A Clinical and Microbiological Comparison of *Staphylococcus aureus* Toxic Shock and Scalded Skin Syndromes in Children

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**Background.** This study was designed to compare the clinical characteristics, toxin expression, virulence factors, and antimicrobial susceptibilities of staphylococci isolated from Taiwanese children with staphylococcal toxic shock syndrome (STSS) and staphylococcal scalded skin syndrome (SSSS).

**Methods.** Demographic characteristics, hospital course, and outcomes of the children were analyzed. Toxic-specific and virulence genes of the staphylococci were detected by polymerase chain reaction amplification. Antimicrobial susceptibilities were determined by disk diffusion and the Etest.

**Results.** *Staphylococcus aureus* was isolated from 16 children (6 in the STSS group and 10 in the SSSS group). Children with STSS tended to be older than those with SSSS, had a longer duration of hospitalization, and a much higher mortality rate. Community-associated methicillin-resistant *S. aureus* was isolated from 11 (68.8%) of 16 children. All of these isolates contained the *ermB* and *mecA* genes, but none had the *mefA* gene. All 16 isolates tested positive for the *fnbA* gene. The *pvl* and *seb* genes were more frequently found among *S. aureus* from the STSS group, compared with *S. aureus* from the SSSS group. We found that 67% (4 of 6) of the STSS isolates were genetically related. All of the *S. aureus* isolates were susceptible to vancomycin, gentamicin, doxycycline, and trimethoprim-sulfamethoxazole. Most isolates were resistant to clindamycin (63%), oxacillin (69%), and clarithromycin (81%).

**Conclusions.** The most distinguishing feature of these isolates is the greater frequency of *pvl* and *seb* carriage among those from the STSS group. Most of the isolates were community-associated methicillin-resistant *S. aureus* that were highly resistant to macrolides but susceptible to trimethoprim-sulfamethoxazole. Vancomycin remains the initial drug of choice for treatment of STSS and SSSS. More studies are needed to determine the efficacy of trimethoprim-sulfamethoxazole in children with these syndromes.

*Staphylococcus aureus* causes a variety of infectious diseases, ranging from superficial skin infections to severe, toxin-mediated systemic infections. *S. aureus* produces many extracellular products, including toxins, that affect host cell function or morphology. Staphylococcal toxic shock syndrome (STSS) and staphylococcal scalded skin syndrome (SSSS) are 2 distinct toxin-mediated diseases with very distinct cutaneous features.

STSS was first described in 1978 [1]. Toxic production is associated with fever, a desquamative skin rash, hypotension, and multiorgan system failure [2]. During the 1980s, most cases of STSS were associated with menstruation [3]. Currently, nonmenstrual cases of STSS occur approximately as often as the classic menstrual cases. The nonmenstrual cases are usually caused by secondary colonization by toxin-producing strains of *S. aureus* in traumatized skin or on mucosal surfaces [2]. TSST-1, the most common STSS toxin, is responsible for ~75% of the STSS cases. The remaining cases, particularly those of the nonmenstrual variety, are more commonly associated with enterotoxins [4–6]. TSST-1 and 3 other toxins produced by *S. aureus* (Panton-Valentine leukocidin [PVL] and 2 fibronectin-binding proteins [FnbA and FnbB]) have been identified, and their corresponding genes have been described [7–10].
The presence of the genes encoding these toxins is associated with a severe clinical course [11–13].

SSSS is characterized by the appearance of bullae and the separation of extended areas of epidermis after infection by exfoliative, exotoxin-producing staphylococci [14]. Two human variants of the toxins, exfoliative toxins A and B (ETA and ETB, respectively), were described in 1975 [15]. These exotoxins cause intraepidermal cleavage, bullae formation, and a positive Nikolsky sign. S. aureus is not present in the skin lesions but is confined to the primary site of infection, usually the nasopharynx. There may be bacteremia, as well. This study was designed to correlate the clinical syndromes of STSS and SSSS in children infected or colonized with S. aureus strains positive for genes encoding for toxins, virulence factors, and antimicrobial resistance. We also analyzed the antimicrobial resistance patterns among the isolates.

PATIENTS, MATERIALS, AND METHODS

Study design and case definition. Children who had clinically defined STSS and SSSS between May 2001 and August 2004 were included in the study. All patients had a specimen from the infected site, a nasopharyngeal swab specimen, and/or a blood specimen that yielded S. aureus on culture. We obtained demographic and clinical information, including age, sex, site of the cultured specimen, initial and definitive antimicrobial therapy, dates of hospital admission and discharge, duration of hospital stay, surgical intervention, underlying medical conditions, and outcome. STSS was defined as fever, hypotension, and rash followed by desquamation. SSSS was defined as large superficial bullae that exfoliated with a positive Nikolsky sign. Community-associated infection was defined as isolation of S. aureus from nonhospitalized patients or within 72 h after hospitalization.

Bacterial strains and antimicrobial susceptibility. S. aureus was identified by the standard microbiologic methods. Susceptibility testing was performed by disk-diffusion in accordance with NCCLS guidelines [16]. MICs of oxacillin, clarithromycin, clindamycin, doxycycline, gentamicin, vancomycin, and trimethoprim-sulfamethoxazole were further determined by the Etest (AB Biodisk), according to the manufacturer’s instructions.

Detection of genes encoding for toxins and virulence factors. Genomic DNA was obtained by means of a Qiapm DNA Mini Kit protocol (Qiagen). The synthetic oligonucleotide primers for the PCR amplification of the sea, seb, sec, sed, see, seg, seh, sei, seq, eta, etb, pvl, fnbA, fnbB, and tst genes encoding SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SEQ, ETA, ETB, PVL, FnBA, FnBB, and TSST-1, respectively, have been described elsewhere [7, 17, 18].

PCR amplification of drug resistance genes. PCR for meca was performed using published sequences and temperature conditions. The presence of macrolide-lincosamide-streptogramin B and M phenotype resistance genes (ermB and mefA) were determined according to the methods described elsewhere [19–22].

PFGE. Six S. aureus isolates from children with STSS were available for further PFGE analysis, with PFGE of the chromosomal DNA performed using Smal. The relatedness of strains was determined by comparison of restriction fragment–length polymorphism findings, in accordance with the guidelines published by Tenover et al. [23]. PFGE patterns resulting in 2–3-band differences were considered to be closely related, those with 4–6-band differences were considered to be possibly related, and those with >7-band differences were considered to be unrelated [23].

Statistical analysis. The Mann-Whitney U test was used for continuous variables without normal distribution, and the χ² test or Fisher’s exact test was used for dichotomous variables (SPSS software, version 11.5; SPSS). A P value of <.05 was considered to be statistically significant.

RESULTS

Patient characteristics. Sixteen children were identified as having either STSS (n = 6) or SSSS (n = 10) due to community-associated S. aureus. Demographic and clinical characteristics of the children are summarized in table 1. Children with STSS were significantly older than those with SSSS (median age, 12.6 vs. 4.2 years; P = .03). The sites of clinical infections differed between the STSS group and the SSSS group. Bloodstream infections were more common in the STSS group (33% vs. 0%; P = .13). The sex ratios in the study groups were similar. The duration of hospital stay was significantly longer in the STSS group (median duration, 8 vs. 4 days; P = .02). Two children in the STSS group received surgical intervention for osteomyelitis and necrotizing fasciitis, and another child from this group died. In contrast, all children in the SSSS group...
survived without long-term sequelae. The case-fatality rates in the STSS and SSSS groups were 17% (1 of 6 children) and 0%, respectively.

**Toxins and virulence factors.** The distribution of genes encoding toxins and virulence factors is shown in Table 2. Of the 6 isolates from patients with STSS, 2 carried the *pvl*, *sea*, *seb*, and *etb* genes; 1 carried the *fnbA*, *sea*, and *seb* genes; 1 carried the *fnbA*, *seb*, and *etb* genes; 1 carried the *sea*, *eta*, and *etb* genes; and only 1 carried the *tst* gene. A comparison of the frequency of carriage of genes encoding for toxins and virulence factors revealed that a greater proportion of isolates from the STSS group carried the *pvl* and *seb* genes (*P* = .008) (Table 2). The frequency of carriage of other genes did not significantly differ between the groups. A greater percentage of isolates from children with SSSS carried the *fnbB* gene (50% vs. 0%; *P* = .09). All 16 isolates tested positive for the *fnbA* gene, and a large percentage of isolates from both groups carried the *etb* gene (83% in the STSS group vs. 90% in the SSSS group; *P* = .5).

**Antimicrobial susceptibility pattern.** All community-associated methicillin-resistant *S. aureus* (MRSA) isolates were *mecA* positive, and all 16 isolates in the study carried the *ermB* gene. No isolates carried the *ermA* gene (Table 3). The proportion of MRSA isolates was similar for both groups (*P* = 1.0). Table 4 summarizes the results of tests for susceptibility to 7 antimicrobial agents for all isolates. There were no significant differences in antibiotic susceptibility patterns for the staphylococci isolated from the 2 groups.

**PFGE typing.** Isolates from 5 children in the STSS group underwent PFGE typing and were divided into 3 types (figure 1). On the basis of the interpretable phylogenetic tree, it appeared that 1 set of isolates (4 [67%] of 6) was clustered, with >80% homology (data not shown).

**DISCUSSION**

*S. aureus* can produce and secrete a variety of enzymes and toxins. Several of these have been shown to be associated with the pathogenesis of staphylococcal skin infections. TSST-1 and enterotoxins are associated with STSS. In contrast, the exfoliative toxins are more closely associated with SSSS [14, 15]. These toxins behave as superantigens by releasing cytokines that stimulate T lymphocytes [24].

In this study, we found that the *seb* gene appears to be an important factor for the development of nonmenstrual STSS in children. We were unable to demonstrate a statistically significant difference in the frequency of exfoliatin genes *eta* and *etb* among *S. aureus* isolates recovered in each group. Previous studies have shown that STSS is associated with the presence of TSST-1 or enterotoxins [4–6]. We have no ready explanation for the difference between our findings and those in other reports. In our patients, there might have been correlation between the presence of exfoliatin toxins and distinct clinical features of STSS, such as rash, desquamation, and even shock.

All isolates from patients in the STSS and SSSS groups carried the *fnbA* gene. Nashev et al. [15] reported that persistent nasal carriage of strains harboring *fnbA* or *fnbB* genes increases the risk for subsequent invasive infections. Peacock et al. [12] found that isolates from healthy carriers were somewhat less likely to harbor *fnbA* (87% of isolates) than were isolates from persons with invasive disease (98% of isolates). The primary action of FnB proteins is to bind to fibronectin in the extracellular matrix, thus facilitating the adhesion of *S. aureus* to the host cell [25]. Staphylococcal endocarditis, primary septic arthritis, and osteomyelitis have been reported to be associated with the *fnbA* gene [13]. In the current study, we found a similar association among isolates from patients with STSS or SSSS. We also found that isolates from the SSSS group had a greater frequency of *fnbB* gene carriage. The relationship between the *fnbB* gene and disease severity still requires much further study.

**Table 2.** Genes encoding for toxins and virulence factors in community-associated *Staphylococcus aureus* isolates from children with staphylococcal toxic-shock syndrome (STSS) or staphylococcal scalded skin syndrome (SSSS).

<table>
<thead>
<tr>
<th>Gene(s)</th>
<th>No. (%) of isolates with the gene(s)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>STSS group (n = 6)</td>
</tr>
<tr>
<td>sea</td>
<td>5 (83)</td>
</tr>
<tr>
<td>seb</td>
<td>4 (67)</td>
</tr>
<tr>
<td>sec</td>
<td>1 (17)</td>
</tr>
<tr>
<td>seg</td>
<td>1 (17)</td>
</tr>
<tr>
<td>sei</td>
<td>1 (17)</td>
</tr>
<tr>
<td>seg, sei</td>
<td>1 (17)</td>
</tr>
<tr>
<td>eta</td>
<td>1 (17)</td>
</tr>
<tr>
<td>etb</td>
<td>5 (83)</td>
</tr>
<tr>
<td>pvl</td>
<td>4 (67)</td>
</tr>
<tr>
<td>fnbA</td>
<td>6 (100)</td>
</tr>
<tr>
<td>fnbB</td>
<td>0</td>
</tr>
<tr>
<td>tst</td>
<td>1 (17)</td>
</tr>
</tbody>
</table>

**NOTE.** The following genes were not detected: *sed, see, seh, and sej.*
Table 4. Antimicrobial susceptibility profiles for 16 community-associated *Staphylococcus aureus* isolates.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>MIC(90) (range), (\mu \text{g/mL})</th>
<th>No. (%) of resistant isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin</td>
<td>&gt;256 (0.75 to &gt;256)</td>
<td>11 (69)</td>
</tr>
<tr>
<td>Clarithromycin</td>
<td>&gt;256 (0.25 to &gt;256)</td>
<td>13 (81)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>&gt;256 (0.125 to &gt;256)</td>
<td>10 (63)</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>4 (0.25–4)</td>
<td>0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1.5 (0.5–1.5)</td>
<td>0</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>2 (1.0–2)</td>
<td>0</td>
</tr>
<tr>
<td>TMP-SMX</td>
<td>0.94 (0.047–1)</td>
<td>0</td>
</tr>
</tbody>
</table>

**NOTE.** TMP-SMX, trimethoprim-sulfamethoxazole.

These toxins create lytic pores on the cell membrane of neutrophils [7, 8]. The production of PVL may contribute to the unique pathogenicity of community-associated MRSA. Previous studies have shown that binding of PVL component to neutrophils induces the release of neutrophil chemotactic factors (i.e., IL-8 and leukotriene B4) [26]. The combination of neutrophil chemotaxis, release of inflammatory mediators, and karyorrhexis promotes tissue necrosis and abscess formation. In studies reported from Europe, the *pvl* gene was associated with community-associated staphylococcal skin infections and necrotizing pneumonia [8, 11, 27]. We found that nonmenstrual STSS but not SSSS in children was also closely associated with the *pvl* gene. To our knowledge, this is the first observation that suggests a possible association between the *pvl* gene and the development of STSS in children. Further studies are needed to clarify the role of the *pvl* gene in STSS and SSSS [28].

In this report, we describe several genetic characteristics of *S. aureus* isolated from children with STSS. It is likely that there are strains circulating in our community that carry STSS-associated genes. Genetic exchange and spread of these strains may lead to outbreaks of STSS. Continuous monitoring of staphylococci from various parts of Taiwan, combined with molecular studies, is needed to assess this potential risk.

We found no statistically significant difference in the frequency of MRSA in the STSS and SSSS groups \((P = 1.0)\). In Taiwan, the strikingly high prevalence of macrolide resistance among clinical isolates of *Streptococcus pyogenes* and *Streptococcus pneumoniae* is associated with the excessive use of antibiotics [29]. Excessive use of macrolides probably accounts for the remarkably high incidence of erythromycin resistance among the *S. aureus* isolates from children in this country. All community-associated MRSA isolates in this study were sus-

**Figure 1.** PFGE of *Sma*I-digested genomic DNA of 5 community-associated *Staphylococcus aureus* isolates from 5 children with staphylococcal toxic-shock syndrome. An isolate from another child with staphylococcal toxic-shock syndrome could not be analyzed despite repeated attempts and is not included.
ceptible to trimethoprim-sulfamethoxazole. A clinical trial is
needed to establish the efficacy of trimethoprim-sulfameth-
ozole for treatment of staphylococcal skin infection in chil-
dren. Although empirical treatment with broad-spectrum an-
timicrobials and de-escalation of therapy after the pathogen
is identified are justified when there is a suspicion of *S. aureus*
harboring the *pvl* gene [30], it should be noted that PVL pro-
duction is not a specific attribute of community-acquired
MRSA. Whether routine testing for the *pvl* gene would influ-
ence treatment decisions needs further evaluation.

In conclusion, the present study demonstrates that *S. aureus*
harboring the *pvl* and *seb* genes are closely associated with STSS
in previously healthy children. The *fsbA* and *etb* genes were
expressed with a high frequency among isolates from the STSS
and SSSS groups. Early detection of these strains in the com-
munity, measures to control their spread, and development of
drugs that block the virulence factors may be needed to prevent
outbreaks of STSS. Additional studies are needed to determine
the expression of toxins in strains carrying virulence genes for
STSS and SSSS.

Acknowledgments

We thank Dr. Calvin M. Kunin for providing invaluable suggestions and
a critical review of the manuscript. We also thank Ya-Lan Lin and Li-Rong
Wang for skillful technical assistance during this study.

Financial support. National Science Council, Taiwan (NSC93-2314-B-
006-059) and National Health Research Institutes (NHRI-EX94-9429SP).

Potential conflicts of interest. All authors: no conflicts.

References

The staphylococci in human disease. New York, NY: Churchill Liv-
3. Berkley SF, Hightower AW, Broome CV, Reingold AL. The relationship
of tampon characteristics to menstrual toxic shock syndrome. JAMA
5. Schlievert PM. Staphylococcal enterotoxin B and toxic-shock syndrome
toxin-1 are significantly associated with non-menstrual TSS. Lancet
lococcal enterotoxin A and C causing toxic shock syndrome [letter].
ylococcus aureus* strains producing synergohemotoxin toxins. J Med
Valentine leukocidin–producing *Staphylococcus aureus* in primary skin
gene for a fibronectin-binding protein from *Staphylococcus aureus*.
10. Jonsson K, Signas C, Muller HP, Lindberg M. Two different genes encode
fibronectin binding proteins in *Staphylococcus aureus*; the com-
plete nucleotide sequence and characterization of the second gene. Eur
aureus* strains carrying gene for Panton-Valentine leukocidin and highly
lethal necrotising pneumonia in young immunocompetent patients.
hesin and toxin genes in natural populations of *Staphylococcus aureus*.
13. Peacock SJ, Day NP, Thomas MG, Berendt AR, Foster TJ. Clinical
isolates of *Staphylococcus aureus* exhibit diversity in *fsc* genes and
15. Kondo I, Sakurai S, Sarai Y, Futaki S. Two serotypes of exfoliatin and
their distribution in staphylococcal isolates from patients with
16. NCCLS. Performance standards for antimicrobial susceptibility testing.
17. Martinez-Aguilar G, Avalos-Mishaan A, Hulten K, Hammerman W,
Mason EO Jr, Kaplan SL. Community-acquired, methicillin-resistant
and methicillin-susceptible *Staphylococcus aureus* musculoskeletal in-
M. Distribution of virulence genes of *Staphylococcus aureus* isolated
strains of methicillin-resistant *Staphylococcus aureus* using polymerase
20. Mongkolrattanothai K, Boyle S, Kahana MD, Daum RS. Severe *Staph-
ylococcus aureus* infections caused by clonally related community-ac-
mquired methicillin-susceptible and methicillin-resistant isolates. Clin
and molecular characterization of community-acquired methicillin-
resistant *Staphylococcus aureus*. Diagn Microbiol Infect Dis 2002; 43:
225–32.
22. Shorttirde KD, Flamm CK, Ramer N, Beyer J, Tanaka SK. Novel mech-
anism of macrolide resistance in *Streptococcus pneumoniae*. Diagn Mi-
DNA restriction patterns produced by pulsed-field gel electrophoresis:
24. Keb M. Bacterial pyrogenic exotoxins as superantigens. Clin Microbiol
25. Dziewanowska K, Patti JM, Deobald CF, Bayles KW, Trumble WR,
Bohach GA. Fibronectin binding protein and host cell tyrosine kinase
are required for internalization of *Staphylococcus aureus* by epithelial
aureus* leukocidins on inflammatory mediator release from human
resistant *Staphylococcus aureus* infections in France: emergence of a
single clone that produces Panton-Valentine leukocidin. Clin Infect Dis
28. Gorak EJ, Yamada SM, Brown JD. Community-acquired methicillin-
resistant *Staphylococcus aureus* in hospitalized adults and children with-
29. Husek PR, Liu CY, Luh KT. Current status of antimicrobial resistance
30. Wargo KA, Eiland EH. Appropriate antimicrobial therapy for com-
munity-acquired methicillin-resistant *Staphylococcus aureus* carrying