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Infant colonization by *Staphylococcus aureus*: role of maternal carriage

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Abstract

Infant colonization by *Staphylococcus aureus* has not been adequately investigated. In this study we aimed to define determinants associated with carriage of *S. aureus* in early infancy. Serial nasal swabs were collected from 128 infants and their mothers at months 0, 6 and 12 postpartum. *S. aureus* isolates were characterized by pulsed-field gel electrophoresis, multilocus sequence typing, *spa* typing, and presence of chromosomal *mecA* and of Panton-Valentine leukocidin (PVL) genes. *S. aureus* was isolated in 17.7% and 15.7% swabs from infants and mothers, respectively. Carriage rates were higher in infants with carrier mothers, non-smoking mothers, and many siblings. Persistent carriage rates were higher in infants with carrier or non-smoking mothers. *S. aureus* typing revealed identical strains in 10/15 investigated infant-mother pairs. Among 19 investigated *S. aureus* isolates from infants, 10 harbored *mecA* and 2 *PVL* genes, and these determinants were concomitantly present in isolates from mothers. Resistance to methicillin was 43.6% among all isolates from infants. In conclusion, isolates from infants were commonly identical to isolates from their mothers, pointing to a principal role of maternal carriage in *S. aureus* colonization in infants.

Keywords: Carriage; Colonization; Infant; MRSA; *Staphylococcus aureus*; Virulence

Introduction

Staphylococcus aureus is a major pathogen in both community-acquired and nosocomial infections with alarming rates of resistance to antibiotics [1]. *S. aureus* can colonize several human epithelial surfaces, of which the anterior nares represent the dominant ecological niche [1-3]. There seems to exist a link between *S. aureus* nasal carriage and infection, since nasal and infection strains commonly share the same genotype, rates of infection are higher in carriers than in non-carriers, and eradication of carriage has been shown to reduce nosocomial infection [1, 2, 4]. About 30% of the population carries *S. aureus* at any one time; this includes 20% who are persistent and 10% who are transient carriers [1, 3].

Children are a major source of transmission of many infections. Carriage of *S. aureus* in childhood is often reported to be higher than in adulthood [2, 3, 5-12]. Given the morbidity of staphylococcal infections, a better understanding of the timing and the source of acquisition in infancy is required. Maternal carriage may reasonably affect infant colonization, but this hypothesis has been little tested [2]. The objective of the present study was to explore determinants associated with intermittent and persistent carriage of *S. aureus* in infants and the molecular epidemiology of *S. aureus* strains isolated from infants and their mothers in a well defined area, i.e., the island of Crete, Greece.

Patients and methods

Study design. *S. aureus* nasal colonization was investigated in a cohort of 128 consecutively recruited newborn infants and their mothers at Venizeleion General Hospital in Heraklion, Crete, Greece between November 2005 and May 2006. Mothers were recruited through the Maternity Ward registry and contacted by the

same investigator before newborn discharge either from the Maternity or the Neonatology Ward. Nasal swabs were obtained from mothers and infants before discharge and after 6 and 12 months. A series of determinants related to infant, mother, father and family were investigated including infant's gender, gestational age, delivery mode, birth weight, admission to neonatology ward, feeding mode; parental ethnicity, age, education, work, and smoking habits; and family size (Table 1). Information was obtained from hospital registries and mother-retrieved questionnaires. Ethical approval for the study was obtained from the Institutional Committee of the Faculty of Medicine, University of Crete, Greece.

Nasal swabs, culture, identification, and antimicrobial susceptibility testing.

Nasal samples were obtained with a sterile fiber-tipped swab from both anterior nares of each enrolled individual. The swabs were immediately placed in Amies transport medium (BioMerieux, Marcy L'Etoile, France) and kept at 4° C until processed. Infants on antibiotics in the preceding 2 days were excluded from swabbing. The sample was inoculated onto mannitol salt agar and Columbia agar with 5% sheep blood. The culture plates were incubated at 36° C for 48 hours. *S. aureus* was identified on the basis of colony morphology, Gram stain, catalase and coagulase test, and the API 20 Staph system (BioMerieux). Antimicrobial susceptibility tests against oxacillin, erythromycin and clindamycin were performed by employing the disk diffusion method and the results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) criteria. For determination of methicillin resistance, oxacillin was the preferred class compound for testing since it is more resistant to degradation in storage, and because it is more likely to detect heteroresistant strains. In addition, the presence of *mecA* gene was investigated by specific PCR as described below. Three patterns of carriage were defined, i.e.,

persistent noncarriers in case all 3 swabs were negative, intermittent carriers in case 1 swab was positive, and persistent carriers in case 2 or 3 swabs were positive [3, 4].

Pulsed-field gel electrophoresis (PFGE). *S. aureus* isolates were genotyped using the restriction enzyme *Sma*I [2, 3, 13, 14]. Bacteria were grown overnight in 5 ml of brain heart infusion broth, and 0.2 ml of broth culture (optical density, 0.55-0.7 at 610 nm) was harvested by centrifugation (7,000 rpm for 2 min). Cells were washed once in 0.4 ml of sterile TEN buffer (0.1 M Tris Cl, 0.15 M NaCl, 0.1 M EDTA), centrifuged, resuspended in 0.2 ml of sterile EC buffer (6 mM TrisCl, 1 M NaCl, 0.1 M EDTA, 0.5% Brij 58, 0.2% deoxycholate, 0.5% Sarkosyl), and embedded in 0.6 ml of 0.9% agarose (pulsed field certified agarose, Biorad) containing 4 µg of lysostaphin (Sigma-Aldrich). The suspension was gently mixed, pipetted into a plug mold and allowed to solidify for about 10 min before being placed in a tube containing 3 ml of EC buffer. Cell lysis was achieved at 37°C for 4h and the plug was washed 4 times in 3 ml of TE buffer (10 mM TrisCl, 0.5 mM EDTA) for 1h at 55°C and transferred to 5 ml of fresh TE buffer for storage at 4°C. For the electrophoresis, the plug was cut into small slices of 2 by 5 mm. Each slice was equilibrated in 150 µl restriction buffer for 30 min at room temperature, and then placed in 150 µl of restriction enzyme mixture containing 50 U of *Sma*I (Takara). After a 4-hour incubation at 30°C, the plug was mounted on 1% Agarose Gel (Biorad) and electrophoresis proceeded in a CHEF-DRIII apparatus (Bio-Rad Laboratories, Mississauga, Ontario, Canada) at 0.5x Tris-borate-EDTA running buffer. Running parameters consisted of pulse times from 5 to 40 s, at 6 V/cm for 20 hours at 14°C.

Multi locus sequence typing (ST). Chromosomal bacterial DNA was extracted by GFX genomic blood DNA purification kit (Amersham Bioscience). Sequencing was performed using the Thermo Sequenase Primer Cycle sequencing kit (GE

Healthcare). The primers were used as indicated in the <http://www.mlst.net> site except that they were extended at the 5' end with the sequencing primers T7–TATAATACGACTCACTATAGGG and M13 reverse–TACAGGAAACAGCTATGACC. Sequencing primers were labelled with the 700IRD and 800IRD fluorescent labels and fragment sequences were resolved in a 60 cm long 6% RapidGel-XL (USB Cleveland USA) gel on a LI-COR 4200 dual band automated sequencer. Sequences were submitted for the ST assignments to the <http://www.mlst.net> site.

Detection of *mecA*. Presence of *mecA* was determined by using the PCR primers Sa5b–GCGATAATGGTGAAGTAG and Sa6b–TCTTGTAACGTTGTAACCAC to amplify a 1,171-bp internal fragment of the gene. PCR was performed with PureTaq ready-to-go beads (GE Healthcare). A denaturation step of 5 min at 94°C was followed by 30 cycles of 25 sec at 94°C, 25 sec at 55°C and 45 sec at 72°C. The PCR program ended by an elongation step of 7 min at 72°C. PCR products were separated in 1% agarose gel and visualised in UV light after staining with Gel star (Cambrex Rockland USA).

Detection of Pantón-Valentine leukocidine. Presence of *lukS-PV* and *lukF-PV* genes encoding PVL components were determined by primers ATCATTA–GGTAAAATGTCTGGACATGATCCA–3' (forward), and GCATCAAGTGTATTGGA–TAGCAAAAGC (reverse) [12, 15]. PCR conditions and product visualization were as described for the *mecA* gene.

Ribotyping and *spa* typing. Isolates were genotyped by automated ribotyping on the Riboprinter (Qualicon Inc., Wilmington, DE) using *EcoRI*. Analysis was performed using the Qualicon software. Polymorphic X-region of the protein A gene (*spa*) was amplified and sequenced according to the Ridom StaphType protocol

(www.ridom.org). PCR was performed with Platinum pfx Taq DNA Polymerase (Invitrogen) in 50 µl of reaction mixture containing 2.5 mM MgSO₄, 80 nM of each of the primers, 200 µM dNTPs and 1x buffer. PCR parameters were: denaturation of 10 min at 95°C 30 cycles of 30 sec at 95°C, 30 sec at 60°C and 45 sec at 72°C and 1 cycle of 10 min at 72°C. As described for ST, primers were extended with T7 and M13 reverse and sequencing was performed as with ST except that sequences were resolved on a 45 cm long gel. The resulting *spa*-types were assigned by using the Ridom StaphType software (Ridom GmbH, Würzburg, Germany). Cluster analysis of *spa* types was based on the BURP algorithm as implemented in the Ridom StaphType software [16].

Statistical analysis. Only infant-mother pairs successfully followed-up for 12 months were included in the analysis of carriage. Nominal variables were analyzed by two-tailed Fisher's exact test, and odds ratios with 95% confidence intervals were calculated. Continuous variables were compared by the two-tailed t-test and the Pearson correlation coefficient. A *P* value of <0.05 was defined as significant. A multivariate regression analysis was performed, including variables with *P* <0.10 in the univariate analysis.

Results

Population. The study population consisted of 128 infants paired with 124 mothers (there were 4 sets of twins). Mothers who declined to participate were not included in the study. From the 128 enrolled mother-infant pairs, 82 (64.2%) completed the 3 points of follow-up. Of the 128 infants, 98 (76.6%) were recruited from the Maternity Ward and 30 (23.4%) from the Neonatology Ward. Infants from the Neonatology Ward were of less gestational age (36.1 vs. 38.6 weeks, *P*<0.0001) and birth weight

(2.546 vs. 3.332 kg; $P < 0.0001$), and were delivered more often by caesarean section (70% vs. 30.6%; OR 5.29; 95% CI 2.17-12.9; $P < 0.001$).

***S. aureus* carriage all through the first year of life.** A total of 615 nasal swabs were obtained from 310 infant-mother pairs, including 10 cases of sampling in twins. Positive for *S. aureus* were 55 (17.7%) samples from infants and 47 (15.4%) samples from mothers. Carriage rates by *S. aureus* in infants and mothers were positively related (Pearson r 0.261; P 0.003). Pairs of infant-mother carriers were found in 15 occasions (4.8%), almost twice the rate expected by chance (8.3 pairs, 2.7%). Of the infants with *S. aureus* carrier-mothers, 15/47 (31.9%) were *S. aureus* carriers themselves, while only 40/263 (15.2%) of infants to noncarrier-mothers were carriers (OR 2.61, 95% CI 1.30-5.26; P 0.008). The single documented case of staphylococcal infection during follow-up, i.e., a case of osteomyelitis, was not related to previously observed *S. aureus* nasal carriage.

***S. aureus* carriage at months 0, 6, and 12.** In the initial swabbing at the first week of life, carriage of *S. aureus* was found in 20/128 infants (15.6%) and 13/124 (10.5%) mothers. Swabbing at month 6 was achieved for 100/128 (78.1%) infant-mother pairs (3 sets of twins) and revealed carriage in 22 (22%) infants and 23 (23.7%) mothers. Swabbing at month 12 was achieved for 82/128 (64.1%) infant-mother pairs (3 sets of twins) and revealed carriage in 13 (15.9%) infants and 11 (13.9%) mothers. Relationship between infant colonization and maternal colonization, maternal smoking and number of siblings in the family is depicted in Table 2. No significant relationships were observed for the remaining variables of Table 1 in all 3 samplings.

***S. aureus* carriage through the first year of life.** Of the 82 infants who had all 3 swabs, 47 (57.3%) were persistent *S. aureus* noncarriers, 23 (28%) infants were positive once, and 12 (14.6%) were positive at least twice; of the latter, one infant

(1.2%) was positive in all 3 samplings. Of the 79 mothers who had all 3 swabs, 53 (67.1%) were *S. aureus* noncarriers, 15 (19%) were positive once and 11 (13.9%) were positive at least twice; of the latter, four mothers (5.1%) were positive in all 3 samplings.

In the group of 82 infant-mother pairs with 12-month follow-up, carriage rates in infants at any sampling were positively related to maternal carriage rates (Pearson $r = 0.246$; $P 0.026$) and to non-smoking mothers (53.8% vs. 23.3%; OR 3.83, 95% CI 1.40 to 10.5; $P 0.010$). Persistent carriage rates were further higher in infants born to non-smoking mothers (29.4% vs. 8.0%; OR 4.79, 95% CI 0.95-24.3; $P 0.054$), and having more siblings (1.75 vs. 1.01, $P 0.044$) than infants with no carriage. Logistic regression analysis confirmed the effect of maternal non-smoking and maternal *S. aureus* carriage but not of the number of siblings on *S. aureus* persistent carriage in infants ($P 0.028$, $P 0.023$, and $P 0.077$, respectively).

Concomitant carriage in infant and mother. Of the 82 infants who had all 3 swabs, 35 presented with at least one positive swab and a total of 48 positive swabs. In 15 of these 35 infants (42.9%), mother was found concomitantly colonized at least once. There was a substantial degree of concordance for *S. aureus* carriage between mothers and infants with 15/48 concordant pairs (31.2%). In only 5 cases was the infant a noncarrier despite the mother being a persistent carrier. Isolates were further typed in the 15 infant-mother pairs and were found to be identical at the same time point in 8 pairs (53.3%, Figure 1); in 2 further pairs the same isolate was observed in infant and mother though in different sampling time. All identical *S. aureus* strains were found in infant-mother pairs without history of admission to Neonatal Ward, and almost all identical pairs (7/8) were observed in the 6-month sampling. Both infants with persistent carriage of the same strain, including the one with 3 positive samples, had

mothers colonized by an identical strain (methicillin-resistant PFGE 2b, ST 30; and methicillin-resistant, PFGE 2e, ST 30, respectively).

Genetic Determinants. Genetic determinants were investigated in 41 *S. aureus* isolates, i.e., in all 15 mother-infant pairs with simultaneous isolation, and in all cases *S. aureus* was isolated in the mother-infant pair not spontaneously but at a different sampling. The genetic relatedness of the isolates is shown in Figure 2. Isolates from infants principally belonged to ST types 30 (8/19) and to PFGE type 2 (6/19). Furthermore, 10/19 isolates harbored *mecA* gene and 2/19 isolates *PVL* gene. Both *PVL* positive and 8/10 *mecA* isolates from infants were paired with isolates with the same determinants in mothers. All four *PVL* positive isolates were *mecA* positive and belonged to ST type 80.

Resistance. Resistance rates for methicillin, erythromycin and clindamycin were 43.6%, 5.4%, and 3.6% respectively in isolates from infants and 42.5%, 8.5%, and 0% in isolates from mothers. No significant difference was observed in resistance between isolates from infants and mothers. The majority of erythromycin resistant isolates (4/6) were methicillin resistant as well. Resistance to methicillin was more frequent in isolates from Neonatology Ward than Maternity Ward infants (72.7% vs. 36.4%; OR 4.67, 95% CI 1.08-20.1; *P* 0.042).

Discussion

Our findings, derived from a prospective follow-up of infant-mother pairs from birth through the first year of life, suggest that strains carried by young infants are commonly identical to their mothers' strains. Therefore, *S. aureus* transmission in infancy is from parent to child, in contrast to other pathogens where transmission is from child to parents. Regarding the issue of whether infants are colonized through

vertical transmission or close contact [17, 18], our findings favor the close contact scenario, given that very few infant-mother pairs were colonized in the first days of life, the vast majority of identical infant-mother isolates were observed in month 6 of life, children admitted to Neonatology Ward developed a different carriage profile, and as similar carriage rates were found in infants after vaginal delivery or caesarean section. Colonization rates at months 6 and 12 were close to those already reported [2, 3, 10]. In our study infants were less colonized at month 0 than elsewhere [2, 3, 6, 8, 9], possibly because they were swabbed earlier in their life. Peak infant colonization seems thus to occur early in life but not very close to birth. The decline in carriage later in infancy may reflect the development of immune response, less contact to mother or other caring individuals, or colonization competition between *S. aureus* and other bacteria such as *S. pneumoniae* [2, 3, 5, 6, 8].

Persistent carriage rates were similar to those reported previously [3]. Although adults may carry the same strain for years [1], only a few infants were found in our study to carry the same strain in repeated samplings. This finding has been attributed to different pathogen-and-host match in *S. aureus* carriage in different ages [3]. Interestingly, both infants with persistent carriage of the same strain had mothers carrying identical strains. This finding has not been reported previously and points to the role of maternal colonization in the persistent carriage in the infant.

Determinants that have been suggested to influence carriage of *S. aureus* in children include gender, age, history of antibiotics or hospitalization, number of siblings and family size, day care center attendance, passive smoking and breastfeeding, education of mother, birth weight and gestational age; however results have often been inconsistent [2, 3, 5, 7, 8, 11]. We found that maternal non-smoking, maternal colonization, and many siblings were related to higher rates of *S. aureus*

colonization in the infant, though at different time points in infancy. In our study *S. aureus* carriage, both intermittent and persistent, was less common in infants of smoking mothers. This interesting finding is in accordance to previous studies reporting decreased rates of *S. aureus* carriage in smokers themselves [19, 20]. The association between smoking and *S. aureus* carriage is not well understood yet. It has been suggested that active smoking protects against colonization, but passive smoking is associated with increased colonization rates and with carriage of a specific *S. aureus* cluster [5, 21]. Infants born to smoking mothers are both passive smokers and at risk to be colonized by strains of their smoking mothers. Therefore, our finding of less *S. aureus* carriage among infants of smoking mothers needs careful explanation and further investigation. Presence of siblings has been related to *S. aureus* carriage [2, 3, 5], but in our study siblings were found to predispose infants for persistent carriage in late rather than in early infancy.

Concordance in carriage in mother-infant pairs in this study points to both common environment and host genetics. Identical typing patterns and resistance profiles have been reported to be common in isolates from healthy children and children with atopic dermatitis and cystic fibrosis and their household contacts [6, 8-10, 13, 14, 22]. *PVL* and *mecA* positive isolates from infants were simultaneously paired with isolates with the same genetic determinants in mothers. Infants having been admitted to NICU were not colonized by maternal strains suggesting that hospitalization in early life crucially influences carriage.

Presence of virulent determinants was not rare in our study, although the majority of isolates were obviously community acquired. Interestingly, isolates commonly belonged to the MLS type 30 and not to the European type ST80 [15];

however all four PVL positive strains did belong to ST 80. Methicillin-resistance rates were alarming. High resistance rates are well known for *S. aureus* in Greece [15, 23].

Our study was designed to cover an extended period and this prolonged period of follow-up allowed for estimation of persistent carriage but not for defining whether common host genetics or close contact are the cause of concomitant carriage between mother and infant. We did not culture other body sites and we only focused on mothers; investigation of carriage in other family members would further elucidate colonization by *S. aureus* in infancy. Similarly, we did not culture potential sources of hospital transmission in the group of infants admitted to the Neonatology Ward.

In conclusion, our findings suggest that environmental determinants, principally maternal colonization, presence of siblings and early hospitalization significantly influence carriage and determine virulence and resistance of *S. aureus* strains in infancy. Our data may have considerable implications for strategies of infection control, treatment and eradication of *S. aureus*. In an era of increasing resistant staphylococcal infections in the community, better understanding of carriage in infants is of great importance

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Potential conflicts of interest. All authors: no conflicts.

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Table 1 Baseline demographic and clinical characteristics in 128 infants investigated for *S. aureus* nasal colonization

Variable	Findings
Infant	
Gender (male; female)	63; 65
Gestational age in weeks (mean; range)	38; 30-40
Delivery mode (vaginal; caesarean)	77; 51
Birth weight in kg (mean; range)	3.15; 1.04-4.5
Admission to Neonatology Ward (yes; no)	30; 98
Breastfeeding (yes, any; no)	69; 32
Breastfeeding duration in months (mean; range)	3; 0-12
Mother	
Ethnicity (indigenous; immigrants)	105; 23
Age in years (mean; range)	28; 19-42
Education in years (mean; range)	11; 0-18
Antibiotics during pregnancy/labor (yes; no)	32; 96
Employment during pregnancy (yes; no)	56; 72
Tobacco use (smoking; non smoking)	37; 70
Father	
Age in years (mean; range)	32; 21-44
Education in years (mean; range)	10; 0-18
Tobacco use (smoking; non smoking)	61; 42
Family	
Family size, total members (mean; range)	4; 3-8

Table 2 Association between *S. aureus* colonization in infants and maternal colonization, maternal smoking, and number of siblings in the family

Colonized infants	Colonized mother		Smoking mother		Number of siblings	
	yes	no	yes	no	colonized infant	not colonized infant
Week 1 of life	23.1% ^{ns}	14.8%	5.41% ^a	24.3%	1.33 ^{ns}	0.94
Month 6 of life	39.1% ^b	16.9%	16.2% ^{ns}	25.4%	1.01 ^{ns}	0.95
Month 12 of life	27.3% ^{ns}	14.1%	21.2% ^{ns}	6.67%	1.62 ^c	0.94

^a *S. aureus* colonization more frequent in infants with non-smoking mothers (OR 4.37, 95% CI 0.94 to 20.4; *P* 0.049).

^b *S. aureus* colonization more frequent in infants with colonized mothers (OR 3.02; 95% CI 1.08 to 8.44; *P* 0.045).

^c *S. aureus* colonization more frequent in infants with many siblings (*P* 0.028).

^{ns} not significant difference.

Figure 1 PFGE patterns of 8 matched (pairs 1-7) and of 6 non-matched (pairs 8-13) *S aureus* nasal isolates from mother (left) and infant (right).

Figure 2 Dendrogram indicating genetic relatedness of *S. aureus* isolates as deduced by the sequences of the 7 allelic gene fragments used for the ST. Isolates were distributed in 12 strains. Of the 41 isolates, 23 were clustered in the first group (including the common ST 30 strain B and the ST 45 strain C). From the 18 remaining isolates, 12 clustered in subgroup 2 (strains A, D, K, E) and 6 in subgroup 3 (strains G, H, I, J, L)