

Correlation Between Serum 25-Hydroxyvitamin D and Virulence Genes of *Staphylococcus aureus* Isolates Colonizing Children with Atopic Dermatitis

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Abstract: The skin of children with atopic dermatitis (AD) is colonized with *Staphylococcus aureus* more frequently than that of their peers. We investigated the prevalence of skin and nares colonization by *S. aureus* in children with AD, the virulence genes of the isolates, and their association with allergy, AD severity, and serum vitamin D (25(OH)D). This was an observational, cross-sectional study in a sample of children diagnosed with AD in two settings in Spain. The samples were collected in 2012. Swabs from affected skin and nares were taken for microbiologic culture. The prevalence of *S. aureus* and presence of 17 staphylococcal virulence genes were studied using polymerase chain reaction. A total of 114 patients with a mean age of 5.7 ± 4.1 (range 3 mos to 14 yrs) were included in the study. Swabs were taken from the skin of 113 individuals with AD and from the nares of 85; 28.3% had *S. aureus* on the skin, which was significantly associated with positive allergen-specific immunoglobulin E antibodies and higher Scoring Atopic Dermatitis (SCORAD) scores in the multivariate analysis. The presence of virulence factors *tsst-1*, *eta*, *cna*, *aur*, and *sec* in cutaneous *S. aureus* isolates was associated with lower serum levels of 25(OH)D. *S. aureus* on nasal swabs correlated with its presence on the skin and was associated with lower 25(OH)D levels. In conclusion, *S. aureus* colonization is associated with allergy and severity

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in AD, whereas certain virulence genes are associated with lower serum 25(OH)D levels.

The clinical phenotype of atopic dermatitis (AD) results from interactions between genetic defects in the cutaneous barrier (abnormalities in filaggrin or proteins of the epidermis differentiation complex) and dysfunction of the innate and adaptive immune responses (1). Recent data have also provided further insights into the important role of immune responses in the pathogenesis of AD (2). The eczematous skin of patients is highly susceptible to bacterial colonization, and the composition of skin microbiota changes during AD flares and depends on the presence or absence of recent AD treatments (3). *Staphylococcus aureus* is the most frequent microorganism on the skin of patients with AD (4). In addition, the proportion of genetic sequences of *Staphylococcus* spp., particularly of *S. aureus*, is greater during disease flares than at baseline or after treatment and is correlated with disease severity (3).

S. aureus is a gram-positive bacterium that, although part of the normal microbiota of the skin, can give rise to virulent strains characterized by the expression of virulence factors and the acquisition of resistance to a number of different antimicrobial agents, including β -lactams (5). *S. aureus* colonization can produce a myriad of proinflammatory factors that may trigger the cutaneous immune system and might play a vital role in the perpetuation of the inflammatory phenomena. *S. aureus* toxins act as superantigens (e.g., staphylococcal enterotoxin sea, seb, sec, sed, and toxic shock syndrome toxin (tsst-1)) and may induce monocyte and lymphocyte activation, which in turn are stimulated to produce several inflammatory cytokines (6).

Evidence exists that vitamin D may have antimicrobial activity. It boosts innate immunity, modulating the production of antimicrobial peptides and cytokine response (7). Cathelicidin, an antimicrobial peptide, is known to be associated with AD. Vitamin D has been shown to suppress inflammatory responses, enhance antimicrobial peptide activity, and promote the integrity of the epidermal barrier (8), especially in patients with AD (9). Additionally, Liu et al (10) demonstrated a link between vitamin D-mediated activation of Toll-like receptors, the production of cathelicidin, and lower sensitivity to bacterial infections.

The aim of this study was to investigate the prevalence of colonization by *S. aureus* in skin lesions and the anterior nares of children with AD, as well as the presence of virulence genes in recovered isolates.

These data were analyzed in relation to serum 25-hydroxyvitamin D (25(OH)D), clinical severity, and the allergy status of patients.

PATIENTS AND METHODS

Study Design

A cross-sectional study was performed in patients diagnosed with AD ages 0 to 14 of both sexes at the Departments of Dermatology of San Jorge Hospital, Huesca, Spain, and Children University Hospital Niño Jesús, Madrid, Spain. All patients fulfilled the diagnostic criteria of Hanifin and Rajka and were on flare (11). Patients were recruited consecutively for 1 year, between January and December 2012. Patients who had undergone vitamin supplementation in the last 6 months were excluded.

The variables studied included age, sex, phototype, season, AD severity, allergy severity, serum 25(OH)D, microbiologic skin and nares colonization by *S. aureus*, and virulence genes of recovered isolates.

The severity of AD was measured using the objective Scoring Atopic Dermatitis (SCORAD) score of the European Task Force on Atopic Dermatitis and classified as mild (<15), moderate (15–40), or severe (≥ 40) (12).

Allergen-specific immunoglobulin E (IgE) antibodies (ImmunoCAP Phadiatop, fx5, and ImmunoCAP Phadiatop Infant test (Thermo Fisher Scientific; Sant Cugat del Vallés, Barcelona, Spain)) and total serum IgE were used to evaluate allergy. The Phadiatop Infant test is designed to detect allergen-specific IgE antibodies to food and inhalant allergens that are relevant in the development of IgE-mediated disease in children (13). Results greater than 0.35 UI/L were considered positive. Serum 25(OH)D level (electrochemiluminescence, Roche (Madrid, Spain)) was determined when the clinical examination was performed and the microbiologic samples gathered. Vitamin D deficiency was defined as serum 25(OH)D of 20 ng/mL or less and vitamin D insufficiency as less than 30 ng/mL (14).

Microbiologic Analysis

Sampling was conducted in the dermatology clinics by rolling a cotton swab on clinically uninfected lesional

skin (preferably from the antecubital or popliteal areas) and nares. The samples were sent in Cary Blair medium to microbiology laboratories, where swabs were seeded on solid media (blood agar and Colistine Nalidixic Agar, CNA agar, Oxoid, Basingstoke Hampshire, United Kingdom) and processed routinely. The strains were identified using biochemical and molecular species-specific methods (15).

Seventeen virulence genes were analyzed using polymerase chain reaction (PCR) in recovered *S. aureus* isolates (16). The genes and the encoded virulence factors tested were Panton-Valentine leukocidin (lukF/lukS-PV); toxic shock syndrome toxin (tsst-1); exfoliative toxins A, B, and D (eta, etb, etd, respectively); alpha-, beta-, delta-, gamma-, and gamma-hemolysin variant (hla, hlb, hld, hlg and hlgv, respectively); leukocidins DE and M (luk-DE and lukM, respectively); aureolysin (aur); biofilm-associated protein (bap); adhesion factor (cna); and enterotoxin B and C (seb and sec, respectively).

Ethics

Written voluntary informed consent was obtained from all parents or legal guardians. The ethics committees for Clinical Investigation of Aragón and the Children University Hospital Niño Jesús approved the study.

Statistics

Continuous variables were described as means and standard deviations. Dichotomous variables were described in terms of proportions. Associations between qualitative variables were assessed using the Pearson chi-square or Fisher exact test. Parametric (Student *t* or analysis of variance) or nonparametric (Mann-Whitney U or Kruskal-Wallis) statistical tests were used after normality criteria as assessed using the Kolmogorov-Smirnov test. Associations between *S. aureus* colonization and epidemiologic factors, variables related to AD severity (SCORAD) and allergy (Phadiatop), and serum IgE and 25(OH)D levels were evaluated using univariate analysis and stepwise logistic regression. The threshold for statistical significance was set as $p < 0.05$. Analysis was performed using SPSS version 19.0 (IBM, Armonk, NY).

RESULTS

Patient Characteristics

A total of 114 patients diagnosed with AD were included in the study, 70 from Huesca and 44 from

Madrid. Most were enrolled in winter (54.4%), followed by summer (25.4%), spring (10.5%), and autumn (7%). Epidemiologic and clinical characteristics of the sample are summarized in Table 1. Fifty-three (46.5%) were boys and 61 (53.5%) girls, with a mean age of 5.7 ± 4.1 (range 3 mos–14 yrs). Most of the children had skin phototype III (33.3%), II (24.6%), and IV (19.3%). Fourteen (12.3%) had mild AD severity according to SCORAD, 77 (67.5%) moderate, and 21 (18.4%) severe. Fifty-eight children (50.9%) had a positive Phadiatop Infant test and 51 (44.7%) negative.

S. aureus Colonization

Table 2 summarizes the microbiologic results of *S. aureus* colonization related to the epidemiologic and clinical characteristics of the patients. One hundred thirteen patients with AD had a swab from the skin, 84 also had a swab from the nares, and 1 had only a swab from the nares ($n = 198$ total microbiologic samples). Thirty-two of 113 patients (28.3%) were found to harbor *S. aureus* on the skin, 20 of 85 (23.5%) in the nares, and 7 in both locations. All *S. aureus* isolates recovered were methicillin-susceptible (MSSA) except one from the skin that was methicillin-resistant (MRSA; prevalence 0.9%).

TABLE 1. Demographic and Clinical Characteristics of Patients with Atopic Dermatitis ($N = 114$)

Characteristic	Value
Age, mean $\pm$ standard deviation	5.7 $\pm$ 4.1
Sex, n (%)	
Male	53 (46.5)
Female	61 (53.5)
Phototype, n (%)	
I	2 (1.7)
II	28 (24.6)
III	38 (33.3)
IV	22 (19.3)
V	10 (8.8)
VI	1 (0.9)
Unknown	13 (11.4)
Season, n (%)	
Winter	62 (54.5)
Spring	12 (10.5)
Summer	29 (25.4)
Autumn	8 (7)
Unknown	3 (2.6)
Scoring Atopic Dermatitis score, n (%)	
<15	14 (12.3)
15–39	77 (67.5)
≥ 40	21 (18.4)
Unknown	2 (1.8)
Phadiatop Infant test, n (%)	
Positive	58 (50.9)
Negative	51 (44.7)
Unknown	5 (4.4)

TABLE 2. Association Between *Staphylococcus aureus* Isolates from the Skin and Nares Samples and the Epidemiologic and Clinical Characteristics of Patients with Atopic Dermatitis

Characteristic	Cutaneous swab (n = 113)			Nare swab (n = 85)		
	Positive	Negative	p-Value	Positive	Negative	p-Value
Age, mean $\pm$ SD	7.8 $\pm$ 4.2	4.9 $\pm$ 3.8	0.001	5.2 $\pm$ 3.5	5.1 $\pm$ 4.1	0.91
Sex, n (%)						
Male	15 (28.8)	37 (71.2)	>0.99	13 (31.0)	29 (69.0)	0.13
Female	17 (27.9)	44 (72.1)		7 (16.3)	36 (83.7)	
Season, n (%)						
Winter	16 (25.8)	46 (74.2)	0.03	14 (25.0)	42 (75.0)	0.76
Spring	1 (8.3)	11 (91.7)		4 (33.3)	8 (66.7)	
Summer	14 (48.3)	15 (51.7)		1 (16.)	5 (84)	
Autumn	1 (12.5)	7 (87.5)		1 (12.5)	7 (87.5)	
Unknown	0	2		0	3	
Phototype, n (%)						
I	0 (0)	2 (100)	0.23	0 (0)	2 (100)	0.84
II	10 (35.7)	18 (64.3)		4 (25.0)	12 (75.0)	
III	16 (43.2)	21 (56.8)		7 (28.0)	18 (72.0)	
IV	5 (22.7)	17 (77.3)		5 (26.3)	14 (73.7)	
V	1 (10.0)	9 (90.0)		1 (11.1)	8 (88.9)	
VI	0 (0)	1 (100)		0 (0)	1 (100)	
Unknown	0	13		3	10	
Phadiatop Infant test, n (%)						
Positive	27 (46.5)	31 (53.5)	<0.001	11 (36.7)	22 (63.3)	0.05
Negative	2 (3.9)	49 (96.1)		8 (16.3)	41 (83.7)	
Unknown	3	1		2	7	
Scoring Atopic Dermatitis score, mean $\pm$ SD ^a	38.7 $\pm$ 16.3	23.8 $\pm$ 11.2	<0.001	28.5 $\pm$ 15.7	26.0 $\pm$ 13.5	0.49 ^b
N	31	80		20	64	
Unknown	1	1		0	1	
Immunoglobulin E, UI/L, mean $\pm$ SD	662.6 $\pm$ 1,043.0	195.1 $\pm$ 540.1	0.04	363.9 $\pm$ 708.1	137.8 $\pm$ 409.3	0.20 ^b
N	25	73		19	62	
Unknown	7	8		1	3	
25(OH)D ₃ , ng/mL, mean $\pm$ SD	29.7 $\pm$ 15.0	29.1 $\pm$ 10.9	0.87	22.8 $\pm$ 9.4	28.1 $\pm$ 10.2	0.04 ^b
N	30	78		20	62	
Unknown	2	3		0	3	
Total	32 (28.3%)	81 (71.7%)		20 (23.5%)	65 (76.5%)	

^aMild < 15, moderate 15–39, severe $\geq$ 40.^bStudent *t* test was used for quantitative variables.

SD, standard deviation.

Regarding skin colonization, the mean age of children harboring *S. aureus* was 7.8 $\pm$ 4.2 years, significantly higher than those without it (4.9 $\pm$ 3.8 years) (p = 0.001). The SCORAD score was higher in those children whose skin was colonized by *S. aureus* (38.7 $\pm$ 16.3) than those not colonized (23.8 $\pm$ 11.2, p < 0.001). *S. aureus* colonized the skin of 7.1% of patients with mild AD, 19.5% with moderate AD, and 71.4% with severe AD (p < 0.001). Forty-six percent (n = 27) of children with a positive Phadiatop test had *S. aureus* on cutaneous swabs, compared with 3.9% (n = 2) of those with a negative Phadiatop test (p < 0.001). There were differences in the frequency of colonization depending on the season, being significantly higher in summer (48.3% of summer samples) than in the rest of the year (p = 0.02). There was no relationship between cutaneous *S. aureus* colonization and sex, phototype, or serum 25(OH)D level. Multivariate analysis identified a positive Phadiatop test (odds ratio [OR] = 8.66 [95% confi-

dence interval [CI] 1.70, 44.09], p = 0.009) and severe AD (OR = 22.15 [95% CI 2.04, 240.9], p = 0.011) as risk factors for cutaneous *S. aureus* colonization in children with AD.

Nares colonization was more frequent in children with a positive Phadiatop test (36.7%) than in those without (16.3%) (p = 0.05). Children with nares colonization also had higher mean serum IgE levels (363 UI/L vs 137 UI/L), although this was not statistically significant (p = 0.20). The most remarkable finding was that mean serum 25(OH)D levels were lower in the group with nares colonization (22.8 $\pm$ 9.4 ng/ml) than in those without (28.1 $\pm$ 10.2 ng/ml) (p = 0.04). There was a significant association between nares and skin *S. aureus* colonization (p = 0.04).

Virulence Genes and Correlation with Clinical Variables

Forty of the 52 initial *S. aureus* isolates were characterized to determine their virulence genes: 26 from

TABLE 3. Frequency of Virulence Genes in the *Staphylococcus aureus* Recovered from Patients with Atopic Dermatitis

Virulence gene	Skin and nasal isolates (n = 40) N (%)	Skin isolates (n = 26) N (%)
Panton-Valentine leukocidin	0	0
Leukocidin DE	34 (85%)	25 (96.1%)
Leukocidin M	0	0
Toxic shock syndrome toxin	22 (55%)	13 (50%)
Exfoliative toxin A	5 (12.5%)	4 (15.4%)
Exfoliative toxin B	0	0
Exfoliative toxin D	0	0
Alpha-hemolysin	40 (100%)	26 (100%)
Beta-hemolysin	21 (52.5%)	17 (65.4%)
Delta-hemolysin	0	0
Gamma-hemolysin	15 (37.5%)	8 (30.8%)
Gamma-hemolysin variant	25 (62.5%)	18 (69.2%)
Gamma-hemolysin or gamma-hemolysin variant	40 (100%)	26 (100%)
Adhesion factor	16 (40%)	10 (38.5%)
Aureolysin	21 (52.5%)	11 (42.3%)
Biofilm-associated protein	0	0
Enterotoxin C	2 (5%)	2 (7.7%)
Enterotoxin B	1 (2.5%)	0

skin and 14 from nares. The other 12 isolates were not analyzed because of problems with their preservation. Table 3 shows the frequency of virulence genes in the 40 skin and nares isolates. Some virulence genes, such as hla (encoding alpha-hemolysin) and hlg or hlg_v (encoding gamma- or gamma-variant-hemolysin), were detected in all isolates; others, such as lukF/lukS-PV, lukM, etb, etd, hld, and bap, were not identified in our staphylococcal collection.

The virulence genes detected in the cutaneous isolates were hla and hlg/hlg_v (26, 100%), hlb (17, 65.5%), lukDE (25, 96.1%), tsst-1 (13, 50%), aur (11, 42.3%), cna (10, 38.5%), eta (4, 15.4%), and sec (2, 7.7%) (Table 3). All of the virulence genes were less frequently present in the isolates taken in summer than in those taken in winter except for the sec gene, although the differences were statistically significant only for the aur gene, present in one case of the isolates in the summer (7.7%) and nine in the winter (81.1%) ($p = 0.001$). No association was found between phototype and the virulence genes detected.

If we consider the presence of *S. aureus* in skin samples, mean serum 25(OH)D levels were lower in children with skin *S. aureus* harboring some virulence genes than in those without them. This difference was statistically significant for tsst1 (24.6 ± 12.0 vs 36.7 ± 14.6 ng/mL, $p = 0.001$) and aur (19.5 ± 8.7 vs 37.2 ± 14.2 ng/mL, $p = 0.001$) (Table 4). No significant association was found between the presence

TABLE 4. Association Between Virulence Genes of *Staphylococcus aureus* Isolates of Skin Origin and Clinical Characteristics

Gene		N	Mean $\pm$ standard deviation	p-Value*
Exfoliative toxin A		26		
Age	Positive	4	8.3 ± 4.0	>0.99
	Negative	22	8.0 ± 4.5	
SCORAD	Positive	4	44.1 ± 23.9	0.59
	Negative	22	39.6 ± 16.4	
Vitamin D level, ng/mL	Positive	4	19.5 ± 8.4	0.59
	Negative	21	31.3 ± 15.2	
	Unknown	1		
IgE, IU/L	Positive	3	98.7 ± 30.4	0.21
	Negative	17	$857.2 \pm 1,222.0$	
	Unknown	2		
Toxic shock syndrome toxin		26		
Age	Positive	13	7.4 ± 4.4	>0.99
	Negative	13	8.6 ± 4.5	
SCORAD	Positive	13	38.8 ± 18.4	>0.99
	Negative	13	41.8 ± 16.6	
Vitamin D level, ng/mL	Positive	13	24.6 ± 12.0	0.001
	Negative	12	36.7 ± 14.6	
	Unknown	1		
IgE, IU/L	Positive	9	$1,059.1 \pm 1,624.6$	>0.99
	Negative	11	485.2 ± 511.4	
	Unknown	6		
Aureolysin		26		
Age	Positive	11	5.8 ± 4.1	0.13
	Negative	15	9.6 ± 4.0	
SCORAD	Positive	11	36.9 ± 18.6	0.43
	Negative	15	42.8 ± 16.4	
Vitamin D level, ng/mL	Positive	11	19.5 ± 8.7	0.001
	Negative	14	37.2 ± 14.2	
	Unknown	1		
IgE, IU/L	Positive	10	271.3 ± 233.9	0.66
	Negative	10	$1,215.6 \pm 1,506.1$	
	Unknown	6		
Enterotoxin C		26		
Age	Positive	2	5.0 ± 1.4	0.48
	Negative	24	8.3 ± 4.5	
SCORAD	Positive	2	49.0 ± 7.1	0.48
	Negative	24	39.7 ± 17.7	
Vitamin D level, ng/mL	Positive	2	28.2 ± 20.3	>0.99
	Negative	23	29.5 ± 14.9	
	Unknown	1		
IgE, IU/L	Positive	1	1,558.0	>0.99
	Negative	19	$700.6 \pm 1,170.5$	
	Unknown	6		
Adhesion factor		26		
Age	Positive	10	7.6 ± 4.4	0.70
	Negative	16	8.3 ± 4.5	
SCORAD	Positive	10	39.0 ± 13.7	>0.99
	Negative	16	41.1 ± 19.5	
Vitamin D level, ng/mL	Positive	9	23.1 ± 9.5	0.10
	Negative	16	32.9 ± 16.4	
	Unknown	1		
IgE, IU/L	Positive	8	488.3 ± 484.1	0.65
	Negative	12	$913.6 \pm 1,441.3$	
	Unknown	6		

SCORAD, Scoring Atopic Dermatitis; Ig, immunoglobulin.

of any of the genes studied and the SCORAD score or positive Phadiatop test, although the mean IgE level in the children with *S. aureus* harboring *tsst-1* was double (mean 1059.11 IU/mL) that of the *tsst-1*-negative group (mean 485.18 IU/mL) ($p = 0.21$).

With regard to the nares isolates, no association was found between any of the virulence genes and the different variables studied.

DISCUSSION

S. aureus and vitamin D are two factors implicated in the pathophysiology of AD. The data show that the presence of some virulence genes in *S. aureus* that colonize individuals with AD was associated with lower serum 25(OH)D levels.

The present study shows the prevalence of *S. aureus* colonization in children with AD in Spain. This prevalence is lower (28.3%) than reported in previous publications in other countries (46.1%–100%) (17–19). The fact that only 18.4% of the individuals with AD had severe disease can explain this low colonization rate, and it is known that *S. aureus* colonization correlates with AD severity (19,20); 71.4% of our patients with severe AD (SCORAD ≥ 40) were colonized by *S. aureus*.

The prevalence of MRSA in our collection of *S. aureus* isolates was low (<1%), similar to that found in other studies conducted in our area (21). In addition, this finding agrees with previous reports that conclude that patients with AD do not seem to have greater susceptibility to infection or colonization with resistant strains of *S. aureus* than the general population (22,23).

According to our multivariate analysis, allergies and AD severity are significantly associated with cutaneous *S. aureus* colonization in children with AD. Breuer et al proposed that IgE-mediated hypersensitivity against enterotoxin and exotoxins may be one of the mechanisms by which *S. aureus* exacerbates AD (24,25), although a positive correlation between disease severity and *S. aureus* colonization of lesional and nonlesional skin has been reported (18,19,26).

The serum 25(OH)D level was the only variable associated with nares *S. aureus* colonization in our sample. Serum 25(OH)D is a determinant of *S. aureus* colonization in nonsmoking adults, with a 6.6% lower probability of colonization with each 5 nmol/L increase in serum 25(OH)D (27).

Like Williams et al (28), we found an association between recovery of *S. aureus* from lesional skin and nares in children with AD. Nevertheless, only skin colonization, not nasal, was independently associated

with AD severity, as previously described (20,29), although some authors have shown that significant nasal *S. aureus* colonization is clinically associated with more extensive lesions and signs of cutaneous infection in children with AD (30).

The most common virulence gene detected in our *S. aureus* isolates was *tsst-1*, similar to other studies from southern Europe (31), whereas in Asia, the staphylococcal enterotoxins A and B (sea and seb) were most frequently found in patients with AD (17,18,32). Some of these studies found an association between any of these virulence factors and AD severity, but we did not find a significant association between any of the virulence genes with severity or with allergy, although mean IgE level in the group of children with *S. aureus* harboring *tsst-1* was twice (mean 1059.11 IU/mL) that in the *tsst-1*-negative group (mean 485.18 IU/mL).

There is debate regarding optimal vitamin D serum levels for health. For the Institute of Medicine, 20 ng/mL is the upper range of human requirements (33) whereas, the American Endocrine Society states in its guidelines that the benefits of vitamin D occur only when serum 25(OH)D levels are 30 ng/mL and above (34). Additionally, the importance of bioavailable 25(OH)D, the part of serum vitamin D not bound to proteins, and the vitamin D-binding protein genotypes has recently been proposed (35). Our most remarkable finding was the association between lower serum 25(OH)D levels and some virulence genes. Whereas some studies found a correlation between AD severity and serum 25(OH)D concentration (36) or showed improvement after supplementation with oral vitamin D (37,38), others did not (39). Maybe this correlation occurs only in patients with allergic sensitizations, as has been recently proposed (40). On the other hand, Samochocki et al (41) found a significant association between bacterial skin infections in patients with AD and serum 25(OH)D levels lower than 30 ng/mL. Although our work did not study infection, we found no association between cutaneous *S. aureus* colonization and vitamin D insufficiency, although we found it with nares colonization.

Vitamin D deficiency has been linked to greater infectious diseases rates (42). Hata et al (9) demonstrated that supplementation with oral vitamin D can result in the correction of defects in cathelicidins of subjects with AD, and cathelicidin LL-37 is one of the few human bactericidal peptides with potent anti-staphylococcal activity. On the other hand, it has been proposed that seasonality may be a potent contributor to cathelicidin induction through its effect

on 25(OH)D levels, and our results might reinforce this hypothesis (9). In this sense, *S. aureus* with genetic expression of *aur* synthesizes aureolysin, which inactivates cathelicidins, which are low in the skin of patients with AD. According to our results, this virulence factor gene is related to low levels of 25(OH)D and is more frequent in winter isolates, although this could be in contradiction to the finding that *S. aureus* colonization was more prevalent in the summer than in the winter. Infection or colonization of the skin by *S. aureus* is more frequent in the summer (43). Ogawa et al (44) found significantly more *S. aureus* colonies in skin lesions of patients with AD and in more patients in the summer than in the winter, as we found. Other factors apart from cathelicidin status influence staphylococcal colonization of epithelial cells. Elegbe (45) proposes that local skin temperature and humidity due to perspiration in summer are highly conducive to the overgrowth of *S. aureus*.

Our study has the limitation that not all of the recovered isolates were studied to determine their virulence genes. This reduced the sample size, which could decrease the internal validity of the results. Another limitation is that the CIs of the ORs obtained in the multivariate analysis were wide; a larger sample would be needed to make them more precise. Finally, there are some missing data, especially phototypes (11.4%).

In conclusion, this is the first study to establish a relationship between lower serum 25(OH)D levels and some *S. aureus* virulence genes. The way this genetic information influences AD is unknown, but our findings contribute to further understanding how vitamin D influences AD.

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