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Reduced vancomycin susceptibility and staphylococcal cassette chromosome *mec* (SCC*mec*) type distribution in methicillin-resistant *Staphylococcus aureus* bacteraemia

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Objectives: Recent epidemiological evidence suggests that genotypic and phenotypic characteristics that have typically distinguished community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) and healthcare-associated MRSA strains may be evolving. The objective of this study was to examine the association between reduced vancomycin susceptibility (RVS) and staphylococcal cassette chromosome *mec* (SCC*mec*) type in MRSA bloodstream isolates.

Methods: A cohort study of patients who were hospitalized from 2007 to 2009 with *S. aureus* bacteraemia was conducted within a university health system. Bivariable analyses were conducted to determine the association between RVS and *SCCmec* type, as well as other microbiological characteristics including Panton–Valentine leucocidin, accessory gene regulator (*agr*) dysfunction and vancomycin heteroresistance.

Results: A total of 188 patients with MRSA bacteraemia were identified: 116 (61.7%) and 72 (38.3%) patients had infections due to healthcare-associated MRSA and community-associated MRSA, respectively. As defined by a vancomycin Etest MIC >1.0 mg/L, the prevalence of RVS was 40.4%. Isolates with RVS were significantly more likely to be associated with SCC*mec* II compared with isolates without RVS (74.7% and 47.3%, respectively, $P<0.001$), but not with Panton–Valentine leucocidin ($P=0.10$), *agr* dysfunction ($P=0.19$) or healthcare-associated infection ($P=0.36$).

Conclusions: The results of our study demonstrate important microbiological characteristics among MRSA isolates characterized by RVS, including a significant association between SCC*mec* II and elevated vancomycin MIC. It is clear that the clinical and molecular epidemiology of MRSA is evolving, and further understanding of factors determining virulence will be important for the elucidation of optimal treatment approaches for associated infections.

Keywords: MRSA, virulence factors, antimicrobial resistance, epidemiology

Introduction

The increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in both the healthcare and community setting is of major public health concern.[1] A recently recognized phenomenon of increasing vancomycin MICs over time has been described among vancomycin-susceptible *S. aureus* isolates.[2] This reduced vancomycin susceptibility (RVS) has been associated with prior vancomycin exposure,[3] as well as with increased mortality in the setting of MRSA bacteraemia.[4]

Community-associated MRSA (CA-MRSA) has usually referred to strains causing infections in patients without recent contact with the healthcare environment.[1] CA-MRSA has typically been distinguished from healthcare-associated MRSA (HA-MRSA) by the staphylococcal cassette chromosome *mec* (SCC*mec*) element, with SCC*mec* IV and V predominating in CA-MRSA strains, and SCC*mec* I, II and III predominating in HA-MRSA strains. However, recent epidemiological evidence indicates that CA-MRSA and HA-MRSA strains are increasingly mixing in both community and healthcare settings.[1] It is possible that

the genotypic and phenotypic characteristics that have typically distinguished CA-MRSA and HA-MRSA strains may be evolving, and that selection pressure for RVS may be increasingly similar among CA-MRSA and HA-MRSA. However, to our knowledge, there are few studies that have primarily evaluated the association between RVS in MRSA and SCC*mec* type.[5–7] Furthermore, these studies have focused on study populations characterized by persistent bacteraemia and enriched for vancomycin treatment failure,[6] a low prevalence of bacteraemia[5] or outside of the USA, where different SCC*mec* types predominate.[7]

We conducted this study to determine the association between RVS and SCC*mec* type in MRSA bloodstream isolates. We also sought to comprehensively characterize the genotypic and phenotypic characteristics of MRSA bloodstream isolates with and without RVS, including the presence of Panton–Valentine leucocidin (PVL), accessory gene regulator (*agr*) dysfunction and vancomycin heteroresistance.

Patients and methods

This study was conducted at two hospitals in the University of Pennsylvania Health System (UPHS) in Philadelphia.[8] All inpatients with an episode of MRSA bacteraemia occurring between 1 December 2007 and 31 May 2009 were identified through the Hospital of the University of Pennsylvania Clinical Microbiology Laboratory. For patients with multiple episodes of MRSA bacteraemia, only the first episode was included. The study was approved by the institutional review board of the University of Pennsylvania and based on a database constructed for a prior study.[8]

Identification and susceptibility testing of *S. aureus* was performed and interpreted according to CLSI guidelines. The vancomycin MIC of the isolates was determined by the Etest and broth microdilution methods as previously described,[8] with RVS defined as a vancomycin MIC >1.0 and ≥ 1.0 mg/L, respectively.[4,6] Vancomycin heteroresistance was screened for and confirmed as previously described.[8] Detection of the genes encoding PVL was performed using real-time PCR.[9] Isolates were evaluated for *agr* dysfunction by delta-haemolysin production as previously described.[10] SCC*mec* typing was performed using previously described methods.[11] Isolates were compared quarterly (six at 3 month intervals) to determine whether there were significant changes in the proportion of a given characteristic over the study period.

Baseline demographic and hospitalization data were abstracted from the Pennsylvania Integrated Clinical and Administrative Research Database, as previously described.[8] Infections were classified as HA-MRSA if the date of the first positive blood culture was ≥ 48 h from the date of admission or if the patient was admitted as a transfer from another institution or had been hospitalized at UPHS in the 30 days prior to the culture date. Otherwise, the infection was classified as CA-MRSA.

Continuous variables were compared using the Wilcoxon rank-sum test, and categorical variables were compared using the χ^2 or Fisher's exact test, including the χ^2 test for trend to determine temporal changes. For all calculations, a two-tailed *P*-value <0.05 was considered significant. All statistical calculations were performed using commercially available software (STATA version 11.0; StataCorp LP, College Station, TX, USA).

Results

Study population

A total of 188 patients with MRSA bacteraemia were identified during the study period. The mean age of patients was 60 years (standard deviation, 17), 76 (40.4%) were female, with 116 (61.7%) and 72 (38.3%) patients classified as having infections due to HA-MRSA and CA-MRSA, respectively. Of 171 patients for whom race was indicated, 100 (58.5%) were white and 70 (40.9%) were African-American.

Microbiological characteristics

The distribution of vancomycin MICs among isolates as determined by Etest was as follows: 11 (5.9%) with MIC ≤ 0.5 mg/L, 34 (18.1%) with MIC $=0.75$ mg/L, 67 (35.6%) with MIC $=1.0$ mg/L, 73 (38.8%) with MIC $=1.5$ mg/L and 3 (1.6%) with MIC $=2.0$ mg/L. Accordingly, 40.4% of the MRSA bloodstream isolates demonstrated RVS.[8] There was no significant change in the proportion of isolates with RVS over time during the study period ($P=0.31$). Table 1 reports the microbiological characteristics of MRSA isolates with and without RVS.

The distribution of SCC*mec* types among isolates was as follows: 109 (58.0%) with type II, 75 (40.0%) with type IV, 1 (0.5%) with type III, 1 (0.5%) with type V, 1 (0.5%) with type VIII and 1 (0.5%) was untypeable. The distribution of SCC*mec* types among isolates over time did not significantly change during the study period ($P=0.41$). Figure 1 compares SCC*mec* type distribution among isolates with and without RVS. Isolates characterized as RVS-positive were significantly more likely to be associated with SCC*mec* II compared with isolates without RVS (74.7% and 47.3%, respectively; $P<0.001$). Conversely, isolates without RVS were more likely to be associated with SCC*mec* type IV compared with isolates with RVS (51.8% and 22.7%, respectively; $P<0.001$).

On secondary analyses utilizing broth microdilution to determine RVS, 48 (25.5%) of isolates were characterized as demonstrating RVS. SCC*mec* type distribution among isolates was similar to that seen with Etest, with isolates characterized by RVS more likely to be associated with SCC*mec* II compared with isolates without RVS (80.9% and 50.7%, respectively; $P<0.001$).

Finally, HA-MRSA isolates were significantly more likely than CA-MRSA isolates to be associated with SCC*mec* type II (66.1% versus 45.8%, respectively; $P=0.03$) and absence of PVL (80.2% versus 59.7%, respectively; $P=0.004$). However, there was no significant difference in *agr* dysfunction among HA-MRSA compared

Table 1. Characteristics of MRSA isolates with and without RVS

Characteristic	RVS ($n=76$)[a]	No RVS ($n=112$)[a]	*P*-value
SCC*mec* type			
II	56 (74.7)	53 (47.3)	<0.001
III	1 (1.3)	0 (0.0)	0.40
IV	17 (22.7)	58 (51.8)	<0.001
V	1 (1.3)	0 (0.0)	0.40
VIII	0 (0.0)	1 (0.9)	>0.99
Panton–Valentine leucocidin	16 (21.1)	36 (32.1)	0.10
agr dysfunction	14 (18.4)	13 (11.6)	0.19
hGISA	11 (14.5)	1 (0.9)	0.001

hGISA, heteroresistant glycopeptide-intermediate *S. aureus*.
[a]Data are presented as numbers (percentages).

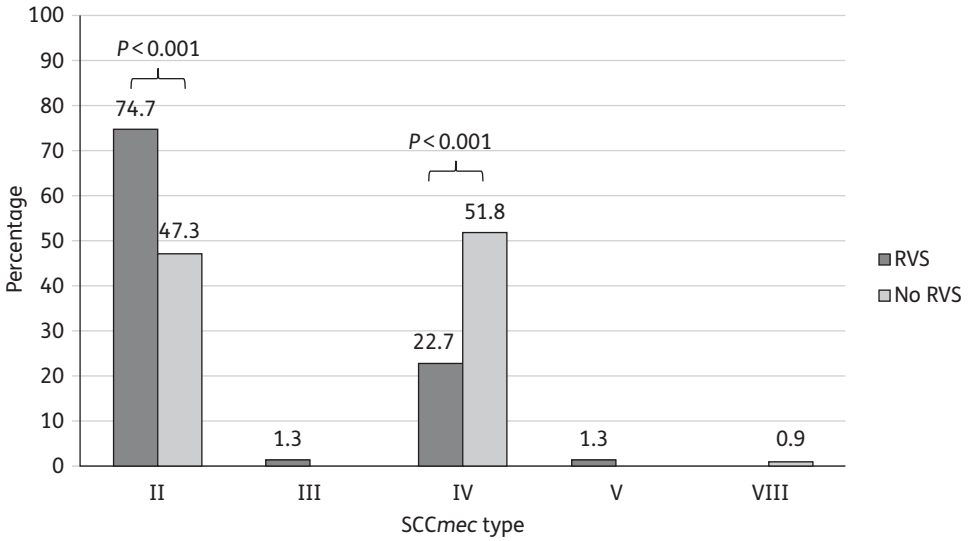

Figure 1. Distribution of SCC*mec* types among MRSA isolates with and without RVS. Fisher's exact test was used for all comparisons.

with CA-MRSA isolates (12.1% versus 18.1%, respectively; $P=0.29$). There was no significant difference in the proportion of isolates classified as HA-MRSA, compared with CA-MRSA, that demonstrated RVS by Etest (43.1% versus 36.1%, respectively; $P=0.36$).

Discussion

The prevalence of RVS among 188 MRSA bloodstream isolates in the present study was 40.4% as defined by Etest. MRSA isolates characterized by RVS were significantly more likely to be associated with SCC*mec* type II but not with *agr* dysfunction or absence of PVL.

Previous studies have implicated prior exposure to vancomycin as a risk factor for RVS,[3] most likely as a result of antimicrobial selection pressure. However, the relationship between specific microbiological factors with RVS in MRSA is unclear, including microbial virulence determinants. Similarly to prior studies,[5,6] we found that MRSA isolates with RVS were more likely to harbour SCC*mec* II. However, unlike previous studies, our patient population was not enriched for vancomycin failure or persistent bacteraemia,[6] and the samples consisted solely of bloodstream isolates.[5] Furthermore, MRSA isolates without RVS in the present study were associated with SCC*mec* IV. SCC*mec* II has been associated with increased mortality in MRSA bacteraemia.[1] Similarly, studies suggest that RVS is associated with poor clinical outcomes in the setting of MRSA bacteraemia.[4] Given this, the relatively high prevalence of RVS found in our study is of concern, and further research on optimal treatment strategies for infections due to MRSA with RVS is urgently needed.

SCC*mec* II has also typically been a marker for healthcare-associated infections due to MRSA. However, similarly to a previous study,[5] there was no significant association between RVS and HA-MRSA in our study. While our definition of HA-MRSA differed from the CDC definition, this finding suggests that risk factors or antimicrobial selection pressure, apart from those seen with recent healthcare exposure, may contribute to the development of RVS. Finally, while elevated vancomycin MIC has been postulated to be a marker for an as yet unidentified organism virulence factor,[4] the present study demonstrated no significant association between RVS and *agr* dysfunction or PVL.

There are potential limitations of our study. Selection bias is a potential concern; however, patients were identified through the Clinical Microbiology Laboratory, which processed and cultured all specimens obtained during the study period, thereby minimizing the likelihood of excluding potential isolates. The present study was conducted in a single healthcare system, and these results may not be generalizable to other institutions, for example, those with differences in MRSA prevalence.

In conclusion, the results of our study demonstrate important microbiological characteristics among MRSA isolates characterized by RVS, including a significant association between SCC*mec* II and elevated vancomycin MIC. It is clear that the clinical and molecular epidemiology of MRSA is evolving, and further understanding of organism factors determining virulence and fitness will be important for elucidation of optimal treatment and preventive approaches for associated infections.

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Transparency declarations

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