MAJOR ARTICLE

Accessory Gene Regulator Group II Polymorphism in Methicillin-Resistant *Staphylococcus aureus* Is Predictive of Failure of Vancomycin Therapy

Pamela A. Moise-Broder,[1] George Sakoulas,[2] George M. Eliopoulos,[3] Jerome J. Schentag,[1] Alan Forrest,[1] and Robert C. Moellering, Jr.[3]

[1]School of Pharmacy and Pharmaceutical Sciences, State University of New York at Buffalo, CPL Associates, Amherst, and [2]Crystal Run Healthcare, Middletown, New York; and [3]Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, Massachusetts

We studied methicillin-resistant *Staphylococcus aureus* (MRSA) isolates to determine if the group II polymorphism at the accessory gene regulator *(agr)* locus demonstrated any relationship with the clinical efficacy of vancomycin. One hundred twenty-two MRSA isolates from 87 patients treated with vancomycin were evaluated. Forty-five of 87 patients had no clinical or bacteriological response to vancomycin. Among the 36 clinically evaluable patients with the *agr* group II polymorphism, 31 had an infection that failed to respond to vancomycin, whereas only 5 had an infection that responded successfully to vancomycin. This finding is of interest in light of our previous findings that glycopeptide–intermediately resistant *S. aureus* (GISA) and hetero-GISA clinical isolates in the United States and Japan are enriched for the *agr* group II polymorphism, and it suggests a possible intrinsic survival advantage of some *S. aureus* clones with this genetic marker under vancomycin selective pressure.

In the United States, methicillin-resistant *Staphylococcus aureus* (MRSA) has become a prevalent nosocomial pathogen, and it is a frequent cause of serious infections, such as endocarditis, pneumonia, osteomyelitis, and bacteremia [1–4]. In addition, several case reports have described *S. aureus* clinical isolates with reduced susceptibility to vancomycin [5–10]. There have also been reports suggesting that results of vancomycin treatment can be quite variable and that treatment with this antibiotic may sometimes be ineffective, despite laboratory susceptibility of the organism to vancomycin [11–14].

The accessory gene regulator *(agr)* operon of *S. aureus* is a key global regulon that coordinately controls many critical virulence pathways in this organism, including those involved in exoprotein, exotoxin, and adhesin expression. In general, *agr* upregulates production of secreted virulence factors, such as hemolysins and proteases, and downregulates production of virulence factors expressed on the staphylococcal cell surface [15, 16]. DNA sequence polymorphisms at this locus comprise 4 *S. aureus agr* groups. Recent studies have demonstrated that some *agr* specificity groups are associated with distinct clinical situations. For example, community-acquired MRSA usually have the *agr* group III polymorphism [17–19], glycopeptide–intermediately resistant *S. aureus* (GISA) are highly enriched for the *agr* group II polymorphism [20], and exfoliatin-producing strains are enriched for the *agr* group IV polymorphism [21]. In light of our previous findings that *agr* group II predominates among GISA isolates [20, 22], we studied clinical MRSA isolates to determine if the group II *agr* polymorphism demonstrated any relationship to the clinical efficacy of vancomycin.

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Reprints or correspondence: Dr. Pamela A. Moise-Broder, CPL Associates, 3980 Sheridan Dr., Ste. 501, Amherst, NY 14226 (pmoise@cplassociates.com).

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PATIENTS, MATERIALS, AND METHODS

Patients

All patients with viable MRSA isolates available in our frozen MRSA repository were eligible for enrollment. The MRSA repository contains ~400 MRSA isolates from ~200 patients. To be included in the present study, patients were also required to have been a participant in a larger multicenter, phase III and IV prospective study from July 1998 through November 2001 and to have received vancomycin for treatment of MRSA infection. For each study, appropriate ethical regulations were followed, and the study was approved by the ethics committee or institutional review board at each participating institution.

The 87 patients included in this analysis were from 24 hospitals, representing Connecticut, Delaware, Florida, Hawaii, Iowa, Illinois, Louisiana, Massachusetts, Maryland, New Jersey, New York, Ohio, Pennsylvania, Rhode Island, Texas, and Washington. Patients were classified as having a clinically significant MRSA infection, as defined elsewhere [23]. As part of the phase III and IV studies, patients were required to have documented signs and symptoms of a serious infection, such as fever, shaking chills, leukocytosis with a prominent shift to the left, and significant changes in vital signs. Patients who were merely colonized with MRSA were excluded from analysis. Forty-five of 87 patients had infections that failed to respond clinically and bacteriologically to vancomycin.

Definition of vancomycin treatment failure. Vancomycin treatment failure was defined for patients who had received an appropriate vancomycin dosage for at least 5 days, with persistently positive culture results and $\geqslant 1$ of the following: (1) persistence of signs and symptoms of infection observed at baseline, (2) appearance of new signs or symptoms, and (3) worsening of a sign or symptom observed at baseline. Signs and symptoms of infection used in this evaluation included fever, shaking chills, leukocytosis with a prominent shift to the left, and significant changes in vital signs.

Evaluable populations. MRSA isolates were recovered from 87 patients treated with vancomycin, 63 of whom were clinically and microbiologically evaluable for response to that agent. Patients were considered to be evaluable if they had received vancomycin for at least 5 days, the response of infection to vancomycin therapy was classified as being either a treatment success or a treatment failure, a test-of-cure assessment was performed after vancomycin treatment had been discontinued, there were no protocol violations precluding assessment of clinical outcome, and the infection outcome was not classified as being indeterminate because of confounding circumstances that precluded classification into either the treatment success or treatment failure category.

Mortality. Thirty-day mortality was based on the patients' status 30 days after the onset of MRSA infection.

Organisms

Clinical MRSA isolates, which had been stored at −70°C, were grown and maintained on trypticase-soy blood agar for subsequent testing. Vancomycin susceptibility testing was performed at a central laboratory by broth dilution, according to NCCLS guidelines.

PCR for agr Group II–Specific Primers

Clinical MRSA isolates were analyzed by PCR using *agr* II–specific primers, as reported elsewhere [24]. The 5′ primer sequence (GenBank accession no. AF001782; bp 155–163) was 5′-GTAGAGCCGTATTGATTCC-3′, and the 3′ primer sequence (GenBank accession no. AF001782; bp 600–618) was 5′-GTATTTTCATCTCTTTAAGG-3′. Reaction conditions are described elsewhere [20, 25]. In brief, 2–3 colonies of bacteria were randomly selected from an inoculum on a blood agar plate and resuspended in 100 μL of sterile water. One microliter of this suspension was added to 29 μL of PCR reaction mixture. The PCR program comprised an initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 60 s, as well as a final extension step at 72°C for 10 min [24]. The analysis of the clinical strains was done in groups of 20, and each experiment was accompanied by a positive control strain (*agr* group II prototype strain RN6607) and a negative control strain (*agr* group I prototype strain RN6390b). Both strains are well characterized elsewhere [26]. Products were analyzed using standard methods of agarose gel electrophoresis [20, 25].

Statistical Methods

Dichotomous variables were compared by means of χ^2 analysis or Fisher's exact test, when appropriate. Continuous variables were analyzed with the Kruskal-Wallis analysis of variance. Multivariate analysis was done using logistic regression. Significance was defined as $P < .05$. Statistical analysis of the data was performed using SAS software, version 8.02 (SAS Institute).

To avoid the introduction of bias into the evaluation of this study, investigators classified the patient's infection response to vancomycin before PCR was performed. In addition, the individuals who performed PCR analysis were blinded to the infection response to vancomycin, as well as to any other information linking isolates to patients. Each isolate was coded with a unique number and labeled only with that number when shipped for PCR testing. Efficacy responses and the presence of the group II polymorphism at the *agr* locus were both determined prior to unblinding the study.

RESULTS

From July 1998 through November 2001, 87 patients treated with vancomycin for an infection due to MRSA were identified.

They ranged in age from 24 to 99 years, with a mean age (±SD) of 65.7 ± 16.3 years. There were slightly more men (56.3%) than women. The mean baseline creatinine clearance rate (±SD) was 64.1 ± 38.3 mL/min (median, 58.6 mL/min). At baseline, 36 (41.4%) of 87 patients were receiving mechanical ventilation, and 40 (46.0%) of 87 were in an intensive care unit. Many patients had significant comorbidities, such as congestive heart failure (25 patients), coronary artery disease (25), chronic obstructive pulmonary disease (25), diabetes (24), dialysis-dependent renal failure (10), and malignancy (10).

Table 1 contains a description of the types of infections treated with vancomycin. Of the 87 patients who received vancomycin, 63 were clinically and bacteriologically evaluable. Respiratory infections were found in 34 (39.1%) of 87 patients who received vancomycin and 22 (34.9%) of 63 patients who were clinically and bacteriologically evaluable. Central catheter–related bacteremia infections were observed in 19 (21.8%) of 87 vancomycin recipients and in 13 (20.6%) of 63 evaluable patients. MRSA bacteremia was identified in 40 (46%) of 87 patients who received vancomycin and in 34 (54.0%) of 63 patients who were clinically and bacteriologically evaluable. Of patients clinically and bacteriologically evaluable, MRSA was isolated from blood cultures of patients with bone and joint, intraabdominal, respiratory tract, and skin and skin structure infections; central catheter–related bacteremia; and endocarditis. Infection response rates to vancomycin by population are also shown in table 1.

MICs of vancomycin and treatment outcome. Table 2 reveals results of univariate analysis of variables and vancomycin treatment outcome. Despite the fact that all organisms were susceptible to vancomycin by MIC testing (i.e., MICs ≤ 4 μg/mL), univariate analysis demonstrated that, as MICs of vancomycin increased within the susceptible range, there was a statistically significant association with vancomycin treatment failure.

Relationship between clinical characteristics of MRSA with the* agr *group II polymorphism and vancomycin efficacy. In the univariate analysis, there was decreased vancomycin efficacy against MRSA infection when the infecting isolate contained the group II polymorphism at the *agr* locus (table 2). Thirty-one (68.9%) of the 45 patients in the treatment failure group had isolates with the *agr* group II polymorphism, compared with only 5 (27.8%) of the 18 patients whose infection responded successfully to vancomycin. Of the 24 patients whose infection response was classified as indeterminate, 16 (66.7%) had *agr* group II isolates.

Significant univariate predictors of infection response to vancomycin were entered into a logistic regression analysis (table 3). Creatinine clearance rate and the group II *agr* polymorphism remained important predictors of the response of MRSA infection to vancomycin. For every 10-mL/min decrease in the clearance rate, the odds of a successful response to vancomycin decreased by ~20%. The odds of successful vancomycin treatment against infections with isolates that had the *agr* group II polymorphism were ~6.9 times higher than against infections due to *agr* group II isolates.

Table 1. Types and sites of methicillin-resistant *Staphylococcus aureus* infection.

Infection type or site	Response to vancomycin therapy, no. of patients		
	Success (n = 18)	Failure (n = 45)	Indeterminate (n = 24)
Central catheter–related bacteremia	9	4	6
Bacteremia of unknown origin	1	5	0
Bone and joint	1	7	0
Device	0	1	0
Endocarditis	0	3	0
Intraabdominal	1	1	0
Respiratory	5	17	12
Skin and skin structure	1	6	6
Urinoma[a]	0	1	0

[a] Renal allograft pyelonephritis with infected extrarenal urine collection.

Relationship between the group II* agr *polymorphism, infection site, prior vancomycin use, and year of isolate recovery. Thirty-six (57.1%) of the 63 clinically and microbiologically evaluable patients had MRSA infections with *agr* group II isolates. As stated above in the Patients subsection, these 63 patients had a variety of infections and were identified from July 1998 through November 2001. There was no statistically significant relationship between the prevalence of infecting isolates with the *agr* group II polymorphism and the site of MRSA infections in the evaluable population ($P = .3731$). There was also no significant relationship between the prevalence of infecting isolates with the *agr* group II polymorphism and the following durations of vancomycin treatment before performance of culture: 0 days, 1–4 days, 5–14 days, 15–28 days, and >28 days ($P = .7738$). Finally, there was no significant relationship between the year in which isolates were recovered (1998, 1999, 2000, or 2001) and the prevalence of infecting isolates with the *agr* group II polymorphism ($P = .2317$).

Relationship between the* agr *group II polymorphism and 30-day mortality. Thirty-day all-cause mortality was not associated with isolates with the *agr* specificity group II polymorphism. Overall 30-day mortality involved 8 (22.2%) of 36 patients with *agr* group II MRSA isolates and 6 (22.2%) of 27 patients with non–*agr* group II isolates. No attempt was made

Table 2. Univariate analysis of factors associated with response to vancomycin therapy.

Factor	Response to vancomycin therapy		
	Success (n = 18)	Failure (n = 45)	P
Demographic/clinical characteristic			
Age, years	65.5 ± 17.2 (71.5)	66.5 ± 16.2 (70.0)	.99
Baseline creatinine clearance rate, mL/min	73.3 ± 33.3 (74.8)	55.9 ± 34.7 (54.0)	.038
Male sex	12 (67)	26 (58)	.51
Located in an ICU on day 1	9 (50)	19 (42)	.57
Receipt of mechanical ventilation	10 (56)	15 (35)	.13
Comorbid disease or condition			
Diabetes	4 (22)	15 (33)	.55
Congestive heart failure	4 (22)	17 (38)	.38
Coronary artery disease	3 (17)	20 (44)	.047
Peripheral vascular disease	4 (22)	10 (22)	1.0
Dialysis-dependent renal failure	1 (6)	8 (18)	.43
COPD	6 (33)	14 (31)	.86
Cirrhosis	0 (0)	2 (4)	1.0
Malignancy	1 (6)	8 (18)	.43
Alcoholism	2 (11)	1 (2)	.19
Cystic fibrosis	0 (0)	1 (2)	1.0
Steroid use	5 (28)	9 (20)	.52
HIV infection	0 (0)	1 (2)	1.0
Transplant recipient	1 (6)	1 (2)	.49
Isolate characteristic			
Vancomycin susceptibility, MIC			.004
0.5	11 (61)	10 (22)	
1.0	5 (28)	12 (27)	
2.0	2 (11)	23 (51)	
Group II *agr* specificity	5 (28)	31 (69)	.003

NOTE. Data are no. (%) of patients or mean value ± SD. *agr,* accessory gene regulator; COPD, chronic obstructive pulmonary disease; ICU, intensive care unit.

to determine if death was directly attributable to MRSA infection.

DISCUSSION

Infections with *S. aureus* are associated with mortality rates of 15%–60%, and a recent meta-analysis of published reports on *S. aureus* bacteremia suggests that MRSA bacteremia has a mortality risk that is almost twice that of MSSA bacteremia [27]. One of several possible explanations for this observation is that antimicrobial therapy for treatment of MRSA infection is not as effective as therapy for MSSA infection. Vancomycin has been considered to be less effective against MRSA infection than β-lactams have been against MSSA infection [28, 29].

At the present time, vancomycin remains the standard treatment for infections due to MRSA. However, vancomycin treatment failures have been reported even when isolates were determined to be susceptible using current clinical microbiological methods [11–13]. Bactericidal therapy is believed to be critical in the treatment of bloodstream infections, and, therefore, clinical failures with vancomycin may be at least partially explained by tolerance to vancomycin by some MRSA isolates, as some of our preliminary data suggests (G. Sakoulas, P. Moise-Broder, G. Eliopoulos, et al.; unpublished data).

We believe that clinical GISA isolates represent the final evolutionary step in a multiple-stage process that MRSA has undergone because of vancomycin selection pressure. MRSA organisms in the hospital have developed the capability to survive in a glycopeptide-rich environment. As such, we found it to be very interesting that ~55% to 60% of MRSA isolates and all 6 of the Centers for Disease Control and Prevention–confirmed GISA isolates in the United States that we evaluated have the *agr* group II polymorphism. Our previous studies

Table 3. Multivariate analysis of factors associated with successful vancomycin treatment.

Factor	OR (95% CI)	*P*
Baseline creatinine clearance rate	1.198 (1.002–1.433)[a]	.047
Non–group II *agr*	6.935 (1.774–27.112)	.005

[a] Per 10 mL/min change in clearance. *agr,* accessory gene regulator.

suggested that the glycopeptide-influenced selection process for *S. aureus* may begin with the clonal background of MRSA [20, 22]. We undertook the present study, therefore, to determine if the group II *agr* polymorphism would confer any clinical significance with respect to vancomycin efficacy.

Our study showed a very strong statistical relationship between vancomycin treatment failure and infection due to MRSA with the *agr* group II polymorphism. The *agr* group II polymorphism was found to be an independent predictor of vancomycin treatment failure in patients with MRSA infection. This is the first report linking a specific genetic polymorphism in *S. aureus* to antimicrobial treatment failure. These findings may serve as an initial step toward providing clinicians with useful information for guiding treatment of patients with MRSA infection. The explanation behind the findings in the present article is not clear, but we feel that these results lend support to our previous findings of possible selective advantages of some clonal types of *S. aureus* with the *agr* group II polymorphism under glycopeptide selection pressure [22]. It is clear that more-detailed genetic and biochemical testing, as well as epidemiological studies of MRSA, in the setting of increasing vancomycin exposure will be necessary to lend further support to these findings.

At the time of writing, we suspect that it may not be the *agr* specificity group itself but, rather, select clonal backgrounds within this *agr* group that may have the intrinsic advantages under vancomycin selection and may, therefore, be more likely responsible for the failure of vancomycin therapy. With further study of the genetic backgrounds that comprise *agr* group II *S. aureus,* perhaps using the more discriminatory method of multilocus sequence typing, one may be able to determine even more-specific markers for MRSA bacteremia treatment failure.

The strong correlation between infection with *agr* group II MRSA and vancomycin treatment failure did not translate into a difference in mortality rate. However, this study was not reliable in its evaluation of mortality, because most of the patients who did not respond to vancomycin therapy received salvage therapy with newer antimicrobial agents in accordance with compassionate-use protocols. Whether vancomycin treatment failure leading to prolonged bacteremia translates into increased morbidity in the form of metastatic complications would be an interesting subject for future study.

Finally, we found diminished creatinine clearance rate to be the only other variable with a statistically significant association with vancomycin treatment failure. We can only speculate, but this finding suggests that perhaps there are mechanisms of the antistaphylococcal host defense that are impaired in uremic patients. For example, it is known that the uremic state is characterized by impaired platelet aggregation, perhaps through circulating fibrinogen fragments [30]. Evidence suggests that platelet aggregation may serve an integral role in host defense against *S. aureus* bloodstream infections [31, 32].

In summary, we have demonstrated a strong relationship between vancomycin treatment failure and MRSA isolates with the group II polymorphism at the *agr* locus. Together with our previous findings that the *agr* group II polymorphism is common among GISA [20, 22], these observations suggest that *S. aureus*—particularly MRSA—may be evolving at the clonal level (because of increasing glycopeptide use) to maximize survival.

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