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Original Article

Distribution of virulence genes in bacteremic methicillin-resistant *Staphylococcus aureus* isolates from various sources

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Abstract *Background/purpose:* Methicillin-resistant *Staphylococcus aureus* (MRSA) can encode proteins which directly bind bacteria to many tissues and medical devices or catheters to trigger pathogenesis. However, the relationship between genetic backgrounds and virulent factors in MRSA isolates remained incompletely understood yet.
Methods: MRSA isolates were collected from blood cultures of patients with infective endocarditis, bone/joint infection, skin/soft tissue infection, or catheter-related bacteremia in hemodialysis at a tertiary medical center between 2005 and 2011. MRSA isolates were characterized by the methods of *spa*, multilocus sequence, and staphylococcal cassette chromosome *mec* (SCC*mec*) typing. Identification of virulence gene expression was measured by Power SYBR Green PCR Master Mix.
Results: Overall collected were 136 MRSA bacteremic isolates, including those from the cases of infective endocarditis (n = 23), bone/joint infection (n = 49), skin/soft tissue infection (n = 20), or catheter-related bacteremia in patients with acute kidney injury or end-stage renal stage receiving hemodialysis (n = 54). CC8-ST239-MRSA-SCC*mec* type III-*spa* type t037 was the most prevalent type observed in all of 136 MRSA bacteremic isolates. The prevalent genes in the group of infective endocarditis were *clfA*, *clfB*, *fnbA*, *ebpS*, *eap*, *emp*, *sae*, and *eno*; bone/joint infections *clfA*, *emp*, *sae*, and *eno*; skin/soft tissue infection *eno*; hemodialysis catheter-related bacteremia *clfA* and *sae*. The distribution of each gene was not statically different among four groups.
Conclusions: A major MRSA lineage, CC8-ST239-MRSA-SCC*mec* type III-*spa* type t037, is noted among bacteremic MRSA isolates. No disease-specific virulent genes can be identified.

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Introduction

Staphylococcus aureus is a virulent Gram-positive coccus and is well known for its disease-causing capabilities. Irrespective of methicillin-resistant (MRSA) or methicillin-susceptible (MSSA) phenotype, it can cause numerous infections in humans, such as superficial lesions, deep-seated infections, osteomyelitis, and infective endocarditis.[1–3]

MRSA exhibits antibiotic resistance that is mediated by extrachromosomal genetic elements, *i.e.*, staphylococcal cassette chromosome *mec* (SCC*mec*). It includes an antibiotic resistance gene, *mec*A, which encodes a protein that stops β-lactam antibiotics from inactivating transpeptidases that are critical for cell wall synthesis.[4] To compare the genetic background of MRSA, multilocus sequence typing (MLST), SCC*mec* typing, and *spa* typing have been effective in distinguishing different *S. aureus* lineages.[4–6]

The virulence factors of *S. aureus* are extensive with both structural proteins and secreted products, which play a role in pathogenesis of infections. *S. aureus* expresses microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) that mediate adherence to host tissues.[7] Those adhesion proteins include clumping factors A and B (*clfA* and *clfB*), fibronectin-binding proteins (*fnBPs*), collagen-binding adhesin (*cna*), serine-aspartate repeat proteins (*sdr*), elastin-binding protein (*ebpS*), and enolase (*eno*).[7–13] Other adhesins are extracellular adherence protein (Eap) and extracellular matrix protein-binding protein (Emp), which have the ability to bind to extracellular matrix proteins.[14–17] *S. aureus* exoprotein expression (*sae*) system can regulate the expression of genes involved in adhesion and invasion (such as those encoding fibronectin-binding proteins and fibrinogen-binding proteins).[18] Variable *S. aureus* strains may express different MSCRAMMs, which play a key role in the initiation of endovascular infection, bone/joint infection, and prosthetic device-associated infection.[14,19]

This study aimed to investigate the molecular features of various MRSA strains and to determine the genetic background and distribution of virulent genes among MRSA bacteremic isolates from different infectious sources.

Methods

MRSA bacteremic isolates

This study was conducted at a 2900-bed tertiary medical center, Taipei Veterans General Hospital, between 2005 and 2011. Patients equal to or older than 20 years of age with MRSA bacteremia and infective endocarditis, bone/joint infection, skin/soft tissue infection, or catheter-related bacteremia in patients with acute kidney injury or end-stage renal stage receiving hemodialysis were included. Infective endocarditis was diagnosed according to the modified Duke criteria.[20] Bone/Joint infection was diagnosed by radiology, nuclear medicine, or pathology. Skin/Soft tissue infection was impressed by the presence of redness, swelling, heat, and tenderness. Catheter-related bacteremia was referred to the presence of Permcath-related or central venous catheter-related bacteremia in patients receiving emergent or long-term hemodialysis.[21] Patients who met the criteria of more than one above-mentioned diseases were excluded. In those suffering two or more episodes of MRSA bacteremia during the study period, only the first episode was included in our analysis. This study was approved by the Institution Review Board of Taipei Veterans General Hospital, and informed consent was waived.

Multi-locus sequence typing (MLST)

The MRSA MLST was performed using internal fragments of seven housekeeping genes, including the genes encoding carbamate kinase (*arcC*), shikimate dehydrogenase (*aroE*), glycerol kinase (*glpF*), guanylate kinase (*gmk*), phosphate acetyltransferase (*pta*), triosephosphate isomerase(*tpi*), and acetyl coenzyme A acetyltransferase (*yqiL*). The primers were synthesized according to the MLST database accessible via http://www.mlst.net.

Multiplex polymerase chain reaction (PCR) for MRSA SCC*mec* types I–V

MRSA isolates were examined for SCC*mec* types by multiplex PCR developed by Boye et al.[22] The PCR reaction was run in a thermal cycler (Perkin-Elmer Applied Biosystems) under the following conditions: 200 ng/μl chromosomal DNA, 1X GoTag buffer, 0.25 μl GoTaq DNA polymerase (Promega, United Kingdom), 3 mM $MgCl_2$, 0.2 mM dNTP. Primer concentrations were as follows: 0.2 μM primers β and α3 for each; 0.25 μM ccrCF and ccrCR for each; 0.08 μM 1272F1 and 1272R1 for each; and 0.1 μM 5RmecA and 5R431 for each. DNA electrophoresis of PCR products was run on 1.5% w/v agarose gel, then stained with ethidium bromide. The primers were designed according to Boye et al.[22] and Ito et al.[23,24]

Spa typing

The *spa* locus was amplified by PCR using *spa* forward primer 5′-AGACGATCCTTCGGTGAGC-3′ and *spa* reverse primer 5′-GCTTTTGCAATGTCATTTACTG-3’. PCR reaction was performed in a thermal cycler (Perkin-Elmer Applied Biosystems) under the following conditions: 200 ng/μl chromosomal DNA, 1X GoTag buffer, 0.25 μl GoTaq DNA

polymerase (Promega, United Kingdom), 3 mM $MgCl_2$, 0.2 mM dNTP, 0.2 μM primer. The PCR product was sequenced with the ABI DNA sequencer. The *spa* type and BURP (based upon repeat patterns) were performed using the Ridom StaphType, version 1.5 (Ridom, Wurzburg, Germany), software package (http://www.ridom.de). Those data were combined with MLST results and were analyzed in the Ridom SpaServer (http://spaserver.ridom.de).

Identification of MRSA virulence genes

Identification of MRSA virulence gene expression was performed using Power SYBR® Green PCR Master Mix (ABI; USA). The adhesion and virulence factors included encoding *clfA* and *clfB* (encoding clumping factors A and B); *fnbA* and *fnbB* (encoding fibronectin binding proteins A); *cna* (encoding collagen binding protein); *ebpS* (encoding elastin binding protein); *eap* (encoding extracellular adherence protein); *emp* (encoding extracellular matrix protein-binding protein); *sae* (encoding *S. aureus* exoprotein expression); *sdrC*, *sdrD* and *sdrE* (encoding *S. aureus* Serine-aspartate repeat-containing protein C,D and E); *eno* (encoding laminin binding protein). Primers used for these genes were designed according to previous literature.[25–30] The PCR was performed in 20-μl reaction volumes containing 1 μl cDNA, 0.2 μM primers, and 10 μl SYBR Green master mix. The thermal program was provided by the kit protocol.

Statistical analysis

The prevalence of virulence genes in MRSA bacteremic isolates among different groups was compared by the Chi-square test or Fisher exact test. Two-sided *P* values of less than 0.05 were considered as the threshold for statistical significance. Statistical analyses were performed using SPSS software version 17 (SPSS, Chicago, IL, USA).

Table 1 Combinatorial genotyping with MLST, SCC*mec*, and *spa* typing of methicillin-resistant *Staphylococcus aureus* bacteremic isolates and their distribution among four clinical disease groups.

Group	MLST (No./%)	SCC*mec* (No./%)	*spa* (No./%)
I: infective endocarditis (N = 23)	5 (3/13.04%)	Type I (1/4.34%)	t002 (2/8.69%)
	7 (1/4.34%)	Type II (5/21.73%)	**t037 (13/56.52%)**
	59 (2/8.69%)	**Type III (12/52.17%)**	t091 (1/4.34%)
	239 (15/65.21%)	Type IV (3/13.04%)	t164 (1/4.34%)
	965 (1/4.34%)	Type V (2/8.69%)	t437 (1/4.34%)
	1281 (1/4.34%)		t631 (2/8.69%)
			t1084 (1/4.34%)
			t3515 (1/4.34%)
			t5061 (1/4.34%)
II: bone/joint infection (N = 39)	5 (10/25.64%)	Type I (0/0%)	t002 (8/20.51%)
	59 (6/15.38%)	Type II (11/28.21%)	**t037 (18/46.15%)**
	239 (20/51.28%)	**Type III (22/56.41%)**	t233 (1/2.56%)
	241 (2/5.12%)	Type IV (5/12.82%)	t421 (1/2.56%)
	1863 (1/2.56%)	Type V (1/2.56%)	t437 (5/12.82%)
			t987 (1/2.56%)
			t1062 (1/2.56%)
			t1340 (1/2.56%)
			t3592 (1/2.56%)
			t5490 (1/2.56%)
			t10401 (1/2.56%)
III: skin/soft tissue infection (N = 20)	5 (1/5.26%)	Type I (0/0%)	**t037 (16/80%)**
	8 (1/5.26%)	Type II (2/10.52%)	t437 (2/10.52%)
	59 (2/10.52%)	**Type III (17/85%)**	t3528 (1/5.26%)
	239 (15/75%)	Type IV (0/0%)	t7506 (1/5.26%)
	444 (1/5.26%)	Type V (1/5.26%)	
IV: hemodialysis catheter-related bacteremia (N = 54)	1 (1/1.92%)	Type I (3/5.56%)	t002 (11/21.15%)
	5 (11/21.15%)	Type II (11/21.15%)	**t037 (35/64.81%)**
	45 (1/1.92%)	**Type III (37/68.51%)**	t298 (1/1.92%)
	59 (2/3.84%)	Type IV (2/3.84%)	t437 (3/5.76%)
	239 (36/66.67%)	Type V (1/1.85%)	t1081 (1/1.92%)
	338 (1/1.92%)		t2457 (1/1.92%)
	537 (1/1.92%)		t3525 (1/1.92%)
	573 (1/1.92%)		t3528 (1/1.92%)

MLST, multi-locus sequence typing; SCC*mec*, staphylococcal cassette chromosome *mec*.
The bold letters indicate the most common lineage of MRSA in this current study.

Results

MLST

A total of 136 MRSA bacteremic isolates from 136 patients were studied. These isolates were classified into four groups: group I, infective endocarditis (n = 23); group II, bone/joint infection (n = 39); group III, skin/soft tissue infection (n = 20), and group IV, catheter-related bacteremia in hemodialysis (n = 54). Overall 15 MLST types (sequence types, STs) were identified, and ST239 (MLST type: 2-3-1-1-4-4-3) (86, 63.2%) was the most prevalent MLST type, followed by ST5 (MLST type: 1-4-1-4-12-1-10) (25, 18.4%), and ST59 (MLST type: 19-23-15-2-19-20-15) (12, 8.8%), as shown in Table 1.

Verification of MRSA SCC*mec* types I–V

Of 136 MRSA isolates, SCC*mec* type I isolates accounted for 2.9% (4), type II 21.3% (29), type III 64.7% (88), type IV 7.4% (10), and type V 3.7% (5). Accordingly, SCC*mec* type III was most common among all four groups (52.2% in group I, 56.4% in group II, 85.0% in group III, and 68.5% in group IV).

Spa typing

Overall there were 23 *spa* types, and t037 (repeat succession: 15-12-16-02-25-17-24) (82, 60.3%) was the most common *spa* type, followed by t002 (repeat succession: 26-23-17-34-17-20-17-12-17-16) (21, 15.4%) and t437 (repeat succession: 04-20-17-20-17-25-34) (11, 8.1%). The proportion of other *spa* types was <1.5%.

Correlation of MLST, SCC*mec* typing and *spa* typing

We collected and analyzed MLST data along with the clonal complex information, SCC*mec* and *spa* typing. In the four groups, the most prevalent MRSA lineage was CC8-ST239-MRSA-SCC*mec* type III-*spa* type t037 (55.1%, 75), which accounted for 47.8% (11/23) in the group I, 41% (16/39) group II, 70% (14/20) group III, and 62.9% (34/54) group IV, and followed by CC5-ST5-MRSA-SCC*mec* type II-*spa* type t002 (14.7%, 20).

Distribution of adhesion and virulent genes in different clinical diseases

The distribution of adhesion and virulence genes between MRSA bacteremic isolates in four groups was analyzed. Eight studied adhesion genes (including *clfA*, *clfB*, *fnbA*, *ebpS*, *eap*, *emp*, *sae*, and *eno*) were present in all MRSA isolates of the group I. Likewise, *clfA*, *emp*, *sae*, and *eno* were universally detected in the group II; so was *eno* in the group III, and *clfA* and *sae* in the group IV. Of note, *cna* was most common in ST239 type isolates (98.8%, 85/86), 60% (15/25) in ST5 type, and 41.7% (5/12) ST59 type. In general, 78%–100% of MRSA strains isolated from different infections carried *clfA*, *clfB*, *fnbA*, *fnbB*, *cna*, *ebpS*, *eap*, *emp*, *sae*, *sdrC*, *sdrD*, *sdrE*, or *eno*, whose distribution was not significantly different (Table 2). Of CC8-ST239-MRSA-SCC*mec* type III-*spa* type t037, the predominant genotype, 90%–100% possesses these virulence genes (Table 2).

Discussion

Various investigations have developed several efficient typing methods in order to better identify MRSA clonal lineages. In this study, we examined the distribution of MRSA clonal lineages causing various infectious diseases. Type III was the most common SCC*mec* type among four infection groups in our study, and such a finding was compatible with previous reports in Taiwan.[31,32] In the study hospital, there were three prevalent MLST types (ST239, ST5, and ST59) and three *spa* types (t037, t002, and t437). Previous epidemiological data showed that t037, t002, and t437 were common *spa* types in the cases of community- and hospital-acquired MRSA infections in Taiwan.[1,2,33] Dispersion of MRSA among 11 Asian countries was estimated in the study of Chongtrakool et al., which showed that ST239 accounted for almost 90% of hospital-acquired MRSA cases in Asian countries such as Saudi Arabia, India, Sri Lanka, Singapore, Indonesia, Thailand, Vietnam, and China.[34] In the present study, combined results of three typing methods showed that CC8-ST239-MRSA-SCC*mec* type III-*spa* type t037 was the most common MRSA types, which was consistent with that of a previous study.[35] This predominant MRSA type also showed widespread distribution of these strains in Asian countries, including Thailand, Korea, Vietnam, and India.[33]

ClfA, ClfB, FnbA, FnbB, and Cna are involved in adherence to many cells or tissues and implicated in invasive infections, such as osteomyelitis, infective endocarditis, or septic arthritis.[10,11,36–38] This present study showed that not only *clfA* (95%–100%), *clfB* (92.3%–100%), *fnbA* (95%–100%), *fnbB* (77.8%–90%), *cna* (76.9%–91.3%), but also *sdrC* (79.5%–91.3%), *sdrD* (85%–87.2%), *sdrE* (79.5%–90%), *ebpS* (95%–100%) were commonly present in our MRSA bacteremic isolates, which were obtained from the patients with infective endocarditis, bone/joint infection, skin/soft tissue infection, or catheter-related bacteremia. It indicates that these genes may play an important role in clinical diseases caused by MRSA, despite of no significantly different distribution among various diseases.

According to our data, some genes are universally prevalent in each group. Among the isolates causing infective endocarditis, all had *clfA*, *clfB*, *fnbA*, *ebpS*, *eap*, *emp*, *sae*, and *eno*; all bone/joint infection isolates possessed *clfA*, *emp*, *sae* and *eno*; and skin/soft tissue infection *eno*. In all isolates leading to catheter-related bacteremia, *clfA* and *sae* are noted. However, *clfA* or *fnbA* was more common than *clfB* or *fnbB*, respectively. On the other hand, those genes encoding virulence factors of *S. aureus*, such as *clfA*, *clfB*, *fnbA*, *ebpS*, *eap*, *emp*, *sae*, and *eno*, with the involvement of host cell adhesion, are highly conserved in almost all MRSA isolates and may play a crucial role of invasive *S. aureus* infections. However, though no virulent factors can be linked to specific infection diseases, the development of different MRSA infections in each patient may involve host or microbiological variables, such as underlying conditions of hosts, antimicrobial susceptibility, prescribed antimicrobial agents, adequacy

Table 2 Virulence gene prevalence in bacteremic methicillin-resistant *Staphylococcus aureus* isolates causing infective endocarditis, bone/joint infection, skin/soft tissue infection, and hemodialysis catheter-related bacteremia.

	clfA	*clfB*	*fnbA*	*fnbB*	*cna*	*ebpS*	*eap*	*emp*	*sae*	*sdrC*	*sdrD*	*sdrE*	*eno*
infective endocarditis (N = 23)	23 (100%)	23 (100%)	23 (100%)	20 (86.9%)	21 (91.3%)	23 (100%)	23 (100%)	23 (100%)	23 (100%)	21 (91.3%)	20 (86.9%)	20 (86.9%)	23 (100%)
bone/joint infection (N = 39)	39 (100%)	36 (92.3%)	38 (97.4%)	32 (82.1%)	30 (76.9%)	38 (97.4%)	38 (97.4%)	39 (100%)	39 (100%)	31 (79.5%)	34 (87.2%)	31 (79.5%)	39 (100%)
skin/soft tissue infection (N = 20)	19 (95%)	19 (95%)	19 (95%)	18 (90%)	16 (80%)	19 (95%)	19 (95%)	19 (95%)	19 (95%)	18 (90%)	17 (85%)	18 (90%)	20 (100%)
hemodialysis catheter-related bacteremia (N = 54)	54 (100%)	51 (94.4%)	53 (98.1%)	42 (77.8%)	45 (83.3%)	53 (98.1%)	50 (92.6%)	53 (98.1%)	54 (100%)	45 (83.3%)	46 (85.2%)	44 (81.5%)	52 (96.3%)
P value	0.147	0.623	0.746	0.655	0.544	0.746	0.603	0.479	0.147	0.608	1.000	0.773	0.771
CC8-ST239-MRSA-SCC*mec* type III-*spa* type t037 (N = 75)	75 (100%)	69 (92%)	73 (97.3%)	68 (90.7%)	74 (98.7%)	73 (97.3%)	75 (100%)	75 (100%)	75 (100%)	70 (93.3%)	74 (98.7%)	72 (96%)	75 (100%)

of source control. These variables were not included in our analysis, and hence comprehensive investigations of interacting host and microbiological factors for MRSA-associated diseases are warranted.

In conclusion, a major MRSA lineage, ST239-MRSA-III-t037, is noted in bacteremic MRSA isolates, and the studied genes involving adhesion to host cells or biofilm formation are conserved in MRSA isolates. No infectious disease-specific genes are identified.

Conflicts of interest

All authors declare that they have no conflicts of interest.

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