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Microbiological Characteristics of Community-Associated *Staphylococcus aureus* Causing Uncomplicated Bacteremia and Infective Endocarditis▽†

Chia-Yu Chi,[1,2,3] Shih-Min Wang,[4] Chia-Chun Lin,[1] and Ching-Chuan Liu[3]*

Division of Clinical Research, National Health Research Institutes, Tainan, Taiwan[1]; Graduate Institute of Clinical Medicine, National Cheng Kung University, Tainan, Taiwan[2]; and Departments of Pediatrics[3] and Emergency Medicine,[4] College of Medicine, National Cheng Kung University and Hospital, Tainan, Taiwan

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Staphylococcus aureus **is an important cause of community-associated bacteremia (SAB) and infective endocarditis (IE). No significant differences in distribution or frequency of genes encoding virulence factors, including genes encoding adhesins, were found between isolates from the IE and SAB groups (12 IE and 10 SAB patients).**

Staphylococcus aureus bacteremia (SAB) is a serious infection in both adult and pediatric patients (21). One of the most devastating complications of SAB is infective endocarditis (IE), which carries a high risk of morbidity and mortality despite aggressive therapy (12). *S. aureus* produces many extracellular toxins and virulence factors that elicit immune responses. Exotoxins, such as Panton-Valentine leukocidin (PVL), and exfoliative toxins, toxic shock syndrome toxin 1 (TSST-1), and enterotoxins (SEs) with superantigenic properties cause severe diseases (2, 14). Circulating *S. aureus* can adhere to the undamaged endothelium either directly through adhesion-receptor interactions or by bridging ligands of plasma and extracellular matrix proteins fibrinogen, fibronectin, and collagen. Attached bacteria may then cause further aggregation of platelets, leading to an enlargement of the infected valvular vegetation (15). *S. aureus* produces several adhesins, including collagen-, fibrinogen-, and fibronectin-binding proteins. Collagen-binding adhesin (CNA) is important in the development of endocarditis in animal models (11). Clumping factors A and B (ClfA and -B) for fibrinogen- and fibronectin-binding proteins (FnBP) are mediators of *S. aureus* adherence to platelets (10, 18). Both Clf and FnBP have also been shown to play critical roles in experimental *S. aureus* IE (19).

This study was designed to determine whether there are differences in the distributions of *S. aureus* genes encoding toxins and virulence factors and antimicrobial resistance patterns in patients with uncomplicated SAB compared to IE.

The population consisted of patients with community-associated *S. aureus* bacteremia who were admitted to the National Cheng Kung Hospital from 2000 to 2007. Demographic and clinical information was obtained from hospital records by using a standardized form. Uncomplicated SAB was defined as the isolation of *S. aureus* from the blood of patients without endocarditis, evidence of hematogenous spread or an overt source of infection. *S. aureus* IE was defined according to the modified Duke criteria (13). Community-associated bacteremia was defined as a positive blood culture for *S. aureus* obtained at the time of or within 48 h of admission. Patients were excluded if they had any established hospital-associated risk factors (9). To focus as much as possible on the influence of genetic microbial factors in the pathogenesis of IE, we excluded patients with underlying heart disease.

Antimicrobial susceptibility was determined by the agar dilution method performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines (17). Erythromycin-resistant strains were further studied for inducible clindamycin resistance.

The synthetic oligonucleotide primers for the PCR amplification of genes encoding the toxin, adherence, and virulence factors were used as described elsewhere (16). PCR for *mecA* and macrolide-lincosamide-streptogramin resistance genes (*ermA*, *ermB*, and *ermC*) was performed using published sequences and temperature conditions (1). The characterization of the staphylococcal cassette chromosome *mec* (SCC*mec*) element was performed according to a published multiplex PCR method (3).

Sequence types (ST) were assigned with reference to the multilocus sequence typing database website (http://www.mlst.net). Pulsed-field gel electrophoresis was performed using the enzyme SmaI. The derived patterns were analyzed using GelCompar software (Applied Maths, Kortrijk, Belgium). Results were analyzed using the unweighted-pair group method for arithmetic averages and Dice coefficient with 1.2% band tolerance (6).

The Mann-Whitney U test was used for continuous variables without normal distribution and the χ^2 or Fisher exact test for dichotomous variables (SPSS software, version 11.5). A P value of <0.05 was considered to be statistically significant.

Twenty-two patients were identified as having either *S. aureus* IE ($n = 12$) or uncomplicated SAB ($n = 10$) through blood culture and comprehensive echocardiography examina-

* Corresponding author. Mailing address: Department of Pediatrics, College of Medicine, National Cheng Kung University and Hospital, 138 Sheng-Li Road, Tainan 704, Taiwan. Phone: 886-6-2353535. Fax: 886-6-2753083. E-mail: liucc@mail.ncku.edu.tw.

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TABLE 1. Genes encoding toxins and virulence factors in isolates of community-associated *Staphylococcus aureus* obtained from patients with IE and uncomplicated SAB[a]

Gene	No. (%) of indicated cases with gene sequence		*P* value
	IE (n = 12)	SAB (n = 10)	
sea	4 (33)	1 (10)	0.22
seb	3 (25)	6 (60)	0.11
sec	1 (8)	1 (11)	0.71
seg	1 (8)	0	0.55
sei	1 (8)	0	0.55
seg and *sei*	1 (8)	0	0.55
eta	0	0	
etb	0	0	
pvl	2 (17)	2 (20)	0.63
fnbA	12 (100)	9 (90)	0.46
fnbB	2 (17)	1 (10)	0.57
cna	5 (42)	2 (20)	0.27
clfA	10 (83)	8 (80)	0.63
clfB	12 (100)	10 (100)	
tst	1 (8)	0	0.55

[a] The following genes were not identified among any of the staphylococci: *sed*, *see*, *seh*, and *sej* genes.

tion. The mean ages were similar between groups ($P > 0.05$). Nine patients in the IE group received surgical intervention. The duration of hospital stay was significantly longer for the IE group (36.1 versus 6.8 days; $P < 0.001$).

The frequencies of carriage of the detected virulence genes, including *fnb* and *clf*, did not differ significantly between groups (Table 1). FnBPs and Clf are *S. aureus* microbial surface components reacting with adherence matrix molecules that are thought to participate in the development of experimental endocarditis (19). However, some reference strains of *S. aureus* were found to be invasion deficient because of regulatory defects (20). Cheung et al. demonstrated that the inactivated staphylococcal accessory regulator gene (*sar*) decreased infectivity in the rabbit model of endocarditis because the inactivated mutant has decreased expression of surface FnBPs (4). Therefore the emergence of *S. aureus* strains with certain adhesion genes is not the only factor responsible for *S. aureus* IE. The systems involved in the regulation of transcription of the virulence genes may play a more important role in the pathogenesis of *S. aureus* IE. Exfoliative toxins A and B (ETA and ETB), encoded by *eta* and *etb*, respectively, are frequently associated with staphylococcal scalded skin syndrome (SSSS) (5). None of the isolates in this study harbored an *eta* or *etb* gene. This indicates that exfoliative toxins do not play a role in *S. aureus* bloodstream infections.

Only four (18%) isolates in this study carried *pvl*, and all were methicillin (meticillin)-resistant *S. aureus* (MRSA) strains. Community-acquired MRSA (CA-MRSA) containing *pvl* is associated with severe infections, such as primary skin infections, necrotizing pneumonia, and nonmenstrual staphylococcal toxic shock syndrome (5, 14), but not associated with bacteremia (7, 8). Our finding also supports that *pvl* has no special association with the development of bloodstream infection or its complications, such as IE.

MRSA was isolated from 8/22 (36%) patients. There were no significant differences in antibiotic susceptibility patterns between the two groups (see the supplemental material). Most of the macrolide-resistant isolates were MRSA and possessed the *ermB* gene. One methicillin-susceptible *S. aureus* (MSSA) isolate from the IE group possessed the *ermC* gene and showed inducible clindamycin resistance.

Among eight CA-MRSA isolates, SCC*mec* type IV was identified in four isolates, and the others were SCC*mec* type V_T. On the basis of the interpretable phylogenetic tree, one set of CA-MRSA strains (4/8 [50%]) was clustered, with >80% homology (see the supplemental material). Multilocus ST 59 is the major genetic background of CA-MRSA isolates. This common clone is distinct from other CA-MRSA strains isolated from different countries but is consistent with reports from northern Taiwan (22). They proposed that a single CA-MRSA clone did not spread around the world but that there was simultaneous coevolution of CA-MRSA in different regions.

In conclusion, this study demonstrates that virulence and toxin genes are frequently present in staphylococcal blood isolates. There were no significant differences in frequency of genes encoding such virulence factors studied herein between isolates from patients with IE and those from patients with SAB.

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