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Genotypic and phenotypic relationships among methicillin-resistant *Staphylococcus aureus* from three multicentre bacteraemia studies

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***Background*: At a time when the molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) was changing, we sought to characterize several genotypic markers and glycopeptide susceptibility features of clinical isolates from patients with bacteraemia.**

***Methods*: One hundred and sixty-eight MRSA bloodstream isolates obtained from three multicentre clinical trials were microbiologically and genotypically characterized.**

***Results*: All isolates were susceptible to vancomycin (MIC $\leq$2 mg/L); 38% belonged to accessory gene regulator (*agr*) group I, 52% belonged to group II and 10% belonged to group III. Typing of the staphylococcal cassette chromosome *mec* (SCC*mec*) showed that 67% were type II and 33% were type IV. The *agr* group II polymorphism was associated with SCC*mec* II ($P<0.001$). Fifty-three percent of SCC*mec* II and 27% of SCC*mec* IV isolates had vancomycin MICs $\geq$1 mg/L ($P=0.001$). One hundred percent of *agr* II strains were predicted to be members of clonal complex 5. SCC*mec* II was the genetic marker most predictive of vancomycin MICs of $\geq$1 mg/L. SCC*mec* IV isolates were more likely to have vancomycin MICs $\leq$0.5 mg/L.**

***Conclusions*: Given that SCC*mec* IV is a marker for a community-based organism for which less prior vancomycin exposure is predicted, we conclude that prior antibiotic exposure in *agr* group II organisms may account for their increased vancomycin MICs.**

Keywords: MRSA, SCC*mec* types, clonal types, *Staphylococcus* spp.

Introduction

Vancomycin has served as the cornerstone of therapy for serious methicillin-resistant *Staphylococcus aureus* (MRSA) infections for 50 years.[1] Despite the fact that microbiological resistance to vancomycin in *S. aureus* remains very rare, recent years have seen a shift upwards in vancomycin MICs (i.e. the 'MIC creep') within the susceptible range,[2–4] with consequential effects on vancomycin efficacy in MRSA bacteraemia and pneumonia.[5–8] In addition to microbiological susceptibility phenotype, certain genotypic markers may also serve as a predictor of vancomycin treatment failure in MRSA bacteraemia.[5,9] However, the relationship between the MRSA genotype and glycopeptide susceptibility *in vitro* has not been extensively studied. We evaluated a multicentre collection of 168 MRSA bloodstream isolates compiled from three prior clinical trials to further evaluate the relationship between the MRSA *agr* type, *spa* type, and SCC*mec* type and vancomycin susceptibility and to evaluate for MRSA strain differences between the trials.

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Methods

Isolates were obtained as part of three multicentre clinical trials evaluating MRSA bacteraemia.[10–12] PCR was used to characterize *mec* cassettes and to characterize accessory gene regulator (*agr*) types as described previously.[13–15] *spa* X-repeat polymorphisms were determined by nucleotide sequencing, as described previously.[15,16] A semi-quantitative delta-haemolysin functional assay was performed to assess *agr* function.[17] Vancomycin susceptibility testing was performed by CLSI microdilution methods and was evaluated for differences based on *agr* and SCC*mec* type (MIC $\leq$0.5 versus $\geq$1 mg/L).[18] Ordinal data were compared using Kruskal–Wallis analysis of variance. Categorical data were compared using χ^2 or Fisher's exact test where appropriate. All statistical procedures were performed with Systat 11 (Systat Software Inc., Point Richmond, CA, USA).

Results

One hundred and sixty-eight *S. aureus* isolates from 168 unique patients were studied. All isolates were susceptible to vancomycin, with MICs of 0.25 mg/L (n=1), 0.5 mg/L (n=92), 1.0 mg/L (n=68) and 2.0 mg/L (n=7). The isolates consisted of 64 (38%) *agr* group I, 86 (52%) group II and 18 (10%) group III MRSA. One hundred and thirteen (67%) were staphylococcal cassette chromosome *mec* (SCC*mec*) II and 55 (33%) were SCC*mec* IV. Of the 164 isolates that were tested by *spa* typing, 83 (50%) were predictive of clonal complex 5.

The *agr* group II polymorphism was associated with the presence of SCC*mec* II (P<0.001) (Figure 1); 73% (83/113) of SCC*mec* II were *agr* group II. Eighty-seven percent (48/55) of SCC*mec* IV were *agr* group I. All (100%) of the *agr* group II isolates were predicted to be of clonal complex 5.

Vancomycin MICs were significantly higher among SCC*mec* II MRSA (P=0.001) (Figure 2). Fifty-three percent of SCC*mec* II and 27% of SCC*mec* IV isolates had vancomycin MICs $\geq$1 mg/L. Statistically significant differences in vancomycin MICs were not noted between *agr* groups or *spa* types. Among the isolates showing vancomycin MICs of $\geq$1 mg/L, 49% were *agr* group II, 42% were *agr* group I and 33% were *agr* group III (P=0.24). Thirty-nine of the 83 (47%) isolates whose *spa* typing predicted clonal complex 5 and 33 of 81 (41%) other clonal complex types had vancomycin MICs $\geq$1 mg/L (P=0.661).

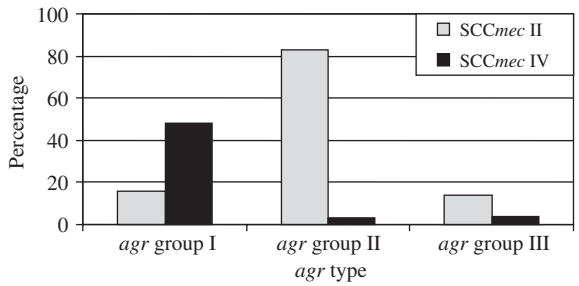

Figure 1. Relationship between accessory gene regulator (*agr*) type and SCC*mec* type in 163 MRSA bloodstream isolates. *agr* group I versus II, P<0.001; *agr* group I versus III, P<0.001; *agr* group II versus III, P=0.004.

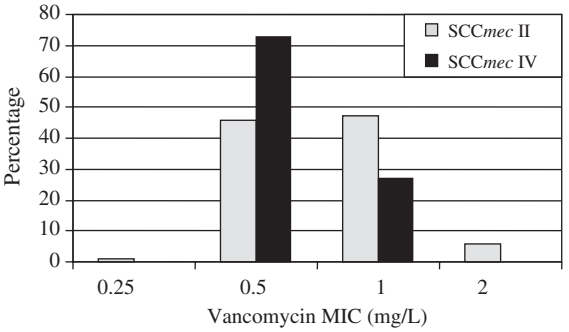

Figure 2. Relationship between SCC*mec* type and vancomycin MIC values in 163 MRSA bloodstream isolates (P=0.001).

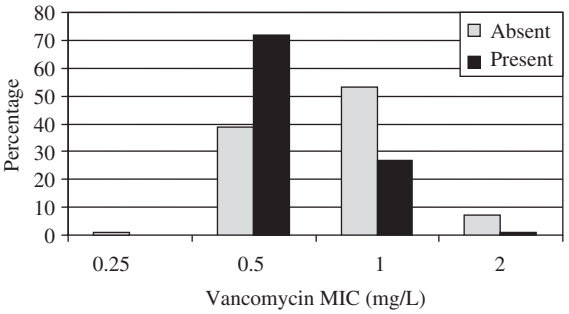

Figure 3. Relationship between delta-haemolysin production (absent or present) and vancomycin MIC values for 163 MRSA bloodstream isolates (P<0.001).

The function at the *agr* locus was significantly more reduced among MRSA with higher MICs within the susceptible range (Figure 3). Decreased delta-haemolysin production was noted in 86%, 66% and 36% of MRSA with vancomycin MICs of 2, 1 and $\leq$0.5 mg/L, respectively (P<0.001).

Analysis of the microbiological and genotypic properties of MRSA was segregated by the clinical studies from which they were obtained (Table 1). The earlier two studies enrolling patients in Phase III/IV protocols from 1998 to 2003 highly weighted towards persistent MRSA bacteraemia (>5 days) and inclusive of patients in renal failure showed a different spectrum of organisms from the randomized Phase III trial enrolling from 2002 to 2005 evaluating daptomycin versus comparator and exclusive of renal failure patients. When compared with the later daptomycin *S. aureus* bacteraemia trial, the earlier trials had MRSA with a lower percentage of *agr* group I strains (25% versus 51%) and SCC*mec* IV strains (88% versus 46%), and a significantly higher percentage of MRSA with vancomycin MICs $\geq$1 mg/L (77% versus 16%) and *agr* dysfunction (75% versus 24%).

Discussion

It is well understood that there is a differential response to the treatment of infection based on host, pathogen and antimicrobial selection. While appearing straightforward, antibiotic susceptibility as measured *in vitro* via the determination of an MIC in a clinical laboratory may be complicated by phenotypes too subtle

Table 1. Shifting molecular epidemiology of MRSA bacteraemia

Characteristic	Sample from studies 1 and 2 ($n=81$)	Sample from study 3 ($n=87$)	P value
Year MRSA isolated	1998–2003	2002–05	
agr group, *n* (%)			
I	20 (25%)	44 (51%)	0.001
II	49 (60%)	38 (44%)	0.029
III	12 (15%)	4 (5%)	0.024
SCC*mec* II, *n* (%)	71 (88%)	40 (46%)	<0.001
Vancomycin MIC ≥1 mg/L, *n* (%)	62 (77%)	14 (16%)	<0.001
δ-lysin 0/1, *n* (%)[a]	61 (75%)	21 (24%)	<0.001

Study 1: Moise-Broder *et al*., 2002. Study continued through year 2003.
Study 2: Moise *et al*., 2002.
Study 3: Fowler *et al*., 2006.
[a]Delta-lysin scoring system: 0, absent; 1, diminished; 2, comparable to *agr* wild-type strain RN6607 (see reference 17); 3, increased; and 4, markedly increased.

to be detected by standard methods. For example, the heterogeneous nature of susceptibility to glycopeptides has resulted in a recently appreciated discordance between microbiological and clinical vancomycin resistance among serious *S. aureus* infections such as bacteraemia and pneumonia.[19]

In addition to vancomycin MIC, *agr* group II MRSA have also been linked to vancomycin treatment failure in one study,[5] but not in another.[9] With the understanding that different MRSA clones with specific genotypic characteristics may predominate in specific epidemiological settings with consequential differences in antimicrobial selection driving the development of reduced susceptibility, we sought to determine the relationships between the MRSA *agr* type, SCC*mec* type and vancomycin susceptibility.

We found that MRSA harbouring SCC*mec* II were more likely to have vancomycin MICs of ≥1 mg/L. SCC*mec* IV isolates were more likely to have vancomycin MICs ≤0.5 mg/L. SCC*mec* II predominated among *agr* group II strains and SCC*mec* IV among *agr* group I strains.

We noted a strong association between increased vancomycin MIC within the susceptible range and *agr* dysfunction shown by reduced delta-haemolysin activity. Loss of *agr* has been associated with the glycopeptide-intermediate *S. aureus* phenotype,[17] glycopeptide tolerance[17,19] and prolonged bacteraemia.[9] Since a vancomycin MIC of 2 mg/L has been associated with prolonged bacteraemia, this new observation is to be expected, based on prior data.

Given that SCC*mec* II has been a marker for a healthcare-associated organism with consequential vancomycin selection pressure, these data suggest that the prior finding of *agr* group II being associated with vancomycin treatment failure may reflect the fact that these clones have predominated in settings of antecedent vancomycin selection pressure. Consistent with this premise is that the *agr* group II, clonal complex 5 USA 100 MRSA clone includes the first US and Japanese VISAs and vancomycin-resistant *S. aureus*.[20,21] This inference is strengthened by the finding of another single-centre study, where *agr* group III MRSA clones predominate, that the *agr* group III genotype was associated with vancomycin treatment failure.[9] Thus, differences in vancomycin response at the genetic level likely reflect microbiological differences rather than intrinsic differences in antibiotic susceptibility. However, the reasons for the establishment of different specific MRSA clones in different settings or even different hospitals are unknown and warrant further study.

In addition to spatial differences in MRSA susceptibility, an evaluation of the microbiological and phenotypic properties of the 168 bacteraemia isolates as they sort out by the previous studies from which they were derived shows temporal shifts in MRSA as well. Collectively, the majority (52%) of MRSA in this study belonged to clonal complex 5 (*agr* group II). However, this genotype was not evenly distributed between the three studies. In the first two studies, where patients were derived from Phase III/IV linezolid and quinupristin/dalfopristin protocols and enriched for patients with prolonged bacteraemia on vancomycin, isolates were heavily weighted towards *agr* group II (60%), contain SCC*mec* II, have vancomycin MICs of at least 1 mg/L and have reduced *agr* function. In the third study, where isolates were more contemporary and excluded patients with renal dysfunction, MRSA were much more likely to be *agr* group I, contain SCC*mec* IV and have lower vancomycin MICs and preserved *agr* function. Only 44% of MRSA from the third study are *agr* group II. These latter attributes are features of MRSA that have generally been seen in community-onset infections. Nevertheless, the 'hospital' and 'community' labels associated with specific MRSA genotypes are certain to break down over time, likely in a fashion similar to that of *S. aureus* with penicillin resistance over the past decades.

The findings from this multicentre study are in agreement with the recent single-centre study from Detroit by Chua *et al*.,[20] showing *agr* group II MRSA to be associated with hospital-onset infection, SCC*mec* II and higher vancomycin MICs, and showing *agr* group I strains to be associated with community-onset infection, SCC*mec* IV and lower vancomycin MICs. Many *agr* group I SCC*mec* IV MRSA have recently been seen in healthcare-onset infections.

In summary, these findings highlight important genotypic and phenotypic characteristics of MRSA bloodstream isolates as they relate to one another, with SCC*mec* II being the strongest

predictor of vancomycin MIC at the upper limit of the susceptibility range. Prior vancomycin exposure in CC5 *agr* II SCC*mec* II organisms may account for their higher vancomycin MICs. Currently, the molecular epidemiology of MRSA is a moving target, both geographically and temporally, and therefore, considerable strain heterogeneity may be found among different clinical studies. Therefore, generalizability of findings from clinical trials on MRSA bacteraemia to individual clinical centres needs to be done with caution.

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