Bacterial Carriage of Genes Encoding Fibronectin-Binding Proteins Is Associated with Long-Term Persistence of *Staphylococcus aureus* in the Nasal and Gut Microbiota of Infants

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ABSTRACT  *Staphylococcus aureus* can colonize both the anterior nares and the gastrointestinal tract. However, colonization at these sites in the same individuals has not been studied, and the traits that facilitate colonization and persistence at these sites have not been compared. Samples from the nostrils and feces collected on 9 occasions from 3 days to 3 years of age in 65 infants were cultured; 54 samples yielded *S. aureus*. The numbers of nasal and fecal *S. aureus* strains increased rapidly during the first weeks and were similar at 1 month of age (>40% of infants colonized). Thereafter, nasal carriage declined, while fecal carriage remained high during the first year of life. Individual strains were identified, and their colonization patterns were related to their carriage of genes encoding adhesins and superantigenic toxins. Strains retrieved from both the nose and gut (*n* = 44) of an infant were 4.5 times more likely to colonize long term (≥3 weeks at both sites) than strains found only in the rectum/feces (*n* = 56) or only in the nose (*n* = 32) (*P* = 0.001). Gut colonization was significantly associated with carriage of the fnbA gene, and long-term colonization at either site was associated with carriage of fnbA and fnbB. In summary, gut colonization by *S. aureus* was more common than nasal carriage by *S. aureus* in the studied infants. Gut strains may provide a reservoir for invasive disease in vulnerable individuals. Fibronectin-binding adhesins and other virulence factors may facilitate commensal colonization and confer pathogenic potential.

IMPORTANCE *S. aureus* may cause severe infections and frequently colonizes the nose. Nasal carriage of *S. aureus* increases 3-fold the risk of invasive *S. aureus* infection. *S. aureus* is also commonly found in the gut microbiota of infants and young children. However, the relationships between the adhesins and other virulence factors of *S. aureus* strains and its abilities to colonize the nostrils and gut of infants are not well understood. Our study explores the simultaneous colonization by *S. aureus* of the nasal and intestinal tracts of newborn infants through 3 years of follow-up. We identify bacterial virulence traits that appear to facilitate persistent colonization of the nose and gut by *S. aureus*. This expands our current knowledge of the interplay between bacterial commensalism and pathogenicity. Moreover, it may contribute to the development of targeted strategies for combating *S. aureus* infection.

KEYWORDS infants, *S. aureus*, adhesins, enterotoxin, fibronectin-binding protein, gut colonization, nasal colonization, persistence, virulence factors

*S. aureus* is one of the most important human pathogens, causing infections that range from minor skin conditions to life-threatening diseases such as sepsicaemia (1). *S. aureus* is also a member of the commensal flora, colonizing the anterior nares of...
approximately 50% of the population, with 20% being persistently colonized (2, 3). Infection by *S. aureus* is generally preceded by commensal colonization (1, 4, 5); being a nasal carrier of *S. aureus* increases the risk of invasive infection 3-fold (6).

*S. aureus* may also colonize the mucosal surfaces of the throat, vagina, and gut (7, 8). During the last few decades, *S. aureus* has been identified as a common colonizer of the gut of infants (7, 9). It has been proposed that this is because improved levels of hygiene have decreased colonization by more “traditional” fecal bacteria (10). Thus, *S. aureus* is found in the feces of approximately 75% of Swedish infants, with individual strains often persisting in an infant for many months (9). In *S. aureus*-colonized infants, the bacterium attains high fecal population levels (10⁷ CFU/g, on average, in 1-month-old infants) (9). The gut has been highlighted as a potentially important reservoir for *S. aureus* strains that cause infections (7, 11, 12). Accordingly, carriage of *S. aureus* in both the nose and gut seems to increase the risk of developing *S. aureus* infection compared to nasal carriage alone (12).

Staphylococcal virulence factors enable the bacterium to cause disease (13). These factors may also enable commensal colonization and persistence by staphylococci (1, 4, 5). Staphylococcal adhesins enable the bacteria to adhere to host matrix components, such as collagen, fibronectin, elastin, laminin, and bone sialoprotein (14). Fibronectin-binding proteins (15–18) and collagen-binding protein (19) have been shown to contribute to virulence in experimental invasive disease models, and their respective genes (*fnb* and *cnb*) are frequently detected in *S. aureus* isolates from the nostrils, from skin infections, and from persons with invasive disease (20, 21). Nasal commensal *S. aureus* strains actively transcribe *fnbA* (22). For gut-colonizing *S. aureus* strains, carriage of the *cnb* gene is associated with increased numbers of *S. aureus* in the feces (23).

The plasma-clotting protein fibrinogen is recognized by several staphylococcal adhesins, i.e., clumping factors A and B (ClfA and ClfB) and fibrinogen-binding proteins A and B. ClfA has been shown to contribute to septicemia in a murine model (24). ClfB also mediates adherence to epidermal cytokeratins (25). *S. aureus* strains from the nostrils spontaneously transcribe *clfB* (22), and *clfB* facilitates experimental nasal colonization in humans (26).

Other virulence factors of *S. aureus* include various toxins. T-cell-mitogenic toxins or superantigens, e.g., *S. aureus* enterotoxin A (SEA), SEB, SEC, etc., and toxic shock syndrome toxin-1 (TSST-1) (27), have been shown to contribute to disease in various experimental models (28). The most commonly detected superantigens, SEG, SEI, SEIM, SEIN, and SEIO, all of which are encoded by the egc gene cluster (29), appear to have lower pathogenic potential (28, 30). Both nasal commensals and skin infection isolates frequently carry genes that encode the superantigens SEIM-SEIO, SEG, and SEI (31). These toxins may play roles in commensal colonization, as *S. aureus* strains that possess the genes for the enterotoxins M/O (encoded by the egc cluster) attain higher fecal numbers than strains that lack these genes (23).

Compared to nasal colonization by *S. aureus*, less is known regarding its gut-colonizing behavior, and the nasal and gut colonization profiles have not been studied in the same individuals. Similarly, the factors that enable colonization of these two separate ecological niches and long-term persistence at these sites have not been elucidated.

Here, we examined the patterns of *S. aureus* nasal and fecal colonization from 3 days to 3 years of age in 54 culture-positive infants who harbored the species in the nose, gut, or both the nose and gut. Individual strains were identified, and their virulence gene patterns were related to the site of colonization and the capacity of the strain to persist at that anatomical site.

**RESULTS**

**Comparison of *S. aureus* colonization rates in the nose and gut of infants.**

Figure 1 compares the rates of *S. aureus* colonization of the nose and gut in the 65 infants between samples taken at 3 days and 3 years of age. Nasal and gut colonization by *S. aureus* increased rapidly in parallel over the first weeks of life (Fig. 1). Thereafter,
nasal colonization declined progressively, from a peak level at 1 month of age (42% of infants colonized) to 14% by 6 months of age and 8% by 18 months (4 months versus 6 months of age, 34% versus 14%, \( P = 0.01 \)). In contrast, the fecal colonization rate remained high, at slightly below 50%, up to 1 year of age (Fig. 1). Thus, \textit{S. aureus} was significantly more frequently retrieved from feces than from nasal swabs at 6, 12, and 18 months of age (\( P = 0.0001, P = 0.002, \) and \( P = 0.01 \), respectively) (Fig. 1).

In total, 54 infants (83%) yielded at least one sample that contained \textit{S. aureus}. The absolute majority (71%, 46/65) had \textit{S. aureus} in both the nose and rectum/feces, while four infants (6%) had \textit{S. aureus} only in the nose and another four infants (6%) had \textit{S. aureus} only in the rectum/feces. The proportion of infants colonized by \textit{S. aureus} on at least one sampling occasion did not differ between infants from farming and nonfarming families, and colonization was not linked to the infant’s sex, delivery mode, or antibiotic treatment (Table 1). Breast-feeding was associated with reduced rate of \textit{S. aureus} colonization in the infants (Table 1).

\textbf{Identification of \textit{S. aureus} strains and characterization of their colonizing behaviors.} In all, 449 \textit{S. aureus} isolates from the 54 colonized infants were analyzed by RAPD to identify distinct strains in individual infants. Figure 2a shows an example of the RAPD patterns of the 9 nasal and 11 gut isolates of an infant. Two strains, designated strains A and B, were found in the nose, and both appeared in the 3 day sample; strain A was present until 2 months of age, and strain B persisted until 1 month of age. Strain A was also found in the gut, where it was present from 1 week to 18 months of age. Strain B was likewise found in the gut (at 1 and 2 months of age). Furthermore, a third strain (strain C) appeared in a single fecal sample, obtained at 4 months of age (Fig. 2a). The colonization patterns of strains A, B, and C are depicted in Fig. 2b. Both strain A and strain B persisted in the nasal microbiota, i.e., were present for \( \geq 3 \) weeks at the colonized site. Both strains were also persistent colonizers of the infant gut, where strain A colonized from 1 week to 18 months of age. No transient strain was identified in this infant, i.e., a strain that colonized for \( < 3 \) weeks. Strain C was deemed “unclassified” with respect to persistence. As it was only present in one sample and this sample was obtained during a period of sampling with long intervals, it could not be classified as either persistent or transient.

Thus, each strain could be classified as persistent, transient, unclassified, or not present at each site, which yielded 15 possible combinations, 13 of which were represented among
TABLE 1 Characteristics of the FARMFLORA cohort

| Parameter                        | No. (%) of infants | All infants (N = 65) | S. aureus-colonized infants (N = 54) | P value* |
|----------------------------------|--------------------|----------------------|--------------------------------------|----------|
| Farmer’s children                |                    | 28 (43)              | 23 (43)                              | 1.0      |
| Pets in householda               |                    | 40 (62)              | 32 (60)                              | 0.5      |
| Vaginal delivery                 |                    | 55 (85)              | 44 (81)                              | 0.2      |
| Exclusive breastfeeding at 4 mo of age |                  | 34 (52)              | 25 (46)                              | 0.04     |
| Partial breastfeeding at 12 mo of age |                | 33 (51)              | 24 (44)                              | 0.01     |
| First-born                       |                    | 29 (45)              | 27 (50)                              | 0.09     |
| Intrapartum antibioticsb         |                    | 10 (15)              | 10 (18)                              | 0.2      |
| Antibiotics, 0–6 mo of agec      |                    | 11 (17)              | 7 (14)                               | 0.09     |
| Antibiotics, >6–12 mo of age     |                    | 11 (17)              | 10 (18)                              | 0.6      |
| Antibiotics, >12–18 mo of age    |                    | 17 (26)              | 16 (30)                              | 0.2      |
| Girls                            |                    | 32 (49)              | 27 (50)                              | 1        |

*Data regarding household pets (cat and/or dog) were missing in one case.

1Antibiotic treatment of the mother during partus. The administered antibiotics included erythromycin (number of infants [N] = 2), cefuroxime (N = 2), metronidazole (N = 2), benzylpenicillin (N = 1), and benzylpenicillin plus cefuroxime (N = 1). Missing data, N = 2.

2Antibiotic treatment of the child at least once in the indicated time period. The following antibiotics were administered: at 0 to 6 months of age, phenoxymethylpenicillin (N = 4), ampicillin (N = 1), cephalosporin (N = 1), and trimethoprim-sulfamethoxazole (N = 3); missing data, N = 2; at 6 to 12 months of age, phenoxymethylpenicillin (N = 10), ampicillin (N = 3), cephalosporin (N = 1), and isoxazolyl penicillin (N = 1); and at 12 to 18 months of age, phenoxymethylpenicillin (N = 13), trimethoprim (N = 1), trimethoprim-sulfamethoxazole (N = 4), ampicillin (N = 1), amoxicillin (N = 2), amoxicillin-clavulanic acid (N = 2), and isoxazolyl penicillin (N = 2).

3P values refer to the comparisons of infants who were colonized and those who were not colonized by S. aureus on at least one sampling occasion.

The 132 identified strains (see Table S1 in the supplemental material). Table 2 summarizes the colonization behaviors of the S. aureus strains: 24% (number of strains [n] = 32) were found only in the nose, 42% (n = 56) only in the gut (rectum/feces), and 33% (n = 44) in both the nose and gut of the same infant. Strains that were retrieved exclusively from one site (nose or gut) were usually transient or unclassified, and only 11% of these strains were persistent colonizers. This was in striking contrast to the strains that appeared both in the nose and gut, among which 50% were persistent at both sites (P = 0.001, Fisher’s exact test) (Table 2). Thus, there appears to be a strong linkage between being able to colonize two different sites and having the capacity to persist in the microbiota.

**Virulence gene pattern in relation to site of colonization.** To determine whether there was any correlation between virulence gene carriage and site of colonization, principal component analysis (PCA) was applied. As shown in Fig. 2c, the agr3 type, the toxin gene tst, and the adhesin gene bsp appeared to be enriched in strains that were found only in the nose. Furthermore, strains that were found in both the nose and gut appeared more similar to those found in the gut only, and the fnbA gene seemed to be associated with the gut-colonizing strains (Fig. 2c).

This aspect was further explored using orthogonal projection onto latent structures-discriminant analysis (OPLS-DA), which is a regression variety of PCA, whereby virulence gene carriage (X variables) was related to the site of bacterial isolation (Y variables). This analysis confirmed that the “nose & gut” and “gut-only” strains were similar, as they clustered together at the right side of the plot, and that they were enriched for the fnbA and fnbB genes (Fig. 2d). Conversely, the “nose-only” strains were characterized as having the agr3 allele and carrying the adhesin gene bsp as well as the genes for the superantigens TSST-1 and SEH.

In the univariate analyses (Fisher’s exact test), the “nose-only” strains (n = 32) were compared with strains that colonized the gut, regardless of whether they were also isolated from the nose (n = 100). Confirming the multivariable analyses, “nose-only” strains were found to be significantly more likely to carry the superantigen gene seh (corrected P value [Pc] of 0.043) and tended to more frequently carry the tst gene (Pc = 0.055) and the agr3 allele (Pc = 0.09) compared to strains obtained from the gut (Table 3). Conversely, the gut-colonizing
FIG 2 (a) RAPD patterns of 9 nasal and 11 gut *S. aureus* isolates obtained on different occasions from a single infant. These isolates were subjected to RAPD, and the patterns were visualized by silver staining. Isolates that show identical RAPD patterns are considered to belong to the same strain. Three individual strains were identified, termed A, B, and C. Strains A and B were repeatedly isolated from both the nose and gut, while strain C was only isolated once, from the gut. (b) Visualization of
TABLE 2 Colonization behaviors of the 132 \emph{S. aureus} strains retrieved from the nose and/or gut of infants who were monitored from 3 days to 3 years of age
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| Colonization site | Persistent behavior [n (%)] by strain type | Transient behavior [n (%)] by strain type | Unclassified behavior [n (%)] |
|-------------------|------------------------------------------|------------------------------------------|-----------------------------|
| Nose only (n = 32) | Nose (4 (12.5)) Gut (12 (37.5)) Both (16 (50)) | Nose (20 (36)) Gut (2 (4.5)) Both (1 (2)) | Nose (30 (54)) Gut (9 (20)) Both (4 (9)) |
| Gut only (n = 56)  | Nose (6 (11)) Gut (33 (75)) Both (22 (50)) | Nose (6 (14)) Gut (2 (4.5)) Both (1 (2)) | Nose (30 (54)) Gut (9 (20)) Both (4 (9)) |
| Nose and gut (n = 44) | Nose (28 (64)) Gut (33 (75)) Both (22 (50)) | Nose (6 (14)) Gut (2 (4.5)) Both (1 (2)) | Nose (30 (54)) Gut (9 (20)) Both (4 (9)) |

*Persistent strains appeared in several nasal and/or gut samples over a long period of 3 weeks or more. Transient strains colonized the nose and/or gut of an infant for <3 weeks or were present only once at either 1 month or 2 months of age. Unclassified strains were those isolated on a single sampling occasion during a period of long sampling intervals (at 4, 6, 12, 18, or 36 months of age). These strains could not be defined as either persistent or transient.

strains significantly more frequently carried the \textit{fnbA} gene compared to the “nose-only” strains ($P_r = 0.027$) (Table 3).

No significant differences in virulence gene carriage were found between the “gut-only” and “nose & gut” strains in the univariate analysis (data not shown).

A virulence score was calculated for each strain by summing the number of virulence genes carried (among those genes that were screened). The “nose-only” strains did not differ significantly from the strains colonizing the gut (mean, 9.7 versus 10, $P = 0.2$; Mann-Whitney \textit{U} test). Furthermore, the “gut-only” and “nose & gut” strains did not differ from one another in terms of virulence score (10 versus 10, $P = 0.3$).

**Virulence gene patterns and capacities to persist in the nose or gut microbiota.**

We next examined whether persistence in the nose or gut was associated with any particular virulence traits by comparing persistent and transient strains at these sites (Table 2). PCA indicated that persistent strains resembled one another, regardless of their site of isolation, and that they were enriched for \textit{fnbA} and \textit{fnbB} (Fig. 3a). Conversely, transient strains, regardless of their site of isolation, belonged to the \textit{agr3} allele and carried the \textit{bsp} and \textit{tst} virulence genes (Fig. 3a). It is noteworthy that these same traits were enriched also in the “nose-only” strains (Fig. 2b).

The OPLS-DA analysis revealed that \textit{fnbA} and \textit{fnbB} characterized persistent strains in both the nose (Fig. 3b) and the gut (Fig. 3c). The contribution of \textit{fnbB} to persistence was of similar magnitude at both sites, although it was only significant for gut strains (Fig. 3b and c). This finding might be attributable to the slightly higher numbers of gut strains. Interestingly, nasal-transient strains (left side of diagram in Fig. 3b) tended to...
belong to the agr3 allele and to carry the tst, bsp, and seh genes, i.e., the same traits that characterized the "nose-only" strains (Fig. 2d).

We speculated that the poor capacity of tst-agr3-bsp-seh strains to persist could be due to a lack of genes that promoted persistence, e.g., fnbAB. Therefore, we analyzed the association between fnbA or fnbB on the one hand and tst, bsp, seh, and the agr3 allelic variety on the other hand (n = 132 strains). There was, indeed, a strong inverse relationship between tst and fnbA carriage, i.e., 21% of the tst-positive strains (6/28) carried fnbA, while this also was true in the case 86% of tst-negative strains (90/104, P = 0.0001) and between tst and fnbB carriage (71% versus 95%, P = 0.001). The same correlations were seen for agr3 and fnbA (26% versus 87%, P = 0.0001), agr3 and fnbB (78% versus 95%, P = 0.01), and bsp and fnbA (41% versus 83%, P = 0.0001) but not for bsp and fnbB (84% versus 92%, P = 0.3). There were no significant associations between fnbA or fnbB carriage and seh carriage.

As the capacity to persist for both gut-colonizing and nose-colonizing strains seemed to be linked to the same traits, we compared strains that were always persistent colonizers to those that always displayed a transient colonization pattern, regardless of their colonization site. We identified 43 strains that were "always persistent," i.e., they were persistent at one site or both and not transient at any site (they could be unclassified or absent at the other site). Of these, 22 were persistent both in the nose and gut, and 9 were present only in the nose and 12 only in the gut (Table 51). We compared these with 33 "always transient" strains (defined as strains that were transient at one site and transient or absent at the other site), and 12 of these strains were found (transiently) only in the nose, 20 only in the gut,

| Virulence trait                                | Gene    | Carriage rate (% of strains) | P value          |
|------------------------------------------------|---------|------------------------------|-----------------|
|                                                 |         | Only nose (n = 32) | Gut (n = 100) | Fisher’s | Corrected |
| Bone sialoprotein-binding protein                | bsp     | 41                           | 19              | 0.018    | 0.16      |
| Collagen-binding protein                         | cbp     | 59                           | 64              |          |           |
| Elastin-binding protein                          | ebp     | 80                           | 68              |          |           |
| Fibrinogen-binding protein                       | fi      | 55                           | 58              |          |           |
| Fibronectin-binding protein A                    | fnbA    | 50                           | 80              | 0.002    | 0.027     |
| Fibronectin-binding protein B                    | fnbB    | 81                           | 93              |          |           |
| Superantigens                                    |         |                              |                 |          |           |
| Enterotoxin A                                    | sea     | 6                            | 6               |          |           |
| Enterotoxin C                                    | sec     | 19                           | 29              |          |           |
| Enterotoxin H                                    | seh     | 31                           | 9               | 0.003    | 0.043     |
| Enterotoxin K                                    | selk    | 3                            | 8               |          |           |
| Enterotoxin L                                    | sel    | 31                           | 37              |          |           |
| Enterotoxin M                                    | selm    | 56                           | 63              |          |           |
| Enterotoxin O                                    | sola    | 71                           | 71              |          |           |
| Enterotoxin P                                    | selp    | 0                            | 12              |          |           |
| Enterotoxin R                                    | selr    | 9                            | 7               |          |           |
| Toxic shock syndrome toxin TSST-1                | tst     | 41                           | 15              | 0.005    | 0.055     |
| No superantigen                                  |         | 12                           | 13              |          |           |
| Other toxins                                     |         |                              |                 |          |           |
| Beta-hemolysin                                   | hlb     | 44                           | 64              |          |           |
| Epidermal cell differentiation inhibitor toxin   | edin    | 3                            | 10              |          |           |

<sup>a</sup>S. aureus Long-Term Persistence in Infants

<sup>a</sup>Subjects were infants who were monitored from 3 days to 3 years of age. Rates of virulence gene carriage by S. aureus strains that were found exclusively in the nose (n = 32) or in the gut (either only in the gut or in both the nose and gut, n = 100), isolated from 54 Swedish infants, are shown. Proportions were compared using Fisher’s exact test. The column heading P value corrected refers to the P values derived after correction for multiple inferences using the Westfall-Young permutation method. No superantigen refers to a lack of carriage of one of all the screened superantigen genes. The following toxin genes were identified in <5% of the strains: sed (enterotoxin D), eta, etb, and etd (exfoliative toxins A, B and D), pvl (Panton-Valentine leukocidin), and lukM (leukotoxin M). No strain carried the toxin gene seb or selq. The following adhesin genes were carried by ≥98% of the strains: clfA and clfB (clumping factors A and B, respectively) and ffb (laminin-binding protein).

TABLE 3 Carriage rates of genes that encode toxins and adhesins in S. aureus strains that colonize only the nose and those that colonize the gut or both the gut and nose<sup>a</sup>
and 1 transient in both the nose and gut (Table 2). As shown in Fig. 3d, the fnbA and/or fnbB genes were enriched in the strains that were "always persistent," and this was confirmed in the univariate analysis (Table 4). In fact, all of the strains that were classified as "always persistent" carried the fnbB gene (Table 4). The association between a strain always being transient and its carriage of agr3 and tst was found to be not significant in the univariate analysis (Table 4).

We also compared the virulence scores for the "always persistent" and "always transient" strains. The "always persistent" strains had significantly higher virulence scores than the "always transient" strains (mean, 11 versus 9; $P = 0.002$).

**Duration of colonization of persistent S. aureus strains in the nose and gut.** We compared the duration of colonization of those strains that were persistent colonizers of the nose or gut. Overall, persistent colonization was noted for 32 strains in the nose and 39 strains in the gut (Table 2). The average duration of colonization of the persistent nasal strains was $\geq 26$ weeks, compared to $\geq 42$ weeks for persistent strains in the gut ($P = 0.001$).

Furthermore, among the strains that persisted at both sites ($n = 22$; Table 2), the duration of persistence was significantly longer in the gut (mean, $\geq 49$ weeks) than in the nose (mean, $\geq 20$ weeks) ($P = 0.003$).

**Fecal population counts of gut-colonizing S. aureus strains in relation to gut persistence and virulence gene carriage.** The S. aureus counts at different fecal sampling occasions were compared between persistent and transient gut-colonizing strains, and no
significant differences were observed. Carriage of \(\text{fnbA}\) or \(\text{fnbB}\) was not associated with increased fecal counts at any of these time points. Since carriage of genes that encode collagen-binding protein, enterotoxin \(M\), or enterotoxin \(O\) was previously linked to high fecal counts of \(S.\ aureus\) (23), we also analyzed these genes. However, no significant associations were observed. Furthermore, no correlation was found between the virulence score of a strain and its fecal population counts (data not shown).

**S. aureus strain characteristics in relation to the lifestyle factors and clinical histories of the colonized infants.** The following lifestyle factors and clinical histories were analyzed in relation to \(S.\ aureus\) strain virulence and colonization characteristics: gender, delivery mode, intrapartum antibiotic exposure, antibiotic treatment (at least once between birth and 18 months of age), exclusive breastfeeding for at least 4 months, having elder siblings, being from farming families, and pet exposure (Table 1). Carriage of \(\text{fnbA}\) or \(\text{fnbB}\) or the virulence score of the \(S.\ aureus\) strain did not correlate significantly with any of these factors. Furthermore, none of these factors significantly affected the proportions of persistent versus transient strains in the nose and gut of colonized infants. Regarding the long-term persistence of \(S.\ aureus\) strains, the duration of colonization for persistent nasal strains was significantly shorter in infants with elder siblings than in infants without siblings (mean, 7 versus 30 weeks, \(P = 0.01\)); the same was true for persistent gut strains (mean, 26 versus 51 weeks, \(P = 0.02\)). However, the statistical significance disappeared following multiple inference correction.

**DISCUSSION**

In this study, we monitored nasal and gut colonization by \(S.\ aureus\) in parallel in a birth cohort of 65 infants who were monitored from 3 days to 3 years of age.
In total, 83% of the infants yielded at least one positive sample, and 71% had *S. aureus* at both sites. Although nasal and gut colonization increased in parallel during the first month of life, nasal colonization declined thereafter, and between 6 and 18 months of age, *S. aureus* was isolated 2 to 4 times more frequently from the feces than from the anterior nares.

Nasal samples were obtained using swabs. This method was also used for assessing rectal colonization on day 3 after delivery. Thereafter, gut colonization was assessed by quantitative culturing of feces. The latter method could be considered more sensitive, although in the case of enteric pathogens the culturing of rectal swabs is as sensitive as the culturing of feces (32). There is no reason to believe that this is not also true for *S. aureus*. Furthermore, there was no difference in the nasal and gut colonization rates up to 1 month of age, and thereafter nasal colonization decreased while gut colonization remained high. A decline in nasal colonization by *S. aureus* after the first weeks of life has been noted previously (33). The observed decrease may relate to anatomical changes, increased production of antimicrobial peptides, and increased competition from other skin colonizers. Gut colonization by *S. aureus* decreased at a much later stage, after 1 year of age, and this probably reflects increased competition from the more complex gut microbiota (34).

Other groups have suggested that the gut is the main habitat of *S. aureus* strains under certain circumstances (35). Piewngam and Otto have even suggested that gut colonization by *S. aureus* is the primary event and nasal colonization is secondary and transient, resulting from inoculation of the nose with gut-colonizing strains, representing anorectal-nasopharyngeal transmission (35). Such transmission might explain the failure of topical decolonization when only the nose is targeted (7, 36).

Our previous and present results confirm that successful isolation of *S. aureus* from the feces is not simply the result of the gut being seeded by the nasal microbiota. First, the high population counts of *S. aureus* during early infancy, 10^7 CFU/g feces on average (9), can hardly be achieved without the multiplication of bacteria indigenous to the gut. Second, in the present study, we identified almost twice as many strains that were found only in the feces (n = 56) than strains that were found only in the nose (n = 32). Third, strains that colonized, for at least 3 weeks, both the gut and the nose of the same infant persisted, on average, more than twice as long in the gut as in the nose (≥49 weeks compared to ≥20 weeks, on average). Fourth, the strains that colonized both the gut and the nose of the same individual displayed fecal population counts that were no higher than those of the strains that colonized only the gut of an infant (our unpublished observations). We conclude that *S. aureus* is a true gut colonizer of young infants and that, at least in infants and young children, gut colonization by *S. aureus* is as common as or more common than nasal colonization.

Persistent carriage in both the nose and gut of the same *S. aureus* strain was frequently observed in our cohort. Interestingly, 50% of the strains that were retrieved from both the nose and gut of an infant were persistent at both sites, while only 13% of the strains found exclusively in the nose and 11% of the strains found exclusively in the gut colonized that site for ≥3 weeks (P = 0.001). Thus, for *S. aureus*, there is a strong correlation between being able to colonize two different ecological niches (nose and gut) and being able to persist for extended periods of time in the microbiota.

Fibronectin-binding proteins A and B appeared to be associated with long-term persistence within either the nasal or gut microbiota. In fact, when we defined a subset of strains that were always persistent, whether they colonized the nose or the gut or both, and never appeared transiently at any of these sites, 100% of them carried the *fnbB* gene. Moreover, *fnbB* gene carriage was also significantly associated with persistence among the gut-colonizing strains. We regard the higher prevalence rates of the *fnbA* and *fnbB* genes among persistent strains as indicating that *S. aureus* exploits these proteins during long-term colonization of the nose and/or gut of infants. Accordingly, preferential transcription of the *fnbA* gene was observed in *S. aureus* strains in the noses of healthy carriers (22). As fibronectin is an extracellular matrix protein, it is not thought to be present on intact epithelia. However, the fibronectin-binding adhesin may recognize other (as-yet unknown) receptors,
given that *S. aureus* binds to intact mammary gland epithelial cells via fibronectin-binding protein in a manner that can be inhibited by soluble fibronectin (37). To the best of our knowledge, the importance of fibronectin-binding protein for persistent colonization of *S. aureus* in the nose and gut has not previously been demonstrated. Animal studies have shown that fibronectin-binding proteins play essential roles in the capacities of *S. aureus* to cause septicemia, septic arthritis, and endocarditis (15, 17, 18).

In the present study, carriage of the *fnbA* gene was also significantly linked to the ability of *S. aureus* strains to colonize the gut. Strains that were restricted to the nose were instead enriched for the *tst*, *bsp*, and *seh* genes and often belonged to the *agr3* allelic genotype. These traits were, however, not associated with persistent colonization of the nose but rather with a transient colonization behavior. Strains that produce TSST-1 are mostly of the *agr3* allelic variety and originate from a single clone (38). This clone may be a poor colonizer of infants, perhaps being particularly inept at surviving in the gut milieu. This could relate to lacking the *fnbA* gene, as there was a significant inverse relationship between the carriage of *tst* and the carriage of *fnbA*: only about 20% of the *tst*-positive strains carried the *fnbA* gene, while 86% of the *tst*-negative strains carried the *fnbA* gene. TSST-1 itself is unlikely to function in the gut, as it is susceptible to cleavage by pepsin and, therefore, is likely to be structurally unstable in this environment (39, 40).

Nasal colonization by *S. aureus* has previously been linked to the presence of ClfB (25, 26), which promotes staphylococcal adherence to human type I cytokeratin (25). In the present study, 99% of all the nasal and intestinal *S. aureus* strains carried the genes for clumping factors A and B, making it impossible to assess the impact of ClfB on persistent colonization by *S. aureus* strains.

The superantigens SEIM, SEIO, SEG, and SEI, which are all encoded by the *egc* locus, have been suggested to play a role in *S. aureus* nasal colonization (20, 31). We have previously observed that strains carrying the genes for SEIM or SEIO attained higher counts in the feces of colonized infants than strains that are negative for these genes (23). In the present study, carriage of *selm* was more common in strains that were “always persistent” at the site that they colonized than in strains that were “always transient” at the site where they appeared. However, the statistical significance of this difference was lost after correction for multiple comparisons.

Interestingly, we found that the “virulence score” of a strain, i.e., the number of different virulence genes identified in the strain, was significantly positively related to the capacity of that strain to persist at the site that it colonized, either in the gut or the nose. This supports the notions that virulence factors have evolved primarily to facilitate commensal colonization and that successful commensal colonization and pathogenicity are linked.

Although nasal colonization increases the risk for invasive infection 3-fold (6), gut carriage is also an important risk factor for *S. aureus* infections (7, 11, 12). Interestingly, patients who are colonized by *S. aureus* in both the nose and gut are more likely to contract *S. aureus* infection than patients who are colonized only in the nose (12). This accords with our observations of a strong link between the capacity of a strain to colonize both sites and its ability to establish long-term persistence, which, in turn, is linked to a high “virulence score,” i.e., the possession of not only *fnbA* and *fnbB* but also other virulence traits.

Our examined cohort is relatively small and not designed to elucidate potential connections between *S. aureus* carriage and infection. Although we do not have access to hospital records, two infants (out of 65) received treatment with isoxazolyl penicillin (one of them twice) during the first 18 months of life, indicating *S. aureus* infection. One of these infants, who received treatment with this antibiotic on one occasion between the ages of 12 and 18 months, carried one nasal-persistent and one gut-persistent *S. aureus* strain that colonized the nose/gut from 1 month of age up to 3 years of age. The other infant was not colonized with *S. aureus*.

*S. aureus* colonization may be affected by different demographic, clinical, and behavioral parameters. Indeed, we found a negative association between being breastfed
and yielding at least one sample (nasal or rectal/fecal) that was positive for *S. aureus*. This might be explained by the array of antibacterial factors, such as lysozyme and lactoferrin, present in breast milk. In a separate study based on the same birth cohort, we have uncovered a relationship between different lifestyle parameters and colonization by various gut bacteria, including *S. aureus* (A. Ljung, B. Hesselmar, H. Rabe, F. L. Nowrouzian, S. Johansen, A. E. Wold, and I. Adlerberth, unpublished data). For example, being the first-born child was found to be associated with an increased likelihood of *S. aureus* gut colonization. In the present study, we have focused on the colonization behaviors of *S. aureus* strains and noted that persistent nasal and gut strains resided at these sites for a longer period in first-born infants than in infants with elder siblings. These two observations are in agreement and suggest that *S. aureus* has greater difficulties colonizing and persisting in infants who have elder siblings. This indicates a more rapid maturation of the commensal microbiota in infants with elder siblings, leading to an ecological pressure on, for example, *S. aureus*, which may reduce the ability of strains to persist at mucosal sites.

We have previously observed that certain adhesins and other traits that are commonly regarded as virulence factors in uropathogenic *Escherichia coli* also promote the commensal persistence of *E. coli* strains in the infantile gut (41–43). *S. aureus* fibronectin-binding proteins, and probably other virulence factors, may well exert such a dual function, initially enhancing commensal nasal and gut colonization and thereafter aggravating disease when there is invasion.

**MATERIALS AND METHODS**

**Subjects.** The FARMFLORA birth cohort comprises 65 infants from rural areas of southwest Sweden. The cohort was recruited and monitored to identify factors including early microbiota colonization associated with farm living and contributing to protection against allergy development; 28 of the infants were from farming families, and 37 were from nonfarming control families (44). The study was approved by the Ethics Committee of the University of Gothenburg (Dnr. 363-05). The characteristics of the cohort are listed in Table 1.

**Sampling and isolation of *S. aureus* from infant nasal and gut microbiota.** Briefly, the nasal microbiota was sampled using a cotton-tipped swab (Copan Diagnostics Inc., CA, USA) at the following time points: 3 days; 1, 2, 4, and 8 weeks; and 4, 6, 12, 18 and 36 months of age. On the same occasions, the gut microbiota was sampled, either through the use of a rectal swab (at 3 days of age) or through the collection of fecal samples (all other occasions). The methodology used for the culturing and identification of *S. aureus* has been described previously (45). In brief, swabs were streaked onto *Staphylococcus* agar plates, while a calibrated spoonful of feces was serially diluted in sterile peptone water in 10-fold steps and the dilutions were plated onto *Staphylococcus* agar (10). After incubation aerobically at 37°C for 48 h, colonies that differed with respect to size, shape, or color were separately enumerated from plates and identified as *Staphylococcus* based on Gram staining and catalase production. *S. aureus* was identified by a positive coagulase test. The lowest level of detection was 330 CFU/g feces. The rectal swab cultures were not quantified. The *S. aureus* fecal population counts from 1 week to 18 months of age are reported elsewhere (Ljung et al., unpublished). Here, the fecal population counts for each individual *S. aureus* strain in each stool sample were determined.

**Strain typing of *S. aureus*.** Overall, 83% (54/65) of the infants yielded at least one positive *S. aureus* culture. In total, 449 *S. aureus* isolates were obtained from these infants: 186 from nasal swabs and 263 from rectal swabs/fecal samples. Random amplified polymorphic DNA (RAPD) analysis was used to identify different strains in the same individual, as described previously (9, 23). All the *S. aureus* nasal and gut isolates from an individual infant were assessed together in a single run or two consecutive runs. Isolates that yielded identical RAPD patterns were regarded as belonging to a single strain, regardless of the site of isolation, and they were labeled with the infant number and a letter (strains 1A, 1B, and 1C in infant 1, strains 2A to D in infant 2, and so forth; the RAPD patterns were not compared between infants).

Strains were designated “persistent” at one site if they appeared in several samples from that site (nose or gut) over a period of 3 weeks or longer. The length of the persistence period was calculated as the amount of time that elapsed between the first and last sample from which the strain was retrieved. “Transient” strains were defined as strains that were present at a site for <3 weeks, while strains that were retrieved only once at either 1 month or 2 months of age were also defined as transient. “Unclassified strains” were those that appeared in a single sample obtained at 4, 6, 12, 18, or 36 months of age, as they could not be unambiguously categorized as either persistent or transient due to the long interval between the sampling occasions. This classification of strains has been used in previous studies (9, 23, 46).

**S. aureus virulence genes.** A representative isolate of each strain was analyzed using multiplex PCRs (23) for the accessory gene regulatory *(agr)* to –4 locus and genes encoding the following adhesins: fibrinogen-binding protein, CIFA and CIBM, elastin binding-protein, laminin-binding protein,
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AB, a small research-driven company investigating potential preventative treatments for other associations.

The virulence gene profiles of the S. aureus strains that colonized the present cohort between 3 days and 2 months of life have previously been reported and analyzed in relation to the development of eczema in colonized infants (45).

Statistical analysis. Proportions were compared using Fisher’s exact test. The Westfall-Young permutation method was used to correct for multiple inferences (47).

The Mann-Whitney U test was used to compare the S. aureus fecal population counts or the virulence scores between different groups of strains. This test also was used to compare the time of colonization (in weeks) of persistent strains at different body sites. Spearman’s correlation was used to test for correlation between the S. aureus fecal population counts on different sampling occasions and virulence scores (SPSS software; IBM Corp., NY, USA).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1

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