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ARTICLE

Prevalence of isolates with reduced glycopeptide susceptibility in orthopedic device-related infections due to methicillin-resistant *Staphylococcus aureus*

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Abstract We evaluated, by an improved susceptibility testing method, the prevalence and significance of low-level glycopeptide resistance in methicillin-resistant *Staphylococcus aureus* (MRSA) isolates, which belonged to a previously described, retrospective cohort of patients treated for orthopedic device-related infections (ODRI) at the Geneva University Hospital between 2000 and 2008. Fifty-seven individual or multiple isolates were retrieved from 41 ODRI patients for glycopeptide susceptibility and clonality studies, including 20 patients with prosthetic joint (PJ) and 21 with osteosynthesis (OS) MRSA infections. Low-level glycopeptide resistance was detected by elevated teicoplanin or/and vancomycin minimum inhibitory concentrations (MICs ≥4 mg/L), as determined by a previously validated combination of macrodilution and agar dilution assays of improved sensitivity. MRSA isolates with elevated teicoplanin MICs were detected in 20/41 (49 %) ODRI patients at the onset or during the course of glycopeptide therapy, namely, in 10 of 20 patients with PJ and 10 of 21 patients with OS infections. Only one isolate developed a concomitant increase in vancomycin MIC during therapy. 13/20 (65 %) glycopeptide-intermediate *S. aureus* (GISA)-infected patients, including 7/10 (70 %) with PJ and 6/10 (60 %) with OS, experienced treatment failure. In contrast, therapy failed in only 5/21 (24 %) ODRI patients with non-GISA isolates ($p=0.012$), including 2/10 (20 %) with PJ and 3/11 (27 %) with OS infections. The emergence of low-level teicoplanin resistance could not be explained by teicoplanin administration, since only four patients received teicoplanin. The evaluation of

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low-level teicoplanin resistance may improve the detection of GISA isolates. Further studies are warranted to evaluate the impact of low-level teicoplanin resistance on the outcome of glycopeptide therapy.

Introduction

Factors reported to increase the risk of glycopeptide treatment failure against invasive methicillin-resistant *Staphylococcus aureus* (MRSA) infections are: (1) the difficulty to reach adequate tissue levels at the true sites of MRSA infections, (2) the moderate bactericidal activity of glycopeptides, and (3) the emergence of glycopeptide resistance [1–8].

Phenotypic detection of vancomycin-intermediate *S. aureus* (VISA) isolates, which are defined by vancomycin minimum inhibitory concentration (MIC) breakpoints of ≥4 mg/L and <16 mg/L, and the absence of any vancomycin or teicoplanin resistance determinants (*vanA*, *vanB*, or *vanC*) found in vancomycin-resistant *Enterococcus faecalis* or high-level vancomycin-resistant (vancomycin MIC: 16 mg/L) *S. aureus* isolates (VRSA) [5–12] is frequently problematic [1, 2, 4–8]. Since VISA isolates are generally cross-resistant to teicoplanin [4, 13], they are also designated glycopeptide-intermediate *S. aureus* (GISA). In contrast to vancomycin, teicoplanin susceptibility breakpoints in *S. aureus* vary from 2 mg/L by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [14] to 8 mg/L by the Clinical and Laboratory Standards Institute (CLSI) [15].

Detection of the GISA phenotype is particularly difficult for isolates displaying heterogeneous resistance to either or both glycopeptide(s) (hGISA), in which only a subset of the microbial population can express glycopeptide resistance [7, 9, 14–18], and which are likely precursors of GISA isolates under the selective pressure of glycopeptides [6, 19]. The unsuccessful detection of hGISA isolates by standard microbiological methods has triggered the development of alternative assays, such as the modified population analysis profile (PAP) area under the curve (AUC), the Etest macromethod, and the glycopeptide resistance determination (GRD) test [6, 7, 20–22].

The predictive value of vancomycin and teicoplanin susceptibility breakpoints on the outcome of glycopeptide therapy is still debated, despite their recent adjustment by the CLSI and EUCAST [8, 16]. While higher rates of vancomycin treatment failures were frequently reported in bacteremic patients infected with MRSA isolates for which vancomycin MICs were 2 mg/L, compared to those with lower vancomycin MICs (<2 mg/L) [1, 2, 8, 16, 23–26], as confirmed by a recent meta-analysis [27], these data might be explained, at least in part, by the low sensitivity of some MIC testing methods, such as the broth microdilution and agar dilution assays, which lead to a significant underdetection of some GISA or hGISA isolates [28–30].

While numerous studies evaluated the impact of glycopeptide MICs and low-level resistance on the outcome of glycopeptide therapy in MRSA bacteremic patients [5–8, 24–26, 31], a single report linked the presence of GISA/hGISA isolates with a negative outcome of vancomycin therapy for MRSA-infected, orthopedic patients [32].

We recently reported a high prevalence of GISA isolates in patients with persistent or recurrent MRSA bacteremia [33], in which low-level glycopeptide resistance was detected by elevated teicoplanin or/and vancomycin MICs (≥4 mg/L), as determined by a previously validated combination of macrodilution and agar dilution assays, allowing more sensitive detection of slow-growing, glycopeptide-"resistant" subpopulations [30]. Using this improved susceptibility testing method, we now present data on the prevalence and potential significance of low-level glycopeptide resistance in MRSA isolates from patients treated for orthopedic device-related infections (ODRI) at the Geneva University Hospital [34].

Materials and methods

Clinical and microbiological data collection

Fifty-seven MRSA isolates from 41 patients with MRSA ODRI, who were treated at the Geneva University Hospital between 2000 and 2008, were routinely stored in skimmed milk/glycerol at −80 °C [34]. These ODRI patients belonged to a retrospective cohort study, and their major clinical characteristics and risk factors for treatment failure have been previously described in detail [34]. MRSA isolates were essentially intra-operative specimens and aspirated synovial fluid [34]. In five patients, blood isolates that were clonally related to intra-operative isolates were also analyzed.

Twenty of the 41 ODRI patients had prosthetic joint (PJ) and 21 osteosynthesis (OS) MRSA infection [34]. MRSA infections were considered persistent if the patient's clinical status required further surgery 5 days after the initiation of antimicrobial therapy, with isolation of the same MRSA isolate by intra-operative specimen [34]. Recurrence was defined as resurgence of the infection with a clonally related MRSA isolate after the end of antimicrobial therapy [34].

Determination of glycopeptide MICs

Vancomycin and teicoplanin MICs were determined by a slightly modified, previously described tube macrodilution assay, using cation-adjusted Mueller–Hinton broth and standardized inocula of 10^6 colony-forming units (CFU)/mL [30]. MIC endpoints were read after 48 h incubation at 37 °C, to improve the detection of slow-growing, glycopeptide-"resistant" subpopulations [30]. This procedure was combined with

a modified agar MIC testing method, which was used for confirmatory testing of glycopeptide MICs recorded by macrodilution, as previously described [30, 33]. In this modified agar MIC testing method, residual viable counts of each antibiotic-containing agar plate inoculated with ca. 10^6 CFU were scored after 48 h incubation at 37 °C in a semi-quantitative manner as confluent, semi-confluent, or $\leq 10^3$ CFU, as previously described [30]. Glycopeptide MIC was defined as the lowest antibiotic concentration leading to a ≥99.9 % reduction in viable counts ($\leq 10^3$ CFU) on brain–heart infusion agar from the uniformly applied inoculum of 10^6 CFU, as previously described [30].

To be scored as GISA, all MRSA isolates had to display elevated teicoplanin or/and vancomycin MICs (≥4 mg/L) by both modified macrodilution and agar testing assays [30, 33].

Molecular typing

The clonality of consecutive MRSA isolates from patients with persistent or recurrent ODRI was assessed by a variable-number tandem repeat (VNTR) genotyping method [35], as previously described [34]. Strain pairs with >85 % similarity in the dendrogram were considered to be clonally related (Bioanalyzer Experiments Clustering Software) [35].

Statistical analyses

Microbiological, demographic, and clinical characteristics of GISA- and non-GISA-infected ODRI patients were compared by the Fisher's exact test for categorical variables or the Mann–Whitney test for continuous variables (http://vassarstats.net/). Relationships were considered to be significant when the two-sided p-value was ≤0.05.

Results

Prevalence of GISA in ODRI patients

MRSA isolates showing elevated teicoplanin MICs (≥4 mg/L) were detected in 20 (49 %) of the 41 ODRI patients, including 10 of 20 (50 %) PJ-infected and 10 of 21 (48 %) OS-infected patients. In contrast, none of the 41 ODRI pretherapy isolates displayed elevated vancomycin MICs (≥4 mg/L).

For two patients, teicoplanin MICs were 2 mg/L in pretherapy isolates and increased to 4 mg/L in subsequent isolates. For the remaining 18 patients, teicoplanin MICs were already elevated in pretherapy isolates and remained constant in subsequent isolates, except for two patients in whom teicoplanin MICs increased from 4 to 8 mg/L in consecutive isolates. In a single patient with low-level teicoplanin-resistant isolates, vancomycin MICs increased from 2 to 4 mg/L from baseline to subsequent isolates.

Clonality of GISA isolates in ODRI patients

All MRSA isolates with elevated teicoplanin MICs belonged to the hospital-acquired South German MRSA clone ST228 (SCC*mec* type 1 and *agr* type 2), which became predominant in our institution after 1998 [36]. This MRSA clone was previously reported to have infected 35 of the 41 ODRI patients (85 %) by *spa* and VNTR typing methods [34]. All available sequential isolates from patients with persistent or recurrent ODRI were clonally related (data not shown). In contrast, none of the six residual isolates belonging to other clonotypes (ST1, ST5, ST8, ST80, ST239), including other SSC*mec* (III, IV, V) and *agr* (1, 3) types [34], exhibited low-level glycopeptide resistance. The prevalence of isolates displaying low-level glycopeptide resistance was significantly higher in the ST228 clonotype compared to other clonotypes (p=0.021; Table 1).

Prevalence of GISA isolates and treatment outcomes in different subgroups of ODRI patients

Thirteen of 20 GISA-infected ODRI patients (65 %) experienced MRSA-linked treatment failure compared to 5/21 (24 %) patients infected with non-GISA isolates (p=0.012) (Table 1). Treatment failure was significantly (p=0.032) higher in GISA-infected patients with persistent ODRI episodes compared to non-GISA isolates. There were also non-significant trends toward higher rates of recurrent ODRI episodes or multiple treatment failures in GISA-infected compared to non-GISA-infected patients.

Higher failure rates were observed in both subgroups of GISA-associated ODRI patients, namely, in 7/10 (70 %) PJ and 6/10 (60 %) OS patients, compared to 2/10 (20 %) PJ (p=0.070) and 3/11 (27 %) OS patients with non-GISA infections (p=0.198; Table 1). Higher treatment failure rates were recorded in GISA- compared to non-GISA-infected ODRI patients with implant retention, as well as in patients with implant removal (Table 1), but these differences did not reach statistical significance due to the small sample sizes.

In brief, there was a consistent trend toward higher treatment failure rates in all subgroups of GISA- compared to non-GISA-infected ODRI patients.

Comparison of GISA- and non-GISA-infected patients

The major demographic and clinical characteristics of ODRI-treated patients are presented in Table 2. Noteworthy, GISA-infected patients were significantly younger (p=0.037) than non-GISA-infected patients. While underlying conditions and rates of surgical debridement were similar in GISA- and non-GISA-infected patients, there was a trend for longer duration of hospital stay, which reached significance for the cumulated duration of hospital stay (p=0.036) in GISA- versus non-

Table 1 Prevalence of glycopeptide-intermediate *Staphylococcus aureus* (GISA) isolates and treatment outcomes in different subgroups of orthopedic device-related infection (ODRI) patients

Characteristics	No. of ODRI patients with indicated type of isolate		
	GISA (n=20)	Non-GISA (n=21)	p- value[a]
Microbiological characteristics			
No. (%) of isolates belonging to the ST228 clonotype	20 (100)	15 (71)	0.021*
No. (%) of ODRI patients with MRSA-linked treatment failures	13 (65)	5 (24)	0.012*
No. (%) of patients with persistent ODRI episodes	8 (40)	2 (10)	0.032*
No. (%) of patients with recurrent ODRI episodes	5 (25)	3 (14)	0.454
No. (%) of patients with multiple treatment failures	7 (35)	3 (14)	0.159
No. (%) of PJ patients	10 (50)	10 (50)	1.000
No. (%) of PJ patients with MRSA-linked treatment failures	7 (70)	2 (20)	0.070
No. (%) of PJ patients with removed implants	4 (57)	3 (43)	1.000
No. (%) of treatment failures	3 (75)	0 (<25)	0.142
No. (%) of PJ patients with implant retention	6 (46)	7 (54)	1.000
No. (%) of treatment failures	4 (75)	2 (29)	0.286
No. (%) of OS patients	10 (48)	11 (52)	1.000
No. (%) of OS patients with MRSA-linked treatment failures	6 (60)	3 (27)	0.198
No. (%) of OS patients without debridement	2 (10)	2 (10)	1.000
No. (%) of OS patients with removed implants	3 (43)	4 (57)	1.000
No. (%) of treatment failures	1 (25)	0 (<25)	0.429
No. (%) of OS patients with implant retention	5 (50)	5 (50)	1.000
No. (%) of treatment failures	3 (60)	1 (20)	0.524

[a]Significance of differences in the characteristics of GISA- versus non-GISA-infected ODRI patients

*p<0.05

GISA-infected patients. While the rates of bacteremic episodes concomitant to ODRI were not significantly different in GISA- and non-GISA-infected patients, there was a non-significant trend (p=0.180) for a higher overall mortality in GISA-infected (40 %; n=8) compared to non-GISA-infected (19 %; n=4) patients. Most of those deaths were unrelated to MRSA-linked ODRI or bacteremia. Only two GISA-infected patients died from MRSA-linked ODRI or bacteremia, but death occurred only 77 and 185 days, respectively, after ODRI onset.

Treatment modalities for GISA- and non-GISA-infected patients

The potential impact of therapeutic regimens on the treatment outcomes of GISA- compared to non-GISA-infected ODRI patients was evaluated. Besides one patient who was treated with trimethoprim–sulfamethoxazole, all other patients initially received intravenous regimens of vancomycin, either in monotherapy (n=17) or combined with other agents (n=23). Altogether, the administration of additional antibiotic regimens to ODRI patients after initial intravenous therapy led to a total of eight different antimicrobial regimens (Table 3). This great diversity and the small sample size in each group precluded any detailed comparison of the impact of each antimicrobial regimen on GISA- compared to non-GISA-infected ODRI patients.

There was a trend for higher failure rates recorded in GISA-infected patients (78 %) initially treated with vancomycin monotherapy (n=9), with or without additional antimicrobial regimens, compared to the rate (38 %) in non-GISA-infected patients (n=8), which did not reach significance (p=0.153). In contrast, there were no significant differences in the daily doses and durations of antimicrobial regimens (data not shown) administered to GISA- versus non-GISA-infected patients.

Several regimens of combined therapy involving vancomycin associated with either rifampin, trimethoprim–sulfamethoxazole, or fusidic acid were administered to 23 ODRI patients (Table 3). The impact of each therapeutic regimen on the outcome of GISA- versus non-GISA-infected ODRI patients could not be analyzed in detail due to low sample sizes.

Interestingly, teicoplanin administration was shown to play no significant role in the presence of GISA isolates in ODRI patients. While only four ODRI patients received teicoplanin as subsequent antimicrobial therapy (Table 3), none of these patients were infected with GISA isolates.

Discussion

Despite a continuously increasing number of clinical and microbiological reports, as summarized in [6, 7, 27], the

Table 2 Demographic and clinical characteristics of patients with GISA and non-GISA ODRI episodes

Characteristics	No. of ODRI patients with indicated type of isolate		
	GISA (n=20)	Non-GISA (n=21)	p- value[a]
Demographic characteristics			
Age [median years (range)]	62 (35–78)	78 (22–82)	0.037*
Female gender	12 (60)	11 (52)	0.756
Underlying conditions			
At least one underlying illness	12 (60)	15 (71)	0.520
Diabetes mellitus	1 (5)	4 (19)	0.343
Charlson comorbidity index (mean±SE)	1.47 (0.35)	1.38 (0.29)	0.800
No. (%) of patients with surgical debridement	18 (90)	19 (90)	1.000
Median no. (range) of surgical interventions	3 (1–10)	1 (1–5)	0.025*
Duration [median no. of days (range) of initial hospital stay]	42 (9–195)	32 (1–88)	0.234
Median no. (range) of hospitalizations	1.5 (1–7)	1 (1–2)	0.052
Duration [median no. of days (range) of cumulated hospital stay]	53.5 (23–195)	34 (1–108)	0.036*
No. (%) of patients with concomitant bacteremia	3 (15)	6 (28)	0.453
No. (%) of patients with exitus	8 (40)	4 (19)	0.180

[a]Significance of differences in the characteristics of GISA- versus non-GISA-infected ODRI patients

*$p<0.05$

impact of low-level glycopeptide resistance on the outcome of GISA infections is still debated. This unclear situation mostly results from technical issues in characterizing the low-level glycopeptide resistance phenotype in MRSA isolates, which is frequently heterogeneous and, thus, escapes detection by standard MIC assays. Furthermore, there is no molecular assay for detecting low-level glycopeptide resistance, whose molecular basis is likely multifactorial and may even display some strain-specific variability [37]. While a recent meta-analysis supports the concept that slightly elevated vancomycin MICs, scored at the higher end of the susceptibility range by microdilution or Etest, were associated with worse outcomes of glycopeptide therapy in MRSA bloodstream infections,

Table 3 Treatment regimens of patients with GISA and non-GISA ODRI episodes

Initial antibiotic regimen	No. of ODRI patients with indicated type of isolate		
	GISA (n=20)	Non-GISA (n=21)	p- value[a]
No. (%) of patients with VAN monotherapy	9 (45)	8 (38)	1.000
Failure rate (%)	7 (78)	3 (38)	0.153
No. (%) of patients with VAN alone	7 (35)	3 (14)	0.159
Failure rate (%)	5 (71)	1 (33)	0.500
No. (%) of patients with VAN + subsequent antibiotic therapy[b]	2 (10)	5 (24)	0.410
Failure rate (%)	2 (100)	2 (40)	0.429
No. (%) of patients with SXT monotherapy	1 (5)	0 (<5)	NA
Failure rate (%)	1 (100)	0 (NA)	NA
No. (%) of patients with VAN in combination	10 (50)	13 (62)	0.538
Failure rate (%)	5 (50)	2 (15)	0.169
No. (%) of patients with combined VAN + SXT therapy	1 (5)	1 (5)	NA
Failure rate (%)	1 (100)	1 (100)	NA
No. (%) of patients with combined VAN + RIF therapy	9 (45)	12 (57)	0.538
Failure rate (%)	4 (44)	1 (8)	0.119
No. (%) of patients with VAN + RIF + subsequent oral antibiotic therapy[c]	4 (20)	10 (48)	0.100
Failure rate (%)	2 (40)	0 (<10)	0.066

NA not applicable; *LZD* linezolid; *RIF* rifampin; *SXT* trimethoprim–sulfamethoxazole; *VAN* vancomycin; *TEC* teicoplanin

[a]Significance of differences in the treatment regimens and outcomes of GISA- and non-GISA-infected ODRI patients

[b]Two GISA and two non-GISA-infected patients received SXT and three non-GISA-infected patients received TEC

[c]Two GISA and one non-GISA-infected patients received SXT, two GISA and eight non-GISA-infected patients RIF + fusidic acid, one non-GISA patient RIF + LZD, and one non-GISA patient received TEC

the contribution of hGISA could not be evaluated in this context [27].

To facilitate the detection of hGISA or GISA by standard MIC criteria, we developed a modified macrodilution MIC assay by using higher inocula than broth microdilution MIC and extending the incubation period to 48 h at 37 °C, which is mandatory for detecting the slowly growing resistant subpopulations [30]. The increased sensitivities of the modified macrodilution assay combined with a modified agar MIC method markedly increased the detection rates of isolates with slightly elevated teicoplanin and vancomycin MICs (≥4 mg/L), by ca. 10-fold and 4-fold, respectively, compared to broth microdilution [30]. Most recently, the modified macrodilution MIC assay allowed detecting a higher prevalence of MRSA isolates with slightly elevated vancomycin or teicoplanin MICs in patients with persistent or recurrent episodes of MRSA bacteremia compared to those with single episodes [33]. Collectively, these data strongly suggest that a significant proportion of MRSA bloodstream isolates scored with slightly elevated vancomycin MICs (2 mg/L) by microdilution in previous studies [24, 27, 38] could have been undetected VISA or hVISA.

Since our previous contributions indicated that >95 % of isolates with elevated vancomycin MICs displayed cross-resistance to teicoplanin, elevated teicoplanin MIC was considered to be a reliable marker of the GISA phenotype, which could facilitate the screening of isolates with low-level vancomycin resistance [13, 30]. Indeed, in our recent study of patients with persistent or recurrent episodes of MRSA bacteremia, 63 % of isolates displaying low-level resistance to teicoplanin were concomitantly resistant to vancomycin [33].

In contrast to patients with MRSA bloodstream infections, vancomycin cross-resistance was a rare event in teicoplanin-resistant isolates from ODRI patients. Indeed, only one patient among all of the teicoplanin-resistant isolates detected in 20 patients displayed concomitant resistance to vancomycin. Noteworthy, teicoplanin-resistant isolates were already present in 90 % of GISA-infected ODRI patients at the onset of antimicrobial therapy, while the emergence of low-level teicoplanin resistance during therapy occurred in only 10 % of the patients. Remarkably, the administration of teicoplanin therapy was not associated with the emergence of low-level teicoplanin-resistant isolates, since none of the four teicoplanin-treated patients were infected with resistant isolates.

A most intriguing finding of this study was that ODRI patients infected with MRSA isolates displaying low-level teicoplanin resistance but still showing in vitro susceptibility to vancomycin seemed to respond poorly to antimicrobial regimens involving vancomycin compared to non-GISA-infected patients These observations lead us to speculate that in vivo conditions prevailing in ODRI patients might significantly alter the metabolic parameters of MRSA in such a way that teicoplanin resistance mechanisms may compromise MRSA susceptibility to vancomycin therapy. Previous studies performed in an animal model of chronic, implant-associated MRSA infection indicated a drastic loss of susceptibility to antibiotic killing in bacteria directly removed from infected foci [39–42]. Other important parameters that may contribute to bacterial survival in MRSA chronic infections are intracellular persistence [43], which may be further promoted by teicoplanin resistance determinants [44], and bacterial adherence, leading to biofilm formation and colonization of artificial surfaces [41, 45, 46].

Recent molecular studies indicated that the emergence of vancomycin and teicoplanin endogenous resistance is likely a stepwise process involving several mutations in key regulatory genes [6, 13, 37]. While the molecular differences between isolates displaying combined low-level resistance to both teicoplanin and vancomycin versus those resistant to teicoplanin alone have not been elucidated, molecular studies performed in our laboratory suggest that resistance to teicoplanin alone might represent an early step of low-level glycopeptide resistance, which may eventually lead to vancomycin resistance via additional mutations [37]. In this context, the exclusive presence of GISA isolates in a single MRSA clonotype, namely, the South German clone ST228 or relatives, which became predominant in ODRI MRSA infections reported in our institution from 2000 to 2008 [34, 36], as opposed to its absence in other clonotypes, is remarkable. It is possible that the ST228 clonal family, which is characterized by SCC*mec* type 1 and *agr* type 2, may contain some phenotypically silent mutations predisposing to the emergence of glycopeptide resistance.

The complexity of the clinical data, in particular, the high diversity of surgical procedures and antimicrobial regimens administered to ODRI patients, did not allow a detailed risk factor analysis of GISA- compared to non-GISA-infected ODRI patients. Our previous report analyzing the overall impact of antimicrobial therapy on the outcome of all MRSA-infected ODRI patients revealed the significant benefit on patient outcome of a combined rifampin–fusidic acid regimen administered after initial rifampin–vancomycin therapy [34]. Controlled trials involving larger groups of MRSA ODRI patients are needed in order to evaluate the efficacy of the rifampin–fusidic acid combination over other regimens, against GISA- as well as non-GISA-infected patients.

This study has some limitations. This was a single-center, retrospective cohort study, which required extended follow-up periods for the clinical and microbiological evaluations of ODRI patients. Failure of glycopeptide therapy in MRSA ODRI patients is clearly multifactorial, as found in several studies, being influenced by several demographic and clinical risk factors in addition to the emergence of reduced

susceptibility to glycopeptides. The difficulty in recruiting adequate numbers of GISA- and non-GISA-infected ODRI patients for comparative studies leads to small sample sizes that prevent detailed analysis of risk factors. In some parts of our study, both OS and PJ patients with persistent and recurrent GISA ODRI episodes had to be analyzed collectively in view of the small numbers.

In conclusion, the development of simple, more sensitive MIC assays for detecting GISA isolates should prompt the development of a multicenter, prospective study for evaluating the impact of the GISA phenotype and clinical risk factors on the outcome of glycopeptide therapy in MRSA ODRI patients. In particular, multicenter controlled studies are needed so as to define the most adequate antibiotic regimen(s) for such infections.

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Conflict of interest There is no conflict of interest.

References

1. Deresinski S (2007) Counterpoint: vancomycin and *Staphylococcus aureus*—an antibiotic enters obsolescence. Clin Infect Dis 44:1543–1548
2. Mohr JF, Murray BE (2007) Point: vancomycin is not obsolete for the treatment of infection caused by methicillin-resistant *Staphylococcus aureus*. Clin Infect Dis 44:1536–1542
3. Moise PA, Sakoulas G, Forrest A, Schentag JJ (2007) Vancomycin in vitro bactericidal activity and its relationship to efficacy in clearance of methicillin-resistant *Staphylococcus aureus* bacteremia. Antimicrob Agents Chemother 51:2582–2586
4. Liu C, Chambers HF (2003) *Staphylococcus aureus* with heterogeneous resistance to vancomycin: epidemiology, clinical significance, and critical assessment of diagnostic methods. Antimicrob Agents Chemother 47:3040–3045
5. de Kraker ME, Wolkewitz M, Davey PG, Koller W, Berger J, Nagler J, Icket C, Kalenic S, Horvatic J, Seifert H, Kaasch AJ, Paniara O, Argyropoulou A, Bompola M, Smyth E, Skally M, Raglio A, Dumpis U, Kelmere AM, Borg M, Xuereb D, Ghita MC, Noble M, Kolman J, Grabljevec S, Turner D, Lansbury L, Grundmann H; BURDEN Study Group (2011) Clinical impact of antimicrobial resistance in European hospitals: excess mortality and length of hospital stay related to methicillin-resistant *Staphylococcus aureus* bloodstream infections. Antimicrob Agents Chemother 55:1598–1605
6. Howden BP, Davies JK, Johnson PD, Stinear TP, Grayson ML (2010) Reduced vancomycin susceptibility in *Staphylococcus aureus*, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. Clin Microbiol Rev 23:99–139
7. van Hal SJ, Paterson DL (2011) Systematic review and meta-analysis of the significance of heterogeneous vancomycin-intermediate *Staphylococcus aureus* isolates. Antimicrob Agents Chemother 55:405–410
8. Gould IM, Cauda R, Esposito S, Gudiol F, Mazzei T, Garau J (2011) Management of serious meticillin-resistant *Staphylococcus aureus* infections: what are the limits? Int J Antimicrob Agents 37:202–209
9. Hiramatsu K, Aritaka N, Hanaki H, Kawasaki S, Hosoda Y, Hori S, Fukuchi Y, Kobayashi I (1997) Dissemination in Japanese hospitals of strains of *Staphylococcus aureus* heterogeneously resistant to vancomycin. Lancet 350:1670–1673
10. Tenover FC, Lancaster MV, Hill BC, Steward CD, Stocker SA, Hancock GA, O'Hara CM, McAllister SK, Clark NC, Hiramatsu K (1998) Characterization of staphylococci with reduced susceptibilities to vancomycin and other glycopeptides. J Clin Microbiol 36:1020–1027
11. Hiramatsu K (2001) Vancomycin-resistant *Staphylococcus aureus*: a new model of antibiotic resistance. Lancet Infect Dis 1:147–155
12. Fridkin SK, Hageman J, McDougal LK, Mohammed J, Jarvis WR, Perl TM, Tenover FC; Vancomycin-Intermediate Staphylococcus aureus Epidemiology Study Group (2003) Epidemiological and microbiological characterization of infections caused by *Staphylococcus aureus* with reduced susceptibility to vancomycin, United States, 1997–2001. Clin Infect Dis 36:429–439
13. Renzoni A, Kelley WL, Vaudaux P, Cheung AL, Lew DP (2010) Exploring innate glycopeptide resistance mechanisms in *Staphylococcus aureus*. Trends Microbiol 18:55–56
14. European Committee on Antimicrobial Susceptibility Testing (EUCAST) (2009) Glycopeptides—EUCAST clinical MIC breakpoints, 2009-09-29 (v 2.0). EUCAST, Basel, Switzerland. http://www.srga.org/eucastwt/mictab/MICglycopeptides_v2.html
15. Clinical and Laboratory Standards Institute (CLSI) (2009) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. M07-A8, 8th edn. CLSI, Wayne
16. Tenover FC, Moellering RC Jr (2007) The rationale for revising the Clinical and Laboratory Standards Institute vancomycin minimal inhibitory concentration interpretive criteria for *Staphylococcus aureus*. Clin Infect Dis 44:1208–1215
17. Clinical and Laboratory Standards Institute (CLSI) (2009) Performance standards for antimicrobial susceptibility testing, 19th informational supplement. M100-S19. CLSI, Wayne
18. van Hal SJ, Wehrhahn MC, Barbagiannakos T, Mercer J, Chen D, Paterson DL, Gosbell IB (2011) Performance of various testing methodologies for detection of heteroresistant vancomycin-intermediate *Staphylococcus aureus* in bloodstream isolates. J Clin Microbiol 49:1489–1494
19. Maor Y, Rahav G, Belausov N, Ben-David D, Smollan G, Keller N (2007) Prevalence and characteristics of heteroresistant vancomycin-intermediate *Staphylococcus aureus* bacteremia in a tertiary care center. J Clin Microbiol 45:1511–1514
20. Walsh TR, Bolmström A, Qwärnström A, Ho P, Wootton M, Howe RA, MacGowan AP, Diekema D (2001) Evaluation of current methods for detection of staphylococci with reduced susceptibility to glycopeptides. J Clin Microbiol 39:2439–2444
21. Wootton M, Howe RA, Hillman R, Walsh TR, Bennett PM, MacGowan AP (2001) A modified population analysis profile (PAP) method to detect hetero-resistance to vancomycin in *Staphylococcus aureus* in a UK hospital. J Antimicrob Chemother 47:399–403
22. Yusof A, Engelhardt A, Karlsson A, Bylund L, Vidh P, Mills K, Wootton M, Walsh TR (2008) Evaluation of a new Etest vancomycin–teicoplanin strip for detection of glycopeptide-intermediate *Staphylococcus aureus* (GISA), in particular, heterogeneous GISA. J Clin Microbiol 46:3042–3047

23. Hidayat LK, Hsu DI, Quist R, Shriner KA, Wong-Beringer A (2006) High-dose vancomycin therapy for methicillin-resistant *Staphylococcus aureus* infections: efficacy and toxicity. Arch Intern Med 166:2138–2144
24. Sakoulas G, Moise-Broder PA, Schentag J, Forrest A, Moellering RC Jr, Eliopoulos GM (2004) Relationship of MIC and bactericidal activity to efficacy of vancomycin for treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. J Clin Microbiol 42:2398–2402
25. Lodise TP, Graves J, Evans A, Graffunder E, Helmecke M, Lomaestro BM, Stellrecht K (2008) Relationship between vancomycin MIC and failure among patients with methicillin-resistant *Staphylococcus aureus* bacteremia treated with vancomycin. Antimicrob Agents Chemother 52:3315–3320
26. Soriano A, Marco F, Martínez JA, Pisos E, Almela M, Dimova VP, Alamo D, Ortega M, Lopez J, Mensa J (2008) Influence of vancomycin minimum inhibitory concentration on the treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. Clin Infect Dis 46:193–200
27. van Hal SJ, Lodise TP, Paterson DL (2012) The clinical significance of vancomycin minimum inhibitory concentration in *Staphylococcus aureus* infections: a systematic review and meta-analysis. Clin Infect Dis 54:755–771. doi:10.1093/cid/cir935
28. Hsu DI, Hidayat LK, Quist R, Hindler J, Karlsson A, Yusof A, Wong-Beringer A (2008) Comparison of method-specific vancomycin minimum inhibitory concentration values and their predictability for treatment outcome of meticillin-resistant *Staphylococcus aureus* (MRSA) infections. Int J Antimicrob Agents 32:378–385
29. Prakash V, Lewis JS 2nd, Jorgensen JH (2008) Vancomycin MICs for methicillin-resistant *Staphylococcus aureus* isolates differ based upon the susceptibility test method used. Antimicrob Agents Chemother 52:4528
30. Vaudaux P, Huggler E, Bernard L, Ferry T, Renzoni A, Lew DP (2010) Underestimation of vancomycin and teicoplanin MICs by broth microdilution leads to underdetection of glycopeptide-intermediate isolates of *Staphylococcus aureus*. Antimicrob Agents Chemother 54:3861–3870
31. Maclayton DO, Suda KJ, Coval KA, York CB, Garey KW (2006) Case–control study of the relationship between MRSA bacteremia with a vancomycin MIC of 2 microg/mL and risk factors, costs, and outcomes in inpatients undergoing hemodialysis. Clin Ther 28:1208–1216
32. Ariza J, Pujol M, Cabo J, Peña C, Fernández N, Liñares J, Ayats J, Gudiol F (1999) Vancomycin in surgical infections due to methicillin-resistant *Staphylococcus aureus* with heterogeneous resistance to vancomycin. Lancet 353:1587–1588
33. Uçkay I, Bernard L, Buzzi M, Harbarth S, François P, Huggler E, Ferry T, Schrenzel J, Renzoni A, Vaudaux P, Lew DP (2012) High prevalence of isolates with reduced glycopeptide susceptibility in persistent or recurrent bloodstream infections due to methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 56:1258–1264. doi:10.1128/AAC.05808-11
34. Ferry T, Uçkay I, Vaudaux P, François P, Schrenzel J, Harbarth S, Laurent F, Bernard L, Vandenesch F, Etienne J, Hoffmeyer P, Lew D (2010) Risk factors for treatment failure in orthopedic device-related methicillin-resistant *Staphylococcus aureus* infection. Eur J Clin Microbiol Infect Dis 29:171–180
35. François P, Huyghe A, Charbonnier Y, Bento M, Herzig S, Topolski I, Fleury B, Lew D, Vaudaux P, Harbarth S, van Leeuwen W, van Belkum A, Blanc DS, Pittet D, Schrenzel J (2005) Use of an automated multiple-locus, variable-number tandem repeat-based method for rapid and high-throughput genotyping of *Staphylococcus aureus* isolates. J Clin Microbiol 43:3346–3355
36. François P, Harbarth S, Huyghe A, Renzi G, Bento M, Gervaix A, Pittet D, Schrenzel J (2008) Methicillin-resistant *Staphylococcus aureus*, Geneva, Switzerland, 1993–2005. Emerg Infect Dis 14:304–307
37. Renzoni A, Andrey DO, Jousselin A, Barras C, Monod A, Vaudaux P, Lew D, Kelley WL (2011) Whole genome sequencing and complete genetic analysis reveals novel pathways to glycopeptide resistance in *Staphylococcus aureus*. PLoS One 6:e21577
38. Moise-Broder PA, Forrest A, Birmingham MC, Schentag JJ (2004) Pharmacodynamics of vancomycin and other antimicrobials in patients with *Staphylococcus aureus* lower respiratory tract infections. Clin Pharmacokinet 43:925–942
39. Chuard C, Lucet JC, Rohner P, Herrmann M, Auckenthaler R, Waldvogel FA, Lew DP (1991) Resistance of *Staphylococcus aureus* recovered from infected foreign body in vivo to killing by antimicrobials. J Infect Dis 163:1369–1373
40. Schaad HJ, Chuard C, Vaudaux P, Waldvogel FA, Lew DP (1994) Teicoplanin alone or combined with rifampin compared with vancomycin for prophylaxis and treatment of experimental foreign body infection by methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 38:1703–1710
41. Vaudaux P (1998) Phenotypic antibiotic tolerance of *Staphylococcus aureus* in implant-related infections: relationship with in vitro colonization of artificial surfaces. Drug Resist Updat 1:352–357
42. Vaudaux P, François P, Berger-Bächi B, Lew DP (2001) In vivo emergence of subpopulations expressing teicoplanin or vancomycin resistance phenotypes in a glycopeptide-susceptible, methicillin-resistant strain of *Staphylococcus aureus*. J Antimicrob Chemother 47:163–170
43. Clement S, Vaudaux P, François P, Schrenzel J, Huggler E, Kampf S, Chaponnier C, Lew D, Lacroix JS (2005) Evidence of an intracellular reservoir in the nasal mucosa of patients with recurrent *Staphylococcus aureus* rhinosinusitis. J Infect Dis 192:1023–1028
44. Renzoni A, Huggler E, Kelley WL, Lew D, Vaudaux P (2009) Increased uptake and improved intracellular survival of a teicoplanin-resistant mutant of methicillin-resistant *Staphylococcus aureus* in non-professional phagocytes. Chemotherapy 55:183–188
45. Chuard C, Vaudaux P, Waldvogel FA, Lew DP (1993) Susceptibility of *Staphylococcus aureus* growing on fibronectin-coated surfaces to bactericidal antibiotics. Antimicrob Agents Chemother 37:625–632
46. Chuard C, Vaudaux PE, Proctor RA, Lew DP (1997) Decreased susceptibility to antibiotic killing of a stable small colony variant of *Staphylococcus aureus* in fluid phase and on fibronectin-coated surfaces. J Antimicrob Chemother 39:603–608