation.** Surveillance cultures were collected from newborns in the NBSC between January 1989 and June 1989 following the identification of five newborn colonized or infected with methicillin-susceptible *S. aureus.* Cultures of samples obtained from the nares and either the umbilicus or the groin area by swabbing were performed for all newborns in the unit, and newborns whose samples from the nares and either of the two other sites were culture positive were considered to be colonized. Surprisingly, the majority of positive cultures revealed MRSA, not methicillin-susceptible *S. aureus.* Furthermore, these isolates shared certain unusual features suggesting the presence of a single strain. Surveillance was expanded to include newborns in the TNIC. Additionally, cultures of samples from the nares...
of all personnel in contact with patients in either of these two units during the month of June were collected. Personnel found to be colonized with MRSA were treated with trimethoprim-sulfamethoxazole and rifampin for 5 days, and samples from these individuals were recultured in 3 weeks. Subsequently, efforts were made to culture samples from all newborns upon admission to either of the units and to place colonized or infected newborns in a cohort in an area of the unit separate from the other patients. Colonized newborns were defined as those from whom MRSA was recovered from cultures of groin, nares, or umbilicus samples, with all other cultures of samples from all other sites being negative and with no clinical signs of infection. Strict enforcement of hand-washing procedures was also implemented.

Identification of MRSA. Specimens were plated onto trypticase soy agar with 5% sheep erythrocytes and were incubated for 24 h at 35°C. Staphylococci were identified by colony morphology, latex agglutination (Staphaurex; Wellcome Diagnostics; and Staphylococcal Innervation, Innovative Diagnostics), heat-stable nuclease activity (Remel), and slide and tube coagulase production. All isolates were stored in 2× skim milk at −70°C for further testing. MRSA isolates from newborns at the two other hospitals in the city were kindly supplied by Carol Wilson and Carol Baker, Houston, Tex.

Antibiotic susceptibility testing. All isolates were tested for their susceptibilities to oxacillin by the standardized disk diffusion method (16). MICs of 24 antibiotics were determined for selected isolates by the broth microbiidilution method (17; Pasco; Difco Laboratories, Detroit, Mich.), and an expanded antibiogram was obtained in order to provide epidemiological information about the relatedness of the isolates.

Staphylococcal bacteriophage and capsular typing. Selected isolates from colonized newborns were sent to the Texas Department of Health in Austin for staphylococcal bacteriophage typing. The same isolates were also sent to J. Fourmier at the Pasteur Institute for typing of capsular polysaccharides (3).

Plasmid pattern analysis. Plasmids were isolated from MRSA strains by a modification of the method described by Lyon et al. (14). Staphylococcal isolates were grown overnight in 10 ml of trypticase soy broth. Cells were washed with 1.0 ml of 50 mM Tris hydrochloride-5 mM EDTA (pH 8.0) and were resuspended in 450 μl of 10 mM Tris hydrochloride-1 mM EDTA-25% sucrose (pH 8.0); 5 μl of lysostaphin (10 mg/ml in sterile distilled water) was then added. Cells were lysed by incubation at 37°C for 45 min; this was followed by the addition of 75 μl of lysis solution (10% sodium dodecyl sulfate, 50 mM Tris, 10 mM EDTA [pH 8.0]) and incubation at room temperature for 10 min. Following the addition of 400 μl of 5 M potassium acetate, the mixture was incubated on ice for 30 min. The cleared lysate (supernatant) was treated with 5 μl of RNase (10 mg/ml in water) for 30 min at 37°C, after which time the DNA was precipitated with ethanol and a 1/10 volume of sodium acetate. The DNA was resuspended in 40 μl of 10 mM Tris hydrochloride-1 mM EDTA (pH 8.0), and 20 μl was electrophoresed on a 0.7% agarose gel. Two staphylococcal isolates each containing a plasmid with a known molecular weight were included on the gel as size standards. Crude preparations of staphylococcal plasmid DNA were also digested with the restriction endonuclease EcoRI (Boehringer Mannheim) for 2 h at 37°C. Digested DNA was electrophoresed on an agarose gel as described above for whole plasmid DNA.

Genomic DNA analysis by PFGE. Genomic DNA was prepared as described by Murray et al. (15), with minor modifications. Briefly, the staphylococci were grown overnight in brain heart infusion broth at 37°C. Pelleted cells were resuspended in 1 M NaCl-10 mM Tris hydrochloride (pH 7.6) mixed with low-melting-temperature agarose (In Cert Agarose; FMC Corp.) and were put into plug forms. The agarose plugs were added to lysis solution (6 mM Tris hydrochloride [pH 7.6], 1 M NaCl, 100 mM EDTA [pH 7.5], 0.5% Brij 58, 0.2% deoxycholate, 0.5% sodium lauryl sarcosine, 20 μg of RNase per ml, 30 U of lysostaphin per ml) and were incubated overnight at 37°C. Lysis was continued overnight by incubation at 50°C in ESP solution (0.5 mM EDTA [pH 9 to 9.5], 1% sodium lauryl sarcosine, 50 μg of proteinase K per ml). After thorough washing with 10 mM Tris hydrochloride (pH 7.5)-0.1 mM EDTA, plugs were digested with Smal and electrophoresed by pulsed-field gel electrophoresis (PFGE; Bio-Rad CHEF DR II).

RESULTS

Epidemiological investigation. The NBSC is physically divided into two sections, each containing 14 to 18 beds and supervised by 46 to 50 staff members on a rotating basis between the two sections. The children range in age from newborn to approximately 6 months and have minimal interaction with non-staff personnel because of limited access of non-staff personnel to the unit. The TNIC has approximately 12 to 15 beds, supervision is by 20 to 25 staff members, and the age range and non-staff member interactions are as stated above.

Figure 1 shows the number of newborns colonized or infected with MRSA (in both the NBSC and TNIC) during 1989 and 1990. MRSA was cultured from samples from the nares, groin, or umbilicus of seven (20%) of the newborns in the NBSC during January 1989. One of the culture-positive infants had been transferred from another hospital in Houston and was felt to be the index case, although definitive evidence is lacking. Prior to that time, colonization with MRSA had not been found in the NBSC. Following the identification of four MRSA-infected newborns in the TNIC and two in the NBSC in May and June of 1989, additional samples for surveillance cultures were obtained from newborns in both units. Again, the extent of MRSA colonization was approximately 20% in each unit. For surveillance cultures of staff members, samples from personnel working in either unit during June 1989 revealed MRSA in the nares of 4 of 62 personnel members in the NBSC and 0 of 52 staff members in the TNIC. Follow-up samples from staff members after chemotherapeutic decolonization were negative for MRSA on culture. Continued surveillance culturing was used in order to monitor the colonization rate of newborns in each unit.

Despite aggressive enforcement of maintaining colonized and infected infants in cohorts and strict enforcement of hand-washing procedures, follow-up surveillance in February 1990 once again revealed that 2 infants in the TNIC and 10 infants in the NBSC were colonized with MRSA. At that time, samples from the nares of all personnel entering either newborn unit (including students, nurses, and primary and consulting physicians) were cultured. Of the 324 personnel from whom samples for culture were obtained, 5 were found to have nasal MRSA colonization. Colonized individuals were treated orally with trimethoprim-sulfamethoxazole and rifampin, and cultures of samples from all individuals were negative upon repeat culturing at 3 weeks posttreatment.

Concurrent with the decolonization efforts, a strict hand-washing and mandatory glove-wearing policy was instituted.
FIG. 1. Number of pediatric patients infected or colonized with MRSA in the NBSC (A) and TNIC (B) during 1989 and in the NBSC (C) and TNIC (D) during 1990. ■, infected patients; □, colonized patients.

for all personnel having contact with newborns in the units. Subsequent surveillance surveys in April 1990 identified no colonized newborns in the TNIC.

Laboratory investigations. All the MRSA isolates from patients and personnel were noted to be unreactive by both latex agglutination and slide coagulase analyses and were subsequently identified as *S. aureus* on the basis of colony morphology, tube coagulase reactivity, and DNase activity. All of these isolates produced identical disk diffusion susceptibility results and were unique among other MRSA isolated in the hospital in that they were susceptible to clindamycin. Since the isolates shared two unusual characteristics (latex nonreactivity and clindamycin susceptibility), attempts were made to determine their relatedness by using several epidemiological methods, including antibiogram, bacteriophage typing, capsular serotyping, and DNA analysis. During the course of our study, two other hospitals in Houston reported the isolation of MRSA from pediatric patients; the isolates proved to be phenotypically identical to our strains and were, henceforth, included in our studies.

Broth microdilution MICs of a panel of 24 antibiotics were determined for 12 isolates recovered from the seven infants found to be colonized in January 1989 (samples from multiple sites were cultured for some infants). The results confirmed the susceptibilities of the isolates to clindamycin, giving further evidence of their relatedness. Results for all other antibiotics were identical except for a few minor differences (Table 1).

Staphylococcal bacteriophage typing demonstrated that the majority of isolates were nontypeable. Capsular polysaccharide typing revealed that 14 of 30 isolates were reactive with monoclonal antibodies to capsular polysaccharide serotype 5. Fournier and colleagues (9) have shown that MRSA which were nonreactive in latex agglutination assays were of capsular serotype 5.

Plasmid pattern analysis revealed a single plasmid with an approximate molecular weight of 100 kb in each of the MRSA isolates (Fig. 2). Restriction enzyme digestion of these plasmids demonstrated patterns which again suggested their relatedness (data not shown). Plasmid pattern analysis of 30 MRSA strains recovered from adult patients in the same hospital revealed the presence of multiple plasmids, none of which resembled the pattern found in the isolates from the NBSC (data not shown). All MRSA isolates from the NBSC and TNIC as well as those from pediatric patients in two other hospitals in Houston proved to be identical by using PFGE to compare restriction digests of genomic DNAs (Fig. 3). One of those two hospitals was the source of the transferred infant who was presumed to be the index case of the outbreak. The patterns that these isolates exhibited by PFGE were, however, distinct from those seen with isolates of MRSA collected from other patients within the same hospital.

**DISCUSSION**

An unusual strain of MRSA was isolated from pediatric patients in an NBSC in January 1989. Despite the significant occurrence of MRSA in adult patients in the critical care units of this same hospital (4), it has been absent from the NBSC prior to that time. While estimates of the colonization rates of MRSA in hospitals that serve neonates and pediatric patients are not available, few outbreaks of MRSA in neonatal intensive care units have been reported (6, 11, 22).
Isolates from the NBSC were nonreactive in the latex agglutination assay routinely used by our clinical laboratory for the identification of S. aureus. Despite this nonreactivity, colony morphology and hemolytic reactivity on trypticase soy agar with 5% sheep erythrocytes prompted laboratory personnel to perform further tests and thus accurately identify the organism as S. aureus. Even so, it is impossible to rule out the existence of MRSA in the NBSC prior to January 1989, since incorrect identification of isolates by latex agglutination could have masked its presence. The organism’s antibiogram, however, was recognized as not being typical of the hospital’s nosocomial strains of staphylococci. Moreover, in a 1988 in-house evaluation of latex agglutination versus tube coagulase testing for the identification of clinical isolates of S. aureus, including MRSA, latex agglutination showed a sensitivity of >95% (unpublished data). This evidence suggests the absence of the latex agglutination-negative strain in the NBSC before the outbreak. Nonetheless, the lower sensitivities of latex agglutination reagents, particularly for MRSA, have been reported by other investigators (19, 20, 24).

The MRSA strains from the NBSC were susceptible to clindamycin, a finding not frequently seen in MRSA isolates in our hospital. In a recent review of MRSA in pediatric patients, Kline and Mason (21) described a number of isolates from pediatric patients as being resistant to multiple antibiotics, including erythromycin and clindamycin. In contrast, a case report of pediatric community-acquired MRSA described an isolate that was susceptible to clindamycin by microdilution susceptibility testing. The patient was treated with clindamycin, with good clinical results and a bacterioidal titer in serum of >1:128 (21). Current recommendations, however, do not include clindamycin as an empirically useful agent for the treatment of MRSA infections (7).

After isolating the unusual strain of MRSA from our NBSC, we learned of two other hospitals in the city which had also recovered MRSA from pediatric patients. Those strains were nonreactive by latex agglutination and were susceptible to clindamycin. Initial attempts at comparing the isolates from the NBSC with each other and with the isolates collected from the other hospitals by bacteriophage typing

**TABLE 1. Antibiogram of the MRSA isolates examined in this study**

| Isolate | AM | CFM | CFZ | CX | CFX | CL | C | CIP | CC | E | GM | OX | P | RF | SSS | TE | TS | NN | VA |
|---------|----|-----|-----|----|-----|----|---|----|----|---|----|----|---|---|----|----|----|----|----|----|
| 1       | R  | S   | MS  | R  | R   | S  | S | S  | S  | R | R  | R  | R  | S | R  | S  | S  | R  | S  |
| 2       | R  | S   | R   | MS | R   | S  | S | S  | S  | R | R  | R  | R  | S | R  | S  | S  | R  | S  |
| 3       | R  | MS  | MS  | R  | S   | S  | S | S  | S  | R | R  | R  | R  | S | S  | R  | S  | S  | S  |
| 4       | R  | S   | MS  | MS | R   | S  | S | S  | S  | R | R  | R  | R  | S | S  | R  | S  | S  | S  |
| 5       | R  | S   | R   | MS | R   | S  | S | S  | S  | R | R  | R  | R  | S | R  | S  | S  | R  | S  |
| 6       | R  | S   | MS  | R   | MS | R  | S  | S  | S  | R | R  | R  | R  | S | R  | S  | S  | R  | S  |
| 7       | R  | S   | MS  | R   | MS | R  | S  | S  | S  | R | R  | R  | R  | S | R  | S  | S  | S  | S  |
| 8       | R  | S   | R   | MS | R   | MS | S  | S  | S  | R | R  | R  | R  | S | S  | S  | S  | R  | S  |
| 9       | R  | S   | MS  | R   | MS | R  | S  | S  | S  | R | R  | R  | R  | S | R  | S  | S  | R  | S  |
| 10      | R  | S   | R   | R   | R   | S  | S | S  | S  | R | R  | R  | R  | S | S  | R  | S  | S  | S  |
| 11      | R  | S   | MS  | MS | R   | MS | S  | S  | S  | R | R  | R  | R  | S | R  | S  | S  | S  | S  |
| 12      | R  | S   | MS  | MS | S   | S  | S | S  | S  | R | R  | R  | R  | S | S  | S  | S  | S  | S  |

* AM, ampicillin; CFM, cefamandole; CFZ, cefazolin; CX, cefoxitin; CFX, cefuroxime; CL, cephalothin; C, chloramphenicol; CIP, ciprofloxacin; CC, clindamycin; E, erythromycin; GM, gentamicin; OX, oxacillin; P, penicillin; RF, rifampin; SSS, triple sulfonamide; TE, tetracycline; TS, trimethoprim-sulfamethoxazole; NN, tobramycin; VA, vancomycin; R, resistant; S, susceptible; MS, moderately susceptible.

**FIG. 2.** Plasmid DNA from MRSA isolates were electrophoresed on a 0.7% gel. Lanes A, B, and G, MRSA isolated from adult patients; lanes C to F and H to K, MRSA isolated from patients in NBSC; lanes L and M, MRSA from pediatric patients in two other hospitals in Houston.

**FIG. 3.** Genomic DNAs from MRSA isolates were digested with *SmaI* and were separated by PFGE. Lanes A to C and H to J, genomic DNAs of MRSA isolates from pediatric patients in the TNIC and NBSC; the isolates in lanes A to C were collected in January 1989; those in lanes H to J were collected in June 1989. Unlike the other susceptible isolates, the isolate in lane D was resistant to clindamycin. The isolates in lanes E and F were from pediatric patients in two other hospitals in Houston.
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were unsuccessful since the majority of the isolates were nontypeable. This finding is in agreement with the findings presented in previous reports that bacteriophage typing cannot be used to determine relatedness among isolates of MRSA (2, 5).

Capsular polysaccharides have been characterized in clinical isolates of S. aureus, including methicillin-resistant strains. French investigators have shown a predominance of capsular serotypes 5 and 8 in human clinical isolates of S. aureus and, more specifically, the predominance of serotype 5 in MRSA (3, 10). Moreover, in a recent study of 183 isolates of S. aureus from France, 7 isolates could not be identified by latex agglutination (9). Six of the seven isolates were resistant to methicillin, and all were of capsular serotype 5. Thirty isolates in the present investigation were tested against monoclonal antibodies to capsular serotypes 5 and 8. Fourteen isolates were identified as serotype 5, thus confirming the previous observations (9) on the association between the serotype and latex agglutination nonreactivity. The remaining 16 isolates did not react with monoclonal antibodies to serotype 5 or 8. The isolates either belong to another capsular serotype or, alternatively, may not produce detectable capsular polysaccharide. Studies are under way to resolve this question (8).

Since the NBSC isolates contained only a single plasmid, no definitive conclusions about relatedness could be made solely on the basis of plasmid pattern analysis. This method has been shown by others, however, to be reliable in determining the relatedness of isolates involved in nosocomial outbreaks, including isolates of MRSA (5, 13, 23). Plasmids have often proven to be a demonstrable and stable characteristic among the strains of MRSA analyzed (26).

Analysis of genomic DNA by PFGE proved to be a useful technique, demonstrating the presence of multiple identical bands in all of the isolates from the TNIC and NBSC as well as the isolates from the other two hospitals. The characteristic pattern of these isolates was different from that of other isolates of MRSA collected from patients in our hospital. PFGE has been used by other investigators and has proven to be a useful epidemiological tool (15). The major advantage of this technique lies in the ability of the investigator to reproducibly resolve a wide range of sizes of genomic DNA restriction fragments on a single gel (1).

Surveillance culturing, which continued throughout 1990, demonstrated only a single colonized infant. This dramatic decline appears to have occurred only after demanding the attention of all personnel, by obtaining samples from their nates for culture, and enforcing hand-washing and glove-wearing procedures for all care.

In summary, we identified an unusual strain of MRSA from newborn and pediatric patients in three hospitals in Houston. All isolates were susceptible to clindamycin and were unreactive in a latex agglutination test. These two characteristics, which have not previously been found in MRSA isolates from those institutions, coupled with the results of antibiogram and DNA analyses suggest identity among the isolates.

ACKNOWLEDGMENTS

We thank Barbara Murray for assistance with the genomic DNA analysis and critical review of the manuscript and Lisa Armitage for editorial assistance.

REFERENCES


