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Distribution of virulence genes among colonising and invasive isolates of meticillin- resistant *Staphylococcus aureus*, a pilot study.

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Running title: virulence genes in MRSA

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We were interested to read of the role of superantigenic strains in the prognosis of community-acquired meticillin-susceptible *Staphylococcus aureus* (CA-MSSA) [1]. The authors reported that 73 % of CA-MSSA isolates recovered from 37 immunocompetent patients with CA-MSSA, produced at least one superantigen but carriage of superantigens by *S. aureus* did not effect mortality in these patients. We would like to report and comment on similar findings regarding the relationship between virulence genes amongst meticillin-resistant *S. aureus* (MRSA) and the potential for invasive infection. We determined the occurrence of eighteen virulence determinants (including adhesins and toxins) in 26 invasive and 20 colonising isolates of MRSA, using multiplex PCR.

Twenty six isolates were recovered from patients with device-related bloodstream infections between January 2002 and June 2005 and were characterised as part of a previous study from this group [2]. Twenty isolates were from nasal swabs, collected from patients that were MRSA positive on first admission (between September 2006-June 2007) but with no apparent signs of infection, and were therefore considered to be colonising or carriage isolates. Reference *S. aureus* strains, that were positive for one or more of the virulence genes studied, were used as positive controls. Eighteen virulence factors were detected using six separate multiplex reactions follows; multiplex 1; *hlg, lukPV, seg, sei,* multiplex 2; *tst, sec, sed,* multiplex 3; *ica, seb, see, 16SRNA* (internal control), multiplex 4; *sdrE, fnb,* multiplex 5; *seq, sek, cna,* multiplex 6; *sea, seg, seh.* The primer sequences used were either published previously [2-7] or were designed for this study, using the Primer 3 free online tool (http://frodo.wi.mit.edu/) and had the following sequences ; *cna*-F TGGACGACAAGACAATCA, *cna*-R GTTGTTCGTTTTCCGTCTTG, *fnbA*-F CCA

GGTGGTGGTCAGGTTAC, *fnbA*-R TTTCCTCGACTGGTCCTTGT, *sdrE*-F GGCGACGGTACTGTTAAACC, *sdrE*-R ATTCTGGCTCATTTGCATC. PCR reactions were carried out using GoTaq™ Green Master Mix (Promega, UK), which was supplemented to give a final $MgCl_2$ concentration of 3 mM. PCR conditions for multiplexes 1-4 were 30 cycles of 95°C, 1 min, 55°C, 1 min, 72°C, 2 min. For multiplexes 5 and 6, conditions were 35 cycles of 95°C, 1 min, 55°C, 1 min, 72°C, 2.5 min. For all multiplex reactions, cycles were preceded by a melting step of 95°C for 1 min and followed by an extension step at 72°C for 2 min. For some multiplex reactions, the presence of specific genes was confirmed using a single gene detection PCR reaction.

As shown in Table 1, of the 18 virulence genes investigated, *sea, sec, sei, hlg, cna, fnbA*, and *ica* were present in all 46 isolates and *seb, sed, see, sej, sek, seq, lukPV* were not detected in any isolates. We found no significant correlation between the carriage of almost all the virulence genes and the invasiveness of isolates. The gene *seh* was harboured by 5 % of colonising isolates and 19.2 % of invasive isolates but this finding was not statistically significant using Fisher's exact test (p value = 0.212). We found a greater frequency of *seg* in colonising isolates compared to invasive isolates (100 % and 73 %), which was statistically significant by Fisher's exact test (p value = 0.028). Isolates that were positive for *sei*, were not always positive for *seg*, even though these genes are part of the *egc* gene cluster suggesting some variability in this cluster. The invasive isolates used for this study belonged to either one of two genotypes ST8-MRSA-II (35.4 %) or ST22-MRSA-IV (64.6 %) reflecting the predominant lineages found in Ireland recently [8]. The presence of *seg* was more strongly associated with ST22-MRSA-IV (95 %) than ST8-MRSA-II (44 %), *seh* was less prevalent in ST22-MRSA-IV (6 %) than in ST8-MRSA-II (44 %) and for all

other genes there was no significant difference in prevalence between these two lineages among the invasive isolates.

Nashev *et al* [9] also reported an increased frequency of *seg* in colonising isolates compared to invasive isolates. (73 % and 52 % [9]) and an increased frequency of *seh* in invasive isolates (5 % and 19.2 %). However *sea* which has been previously found to correlate with severity of infection for patients with septic shock versus patients without septic shock [10] was found in all isolates in our pilot study. The lack of association of specific genes or combinations of genes with invasive isolates in our study is in agreement with the absence of a role for superantigens in the prognosis of patients with sepsis due to *S. aureus* reported by Desachy *et al.* [1]

Characterisation of the constitutive features that contribute to the potential of MRSA for pathogenicity in hospital settings has been the subject of several recent studies. [1, 9-11] Although it appears that carriage of virulence genes by *S. aureus* does not influence the infection prognosis in general, it is clear that the carriage of certain toxin genes is associated with toxin-mediated diseases such as toxic shock syndrome and scalded skin syndrome [12, 13]. However, as highlighted by Desachy *et al*, [1] other studies, [9-11] and our findings, the inability to find a definitive correlation between invasive *S. aureus* strains and the carriage of virulence genes, suggests that either it is the expression of virulence determinants *in-vivo*, rather than the presence of the genes themselves, that mediates pathogenicity, or that host immune factors may play a significant role in the disease outcome. The mechanisms by which compromised immunity, facilitates MRSA infection, are complex and are likely to involve several aspects of innate immunity such as compromised neutrophil function, variations in cytokine or antimicrobial peptide expression as well as the possible involvement of some components of acquired immunity. The elucidation of

these mechanisms in colonised patients versus patients with MRSA infections will contribute to our understanding of the complexities of *S. aureus* pathogenesis.

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Table 1. Prevalence of eighteen virulence determinants in colonising and invasive MRSA isolates.

Gene name	% prevalence of virulence gene	
	Colonising isolates (n=20)	Invasive isolates (n=26)
sea	100	100
seb	0	0
sec	100	100
sed	0	0
see	0	0
seg	100	73
seh	5	19.2
sei	100	100
sej	0	0
sek	0	0
seq	0	0
lukPV	0	0
hlg	100	100
tst	20	19.2
cna	100	100
fnbA	90	100
sdrE.	100	100
ica	100	100