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DNA Microarray Genotyping and Virulence and Antimicrobial Resistance Gene Profiling of Methicillin-Resistant *Staphylococcus aureus* Bloodstream Isolates from Renal Patients▽

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Thirty-six methicillin-resistant *Staphylococcus aureus* (MRSA) bloodstream isolates from renal patients were genetically characterized by DNA microarray analysis and *spa* typing. The isolates were highly clonal, belonging mainly to ST22-MRSA-IV. The immune evasion and enterotoxin gene clusters were found in 29/36 (80%) and 33/36 (92%) isolates, respectively.

Staphylococcus aureus is a frequent cause of bloodstream infections (BSI) worldwide (2, 3, 17). Methicillin-resistant *Staphylococcus aureus* (MRSA) has accounted for 20 to 50% of *S. aureus* BSI in our hospital over the past 5 years (7). Renal patients are at greater risk of MRSA BSI due to impaired immune function, regular contact with health care facilities, and the presence of central venous catheters (CVCs). We investigated the virulence gene profiles of MRSA BSI isolates from renal patients by using DNA microarray analysis. The study was carried out in Beaumont Hospital, Dublin, Ireland, an 820-bed tertiary referral center harboring the national referral center for renal and pancreatic transplantation and responsible for approximately 200 hemodialysis patients at any given time. Many studies have investigated the sources and outcomes of *S. aureus* bacteremia among renal patients; however, this is the first study, to our knowledge, to genetically characterize MRSA BSI isolates from renal patients (4, 8, 10).

MRSA BSI isolates from renal patients were prospectively collected from 2005 to 2009. Patient details were collected from European Antimicrobial Resistance Surveillance Network (EARS-Net) data and review of their medical notes. Genomic DNA was extracted using a DNeasy blood and tissue kit (Qiagen, Crawley, United Kingdom). *spa* typing, which involves PCR amplification and sequencing of the polymorphic 24-bp variable-number tandem-repeat region within the 3′ end of the protein A gene *spa*, was carried out according to methods described on the SeqNet website (http://www.seqnet.org). Sequencing was performed by Beckman Coulter Genomics (Takeley, United Kingdom) and Source BioScience (Dublin, Ireland). Genetic characterization of isolates was undertaken using the StaphyType kit (Alere Technologies, Germany) as previously described (12, 13). The StaphyType kit is a DNA microarray system that detects 334 *S. aureus* gene sequences, including those encoding (i) species markers (*nuc*, *spa*, *coa*, *femA*, *gapA*, *sbi*, and *sarA*), (ii) antimicrobial resistance genes (e.g., genes encoding resistance to β-lactams, macrolides, tetracyclines, lincosamides, streptogramins, aminoglycosides, and glycopeptides), (iii) genes encoding staphylococcal enterotoxins, toxic shock toxin, exfoliative toxins, Panton-Valentine leukocidin, the immune evasion complex (IEC; *sak*, *chp*, *scn*, *sea*, and *sep*), and the arginine catabolic mobile element (ACME), (iv) microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), adhesion and biofilm genes (e.g., *icaA*, *-C*, and *-D*, *cna*, *fnbA*, *fnbB*, *map*, *cna*, *ebh*, and *bbp*), (v) SCC and SCC*mec*-associated genes and sequences, and (vi) capsule (types 1, 5, and 8) and *agr* (types I to IV) typing markers (13). The DNA microarray can also assign *S. aureus* isolates to multilocus sequence types and/or clonal complexes (CCs) (14).

Thirty-six MRSA BSI isolates recovered from renal patients (19 female, 17 male) were investigated. The median age was 68 years, and 28 patients (78%) were on hemodialysis. The sources of BSI are listed in Table 1. For the majority of patients (26/36; 72.2%), a CVC was the source of the BSI. Six patients (16.7%) developed a secondary focus of infection, and these are listed in Table 1. The majority of isolates belonged to ST22-MRSA-IV (27/36; 75%), consisting of nine *spa* types, with t032 predominating (12/27; 44.4%) (Table 2). Five isolates (5/36; 13.9%) were ST5-MRSA-II and *spa* type t463, three (3/36; 8.3%) were ST8, *spa* type t190, and harbored SCC*mec* IIE and *ccrAB4* or a possible novel SCC*mec* II subtype, and 1 isolate belonged to ST30-MRSA-IV and *spa* type t1662.

All MRSA BSI isolates from renal patients recovered since 2008 belonged to ST22-MRSA-IV, whereas in the previous 3 years 81% had been found to belong to ST22-MRSA-IV, with the remainder consisting of several minor clones (Table 2). ST22-MRSA-IV is the predominant clone in Irish hospitals,

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TABLE 1. Infection types in renal patients with MRSA BSI in the present study

Infection type	No. (%) of isolates
Source of BSI	
Central venous catheter	26 (72.2)
Skin and soft tissue infection	2 (5.6)
Infected peripheral vascular catheter	2 (5.6)
Infective endocarditis	1 (2.8)
Surgical site infection	1 (2.8)
Intra-abdominal infection	1 (2.8)
Not identified	3 (8.3)
Secondary focus of infection	
Osteomyelitis	1 (2.8)
Infective endocarditis	3 (8.3)
Implantable cardiac rhythm device	2 (5.6)

accounting for 85% of MRSA BSI isolates in Ireland in 2009 (15). The enterotoxin gene cluster *egc* (*seg*, *sei*, *sem*, *sen*, *seo*, and *seu*) was found in all isolates except ST8 isolates (33/36; 92%). The toxic shock toxin gene (*tst*) was found in all ST5-MRSA-II and ST30-MRSA-IV isolates. The gene combination *tst*, *sea*, *sed*, *sej*, and *ser* was exclusive to ST5-MRSA-II isolates, and this ST carried more enterotoxin genes than the others. The *sec*/*sel* cluster was present in 16/27 (59.3%) ST22-MRSA-IV isolates but in no other STs. The IEC genes are important virulence factors of *S. aureus* (18). An IEC variant was detected in 80% of isolates (29/36), including 22/27 (81%) ST22-MRSA-IV, 1/3 (33.3%) ST8, and all ST5-MRSA-II and ST30-MRSA-IV isolates (Table 2).

We sought to determine the relationship between the genetic characteristics of the infecting isolate and the type of infection, infection complications, or clinical outcome. MRSA BSI with an ST22-MRSA-IV isolate was a cause of death in one patient. In six patients who developed a secondary focus of infection, the infecting isolates belonged to ST22-MRSA-IV (4/6; 66.6%) or ST5-MRSA-II (2/6; 33.3%). Development of a secondary focus of infection was not significantly associated with any particular ST; however, the highest rate of secondary infections involved ST5-MRSA-II (2/5 isolates; 40%), compared to ST22-MRSA-IV (4/27 isolates; 15%). This clone carried the most enterotoxin genes, including *sea*, and has been shown to be significantly associated with more severe *S. aureus* infection (1, 5, 6). Interestingly, the ST5-MRSA-II isolates harbored more antimicrobial resistance genes than ST22-MRSA-IV, but ST8 isolates harbored the greatest number of resistance genes (Table 2). The antibiotic resistance genes *fosB* and *tetefflux* were present in ST5-MRSA-II, ST8, and ST30 isolates. While nine of the MSCRAMM, adhesion, and biofilm genes investigated were detected in all isolates, only ST22-MRSA-IV and ST30-MRSA-IV isolates harbored the collagen binding adhesin gene *cna* and lacked the genes encoding the fibrinogen binding protein *fib* and the fibronectin binding protein *fnbB* (Table 2). ST22-MRSA-IV isolates also lacked the extracellular matrix binding protein *ebh* (Table 2).

Recent characterization of other *S. aureus* isolate collections indicated a strong clonal association of virulence genes, including the *egc* cluster and IEC variants (11, 16), and these correlations were also evident in the present study. The correlation

TABLE 2. Molecular characteristics of 36 MRSA bloodstream isolates recovered from renal patients between 2005 and 2009

ST	SCC*mec* type (*n*)	*spa* type(s) (*n*)	*agr*/capsule types	Antimicrobial resistance genes[a]	Virulence-associated genes[a]	MSCRAMM, adhesion, and biofilm genes[a]
22	IV (27)	t025 (1), t032 (12), t515 (3), t557 (3), t1214 (3), t2945 (2), t3185 (1), t5420 (1), t7636 (1)	I/5	*erm*(C) (21), *lnu*(A), *aacA-aphD*, *aadD*, *mupA* (1)	*seb* (2), *sec*/*l* (16), *egc*, *sak-chp-scn* (22; IEC type B), ACME (1)	*bbp* (25), *cna*, *map*, *sdrC*, *sdrD* (26), *sasG*
5	II (5)	t463 (5)	II/5	*erm*(A), *aadD*, *tetefflux*, *fosB*, *merA*, *merB* (1)	*tst*, *sed*/*j*/*r*, *egc*, *sea-sak-chp-scn* (IEC type A)	*bbp*, *ebh*, *fib*, *fnbB*, *map*, *sdrC*, *sdrD*, *sasG*
8	IIE and *ccrAB4* (2), novel II subtype (1)[b]	t190 (3)	I/5	*erm*(A), *tetefflux*, *fosB*, *qacA*, *aacA-aphD*, *aadD*, *aphA3-sat* (2), *merA*, *merB* (2)	*sea-sak-scn* (IEC type D)	*bbp*, *ebh*, *fib*, *fnbB* (2), *map* (2), *sdrD*
30	IV (1)	t1662 (1)	III/8	Q6GD50 (*fusC*), *tetefflux*, *fosB*	*tst*, *egc*, *sak-chp-scn* (IEC type B)	*bbp*, *cna*, *ebh*, *fib*, *map*, *sdrC*, *sdrD*

[a] The number of positive isolates is indicated in parentheses if not all isolates within a genotype were positive for the gene indicated. All isolates harbored the beta-lactamase resistance gene *blaZ* and the MSCRAMM, adhesion, and biofilm genes *icaA*, *icaC* and *icaD*, *clfB*, *ebpS*, *eno*, *fnbA*, and *vwb*.

[b] Possible novel SCC*mec* II subtype identified in one ST8 MRSA isolate that yielded signals for class A *mec* complex *ccrAB2* but lacked signals for *kdp* and *aadD*(pUB110).

between carriage of specific virulence genes and clinical outcome remains unclear, because host factors are also involved. For example, there are reports of a negative or positive correlation between *egc* gene carriage and infection severity in different isolate collections (5, 9), but how these genes limit or contribute to clinical complications is difficult to establish. Virulence gene expression may also affect the clinical outcome, but it is technically challenging to reliably determine gene expression that reflects the *in vivo* setting. The detailed characterization of virulence genes described here supports the clonal distribution of virulence-associated genes in a specific patient group with increased risk for multiple episodes of *S. aureus* infection. Although the small sample size excludes a statistically robust evaluation of the relationship between virulence gene carriage and clinical outcome, carriage of *egc* genes, at least, is apparently independent of the development of clinical complications in these patients.

In conclusion, this is the first report of MRSA BSI isolates in renal patients for which the isolates have been typed and characterized in detail with a DNA microarray. DNA microarray analysis is a useful, rapid, and convenient tool for more comprehensive analysis of virulence and antimicrobial resistance genes in *S. aureus*.

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