MAJOR ARTICLE

Epidemiological Typing of Community-Acquired Methicillin-Resistant *Staphylococcus aureus* Isolates from Children in Taiwan

Chih-Chien Wang,[1] Wen-Tsung Lo,[1] Mong-Ling Chu,[1] and L. K. Siu[2]

[1]Department of Pediatrics, Tri-Service General Hospital, National Defense Medical Center, and [2]Division of Clinical Research, National Health Research Institute, Taipei, Taiwan

Background. A 1400-bed tertiary medical center in northern Taiwan was used to conduct an epidemiological study of children hospitalized with community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infection during a 5-year period.

Methods. Nineteen previously healthy children with predominantly skin and soft-tissue CA-MRSA infections were enrolled into the study. Seventeen CA-MRSA isolates were examined for antimicrobial susceptibility and molecular typing.

Results. A comparison of our results with the reported resistance rates among CA-MRSA isolates from other countries showed uniformly high macrolide resistance (100%). Of the 17 MRSA isolates in our study, all had the macrolide-lincosamide-streptogramin–constitutive phenotype and the *ermB* gene. Moreover, on the basis of molecular typing results, 11 (65%) of 17 CA-MRSA isolates were genetically related (as determined by pulsed-field gel electrophoresis), and multilocus sequence typing revealed a sequence type of 59 in all isolates. Staphylococcal toxin genes *lukS-PV* and *lukF-PV* were detected in all isolates. However, staphylococcal cassette chromosome *mec* type IV was only detected in 3 (17.6%) of 17 isolates; the remaining 14 isolates were untypeable.

Conclusions. Analysis of our data suggests the predominance of a single endemic CA-MRSA strain with high macrolide resistance in our community. Clinical improvement with incision and drainage was noted for most patients, despite treatment with an ineffective antibiotic, so the need for a change in treatment guidelines should be addressed.

The first report of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infection in the United States, which occurred in 1980, challenged the accepted view that, for patients with well-described risk factors, methicillin-resistant *S. aureus* (MRSA) is a pathogen confined to the hospital environment [1]. Risk factors for CA-MRSA infection include recent hospitalization, admission from another hospital, nursing-home residence, injection drug use, previous antimicrobial treatment, and underlying illnesses, such as cardiovascular and pulmonary disease, diabetes, malignancy, and chronic skin disease [2–8].

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Reprints or correspondence: Dr. Chih-Chien Wang, Dept. of Pediatrics, Tri-Service General Hospital, National Defense Medical Ctr., No. 325 Cheng-Kung Rd., Sec. 2, Neihu 114, Taipei, Taiwan (ndmcccw@yahoo.com.tw).

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Recent reports have described an increasing incidence of CA-MRSA infection, mostly in adult patients, with corresponding increases in the numbers of such isolates from patients without the usual risk factors for MRSA infection and colonization [2, 9–15]. Furthermore, only a few investigators in the United States and other countries have reported CA-MRSA infections in otherwise healthy children who lack the risk factors for MRSA infection [10, 16–21]. Therefore, MRSA isolates from pediatric patients with CA-MRSA infection who were admitted to our facility during the period of September 1997 through August 2002 were retrospectively evaluated to determine clinical features and outcomes. In addition, we also characterized these CA-MRSA strains and compared them with nosocomially acquired MRSA (NA-MRSA) isolates by means of molecular analyses.

MATERIALS AND METHODS

Study design and case definition. A list of all patients <18 years of age hospitalized with an *S. aureus* infection

during the period of September 1997 through August 2002 was compiled from records at the clinical microbiology laboratory at the Tri-Service General Hospital (Taipei, Taiwan). Laboratory records of susceptibility data for all *S. aureus* isolates were used to define the subset of MRSA. Duplicate isolates from the same patient were excluded. A case was considered to be community acquired if MRSA was isolated from cultures of specimens obtained within 72 h after admission to our hospital. An isolate was deemed to be nosocomially acquired when it was recovered from a specimen obtained beyond that time [16]. Risk factors for MRSA colonization or infection included the following: (1) hospitalization within 6 months before the date of MRSA isolation, (2) history of any surgical procedure, (3) history of endotracheal intubation, (4) underlying chronic disorder, (5) antimicrobial therapy within 6 months before the date of MRSA isolation, (6) presence of an indwelling venous or urinary catheter, or (7) household contact with an individual with an identified risk factor or a worker in a health care environment [20].

Hospital records were also reviewed to obtain other relevant information, including patient age, sex, site of the specimen obtained for culture, initial and definitive antimicrobial therapy, surgical intervention, duration of hospitalization, family member and/or close contact with pyoderma, previous antimicrobial therapy, antimicrobial susceptibilities of isolates, indwelling catheter or prosthetic device present at admission, and underlying diseases.

Bacterial strains and antimicrobial susceptibility. All cultures were processed at the clinical microbiology laboratory. Staphylococci were identified on the basis of colonial morphology and catalase, tube-coagulase, DNase reaction, mannitol fermentation, tellurite reduction, and oxidation-fermentation testing. Methicillin resistance was evaluated by measuring the zone of inhibition surrounding a disk containing 1 μg of oxacillin placed on Mueller-Hinton agar, according to the guidelines of the NCCLS [22]. An isolate with a zone diameter of $\leq$10 mm was considered to be resistant to oxacillin and, therefore, to methicillin. A zone diameter of >10 mm and <13 mm defined intermediate resistance, and an isolate with a zone diameter of $\geq$13 mm was considered to be susceptible [22]. The oxacillin MICs for 21 isolates—17 were CA-MRSA and 4 were NA-MRSA—were further determined using the Etest (AB Biodisk), according to the manufacturer's instructions. Methicillin-susceptible *S. aureus* (MSSA) isolates were defined having MICs of oxacillin of $\leq$2 μg/mL, and MRSA isolates were defined as having MICs of oxacillin of $\geq$8 μg/mL. Borderline-resistant *S. aureus* organisms had an oxacillin MIC at the susceptible-resistant interface of 2–8 μg/mL [23, 24]. In vitro macrolide-lincosamide-streptogramin–inducible (MLSi) phenotypes were detected by the double-disk diffusion assay [25].

During the study period, 19 consecutive children with CA-MRSA infection were compared with 20 consecutive control subjects with NA-MRSA infection. Susceptibility to clindamycin, gentamicin, erythromycin, penicillin G, vancomycin, chloramphenicol, trimethoprim-sulfamethoxazole, fusidic acid, and ciprofloxacin was determined using the disk-diffusion method.

PFGE. Seventeen CA-MRSA and 4 NA-MRSA isolates were available for further PFGE analysis, with PFGE of the chromosomal DNA performed using the enzyme *Sma*I (New England Biolabs). DNA was separated using 0.9% agarose gels at 14°C in 0.5× TBE buffer (45mM Tris, 45 mM boric acid, and 1.0 mM EDTA; pH 8.0) with a CHEF mapper XA system (Bio-Rad Laboratories) for 31.5 h, with initial and final switching times of 2 and 30 s, respectively. Gels were stained with ethidium bromide and were photographed under UV illumination. The derived patterns were analyzed using GelCompar software (Applied Maths). Results were analyzed using the unweighted pair group method for arithmetic averages and the Dice coefficient [26] with 1.2% band tolerance. MRSA isolates sharing identical or closely related PFGE profiles were considered to be the same strain type.

Multilocus sequence typing (MLST). MLST was performed as described elsewhere [27]. The allelic profiles of *S. aureus* isolates were assigned on the basis of their MLST type.

Staphylococcal cassette chromosome* mec *(SCC*mec*) typing. SCC*mec* typing was performed by PCR using sets of region-specific primers, as described elsewhere [28, 29].

***PCR amplification of* mecA, lukS-PV, lukF-PV, ermA, ermB, ermC *and* msrA.** PCR for *mecA* was performed using published sequences and temperature parameters [30]. The PCR amplification of the *lukS-PV* and *lukF-PV* genes encoding Panton-Valentine leukocidin (PVL) components is described elsewhere [31]. The presence of MLS resistance genes *(ermA, ermB, ermC,* and *msrA)* was determined according to the methods described elsewhere [32, 33].

Statistical analysis. Data collected retrospectively were analyzed with Fisher's exact test by means of SPSS software, version 10.0 (SPSS). A P value of $<.05$ was considered to be statistically significant.

RESULTS

Patient characteristics. Twenty-six patients with MRSA infection were admitted to our hospital from 1997 to 2002, with 19 (73%) meeting the inclusion criteria. It appears that isolates were more frequent in the later years of the study. All patients were clinically infected with MRSA, without any apparent epidemiological links. The characteristics of the MRSA-infected patients are presented in table 1. The median age of the 19 patients was 3 years, 8 months (range, 7 months to 10 years, 5 months), and 13 (68%) were male. None of the patients had

Table 1. Characteristics of children with community-acquired infection due to methicillin-resistant *Staphylococcus aureus,* 1997–2002.

Patient	Age	Sex	Source	Initial therapy at admission	Definitive antimicrobial therapy	Oxacillin MIC, μg/mL[a]
1	6 years, 11 months	M	Left knee carbuncle	Oxacillin and ID	Oxacillin	3
2	8 years, 10 months	M	Left knee carbuncle	Oxacillin and ID	Oxacillin	4
3	8 months	F	Forehead carbuncle	Oxacillin	Oxacillin	NA/NM
4	1 year, 7 months	M	Forehead carbuncle	Oxacillin and ID	Oxacillin	4
5	7 months	M	Right axillary abscess	Oxacillin and ID	Oxacillin	4
6	2 years, 8 months	F	Left elbow furuncle	Oxacillin and ID	Oxacillin	16
7	2 years, 6 months	M	Perirectal abscess	Oxacillin and ID	Oxacillin	3
8	10 months	F	Parapharyngeal abscess	Amox/Clv and ID	Vancomycin	NA/NM
9	9 years, 3 months	F	Multiple pyomyositis	Oxacillin and ID	Vancomycin	4
10	10 year, 5 months	M	Right big toe carbuncle	Oxacillin and ID	Oxacillin	3
11	1 year, 4 months	F	Left submandibular abscess	Oxacillin and ID	Vancomycin	4
12	5 years, 6 months	F	Perirectal abscess	Oxacillin and ID	Oxacillin	24
13	9 months	M	Nasal furuncle	Oxacillin	Oxacillin	4
14	5 years, 11 months	M	Perirectal carbuncle	Oxacillin and ID	Oxacillin	32
15	5 years, 8 months	M	Right maxillary abscess	Oxacillin	Oxacillin	3
16	2 years, 4 months	M	Perirectal carbuncle	Oxacillin and ID	Oxacillin	4
17	7 months	M	Forehead furuncle	Oxacillin and ID	Oxacillin	256
18	2 years, 4 months	M	Perirectal abscess	Oxacillin and ID	Oxacillin	32
19	1 year, 6 months	M	Forehead carbuncle	Oxacillin and ID	Oxacillin	24

NOTE. All patients were cured. Amox, amoxicillin; Clv, clavulanate; ID, incision and drainage; NA, not applicable; NM, not measured.

[a] By the Etest (AB Biodisk).

the risk factors or underlying health problems typical of MRSA infection.

CA-MRSA infection sites and treatment. Sixteen children (84%) had superficial skin or soft-tissue infections, of which 4 were extremity furuncles or carbuncles, 5 were perirectal abscesses, 5 were facial abscesses, and 2 were axillary abscesses. The remaining 3 children had deep-seated infections including multiple pyomyositis and parapharyngeal and left submandibular abscess.

Eighteen patients (95%) were initially treated for MSSA infection, with 16 patients (84%) undergoing surgical intervention as part of their initial therapy. Subsequent definitive antimicrobial therapy included administration of oxacillin to 16 patients and vancomycin to 3 patients. There were no deaths or serious long-term complications.

MRSA susceptibility patterns. Of the 17 CA-MRSA isolates obtained for determination of oxacillin MIC, only 6 (35%) were resistant to high levels of oxacillin (MIC, $\geq$8 μg/mL), with borderline resistance determined for 11 (65%; table 1). For the NA-MRSA isolates, all oxacillin MICs were $\geq$256 μg/mL. Irrespective of identified predisposing risk factors, the CA-MRSA isolates obtained from the children were more likely to be susceptible to gentamicin (89% vs. 0%; $P < .05$), chloramphenicol (43% vs. 0%; $P < .05$), trimethoprim-sulfamethoxazole (100% vs. 5%; $P < .05$), fusidic acid (100% vs. 40%; $P < .05$), and ciprofloxacin (100% vs. 0%; $P < .05$) than were the NA-MRSA isolates (table 2). No statistically significant differences were noted between the 2 groups with respect to susceptibilities to clindamycin and erythromycin. All 39 isolates were resistant to penicillin G, and none had reduced susceptibility to vancomycin. Of the 17 CA-MRSA isolates tested using the double-disk diffusion method and PCR for macrolide resistance gene detection, all strains had the MLS-constitutive (MLSc) phenotype and the *ermB* gene.

Molecular typing of the isolates. The 21 isolates (17 of which were CA-MRSA and 4 of which were NA-MRSA) collected for PFGE typing were divided into 6 pulsotypes (figure 1). On the basis of the interpretable phylogenetic tree, it appeared that 1 set of strains (11 [65%] of 17) was clustered, with homology percentages of >80%. No common epidemiological exposures were determined among patients with the same CA-MRSA strain. The pulsotypes of all 17 CA-MRSA isolates tested differed from those of the 4 NA-MRSA isolates (figure 1). All of the 21 strains tested positive for the *mecA* gene. MLST revealed that all 17 CA-MRSA strains were typed ST 59. SCC*mec* type IV was identified in only 3 of the isolates. There was no evidence of any other SCC*mec* type (type I, II, or III) in any

Table 2. Antibiotic susceptibility of community-acquired (CA) and nosocomially acquired (NA) methicillin-resistant *Staphylococcus aureus* (MRSA) isolates.

	No. (%) of CA-MRSA (n = 19)		No. (%) of NA-MRSA (n = 20)			
Antibiotic	Susceptible	Resistant	Susceptible	Resistant	95% CI	*P*
Penicillin	0	19 (100)	0	20 (100)	NA/NM	NA/NM
Erythromycin	0	19 (100)	0	20 (100)	NA/NM	NA/NM
Clindamycin	0	19 (100)	0	20 (100)	NA/NM	NA/NM
Gentamicin	17 (89)	2 (11)	0	20 (100)	2.93–41.24	<.05
Vancomycin	19 (100)	0	20 (100)	0	NA/NM	NA/NM
Chloramphenicol	3 (43)	4 (57)	0	20 (100)	2.45–14.68	.01
TMP-SMX	12 (100)	0	1 (5)	19 (95)	0.01–0.51	<.05
Fusidic acid	13 (100)	0	8 (40)	12 (60)	0.22–0.66	.001
Ciprofloxacin	12 (100)	0	0	20 (100)	NA/NM	<.05

NOTE. NA, not applicable; NM, not measured; TMP-SMX, trimethoprim-sulfamethoxazole.

of the other isolates (data not shown). PCR amplification of the PVL toxin gene was detected for all isolates.

DISCUSSION

In this study, CA-MRSA infections were demonstrated in previously healthy children without known risk factors for MRSA infection. Recent retrospective and prospective observational studies of children at the University of Chicago Children's Hospital (Chicago, IL) have also documented pediatric CA-MRSA infections in patients for which there appeared to be no identified predisposing risk [16, 20]. In both the present study and in previous investigations, skin and soft-tissue infections were the most common sources of CA-MRSA culture [2, 5–8, 14–16, 20, 34].

The major differences with respect to antibiotic susceptibility patterns between this study and others [10, 16, 18–21] are that erythromycin and clindamycin resistance were extraordinarily high in our CA-MRSA isolates. In staphylococci, there are 2 distinct phenotypes based on erythromycin resistance [35], one of which is typically related to constitutive expression of the ribosomal methylation mechanism (MLSc phenotype), whereas the other is associated with inducible expression of this mechanism (MLSi phenotype) [10, 16, 18–21]. All 17 CA-MRSA isolates had the MLSc phenotype and the *ermB* gene, a finding that differed from the report of Almer et al. [32], which showed that MRSA isolates in their study had the MLSc phenotype and the *ermA* or *erm C* genes, with no *ermB* isolates noted. In Taiwan, the strikingly high prevalence of macrolide resistance in clinical isolates of *Streptococcus pyogenes* appears to be associated with the widespread medical use of these agents [36]. This continuous and widespread utilization may have contributed to the remarkably high incidence of erythromycin and clindamycin resistance among the CA-MRSA isolates from children in this area. Although all 17 of our CA-MRSA isolates containing the *mecA* gene were subsequently confirmed as truly MRSA, only borderline resistance was demonstrated for 11 CA-MRSA isolates (65%), based on the oxacillin-MIC data [23, 24, 30]. This pattern has also been described in the study of Okuma et al. [37], in which a low oxacillin MIC was demonstrated for their CA-MRSA strains.

Although recent reports have suggested that MRSA epidemiology may be changing, with significant community transmission and infection now observed [16], it remains unclear whether this traditionally nosocomial pathogen is arising de novo in the community or merely being spread after initial transmission from hospitals or other high-risk facilities [21]. The antibiograms for our CA-MRSA isolates from patients without identifiable risk factors are clearly different from those for the analogous NA-MRSA isolates. Furthermore, the genetic background of the strains was analyzed by PFGE and MLST, as in previous reports [2, 3, 16, 21, 38]. Our PFGE study has demonstrated a difference in DNA patterns between CA-MRSA and NA-MRSA isolates, although both types contain the *mecA* gene, as determined by PCR. With regard to MLST, our CA-MRSA isolates are all of a single type (ST59), which distinguishes them from other CA-MRSA isolates from different continents (i.e., ST1 for the US clone, ST 30 for the southwest Pacific clone, and ST 80 for the European clone). This is in line with the report of Vandenesch et al. [39], who propose that there was no dissemination of a single CA-MRSA clone around the world but, rather, a simultaneous coevolution of CA-MRSA in different areas. The dendrogram of pulsed-field and MLST-determined types in this study differ from those of the MRSA isolates from the United States described by McDougal et al. [40]. This reinforces the notion that our CA-MRSA strains are indeed distinct in Taiwan and originate in the community, suggesting that the CA-MRSA strains did not emerge from the local NA-MRSA analogues, but most probably

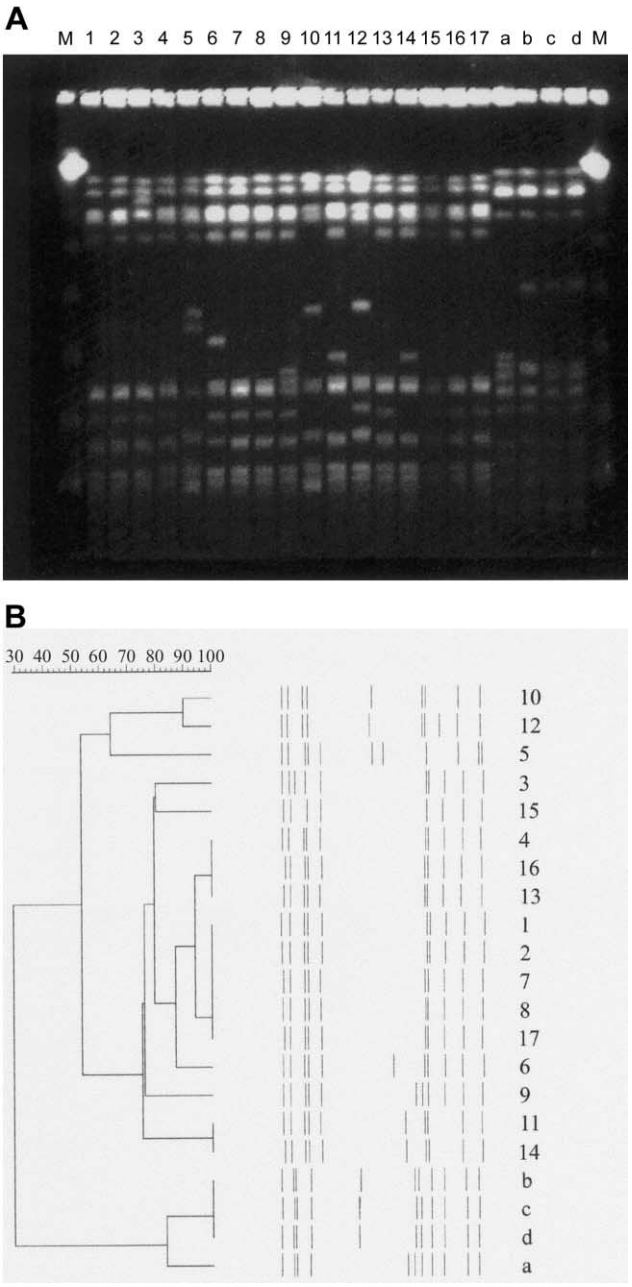

Figure 1. *A,* PFGE profiles of whole cell DNA from 17 community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) isolates *(lanes 1–17)* and 4 nosocomially acquired MRSA (NA-MRSA) isolates *(lanes a, b, c,* and *d)* digested with *Sma*I. The first and last lanes *(M)* are molecular-weight size standards. *B,* Schematic representation *(right)* of PFGE pulsotypes of 17 CA-MRSA isolates *(lanes 1–17)* and 4 NA-MRSA isolates *(lanes a, b, c,* and *d),* together with a dendrogram *(left)* showing percentage similarity for the patterns.

emerged as a result of increased selective pressure resulting from general antibiotic exposure [21, 41].

Furthermore, our CA-MRSA strains all contained the gene encoding PVL. According to the study by Vandenesch et al. [39], this toxin gene is quite specific to the CA-MRSA from each continent. There appears to be something unusual about our strains, however, with SCC*mec* type IV identified in only 3 of the CA-MRSA isolates and no evidence of any other SCC*mec* type. We are continuing to explore the possibility that a novel SCC*mec* type is present in our strains.

Only 17 CA-MRSA isolates were available for genetic background analysis in the present study. Of note, the results of this analysis indicate that most of the CA-MRSA isolates from our hospital were of a common clonal origin, with 11 (65%) of 17 in the group undistinguishable by PFGE and all having the same MLST type. On the basis of our study, it appears that 1 predominant endemic CA-MRSA strain, which is of a different form from those in other countries, is now circulating in the community and that it may be colonizing individuals not previously believed to be at risk. The most plausible explanation for this finding is that the CA-MRSA isolates in pediatric cases in northern Taiwan may have a common clonal origin. Continuous sampling of more isolates from various parts of Taiwan is required, and further molecular studies should be conducted to clarify this phenomenon.

Compared with the NA-MRSA isolates, CA-MRSA analogues obtained from children without known risk factors have tended to be more susceptible to several antimicrobials, such as gentamicin, chloramphenicol, trimethoprim-sulfamethoxazole, fusidic acid, and ciprofloxacin. Thus, the emergence of CA-MRSA as a cause of common infections may warrant a change in the initial selection of antibiotics to ensure appropriate coverage. Most authorities [16, 20, 21] recommend clindamycin as the initial empirical therapy for non–critically ill patients with suspected CA-MRSA infection waiting for susceptibility testing. This strategy is imperfect, however, because most of the CA-MRSA isolates in this study were resistant to clindamycin. From a management standpoint, our findings indicate that skin and soft-tissue infections may resolve well in healthy children with incision and drainage, despite ineffective antibiotic therapy. On the basis of statistical analysis of our sample population, no significant association was demonstrated between ineffective antimicrobial therapy and clinical outcome. This confirms the findings of Lee et al. [42], who concluded similarly that clinical improvement with surgical drainage was demonstrated in most patients, despite treatment with nonsusceptible antibiotics. Thus, the role of surgery and host defenses in the resolution of staphylococcal skin infections is also important.

Of particular importance is the future impact of CA-MRSA in our community or, indeed, elsewhere, should this organism become more prevalent. These MRSA strains can complicate management of pediatric infection because they occur in unexpected circumstances [16, 19, 43]. The true prevalence of CA-MRSA remains unknown, however, because no systematic, population-based study of community isolates of *S. aureus* exists [44]. Furthermore, significant regional variation is likely.

Thus, a significant increase in incidence would necessitate changes to the prescribing guidelines for CA-MRSA infections. Although most CA-MRSA isolates were susceptible to several antimicrobial agents, selection of alternative agents must remain dependent on local susceptibility patterns. The clinicians should be aware that β-lactam antimicrobials can no longer be relied on as empirical therapy for invasive CA-MRSA infections, and the treatment of noninvasive CA-MRSA infections should not routinely require the use of vancomycin [41]. Our results also support those of Lee et al. [42], who indicated that surgical drainage without adjunctive antibiotic therapy was effective for management of small CA-MRSA skin and soft-tissue abscesses in healthy children.

In conclusion, continuing prospective surveillance of CA-MRSA isolates and characterizations of the risk factors for CA-MRSA infection and the nasal carriage rate are required to further analyze this epidemiological trend. Our findings also highlight the importance of rapid diagnosis for identification of CA-MRSA isolates. Clinical improvement with incision and drainage was noted in most patients, despite treatment with an ineffective antibiotic, so the need for a change in treatment guidelines should be addressed.

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