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Prevalence of *agr* Specificity Groups among *Staphylococcus aureus* Strains Colonizing Children and Their Guardians

B. Shopsin,[1] B. Mathema,[2] P. Alcabes,[3] B. Said-Salim,[2] G. Lina,[4] A. Matsuka,[2] J. Martinez,[2] and B. N. Kreiswirth[2]*

Department of Medicine, New York University School of Medicine, New York, New York 10016[1]; Public Health Research Institute Tuberculosis Center, Newark, New Jersey 07103[2]; School of Health Sciences, Hunter College of the City University of New York, New York, New York 10010[3]; and Centre National de Référence des Toxémies à Staphylocoques, INSERM E0230, Faculté de Médecine Laennec, 69372 Lyon Cedex 08, France[4]

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PCR-based assays were used to evaluate *agr* locus nucleotide polymorphism for the identification of *agr* autoinducer receptor specificity groups within a population of *Staphylococcus aureus* isolates colonizing children and their guardians. All isolates could be assigned to one of three major *agr* groups that had similar prevalences, regardless of whether isolates were implicated in transmission of *S. aureus* within families. Among healthy carriers, *agr* groups I to III appear to be equally fit, which may reflect selection for the coexistence of *S. aureus* strains in a population.

In *Staphylococcus aureus*, the accessory gene regulator (*agr*) globally controls the coordinated production of virulence factors. The *agr* signaling system consists of a classical two-component signaling system (AgrC as the signal receptor and AgrA as the response regulator) that serves as a quorum-sensing regulon to autoinduce RNA III, the principal effector of the *agr* response (1). The *agr* signaling system is driven by an autoinducing peptide pheromone encoded within *agrD*. *S. aureus* isolates can be divided into four predominant *agr* groups on the basis of the specificity of the autoinducing peptide for its membrane sensor (AgrC) (5). Isolates of one *agr* group are capable of activating the *agr* response in isolates of the same group, but they usually inhibit it in members of the other groups. Thus, cross-restriction of the *agr* signaling pathway between isolates constitutes a novel form of bacterial interference (4).

It has been suggested that *agr* autoinducer receptor specificity groups may influence host ecology by enhancing or inhibiting the ability of an *S. aureus* isolate to colonize (or compete) in the presence of resident strains (4), including other staphylococci (9). The putative within-host effects of *agr* polymorphism are also likely to affect the host-to-host transmission and epidemiology of *S. aureus* clones (11). If *agr* polymorphism does have these effects, preferential transmission of strains having particular *agr* groups might occur, reflecting those that succeed in competitive within-host situations. To test this idea, we sought linkage between *agr* group and clonal *S. aureus* transmission among family members.

In this study, a rapid PCR-based method was used to identify *agr* specificity groups among a genotypically characterized collection of colonizing *S. aureus* isolates obtained from a healthy population of children and their guardians in New York City, New York (13). Study subjects represented a previously described well-patient population visiting a pediatric outpatient clinic (13). The 500 individuals in the study came from 212 households, which varied in size from two to five persons, with one to four children and either one or two guardians. The majority of families (147 individuals) presented as one child and one guardian (convenience sampling was used, i.e., only family members at the clinic were enrolled). *S. aureus* was isolated (one strain per subject) from 96 of 275 (35%) children and from 64 of 225 (28%) guardians (13). *agr* specificity groups were identified by PCR amplification of the hypervariable domain of the *agr* locus using oligonucleotide primers specific for each of the four major specificity groups (obviating the need for restriction fragment length polymorphism analysis commonly employed in previous studies [7, 10, 14]). A forward primer, pan-*agr* (5′-ATGCACATGGTGCACATGC-3′), corresponding to conserved sequences from the *agrB* gene, was used in all reactions (Fig. 1A) (primer sequences were obtained from GenBank accession numbers X52543, AF001782, AF001783, and AF288215). Four reverse primers, each specific for amplification of a single *agr* group based on *agrD* or *agrC* gene nucleotide polymorphism, were as follows: *agr* I, 5′-GTC ACAAGTACTATAAGCTGCGAT-3′ (in the *agrD* gene); *agr* II, 5′-GTATTACTAATTGAAAAGTGCCATAGC-3′ (in the *agrC* gene); *agr* III, 5′-CTGTTGAAAAAGTCAACTAAAAG CTC-3′ (in the *agrD* gene); and *agr* IV, 5′-CGATAATGCCG TAATAC CCG-3′ (in the *agrC* gene).

agr specificity groups were identified by the expected product sizes (Fig. 1B). PCR was performed by adding 1 μl of a 1:200 dilution of chromosomal template DNA and 24 μl of water to 25 μl of a PCR mixture that includes 2.5 U of AmpliTaq Gold DNA polymerase, 2 mM $MgCl_2$, 350 μM (total) deoxynucleoside triphosphates, and 25 mM KCl in 0.2-ml PCR tubes (Perkin-Elmer Applied Biosystems Division, Norwalk, Conn.). A negative control and control strains from each *agr* specificity group (PHRI *S. aureus* collection) were included. Thermal cycling was performed in a GeneAmp 9600 instrument (Perkin-Elmer Applied Biosystems Division); 25 cycles of PCR were done, with 1 cycle consisting of denaturation (1 min at 94°C),

* Corresponding author. Mailing address: PHRI TB Center, 225 Warren St., Newark, NJ 07103. Phone: (973) 854-3240. Fax: (973) 854-3241. E-mail: barry@phri.org.

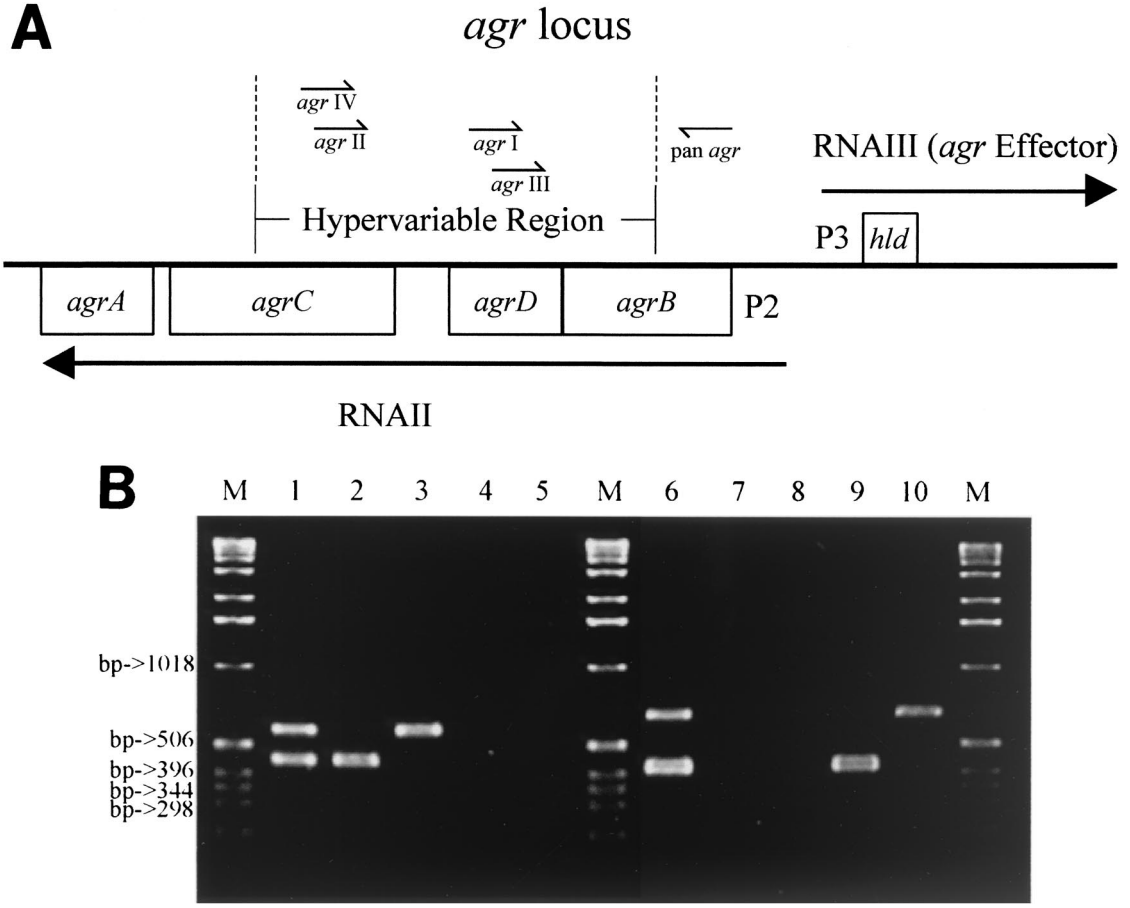

FIG. 1. PCR assay for the identification of *agr* specificity groups. (A) Schematic map of the *S. aureus agr* locus showing the locations of the different primers used for amplification of the hypervariable region. (B) Whole-cell PCR was performed with type strains of each of the four *agr* specificity groups. To distinguish between the similar-sized products of groups I and III and groups II and IV, two duplex PCR amplifications were performed for each isolate as follows. Lane 1, combined products from PCR of *agr* type strains I and II using reverse primers *agr* I and *agr* II; lanes 2 to 5, PCR products using reverse primers *agr* I and *agr* II from *agr* type strains I to IV (440 bp for *agr* I and 572 bp for *agr* II); lane 6, combined products from PCR of *agr* type strains III and IV using reverse primers *agr* III and *agr* IV (406 bp for *agr* III, and 588 bp for *agr* IV); lanes 7 to 10, PCR products using reverse primers *agr* III and *agr* IV from *agr* type strains I to IV. Lanes M contain molecular size markers (sizes in base pairs).

annealing (1 min at 55°C), and extension (1 min at 72°C). PCR products were separated by electrophoresis on 1% agarose gels, which were stained with ethidium bromide (Shelton Scientific, Shelton, Conn.). The lengths of the PCR products were estimated by comparison with the 1-kb DNA ladder molecular size markers (Gibco BRL, Life Technologies, Gaithersburg, Md.).

Genotypes were previously determined for 154 of the *S. aureus* isolates colonizing children and guardians by macrorestriction analysis using pulsed-field gel electrophoresis (PFGE) analysis and DNA sequence analysis of the protein A gene (*spa)* hypervariable region (13). One genetic background accounted for more than 11.7% of the colonizations; the average proportion was 1.7% per strain (range, 0.6 to 11.7%), indicating that overall, genotypic diversity was high in the sample.

S. aureus carriage in family members of carriers was similar to carriage in the overall sample: among 115 households (consisting of 276 individuals) where at least one family member was colonized, *S. aureus* was isolated from only 45 of 154 (29.8%) potential subjects (45 of 154 versus 154 of 500; $P = 0.599$ by one-tailed t test; statistical analysis performed using SAS version 8 [SAS, Cary, N.C.]). Thus, family members of carriers were not more likely to be colonized themselves.

To estimate the frequency of transmission of *S. aureus* in families, we compared *S. aureus* clonal types of host pairs, defined as a colonized child having one colonized guardian. Among the subgroup of 66 isolates from the 33 child-guardian pairs examined, genotypic diversity was high: 29 distinct *spa* strain types were seen. However, in 22 instances (67% of the cases), a child and guardian within a pair had the same *spa* type. This high percentage of concurrence of *spa* types within households when both family members are colonized is greater than would be expected by chance: the kappa statistic equals 0.65 [95% confidence interval of 0.58 to 0.72], where pure-chance concurrence of types would yield a kappa value of 0 and perfect concurrence value of 1.0. Thus, when multiple colonizations do happen, they are likely to occur with S. *aureus* strains of the same *spa* type, indicating transmission. Among

TABLE 1. Frequency of *agr* specificity groups among the *S. aureus* strains carried

agr specificity group	No. of *agr* specificity groups (%)			*P* value[c]
	Nontransmitting isolates[a]	Transmitting isolates[b]	Total	
I	44 (40)	20 (45)	64 (42)	0.589
II	28 (25)	10 (23)	38 (24)	0.837
III	38 (35)	14 (32)	52 (34)	0.851
Total	110 (100)	44 (100)	154 (100)	

[a] *S. aureus* isolates with the indicated *agr* specificity group among households where transmission was not apparent (only one family member was colonized, or family members were colonized by unrelated *S. aureus* strains).
[b] *S. aureus* isolates implicated in staphylococcal transmission in families.
[c] *P* value comparing the number of *agr* specificity groups in the nontransmitting isolates to the number of *agr* specificity groups in the transmitting isolates by Fisher's exact (two-tailed) test.

transmitting isolates, genotypic diversity remained high: 14 types were recovered, one from each of 14 households, and only four *spa* types occurred in two households. Apparently, the ability to transmit is ubiquitous among *S. aureus* strains.

Analysis of *agrD* gene polymorphism showed that all 154 of the *S. aureus* genotypes could be assigned to one of three major *agr* specificity groups. Sixty-four isolates (42%) belonged to *agr* specificity group I, 38 (24%) belonged to group II, and 52 (34%) belonged to group III. Each *agr* group harbored a diversity of *S. aureus* genotypes (not shown). The balanced recovery of three of the four *agr* groups differs from data previously reported (14), perhaps reflecting ecological and geographical structuring (or sampling bias). A fourth *agr* group, which is common among exfoliatin-producing strains (3), was not identified in our population. No association was observed for *agr* group with age, gender (for either child or guardian), previous antibiotic usage, outpatient visits, or known underlying condition. However, among children there was an association between *agr* group I and recent hospitalization ($P = 0.02$).

The results of analyzing hosts implicated in transmission of *S. aureus* within families showed that among the 22 child-guardian pairs colonized with strains of the same *spa* type, the distribution of *agr* specificity groups was no different from that in the overall sample ($P = 0.95$) (Table 1). Thus, there was no correlation between *agr* group and intrafamily staphylococcal transmission.

Frequent exposure to the flora of close contacts may lead to mixed-clone *S. aureus* colonization. Specificity in the *agr* system could then operate to affect the frequency of *agr* group recovery even in the absence of transmission of a predominant colonizing strain. The occurrence of different *spa* types among colonized child-guardian pairs, however, did not predict the *agr* group ($P = 0.87$). Stated differently, among child-guardian pairs colonized by different strains, *agr*-mediated interference did not determine the clonal performance (defined by *agr* allele frequencies) of predominant colonizing isolates.

Congruence was indicated between genetic markers used for clonal analysis and *agr* grouping: any two isolates that were identical on the basis of both PFGE and *spa* typing were invariably of the same *agr* group. The same result was obtained using *spa* type alone in all but four occasions, consistent with the slight decrease in specificity observed for *spa* typing compared to that for PFGE (12).

The rare occurrence of noncongruence between genetic markers and *agr* contrasts with recent reports that recombination rates are high in *S. aureus* (2). This observation may be explained by at least three hypotheses. First, primary differentiation of *agr* group is apparently followed by polymorphism of *spa*. Second, recombination in the short-term will not obscure relationships among many of the isolates that were implicated in family (i.e., recent) transmission. A third but not mutually exclusive hypothesis is that reassortment events involving *agr* frequently occur between identical alleles.

In summary, a study of children and their guardians attending an urban pediatric clinic showed evidence that different family members were frequently colonized by a variety of *agr* types, and the presence of any one *agr* group in a family did not affect the likelihood of recovering isolates with the same (stimulatory) or different (inhibitory) *agr* autoinducer. We did not find a predominance of any particular *agr* group among isolates implicated in transmission of *S. aureus* in families. Apparently, in a population of healthy carriers, *agr* groups I to III affect the transmission of *S. aureus* equally or not at all. Thus, it seems unlikely that one interference group will spread at the expense of another, even among hosts living in close proximity.

The uniform fitness of *S. aureus agr* groups suggests that they also have comparable competitive ability within the host. Rather than promoting exclusion, it may be that *agr*-mediated interference functions to create niche (or microniche) separation between staphylococcal strains so that mixed genotype colonizations can occur. Heterogeneity could enhance the fitness of the total population through increased genetic exchange, variability, host defense costs, and transmissibility.

Our conclusions may not apply when considering populations faced with different ecological constraints (such as carriage of hospital-acquired strains or methicillin-resistant strains), or isolates of *agr* group IV. Despite the unique ability of the group IV pheromone to inhibit the *Staphylococcus epidermidis agr* response (8), the absence of group IV isolates from this study and other studies (6, 14) suggests that competition does not favor these strains.

The results also showed that the family members of *S. aureus* carriers are not more likely to be colonized themselves than the overall population. When both members of a child-guardian pair were colonized with *S. aureus*, however, transmission of staphylococci within the family was indicated, since the colonizing isolates are often (67%) the same strain. Frequency of colonization, but not strain type, is independent of exposure to colonized family members. Thus, among healthy urban carriers, it appears that host-microbe interactions, rather than exposure, determine carrier status.

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