

Adhesin and Superantigen Genes and the Capacity of *Staphylococcus aureus* to Colonize the Infantile Gut

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Staphylococcus aureus is a pathogen and a skin commensal that is today also common in the infant gut flora. We examine the role of *S. aureus* virulence factors for gut colonization. *S. aureus* isolated from quantitative stool cultures of 49 Swedish infants followed from birth to 12 months of age were assessed for 30 virulence-associated genes, *spa* type, and *agr* allele by serial polymerase chain reaction (PCR) assays. Strains carrying genes encoding collagen-binding protein, and the superantigens *S. aureus* enterotoxin O/M (SEO/SEM) had higher stool counts than strains lacking these genes, whereas genes for *S. aureus* enterotoxin A (SEA) were associated with low counts. A cluster of strains belonging to *agr* allele I and the *spa* clonal cluster 630 (*spa*-CC 630) that carried genes encoding SEO/SEM, SEC, collagen-binding protein, and elastin-binding protein were all long-time colonizers. Thus, certain *S. aureus* virulence factors might promote gut colonization.

Staphylococcus aureus is a versatile pathogen that causes local and invasive infections, including abscesses, septicemia, osteitis, pneumonia, and endocarditis [1]. *S. aureus* virulence factors include adhesins for extracellular matrix molecules, such as collagen, elastin, laminin, and bone sialoprotein, and for fibrinogen (fibrinogen-binding protein, clumping factors A, B) [2, 3]. Toxins include hemolysins (α , β , γ , and δ), the Panton–Valentine leukocidin, and exfoliative toxins [4] as well as superantigens, which vigorously activate T cells causing vomiting, diarrhea, and shock. Superantigens include >20 *S. aureus* enterotoxins (SEs) as well as toxic shock syndrome toxin 1 (TSST-1) [5]. *S. aureus* produces a polysaccharide capsule, of which

serotypes 5 and 8 are most prevalent in clinical isolates [6]. The *agr* operon encodes a quorum-sensing system that regulates several *S. aureus* virulence genes. It exists in 4 allele variants, termed *agr* I–IV that might be linked to pathogenicity [7]. Resistance to staphylococcal penicillins is encoded by the *mecA* gene.

S. aureus is, however, foremost a commensal colonizing the skin of the anterior nares in some 30% of children and adults [8–10] and, less commonly, the mucosal surfaces of the oropharynx and vagina [11]. During the last decades, we and others have reported an increased rate of isolation of *S. aureus* from infants' stools. High population counts, especially during the first postnatal weeks, combined with persistence of individual strains for many months in individual infants strongly suggest true colonization [12–14], and that *S. aureus* is a member of the infantile gut microbiota.

Infection by *S. aureus* is generally preceded by commensal colonization [1, 15, 16], with nasal carriage tripling the risk of infection [17]. Several staphylococcal toxins and adhesins are thought to be essential for nasal colonization by *S. aureus* [1, 15, 16] as well as for invasive disease [18]. For example, both *S. aureus* nasal and skin infection isolates carry the genes encoding

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fibronectin-binding and collagen-binding adhesins [19] as well as the superantigen toxins SEM-SEO, SEG, and SEI that are collectively encoded by the *egc* cluster [20].

Whether commensal carriage of *S. aureus* in the gut increases the risk for infection has not been studied. We have previously observed that traits that enable *E. coli* to cause urinary tract infections also promote long-term colonization in the gut, which may be the reason why they evolved [21–23]. Thus, we hypothesized that pathogenic traits could also contribute to *S. aureus* gut colonization.

Here, we examined carriage of genes for toxins, adhesins, capsular antigens, and *agr* alleles in *S. aureus* strains from a longitudinal study of Swedish infants. The time of persistence of each strain in the gut of the colonized infant was previously known, as was its fecal population counts on each sampling occasion. Our results indicate that certain adhesins and superantigenic toxins confer fitness in the infant gut.

MATERIALS AND METHODS

S. aureus Strains

The 68 *S. aureus* strains examined here were previously isolated from fecal samples obtained from 49 Swedish full-term healthy infants born in Göteborg, Sweden in 1998–1999. The infants participated in the ALLERGYFLORA birth-cohort study investigating the relation between gut microbiota and allergy development [24]. The methods used for their culture, speciation, and strain-typing have been described previously [14]. Rectal swabs were collected 3 days after birth and cultured semi-quantitatively under aerobic conditions, although fecal samples obtained at 1, 2, 4, and 8 weeks and at 6 and 12 months of age were serially diluted and cultured quantitatively on Staphylococcus agar under aerobic conditions. The limit of detection was 330 CFU/g feces. Staphylococcal colonies of different size, shape, color, or transparency were individually enumerated and subcultured for purity. *S. aureus* was identified with coagulase and catalase tests (bioMérieux). All *S. aureus* isolates found in 1 infant were analyzed by random amplified polymorphic DNA (RAPD) for identification of different strains [14]. No comparisons were made between infants. All isolates have been frozen at –80°C until use in this study.

One isolate of each RAPD profile was *spa*-typed, ie the short repeats in the *S. aureus* protein A (*spa*) gene region were sequenced [25]. The Ridom Staph Type® software was used (Ridom GmbH and <http://spaserver.ridom.de/>).

Detection of Virulence Genes and *agr* Alleles I–IV by Multiplex PCR

Genomic DNA was extracted using the Insta Gene protocol (Bio-Rad) with slight modifications [14]. The *mecA* gene encoding methicillin resistance and the *gyrA* housekeeping gene (internal positive control) were detected in a duplex PCR [26]

and the *agr* allele variety (I–IV) in another PCR [7]. Genes encoding *S. aureus* enterotoxins SEA-D (*sea-d*); SEH (*seh*); enterotoxin-like SEIK (*selk*); SEIL (*sell*); SEIM (*selm*); SEIO (*selo*); SEIR (*selr*); SEIP (*selp*); SEIQ (*selq*); toxic shock syndrome TSST-1 (*tst*); exfoliative toxins A, B, and D (*eta*, *etb*, and *etd*); beta-hemolysin (*hlyB*); epidermal cell differentiation inhibitor toxin A-C (*edin*); Panton–Valentine leukocidin (*pvl*); and leukotoxin M (*lukM*) were detected by 6 different multiplex polymerase chain reaction (PCR) assays [7]. The genes encoding fibrinogen-binding protein (*fib*), clumping factors A and B (*clfA* and *clfB*), elastin-binding protein (*ebp*), laminin-binding protein (*lbp*), collagen-binding protein (*cna*), and bone sialoprotein-binding protein (*bbp*) were detected in another 2 PCRs [3]. The genes for fibronectin-binding proteins A and B (*fnbA* and *fnbB*) were identified using 2 separate PCRs [27]. Genes for capsular types 1, 5, and 8 were detected using a triplex PCR [6].

Statistical Analysis

Proportions were compared using the Fisher's exact test. Stool population counts of strains carrying or lacking a certain virulence gene were compared using Mann–Whitney U-test with correction according to the Holm–Bonferroni method. Multivariate logistic regression models were used to evaluate the independent contributions of genes identified in univariate analysis to population numbers of *S. aureus* strains in stool samples collected from infants at 1, 2, and 4 weeks of age (SPSS 15 analytics).

Multivariate analysis was performed using the SIMCA-P (Umetrics AB). Principal component analysis (PCA) was used to reveal clustering of strains based on virulence factor combinations. Orthogonal projection onto latent structures (O-PLS) was used to reveal the relationship between virulence genes and persistence in the gut flora. This method is a regression development of PCA in which the clustering data is related to a Y variable, in this case persistence in the gut flora [28, 29].

Ethics Permission

This study was approved by the Ethics committee of the University of Gothenburg.

RESULTS

Genetic Diversity

The 68 *S. aureus* strains examined here were previously isolated from the gut flora of 49 infants via regular quantitative stool cultures during their first year of life. The strains were distributed within 36 different *spa* types, and their *agr* allele distribution was as follows: *agr* I (44%), *agr* II (31%), *agr* III (24%), and *agr* IV (1.5%).

Virulence Gene Carriage in Infantile Intestinal *S. aureus* Strains

Toxin gene carriage among the gut commensal *S. aureus* strains is shown in Table 1. The most prevalent genes were *selo* and *selm*, both encoded by the enterotoxin gene cluster, *egc* [30], that were

found in two-thirds of the strains. Two strains carried *selo* but not *selm*, attesting to the variability of *egc* [31]. The prevalence of *sea*, *sec*, *sell*, and *tst* was more moderate (18–22%), although *seb* and other superantigen genes were infrequent (Table 1). All *sec*-positive strains also carried *sell*. Altogether, 74% of the strains carried ≥ 2 superantigen genes, 49% ≥ 3 and 37% ≥ 4 , although 24% possessed none of the 13 screened superantigen genes.

Regarding other toxin genes, 10% of strains had *hlyB*, 4% *eta*, and 1% *pvl* whereas none had *etb*, *etd*, *edin*, and *lukM*. No strain was *mecA* positive.

Adhesin gene carriage is shown in Table 2. Genes encoding laminin-binding protein (*lbp*), fibronectin-binding protein B (*fnbB*), and clumping factors A and B (*clfA*, *clfB*) were almost ubiquitous and many other adhesin genes were carried by most strains. Only bone sialoprotein-binding protein (*bsp*) was found in a minority of strains.

Capsular antigen type 8 was most frequent (81%), followed by type 5 (19%). None of the strains had the type 1 capsule variety.

Principal Component Analysis (PCA)

To reveal clustering of the 68 *S. aureus* strains based on their virulence traits and *agr* allele variants, we performed principal component analysis (PCA). The first principal component, which accounts for the major spread in the material, is represented by the *x*-axis and the second principal component by the *y*-axis. As shown in Figure 1A, 4 dense clusters of strains were seen. The virulence factor genes that generated the separation are

Table 1. Prevalence of Genes Encoding Various Toxins in Intestinal *Staphylococcus aureus* Strains

Toxin	Gene	Positive strains, %
Superantigens		
Enterotoxin A	<i>sea</i>	18
Enterotoxin B	<i>seb</i>	6
Enterotoxin C	<i>sec</i>	19
Enterotoxin D	<i>sed</i>	1
Enterotoxin H	<i>seh</i>	3
Enterotoxin K	<i>selk</i>	6
Enterotoxin L	<i>sell</i>	19
Enterotoxin gene cluster (<i>egc</i>)		
Enterotoxin M	<i>selm</i>	66
Enterotoxin O	<i>selo</i>	69
Enterotoxin P	<i>seip</i>	4
Enterotoxin Q	<i>selq</i>	6
Enterotoxin R	<i>ser</i>	1
Toxic shock syndrome TSST-1	<i>tst</i>	22
Others		
Beta-hemolysin	<i>hlyB</i>	10
Exfoliative toxin A	<i>eta</i>	4
Panton–Valentine leukocidin	<i>pvl</i>	1

NOTE. The carriage rate of the toxin genes was assessed by serial polymerase chain reaction assays in 68 *S. aureus* strains obtained from the intestinal microbiota of 49 Swedish infants followed during the first year of life.

shown in Figure 1B. Separation along the *x*-axis was generated by a cluster of virulence factors (*selm*, *selo*, *ebp*, and *cna*) pulling the strains to the right side. Alternatively, having none of the investigated superantigenic genes and belonging to the *agr* allele II and to the *spa* clonal cluster 084 (*spa*-CC 084) characterized strains gathered to the left. Separation according to the second principal component was generated by, for example, *agr* allele I upwards and *agr* II or III downwards. We observed several clusters, such as *sec* and *spa*-CC 630, or *agr* III, *spa*-CC 012 and *bsp*. All 13 strains carrying the *sec* gene belonged to *agr* I ($P < .0001$, Fisher's exact test, compared with other *agr* groups). Conversely, *sea* (9/12) and *tst* (12/15) were linked to *agr* III ($P < .0001$, for both analyses, Fisher's exact test).

Persistence of *S. aureus* Strains in Relation to Virulence Gene Carriage

Strains that persisted in the microflora for at least 3 weeks were classified as resident ($n = 40$). Strains appearing for shorter periods were defined as transient ($n = 10$). Strains appearing only in the 2-, 6-, or 12-month sample ($n = 18$) could not be classified, because too much time passed between the sampling occasions. To reveal whether the clusters observed by PCA analysis were linked to capacity to persist in the gut, we applied O-PLS, a regression variety of PCA [28, 29] to compare virulence gene pattern between resident and transient strains. Figure 1C shows the variables associated with persistence on the right side of the diagram, whereas genes enriched in transient strains appear on the left. The larger the bar, the greater the contribution and the smaller error bar, the more reliable the contribution. As shown in the figure, *spa*-CC 630, the *agr* I, and *sec* were strongly associated with persistence in the gut. In accordance, the 15 strains that belonged to *spa*-CC 630 and belonged to *agr* I, were either resident ($n = 8$) or unclassified ($n = 7$). These strains also carried *selo*/*selm* and *cna*, and 11 and 14 strains had *sec* and *ebp*, respectively.

Table 2. Prevalence of Genes Encoding Various Adhesins in the Intestinal *Staphylococcus aureus* Strains

Adhesin	Gene	Positive strains, %
Bone sialoprotein-binding protein	<i>bsp</i>	22
Clumping factor A	<i>clfA</i>	99
Clumping factor B	<i>clfB</i>	99
Collagen-binding protein	<i>cna</i>	50
Elastin-binding protein	<i>ebp</i>	62
Fibrinogen-binding protein	<i>fib</i>	74
Fibronectin-binding protein A	<i>fnbA</i>	78
Fibronectin-binding protein B	<i>fnbB</i>	99
Laminin-binding protein	<i>lbp</i>	100

NOTE. The carriage rate of genes encoding a range of adhesins was assessed by serial polymerase chain reactions in 68 *S. aureus* strains obtained from 49 Swedish infants followed during the first year of life.

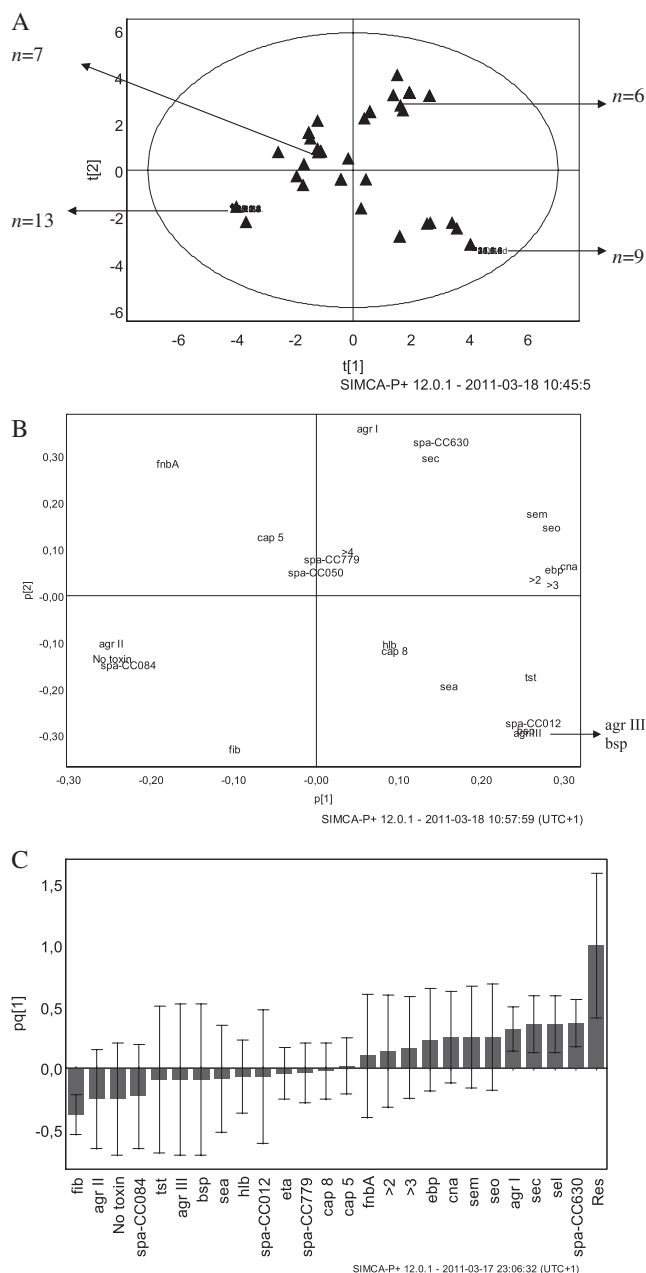


Figure 1. Principal component analysis of 68 *Staphylococcus aureus* strains obtained from the intestinal flora of infants followed during the first year of life. **A**, Position of observations (strains) along the first (x-axis) and second (y-axis) principal component. As a result of overlapping of strains, certain triangles represents >1 strain. **B**, Loadings, ie, the variables generating the observed separation. Virulence genes encoding toxins, adhesins, and genetic background (four *agr* alleles) are included, as well as carriage of ≥ 2 , or ≥ 3 genes encoding superantigen toxins. "No toxin" denotes absence of all screened superantigen toxin genes. Loadings and observations can be superimposed, observations position in the lower right corner are characterized by virulence genes located in that position and a relative absence of genes located in the upper left corner. **C**, Orthogonal projection onto latent structures (OPLS) showing the relation between virulence gene carriage and persistence in the gut flora. Persistence ≥ 3 weeks in the microbiota is modeled as y and the virulence genes associated with this trait

Although the *egc* gene clusters (*selo* and *selm*), *cna*, and *ebp* tended to be associated with persistence (Figure 1C), these genes were also common among transient strains (*selo*: 68% of resident vs 50% of transient strains; *cna*: 53% vs 30%; *ebp*: 65% vs 40%).

Conversely, carriage of the *fib* gene was inversely related to persistence (Figure 1C). This gene was found in all transient strains and in 73% of the resident strains. The *sea* gene was twice as frequent among transient strains (30%) as among resident strains (15%). The *agr* type II tended to be more prevalent among transient than resident strains (50% vs 33%; none significant) (Figure 1C).

Population Counts of *S. aureus* in Relation to Virulence Gene Carriage

The fecal population counts of each *S. aureus* strain in each stool sample were determined previously. *S. aureus* strains had high population counts in the first week of life (10^8 CFU/g on average), but their counts declined steadily and averaged 10^4 CFU/g by 12 months of age, reflecting the competition from an increasingly complex gut flora [14].

Fecal population levels were compared at each sampling occasion between strains carrying a certain virulence gene, and strains lacking this gene during the first 6 months of age (Figure 2). Strains carrying the *selo* or *selm* gene had significantly higher population counts at 1 week of age than strains lacking these genes (Figure 2A). Strains that were positive for *sec* gene tended to be associated with higher bacterial counts but the difference was not significant (Figure 2B). In contrast, at 2 weeks of age, *S. aureus* strains carrying the *sea* gene had considerably lower population counts than strains lacking this gene (Figure 2C).

Among adhesin genes, *cna* ($P = .001$ at 1 week of age) and *fmbA* were linked to higher population counts (Figure 2D and E), whereas strains carrying the *fib* gene had considerably lower stool population counts than strains lacking this gene (Figure 2F). Other virulence genes were not associated with fecal population levels.

Virulence Gene Carriage as Determinant of Population Counts in a Logistic Regression Model

Multivariate linear regression was used to investigate the independent contribution of virulence genes to population counts. Separate models were made for 1, 2, 4, and 8 weeks of age. As seen in Table 3, the superantigen toxin gene *selo* was associated with high counts in 4-week-old infants and the *sea* gene with low counts in infants at 2 and 4 weeks of age. Among adhesin genes, *cna* and *fmbA* were independent determinants of high *S. aureus* population counts at 1 and 4 weeks of age, respectively, whereas *ebp* was a determinant of low population counts in 8-week-old infants.

appear on the right side of the diagram. Conversely, genes enriched in transient strains appear on the left. The bar height shows the explanatory power of the variable and the error bar the uncertainty of its contribution.

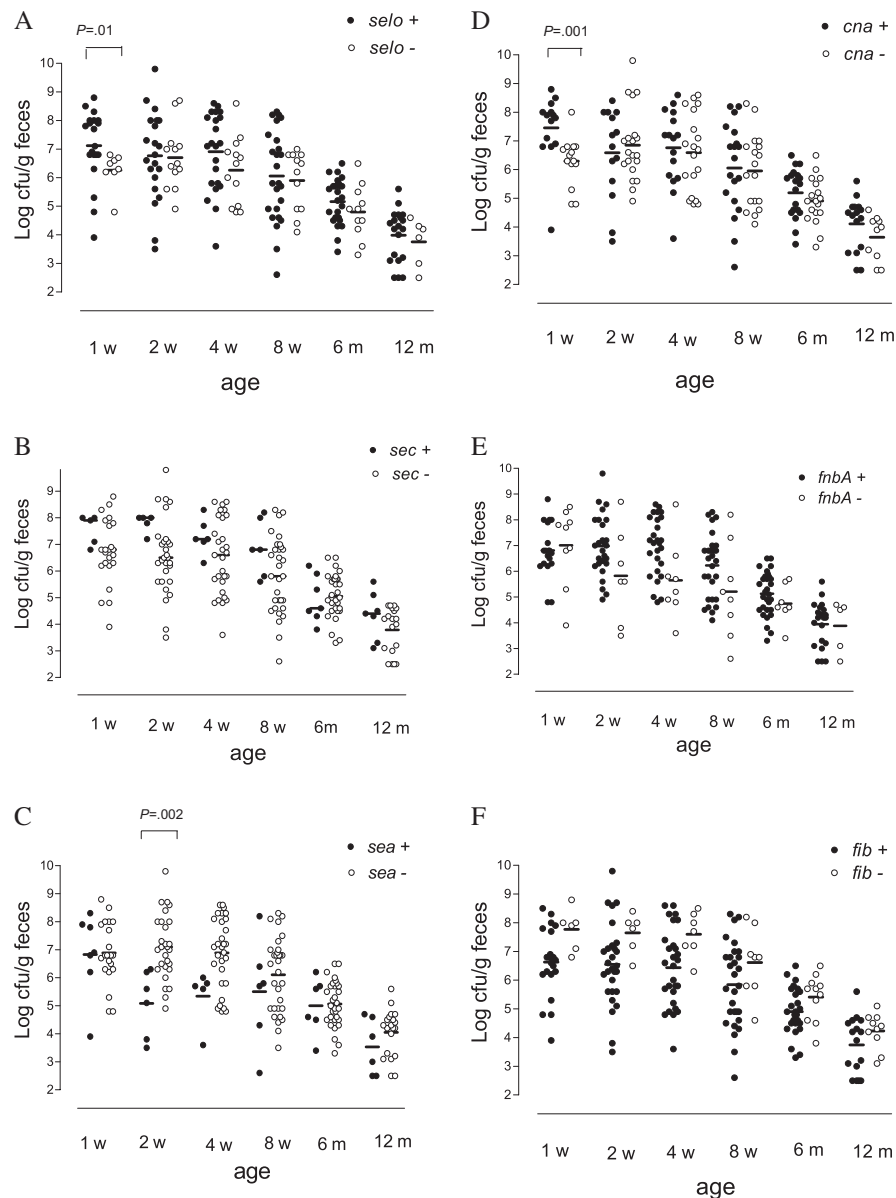


Figure 2. Stool population counts of 68 *Staphylococcus aureus* strains colonizing the intestines of 49 Swedish infants during the first year of life. The figure depicts the population levels at 1, 2, 4, and 8 weeks of age, as well as 6 and 12 months of age, of strains having or lacking the following genes: (A) *selO*, (B) *sec*, (C) *sea*, (D) *cna*, (E) *fnbA*, and (F) *fib*. Filled symbols represent the population numbers of strains carrying the gene in question and open symbols represent the counts of strains lacking this gene; horizontal bars indicate mean values. Difference in population counts during the first 6 months of age between strains having or lacking the gene in question were compared using the Mann–Whitney *U* test. Significant values ($P < .01$, adjusted by the Holm test) are shown in the figure.

DISCUSSION

S. aureus has recently emerged as a prevalent colonizer of the infantile gut; approximately 75% of Swedish infants born today harbor *S. aureus* in the gut flora at some point during the first year of life, and *S. aureus* is as prevalent as *E. coli* in the early microflora [14]. Most infants who harbor *S. aureus* in the gut flora carry a single strain for many months [14]. In the absence of a competing complex flora, *S. aureus* attains high population counts (10^8 colony-forming units (CFU)/g on average), which

contrasts sharply with the level of 10^3 – 10^4 CFU/g found in adults harboring these bacteria [32]. Although we found no direct adverse effect of colonization by superantigen producing *S. aureus* [14], such colonization theoretically could be a risk factor for invasion, transmission, or carriage of antibiotic resistance genes. It is therefore important to investigate the relationship between colonization success in the gut and traits linked to pathogenicity. Here, we investigated *S. aureus* strains obtained from quantitatively cultured stool samples of Swedish infants followed longitudinally during their first year of life. Carriage of genes for a wide

Table 3. Relative Contribution of the Carriage of Toxin Genes to Population Counts of *Staphylococcus aureus* in the Intestine at Different Times

Staphylococcal virulence factors	1 w			2 w			4 w			8 w		
	<i>B</i>	<i>SE</i>	<i>P</i>	<i>B</i>	<i>SE</i>	<i>P</i>	<i>B</i>	<i>SE</i>	<i>P</i>	<i>B</i>	<i>SE</i>	<i>P</i>
Constant	5.896	1.571	...	6.977	1.167	...	5.251	1.009	...	3.747	1.512	...
<i>selo</i>	0.393	0.772	.6	0.251	0.603	.7	1.408	0.563	.02*	1.138	0.726	.1
<i>sec</i>	−1.139	0.970	.3	0.651	0.925	.5	−0.978	0.775	.2	0.452	0.888	.6
<i>sea</i>	−0.297	0.580	.6	−1.803	0.748	.02*	−1.522	0.597	.02*	−0.236	0.706	.7
<i>cna</i>	2.128	0.933	.03*	−0.036	0.818	1.0	0.121	0.668	.1	1.684	0.934	.08
<i>fnbA</i>	0.942	0.962	.3	0.106	0.668	.9	1.539	0.550	.01*	1.661	0.882	.07
<i>ebp</i>	−0.575	0.763	.5	−0.106	0.728	.9	−1.004	0.655	.1	−1.931	0.837	.03*
<i>fib</i>	−0.430	0.812	.6	−0.217	0.821	.8	−0.225	0.709	.8	0.693	0.843	.4

NOTE. Multivariate logistic regression models were used to evaluate the independent contributions of virulence genes to population number of *S. aureus* strains. Separate models were made for 1, 2, 4, and 8 weeks of age because the population counts of *S. aureus* decrease with time (see Figure 2). Virulence factor genes included in the analysis were those shown to contribute to population levels in univariate analysis ($P < .10$ on at least 1 time-point) (SPSS 15 analytics). Significant results ($P < .05$) are shown in bold.

range of virulence determinants including enterotoxins, adhesins, capsular antigens, and different *agr* alleles were related to the capacity of the strains to persist for ≥ 3 weeks in the gut flora, and to their fecal population counts at different time points. The latter was determined individually for each sampling occasion, as *S. aureus* numbers decrease 10 000-fold on average between 1 week and 1 year of infant age in response to increased competition from a more complex commensal flora [14]. Multivariate analysis revealed that certain virulence factor genes including *selo/selm*, *cna*, and *fnbA* were associated with higher population counts. In contrast, *S. aureus* strains carrying the genes for *sea* and *ebp* had lower population counts compared with strains lacking these traits.

Spa typing based on protein A polymorphism revealed a diverse pattern, indicating genetic heterogeneity among the strains studied. There was no correlation between any specific *spa* type and high population counts or persistence of *S. aureus* strains. However, we observed associations among certain *spa*-CCs, virulence genes, and capacity to persist in the microbiota. Indeed, 15 strains were found to belong to *spa*-CC 630 and to the *agr* group I. No transient strains were found in this group, but 8 long-time residents and 7 strains whose capacity to persist could not be adequately determined because they were found only in 1 sample during a period of long sampling intervals. These strains all carried the genes for *selo/selm*, *cna*, and *ebp* and 6 of them also had the *sec* and *sell* genes. This might suggest that certain clones of *S. aureus* have pronounced capacity for long-term colonization of the gut mucosa. The matter remains to be explored further to identify which factors are directly responsible for the capacity to persist. Conversely, *S. aureus* strains colonizing human nasal epithelium show high genetic diversity, and no single clonal lineage has been linked to nasal colonization [33].

It is easy to conceive that collagen-binding and fibronectin-binding proteins contribute to fitness in the gut flora by

conferring adherence to mucosal structures. *S. aureus* adhere to intestinal epithelial cells (unpublished observations) and to airway epithelial cells [34]. Nasal colonization is associated with carriage of clumping factor B (ClfB) [33], which promotes adherence to human type I cytokeratin [35]. Whether clumping factors play a role in gut colonization could not be revealed here, because 99% of all strains carried genes for this adhesin. The same was true of fibronectin-binding protein B.

The *egc* cluster, which harbors the *selo* and *selm* genes, is highly prevalent among *S. aureus* strains and seemed to confer an ability to expand in the infantile gut microflora. It is unclear how superantigen production may enhance fitness on a mucosal surface. However, *S. aureus* has multiple mechanisms for evading both innate immunity mediated by leukocytes [36, 37] and acquired immunity mediated by B and T cells [38]. We cannot exclude that long-term colonizing ability depends on avoidance of defense systems conferred by the effect of superantigens on the immune system [39]. It is notable that antibodies are rarely produced against the enterotoxins encoded by the *egc* cluster on natural colonization in contrast with other enterotoxins [40]. Thus, these toxins may exert their function in colonization unhampered by neutralizing antibodies.

The gene encoding SEC was the second most common enterotoxin gene and all strains carrying this gene were resident in the microflora and belonged to *agr* type I. SEC was, however, not directly linked to the capacity to produce large populations in the gut. This is in accordance with our findings regarding *E. coli* strains, where capacity for long-term persistence is unrelated to fecal population counts [41].

The *agr* allele III was associated with a specific virulence profile (genes for *sea*, *tst*, and *bsp*). These factors were weakly negatively related to long-term commensal persistence, and *sea* was a significant independent factor contributing to low fecal counts. Enterotoxin A is more common and *egc* less common in

S. aureus strains with potential infectious capacity [42]. *S. aureus* enterotoxin A is the most potent enterotoxin, and one may speculate that the inflammation associated with its secretion by strains colonizing a mucosal surface may facilitate their removal. Some recent data suggest that *S. aureus* enterotoxins differ with respect to cytokine profile evoked [42, 43].

Our findings suggest that certain bacterial determinants, including toxins and adhesins, contribute to colonizing success of *S. aureus* strains in the gut that is in accordance with our observations that certain virulence traits confer commensal colonizing capacity of *E. coli* [21, 22, 41]. Persistence as well as high population counts on a mucosal surface might be a risk factor for invasive infection with *S. aureus*, although we found no evidence of staphylococcal disease in the infants examined here. Thus, traits considered virulence factors might serve a dual purpose; first by enhancing commensal colonization, then by aggravating disease in case of invasion.

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