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Accessory Gene Regulator (*agr*) Dysfunction in *Staphylococcus aureus* Bloodstream Isolates from South Korean Patients

Yong Pil Chong,[a,d] Eun Sil Kim,[a,d] Su-Jin Park,[a,d] Ki-Ho Park,[a,d] Tark Kim,[a,d] Mi-Na Kim,[b] Sung-Han Kim,[a] Sang-Oh Lee,[a] Sang-Ho Choi,[a] Jun Hee Woo,[a] Jin-Yong Jeong,[c,d] Yang Soo Kim[a,d]

Department of Infectious Diseases,[a] Department of Laboratory Medicine,[b] and Asan Institute for Life Sciences,[c] Asan Medical Center, University of Ulsan College of Medicine, Seoul, Republic of Korea; Center for Antimicrobial Resistance and Microbial Genetics, University of Ulsan College of Medicine, Seoul, Republic of Korea[d]

We describe the genetic and microbiological characteristics of methicillin-resistant *Staphylococcus aureus* (MRSA) bloodstream isolates with *agr* dysfunction from a tertiary-care hospital in Korea. Of these, ST5-SCC*mec* type II-*agr* group II MRSA isolates, which are known to be prevalent in hospital-acquired infections in Korea, were the most abundant, because of the clonal spread of a specific *agr*-defective lineage. This finding suggests that the loss of *agr* function may confer a potential advantage in a hospital setting. Clonal spread of a specific defective-*agr* strain was not observed among community-associated MRSA or methicillin-susceptible *S. aureus* clones, regardless of community or hospital acquisition of infection. *agr*-defective clones, including ST5 and ST239 MRSA, were enriched for heteroresistant vancomycin-intermediate *S. aureus*.

The accessory gene regulator (*agr*) is a global regulator that controls staphylococcal virulence factors and other accessory gene functions (1). Genetically engineered mutants of *Staphylococcus aureus* with defective *agr* have substantially reduced virulence (2). Notwithstanding the importance of *agr* for staphylococcal virulence, several reports suggest that *agr* dysfunction confers potential advantages in a health care setting and a survival advantage under vancomycin selection pressure (3, 4). *agr* dysfunction has also been associated with persistent bacteremia (5). In this study, we compare the prevalence of *agr* dysfunction within and between methicillin-resistant *S. aureus* (MRSA) and methicillin-susceptible *S. aureus* (MSSA) populations and describe the genetic and microbiological characteristics of *agr*-defective strains in Korea.

Between August 2008 and September 2011, 639 nonduplicate, consecutive *S. aureus* isolates were prospectively collected from patients with bacteremia at the Asan Medical Center, a 2,700-bed tertiary-care teaching hospital in Seoul, South Korea. Of 639 *S. aureus* isolates, 407 (64%) were MRSA and 232 (36%) were MSSA. We used δ-hemolysin activity to determine *agr* functionality as described previously (6) because *agr* dysfunction results in a defect in δ-hemolysin production. The *agr* genotype was determined by using a rapid, PCR-based assay that has been described previously (7). Multilocus sequence type (MLST) determination and staphylococcal cassette chromosome *mec* (SCC*mec*) typing were performed as previously described (8, 9).

One hundred twenty-three (30%) *agr* group I, 276 (68%) *agr* group II, and 3 (1%) *agr* group III MRSA isolates were studied. The most frequent SCC*mec* type was II (282 isolates, 70%) followed by IV (97 isolates, 24%). Among the MRSA isolates, the most frequent sequence types (STs) were ST5 (276, 70%) and ST72 (96, 24%). The most common MRSA clone was ST5-SCC*mec* type II-*agr* group II (274/407 [67%]), followed by ST72-SCC*mec* type IV-*agr* group I (93/407 [23%]). One hundred thirty-three (57%) *agr* group I, 32 (14%) *agr* group II, 52 (22%) *agr* group III, and 6 (3%) *agr* group IV MSSA isolates were studied. Twenty-seven different STs were found among the MSSA isolates. The major STs were ST188 (46 isolates, 20%), ST72 (29, 13%), ST1 (27, 12%), ST30 (25, 11%), ST6 (19, 8%), and ST5 (16, 7%). Similar to the MRSA isolates, each ST in the MSSA isolates had a particular *agr* genotype.

Table 1 shows the differences in genotypic characteristics between *S. aureus* bloodstream isolates with functional *agr* and isolates with dysfunctional *agr*. Of 407 MRSA isolates, 302 (74%) had dysfunctional *agr* (nonhemolytic strains) and 105 (26%) had functional *agr* (hemolytic strains). Strikingly, nearly all of the MRSA isolates with *agr* dysfunction were ST5 (270/302 [89%]) and nearly all of the ST5 isolates had *agr* dysfunction (270/276 [98%]), raising the possibility of clonal spread. In contrast to the results for the MRSA isolates, only 13% (29/232) of the MSSA isolates had *agr* dysfunction and no predominant STs were found in MSSA isolates with *agr* dysfunction.

The analysis of combined genotypes of *S. aureus* isolates according to *agr* functionality showed that whereas the ST5-SCC*mec* type II-*agr* group II genotype was the most prevalent among MRSA isolates with *agr* dysfunction, the ST72-SCC*mec* type IV-*agr* group I genotype predominated among MRSA isolates with functional *agr* (Table 2). In addition, nearly all of the MRSA isolates (15/16) with the ST239-SCC*mec* type III-*agr* group I genotype had *agr* dysfunction. Among the MSSA isolates, predominant clones were not found in either the dysfunctional-*agr* or the functional-*agr* group. However, one-third of the MSSA isolates with ST5 *agr* group II and ST30 *agr* group III genotype had *agr* dysfunction. In Korea, ST5-SCC*mec* type II-*agr* group II and ST239-SC-

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Address correspondence to Yang Soo Kim, yskim@amc.seoul.kr, or Jin-Yong Jeong, jyjeong@amc.seoul.kr.
Y.P.C. and E.S.K. contributed equally to this work.

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TABLE 1 Genotypic characteristics of *S. aureus* bloodstream isolates stratified by *agr* functionality

Genotype	No. (%) of isolates		*P* value[a]
	Dysfunctional *agr*	Functional *agr*	
MRSA ($n = 407$)	302	105	
agr genotype			<0.001
I	29 (9.6)	94 (89.5)	
II	270 (89.4)	6 (5.7)	
III	2 (0.7)	1 (1.0)	
ND	1 (0.3)	4 (3.8)	
SCCmec type			<0.001
I	2 (0.7)	3 (2.9)	
II	274 (90.7)	8 (7.6)	
III	15 (5.0)	2 (1.9)	
IV	10 (3.3)	87 (82.9)	
ND[b]	1 (0.3)	5 (4.8)	
MLST			<0.001
ST1		2 (1.9)	
ST5	270 (89.4)	6 (5.7)	
ST72	10 (3.3)	86 (81.9)	
ST239	17 (5.6)	1 (1.0)	
Other[c]	5 (1.7)	10 (9.5)	
MSSA ($n = 232$)	29	203	
agr genotype			0.024
I	10 (34.5)	123 (60.6)	
II	6 (20.7)	26 (12.8)	
III	10 (34.5)	42 (20.7)	
IV		6 (2.9)	
ND	3 (10.3)	6 (2.9)	
MLST			0.030
ST1	3 (10.3)	24 (11.8)	
ST5	5 (17.2)	11 (5.4)	
ST30	7 (24.1)	18 (8.9)	
ST72	3 (10.3)	26 (12.8)	
ST188	4 (13.8)	42 (20.7)	
Other[d]	7 (24.1)	82 (40.4)	

[a] *P* values comparing the values for the two groups were determined with a two-sided Fisher exact test.
[b] ND, not determined.
[c] The other MLSTs were ST254 (2 isolates), ST291 (1 isolates), and ST89 (2 isolates) in the dysfunctional-*agr* group and ST101 (2 isolates), ST188 (1 isolate), ST199 (1 isolate), ST254 (2 isolates), ST6 (1 isolate), ST8 (2 isolates), and ND (1 isolate) in the functional-*agr* group.
[d] The other MLSTs were ST6 (1 isolate), ST8 (1 isolate), ST59 (1 isolate), ST101 (1 isolate), ST378 (1 isolate), ST1153 (1 isolate), and ST1156 (1 isolate) in the dysfunctional-*agr* group and ST6 (18 isolates), ST8 (2 isolates), ST15 (11 isolates), ST25 (1 isolate), ST45 (1 isolate), ST59 (3 isolates), ST96 (1 isolate), ST97 (7 isolates), ST101 (3 isolates), ST121 (7 isolates), ST217 (1 isolate), ST513 (10 isolates), ST573 (1 isolate), ST580 (1 isolate), ST587 (3 isolates), ST630 (9 isolates), ST883 (1 isolate), ST1821 (1 isolate), and ST2238 (1 isolate) in the functional-*agr* group.

C*mec* type III-*agr* group I MRSA strains account for the majority of hospital-acquired MRSA infections (10, 11). The major types in community-associated MRSA infections are ST72-SCC*mec* type IV-*agr* group I and ST1-SCC*mec* type IV-*agr* group III (11, 12). Our results suggest that ST5-SCC*mec* type II-*agr* group II and ST239-SCC*mec* type III-*agr* group I clones with *agr* dysfunction were clonally spread in the hospital.

TABLE 2 Distribution of combined genotypes in *S. aureus* bloodstream isolates stratified by *agr* functionality

Genotype	No. of isolates	
	Dysfunctional *agr*	Functional *agr*
MRSA ($n = 407$)	302	105
ST5-SCC*mec* II-*agr* II	270	4
ST72-SCC*mec* IV-*agr* I	10	83
ST89-SCC*mec* II-*agr* III	2	
ST239-SCC*mec* III-*agr* I	15	1
ST239-SCC*mec* II-*agr* I	2	
ST254-SCC*mec* I-*agr* I	1	2
Other	2	15
MSSA ($n = 232$)	29	203
ST1-*agr* III	2	24
ST5-*agr* II	5	11
ST6-*agr* I		18
ST30-*agr* III	7	17
ST72-*agr* I	3	25
ST188-*agr* I	4	41
Other	8	67

Sequencing of the 3′ end of *agrA* was performed as described previously (13). Among 270 ST5-SCC*mec* type II-*agr* group II isolates with *agr* dysfunction, 238 (88%) had a nine-adenine mutation (referred to as *agrA*-9A), 14 (5%) had an *agrA*-8A mutation, and 18 (7%) had wild-type *agrA*-7A. This suggests that there was clonal spread of at least two ST5 lineages. The Mu3 and Mu50 strains of *S. aureus* have this *agrA*-9A mutation without any other mutations in the *agr* locus (14). Given that both of these strains are nonhemolytic (*agr* dysfunctional), the *agrA*-9A mutation may be important for *agr* functionality. The *agrA*-8A mutation was known as a cause of *agr* dysfunction (6, 13). In our ST5 MRSA isolates, the *agrA*-9A or *agrA*-8A mutation would be a main cause of *agr* dysfunction. All ST239-SCC*mec* type III-*agr* group I isolates with *agr* dysfunction had wild-type *agrA*-7A. Most of *agr*-defective MSSA isolates (25/29 [83%]) had *agrA*-7A. *agr* dysfunction in these isolates would result from various mutations in other regions of the *agr* locus (3). Shopsin et al. (15) reported *agr* functional analysis with *S. aureus* isolates recovered from colonized individuals and suggested that isolates with *agr* dysfunction were capable of transmission. They also suggested that although *agr* dysfunction is adaptive for survival in the infected host, it appears to confer only a short-term survival advantage (3). However, in ST5-SCC*mec* type II-*agr* group II and ST239-SCC*mec* type III-*agr* group I MRSA, *agr* dysfunction may confer long-term advantages in transmission and survival in the hospital environment. Since there were no case clusters or outbreaks of these clones in our hospital during the study period, this suggests that those were endemic. The predominance of the ST5-SCC*mec* type II-*agr* group II clone among MRSA isolates with *agr* dysfunction needs to be further verified in a multicenter study in Korea.

Previous studies suggested that *agr* dysfunction in MRSA is associated with attenuated vancomycin activity, vancomycin heteroresistance, and an increased proclivity toward vancomycin intermediate resistance (4, 14, 16). To investigate the relationships between *agr* functionality and vancomycin resistance, we estimated the vancomycin susceptibility by using the

TABLE 3 Microbiological characteristics and outcomes of *S. aureus* bacteremia stratified by *agr* functionality

Genotype	No. (%) of isolates		*P* value
	Dysfunctional *agr*	Functional *agr*	
MRSA ($n = 407$)	302	105	
Vancomycin MIC (Etest) results:			0.068
≤1	97 (32.1)	21 (20.0)	
1.5	135 (44.7)	57 (54.3)	
2	62 (20.5)	26 (24.8)	
3	8 (2.6)	1 (1.0)	
hVISA phenotype	107 (35.4)	20 (19.0)	0.002
Persistent bacteremia	79 (26.2)	19 (18.1)	0.096
MSSA ($n = 232$)	29	203	
Persistent bacteremia		14 (6.9)	0.226

Etest (AB Biodisk, Solna, Sweden) and the heteroresistant vancomycin-intermediate *S. aureus* (hVISA) phenotype by using population analysis profiling of MRSA isolates as previously described (17). An isolate was identified as hVISA if the ratio of the AUC of the test isolate to that of the reference strain (Mu3; ATCC 700698) was ≥0.9. The vancomycin MICs for MRSA isolates ranged from 0.5 to 3 μg/ml. Of 407 MRSA isolates, 127 (31%) were hVISA. The most common STs of hVISA isolates were ST5 (72%), followed by ST72 (13%) and ST239 (12%). Most of the hVISA isolates (107/127, 84%) were *agr* defective. The hVISA phenotype was significantly more common in isolates with dysfunctional *agr* than in those with functional *agr* (107/302 [35%] versus 20/105 [19%], $P < 0.002$) (Table 3). However, *agr* dysfunction was not significantly associated with high vancomycin MICs. Among 107 hVISA isolates with *agr* dysfunction, 91 (85%) were ST5-SCC*mec* type II-*agr* group II, 12 (11%) were ST239-SCC*mec* type III-*agr* group I, 2 (2%) were ST239-SCC*mec* type II-*agr* group I, 1 (1%) was ST254-SCC*mec* type I-*agr* group I, and 1 (1%) was ST72-SCC*mec* type IV-*agr* group I. The higher prevalence of hVISA in the present study (31%) than in previous studies (overall, 1.3%) (18) could be explained by the clonal spread of specific *agr*-defective strains (ST5 and ST239 clones) with the hVISA phenotype.

We also investigated the association of *agr* dysfunction with persistent bacteremia (Table 3). Persistent bacteremia was defined as bacteremia for ≥7 days while the patient was receiving appropriate antibiotic therapy (5, 19) and was compared with nonpersistent bacteremia with regard to *agr* functionality. Of 407 MRSA isolates, 98 (24%) caused persistent bacteremia. Of 232 MSSA isolates, 14 (6%) caused persistent bacteremia. Among the MRSA isolates, *agr* dysfunction was associated with a trend toward persistent bacteremia ($P = 0.096$). However, *agr* dysfunction was not significantly associated with persistent bacteremia among the MSSA isolates ($P = 0.226$).

In conclusion, we showed that *agr* dysfunction was common among the nosocomial MRSA isolates in our institution because of the clonal spread of specific clones with *agr* dysfunction, ST5-SCC*mec* type II-*agr* group II and ST239-SCC*mec* type III-*agr* group I. This suggests that *agr* dysfunction may confer a particular advantage on these clones in hospital settings. In addition, *agr* dysfunction was significantly associated with hVISA.

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