Temporal Variation in *Staphylococcus aureus* Protein A Genotypes from Nose and Skin in Atopic Dermatitis Patients

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Keywords

Atopic dermatitis · Eczema · Microbiome · *Staphylococcus aureus*

Abstract

Background: *Staphylococcus aureus* colonization is associated with disease severity in patients with atopic dermatitis (AD). **Objective:** To investigate temporal variation in *S. aureus* protein A gene (*spa*)-types isolated from the nose and lesional skin and the correlation of *spa*-types with disease severity. **Results:** This study included 96 adult AD patients who were assessed at baseline (T0) and after a strict 2-week follow-up period (T1) in which treatment was standardized with a topical corticosteroid. Fifty-five different *spa*-types were detected in the nose and skin cultures. Seventy-three patients were colonized with *S. aureus* in the nasal cavity at both time points (persistent carriership), 59 of whom (81%) had identical *spa*-types over time. For skin samples, 42 (75%) of the 56 persistent skin carriers had identical *spa*-types over time. The same *spa*-type was carried in the nose and skin in 79 and 77% of the patients at T0 and T1, respectively. More

severe disease was not associated with specific *spa*-types or with temporal variation in *spa*-type. **Conclusion:** *S. aureus* strains in AD are highly heterogeneous between patients. The majority of patients carry the same *spa*-type in the nose and skin without temporal variation, suggesting clonal colonization within individual patients. No predominant *spa*-type or temporal variation is associated with increased disease severity.

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Introduction

Atopic dermatitis (AD) is the most common inflammatory skin disease with a lifetime prevalence of 15–20% and a significant negative impact on quality of life [1]. The pathogenesis of AD is multifactorial, involving skin barrier defects and immunological dysregulation [2]. Moreover, strong evidence links the skin microbiome, characterized by an increased abundance of *Staphylococcus aureus* and less bacterial diversity, to the pathogenesis and disease severity in this group of patients [2–6].

S. aureus is both a human commensal organism and a pathogen [7]. The most frequent carriage site is the nose, which is assumed to be the main reservoir for the spread of the pathogen associated with skin infections [7, 8]. Approximately 20% of the general population is a persistent carrier of *S. aureus* in the nose [9]. Cross-sectional data on AD patients show a prevalence of 62% in nasal *S. aureus* colonization and 70% on the lesional skin [3].

The association between *S. aureus* colonization and disease severity has been thoroughly investigated, but the underlying mechanism resulting in the characteristic AD inflammation remains unclear [10]. One of the difficulties in understanding the role of *S. aureus* in AD is that this bacterium is a common colonizer (in nose and on the skin). When *S. aureus* is present on the skin, it is difficult to understand which specific strains can cause inflammation as it can carry a wide diversity of virulence factors enabling transmission, virulence, and antibiotic resistance [11]. It was shown that the type of inflammation and immune signature characteristic of AD depends on the *S. aureus* strain [12]. Furthermore, it has been proposed that temporal variation in colonizing the *S. aureus* strain is an aggravating factor in AD due to a new trigger that is introduced to the immune system [13, 14]. This emphasizes that strain differences may contribute to the complexity of the disease, encouraging a more extensive investigation into the *S. aureus* populations in AD.

Multiple molecular identification methods are available for discriminating among *S. aureus* isolates. A single-locus typing technique, *spa*-typing, offers a subtyping resolution comparable to more expensive and/or laborious techniques such as multilocus sequence typing (MLST) and pulsed field gel electrophoresis (PFGE) [15]. The *spa*-typing technique targets the polymorphic region of the *S. aureus*-specific staphylococcal protein A gene (*spa*). The encoding region consists of a variable number of repeats with highly variable nucleotide sequences [16]. Since it is sequence-based, the results produced in different labs are easily comparable.

Literature on the temporal variation of *S. aureus* and the association with disease severity is scarce. Due to low patient numbers with multiple treatment regimens and the absence of a strict follow-up time, results are subject to cofounders [13, 14, 17]. This study investigates the temporal variation in *S. aureus spa*-types in adult AD patients with a strict follow-up period of 2 weeks and standardized treatment, which reduced possible confounding of the comparisons over time. Furthermore, we assess similarities in *spa*-types from the nose and skin and cor-

relations with disease severity. Knowledge of the colonization patterns of *S. aureus* in AD might help us to develop new strategies to improve inflammation.

Material and Methods

Study Design

This was a longitudinal study embedded in the MAAS trial (ClinicalTrials.gov NCT02840955). Details of the clinical trial and primary outcomes have been published previously [18, 19]. We included measurements from time point 0 (T0, baseline) and time point 1 (T1). T1 was scheduled after a 2-week run-in period, in which treatment was standardized with triamcinoloneacetonide 0.1% cream according to a corticosteroid dosing regimen.

Study Population

Adult patients (≥ 18 years) with moderate to severe AD, according to the UK Working Party diagnostic criteria for AD, defined by an Eczema Area and Severity Index (EASI) score of 7.1–50.0, were eligible for participation in the study. Other inclusion criteria were the use of any topical corticosteroid (TCS) before enrolment and ability to read and understand the patient information. Patients were ineligible for participation if they had used systemic antibiotics or systemic corticosteroids in the previous 2 months, oral immunosuppressive agents or ultraviolet light therapy in the previous 3 months, or topical antibiotics in the previous week. Another exclusion criterion was the presence of another skin condition that could interfere with the assessment of the AD severity. Patients were characterized by demographic data, and disease severity was assessed at both time points by the research physician using the EASI score. Based on the continuous EASI score, patients were categorized as having almost-clear, mild, moderate, or severe disease, as described previously [20]. This study included patients from whom bacterial cultures were collected at both time points.

Sample Collection and *spa*-Typing

Samples for culture analysis were collected using sterile dry Copan 490CE swabs, which were transferred into liquid Amies medium after collection. Skin swabs were collected from the lesional skin, preferably located at the antecubital fold or the popliteal fold at T0. These lesions did not show signs of impetigo. At T1, the skin swab was collected from the location chosen at T0. Nose swabs were collected from both the anterior nares at T0 and T1.

Bacterial semiquantitative cultures were performed using routine diagnostic culture procedures, blood agar plates, and specific *S. aureus* culture plates (ChromID *S. aureus* Elite agar [SAIDE], Biomérieux, France). Plates were incubated at 35 °C and growth was assessed after 1 and 2 days of culture. Subsequent species determination by MALDI-TOF (Bruker Daltonics, Bremen, Germany) was performed. For each distinct colony morphology of *S. aureus* in a sample, a representative colony was picked to store the strain at –20 °C for future analysis. The semiquantitative *S. aureus* cultures were reported on a categorical scale ranging from 0 (negative) to 4 (strongly positive).

DNA was extracted from the collected *S. aureus* isolates using a MagNA Pure 96 platform in combination with the MagNA Pure DNA and Viral Nucleic Acid Small Volume Kit (Roche Diagnostics, Almere, The Netherlands). PCR reactions were performed in

25-μL reactions using 1 μL of isolated DNA in 1× FastStart PCR Master (Roche) and 0.5 μM of both forward primer (5'-AACAAC-GTAACGGCTTCATCC-3') and reverse primer (5'-GCTTTTG-CAATGTCATTTACTG-3'). Thermal cycling consisted of an initial denaturation step for 10 min at 95 °C, followed by 35 cycles of 30 s at 95 °C, 30 s at 60 °C, and 1 min at 72 °C. After a final extension step of 10 min at 72 °C, reactions were cooled to room temperature. Five microliters of PCR product were analyzed on agarose gel to confirm amplification. The remainder of the PCR product was treated with 2 μL of ExoSAP-IT (Isogen Life Science, De Meern, The Netherlands) for 15 min at 37 °C following inactivation for 15 min at 80 °C. Amplicon sequencing was performed by BaseClear (Leiden, The Netherlands) using the forward amplification primer as a sequencing primer. Electropherograms were analyzed and interpreted using the *spa*-typing plugin in BioNumerics software v7.6 (Applied Maths, Sint-Martens-Latem, Belgium).

Statistical Analysis

Descriptive statistics were used to present patient and sample characteristics. Persistent nose and skin carriers were defined as patients with a positive *S. aureus* culture at both time points for the nose and skin, respectively. Intermittent carriers were defined as having a positive *S. aureus* culture at only 1 time point (T0 or T1), either in the nose or skin. For the difference in EASI score over time, the Wilcoxon signed rank test was used. The Mann-Whitney U test was performed to determine the differences in EASI score for *S. aureus* colonization and temporal variation in *spa*-type. The *S. aureus* load, as determined by semiquantitative culture, between both time points was tested using the χ^2 test. Statistical analysis was performed in SPSS v25. $p \leq 0.05$ was considered statistically significant.

Results

Population Characteristics

A total of 100 patients were eligible for participation in this study. Bacterial cultures were available from 96 patients at both time points and thus included in this study. The median age was 32.0 years (interquartile range [IQR] 25.0–47.0) and 45 (47%) patients were female. The median EASI score at T0 was 13.1 (IQR 9.4–19.0), compared to 8.1 (5.0–13.8) after the 2-week run-in period with triamcinolone ($p < 0.01$; Table 1). We found high colonization rates in both the nose and skin at both time points (Table 1; Fig. 1).

Heterogeneous *S. aureus* among AD Patients with Identical *Spa*-Types in the Nose and Skin

Of the 290 *S. aureus*-positive cultures (nose T0: 79; nose T1: 78; skin T0: 71; skin T1: 62), 285 isolates were available for *spa*-typing. All *S. aureus*-positive cultures contained only 1 distinct morphological colony type of *S. aureus*. Fifty-five different *spa*-types were found, with t002 (10%), t091 (8%), t127 (8%), t571 (7%), and t189

Table 1. Characteristics of 96 patients

Age, years	32.0 (25.0–47.0)
Female	45 (46.9)
Race	
American or Alaska-Native	5 (5.2)
Asian	10 (10.4)
Black or African-American	7 (7.3)
White	74 (77.1)
EASI ¹	
T0	13.1 (9.4–19.0)
T1	8.1 (5.0–13.8)
Positive for <i>S. aureus</i> nose ²	
T0	79 (82.3)
T1	78 (81.3)
Positive for <i>S. aureus</i> skin ³	
T0	71 (74.0)
T1	62 (64.6)

Values are expressed as *n* (%) or median (IQR). EASI, eczema area and severity index; T0, baseline; T1, 2 weeks after baseline.

¹ The EASI score ranges from 0 to 72.

² 73 (76.0%) patients were colonized in the nasal cavity at both time points.

³ 56 (58.3%) patients were colonized on the skin at both time points.

(6%) being the most common, accounting for < 40% of all *spa*-types. Figure 2 shows the prevalence of the 10 most common *spa*-types separated for the nose and skin. The total of 55 *spa*-types is depicted in online supplementary Table 1 and Figure 1 (for all online suppl. material, see www.karger.com/doi/10.1159/000515235). Furthermore, online supplementary Table 1 shows that the *spa*-types found in our study population are also present in previously investigated AD populations and within the general population [21–26].

Sixty-six patients had positive *S. aureus* cultures in both the nose and skin at baseline, including 79% (52/66) with identical *spa*-types at both locations. At T1, the group of patients positive at both sample locations included 59 patients, 46 of whom (78%) carried the same *spa*-type.

High Prevalence of Persistent *S. aureus* Carriers in the Nose and Skin with Identical *Spa*-Types

Our study population comprised 76% (73/96) persistent and 11% (11/96) intermittent nose carriers. Eighty percent (59/73) of the persistent nose carriers were colonized with the same *spa*-type during follow-up. On AD skin, 58% (56/96) patients were persistent carriers and 22% (21/96) intermittent carriers. In the group of persis-

Fig. 1. Temporal *S. aureus* colonization. Of the 84 patients positive at baseline, 66 (79%) were positive in both nose and skin, 13 (15%) only in the nose, and 5 (6%) only on the skin. Of the 81 patients positive at T1, 59 (73%) were positive for both nose and skin, 19 (23%) only in the nose, and 3 (4%) only on the skin.

Fig. 2. Frequency of the 10 most common *spa*-types per location and time point. The 10 most common *spa*-types in the nose samples at T0 represent 48.1% of the total *spa*-types. For the nose samples at T1, the 10 most common *spa*-types represented 53.8% of the total. For the skin samples at T0 and T1, this was 61.9 and 59.8%, respectively. The overview of all *spa*-types is presented in online supplementary Figure 1.

tent skin carriers, 79% (44/56) of the patients had identical *spa*-types over time.

Disease Severity Is Not Associated with the spa-Type Present in the Nose and Skin

Patients who were positive for *S. aureus* (nose and/or skin) at baseline had a significantly higher EASI score at T0 than those who were negative for *S. aureus* ($p = 0.02$). The median EASI score was 14.2 (IQR 9.9–19.2) and 9.1 (IQR 7.7–14.8), respectively. At T1, after the standardized

2-week run-in treatment with triamcinolone, there was no difference in EASI score anymore between *S. aureus*-positive and *S. aureus*-negative patients (EASI 8.5 [IQR 5.0–14.4] and 8.0 [IQR 4.8–12.0], respectively; $p = 0.55$). Interestingly, the EASI score and the *S. aureus* density in the nose and skin at T1 was significantly lower than at T0 (Table 1; online suppl. Table 2). Looking into the abundance of the 5 most common *spa*-types per severity category, we did not find an overrepresentation of a certain *spa*-type (online suppl. Fig. 2). Because of the high num-

ber of *spa*-types with a low prevalence and the fact that these numbers are accounted for by only a few patients, this was not amenable for statistical testing. Between patients with and without a change in *spa*-type over time (in the nose and/or skin), the change in EASI score from T0 to T1 did not differ significantly ($p = 0.11$; online suppl. Fig. 3).

Discussion

Our study showed a heterogeneous *S. aureus* population in AD patients, as seen in the general population. *Spa*-types t002, t091, t127, t571, and t189 were the most common and the remaining types were almost unique to 1 patient. Large proportion of the patients (79% at T0 and 78% at T1) had positive cultures in both the nose and skin and were colonized with only 1 *spa*-type. Eighty percent (59/73) of the persistent nose carriers were colonized with the same *spa*-type compared to 79% (44/56) of the persistent skin carriers. Lastly, we showed that disease severity was not affected by the *spa*-type present or by a change in *spa*-type over time.

Our results are in line with previous publications describing a heterogeneous population structure of *S. aureus* in AD and the absence of a prevailing strain [14, 21, 22]. Moreover, the most common *spa*-types found in our study population are also present in previously investigated AD populations and the general population, although the prevalence point estimates are not the same [14, 21–27]. This study confirmed that there are no specific *spa*-types preferentially colonizing patients with AD compared to healthy individuals. It could be argued that *spa*-typing does not have enough resolution to identify the genetic diversity between strains in AD. Compared to whole-genome sequencing (WGS) techniques, that examine complete genomes, *spa*-typing is based on a single gene [28]. However, WGS remains expensive to conduct in large series of cases and the *spa*-typing technique has proven successful in settings requiring high levels of discrimination [28, 29].

The nose is commonly described as the most important reservoir for *S. aureus* and several studies show identical *S. aureus* strains in the nose and infected skin regions [7, 30, 31]. Moreover, nasal eradication with the application of an anti-staphylococcal agent leads to decolonization at other body sites and can prevent infection in surgical and dialysis patients, suggesting a causal relationship between nasal carriage and infection [32, 33]. Our results show that up to 79% of AD patients carried the

same *spa*-type in the nose and skin, indicating clonal colonization within individual patients and a possible role for endogenous *S. aureus* transmission from nose to skin and/or vice versa. These data form an important lead for future intervention studies investigating the effect of nasal *S. aureus* eradication on skin colonization patterns and their role in current treatment guidelines.

A large proportion of patients were persistent *S. aureus* carriers in the nose (76%) and on the skin (58%). There is no general definition for persistent and intermittent skin carriers or consensus on how many cultures should be taken from a skin site to define these groups [7, 31]. Studies on non-AD patients suggest that persistent carriers are colonized by a single strain in the nasal cavity over time and have a higher *S. aureus* load, with an increased risk of infection [7]. Literature on this subject in AD patients is scarce and include low patient numbers, but seem to confirm these findings [13, 14, 17]. Due to the absence of a general definition on carriership, the comparison between studies investigating *S. aureus* colonization patterns and temporal strain variation is difficult, as different sample frequencies and definitions have been used. Our study, with a strict 2-week follow-up and a large sample size, confirmed high rates of colonization by a single strain per individual in persistent carriers. We did not find a difference in disease severity between patients with and without temporal variation in colonizing *S. aureus* strain. However, more studies with larger populations should follow.

We found a significantly higher EASI score at baseline in patients positive for *S. aureus* in comparison to *S. aureus*-negative patients, which was not observed anymore after the 2-week run-in period with triamcinolone (T1). Furthermore, a significantly lower EASI score and *S. aureus* load were seen at T1 than at T0. The discrepancies in these results could be explained by the standardized TCS treatment, which is known to suppress inflammation in AD skin. Our hypothesis is that, due to a suppression of inflammation, the skin can recover, making it less prone to *S. aureus* overgrowth. Thus, the use of TCS disturbs the correlation at this time point. This was also proposed in a previous study, showing a reduction in *S. aureus* load and a correlation with disease severity after the use of standardized TCS treatment [34].

A major strength of this follow-up study is that all patients were sampled within the same interval of 2 weeks. Because treatment was similar in all patients, our results were not subject to differences in treatment regimens between participants. However, it is likely that the standardized use of TCS affected the disease severity and *S. aureus*

density by inhibiting the inflammatory response, preventing subsequent damage to the skin and making it less prone to *S. aureus* overgrowth.

This study has several limitations. First, due to the lack of control group, we were not able to compare the presence of certain *spa*-types to a well-characterized control population. For this reason, we compared the *spa*-types in our study population with those in previous publications that described genetic populations in the general population and with data from “ridom SpaServer” [26]. Second, as mentioned before, due to the *spa*-typing technique used, we may have not detected changes in other genes that may be important in AD. Last, due to the high heterogeneity and low patient numbers per *spa*-type, statistical testing for differences between the genetic types was difficult. Larger studies using WGS will help to clarify the role of specific *S. aureus* genes rather than specific strains. Furthermore, the lack of association of specific *spa*-types with disease severity encourages further investigation of the potential role of other virulence factors.

In conclusion, our results show that the colonizing *S. aureus* strains are not specific for AD and might rather be caused by host-genetic and environmental factors. The majority of the AD patients were found to be persistent *S. aureus* carrier with identical *spa*-types in the nose and skin. The results of this study underline the relevance of investigating the clinical benefit of anti-staphylococcal treatment in AD patients that are persistently colonized with *S. aureus*.

Key Message

The skin and the nose are mostly colonized by a single strain of *S. aureus*. It is clinically relevant to investigate the benefit of anti-staphylococcal treatment in persistent *S. aureus* carriers.

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Statement of Ethics

This research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki. Study procedures were reviewed and approved by the Medical Ethics Committee of the Erasmus MC University Medical Center Rotterdam, the Netherlands (MEC-2016–233). Signed informed consent was obtained from all participants.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

S.G.M.A.P., J.E.E.T., J.W., and L.M.P. designed the study. M.M.F.M., J.E.E.T., and J.W. collected all the data. B.L.H. supervised the bacterial semi-quantitative culture and storage of colonies. M.C.V. and C.H.W.K. supervised the *spa*-typing. M.M.F.M., C.H.W.K., and L.M.P. performed the data analysis. M.M.F.M. wrote the manuscript. L.M.P. supervised the study. All authors critically commented on the manuscript.

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