

Neonatal colonization with *Staphylococcus aureus* is not associated with development of atopic dermatitis

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Summary

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Conflicts of interest

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Background *Staphylococcus aureus* in atopic skin has been associated with exacerbation of eczema.

Objectives To investigate a possible association between neonatal colonization with *S. aureus* and the risk of atopic dermatitis (AD) during the first 3 years of life.

Materials and methods The study participants were 356 children born of mothers with asthma from the Copenhagen Prospective Study on Asthma in Childhood. Swabs from the vestibulum nasi and the perineum were cultured at 1 month and 1 year, from acute eczema, and from parents (vestibulum nasi and pharynx). AD development and severity were monitored prospectively.

Results Of the neonates, 5.3% had positive swabs for *S. aureus* cultured from the vestibulum nasi (51.3%) and/or the perineum (11.3%). Forty-two per cent developed AD, but without association between colonization with *S. aureus* at 1 month of age and risk of developing AD at 3 years of age. There was a 70% concordance for *S. aureus* carriage between neonates and parents. At 1 year of age 11.3% children had swabs positive for *S. aureus*. Fourteen per cent of children tested at the 1-year visit developed AD after the visit but before 3 years of age, but again, there was no association between colonization with *S. aureus* and the risk of AD. In children seen at acute visits the severity of AD measured by scoring of atopic dermatitis (SCORAD) was significantly higher in children with a positive culture for *S. aureus* in lesions.

Conclusions Colonization with *S. aureus* at 1 month of age is not associated with an increased risk of developing AD during the first 3 years of life.

Staphylococcus aureus is known as an aggravating factor for atopic dermatitis (AD). In patients with AD up to 80–100% of acute lesions are colonized with *S. aureus*^{1,2} and the presence of *S. aureus* is often associated with an exacerbation of the eczema. A correlation between the severity of the eczema and the colonization with *S. aureus* has been reported^{3–5} and studies have suggested a beneficial effect of antibiotics, especially in patients with moderate to severe eczema.^{6,7} *Staphylococcus aureus* is capable of releasing several proinflammatory exotoxins which may induce the eczema of which the superantigens, especially staphylococcal enterotoxin B,⁸ are the most studied. The superantigens strongly activate T cells, stimulate production of IgE and directly activate dendritic cells and keratinocytes in the skin leading to cytokine release,^{9,10} including the

pruritic cytokine interleukin (IL)-31¹¹ and inhibition of regulatory T cells.¹² This may lead to activation of eczema.

However, no studies have looked at colonization with *S. aureus* in patients before the onset of AD. In children at risk of AD, *S. aureus* and the released exotoxins may more easily penetrate the skin due to a barrier defect. Loss-of-function variants in the filaggrin gene¹³ were recently identified as an example of such a skin barrier defect which was identified in up to 50% of patients with AD.

The primary aim of this prospective longitudinal birth cohort study was to determine if colonization with *S. aureus* in neonates increases the risk of developing AD during the first 3 years of life. The secondary aims were to study the association between colonization with *S. aureus* in neonates and

parents, colonization during acute skin problems and the role of superantigen specific IgE.

Materials and methods

The Copenhagen Prospective Study on Asthma in Childhood (COPSAC) is a prospective longitudinal birth cohort study of children born by mothers with a history of asthma. The cohort has been described in detail previously.¹⁴ In brief, mothers with a history of asthma were invited during pregnancy, and the neonates were enrolled during the first month of age. Neonates born before 36 gestational weeks or with known severe congenital diseases were excluded. The main catchment area of the cohort was greater Copenhagen, Denmark. The children were born between August 1998 and December 2001, and 397 were white. All mothers had a history of asthma and 48% had a history of AD. Sixteen per cent of the fathers had a history of asthma and 13% a history of AD. The study followed the guiding principles of the Declaration of Helsinki, and was approved by the Ethics Committee for Copenhagen (KF 02-118/98) and the Danish Data Protection Agency (2008-41-1754) and written informed consent was obtained from the parents.

The participants were seen in the COPSAC Clinical Research Unit (CRU) at age 1 month and at scheduled visits every 6 months thereafter as well as for any acute onset of exacerbation of symptoms from skin or airways.

Sampling methods, bacterial isolation and identification

Swabs were taken from the vestibulum nasi and perineum at scheduled visits at 1 month and 1 year of age. Swabs were obtained from involved skin at acute visits due to eczema-like skin problems. Swabs were also taken from the vestibulum nasi and pharynx from the parents who attended the 1-month visit.

Cultures were taken with charcoal cotton swabs and referred to the clinical microbiology laboratory in Stuart transport media. During weekends and at night the cultures were stored in Stuart transport media in a refrigerator at 5 °C for a maximum of 48 h. In the laboratory the swabs were handled immediately according to standard procedures for the laboratory. In brief, the swabs were inoculated on blood-agar plates (Oxoid, Basingstoke, U.K.) and a plate for antibiotic susceptibility testing (Oxoid). The plates were incubated for 18–24 h at 35 °C. To identify *S. aureus*, *Staphylococcus*-like colonies were tested with Staphaurix[®] (Remel, Dartford, Kent, U.K.) for the presence of clumping factor and/or protein A. If the result was doubtful, an additional test – coagulase production with a test tube containing horse plasma – was done. Colonies positive for *S. aureus* were tested for antibiotic susceptibility by means of agar-diffusion tests with the following antibiotic disks (Oxoid): penicillin G (10 i.u.); oxacillin (1 µg); gentamicin (30 µg); erythromycin (15 µg); clindamycin (15 µg); fusidic acid (10 µg); rifampicin (5 µg); vancomycin (5 µg); tetracycline (30 µg); and chloramphenicol

(30 µg). *Streptococcus*-like colonies with β-haemolysis were grouped with antisera against group A (*S. pyogenes*), B (*S. agalactiae*), C and G.

All isolates were stored at –80 °C. *Staphylococcus aureus* isolates were later referred to the Statens Serum Institute, Copenhagen. Phage typing was performed according to Blair and Williams¹⁵ using the international set of phages. The subdivision into phage groups I–III was performed according to Parker¹⁶ and, additionally, strains of phagetype 95, 94/96 complex, 80 complex and 83A complex, respectively, were recorded separately.

IgE to staphylococcal enterotoxin

Serum samples were collected at the 18-month visit and were assayed for IgE to staphylococcal enterotoxins [staphylococcal enterotoxin A (SEA), staphylococcal enterotoxin B (SEB) and toxic shock syndrome toxin-1 (TSST-1)]. Specific IgE was determined by a commercial assay for IgE (ImmunoCAP Phadiatop Infant, Phadia, Pharmacia Diagnostic AB, Uppsala, Sweden). Values of Phadiatop Infant of 0.35 kU L⁻¹ or more were considered indicative of sensitization.¹⁷

Objective clinical examination

The neonates were given a standardized physical examination at every visit and history was obtained by the doctors at the CRU using structured questions and closed response categories including skin manifestations and medication use.

Skin lesions with typical dermatitis morphological features were described at each visit online according to predefined morphological categories and localization. AD was defined based on the criteria of Hanifin and Rajka.^{14,18} The severity of AD was scored using the scoring of atopic dermatitis (SCORAD) index, ranging from 0 to 103 points,¹⁹ which includes the assessment of (i) extent; (ii) intensity (erythema, oedema/papules, oozing/crust, excoriation, lichenification, and dryness on a scale from 0 to 3); and (iii) subjective symptoms [pruritus and sleeplessness (using a scale from 0 to 10 of the parents' subjective opinion of the child's degree of pruritus and sleeplessness)].

Statistical analysis

The χ^2 test was used to compare colonization and the risk of AD. McNemar's test was used for analyses of association between colonization in neonates and parents. A nonpaired Student's t-test was used to compare SCORAD in children with and without *S. aureus*. $P < 0.05$ was considered significant.

Results

The COPSAC study enrolled 411 neonates at age 1 month, 356 of whom provided complete follow-up data by age 3 years. Only the 356 children who completed the 3-year follow-up were included in the current analyses.

Colonization with *Staphylococcus aureus* at 1 month of age and the risk of atopic dermatitis during the first 3 years of life

Swabs were cultured from 302 neonates at their first visit. The median age of the neonates at the first visit was 4 weeks (range 2–11 weeks). Of these neonates, 161 (53.3%) had at least one swab culture that tested positive for *S. aureus*, mainly from the vestibulum nasi (51.3%), but 11.3% of the swabs from the perineum were also positive. Only six neonates carried *S. aureus* in the perineum and not in vestibulum nasi. Sixty-six (22%) of the neonates were delivered by caesarian section. However, there was no difference in the number of neonates colonized by *S. aureus* between neonates delivered naturally and by caesarian section.

One hundred and twenty-seven (42.1%) infants developed AD before the age of 3 years. However, no association between colonization with *S. aureus* at 1 month of age and risk of developing AD at the age of 3 years was found (Table 1).

Ten of the 302 neonates tested at the 1-month visit had at least one swab positive for β -haemolytic streptococci. One neonate had a positive test for *Streptococcus pyogenes* (vestibulum nasi), eight had a positive test for *Streptococcus agalactiae* (three vestibulum nasi, three perineum and two both places) and one positive for β -haemolytic *Streptococcus* group G (vestibulum nasi). Six of the children colonized with β -haemolytic streptococci at 1 month of age developed AD before age 3 years. Taken together a low number of children were colonized with β -haemolytic streptococci at scheduled visits at 1 month of age and there was no association of colonization with β -haemolytic streptococci and a risk of AD at the age of 3 years.

Colonization with *Staphylococcus aureus* at 1 year of age

Swabs were cultured from 336 of the children seen at the 1-year visit. The median age of the children at this visit was 53 weeks (range 47–64 weeks). In three children, swabs were taken only from the vestibulum nasi. In total 38 (11.3%) of

Table 1 Colonization with *Staphylococcus aureus* at 1 month of age and the association of atopic dermatitis (AD) at age 3 years

Localization	Number	<i>S. aureus</i>		P-value
		Negative (%)	Positive (%)	
All children	302			
AD	127	56 (18.5)	71 (23.5)	0.44
Not AD	175	85 (28.1)	90 (29.8)	
Nasal swabs	302			
AD	127	57 (18.9)	70 (23.2)	0.26
Not AD	175	90 (29.8)	85 (28.1)	
Perineum swabs	301			
AD	126	114 (37.9)	12 (4.0)	0.41
Not AD	175	153 (50.8)	22 (7.3)	

Table 2 Colonization with *Staphylococcus aureus* at 1 year of age

Localization	Number	<i>S. aureus</i>	
		Negative (%)	Positive (%)
All children	336		
AD			
AD at 1 year	99	85 (25.3)	14 (4.2)
AD > 1 year	46	43 (12.5)	4 (1.2)
Not AD	191	171 (50.9)	20 (6.0)
Nasal swabs	336		
AD	145	129 (38.4)	16 (4.8)
Not AD	191	173 (51.5)	18 (5.4)
Perineum swabs	333		
AD	144	141 (42.3)	3 (0.9)
Not AD	189	187 (56.2)	2 (0.6)

AD, atopic dermatitis.

the children tested positive for *S. aureus* in the swabs from the vestibulum nasi (34 children) or perineum (five children) (Table 2). At the 1-year visit, 99 of the 336 (29.5%) included children who had already developed AD and 14 of these children were colonized with *S. aureus* in the vestibulum nasi or perineum.

Forty-six of 336 children (13.7%) tested at the 1-year visit developed AD after the visit but before 3 years of age; again, there was no association between colonization with *S. aureus* at 1 year of age and the risk of developing AD at 3 years of age ($P = 0.72$).

Among the 38 children with a positive swab for *S. aureus* at the 1-year visit, 28 children had a swab test taken at 1 month of age, and 22 of these had a positive test both at the 1-month and 1-year visit. However, only eight of these children developed AD during the first 3 years of life.

Eight of the 336 children tested at the 1-year visit had at least one swab positive for *Streptococcus*. Four children had a positive test for *Streptococcus pyogenes* (vestibulum nasi), three had a positive test for *Streptococcus agalactiae* (perineum) and one was positive for β -haemolytic *Streptococcus* group G (vestibulum nasi). Three of the children colonized with *Streptococcus* at 1 year of age developed AD before the age of 3 years.

Acute visits due to eczema-like skin problems and colonization with staphylococci and streptococci

In the study period 223 children were seen with acute eczema-like skin problems. Each child had a swab taken from one to seven times during the study period. Swabs were taken from one to three of the most severely affected eczema-like lesions at each visit and in total 594 swabs were taken; 134 of the 594 swabs tested positive for either *S. aureus* or streptococci (Table 3). Among the 223 children seen with acute eczema-like skin problems 135 (60.5%) developed AD during the first 3 years of life, whereas 88 (39.5%) did not fulfil the AD diagnosis. Of the culture-positive swabs, 106 were from

Table 3 Positive test for *Staphylococcus aureus* and *Streptococcus* at acute visits due to skin problems and the risk of atopic dermatitis (AD) at age 3

Swabs	Total (n = 594)	Not AD (n = 128)	AD (n = 466)
Positive total	134	19	115
<i>S. aureus</i>	123	17	106
<i>Streptococcus</i>			
<i>S. pyogenes</i>	6	1	5
<i>S. agalactiae</i>	4	1	3
Group C	1	0	1

the 135 children who developed AD and there was a significant increase in the number of children with *S. aureus* among children who developed AD during the first 3 years of life ($P = 0.02$).

Nearly half of the swabs taken (258) at the acute visits were from children who already had a diagnosis of AD and AD severity was measured using SCORAD. Colonization with *S. aureus* was demonstrated in 55 (21.3%) children with AD at the acute visits. Severity of AD measured by SCORAD in children with a positive swab for *S. aureus* was significantly higher than in children with a negative swab [positive swab: $n = 55$, mean $\pm$ SD 31.0 ± 12.7 ; negative swab: $n = 203$ mean $\pm$ SD 22.8 ± 11.1 , mean difference -8.25 (95% CI -12.0 to -4.5)].

At the acute visits 11 swabs were cultured positive for β -haemolytic streptococci, mainly *S. pyogenes* (Table 3). Only one child with an AD diagnosis at the time of the acute visit was positive for *S. pyogenes*.

Phage groups and drug resistance among *Staphylococcus aureus*

The main part of the isolated *S. aureus* was phage typed and the phage groups from the children are shown in Table 4. The distribution of phage groups was comparable with results

from the Danish population in general (Table 4). There was no association between phage groups and the development of AD during the first 3 years of life.

In accordance with the very low prevalence of methicillin-resistant *S. aureus* (MRSA) in Denmark, MRSA was not found in the study. In swabs taken at 1 month and 1 year of age few isolates were resistant to erythromycin and fusidic acid (see Table 5). Twenty *S. aureus* isolates found at acute visits were resistant to fusidic acid. The 20 swabs were taken from eight children of whom six had AD. Five of the eight children with *S. aureus* resistant to fusidic acid had been treated with topical fusidic acid. No children received systemic fusidic acid.

The results demonstrate that the *S. aureus* phage group distribution found was in line with the normal distribution in Denmark. Among the *S. aureus* isolated there was no methicillin resistance and only a few *S. aureus* cultures were resistant to erythromycin or fusidic acid.

Specific IgE to staphylococcal enterotoxins and development of atopic dermatitis

Serum samples collected at 18 months of age were as follows: SEA ($n = 331$), SEB ($n = 328$) and TSST-1 ($n = 330$) and only a few were positive (SEA = 2, SEB = 5 and TSST-1 = 11). Seven of the positive samples were from children with AD at the age of 3 years. The number of positive tests was not increased among children who develop AD before the age of 3 years.

Colonization with *Staphylococcus aureus* in the parents

Swabs were cultured from 300 of the mothers and 260 of the fathers at the 1-month visit; 131 (43.7%) of the mothers and 126 (48.5%) of the fathers had a swab positive for *S. aureus*. In total, 139 (86.3%) of the 161 neonates with a positive test had at least one parent with a positive swab, and in 44 (27.3%) of the neonates both parents had a positive swab for *S. aureus* (Fig. 1). The concordance for *S. aureus* carriage

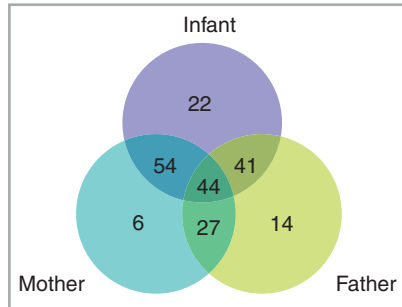
Table 4 Phage group distribution among swabs positive for *Staphylococcus aureus*

Phage group	1 month		1 year		Acute visits		Denmark, 1999 (17 174 isolates) ^a
	Not AD (%)	AD at 3-year (%)	Not AD (%)	AD at 3-year (%)	Not AD (%)	AD at 3-year (%)	
I	16 (19.0)	13 (18.6)	2 (11.1)	1 (6.3)	1 (5.6)	10 (8.9)	14.8
II	13 (15.5)	10 (14.3)	5 (27.8)	2 (12.5)	8 (47.1)	20 (17.9)	22.5
III	6 (7.1)	5 (7.1)	2 (11.1)	2 (12.5)	1 (5.9)	11 (9.8)	12.0
80-complex	1 (1.2)	1 (1.4)	1 (5.6)	0 (0)	1 (5.9)	5 (4.7)	1.8
83A-complex	4 (4.8)	4 (5.7)	3 (16.7)	1 (6.3)	2 (11.8)	4 (3.8)	4.5
94/96	3 (3.6)	3 (4.3)	0 (0)	1 (6.3)	0 (0)	8 (7.1)	5.6
95	18 (21.4)	19 (27.4)	4 (22.2)	4 (25.0)	1 (5.9)	27 (24.1)	17.1
NI (mixed group)	13 (15.5)	5 (7.1)	1 (5.6)	2 (12.5)	1 (5.9)	15 (13.4)	10.1
NT (nontypable)	10 (11.9)	10 (14.3)	0 (0)	3 (18.6)	2 (11.8)	12 (10.7)	11.6
Totals	84 (100)	70 (100.2)	18 (100.1)	16 (100)	17 (99.9)	112 (100.4)	100

^aSee annual report on *Staphylococcus aureus* isolates 1999.²⁶

Table 5 Drug resistance among *Staphylococcus aureus* isolates

Visits	Total swabs	<i>S. aureus</i> isolated, n	Erythromycin-resistant, n	Fusidic acid-resistant, n
1 month	603	189	2	7
1 year	669	39	2	3
Acute	594	123	5	20

**Fig 1.** Concordance of swabs positive for *S. aureus* in neonates, mothers and fathers at the 1-month visit. Number of swabs positive for *S. aureus* from 302 (161 positive) neonates, 300 mothers (131 positive) and 260 fathers (126 positive).

between at least one parent and the neonates was 70% with 210 concordant pairs ($P = 0.012$). The parents' history of AD was not associated with the number of neonate colonized with *S. aureus*.

The isolated *S. aureus* was phage typed and 97 (60%) of the 161 neonates with a positive test for *S. aureus* at the 1-month visit carried the same phage group as one of the parents.

Discussion

The colonization of the skin (vestibulum nasi and/or perineum) with *S. aureus* in neonates at 1 month of age was not associated with an increased risk of developing AD during the first 3 years of life. We found a high number of neonates who carried *S. aureus* at 1 month of age (53.3%), predominantly in the nasal vestibulum (51.3% of 302 swabs cultured). The number of children who carried *S. aureus* decreased markedly during the first year to 11.3% of 336 swabs cultured. There was a 70% concordance for *S. aureus* carriage between parents and neonates. Positive swabs were found significantly more often in children with skin lesions that later were diagnosed as AD compared with skin lesions that did not develop into AD. The severity of AD measured by SCORAD in children with a positive swab for *S. aureus* was significantly higher than in children with a negative swab. The phage type of the identified *S. aureus* resembled the normal distribution for Denmark and only a few *S. aureus* cultures were resistant to erythromycin or fusidic acid.

Colonization with *S. aureus* is increased in patients with AD and the colonization is often associated with exacerbation of the eczema.¹⁻⁵ Exotoxins from *S. aureus*, especially superanti-

gens, have several proinflammatory effects. Whether colonization and superantigen release lead to induction of eczema is debatable. We therefore studied colonization with *S. aureus* in neonates and subsequent development of AD during the first 3 years of life. An association between bacterial colonization and an increased risk of atopy is not unlikely. From the same cohort we have previously shown that neonates colonized in the hypopharyngeal region with *S. pneumoniae*, *Haemophilus influenzae* or *Moraxella catarrhalis* or with a combination of these organisms have an increased risk of recurrent wheeze and asthma early in life.²⁰ However, the current study found no indication of an association between early colonization with *S. aureus* and later development of AD.

We cultured from both the vestibulum nasi and perineum in the children to increase the sensitivity²¹ but identified only a few children who were found to have *S. aureus* in the swabs taken from the perineum. Of the neonates 53.3% were colonized with *S. aureus*, which is in accordance with a previous report of nearly 50% nasal swabs positive for *S. aureus* in healthy neonates at 2 weeks of age.²² The colonization rate fell to 11.3% by age 12 months, which is also in agreement with previous reports.²²

We found a high number of swabs positive for *S. aureus* in the parents. The high level of *S. aureus* colonization may be due to the sampling method (both vestibulum nasi and pharynx²¹), the parental history of AD and the high number of neonates positive for *S. aureus*. We found a high degree of concordance (70%) for *S. aureus* carriage between parents and neonates, which is in agreement with previously published data.²²

Positive swabs taken at acute visits were found significantly more often in children with skin lesions that later developed into AD than skin lesions that did not. In children with established AD 21% were positive for *S. aureus*. This is in agreement with another study²³ but is less than other studies have reported.^{1,2} Our cohort consists of a few cases of severe AD¹⁴ whereas comparative studies primarily consist of moderate to severe cases of AD.²⁴ SCORAD in children with a positive swab for *S. aureus* was significantly higher than in children with a negative swab. This is in agreement with previous findings that the more severe the eczema the higher the colonization rate of *S. aureus*.⁷

β -Haemolytic streptococci have not been associated with AD.²⁵ Accordingly we found only a few swabs positive for *Streptococcus* at both acute and planned visits and no association with positive swabs at 1 month or 1 year and the risk of AD at the age of 3 years.

We did not identify any MRSA and only a few *S. aureus* isolates were resistant to erythromycin or fusidic acid in this study. This is in accordance with the very few problems with antibiotic resistance faced in Danish clinical isolates of *S. aureus*, where resistance to penicillin and susceptibility to other examined antibiotics is by far the predominant pattern.²⁶ Children with AD in this cohort had for the main part mild eczema and were treated with topical steroid and topical fusidic acid if infection was suspected. Despite this, resistance to fusidic acid was not a problem in our cohort.

Staphylococcus aureus phage distribution was comparable with the normal distribution in Denmark. Only a few small studies have previously looked at the phage group of *S. aureus* in AD. Older studies mainly found phage group III;^{25,27} in contrast we found mainly phage group II and NT, confirming more recent findings.²⁸

Serum samples collected at 18 months of age were tested for the presence of specific IgE to three staphylococcal enterotoxins (SEA, SEB and TSST-1). At this early age only a few samples were positive and there was no increased number among children who develop AD before the age of 3 years. An association between eczema and sensitization to *S. aureus*-secreted enterotoxins have been found; however, these studies have been performed in older children and the low number of specific IgE to staphylococcal enterotoxins in our study is probably related to the fact that the samples were taken early, at the age of 18 months.

In conclusion, study of this prospective longitudinal birth cohort produced strong evidence that *S. aureus* colonization and IgE sensitization to staphylococcal enterotoxins are not associated with AD onset, but confirmed *S. aureus* as an aggravating factor in established AD.

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