Research Article   **Open Access**

Nasal Colonization by *Staphylococcus aureus* in Children with Atopic Dermatitis

Berbggal L[1*], Sanchez-Payá J[1], Rodriguez JC[2,3], De Leon FJ[4], Martínez-Miravete MT[5], Galiana AJ[6] and Betlloch I[7]

[1]*Department of Dermatology, Hospital Marina Salud of Denia, Spain*

[2]*Department of Preventive Medicine, ISABIAL General University Hospital of Alicante, Spain*

[3]*Department of Microbiology, ISABIAL General University Hospital of Alicante, Spain*

[4]*Department of Dermatology, Hospital Morales Meseguer of Murcia, Spain*

[5]*Department of Pediatrics, General University Hospital of Alicante, Spain*

[6]*Department of Microbiology, General University Hospital of Elche, Elche, Spain*

[7]*Department of Dermatology, ISABIAL General University Hospital of Alicante, Spain*

*****Corresponding author:** Laura Berbggal De Gracia, Department of Dermatology, Hospital Marina Salud of Denia, Spain, Tel: +966429000; E-mail: laura.berbggal@marinsalud.es

Abstract

Background: The cutaneous and nasal microbiota, especially *Staphylococcus aureus* (*S. aureus*), plays a key role in atopic dermatitis (AD). Although this association is well known, it is difficult to establish whether colonization by these bacteria is a cause or a consequence of the disease.

Objectives: The primary objectives of our study are to determine the prevalence of nasal colonization by *S. aureus* and its relationship with disease severity in children with AD, as well as to describe the magnitude of the association between the two.

Methods: This was an analytical case-control study in children with and without AD. Participants were recruited consecutively upon presenting to the Department of Pediatric Dermatology at the General University Hospital until reaching the estimated sample size of 157 cases and 314 controls (N=471; ratio 1:2).

Results: The prevalence of *S. aureus* nasal colonization was significantly higher in children with AD than in those without (32.5% *vs.* 23.9%; odds ratio (OR) 1.5, 95% CI 1.0, 2.3; p=0.047). Prevalence of colonization was not higher in the children with severe AD than in those with less severe AD. In multivariable analysis, an independent association (borderline significant) between nasal colonization by *S. aureus* and AD persisted after adjusting for all other study variables (OR 1.5; 95% CI 0.9, 2.6; p=0.11).

Conclusions: Our paper provides further evidence of an association between atopy and nasal colonization by *S. aureus*, though not between nasal colonization and AD severity. The role of nasal *S. aureus* and the microbiota in people with AD is a controversial but crucial area of study. Greater knowledge of this topic could be clinically applicable in AD patients.

Keywords: Atopic dermatitis; *Staphylococcus aureus*; Epidermal barrier dysfunction; Nasal colonization

Introduction

Childhood atopic dermatitis (AD) has become a public health issue in developed countries; it affects between 15% and 30% of children and is considered the most prevalent chronic childhood disease [1]. The etiopathogenesis of AD is complex and involves multiple factors, including epidermal barrier dysfunction (especially filaggrin deficiency), immunological and biochemical mechanisms, genetic predisposition, and environmental aspects [2-6]. Many of these factors, and the relationships between them, are poorly understood. Some researchers have associated alterations in the cutaneous and nasal microbiota, especially *Staphylococcus aureus*, with the pathogenesis of this disease.

It is very difficult to determine whether *S. aureus* colonization is a cause or consequence of AD, since the etiopathogenesis of the disease (altered immune system, broken skin, etc.) increases the likelihood of colonization, and the bacteria in turn aggravate the inflammation and the lesions, prolonging healing. Various studies have shown that children with AD are more frequently colonized than those without. In fact, bacterial superinfection of skin lesions by *S. aureus* is the most common complication of AD and is usually present in outbreaks. Whether an association exists between *S. aureus* nasal colonization and eczema severity is still under debate [7-9]. Although antibiotic treatment may reduce the severity of AD and the risk of secondary infection, these benefits are temporary. On the other hand, in the long term the emerging potential for antibiotic resistance is a serious challenge to treatment.

We are continually improving our understanding of the pathogenic and commensal microbiota that inhabits our bodies. The nasal microbiota can include species other than *S. aureus*, and the resulting bacterial competition is thought to affect the prevalence of *S. aureus* nasal colonization. Studies have suggested that people who carry *Corynebacteria*, *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, and *Streptococcus pneumoniae* are less likely to carry *S. aureus*, since these competing bacteria stimulate the production of antimicrobial peptides that prevent *S. aureus* from colonizing their reservoir [10-12].

The primary objectives of our study are to determine the prevalence of nasal colonization by *S. aureus* and its relationship with disease severity in children with AD, as well as to describe the magnitude of the association between the two. Secondary objectives are to describe the antimicrobial sensitivity of isolated strains of *S. aureus* and the frequency of other potentially pathogenic colonizers.

Material and Methods

Study design

This was an analytical case-control study in children with and without AD. Participants were recruited consecutively until reaching the estimated sample size of 157 cases and 314 controls (N=471; ratio 1:2).

Inclusion criteria

We included all children aged 6 months to 16 years who were diagnosed with AD in the Pediatric Dermatology Clinic of Alicante General University Hospital (HGUA) from June 2013 to May 2016. AD diagnosis was based on the criteria of the consensus conference on pediatric atopic dermatitis [13]. Our controls were children in the same age range who came to our clinic in the same time period but who did not have AD or any infectious or dermatological condition that could lead to diagnostic confusion (e.g. contact dermatitis, scabies or childhood frictional dermatitis).

Exclusion criteria

We excluded all patients with any other systemic disease, those treated with antibiotics or immune suppressants in the month prior to recruitment, or a history of infections such as erysipelas or cellulitis.

Data collection

After obtaining informed consent for study inclusion from the child's parent or legal guardian, we recorded the variables in a purpose-designed data extraction form. We took samples from the vestibule of both nostrils with a sterile swab and sent them to the HGUA Microbiology Laboratory for processing. All data were coded, anonymized and stored in a database (SPSS version 22). The HGUA Ethics Committee approved this study.

Statistical analysis

To meet our primary objectives, we calculated the prevalence and 95% confidence intervals (CIs) of nasal colonization in the cases: as a single group; with and without pruritus-induced sleep disorders; and classified according to AD severity on the SCORAD index. We then compared the cases and controls in terms of prevalence of nasal colonization and clinical and epidemiological variables (age, sex, body mass index (BMI), and family history of atopy, personal history of atopy, respiratory and food allergies, and celiac disease). After comparing cases and controls, we determined the homogeneity of the sample by comparing the same clinical and epidemiological variables in colonized and non-colonized participants. For both comparisons, we determined whether the differences reached statistical significance (p<0.05) using the chi-squared test or Fisher's exact test, depending on the conditions of application. For each of the variables, we calculated the magnitude of association with the odds ratio (OR) and corresponding 95% CI. We subsequently performed a multivariable logistic regression analysis to determine whether any variables influenced the association between AD and nasal colonization. We excluded all variables that were not significantly associated and/or for which the OR could not be calculated; these were sex, family history of rhinitis, food allergies and celiac disease.

To meet our secondary objectives, we analysed the prevalence of antimicrobial resistance for each of the isolated strains of *S. aureus*. We then determined the prevalence of nasal colonization by each species of potentially pathogenic bacteria (other than *S. aureus*) in the samples, comparing firstly cases and controls, and secondly colonized and non-colonized participants. To establish whether the differences reached statistical significance, we used the chi-squared test or Fisher's exact test, depending on the conditions of application. The OR and corresponding 95% CI were calculated for each of the variables.

Results

S. aureus nasal colonization was confirmed in 51 of the 157 children with AD, giving a prevalence of 32.5% (95% CI 24.8%, 40.1%) (Figure 1). Seventy-three (46.5%) of the 157 cases had pruritus-induced sleep disorders while the remaining 84 (53.5%) did not. (Figure 2). According to the SCORAD index, 35% of the cases (n=55) were mild, 48.4% (n=76) were moderate and 16.6% (n=26) were severe (Figure 3). All in all, *S. aureus* nasal colonization was confirmed in 26% and 38% of the cases with and without pruritus-induced sleep disorders, respectively (Figure 4). The respective proportions of *S. aureus* nasal colonization in mild, moderate and severe cases were 45.5% (n=25), 22.4% (n=17) and 34.6 (n=9) (Figure 5).
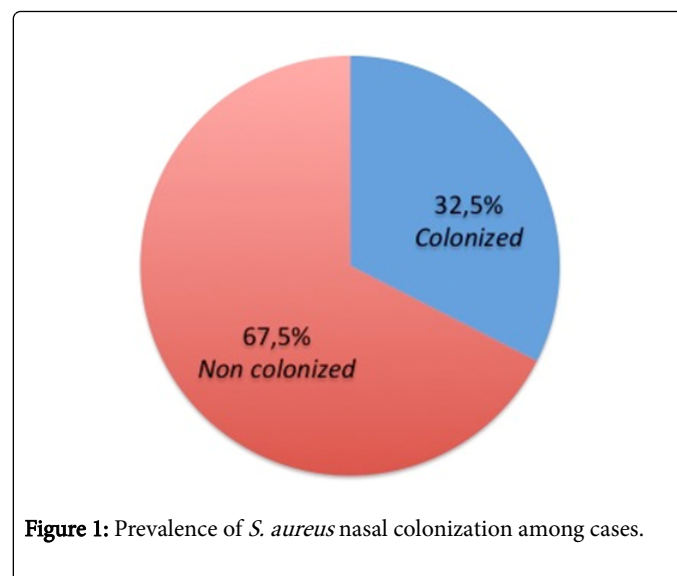

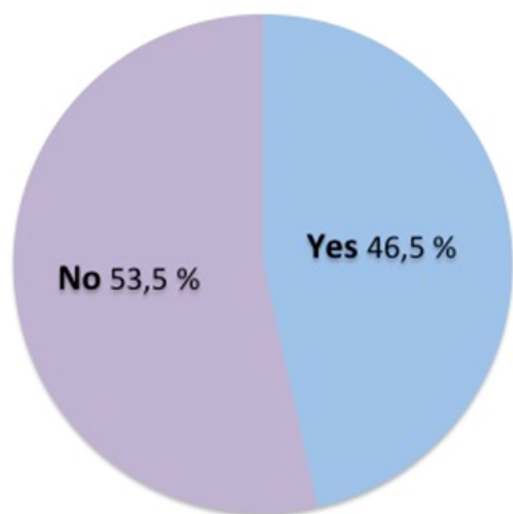**Figure 1:** Prevalence of *S. aureus* nasal colonization among cases.

Figure 2: Prevalence of pruritus-induced sleep disorders among cases.

Table 1 compares the clinical and epidemiological characteristics of the cases and controls. Table 2 includes the same variables, but compares colonized and non-colonized participants. Table 3 displays the results of the multivariable analysis, showing that after adjustment, the OR for *S. aureus* nasal colonization did not change from 1.5, though the corresponding 95% CI (0.9, 2.6) and p value (0.11) did change.
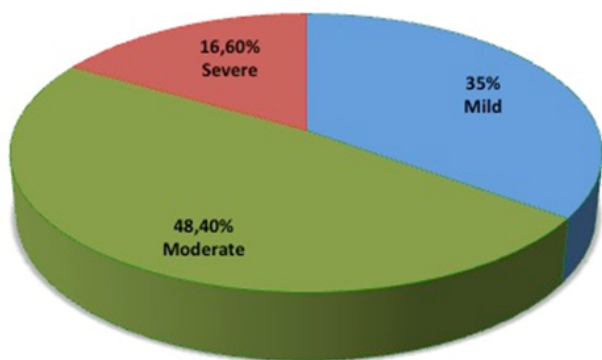

Figure 3: Severity of cases according to SCORAD.

Table 4 shows the profile of *S. aureus* antibiotic resistance in colonized participants. Tables 5a and 5b show the prevalence of participants colonized by potentially pathogenic bacteria other than *S. aureus*, comparing cases and controls (5a), and participants colonized and not colonized by *S. aureus* (5b).
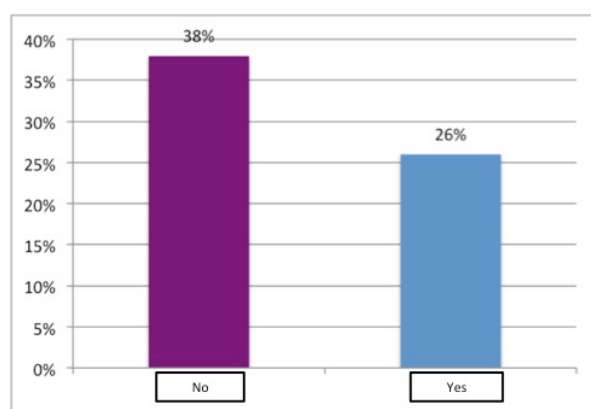
Figure 4: Prevalence of *S. aureus* nasal colonization in cases according to presence or absence of pruritus-induced sleep disorders.
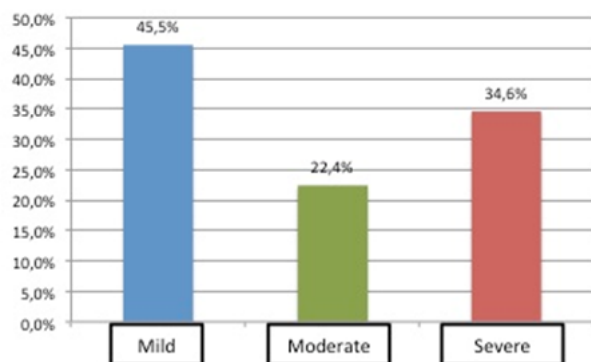
Figure 5: Prevalence of *S. aureus* nasal colonization en cases according to SCORAD severity.

	Case (n=157)		Control (n=314)		OR (95% CI)	P
	%	(n)	%	(n)		
S. aureus nasal col.	32.5	51	23.9	75	1.5 (1.0, 2.3)	0.047
Age						
≤ 2 years	26.8	42	27.1	85	1.1 (0.6, 1.9)	0.722
3-5 years	28.7	45	19.1	60	1.7 (1.0, 2.9)	0.07
6-9 years	22.9	36	29.6	93	0.9 (0.5, 1.5)	0.611
≥ 10 years	21.7	34	24.2	76	1	
Sex (boys)	54.8	86	51.6	162	1.1 (0.8, 1.7)	0.514
BMI						
Underweight	7.6	12	5.1	16	2.6 (1.1, 6.2)	0.035

Healthy weight	59.2	93	50.6	159	2.0 (1.2, 3.4)	0.01
Obese	14.6	23	25.2	79	1	
Fam. hist. rhinitis	16.6	26	13.7	43	1.3 (0.7, 2.1)	0.407
Fam. hist. asthma	14	22	4.1	13	3.8 (1.8, 7.7)	<0.001
Fam. hist. AD	25.5	40	5.1	16	6.4 (3.4, 11.8)	<0.001
Per. hist. rhinitis	21	33	2.9	9	9.0 (4.2, 19.4)	<0.001
Per. hist. asthma	16.7	26	2.2	7	8.8 (3.7, 20.7)	<0.001
RA pollen	9.6	-15	1	3	10.9 (3.1, 38.4)	<0.001
RA animal hair	8.3	-13	0.6	4	14.1 (3.1, 63.2)	<0.001
RA dust mites	13.4	-21	1.3	2	12.0 (4.0, 35.5)	<0.001
FA egg	12.7	-20	0.3	-1	45.7 (6.1, 343.9)	<0.001
FA nuts	9.6	-15	0	0	Incalculable	<0.001
FA peach	4.5	-7	0	0	Incalculable	<0.001
FA kiwi	2.5	-4	0	0	Incalculable	0.012
FA CMP	4.5	-7	0	0	Incalculable	<0.001
FA shellfish	1.9	-3	0.3	-1	6.1 (0.6, 59.1)	0.11
Coeliac disease	2.5	-4	0.6	-2	4.1 (0.7, 22.5)	0.099

Table 1: Comparison of clinical and epidemiological characteristics in cases and controls.

	Colonized (n=126)		Non colonized (n=314)		OR (95%CI)	P
	%	(n)	%	(n)		
Age						
≤ 2 years	10.3	13	33	114	0.2 (0.1, 0.4)	<0.001
3-5 years	18.3	23	23.8	82	1.5 (0.3, 0.9)	0.03
6-9 years	40.5	51	22.6	78	1.2 (0.7, 2.0)	0.517
≥ 10 years	31	39	20.6	71	1	
Sex (boys)	50.8	64	53.3	184	0.9 (0.6-1.4)	0.625
BMI						
Underweight	6.3	8	5.8	20	1.7 (0.7, 4.6)	0.254
Healthy weight	50.8	64	54.5	188	1.5 (0.8, 2.6)	0.175
Overweight	27.8	35	15.7	54	2.8 (1.5, 5.5)	0.002
Obese	15.1	19	24.1	83	1	
Fam. hist. rhinitis	16.7	21	13.9	48	1.2 (0.7, 2.1)	0.454
Fam. hist. asthma	7.1	9	7.5	26	0.9 (0.4, 2.1)	0.885
Fam. hist. AD	13.5	17	11.3	39	1.2 (0.7, 2.3)	0.516
Per. hist. rhinitis	15.1	19	6.7	23	2.5 (1.3, 4.7)	0.005

Per. hist. asthma	9.5	12	6.1	21	1.6 (0.8, 3.4)	0.199
RA pollen	7.1	9	2.6	9	2.9 (1.1, 7.4)	0.03
RA animal hair	4.8	6	2.6	9	1.9 (0.7, 5.4)	0.244
RA dust mites	9.5	12	3.8	13	2.7 (1.2, 6.1)	0.014
FA egg	6.3	8	3.8	13	1.7 (0.7, 4.3)	0.23
FA nuts	4	5	2.9	10	1.4 (0.5, 4.1)	0.559
FA peach	3.2	4	0.9	3	3.7 (0.8, 16.9)	0.086
FA kiwi	0	0	1.2	4	Incalculable	0.578
FA CMP	2.4	3	1.2	4	2.1 (0.5, 9.4)	0.391
FA shellfish	0	0	1.2	4	Incalculable	0.578
Celiac disease	1.6	2	1.2	4	1.4 (0.2, 7.6)	0.661

Table 2: Comparison of clinical and epidemiological characteristics in colonized and non-colonized children.

	ORc (95% CI)	P	ORa (95% CI)	P
S. aureus nasal col.	1.5 (1.0, 2.3)	0.047	1.5 (0.9, 2.6)	0.11
Age				
≤ 2 years	1.1 (0.6, 1.9)	0.722	1.5 (0.8, 3.0)	0.207
3-5 years	1.7 (1.0, 2.9)	0.07	2.3 (1.1, 4.5)	0.018
6-9 years	0.9 (0.5, 1.5)	0.611	0.8 (0.4, 1.6)	0.589
≥ 10 years	1			
BMI				
Underweight	2.6 (1.1, 6.2)	0.035	2.2 (0.8, 6.0)	0.107
Healthy weight	2.0 (1.2, 3.4)	0.01	2.2 (1.2, 4.0)	0.015
Overweight	1.7 (0.9, 3.2)	0.122	1.6 (0.8, 3.5)	0.216
Obese	1			
Fam. hist. asthma	3.8 (1.8, 7.7)	<0.001	2.0 (0.8, 4.8)	0.118
Fam. hist. AD	6.4 (3.4, 11.8)	<0.001	5.5 (2.8, 10.8)	<0.001
Per. hist. rhinitis	9.0 (4.2, 19.4)	<0.001	4.1 (1.1, 15.4)	0.04
Per. hist. asthma	8.8 (3.7, 20.7)	<0.001	4.7 (1.7, 13.0)	0.003
RA pollen	10.9 (3.1, 38.4)	<0.001	1.9 (0.2, 7.1)	0.772
RA animal hair	14.1 (3.1, 63.2)	<0.001	2.1 (0.3, 13.8)	0.454
RA dust mites	12.0 (4.0, 35.5)	<0.001	2.2 (0.5, 10.3)	0.31

ORc: crude odds ratio; ORa adjusted odds ratio; 95% CI: 95% confidence interval; P: level of statistical significance; *S. aureus* nasal col.: nasal colonization by *S. aureus*; BMI: body mass index; Fam. hist.: family history; AD: atopic dermatitis; Per. hist.: personal history; RA: respiratory allergy.

Table 3: Multivariable analysis.

	Total (n=126)	Case (n=51)	Control (n=75)	OR (95% CI)	P
	% (n)	% (n)	% (n)		
Methicillin	15.1 (18/119)	16.3 (8/49)	14.3 (10/70)	1.2 (0.4, 3.2)	0.76
Co-amoxiclav	15.4 (18/117)	18.8 (9/48)	13.0 (9/69)	1.5 (0.6, 4.2)	0.4
Penicillin	91.0 (81/89)	89.2 (33/37)	92.3 (48/52)	0.7 (0.2, 2.9)	0.714
Tetracycline	3.9 (4/103)	9.3 (4/43)	0.0 (0/60)	Incalculable	0.028
Mupirocin	15.9 (17/107)	11.9 (5/42)	18.5 (12/65)	0.6 (0.2, 1.8)	0.365
Fusidic acid	7.1 (3/42)	7.1 (1/14)	7.1 (2/28)	1.0 (0.8, 12.1)	1
Clindamycin	42.7 (44/103)	46.5 (20/43)	40.0 (24/60)	1.3 (0.6, 2.9)	0.51
Erythromycin	31.1 (32/103)	34.9 (15/43)	28.3 (17/60)	1.4 (0.6, 3.1)	0.479
Gentamicin	11.7 (12/103)	16.3 (7/43)	8.3 (5/60)	2.1 (0.6, 7.3)	0.215
Tobramycin	9.8 (10/102)	14.0 (6/43)	6.8 (4/59)	2.2 (0.6, 8.4)	0.315
Amikacin	1.8 (1/57)	4.5 (1/22)	0.0 (0/35)	Incalculable	0.386
Ciprofloxacin	2.0 (2/102)	4.8 (2/42)	0.0 (0/60)	Incalculable	0.167
Levofloxacin	1.9 (2/103)	4.7 (2/43)	0.0 (0/60)	Incalculable	0.172
Co-trimoxazole	0.0 (0/103)	0.0 (0/43)	0.0 (0/60)	Incalculable	-
Rifampicin	0.0 (0/53)	0.0 (0/18)	0.0 (0/35)	Incalculable	-
Vancomycin	0.0 (0/103)	0.0 (0/43)	0.0 (0/60)	Incalculable	-

Table 4: Profile of *S. aureus* antibiotic resistance in colonized participants.

	Total (n=471)		Case (n=157)		Control (n=314)		OR (95% CI)	P
	%	(n)	%	(n)	%	(n)		
Gram+	3.8	18	3.8	6	3.8	12	1.0 (0.4, 2.7)	1
Strep. pneumoniae	3.6	17	3.2	5	3.8	12	0.8 (0.3, 2.4)	0.737
Strep. pyogenes	0.2	1	0.6	1	0	0	Incalculable	0.333
Gram-	13.8	65	19.1	30	11.1	35	1.7 (1.0, 2.9)	0.057
M. catarrhalis	2.1	10	1.9	3	2.2	7	0.9 (0.3, 3.4)	1
H. influenzae	9.6	45	12.7	20	8	25	1.7 (0.9, 3.1)	0.096
Acinetobacter spp.	0.4	2	1.3	2	0	0	Incalculable	0.111
Pseudomonas spp.	0.6	3	1.3	2	0.3	1	4.0 (0.4, 44.9)	0.259
Enterobacter spp.	1.1	5	1.9	3	0.6	2	3.0 (0.5, 18.4)	0.339

OR: Odds Ratio; 95 CI%: 95% confidence interval; P: level of statistical significance; *Strep. pneumoniae*: *Streptococcus pneumoniae*; *Strep. pyogenes*: *Streptococcus pyogenes*; *M. catarrhalis*: Moraxella catarrhalis; *H. influenzae*: Haemophilus influenzae

Table 5a: Prevalence of cases and controls colonized by potentially pathogenic bacteria other than *S. aureus*.

	Colonized (n=126)		Non-colonized (n=345)		OR (95% CI)	P
	%	(n)	%	(n)		
Gram+	1.6	-2	4.6	-16	0.3 (0.1, 1.4)	0.175
Strep. pneumoniae	3.6	-2	4.3	-15	0.4 (0.1, 1.6)	0.262
Strep. pyogenes	0	0	0.3	-1	Incalculable	1
Gram-	4.8	-6	17.1	-59	0.2 (0.1, 0.5)	<0.001
M. catarrhalis	1.6	-2	2.3	-8	0.7 (0.1, 3.2)	1
H. influenzae	1.6	-2	12.5	-43	0.1 (0.0, 0.5)	<0.001
Acinetobacter spp.	0.8	-1	0.3	-1	2.8 (0.2, 44.3)	0.464
Pseudomonas spp.	0	0	0.9	-3	Incalculable	0.568
Enterobacter spp.	0.8	-1	1.2	-4	0.7 (0.1, 16.2)	1

OR: odds ratio; 95 CI%: 95% confidence interval; P: level of statistical significance; *Strep. pneumoniae*: *Streptococcus pneumoniae*; *Strep. pyogenes*: *Streptococcus pyogenes*; *M. catarrhalis*: *Moraxella catarrhalis*; *H. influenzae*: *Haemophilus influenzae*.

Table 5b: Prevalence of patients colonized by potentially pathogenic bacteria other than *S. aureus*, comparing *S. aureus*-colonized and non-colonized patients.

Discussion

In our study, the prevalence of nasal colonization by *S. aureus* was significantly higher in children with AD than in those without (32.5% *vs.* 23.9%; OR 1.5, 95% CI 1.0, 2.3; p=0.047. These figures were consistent with our expectations, as different studies have suggested nasal colonization by *S. aureus* affects 20% to 30% of the general population and is more prevalent in children with DA than in healthy children [14-16]. We did not find a positive association between AD severity (according to SCORAD and presence of pruritus-induced sleep disorders) or rate of colonization. There is a lack of consensus on this point in the literature: some authors have not found an association between nasal colonization and severity, while others have associated nasal colonization by *S. aureus* with more extensive lesions and signs of skin infection [9,17,18].

In our study, 7.6% of the cases were underweight, compared to 5.1% of the controls (OR 2.6, 95% CI 1.1, 6.2; p=0.035). This difference supports the theory that children with AD are usually thinner. Two possible explanations for this trend are that these children cannot sleep properly because of the pruritus, or that they are more likely to have attention deficit hyperactivity disorders and emotional and behavioral disorders, which make them restless and excitable and therefore prevent them from putting on weight. Conversely, recent studies have posited that obesity and metabolic syndrome are associated with AD, as they are with psoriasis [19-22]. This association may be clearer in older children or adolescents; the children in our study may be too young to fit this theory.

In our study, family history of asthma and AD was more common in the cases (14% and 25.5%, respectively) than in the controls (4.1% and 5.1%, respectively), with statistical significance in both comparisons (p<0.001). Differences of this magnitude were to be expected, given the etiopathogenesis and genetic predisposition of atopic disease. Compared with our controls, a higher proportion of cases had a personal history of rhinitis (OR 9.0, 95% CI 4.2, 19.4), asthma (OR 8.8, 95% CI 3.7, 20.7) pollen allergies (OR 10.9, 95% CI 3.1, 38.4), animal hair allergies (OR 14.1, 95% CI 3.1, 63.2) and dust mite allergies (OR 12.0, 95% CI 4.0, 35.5). The p value for all of these differences was below 0.001. These data are consistent with previous findings on the atopic march and the tendency of AD to precede asthma and allergic rhinitis [23]. It is probably true, however, that atopic patients are more closely followed up for allergic sensitization by allergologists than non-atopic people.

A recent study suggested that food allergies could also contribute to the atopic march [24,25]. Of the 157 cases included in our study, 56 (35%) had food allergies. This is in line with the findings of other studies that suggest around 33% of children with moderate to severe AD are positive for IgE antibodies specific to some kind of food protein. Egg protein allergies have been associated with greater severity of atopic eczema [26]. In our study, 12.7% of the cases had an egg allergy, compared to just 0.3% of the controls, with an OR of 47.5 (95% CI 6.1, 343.9; p<0.001). For the remaining food allergies, the OR could not be calculated or did not show statistical significance. Although a larger number of cases than controls had celiac disease (2.5% *vs.* 0.6%), the difference did not reach statistical significance (p=0.099). This is a controversial topic in the literature, and several studies have examined the potential association between celiac disease and immune-mediated diseases like AD.

Concerning age distribution, in our study 71.5% of the colonized participants were aged six years or older, while 55.8% of the non-colonized participants were under six years old. Colonization was therefore associated with older age and non-colonization with younger age. This may be because the youngest children have had the least exposure to the environment, cigarette smoke, colonized people, etc. Another possible explanation is that the responsibility of treating younger children tends to fall on their parents, who are likely to administer anti-inflammatory and antibiotic drugs that may help to reduce colonization. As children grow older and more independent, they take on more responsibility for their own treatment, which they often neglect to adhere to. Or it may be that the microbiota of children under one year old is not yet mature and their nasal reservoir contains many types of commensal and saprophytic bacteria that interfere with *S. aureus* colonization. However, some studies report the opposite trend, suggesting that the highest proportion of *S. aureus* nasal colonization is found in the youngest children [27].

In our study, there was no statistical significance differences in sex between uncolonized participants and colonized participants (OR 0.9, 95% CI 0.6, 1.4; p=0.625). Compared with the uncolonized participants in our study, a significantly higher proportion of the colonized participants had a history of rhinitis (OR 2.5, 95% CI 1.3, 4.7; p=0.005), pollen allergies (OR 2.9, 95% CI 1.1, 7.4; p=0.030), and dust mite allergies (OR 2.7, 95% CI 1.2, 6.1; p=0.014). More of the colonized than uncolonized participants had a history of asthma (OR 1.6, 95% CI 0.8, 3.4; p=0.199) and animal hair allergies (OR 1.9, 95% CI 0.7, 5.4; p=0.244), but the differences did not reach statistical significance. These findings are consistent with other studies that have associated *S. aureus* nasal colonization with the symptoms of allergic rhinitis through super antigens and with the exacerbation of allergic conditions [28,29]. In other studies, antibiotics to which these bacteria are susceptible were administered to people with allergic rhinitis, curing the condition [30,31].

After our multivariable analysis, the rate of nasal colonization by *S. aureus* was 32.5% in the cases and 23.9% in the controls, with an adjusted OR of 1.5 (95% CI 0.9, 2.6; p=0.110). This means adjustment did not affect the OR but did decrease the statistical significance. Had we studied a larger sample, our multivariable analysis may have produced a statistically significant difference. With these data, we can conclude that the increased risk of nasal colonization by *S. aureus* in people with AD depends exclusively on the disease and is unrelated to the rest of the variables studied.

We found no statistically significant differences in *S. aureus* antibiotic resistance between the cases and controls; nor did we expect to, since environmental bacterial strains are the same for children with and without AD. Had the participants with AD been colonized in hospital, the nosocomial *S. aureus* in their samples would probably have been more resistant to antibiotics, especially methicillin.

Some of the participants in our study were colonized by potentially pathogenic bacteria, including *S. pneumoniae* and *H. Influenza*, but had no symptoms, possibly because this colonization was transient and corresponded to asymptomatic carriers. The differences between the

cases and controls in the prevalence of potentially pathogenic germs other than *S. aureus* were not statistically significant. Participants not colonized by *S. aureus* were more likely to be colonized by other potentially pathogenic bacteria, with the exception of *Acinetobacter* spp. This is logical since the nasal vestibule is the primary reservoir of *S. aureus*, which displaces the other bacteria. In our study, then, as in many others, nasal carriage of *S. aureus* was negatively correlated with nasal colonization by other bacteria [11,32,33]. This finding supports the hypothesis that *S. aureus* has an antagonistic relationship with other species in its biological niche, and that carrying other flora is a protective factor against *S. aureus* nasal colonization. In light of our findings, we can conclude that the role of nasal *S. aureus* in people with AD is a controversial but crucial area of research. More epidemiological studies are needed to improve our knowledge on the subject for the clinical benefit of AD sufferers.

References

1. Bieber T (2008) Atopic dermatitis. N Engl J Med 358: 1483-1494.

2. Brown SJ, McLean WH (2012) One remarkable molecule: filaggrin. J Invest Dermatol 132: 751-762.

3. Silverberg NB, Silverberg JI (2015) Inside out or outside in: does atopic dermatitis disrupt barrier function or does disruption of barrier function trigger atopic dermatitis? Cutis 96: 359-361.

4. McAleer MA, Irvine AD (2013) The multifunctional role of filaggrin in allergic skin disease. J Allergy Clin Immunol 131: 280-291.

5. Irvine AD, McLean WH, Leung DY (2011) Filaggrin mutations associated with skin and allergic diseases. N Engl J Med 365: 1315-1327.

6. Homey B, Steinhoff M, Ruzicka T, Leung DY (2006) Cytokines and chemokines orchestrate atopic skin inflammation. J Allergy Clin Immunol 118: 178-189.

7. Semic-Jusufagic A, Bachert C, Gevaert P, Holtappels G, Lowe L, et al. (2007) Staphylococcus aureus sensitization and allergic disease in early childhood: population-based birth cohort study. J Allergy Clin Immunol 119: 930-36.

8. Lomholt H, Andersen KE, Kilian M (2005) Staphylococcus aureus clonal dynamics and virulence factors in children with atopic dermatitis. J Invest Dermatol 125: 977-82.

9. Hon KLE, Lam MCA, Leung TF, Kam WY, Li MC, et al. (2005) Clinical features associated with nasal Staphylococcus aureus colonization in Chinese children with moderate-to-severe atopic dermatitis. Ann Acad Med Singapore 34: 602-605.

10. Konno M, Baba S, Mikawa H, Hara K, Matsumoto F, et al. (2006) Study of upper respiratory tract bacterial flora: first report. Variations in upper respiratory tract bacterial flora in patients with acute upper respiratory tract infection and healthy subjects and variations by subject age. J Infect Chemother 12: 83-96.

11. Cogen AL, Yamasaki K, Sanchez KM, Dorschner RA, Lai Y, et al. (2010) Selective antimicrobial action is provided by phenol-soluble modulins derived from Staphylococcus epidermidis, a normal resident of the skin. J Invest Dermatol 130: 192-200.

12. Zipperer A, Konnerth MC, Laux C, Berscheid A, Janek D, et al. (2016) Human commensals producing a novel antibiotic impair pathogen colonization. Nature 535: 511-516.

13. Eichenfield LF, Hanifin JM, Luger TA, Stevens SR, Pride HB (2003) Consensus conference on pediatric atopic dermatitis. J Am Acad Dermatol 49: 1088-1014.

14. Ammerlaan HS, Kluytmans JA, Wertheim HF, Nouwen JL, Bonten MJ (2009) Eradication of methicillin-resistant Staphylococcus aureus carriage: A systematic review. Clin Infect Dis 48: 922-930.

15. Weidenmaier C, Goerke C, Wolz C (2012) Staphylococcus aureus determinants for nasal colonization. Trends Microbiol 20: 243-250.

16. Lo WT, Wang SR, Tseng MH, Huang CF, Chen SJ, et al. (2010) Comparative molecular analysis of meticillin-resistant Staphylococcus aureus isolates from children with atopic dermatitis and healthy subjects in Taiwan. Br J Dermatol 162: 1110-1116.

17. Chiu LS, Chow VC, Ling JM, Hon KL (2010) Staphylococcus aureus carriage in the anterior nares of close contacts of patients with atopic dermatitis. Arch Dermatol 146: 748-752.

18. Gilani SJ, Gonzalez M, Hussain I, Finlay AY, Patel GK (2005) Staphylococcus aureus re-colonization in atopic dermatitis: beyond the skin. Clin Exp Dermatol 30: 10-13.

19. Holm EA, Wulf HC, Stegmann H, Jemec GB (2006) Life quality assessment among patients with atopic eczema. Br J Dermatol 154: 719-725.

20. Yamanaka K, Mizutani H (2015) "Inflammatory skin march": IL-1-mediated skin inflammation, atopic dermatitis, and psoriasis to cardiovascular events. J Allergy Clin Immunol 136: 823-824.

21. Hjuler KF, Böttcher M, Vestergaard C, Deleuran M, Raaby L, et al. (2015) Increased Prevalence of Coronary Artery Disease in Severe Psoriasis and Severe AtopicDermatitis. Am J Med 128: 1325-1334.

22. Nagel G, Koenig W, Rapp K, Wabitsch M, Zoellner I, et al. (2009) Associations of adipokines with asthma, rhinoconjunctivitis, and eczema in German schoolchildren. Pediatr Allergy Immunol 20: 81-88.

23. Nagel G, Koenig W, Rapp K, Wabitsch M, Zoellner I, et al. (2009) Associations of adipokines with asthma, rhinoconjunctivitis, and eczema in German schoolchildren. Pediatr Allergy Immunol 20: 81-88.

24. Zheng T, Yu J, Oh MH, Zhu Z (2011) The atopic march: Progression from atopic dermatitis to allergic rhinitis and asthma. Allergy Asthma Immunol Res 3: 67-73.

25. Allen KJ, Dharmage SC (2010) The role of food allergy in the atopic march. Clin Exp Allergy 40: 1439-1441.

26. Williams HC (2005) Clinical practice. Atopic dermatitis. N Engl J Med 352: 2314-2324.

27. Ricci G, Patrizi A, Baldi E, Menna G, Tabanelli M, et al. (2006) Long-term follow-up of atopic dermatitis: retrospective analysis of related risk factors and association with concomitant allergic diseases. J Am Acad Dermatol 55: 765-771.

28. Peterson SW, Knox NC, Golding GR, Tyler SD, Tyler AD, et al. (2016) A Study of the Infant Nasal Microbiome Development over the First Year of Life and in Relation to Their Primary Adult Caregivers Using cpn60 Universal Target (UT) as a Phylogenetic Marker. PLoS One 11: 0152493.

29. Clement S, Vaudaux P, Francois P, Schrenzel J, Huggler E, et al. (2005) Evidence of an intracellular reservoir in the nasal mucosa of patients with recurrent Staphylococcus aureus rhinosinusitis. J Infect Dis 192: 1023-1028.

30. Liu T, Wang BQ, Yang PC (2006) A possible link between sinusitis and lower airway hypersensitivity: the role of Staphylococcal enterotoxin B. Clin Mol Allergy 4: 7.

31. Shiomori T, Yoshida S, Miyamoto H, Makishima K (2000) Relationship of nasal carriage of Staphylococcus aureus to pathogenesis of perennial allergic rhinitis. J Allergy Clin Immunol 105: 449-454.

32. Bozzola CM (2003) La colonización nasal por Staphylococcus aureus tiene un papel en la permanencia de los síntomas de la rinitis persistente? AAIC 2: 54-57.

33. Frank DN, Feazel LM, Bessesen MT, Price CS, Janoff EN, et al. (2010) The human nasal microbiota and Staphylococcus aureus carriage. PLoS One 5: e10598.