Antimicrobial Agents and Chemotherapy, Dec. 2011, p. 5433–5437 Vol. 55, No. 12
0066-4804/11/$12.00 doi:10.1128/AAC.00407-11
Copyright © 2011, American Society for Microbiology. All Rights Reserved.

Predictors of *agr* Dysfunction in Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolates among Patients with MRSA Bloodstream Infections[▽]

Jill M. Butterfield,[1] Brian T. Tsuji,[2] Jack Brown,[2] Elizabeth Dodds Ashley,[3] Dwight Hardy,[3] Kristen Brown,[3] Alan Forrest,[2] and Thomas P. Lodise[1,4]*

Albany College of Pharmacy and Health Sciences, Albany, New York[1]; University at Buffalo, School of Pharmacy and Pharmaceutical Sciences, Buffalo, New York[2]; University of Rochester Medical Center, Rochester, New York[3]; and Ordway Research Institute, Albany, New York[4]

Received 28 March 2011/Returned for modification 24 May 2011/Accepted 7 September 2011

Despite emerging evidence that dysfunction in the accessory gene regulator (*agr*) locus is associated with deleterious outcomes among patients treated with vancomycin for methicillin-resistant *Staphylococcus aureus* (MRSA) infections, factors predictive of *agr* dysfunction have not been evaluated. This study describes the epidemiology of *agr* dysfunction, identifies predictors of *agr* dysfunction in MRSA isolates among those with MRSA bloodstream infections, and describes the relationship between *agr* dysfunction and other microbiologic phenotypes. A cross-sectional study of patients with MRSA bloodstream infections at two institutions in upstate New York was performed. Clinical data on demographics, comorbidities, disease severity, hospitalization history, and antibiotic history were collected. Microbiologic phenotypes, including *agr* dysfunction, MIC values by broth microdilution (BMD) and Etest, and vancomycin heteroresistance (hVISA) were tested. Multivariable analyses were performed to identify factors predictive of *agr* dysfunction. Among 200 patients with an MRSA bloodstream infection, the proportion of strains with *agr* dysfunction was 31.5%. The distribution of MICs determined by both BMD and Etest were equivalent across *agr* groups, and there was no association between *agr* dysfunction and the presence of hVISA. Severity of illness, comorbidities, and hospitalization history were comparable between *agr* groups. In the multivariate analysis, prior antibiotic exposure was the only factor of variables studied found to be predictive of *agr* dysfunction. This relationship was predominantly driven by prior beta-lactam and fluoroquinolone administration in the bivariate analysis. Identifying these institution-specific risk factors can be used to develop a process to assess the risk of *agr* dysfunction and guide empirical antibiotic therapy decisions.

The accessory gene regulator (*agr*) is a quorum-sensing operon which coordinates the expression of secreted and cell-associated virulence factors, and it controls several metabolic pathways in *Staphylococcus aureus* in a growth phase-related fashion (7, 30). *In vitro* studies show that alterations in *agr* function result in several phenotypic changes in *S. aureus*, including diminished autolysis (21), attenuated vancomycin activity (20, 22, 29), vancomycin heteroresistance (22), and an increased proclivity for the development of intermediate resistance to vancomycin (7, 22, 29, 30). There is also increasing clinical evidence showing that alterations in *agr* in *S. aureus* are a key risk factor for poor outcomes in patients with *S. aureus* bacteremia (7, 25).

Despite the poor outcomes associated with *agr* dysfunction, factors predictive of *agr* dysfunction have not been well described, and scant literature exists describing the relationship between *agr* dysfunction and other reduced vancomycin susceptibility phenotypes. This study describes the epidemiology of *agr* dysfunction, determines predictors of *agr* dysfunction in methicillin-resistant *Staphylococcus aureus* (MRSA) isolates among those with MRSA bloodstream infections, and describes the relationship between *agr* dysfunction and other microbiologic phenotypes.

MATERIALS AND METHODS

Study design and population. We performed a cross-sectional study of patients with MRSA bloodstream infections at two institutions in upstate New York: Albany Medical Center Hospital (AMCH), a 631-bed tertiary-care academic hospital located in Albany, NY, and Strong Memorial Hospital (SMH), a 765-bed academic medical center located in Rochester, NY. The study included patients at least 18 years of age hospitalized with an MRSA bloodstream infection between 1 January 2007 and 30 June 2009. For those with more than one MRSA bloodstream infection during the study period, only the first episode was considered. For patients with multiple MRSA blood cultures during the study time period, only microbiologic phenotypes of the first isolate were examined. Isolates were shipped to the Laboratory for Antimicrobial Pharmacodynamics at the School of Pharmacy and Pharmaceutical Sciences at the University at Buffalo for processing.

Patient data. Trained reviewers used a structured data collection instrument to collect the following information from patients' medical records: age, sex, weight, height, medical history and comorbidities, health care institution exposure for >72 h within 180 days of hospital admission, receipt of antibiotics in the 30 days prior to the index blood culture collection, length of hospitalization prior to index blood culture, hospital unit at the time of index blood culture collection, creatinine clearance (CL_{CR}) as estimated by the Cockcroft-Gault formula at the time of index blood culture collection (5), illness severity (calculated by using the Acute Physiological and Chronic Health Evaluation [APACHE-II] score) (10), and microbiologic data.

Documentation of the antibiotics used within 30 days of index culture included vancomycin, linezolid, daptomycin, tigecycline, clindamycin, trimethoprim-sulfamethoxazole, metronidazole, rifampin, and all antibiotics within the classes of tetracyclines, beta-lactams, fluoroquinolones, macrolides, and aminoglycosides. Data regarding prior antibiotic use at outside hospitals or in an outpatient setting were not collected because of the difficulty in recovering accurate data. The

* Corresponding author. Mailing address: Department of Pharmacy Practice, Albany College of Pharmacy and Health Sciences, 106 New Scotland Avenue, Albany, NY 12208. Phone: (518) 694-7292. Fax: (518) 694-7202. E-mail: Thomas.lodise@acphs.edu.
▽ Published ahead of print on 19 September 2011.

APACHE-II score was calculated from the worst physiological score within the first 24 h prior to MRSA blood culture sample collection. For non-intensive care unit (ICU) patients with missing data components, APACHE-II was calculated assuming the normal range for the value (27).

Laboratory methods. (i) Bacterial isolates. All bloodstream isolates were identified as *S. aureus* by Gram stain and colony morphology, as well as catalase- and coagulase-positive reactions.

(ii) Determination of *agr* operon function. The function of the *agr* operon was qualitatively assessed for all MRSA bloodstream isolates as previously described, utilizing delta-hemolysin expression as a marker of *agr* function (22, 28). Delta-hemolysin expression was determined by using *S. aureus* RN4420 (obtained from the Network Antimicrobial Resistance in *S. aureus* [NARSA]) as a reference strain that produces a large zone of beta-hemolysin without the interference of alpha- or delta-hemolysins. Changes in *agr* function were characterized by delta-hemolysin activity as absent, severely depressed, easily detectable, increased, and exceptionally high. Isolates which were characterized as absent or severely depressed were defined as *agr* dysfunctional (22, 28).

(iii) Antimicrobial susceptibility testing. The vancomycin MIC was determined by broth microdilution (BMD) in duplicate for all *S. aureus* isolates by methods outlined by the CLSI guidelines (4). Vancomycin MICs also were determined with the Etest (0.016 to 256 mg/liter) (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions. Isolates for which the drug MICs were ≥2 μg/ml by BMD and ≥1.5 μg/ml by Etest were designated the high-MIC group. Those for which the MICs were ≤1 μg/ml by BMD and Etest were considered to be the low-MIC group.

(iv) hVISA determination. Overnight growth cultures of the clinical isolates, control methicillin-susceptible *S. aureus* ATCC 29213, standard hVISA strain Mu3, and Mu50 were suspended in saline solution and evaluated for hVISA status. Evaluation with the Etest glycopeptide-resistant detection (GRD) method was completed according to the manufacturer's instructions. A bacterial suspension corresponding to a 0.5 McFarland standard was lawned on a plate of Mueller-Hinton agar with 5% blood (MHB; Becton, Dickinson and Company, Sparks, MD) and on a Mueller-Hinton agar (MHA; Difco) plate. An Etest GRD strip consisting of a double-sided gradient with vancomycin and teicoplanin was applied to the MHB plate, and a standard vancomycin Etest was applied to the MHA plate. The zone of the Etest GRD strip was read (complete inhibition of growth) at 48 h. The test isolate was considered positive for hVISA if the Etest GRD strip was ≥8 mg/liter for either vancomycin or teicoplanin and the standard vancomycin Etest MIC was ≥4 mg/liter (12).

Statistical analysis. For bivariate analyses, categorical variables were compared by the Pearson chi-square or Fisher's exact test, and continuous variables were compared by the Student's *t* or Mann-Whitney U test. For comparisons involving more than two groups, the Pearson chi-square test was used for categorical variables; for continuous variables, an analysis of variance or the Kruskal-Wallis test was used.

Given the difference in clinical covariates and exposures between community-onset and hospital-onset MRSA bloodstream infections, exposure-response associations were stratified by time to index blood culture collection (<72 versus ≥72 h). Multivariable analyses were performed to identify patients at greatest risk for harboring an isolate with *agr* dysfunction. All variables associated with *agr* dysfunction in the bivariate analysis ($P < 0.2$) were considered for inclusion in the explanatory log-binomial regression model. Log-binomial regression was used, since prevalence ratios (PRs) were estimated; log-binomial regression is better at approximating prevalence than logistic regression (logistic regression estimates odds, not prevalence) (15). Significant interaction terms identified in the analysis stratified by time to index blood culture collection (<72 h versus ≥72 h) also were included at model entry. After all variables were entered in the model, a backward approach was used to delineate the best fitting or most parsimonious model. Interaction terms were evaluated first, and a stepwise approach then was used to derive a parsimonious model. Due to the cross-sectional design of the study, PRs were computed for variables in the final model (15, 26). All potential confounders (variables with $P < 0.2$ in the bivariate analysis) were put back into the model to assess their impact, and those which changed any of the final model PRs by ≥10% were retained. For all analyses, a *P* value of <0.05 was considered significant for two-tailed tests. All calculations were computed using SAS, version 9.2 (SAS, Cary, NC), and SPSS, version 12.0.1 (SPSS, Chicago, IL).

RESULTS

During the study period, 200 patients met the eligibility criteria. While sites were generally well matched with regard to clinical covariates, there were slight differences: AMCH observed lower median CL_{CR} values than SMH (49.1 and 65.0 ml/min, respectively; $P = 0.024$) and had a greater proportion of patients with diabetes mellitus (49.5 and 33.7%, respectively; PR = 1.5; 95% CI = 1.0 to 2.1; $P = 0.023$), hepatic dysfunction (10.1 and 0%, respectively; $P = 0.001$), prior antibiotic exposure (48.5 and 30.7%, respectively; PR = 2.0; 95% CI = 1.3 to 3.2; $P = 0.003$), prior beta-lactam exposure (28.3 and 5.0%, respectively; PR = 5.7; 95% CI = 2.3 to 14.2; $P < 0.001$), prior fluoroquinolone exposure (18.2 and 5.9%, respectively; PR = 3.1; 95% CI = 1.3 to 7.4; $P = 0.008$), and mean number of prior antibiotic exposures (0.88 and 0.26, respectively; $P < 0.001$). The proportion of solid-organ transplants was lower among AMCH than SMH patients (1.0 and 9.9%, respectively; PR = 0.1; 95% CI = 0.0 to 0.8; $P = 0.010$), as was that for decubitus ulcers (15.2 and 30.7%, respectively; PR = 0.5; 95% CI = 0.3 to 0.9; $P = 0.009$) and immunosuppressants (12.1 and 25.7%, respectively; PR = 0.5; 95% CI = 0.3 to 0.9; $P = 0.014$).

The overall proportion of isolates with *agr* dysfunction was 31.5%; a greater proportion was observed at AMCH than at SMH (39.4 and 23.8%, respectively; PR = 1.7; 95% CI = 1.1 to 2.5; $P = 0.02$). In the bivariate analysis, the distribution of MICs was similar across *agr* groups for both testing methods. High MICs (≥2 mg/liter) by BMD were seen for 22 (34.9%) isolates with a dysfunctional *agr* locus and for 42 (30.7%) of isolates with a functional *agr* locus (PR = 1.1; 95% CI = 0.7 to 1.7; $P = 0.62$). Similarly, high MIC values (≥1.5 mg/liter) by Etest were observed for 28 (44.4%) and 48 (35%) of isolates with and without a defect in the *agr* locus, respectively (PR = 1.3; 95% CI = 0.9 to 1.8; $P = 0.21$). A total of 13 (6.5%) isolates demonstrated heteroresistance. The distribution of hVISA isolates also was similar in isolates with *agr* dysfunction versus those without *agr* dysfunction (4.8 and 7.3%, respectively) (PR = 0.7; 95% CI = 0.2 to 2.3; $P = 0.76$). No effect modification on the relative scale was observed when stratifying the relationships between *agr* dysfunction and microbiologic phenotypes by hospital (data not shown).

Table 1 shows the relationship between clinical features and isolates with *agr* dysfunction in the overall population and stratified by length of stay (LOS) prior to culture collection. Overall, no clinical covariates were associated with *agr* dysfunction, and the relationships between clinical features and *agr* dysfunction were consistent between sites (data not shown). In individuals with an LOS of ≥72 h, isolates with *agr* dysfunction were more common among older patients (mean age, 65.6 years versus 57.7 years; $P = 0.04$). For LOS of <72 h, isolates with a dysfunctional *agr* locus were associated with lower mean APACHE II scores than those with a functional *agr* locus (10.8 versus 14.3; $P = 0.01$). No effect modification was observed when stratifying the relationships between *agr* dysfunction and clinical covariates by hospital (data not shown).

A significant relationship between prior antibiotic exposure and isolates with a dysfunctional *agr* locus was noted (Table 1). Although prior antibiotic exposure was more pronounced at AMCH, the magnitude of association between isolates with *agr* dysfunction and prior antibiotic exposure was similar at both sites (no effect modification). When stratified by LOS, the relationship between prior antibiotic exposure within 30 days of culture collection and isolates with *agr* dysfunction was most

TABLE 1. Relationship between clinical covariates and *agr* dysfunctional isolates in the overall population and stratified by LOS

Characteristic	Results by LOS group								
	Overall			LOS of $<$72 h			LOS of $\geq$72 h		
	agr dysfunction (n = 63)	*agr* functional (n = 137)	PR (95% CI)	*agr* dysfunction (n = 32)	*agr* functional (n = 74)	PR (95% CI)	*agr* dysfunction (n = 31)	*agr* functional (n = 63)	PR (95% CI)
Mean (SD) age (yr)	61.2 (15.4)	58.1 (17.8)	NA[d]	56.8 (16.1)	58.5 (17.1)	NA	65.6 (13.5)	57.7 (18.7)	NA
No. (%) male	38 (60.3)	80 (58.4)	1.0 (0.8 to 1.3)	18 (56.3)	44 (59.5)	0.9 (0.7 to 1.4)	20 (64.5)	36 (57.1)	1.1 (0.8 to 1.6)
Median (IQR) prior length of stay (days)	2 (0–14)	2 (0–14)	NA	0 (0–0)	0 (0–1)	NA	14 (7–34)	15 (9–27)	NA
No. (%) in ICU at culture collection	23 (36.5)	53 (38.7)	0.9 (0.6 to 1.4)	2 (6.3)	9 (12.2)	0.5 (0.1 to 2.2)	21 (67.7)	44 (69.8)	1.0 (0.7 to 1.3)
No. (%) with previous hospitalization within 180 days	36 (57.1)	66 (48.2)	1.2 (0.9 to 1.6)	22 (66.8)	36 (48.6)	1.4 (1.0 to 2.0)	14 (45.2)	30 (47.6)	0.9 (0.6 to 1.5)
Median (IQR) no. of comorbidities	2 (1–4)	2 (1–3)	NA	2 (1–3)	2 (1–3)	NA	3 (1–4)	2 (1–4)	NA
No. (%) receiving dialysis	15 (23.8)	41 (29.9)	0.8 (0.5 to 1.3)	8 (25.0)	25 (33.8)	0.7 (0.4 to 1.5)	7 (22.6)	16 (25.4)	0.9 (0.4 to 1.9)
No. (%) with diabetes mellitus	31 (49.2)	52 (38.0)	1.3 (0.9 to 1.8)	17 (53.1)	25 (33.8)	1.6 (1.0 to 2.5)	14 (45.2)	27 (42.9)	1.1 (0.7 to 1.7)
No. (%) with heart failure	25 (39.7)	40 (29.2)	1.4 (0.9 to 2.0)	10 (31.3)	18 (24.3)	1.3 (0.7 to 2.5)	15 (48.4)	22 (34.9)	1.4 (0.8 to 2.3)
No. (%) with chronic obstructive pulmonary disease	20 (31.7)	35 (25.5)	1.2 (0.8 to 2.0)	8 (25.0)	15 (20.3)	1.2 (0.6 to 2.6)	12 (38.7)	20 (31.7)	1.2 (0.7 to 2.2)
No. (%) with hepatic dysfunction	2 (3.2)	8 (5.8)	0.5 (0.1 to 2.5)	0 (0)	3 (4.1)	NA	2 (6.5)	5 (7.9)	0.8 (0.2 to 4.0)
No. (%) with solid-organ transplant	1 (1.6)	10 (7.3)	0.2 (0.0 to 1.7)	1 (3.1)	6 (8.1)	0.4 (0.0 to 3.1)	0 (0)	4 (6.3)	NA
No. (%) with cerebrovascular disease	8 (12.7)	18 (13.1)	1.0 (0.4 to 2.1)	2 (6.3)	9 (12.2)	0.5 (0.1 to 2.2)	6 (19.4)	9 (14.3)	1.4 (0.5 to 3.5)
No. (%) with HIV	1 (1.6)	2 (1.5)	1.1 (0.1 to 11.8)	1 (3.1)	2 (2.7)	1.2 (0.1 to 12.3)	0 (0)	0 (0)	NA
No. (%) with decubitus ulcer	13 (20.6)	33 (24.1)	0.9 (0.5 to 1.5)	7 (21.9)	15 (20.3)	1.1 (0.5 to 2.4)	6 (19.4)	18 (28.6)	0.7 (0.3 to 1.5)
No. (%) with administration of immunosuppressants	12 (19.0)	26 (19.0)	1.0 (0.5 to 1.9)	7 (21.9)	18 (24.3)	0.9 (0.4 to 1.9)	5 (16.1)	8 (12.7)	1.3 (0.5 to 3.6)
No. (%) with surgery $<$30 days prior to onset	15 (23.8)	38 (27.7)	0.9 (0.5 to 1.4)	3 (9.4)	11 (14.9)	0.6 (0.2 to 2.1)	12 (38.7)	27 (42.9)	0.9 (0.5 to 1.5)
Median (IQR) CL_{CR} (ml/min)	55.9 (34.1–93.5)	55 (18.0–93.5)	NA	67.0 (25.0–97.6)	53.6 (16.0–90.4)	NA	52.6 (35.5–89.3)	57.4 (23.7–104.5)	NA
Mean (SD) APACHE-II score at baseline	13.8 (6.8)	14.9 (6.9)	NA	10.8 (5.6)	14.3 (6.4)	NA	16.9 (6.7)	15.5 (7.3)	NA
No. (%) with prior antibiotic exposure of interest									
Prior antibiotic	26 (41.3)	33 (24.1)	1.7 (1.1 to 2.6)[a]	5 (15.6)	9 (12.2)	1.3 (0.5 to 3.5)	21 (67.7)	24 (38.1)	1.8 (1.2 to 2.6)[a]
Prior anti-MRSA antibiotic[b]	9 (14.3)	18 (13.1)	1.1 (0.5 to 2.3)	3 (9.4)	7 (9.5)	1.0 (0.3 to 3.6)	6 (19.4)	11 (17.5)	1.1 (0.5 to 2.7)
Prior vancomycin	3 (4.8)	16 (11.7)	0.4 (0.1 to 1.3)	1 (3.1)	6 (8.1)	0.4 (0.0 to 3.1)	2 (6.5)	10 (15.9)	0.4 (0.1 to 1.7)
Prior beta-lactam[c]	15 (23.8)	18 (13.1)	1.8 (1.0 to 3.4)[a]	4 (12.5)	2 (2.7)	4.6 (0.9 to 24.0)	11 (35.5)	16 (25.4)	1.4 (0.7 to 2.6)
Prior fluoroquinolone	12 (19.0)	12 (8.8)	2.2 (1.0 to 4.6)[a]	0 (0)	1 (1.4)	NA	12 (38.7)	11 (17.5)	2.2 (1.1 to 4.4)[a]
Median no. (IQR) of prior antibiotic exposures	0 (0–1)	0 (0–0)	NA	0 (0–0)	0 (0–0)	NA	1 (0–2)[a]	0 (0–1)[a]	NA

[a] $P < 0.05$.
[b] Prior anti-MRSA antibiotics received included clindamycin (n = 3), linezolid (n = 4), tetracycline (n = 1), tigecycline (n = 1), trimethoprim-sulfamethoxazole (n = 2), and vancomycin (n = 19). Some patients received more than one prior treatment with an anti-MRSA antibiotic.
[c] Prior beta-lactams received included amoxicillin (n = 2), amoxicillin-clavulanate (n = 1), ampicillin-sulbactam (n = 3), aztreonam (n = 1), cefazolin (n = 9), cefepime (n = 4), ertapenem (n = 1), meropenem (n = 6), and piperacillin-tazobactam (n = 22). Some patients received more than one prior treatment with a beta-lactam.
[d] NA, not able to calculate the prevalence ratio.

pronounced in patients with an LOS of ≥72 h ($P = 0.009$). This was driven predominantly by prior beta-lactam and fluoroquinolone administration. For those who developed an MRSA bacteremia within 72 h of hospitalization, nonsignificant trends were found between *agr* dysfunction and prior exposure to antibiotics, particularly beta-lactams. Prior vancomycin exposure was not associated with isolates with *agr* dysfunction in the overall population or when stratified by LOS.

In the log-binomial regression, prior antibiotic exposure was the only factor retained in the final model (PR = 1.7; 95% CI = 1.2 to 2.4). This indicates that for individuals with a prior antibiotic exposure, the probability of being infected with an *agr* dysfunctional isolate at culture collection is 1.7 times higher than for those individuals without prior antibiotic exposure. All variables associated with *agr* functional status among MRSA isolates in the bivariate analysis at $P < 0.2$ were put back into the model, and none changed the final PR for prior antibiotic exposure by 10% or more.

DISCUSSION

This study distinguishes itself by being the first to describe covariates that best predict the presence of *agr* dysfunction among MRSA isolates in patients with MRSA bloodstream infections across two major academic hospitals. It is of greatest clinical interest that a relationship between isolates with *agr* dysfunction and prior antibiotic exposure was observed in the multivariate analysis. Given the results of the bivariate analysis, it is reasonable to surmise that this observed relationship was largely driven by prior administration of beta-lactams and fluoroquinolones in patients with an LOS of ≥72 h. Overall, patients who received a prior beta-lactam or fluoroquinolone were approximately twice as likely to be infected with a dysfunctional versus functional *agr* isolate.

Although this study was not designed to assess causality, these findings are biologically plausible. The prior use of fluoroquinolones and beta-lactam agents has been known to cause collateral damage through the selection of antibiotic-resistant populations in MRSA (1, 6, 8, 9, 11, 14, 17, 18, 24, 34). This phenomenon may be due to the counterselection of *agr* dysfunctional strains (23, 29) and the eradication of *agr* functional strains. In particular, subinhibitory concentrations of fluoroquinolones have been shown to induce mutational mechanisms in *S. aureus* that promote survival, the development of resistance, and bacterial tissue adherence and colonization (2, 3, 13, 16, 19). It also has been shown that *agr* dysfunctional, slow-growing strains that are small colony variants of MRSA display increased resistance to fluoroquinolones and beta-lactam agents (33). Along with recent evidence that suggests that the loss of *agr* function is associated with increased biofilm production, persistence, and prolonged bacteremia, the potential associations with prior exposure to fluoroquinolones and beta-lactams is of interest and suggests a selective survival advantage for *agr* dysfunctional strains which may be driven by these agents (31–33).

Contrary to the prevailing thinking, we did not find an association between *agr* dysfunction and prior exposure to vancomycin or other anti-MRSA antibiotics. Of the 19 patients with a documented prior exposure to vancomycin, only 3 were *agr* dysfunctional. It has been shown that subinhibitory concentrations of vancomycin select for other resistance phenotypes, including decreased autolysis, hVISA, and VISA. This lack of a relationship may be an artifact of our institutions' empirical dosing strategies; our institutions began targeting trough values of 15 to 20 mg/liter in 2007 (20–22, 30). Alternatively, our findings may be a result of incomplete information on prior exposure outside the hospital setting, particularly among those receiving dialysis or those who developed bacteremia within 72 h of admission. Although we cannot exclude the possibility of a type II error given the sample size, our data suggest there may not be a relationship between *agr* dysfunction and prior exposure to vancomycin, particularly in a setting where higher trough concentrations are targeted.

While a relationship between *agr* dysfunction and higher MICs has been presumed, this was not observed in our study. Previous studies also have suggested that defects in the *agr* locus contribute to hVISA development. However, we did not find an association between these variables. Our findings do not refute the relationship between *agr* dysfunction and the development of higher-MIC MRSA isolates and hVISA. Rather, our data suggest that there are other things involved in the causal pathway. In addition, we cannot exclude the possibility of a type II error given the sample size. As an example, there was a nonsignificant trend in the association between *agr* dysfunction and high Etest MICs (PR = 1.3; 95% CI = 0.9 to 1.8; $P = 0.21$). While the definitive explanations for these findings remain unclear, they underscore the need for additional studies to better describe the mechanisms responsible for the development of higher-MIC isolates and hVISA.

Several limitations of this study should be noted. First, we had incomplete information on antibiotic exposure prior to admission, therefore it is possible that subjects who presented with an MRSA bloodstream infection on admission were misclassified on prior antibiotic exposure. We also did not capture the vancomycin troughs maintained in patients previously exposed to vancomycin. Second, we saw a low proportion of hVISA isolates in our study, which may explain why we did not detect an association between this phenotype and *agr* dysfunction. Third, we acknowledge that hVISA is a relatively unstable phenotype, which is no exception concerning the clinical isolates that were included in our study. Fourth, the retrospective laboratory analyses employed in this study may have underestimated the prevalence of the hVISA phenotype at our institutions. Fifth, we did not take into account bacterial density in hVISA detection, as we utilized standard inocula of MRSA according to the manufacturer's recommendations. It has been hypothesized that stationary-phase growth, defective autolysis profiles, biofilm production, and the production of thicker cell walls is facilitated by quorum-sensing mechanisms at high bacterial density, which may increase the proportion of the heteroresistant subpopulations which were not directly studied. Sixth, limited sample size also may have played a role in the lack of association found between *agr* dysfunction and clinical covariates, including comorbidities, critical illness, prior antibiotic exposures, and health care exposure history. Seventh, the source of infection was not delineated in our study. Finally, the influences of the following were not considered: genotype, susceptibility to thrombin-induced platelet microbicidal proteins, and autolytic profile. The presence of different genotypes (e.g., *agr* group, staphylococcal cassette chromosome *mec* type,

and pulsed-field gel electrophoresis pattern) among our isolates may have confounded the associations (or lack thereof) seen between different covariates and *agr* dysfunction.

In conclusion, MRSA isolates with *agr* dysfunction have been described recently as an important clinical factor in outcomes of patients with *S. aureus* bacteremia (7, 25). Our data support the major role of prior antibiotic exposure as a predictor of *agr* dysfunctional isolates, particularly prior exposure to beta-lactams or fluoroquinolones. While clinicians presume there is a relationship between *agr* dysfunction, higher MICs, and hVISA status, we were not able to verify this in our study. Our results do not refute the relationship between *agr* dysfunction and the development of higher-MIC MRSA isolates and hVISA. Rather, they suggest that there are other things involved in the causal pathway and underscore the importance of rigorous studies specifically designed to pinpoint the mechanisms underlying the relationship between MICs and outcomes.

ACKNOWLEDGMENTS

This publication was made possible by grant number UL1 RR024160 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH), and the NIH Roadmap for Medical Research.

The contents of this publication are solely the responsibility of the authors and do not necessarily represent the official view of the NCRR or NIH. Information on the NCRR is available at http://www.ncrr.nih.gov/. Information on Reengineering the Clinical Research Enterprise can be obtained from http://nihroadmap.nih.gov/clinicalresearch/overview-translational.asp.

REFERENCES

1. **Aldeyab, M. A., et al.** 2008. Modelling the impact of antibiotic use and infection control practices on the incidence of hospital-acquired methicillin-resistant Staphylococcus aureus: a time-series analysis. J. Antimicrob. Chemother. **62:**593–600.
2. **Bisognano, C., et al.** 2004. A recA-LexA-dependent pathway mediates ciprofloxacin-induced fibronectin binding in Staphylococcus aureus. J. Biol. Chem. **279:**9064–9071.
3. **Cirz, R. T., et al.** 2007. Complete and SOS-mediated response of Staphylococcus aureus to the antibiotic ciprofloxacin. J. Bacteriol. **189:**531–539.
4. **Clinical and Laboratory Standards Institute.** 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard M7-A7, 8th ed. Clinical and Laboratory Standards Institute, Wayne, PA.
5. **Cockcroft, D. W., and M. H. Gault.** 1976. Prediction of creatinine clearance from serum creatinine. Nephron **16:**31–41.
6. **Crowcroft, N. S., O. Ronveaux, D. L. Monnet, and R. Mertens.** 1999. Methicillin-resistant Staphylococcus aureus and antimicrobial use in Belgian hospitals. Infect. Control Hosp. Epidemiol. **20:**31–36.
7. **Fowler, V. G., Jr., et al.** 2004. Persistent bacteremia due to methicillin-resistant Staphylococcus aureus infection is associated with agr dysfunction and low-level in vitro resistance to thrombin-induced platelet microbicidal protein. J. Infect. Dis. **190:**1140–1149.
8. **Hori, S., R. Sunley, A. Tami, and H. Grundmann.** 2002. The Nottingham Staphylococcus aureus population study: prevalence of MRSA among the elderly in a university hospital. J. Hosp. Infect. **50:**25–29.
9. **Kaier, K., C. Hagist, U. Frank, A. Conrad, and E. Meyer.** 2009. Two time-series analyses of the impact of antibiotic consumption and alcohol-based hand disinfection on the incidences of nosocomial methicillin-resistant Staphylococcus aureus infection and Clostridium difficile infection. Infect. Control Hosp. Epidemiol. **30:**346–353.
10. **Knaus, W. A., E. A. Draper, D. P. Wagner, and J. E. Zimmerman.** 1985. APACHE II: a severity of disease classification system. Crit. Care Med. **13:**818–829.
11. **Landman, D., M. Chockalingam, and J. M. Quale.** 1999. Reduction in the incidence of methicillin-resistant Staphylococcus aureus and ceftazidime-resistant Klebsiella pneumoniae following changes in a hospital antibiotic formulary. Clin. Infect. Dis. **28:**1062–1066.
12. **Leonard, S. N., K. L. Rossi, K. L. Newton, and M. J. Rybak.** 2009. Evaluation of the Etest GRD for the detection of Staphylococcus aureus with reduced susceptibility to glycopeptides. J. Antimicrob. Chemother. **63:**489–492.
13. **Li, D., et al.** 2005. Induction of fibronectin adhesins in quinolone-resistant Staphylococcus aureus by subinhibitory levels of ciprofloxacin or by sigma B transcription factor activity is mediated by two separate pathways. Antimicrob. Agents Chemother. **49:**916–924.
14. **MacDougall, C., et al.** 2005. Pseudomonas aeruginosa, Staphylococcus aureus, and fluoroquinolone use. Emerg. Infect. Dis. **11:**1197–1204.
15. **McNutt, L. A., C. Wu, X. Xue, and J. P. Hafner.** 2003. Estimating the relative risk in cohort studies and clinical trials of common outcomes. Am. J. Epidemiol. **157:**940–943.
16. **Mesak, L. R., and J. Davies.** 2009. Phenotypic changes in ciprofloxacin-resistant Staphylococcus aureus. Res. Microbiol. **160:**785–791.
17. **Muller, A., et al.** 2006. Effect of individual- and group-level antibiotic exposure on MRSA isolation: a multilevel analysis. J. Antimicrob. Chemother. **58:**878–881.
18. **Muller, A. A., et al.** 2003. Relationship between spread of methicillin-resistant Staphylococcus aureus and antimicrobial use in a French university hospital. Clin. Infect. Dis. **36:**971–978.
19. **Nagel, M., T. Reuter, A. Jansen, C. Szekat, and G. Bierbaum.** 2011. Influence of ciprofloxacin and vancomycin on mutation rate and transposition of IS256 in Staphylococcus aureus. Int. J. Med. Microbiol. **301:**229–236.
20. **Sakoulas, G., et al.** 2005. Reduced susceptibility of Staphylococcus aureus to vancomycin and platelet microbicidal protein correlates with defective autolysis and loss of accessory gene regulator (agr) function. Antimicrob. Agents Chemother. **49:**2687–2692.
21. **Sakoulas, G., et al.** 2003. Staphylococcus aureus accessory gene regulator (agr) group II: is there a relationship to the development of intermediate-level glycopeptide resistance? J. Infect. Dis. **187:**929–938.
22. **Sakoulas, G., et al.** 2002. Accessory gene regulator (agr) locus in geographically diverse Staphylococcus aureus isolates with reduced susceptibility to vancomycin. Antimicrob. Agents Chemother. **46:**1492–1502.
23. **Sakoulas, G., P. A. Moise, and M. J. Rybak.** 2009. Accessory gene regulator dysfunction: an advantage for Staphylococcus aureus in health-care settings? J. Infect. Dis. **199:**1558–1559.
24. **Schentag, J. J., et al.** 1998. Genesis of methicillin-resistant Staphylococcus aureus (MRSA), how treatment of MRSA infections has selected for vancomycin-resistant Enterococcus faecium, and the importance of antibiotic management and infection control. Clin. Infect. Dis. **26:**1204–1214.
25. **Schweizer, M. L., et al.** 2011. Increased mortality with accessory gene regulator (agr) dysfunction in Staphylococcus aureus among bacteremic patients. Antimicrob. Agents Chemother. **55:**1082–1087.
26. **Spiegelman, D., and E. Hertzmark.** 2005. Easy SAS calculations for risk or prevalence ratios and differences. Am. J. Epidemiol. **162:**199–200.
27. **Thom, K. A., et al.** 2008. Controlling for severity of illness in outcome studies involving infectious diseases: impact of measurement at different time points. Infect. Control Hosp. Epidemiol. **29:**1048–1053.
28. **Traber, K., and R. Novick.** 2006. A slipped-mispairing mutation in AgrA of laboratory strains and clinical isolates results in delayed activation of agr and failure to translate delta- and alpha-haemolysins. Mol. Microbiol. **59:**1519–1530.
29. **Tsuji, B. T., Y. Harigaya, A. J. Lesse, G. Sakoulas, and J. M. Mylotte.** 2009. Loss of vancomycin bactericidal activity against accessory gene regulator (agr) dysfunctional Staphylococcus aureus under conditions of high bacterial density. Diagn. Microbiol. Infect. Dis. **64:**220–224.
30. **Tsuji, B. T., M. J. Rybak, K. L. Lau, and G. Sakoulas.** 2007. Evaluation of accessory gene regulator (agr) group and function in the proclivity towards vancomycin intermediate resistance in Staphylococcus aureus. Antimicrob. Agents Chemother. **51:**1089–1091.
31. **Tsuji, B. T., C. von Eiff, P. A. Kelchlin, A. Forrest, and P. F. Smith.** 2008. Attenuated vancomycin bactericidal activity against Staphylococcus aureus hemB mutants expressing the small-colony-variant phenotype. Antimicrob. Agents Chemother. **52:**1533–1537.
32. **von Eiff, C., et al.** 2001. Intracellular persistence of Staphylococcus aureus small-colony variants within keratinocytes: a cause for antibiotic treatment failure in a patient with darier's disease. Clin. Infect. Dis. **32:**1643–1647.
33. **von Eiff, C., A. W. Friedrich, K. Becker, and G. Peters.** 2005. Comparative in vitro activity of ceftobiprole against staphylococci displaying normal and small-colony variant phenotypes. Antimicrob. Agents Chemother. **49:**4372–4374.
34. **Washio, M., et al.** 1997. Risk factors for methicillin-resistant Staphylococcus aureus (MRSA) infection in a Japanese geriatric hospital. Public Health **111:**187–190.