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Bacterial Competition for Human Nasal Cavity Colonization: Role of Staphylococcal *agr* Alleles

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We examined the bacterial aerobic nasal flora of 216 healthy volunteers to identify potential competitive interactions among different species, with special emphasis on the influence of staphylococcal *agr* alleles. The *Staphylococcus aureus* colonization rate correlated negatively with the rate of colonization by *Corynebacterium* spp. and non-*aureus* staphylococci, especially *S. epidermidis*, suggesting that both *Corynebacterium* spp. and *S. epidermidis* antagonize *S. aureus* colonization. Most of the *S. aureus* and *S. epidermidis* isolates were *agr* typed by a PCR method. Only one *S. aureus agr* (agr_{Sa}) allele was detected in each carrier. Multiple logistic regression of the two most prevalent agr_{Sa} alleles ($agr\text{-}1_{Sa}$ and $agr\text{-}2_{Sa}$) and the three *S. epidermidis agr* (agr_{Se}) alleles showed a specific influence of the *agr* system. The results of this model did not support conclusions drawn from previous in vitro *agr*-specific cross-inhibition experiments. Our findings suggest that the *agr* alleles, which are strongly linked to the bacterial genetic background, may simply be associated with common biological properties—including mediators of bacterial interference—in the strains that bear them.

Staphylococcus aureus is present in the nasal vestibule of at least 30% of individuals in the normal population, and this carriage is a major risk factor for infection (12). *S. aureus* is an important cause of community- and hospital-acquired infections (14), and strategies for eliminating nasal carriage have been developed (11).

Longitudinal studies of healthy individuals have shown three patterns of *S. aureus* carriage: (i) noncarriage, (ii) intermittent carriage, and (iii) persistent carriage of the same or different strains (23, 24). The differences could be due to host factors and/or to antagonism between members of the nasal flora. Indeed, a lower incidence of *S. aureus* colonization is observed in individuals heavily colonized by *Corynebacterium* spp. (22), and interaction between these two species was confirmed by in vivo experiments showing that experimental colonization by *Corynebacterium* spp. inhibits colonization by *S. aureus* (22). Inconsistent results have been obtained with other species, including non-*aureus* staphylococci (18, 22).

Expression of cell wall-associated and extracellular proteins in staphylococci is controlled by the *agr* locus, which encodes a two-component signaling pathway whose activating ligand is a bacterial-density-sensing peptide (autoinducing peptide [AIP]) which is also encoded by *agr* (10). A polymorphism in the AIP amino acid sequence and in that of its corresponding receptor has been described in staphylococci (4, 7, 9). *S. aureus* strains can be divided into four major groups (designated $agr\text{-}1_{Sa}$ to $agr\text{-}4_{Sa}$), such that within each group, all strains produce a peptide that can activate the *agr* response in the other members of the same group whereas autoinducing peptides are usually mutually inhibitory between members of different groups (7, 9). Functional *agr* loci are present in other staphylococcal species, including *S. epidermidis* ($agr\text{-}1_{Se}$ to $agr\text{-}3_{Se}$) (3, 4, 26), which are different from each other and from agr_{Sa}. The $agr\text{-}1_{Se}$ AIP inhibits the activity of $agr\text{-}1_{Sa}$ to $agr\text{-}3_{Sa}$ but not $agr\text{-}4_{Sa}$, while among *S. aureus* AIPs, only type 4 (weakly) inhibits $agr\text{-}1_{Se}$ activity (20). It has been proposed that $agr\text{-}2_{Sa}$ *S. aureus* strains hinder umbilical stump colonization by $agr\text{-}1_{Sa}$ strains (19). The biological mechanism of this interference is unknown but might be caused by molecular cross-interference between *agr* alleles.

The aim of the present investigation was to determine the qualitative and quantitative composition of the nasal flora of healthy individuals, focusing on *S. aureus*, coagulase-negative staphylococci, and corynebacteria, and to identify potential interactions between these bacteria. Staphylococcal isolates were analyzed at the species and *agr* allele level, and a mathematical model of bacterial nasal interference was constructed.

MATERIALS AND METHODS

Subjects. The nasal floras of 216 healthy volunteer students (defined as subjects with no history of *S. aureus* disease and no current antibiotic use) from four medical and nursing schools (75, 69, 22, and 50 volunteers, respectively) were sampled. The mean age of the volunteers was 21 years (range, 17 to 35 years), and there were 64 males and 152 females.

Estimation of the nasal vestibule flora. The standard cotton swabbing technique was used to sample the nasal vestibule. Swabs were streaked on sheep blood agar and incubated at 37°C in an aerobic atmosphere for 48 h. Bacterial density was estimated by counting CFU in logarithmic graduations. The representative colonies were subcultured and identified using standard methods, as described below. Twenty randomly selected *S. aureus*-positive swabs were inoculated in brain heart broth (bioMérieux) and cultured for 24 h at 37°C. After centrifugation, pellets were harvested and stored at −20°C until used for DNA extraction.

Identification of isolates. *Staphylococcus* species were identified on the basis of conventional phenotypic characteristics, namely, Gram staining, cell morphology and cell arrangement, colony morphology and pigmentation on P agar and Trypticase soy agar (bioMérieux) supplemented with horse blood, catalase ac-

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TABLE 1. Nucleotide sequences of *agr* type-specific oligonucleotide primers used in this study, and anticipated sizes of PCR products

Gene	Primer	Oligonucleotide sequence (5′-3′)[a]	Size of amplified product (bp)[a]	Multiplex PCR set
agr_{Sa}	$agr1$-4_{Sa}-1	ATGCACATGG TGCACATGC		1
agr-1_{Sa}	$agr1_{Sa}$-2	GTCACAAGTA CTATAAGCTG CGAT	439	1
agr-2_{Sa}	$agr2_{Sa}$-2	TATTACTAAT TGAAAAGTGC CATAGC	572	1
agr-3_{Sa}	$agr3_{Sa}$-2	GTAATGTAAT AGCTTGTATA ATAATACCCA G	321	1
agr-4_{Sa}	$agr4_{Sa}$-2	CGATAATGCC GTAATACCCG	657	1
agr-1_{Se}	$agr1_{Se}$-1	GGCATTAGTC GGATTAATTA TTACG	438	2
	$agr1_{Se}$-2	TGTAGGCCTG CAAACGG		2
agr-2_{Se}	$agr2_{Se}$-1	TTTACCATTT GCAGCTATAC AAGTG	575	2
	$agr2_{Se}$-2	ATAACAATAA TATAACCAAA CTCAAAAGTA CAG		2
agr-3_{Se}	$agr3_{Se}$-1	GAAAGAGTGT ATTCAATGGA TGAGC	338	2
	$agr3_{Se}$-2	TAAATATTAT GTATTATATC TTCAGTATAT AAAGAGATGA		2

[a] Nucleotide sequences and anticipated sizes of PCR products were derived from the published sequences of alleles *agr-*1_{Sa} to *agr-*4_{Sa} and *agr-*1_{Se} to *agr-*3_{Se} (GenBank accession numbers X52543, AF001782, AF001783, AF288215, Z49220, AF346724, and AF346725, respectively).

tivity, coagulase production in rabbit plasma (bioMérieux), and production of clumping factor (Pastorex Staph Plus; bioMérieux). For species identification of coagulase-negative staphylococci, we used individual tests (susceptibility to furazolindone [300 μg], bacitracin [0.02 U], desferrioxamine [250 μg], and novobiocin) and the ID32 Staph gallery (bioMérieux). *Corynebacterium* spp. were identified on the basis of colony morphology and pigmentation on Trypticase soy agar supplemented with horse blood and also on the basis of cell morphology and cell arrangement after Gram staining; they were not identified to the species level.

***agr* typing by multiplex PCR.** Genomic DNA was extracted from staphylococci grown on agar plates or in brain heart infusion broth (13) and used as an amplification template with primers (Table 1) designed from the *agr-*1_{Sa} to *agr-*4_{Sa} and *agr-*1_{Se} to *agr-*3_{Se} sequences (GenBank accession numbers X52543, AF001782, AF001783, AF288215, Z49220, AF346724, and AF346725, respectively) to amplify specific *agr* alleles. For multiplex PCR, two primer sets were prepared: one to amplify agr_{Sa} alleles and another to amplify agr_{Se} alleles. Amplification was carried out under the following conditions: an initial 5-min denaturation step at 95°C followed by 25 stringent cycles (1 min of denaturation at 94°C, 1 min of annealing at 55°C, and 1 min of extension at 72°C) and a final extension step at 72°C for 10 min. The quality of the DNA extracts and the absence of PCR inhibitors were confirmed by amplification of *gyrA* (*S. aureus*) or the 16S-23S intergenic region of the ribosomal DNA operon (*S. epidermidis*) (13). PCR products were analyzed by electrophoresis through 0.8% agarose gels (Sigma). The following strains were used to control the specificity of PCR amplification: (i) *S. aureus* RN6390 (*agr-*1_{Sa}), RN6923 (*agr-*2_{Sa}), RN8462 (*agr-*3_{Sa}), and A880740 (*agr-*4_{Sa}) (7); (ii) *S. epidermidis* CCM2124 (*agr-*1_{Se}), N910160 (*agr-*2_{Se}), and N910191 (*agr-*3_{Se}) (4).

Statistical methods. Colony counts were $\log_{10}$ transformed for analysis. Interspecies relationships were first described on a two-by-two basis, looking at the presence or absence of *S. aureus* (of each of the four agr_{Sa} alleles) with respect to the number of colonies (CFU) of one group of bacteria including *Corynebacterium* spp., non-*aureus* staphylococci (including *S. epidermidis*), *S. epidermidis* (of each of the three agr_{Se} alleles), and *S. aureus* (of each of agr_{Sa} alleles), and validated by the χ^2 test. To explain the probability that *S. aureus* is present in terms of the number of colonies of the other species, standard linear regression does not apply. Hence, a multiple logistic regression model was used to analyze simultaneously the influence of *Corynebacterium* spp. and *S. epidermidis agr* alleles on the probability of the presence of *S. aureus agr-*1_{Sa} or *agr-*2_{Sa}. Age, sex, and school of origin were used as candidate covariates for adjustment. Those with a *P* value below 0.05 were finally retained in the final model. In this model, the colony counts were categorized into four groups for corynebacteria ($<10^2$ [reference group], 10^2 to 10^3, 10^3 to 10^4, and $\geq 10^4$) and into two groups for agr_{Se}*-1* to agr_{Se}*-3* ($<10^2$ [reference group] and $\geq 10^2$). Statistical analyses were done with software from SAS Inc.

RESULTS

Aerobic flora of the nasal vestibule. Colony counts in the 216 volunteers ranged from 10^2 to 10^6 CFU/swab (median 10^4). Sixty-five volunteers were positive for *S. aureus* (30%), 209 were positive for non-*aureus* staphylococci (97%), 150 were positive for *Corynebacterium* spp. (69%), 1 was positive for *Streptococcus* (<1%), 2 were positive for *Micrococcus* (1%), and 18 were positive for gram-negative species (8%). Of the 209 non-*aureus Staphylococcus* carriers, 198 were positive for *S. epidermidis* (93%), 12 were positive for *S. capitis* (6%), 9 were positive for *S. haemolyticus* (4%), 9 were positive for *S. warneri* (4%), 8 were positive for *S. hominis* (4%), 4 were positive for *S. lugdunensis* (2%), 3 were positive for *S. cohnii* subsp. *cohnii* (1%), and 1 was positive for *S. auricularis* (<1%); three isolates were not confidently identified to the species level (1%). The observed composition of the aerobic nasal flora was qualitatively and quantitatively similar to that in previous studies (5, 11).

Determination of the *agr* type of staphylococcal isolates. The *agr* type of all isolates belonging to the two most prevalent staphylococcal species, *S. aureus* and *S. epidermidis*, was determined by using specific multiplex PCR. To reduce possible bias of strain cloning, a minimum of 10 colonies of each morphological type were randomly selected for multiplex PCR. As shown in Fig. 1, strong specific signals of the expected sizes were obtained with the reference strains. All 65 *S. aureus* isolates fell into one of the four previously described agr_{Sa} groups (alleles). Only one agr_{Sa} allele was detected in each carrier: 34 isolates belonged to *agr-*1_{Sa}, 19 belonged to *agr-*2_{Sa}, 7 belonged to *agr-*3_{Sa}, and 5 belonged to *agr-*4_{Sa}. To verify that each carrier harbored only one agr_{Sa} allele, PCR was also performed on total DNA extracted from brain heart infusion

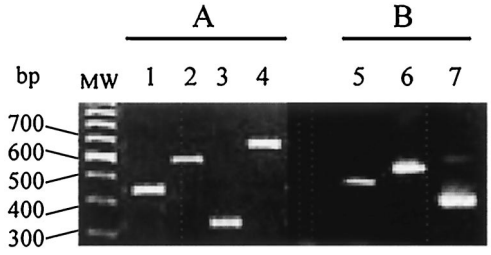

FIG. 1. Multiplex PCR detection of *S. aureus* (A) and *S. epidermidis* (B) *agr* alleles. Lanes 1 to 4, PCR amplicons from primer set agr_{Sa} alleles using DNA from *S. aureus* RN6390 (*agr-*1_{Sa}), RN6923 (*agr-*2_{Sa}), RN8462 (*agr-*3_{Sa}), and A880740 (*agr-*4_{Sa}), respectively; lanes 5 to 7, PCR amplicons from set agr_{Se} alleles using DNA from *S. epidermidis* CCM2124 (*agr-*1_{Se}), N910160 (*agr-*2_{Se}), and N910191 (*agr-*3_{Se}), respectively.

TABLE 2. Incidence of nasal carriage of *S. aureus*, non-*aureus* staphylococci, and *S. epidermidis* according to the degree of colonization by *Corynebacterium* spp.

No. of *Corynebacterium* sp. colonies (CFU)	No. of carriers of *Corynebacterium* spp.	No. (%) of healthy volunteers colonized with[a]:	
		S. aureus	Non-*aureus* staphylococci
0–10^3	142	56 (39)[b]	136 (96)[b]
10^3–10^4	19	3 (16), $P < 0.08$	19 (100), NS[c]
10^4–10^6	55	6 (11), $P < 0.001$	54 (98), NS

[a] A χ^2 test was used to analyze the effect of *Corynebacterium* spp. on the presence of *S. aureus*, *S. epidermidis*, and non-*aureus* staphylococci. The number of colonies was grouped into three categories.
[b] Reference groups.
[c] NS, not significant.

broth cultures of samples from 20 of the *S. aureus* carriers. In all cases, only one agr_{Sa} allele (identical to one of the four previously identified alleles) was detected in each culture by multiplex PCR.

Of the 198 *S. epidermidis* isolates, 194 were positive by PCR for at least one of the previously described agr_{Se} alleles: 96 isolates belonged to *agr-1*$_{Se}$, 63 belonged to *agr-2*$_{Se}$, and 97 belonged to *agr-3*$_{Se}$. Most carriers ($n = 140$) harbored only one agr_{Se} allele (*agr-1*$_{Se}$ in 53 carriers, *agr-2*$_{Se}$ in 34 carriers, and *agr-3*$_{Se}$ in 53 carriers), while two agr_{Se} alleles were detected in 50 carriers (*agr-1*$_{Se}$ and *agr-2*$_{Se}$ in 11 carriers, *agr-1*$_{Se}$ and *agr-3*$_{Se}$ in 27 carriers, and *agr-2*$_{Se}$ and *agr-3*$_{Se}$ in 12 carriers) and three were detected in four carriers.

Interactions between staphylococci and corynebacteria. To detect possible interference between *Corynebacterium* and staphylococcal species in carriers, we examined the number of culture-positive samples for each staphylococcal species as a function of the number of *Corynebacterium* CFU. As shown in Table 2, within the range of 0 to 10^3 *Corynebacterium* CFU, the proportion of samples positive for *S. aureus* was quite similar (34 to 46%), whereas it fell significantly at higher *Corynebacterium* CFU levels (16 and 11% of samples were *S. aureus* positive at 10^4 and $>10^4$ *Corynebacterium* CFU, respectively; $P = 0.08$ and 0.001, respectively). The multiple-regression model (Table 3) confirmed that the probability of *S. aureus* isolation was reduced by about 3-fold when the *Corynebacterium* CFU level rose from $<10^2$ to 10^4 (odds ratio = 0.36; $P = 0.16$) and by more than 10-fold when it rose to $\geq 10^5$ (odds ratio = 0.08; $P = 0.0001$). In contrast, the rate of non-*aureus* staphylococcal colonization (including *S. epidermidis*) was not affected by the degree of *Corynebacterium* colonization (Table 2). These results confirm those of previous studies indicating that *Corynebacterium* spp. specifically inhibit colonization by *S. aureus* but not by non-*aureus* staphylococci (22).

TABLE 3. Influence of the number of *Corynebacterium* or *S. epidermidis* colonies on the probability of *S. aureus* isolation

No. of colonies (CFU)	*S. aureus* odds ratio ($CI_{95\%}$), *P* value[a] for:	
	Corynebacterium spp.	*S. epidermidis*
$<10^2$	1	1
10^2–10^3	1.30 (0.57–2.89), 0.52	0.09 (0.03–0.30), 0.0001
10^3–10^4	0.36 (0.09–1.50), 0.16	0.16 (0.05–0.59), 0.005
$\geq 10^4$	0.08 (0.03–0.27), 0.0001	0.11 (0.04–0.41), 0.0008

[a] A multiple logistic regression model was used to analyze simultaneously the effect of *Corynebacterium* spp. and *S. epidermidis* on the presence of *S. aureus.* The number of colonies was grouped into four categories, as follows: $<10^2$ (reference group, for which only the odds ratio is given), 10^2 to 10^3, 10^3 to 10^4, and $\geq 10^4$ CFU. Corynebacteria and *S. epidermidis* were independently associated with the presence of *S. aureus* ($P = 0.001$). $CI_{95\%}$, 95% confidence interval.

TABLE 4. Incidence of nasal carriage of *S. aureus* according to the degree of colonization by *S. epidermidis*

No. of *S. epidermidis* colonies (CFU)	No. of healthy volunteers colonized with *S. aureus* [no. of cases/total no. (%), *P* value][a]		
	Without adjustment with *Corynebacterium* spp.	In presence of $<10^3$ CFU of *Corynebacterium* spp.	In presence of $\geq 10^3$ CFU of *Corynebacterium* spp.
0–10	12/18 (66)[b]	10/11 (91)[b]	2/7 (26)[b]
10–10^2	6/13 (46)[b]	6/8 (75)[b]	0/5 (0)[b]
10^2–10^3	25/78 (32), 0.004	23/73 (31), 0.001	2/25 (8), NS[c]
10^3–10^4	11/40 (27), 0.004	7/22 (32), 0.001	4/18 (22), NS
10^4–10^6	11/37 (30), 0.004	10/28 (36), 0.001	1/19 (5), NS

[a] A χ^2 test was used to analyze the effect of *S. epidermidis* in the presence of *S. aureus* with and without adjustment with the number of *Corynebacterium* CFU acording to the results of Table 3. The number of *S. epidermidis* colonies was grouped into five categories.
[b] Reference groups.
[c] NS, not significant.

To determine whether *S. aureus*, non-*aureus* staphylococci, or *S. epidermidis* sensu stricto inhibited *Corynebacterium* colonization, we determined the number of *Corynebacterium* culture-positive samples as a function of the staphylococcal CFU level. The percentage of *Corynebacterium* isolates fell from 73 to 42% (not a significant change) when the *S. aureus* CFU value increased from 0 to $>10^5$ but was unaffected by the non-*aureus* staphylococci and *S. epidermidis* CFU values (not shown). Thus, *Corynebacterium* specifically inhibited *S. aureus* colonization but not vice versa.

Relationships between staphylococcal species. To determine if non-*aureus* staphylococci, especially *S. epidermidis*, inhibited *S. aureus* nasal colonization, we examined the number of *S. aureus* culture-positive samples as a function of the non-*aureus* staphylococci (including *S. epidermidis*) and *S. epidermidis* sensu stricto CFU values. The number of *S. aureus*-positive samples fell markedly as the non-*aureus* staphylococcal (not shown) and *S. epidermidis* CFU values increased (from 78 to 23%, $P = 0.001$; and from 66 to 30%, $P = 0.004$, respectively) (Table 4), in particular in the volunter with $<10^3$ UFC of *Corynebacterium* spp. (from 88 to 31%, $P = 0.001$). Multiple-

TABLE 5. Influence of the number of colonies of corynebacteria on the probability of isolating *S. aureus* strains bearing *agr-1*$_{Sa}$ and *agr-2*$_{Sa}$

No. of *Corynebacterium* colonies (CFU)	*S. aureus* odds ratio ($CI_{95\%}$), *P* value[a] for:	
	agr-1$_{Sa}$	*agr-2*$_{Sa}$
$<10^2$	1	1
10^2–10^3	0.93 (0.38–2.19),0.84	1.69 (0.55–5.26), 0.35
10^3–10^4	NI[b]	0.77 (0.08–7.20), 0.81
$\geq 10^4$	0.10 (0.02–0.48),0.004	0.33 (0.06–1.61), 0.17

[a] In this model, the colony counts were categorized into four groups for corynebacteria as follows: $<10^2$ (reference group, for which only the odds ratio is given), 10^2 to 10^3, 10^3 to 10^4, and $\geq 10^4$ CFU. $CI_{95\%}$ 95% confidence interval.
[b] NI, not informative because of the small number of subjects.

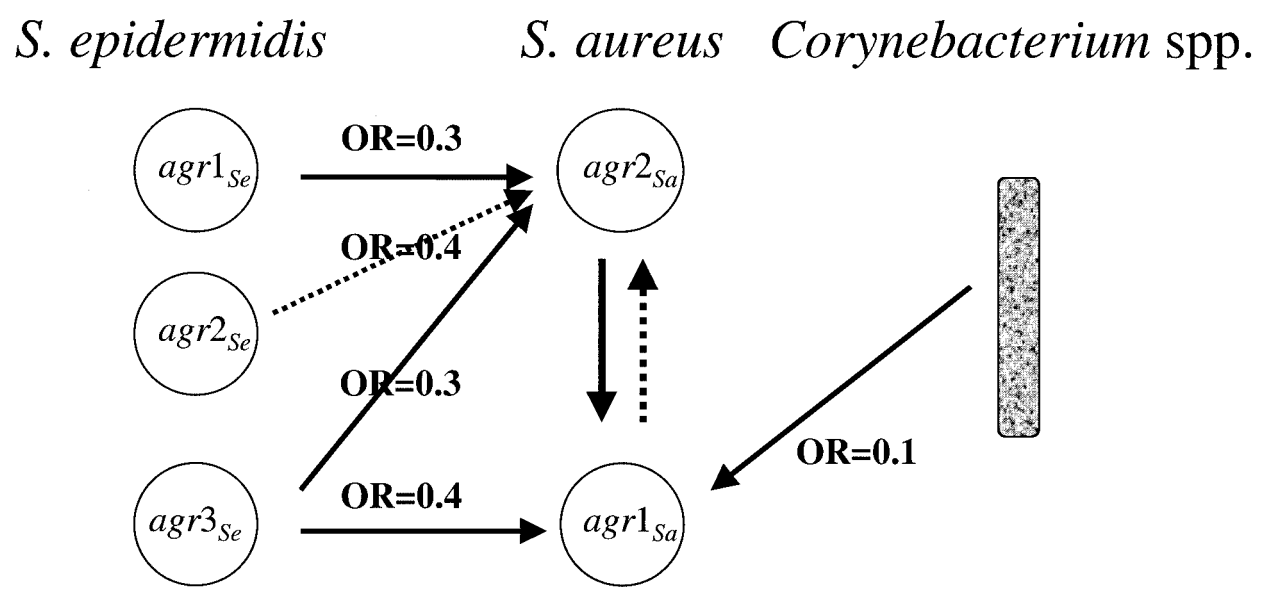

FIG. 2. Model of *agr*-dependent staphylococcal interference. Based on semiquantitative analysis of the aerobic nasal flora, a multiple logistic regression model was used to analyze simultaneously the effect of *S. aureus* agr_{Sa} alleles, *Corynebacterium* spp., and *S. epidermidis* agr_{Se} alleles on *S. aureus* colonization. OR, odds ratio; threshold of 10^2 CFU for *S. epidermidis* and 10^4 CFU for *Corynebacterium* spp.

regression analysis (Table 5) showed that the probability of *S. aureus* isolation fell by about 6- to 10-fold when the *S. epidermidis* CFU value rose from $<10^2$–10^3 to 10^4 and $\geq 10^5$ (odds ratios, 0.1 to 0.16; *P*, 0.0001 to 0.005). This model also showed that the presence of *S. epidermidis* and *Corynebacterium* spp. was independently associated with the presence or absence of *S. aureus* ($P = 0.0001$). Colonization by $>10^3$ non-*aureus* staphylococci, and especially *S. epidermidis*, was clearly protective against *S. aureus* colonization. The rate of non-*aureus* staphylococcal and *S. epidermidis* colonization fell from 100 to 81% and from 99 to 81% as the number of *S. aureus* CFU increased (not shown); however, these results were not statistically significant, probably owing to the small number of volunteers strongly colonized by *S. aureus.* Lastly, some rarely detected staphylococcal species such as *S. warneri*, *S. lugdunensis*, *S. haemolyticus*, and *S. cohnii* (but not *S. capitis* or *S. hominis*) were never isolated simultaneously with *S. aureus.*

Relationships between staphylococci bearing different *agr* alleles. As stated above, even though multiple colonies of *S. aureus* from each swab were typed by PCR, we detected only one agr_{Sa} allele per sample. The presence of *agr-*2_{Sa} was significantly associated with the absence of *agr-*1_{Sa} ($P = 0.02$; Fig. 2). Other agr_{Sa} allele associations were not statistically significant, owing to the small number of subjects colonized by the relevant strains. To determine whether the interaction of *S. epidermidis* with *S. aureus* was linked to *agr* alleles, we used a multiple logistic regression model to analyze the influence of *S. epidermidis agr* alleles (*agr-*1_{Se}, *agr-*2_{Se}, and *agr-*3_{Se}) and *Corynebacterium* spp. (as control) on the presence of the two most frequent *S. aureus agr* alleles (*agr-*1_{Sa} and *agr-*2_{Sa}) (Tables 5 and 6). The probability of isolating *agr-*1_{Sa} *S. aureus* was reduced by about 2.7-fold when the CFU count of *agr-*3_{Se} *S. epidermidis* was $\geq 10^2$ versus $<10^2$ (odds ratio, 0.39; $P = 0.004$), while no statistical differences were observed with other agr_{Se} alleles ($P > 0.35$ or higher). The probability of isolating *agr-*1_{Sa} *S. aureus* was reduced by a factor of about 10 when the number of *Corynebacterium* CFU was $\geq 10^4$ versus $<10^2$ (odds ratio, 0.10; $P = 0.004$), as observed above. In contrast, the probability of isolating *agr-*2_{Sa} *S. aureus* was reduced by a factor of about 3 when the CFU count of *agr-*1_{Se}, *agr-*2_{Se}, or *agr-*3_{Se} *S. epidermidis* was $\geq 10^2$ versus $<10^2$. The results were statistically significant for *agr-*1_{Se} and *agr-*3_{Se} but not for *agr-*2_{Se}, probably because of the small number of isolates (odds ratio, 0.31, $P = 0.06$; odds ratio, 0.37, $P = 0.12$; and odds ratio, 0.33; $P = 0.05$, respectively). Surprisingly, the probability that the *agr-*2_{Sa} *S. aureus* colonization rate fell as the *Corynebacterium* colonization rate rose was not statistically significant, despite the large number of isolates (odds ratio minimal 0.33, $P = 0.17$) (Table

TABLE 6. Influence of the number of colonies of *agr-*1_{Se} to *agr-*3_{Se} allele-bearing *S. epidermidis* on the probability of isolating *S. aureus* strains bearing *agr-*1_{Sa} and *agr-*2_{Sa}

No. of colonies (CFU)	*S. aureus agr-*1_{Sa} odds ratio ($CI_{95\%}$), *P* value, for *S. epidermidis*[a]:			*S. aureus agr-*2_{Sa} odds ratio ($CI_{95\%}$), *P* value, for *S. epidermidis*[a]:		
	agr-1_{Se}	*agr-*2_{Se}	*agr-*3_{Se}	*agr-*1_{Se}	*agr-*2_{Se}	*agr-*3_{Se}
$<10^2$	1	1	1	1	1	1
$\geq 10^2$	0.96 (0.41–2.26), 0.92	0.64 (0.15–2.76), 0.35	0.39 (0.16–0.93), 0.004	0.31 (0.09–1.07), 0.06	0.37 (0.10–1.32), 0.12	0.33 (0.10–1.03), 0.05

[a] In this model, the colony counts were categorized into two groups for *S. epidermidis agr-*1_{Se} to *agr-*3_{Se} ($<10^2$ [reference group, for which only the odds ratio is given] and $\geq 10^2$ CFU). $CI_{95\%}$, 95% confidence interval.

5; Fig. 2). *agr-3*$_{Se}$ *S. epidermidis* and *Corynebacterium* isolation was independently associated with the presence of *agr-1*$_{Sa}$ *S. aureus* ($P = 0.027$ and $P = 0.0002$, respectively), while *agr-1*$_{Se}$ and *agr-2*$_{Se}$ *S. epidermidis* isolation was independently associated with the presence of *agr-2*$_{Sa}$ *S. aureus* ($P = 0.046$ and $P = 0.044$, respectively).

DISCUSSION

We analyzed the composition of the aerobic nasal flora of 216 healthy volunteers to identify potential competitive species interactions, with special emphasis on the influence of staphylococcal *agr* alleles. We found that the *S. aureus* colonization rate in subjects colonized by *Corynebacterium* spp. and/or non-*aureus* staphylococci, especially *S. epidermidis*, was significantly lower than in subjects not colonized by these species, suggesting that both *Corynebacterium* spp. and *S. epidermidis* antagonize nasal colonization by *S. aureus.*

Most of the *S. aureus* and *S. epidermidis* isolates were *agr* typed by PCR. Only one *agr*$_{Sa}$ allele was detected in each individual's nasal flora, as previously described (25). Moreover, multiple logistic regression analysis with the two most prevalent *agr*$_{Sa}$ alleles (*agr-1*$_{Sa}$ and *agr-2*$_{Sa}$) and the three *agr*$_{Se}$ alleles confidently showed *agr*-specific interaction in the nasal vestibule (Fig. 2). Our initial hypothesis was that staphylococcal interaction in the nose would reflect the heteroallelic inhibitory activity previously detected in vitro. In these in vitro experiments, it was shown that the autoinducing peptides produced by the different groups are usually mutually inhibitory on the expression of RNAIII, the effector of the *agr* system, and subsequently on the expression of exoproteins and toxins of *S. aureus*. Hence, *agr*$_{Sa}$ alleles, with the exception of *agr-1*$_{Sa}$ and *agr-4*$_{Sa}$, are all mutually inhibitory in vitro on the expression of RNAIII (7, 9), and *agr-1*$_{Se}$ is also inhibitory for *agr*$_{Sa}$ but not for *agr-4*$_{Sa}$ (20). If these in vitro interferences were relevant in nasal colonization, we would have observed an inhibitory effect of *agr-1*$_{Se}$ on *agr-1*$_{Sa}$ and *agr-2*$_{Sa}$ and frequent simultaneous detection of *agr-4*$_{Sa}$ and *agr-1*$_{Sa}$ or *agr-4*$_{Sa}$ and *agr-1*$_{Se}$. In fact, we found no correlation between in vitro and in vivo data on *agr* alleles. Our results were not in support of a predominant role of the *agr* system in staphylococcal interaction in the human nasal vestibule, as previously observed in several staphylococcal diseases (8). Jarraud et al. (8) examined the possible relationship between *agr* groups and human *S. aureus* disease by studying 198 *S. aureus* strains isolated from patients with suppurative infections and acute toxemia. A relationship between the genetic background, *agr* group, and disease type was observed in most cases of toxin-mediated disease and in several suppurative infections such as infective endocarditis. Jarraud et al concluded that the *agr* type had no direct responsibility for disease initiation and speculated that the preferential association between certain *agr* alleles, certain toxin genes, and a particular genetic background may reflect an ancient evolutionary division of *S. aureus* in terms of this fundamental biology of the species (8).

Hence, the apparent *agr*-dependent in vivo interactions observed in the present study may have been due to other mechanisms. One possibility is the synthesis of antagonists such as bacteriocins, bacteriolytic enzymes, hydrogen peroxide, lactic acids or fatty acids, and ammonia (2). Bactericidal exoproteins have been already detected in staphylococcal and *Corynebacterium* spp. Among the staphylococci, previous studies have identified *S. aureus* bactericiocin against some *Corynebacterium* species (17, 21) and *S. epidermidis* bacteriocin against *S. aureus* (6, 16). However, no bacteriocin-like activity produced by corynebacteria against *S. aureus* or *S. epidermidis* or by one *S. aureus* strain against another has been described. Another possible mechanism of bacterial interference is competition among *Corynebacterium* spp., *S. aureus*, and *S. epidermidis* for specific attachment to epithelial cells (1). Uehara et al. suggested that binding competition might involve the carbohydrate portion of the human nasal mucin support and showed that *Corynebacterium* spp. had higher affinity for mucus than did *S. aureus* and that *S. aureus* had higher affinity than did *S. epidermidis* (22). In our study, *Corynebacterium* spp. inhibited *S. aureus* colonization, but only strains harboring the *agr-1*$_{Sa}$ allele (Table 5). We did not find other concordant results between those reported by Uehara and our in vivo data. The physiological role of mucus is to bind and remove bacteria, not to promote bacterial adhesion to the epithelium. In adhesion experiments with human airway epithelial cells, Mongodin et al. recently showed that *S. aureus* did not adhere in vivo to intact mucus-producing airway epithelium but did adhere to the basolateral plasma membrane of columnar cells, to basal cells, and to the basement membrane (15).

Finally, while we did not identify the precise mechanism of the observed bacterial interference in nasal colonization, our mathematical analysis of ecological data produced a working model of bacterial interactions in the nasal vestibule. Our subsequent experiments aimed at determining these mechanisms will focus on specific interactions identified by the model. Importantly, our results show that the likelihood of nasal colonization by *S. aureus* in healthy subjects varies with the composition of the local flora. The relevance of our model to patients with underlying diseases remains to be tested, but it is noteworthy that most methicillin-resistant *S. aureus* (MRSA) strains harbor *agr-1*$_{Sa}$ (reference 25 and unpublished personal data) and that colonization by *agr-1*$_{Sa}$ strains was specifically associated with a low rate of colonization by *Corynebacterium* spp. and *agr-3*$_{Se}$ *S. epidermidis.* Indeed, our model predicted that the probability of *agr-1*$_{Sa}$ *S. aureus* (probably MRSA) colonization in such cases would be increased by a factor of 33. Larger cross-sectional and longitudinal studies are required to understand how one *S. aureus* strain can displace another, especially in the case of MRSA colonization.

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