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Sinead McNicholas
Royal College of Surgeons in Ireland

Anna C. Shore
Trinity College Dublin

David C. Coleman
Trinity College Dublin

Hilary Humphreys
Royal College of Surgeons in Ireland

Deirdre Fitzgerald-Hughes
Royal College of Surgeons in Ireland, dfitzgeraldhughes@rcsi.ie

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

Sinead McNicholas[1], Anna C. Shore[2], David C. Coleman[2], Hilary Humphreys[1,3], Deirdre Fitzgerald Hughes[1*]

1. Department of Clinical Microbiology, Education and Research Centre, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin 9, Ireland.

2. Microbiology Research Unit, Division of Oral Biosciences, Dublin Dental University Hospital, University of Dublin, Trinity College Dublin, Lincoln Place, Dublin 2, Ireland.

3. Department of Microbiology, Beaumont Hospital, Dublin 9, Ireland

Running title: Characterization of MRSA from Renal Patients

*Corresponding Author

Dr. Deirdre Fitzgerald Hughes, Department of Clinical Microbiology, RCSI Education and Research Centre, Smurfit Building, Beaumont Hospital, Dublin 9, Ireland. Tel. +353 1 8093711, Fax +353 1 8093709, Email dfitzgeraldhughes@rcsi.ie

Abstract

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%) of isolates, respectively.

Staphylococcus aureus is a frequent cause of bloodstream infections (BSI) worldwide (2, 3, 17). Methicillin-resistant *Staphylococcus aureus* (MRSA) accounted for 20-50% of *S. aureus* BSIs in our hospital over the past five years (7). Renal patients are at greater risk of MRSA BSI due to impaired immune function, regular contact with healthcare facilities and the presence of central venous catheters (CVCs). We investigated the virulence gene profiles of MRSA BSI isolates from renal patients by DNA microarray analysis. The study was carried out in Beaumont Hospital, Dublin, Ireland, a 820-bed tertiary referral centre harboring the national referral centre for renal and pancreatic transplantation, 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 2005-2009. Patient details were collected from EARS-Net data and review of their medical notes. Genomic DNA was extracted using a DNeasy® blood and tissue kit (Qiagen, Crawley, UK). *Spa* typing, which involves PCR amplification and sequencing of the polymorphic 24 base pair variable number tandem repeat region within the 3′ end of the protein A gene *spa*, was carried out according to the SeqNet website (http://www.seqnet.org). Sequencing was performed by Beckman Coulter Genomics (Takeley, UK) 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 *selp*) 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, bbp*), (v) SCC and SCC*mec*-associated genes and sequences and (vi) capsule (types 1, 5 and 8) and *agr* (types I-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 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 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 & *ccrAB4* or a possible novel SCC*mec* II subtype and one 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 three years, 81% belonged to ST22-MRSA-IV with the remainder consisting of several minor clones (Table 2). ST22-MRSA-IV is the predominant clone in Irish hospitals, accounting for 85% of MRSA BSI isolates in Ireland in 2009 (15). The enterotoxin gene cluster *egc* (*seg, sei, sem, sen, seo, seu*) was found in all isolates except ST8

(33/36, 92 %) isolates. The toxic-shock-toxin (*tst*) gene 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 a 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 a 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%) and 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 resistant 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 fibronectin binding protein *fnbB*

97 (Table 2). ST22-MRSA-IV isolates also lacked the extracellular matrix binding protein *ebh*
98 (Table 2).

99 Recent characterization of other *S. aureus* isolate collections indicates a strong clonal
100 association of virulence genes including the *egc* cluster and IEC variants (11, 16) and these
101 correlations were also evident in the present study. The correlation between carriage of specific
102 virulence genes and clinical outcome remains unclear because host factors are also involved. For
103 example, there is evidence for negative or positive correlation between *egc* gene carriage and
104 infection severity in different isolate collections (5, 9) but how these genes limit or contribute to
105 clinical complications is difficult to establish. Virulence gene expression may also affect the
106 clinical outcome but it is technically challenging to reliably determine gene expression that
107 reflects the *in vivo* setting. The detailed characterization of virulence genes described here
108 supports the clonal distribution of virulence-associated genes in a specific patient group with
109 increased risk for multiple episodes of *S. aureus* infection. Although the small sample size
110 excludes a statistically robust evaluation of the relationship between virulence gene carriage and
111 clinical outcome, carriage of *egc* genes at least, is apparently independent of the development of
112 clinical complications in these patients.

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

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TABLE 1. Infection types found in renal patients with MRSA bloodstream infections (BSIs) in the present study

Infection type	Number 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 foci of infection	
Osteomyelitis	1 (2.8)
Infective endocarditis	3 (8.3)
Implantable cardiac rhythm device	2 (5.6)

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

ST	SCC*mec* type (n)	*spa* types (n)	*agr*/ capsule type	Antimicrobial resistance genes[a]	Virulence-associated genes[a]	MSCRAMM, adhesion & biofilm genes[a]
ST22	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*
ST5	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*
ST8	IIE & *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*
ST30	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 are indicated in parenthesis 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* & *icaD*, *clfA* & *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).