

Effect of a lotion containing the heat-treated probiotic strain *Lactobacillus johnsonii* NCC 533 on *Staphylococcus aureus* colonization in atopic dermatitis

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Objective: *Staphylococcus aureus* dominates the skin microbiota in patients with atopic dermatitis (AD), with bacterial loads correlating with disease severity. The aim of this exploratory study was to investigate the effect of a cosmetic lotion containing heat-treated *Lactobacillus johnsonii* NCC 533 (HT La1) on *S. aureus* colonization in AD patients.

Methods: This open-label, multicenter study was performed in AD patients in Germany. First, detection of *S. aureus* was performed in all patients using the swab or scrub-wash method of sampling, followed by quantitative culture or quantitative polymerase chain reaction. Repeatability and reproducibility of all method combinations were evaluated to select the best combination of sampling and quantification. Second, a lotion containing HT La1 was applied to lesional skin twice daily for 3 weeks. Scoring using local objective SCORing Atopic Dermatitis (SCORAD), measurement of *S. aureus* load, and lesional microbiome analysis were performed before and after the 3-week treatment period.

Results: Thirty-one patients with AD were included in the study. All sampling and quantification methods were found to be robust, reproducible, and repeatable for assessing *S. aureus* load. For simplicity, a combination of swab and quantitative polymerase chain reaction was chosen to assess the efficacy of HT La1. Following application of a lotion containing HT La1 to AD lesions for 3 weeks, a reduction in *S. aureus* load was observed in patients, which correlated with a decrease in local objective SCORAD. Interestingly, high baseline skin concentrations of *S. aureus* were associated with good responses to the lotion.

Conclusion: This study demonstrated that the application of a lotion containing HT La1 to the lesional skin of patients with AD for 3 weeks controlled *S. aureus* colonization and was associated with local clinical improvement (SCORAD). These findings support further development of topical treatments containing heat-treated nonreplicating beneficial bacteria for patients with AD.

Keywords: atopic dermatitis, *S. aureus*, heat treated La1, HT La1, *L. johnsonii* NCC 533, microbiology

Introduction

Atopic dermatitis (AD) is a chronic pruritic inflammatory skin disease that follows a relapsing course with recurrent eczematous lesions. The prevalence of AD has doubled or tripled in industrialized countries during the past three decades, with 15%–30% children and 2%–10% adults currently affected by this disease.^{1,2}

It is well known that *Staphylococcus aureus* has a high prevalence on the skin of patients with AD,³ and that patients with AD are prone to *S. aureus* infection and skin colonization.^{3–6} Early studies have shown that 85% to 93% of lesional sites in patients with AD are colonized by *S. aureus*, with bacterial loads exceeding 10⁴ colony forming units (CFU) per cm² of skin. In addition, more recent studies have shown that *S. aureus* loads correlate with disease severity and that staphylococci represent 90% of the skin microbiota during untreated AD flares.^{7,8} However, in a recent meta-analysis of 95 selected observational studies, Totté et al concluded that patients with AD were more likely to be colonized with *S. aureus* on lesional skin than healthy controls but found high heterogeneity between studies in the prevalence and load of *S. aureus* colonization.⁹ Overall, these observations indicate that *S. aureus* might play a role in important aspects of AD pathogenesis and that strategies to decrease skin colonization by *S. aureus* in patients with AD would likely help to reduce the frequency and severity of flares.¹⁰

Lactobacillus johnsonii NCC 533 (hereunder designated as La1) is a member of the acidophilus group of intestinal lactobacilli that have been extensively studied for their “probiotic” properties that include pathogen inhibition, epithelial cell attachment, and immunomodulation.¹¹ In vitro experiments on monolayer keratinocytes have shown that La1 can adhere to keratinocytes and impair *S. aureus* attachment to these cells and was one of the most effective among different *Lactobacillus*, *Micrococcus*, and *Bifidobacterium* strains (NRC patent, WO01/45721 A1). Interestingly, when inactivated by heat treatment, *L. johnsonii* La1 (HT La1) maintains its capacity to reduce *S. aureus* attachment on keratinocytes and its in vitro immune profile (Nestlé Research Center data). HT La1 displayed a concentration-dependent inhibitory effect on *S. aureus* adhesion and concentration of 0.3% (0.93×10⁹ CFU/mL), which led to a 54% decrease in *S. aureus* adhesion, was selected for the clinical evaluation in this study. Additionally, our studies (Rosignoli et al, submitted) have shown that HT La1 can increase the gene expression and protein synthesis of certain antimicrobial peptides, including β -defensin 2, which is known to be altered in AD and to be a very important component of innate immunity.¹² All these observations suggest that a topical formulation containing HT La1 may reduce *S. aureus* colonization in AD patients.

Swab sampling to determine skin *S. aureus* load is widely used. However, the scrub wash method described in 1965 by Kligman is unique to cutaneous sampling and may provide a more robust and sensitive technique to recover bacteria.¹³

Determination of skin *S. aureus* load by quantitative culture on a chromogenic medium is the gold standard. However, it is time consuming (24–48 h of bacterial growth), labor intensive, and requires fresh samples. Identification of *S. aureus* by quantitative (real time) polymerase chain reaction (qPCR) has already demonstrated excellent specificity and sensitivity for detecting *S. aureus* in nasal specimens¹⁴ and is easier to implement in a clinical study as it can be performed on frozen samples.

Therefore, this study aims to first compare the robustness and reproducibility of two sampling methods of skin microbiota: swab and scrub wash, as well as to evaluate bacteria quantification methods using culture and qPCR. In the second part of this study, the effect of a topical lotion containing HT La1 on *S. aureus* colonization and its clinical efficacy in AD was assessed.

Materials and methods

Study design

This was an open label exploratory study in patients with AD conducted at three centers in Germany. The study consisted of two parts: 1) an assessment of *S. aureus* sampling and quantification methods and 2) evaluation of a cosmetic lotion containing a heat-treated probiotic. The first part of the study included all enrolled patients (Population 1, N=31) whereas the second part of the study included only those patients who were found to be carriers of *S. aureus* at the start of the study and presented with clinically visible lesions on Day 8 (Population 2, N=21). Both parts of the study are described in detail below. This study was carried out in accordance with the Declaration of Helsinki and in agreement with the ethical guidelines of the European Independent Ethics Committee. The protocol was submitted and approved by ethics committee of La Charité center (number EA1 7121/15) and the Freiburger ethik-kommision international (number 015/1154). All patients gave written informed consent to participate in this study.

Study population

Male and female patients aged 18–75 years diagnosed with AD of mild-to-moderate severity at baseline (Investigator’s Global Assessment scores of 2 or 3) who exhibited at least one AD lesion of >20 cm² were eligible for inclusion in the study.¹⁵ The location of AD lesions had to allow sampling with the scrub wash method.

For the purposes of this study, lesional skin was defined as an active lesion of AD: an erythematous plaque with or without oozing, vesiculation, crusting, excoriations, or

erosions but with no overt signs of infection, located within the antecubital or popliteal fossae, or other region typical of AD.

Exclusion criteria included severe fungal infection or other skin disease that could affect AD diagnosis, any uncontrolled or serious disease, or any condition that could interfere with data interpretation or put the patient at significant risk. Patients who did not respect the appropriate wash-out periods for topical and systemic steroids, topical and systemic antibiotics, and topical antiseptics—the use of which was prohibited during the study—before baseline (Day 1) were also excluded.

Evaluation of sampling and quantification methods

In the first part of the study, enrolled patients were assigned to one of the two groups to compare the swab (Group 1) and scrub wash (Group 2) sampling methods from a 3 cm diameter O-ring area of an AD lesion. At baseline, two swabs (first and second sampling) were taken from adjacent areas on one representative lesion (target lesion) of Group 1 patients (Figure 1) to assess repeatability. Similar scrub wash samplings were performed in patients assigned to Group 2. At Days 3 and 8 (before treatment), target lesions were sampled with both the swab and scrub wash methods from adjacent areas of the target lesion to evaluate the reproducibility of the methods.

The patients were instructed to avoid bathing for 24 h prior to all sampling time points and avoid showers and emollient on the day of the visit.

Swab sampling was carried out using a nylon flocked swab (eSwab 480CE [COPAN ITALIA spa, Brescia, Italy]) dipped in normal saline solution, in a 3 cm diameter O-ring.

Skin microbiota was recovered in 1 mL Amies solution (COPAN ITALIA spa, Brescia, Italy). Scrub wash sampling was also performed using a 3 cm diameter O-ring. Briefly, 3 mL of buffer was added inside the O-ring then skin was scrubbed with a flat-ended rod and wash fluid was collected for *S. aureus* quantification. Both methods of sampling were performed by the study investigator or designee and samples were sent within 24 h at 4°C to an independent center (CHU, Saint-Etienne, France) for *S. aureus* quantification using both qPCR and quantitative plate culture. For qPCR, the Cepheid® Xpert SA Nasal Complete Assay was used with the GeneXpert® System (Cepheid, Maurens-Scopont, France). Quantitative plate culture was performed on selective chromogenic medium (BBL CHROMagar Staph aureus [Becton Dickinson, Le Pont de Claix, France]) using the EasySpiral dilute instrument (Interscience, Saint-Nom-la-Bretèche, France).

Clinical evaluation of HT La1 lotion

Strain La1 is a proprietary strain of the Nestlé Culture Collection. HT La1 was supplied to Galderma R&D in spray dried powder form, with an initial concentration of 3.1×10^{11} equivalent CFU/g (Nestlé PTC, Konolfingen, Switzerland).

For clinical assessment, a lotion (COS daily moisturizing lotion) containing HT La1 at 0.3% (w/w): designated as HT La1 lotion, was developed by Galderma R&D under good manufacturing practice conditions and was supplied to eligible participants. The lotion consisted of oil-in-water emulsion with oil phase being mainly composed of sunflower seed oil, caprylic/capric triglyceride, *Butyrospermum parkii* and the water phase of glycerin and xanthan gum. The complete composition is available in the pending patent PCT/EP2016/074065 (example 2).

On Day 8, patients included in the assessment of HT La1 lotion (i.e., *S. aureus* carriers on the target lesion identified at baseline and target lesion still present at the beginning of the treatment) were asked to apply HT La1 lotion on the target lesion in place of their usual moisturizer twice daily for 21(±1) days, leaving at least 6 h between applications. The contralateral lesion (if available) was either untreated or treated with the patient's usual moisturizer.

For bacterial quantification, samples from target and contralateral lesions (if available) at Days 8 and 28 were analyzed using a combination of swab and qPCR. Samples were also collected from patients using the swab method on the target lesion at Days 8 and 28 and frozen at -20°C prior to analysis of microbiota profile by GenoScreen (Lille, France). Bacteria were identified at the genus level using 16S rRNA gene sequencing of the hypervariable regions V3–V4 with

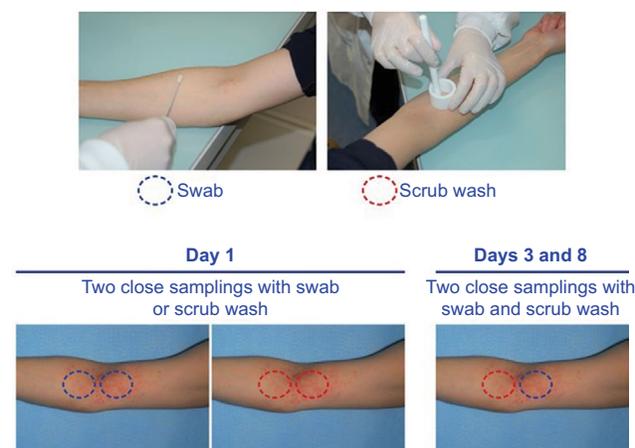


Figure 1 Swab and scrub wash sampling techniques.

454 sequencing technology on a GS FLX+ system (Roche, Basel, Switzerland). The efficacy of HT La1 lotion on disease severity was evaluated by local objective SCORing Atopic Dermatitis (SCORAD) on Days 8 and 28, which included assessment of erythema, edema, or papulation, oozing or crusting, excoriation, lichenification, and dryness of the treated lesion.^{16,17}

Tolerability

Cutaneous tolerability of the sampling method (Population 1, N=31) and HT La1 lotion application (Population 2, N=21) was evaluated at each visit based on signs of erythema, induration or papulation, oozing or crusting, excoriation, lichenification, and pruritus in the target lesion or contralateral lesion (where available). All signs were classified according to intensity (0=none, 1=mild, 2=moderate, and 3=severe).

Statistical analysis

A sample size of 15 patients was deemed sufficient for this study. However, with an expected *S. aureus* carriage rate of 50% in AD patients, at least 30 patients needed to be included at Day 1 (where *S. aureus* carriage was determined) to ensure sufficient patients for HT La1 lotion assessment.

For the methodology part, all patients enrolled in the study from baseline to Day 8 (before treatment) (Population 1, N=31), were included in the corresponding statistical analyses. For HT La1 lotion assessment, patients from Population 2 (N=21) only (i.e., with *S. aureus* colonization at baseline) were included in statistical analyses.

The safety population included all patients who were included in the study.

Patient disposition, baseline characteristics, previous and concomitant therapies were summarized by descriptive statistics.

Pearson's coefficient of correlation was calculated for comparison of quantification methods (qPCR vs culture) and sampling methods (scrub wash vs swab).

Differences in *S. aureus* load between Days 8 and 28 were tested using Wilcoxon signed rank test. A *p*-value of <0.05 was used to indicate significance. Statistical analysis on local objective SCORAD assessment was performed using the paired *t*-test.

Results

Baseline demographics and disease characteristics of all enrolled patients

A total of 31 patients were enrolled and participated in the first part of the study (Population 1). The mean age of patients was

33.5±11.6 years and both genders were equally represented as 16 (51.6% of patients were females [Table 1]). The majority of patients (80.6%) had AD of moderate severity, with a mean target lesion local objective SCORAD of 30.5±8.2 and mean disease duration of 27.1±14.2 years. Target lesions were mostly located on the elbow (in 14 [45.2%] patients) and forearm (in 7 [22.6%] patients). Twenty patients (64.5%) presented with contralateral lesions. Similar to the target lesions, the majority of contralateral lesions were located on the elbow (in 9 [45%] patients) and the forearm (in 6 [30%]).

A total of 21 patients who were *S. aureus*-positive at baseline (for one sample, whatever the quantification method) and still presented clinically visible AD lesions on Day 8 were eligible for treatment and included in the second part of the study (Population 2). Baseline characteristics were similar between the two populations and no statistically significant difference was found between them, except that there was a majority (66.7%) of male patients in Population 2 (Table 1). All patients completed the study.

Table 1 Baseline demographics and disease characteristics

Baseline characteristics	Population 1	Population 2
Patients, n	31	21
Age, years (SD)	33.5 (11.6)	33.0 (12.5)
Female, n (%)	16 (51.6)	7 (33.3)
Male/female ratio	0.94	2
Atopic dermatitis		
Severity, n (%)		
Mild	2 (6.5)	0 (0.0)
Moderate	25 (80.6)	18 (85.7)
Severe	4 (12.9)	3 (14.3)
Atopic dermatitis duration, years (SD)	27.1 (14.2)	26.5 (14.7)
Target lesion SCORAD score (SD)	30.5 (8.2)	32.2 (7.0)
Contralateral lesions, n (%)	20 (64.5)	15 (71.4)
Location of target lesions, n (%)		
Ankle	1 (3.2)	1 (4.8)
Arm	1 (3.2)	0 (0.0)
Elbow	14 (45.2)	11 (52.4)
Forearm	7 (22.6)	4 (19.0)
Knee	3 (9.7)	2 (9.5)
Thigh	1 (3.2)	1 (4.8)
Tibia	3 (9.7)	1 (4.8)
Trunk	1 (3.2)	1 (4.8)
Location of contralateral lesions, n (%)		
Elbow	9 (45.0)	8 (53.3)
Forearm	6 (30.0)	4 (26.7)
Knee	2 (10.0)	1 (6.7)
Thigh	1 (5.0)	1 (6.7)
Tibia	2 (10.0)	1 (6.7)

Notes: Population 1= enrolled patients; Population 2= patients who were found to be carriers of *S. aureus* at the start of the study and presented with clinically visible lesions on Day 8.

Abbreviations: SD, standard deviation; SCORAD, SCORing Atopic Dermatitis.

Evaluation of sampling and quantification methods

At baseline, skin samples (first and second sampling) were collected from the target lesion using the swab or scrub wash methods from a standardized surface (3 cm diameter O-ring area). Presence and levels of *S. aureus* on the target lesion at baseline are presented in Table 2.

More than 50% of the patients in Groups 1 and 2 of Population 1 were *S. aureus* carriers on the target lesion at Day 1 with mean bacterial loads ranging from 1.42 to 2.31 Log₁₀ CFU/cm², depending on the combination of sampling and quantification methods used (Table 2). The two methods of quantification yielded comparable *S. aureus* counts. A good correlation between qPCR and culture (Pearson's correlation coefficient approaching 1 [0.92, $p < 0.001$]) was observed despite a slightly higher bacterial load generally detected following culture compared with qPCR. Overall, no major differences were noted in terms of *S. aureus* concentrations

between swab and scrub wash sampling, although there was less variability using the scrub wash method as shown by the smaller standard deviations.

A good correlation was also found between first and second samples taken from AD lesions of patients at Baseline using both sampling methods (swab or scrub wash) and both quantification methods (culture or qPCR), indicating good repeatability of the methods (Pearson's correlation coefficient ≥ 0.83 [$p < 0.0001$]; Figure 2). In the same way, analysis of the variance of *S. aureus* counts (CFU/cm²) at Days 1, 3, and 8 (before treatment) showed that there was no time effect for swab ($p = 0.340$) and scrub wash ($p = 0.532$) in *S. aureus*-positive patients, suggesting that both sampling methods were reproducible.

Since swab and scrub wash were performed in 2 groups of patients at Day 1 (Figure 1), the direct comparison of the two methods was performed on Day 3. Using scrub wash, the mean *S. aureus* load ($2.08 \text{ Log}_{10} \text{ CFU/cm}^2 \pm 1.70$ for qPCR

Table 2 Presence and concentration of *Staphylococcus aureus* on the target lesion at baseline (Population 1)

Sampling method	Sampling	Quantification method	Presence of <i>S. aureus</i> (%)	Concentration of <i>S. aureus</i> (Log ₁₀ CFU/cm ²)	
				Mean \pm SD	Median (Min, Max)
Swab (Group 1)	First	Culture	8/16 (50.0)	2.16 \pm 2.25	0.73 (0.31, 6.21)
		PCR	8/15 (53.3)	1.56 \pm 2.03	0.57 (0.01, 5.93)
	Second	Culture	8/16 (50.0)	1.71 \pm 1.85	0.81 (0.31, 5.87)
		PCR	8/16 (50.0)	1.42 \pm 1.93	0.37 (0.01, 5.69)
Scrub wash (Group 2)	First	Culture	9/15 (60.0)	2.30 \pm 1.65	2.20 (0.61, 5.25)
		PCR	10/15 (66.7)	1.92 \pm 1.40	2.03 (0.31, 3.90)
	Second	Culture	9/15 (60.0)	2.31 \pm 1.72	1.91 (0.31, 5.02)
		PCR	10/15 (66.7)	2.00 \pm 1.44	2.19 (0.31, 4.35)

Notes: In the absence of *S. aureus* detection, the missing value was replaced by the limit of quantification of the method (culture: 10 CFU/mL; PCR: 5 CFU/mL). Population 1 = enrolled patients.

Abbreviations: CFU, colony forming units; SD, standard deviation; Min, minimum; Max, maximum; PCR, polymerase chain reaction.

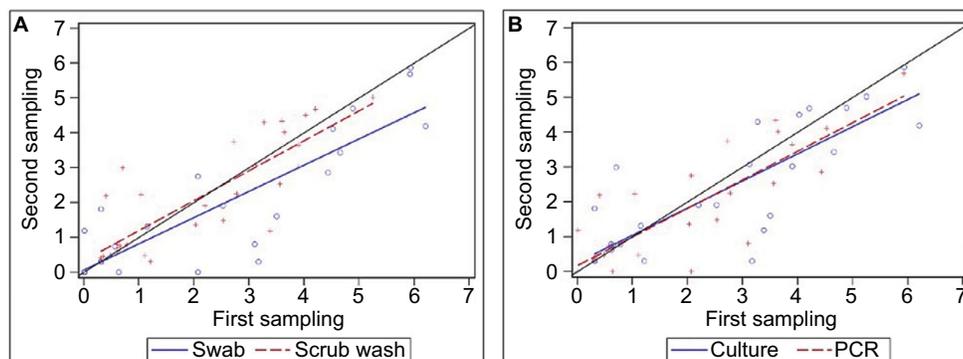


Figure 2 Repeatability (first and second sampling) of *Staphylococcus aureus* sampling (A) and quantification (B) methods at Day 1 for load determination (Log₁₀ CFU/cm²) in target atopic dermatitis lesions.

Notes: Results are presented for both quantification methods (A) and both sampling methods (B). Pearson's correlation coefficient ≥ 0.83 .

Abbreviations: CFU, colony forming units; PCR, polymerase chain reaction.

and $2.21 \text{ Log}_{10} \text{ CFU/cm}^2 \pm 1.80$ for culture) was slightly but significantly higher when compared with swab whatever the quantification method ($1.83 \text{ Log}_{10} \text{ CFU/cm}^2 \pm 1.77$ for qPCR and $1.47 \text{ Log}_{10} \text{ CFU/cm}^2 \pm 1.66$ for culture). Though, the Pearson's coefficient of correlation was equal to 0.77 ($p < 0.0001$), indicating that the two methods are strongly correlated.

In summary, we observed that qPCR and culture were very well correlated and not statistically different; likewise, the sampling methods (swab and scrub wash) were strongly correlated and all 4 couples of methods displayed a good repeatability and reproducibility. Since all methods of sampling and quantification were robust and reliable, the combination of swab and qPCR was selected to test the efficacy of the lotion containing the HT La1 as these techniques are easier to use.

Clinical evaluation of HT La1

COS daily moisturizing lotion containing 0.3% HT La1 was applied twice daily on the target lesions for 21 ± 1 days. Following 3-week application, the lotion was found to control *S. aureus* load of the treated target lesions compared with contralateral lesions not treated with HT La1 lotion ($-1 \text{ Log}_{10} \text{ CFU/cm}^2$, $p < 0.05$). Indeed, in the 15 patients having a contralateral lesion, median *S. aureus* counts remained stable in the treated AD lesions ($1.53 \text{ Log}_{10} \text{ CFU/cm}^2$ on Day 8 to $1.36 \text{ Log}_{10} \text{ CFU/cm}^2$ on Day 28, $p < 0.001$) compared with contralateral lesions not treated with HT La1 lotion ($1.9 \text{ Log}_{10} \text{ CFU/cm}^2$ on Day 8 to $3.3 \text{ Log}_{10} \text{ CFU/cm}^2$ on Day 28) where an increase was observed (Figure 3). Using a logistic model to assess the role of *S. aureus* concentration at Day 8 in patient responsiveness to HT La1 lotion at Day 28, high *S. aureus* skin load at the time of lotion application was found to be a predictive factor of the microbiological response (point estimate: 3.117; Chi-square test: $p < 0.05$). In those patients for whom *S. aureus* levels decreased in response to HT La1 lotion, the minimum *S. aureus* concentration at Day 8 was $2.89 \text{ Log}_{10} \text{ CFU/cm}^2$.

The 16S rRNA gene sequencing-based analysis of microbiota composition and diversity was performed on samples from the target lesions of 15 patients at Days 8 and 28. Considering the most common skin phyla (*Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Bacteroidetes*), the *Firmicutes* phylum was the only one significantly modified by the treatment. A noticeable reduction in the proportion of the Staphylococcaceae family was observed between Days 8 and 28 in AD lesions treated with HT La1 lotion (Figure 4) and was almost the only phyla modified. Also, a slight reduction of the Shannon diversity index was observed between

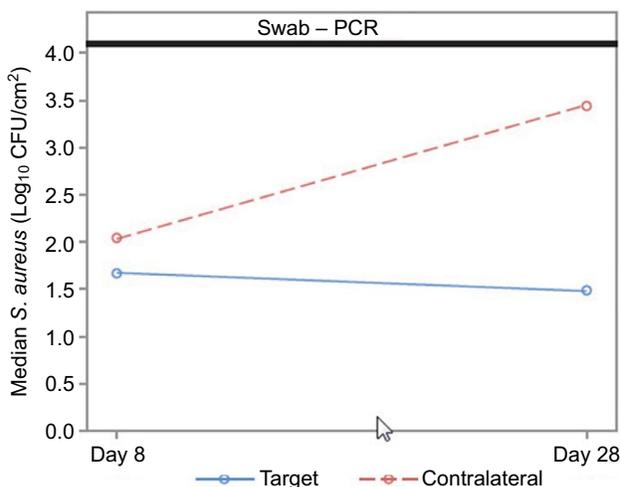


Figure 3 *Staphylococcus aureus* load evolution between Days 8 and 28 in target atopic dermatitis lesions treated with heat-treated *Lactobacillus johnsonii* lotion vs contralateral lesions untreated or treated with patient's usual moisturizer (n=15).

Notes: Bacterial sampling and quantification were performed by swab and quantitative polymerase chain reaction. The difference between *S. aureus* load in target vs contralateral lesions at Day 28 was significant ($p < 0.05$ using Wilcoxon signed rank test).

Abbreviations: CFU, colony forming units; PCR, polymerase chain reaction.

Days 8 and 28, which was not statistically significant (data not shown).

Regarding the disease severity of the target lesion, the use of HT La1 lotion for 3 weeks led to a statistically significant decrease in mean SCORAD values of 4.93 ± 8.23 between Days 8 (31.2 ± 9.2) and 28 (26.2 ± 7.9) ($p = 0.012$; Figure 5).

Tolerability

When applied to the lesional skin of patients with AD, HT La1 lotion was found to be well tolerated.

Discussion

Several clinical studies have demonstrated that patients with AD are more likely to be colonized with *S. aureus* on lesional skin than healthy controls, although the prevalence of *S. aureus* colonization varies among studies.⁹ These variations are likely to be due to differences in the studied populations (e.g., age, ethnicity, disease severity), the control populations and in the sampling methods. Furthermore, patients with AD have higher *S. aureus* titers on their skin than healthy controls, with mean titers of around 10^4 CFU/cm^2 on lesional skin that can reach up to 10^7 CFU/cm^2 in the acute phase, and a correlation between *S. aureus* titers and disease severity has been reported in some studies.^{7,18,19} Moreover, the skin microbiota in AD is dominated by *S. aureus*, especially during flares.⁷

These findings support a role for *S. aureus* in the pathogenesis of AD, although the precise mechanism of

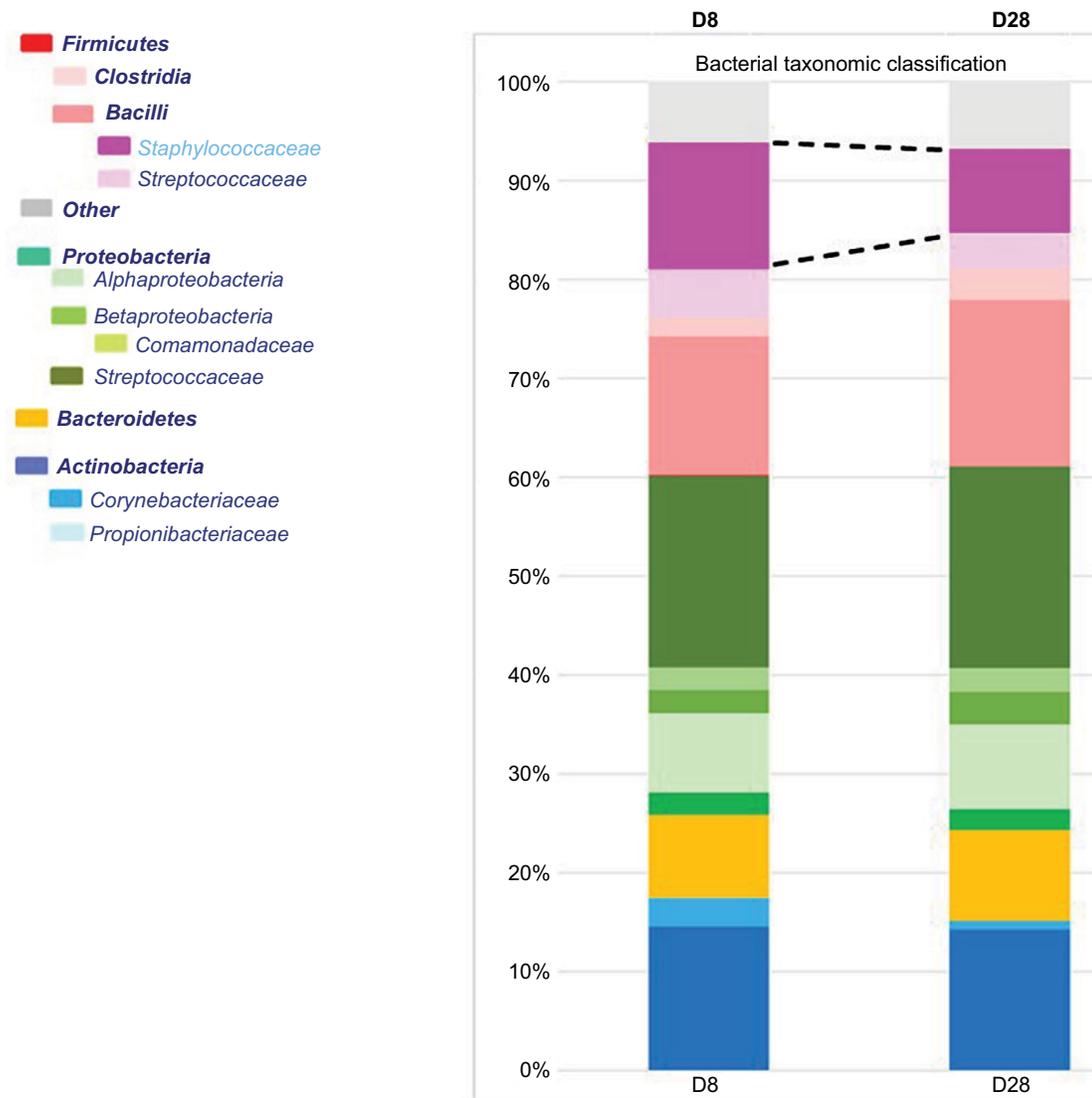


Figure 4 Change in skin microbiota composition between Days 8 and 28 in target atopic dermatitis lesions treated with heat-treated *Lactobacillus johnsonii* lotion.

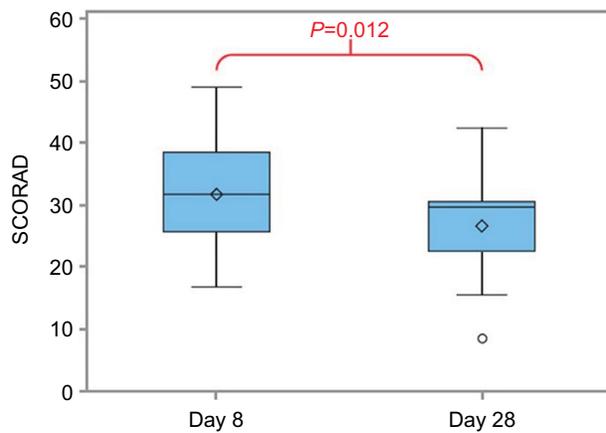


Figure 5 Box plots show local objective SCORAD evolution between Days 8 and 28 in target atopic dermatitis lesions treated with heat-treated *Lactobacillus johnsonii* lotion. Statistical analysis was performed using the paired *t*-test. **Notes:** ◊ indicates mean values and ○ indicates outlier values. **Abbreviation:** SCORAD, SCORing Atopic Dermatitis.

its involvement remains to be determined. They nevertheless suggest that a reduction in *S. aureus* may improve the symptoms of AD.^{9,10}

Previously, there have been many interventional trials aiming to decrease *S. aureus* in patients with atopic eczema. A Cochrane review evaluating these studies failed to find clear evidence that commonly used anti-*S. aureus* interventions are clinically beneficial.^{20,21} However, the studies included in the review were small and poorly reported, *S. aureus* load decrease was not documented in most studies and tested therapies (oral antibiotics, topical antibiotics, or antiseptics in combination with corticosteroids, bleach baths, and antibacterial bars) did not specifically target *S. aureus*.

In order to assess the efficacy of therapies that function by reducing *S. aureus* load on the skin of patients with

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AD, a reliable method for sampling and quantifying *S. aureus* is required. The swab and scrub wash methods of sampling have previously been shown to provide comparable recovery of bacterial species from the skin surface.²² This study showed that the swab and scrub wash methods of skin sampling are both robust, reproducible and repeatable methods of assessing the *S. aureus* load in patients with AD when coupled with either quantitative culture or qPCR. Since we found no major difference between the sampling and quantification methods for simplicity and speed, we elected to assess the effect of a lotion containing a nonreplicating probiotic strain on *S. aureus* levels using the combination of swab and qPCR.

The effect of probiotics on skin health is an interesting field of development and, although such studies generally make use of oral probiotics, there is increasing focus on the potential use of topical probiotics to modulate the cutaneous microbiota.²³ In this study, we evaluated a lotion (COS daily moisturizing lotion) containing 0.3% La1 inactivated by heat treatment (HT La1 lotion) in patients with AD. Results demonstrated that after 3 weeks of twice daily application to target AD lesions, HT La1 lotion significantly lowered *S. aureus* loads compared with contralateral lesions that were untreated or treated with the patient's usual moisturizer ($p < 0.001$). This result is consistent with the antagonistic properties of HT La1 found in studies investigating the attachment of *S. aureus* to keratinocytes using the reconstructed human epidermis model (Nestlé patent, WO01/45721 and internal data [Rosignoli et al, unpublished data, 2017]).

Additionally, a noticeable reduction was found in the Staphylococcaceae family abundance on treated AD lesions between Days 8 and 28 using 16S rRNA gene sequencing for microbiota profiling. *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, and *Proteobacteria* are the four dominant phyla of skin bacteria.²⁴ Treatment with the HT La1 containing lotion mainly affected the *Firmicutes* phylum and, more precisely, the Staphylococcaceae family. This highlights the specificity of action of the HT La1 lotion when compared with antibiotics or bleach bath.

Interestingly, the control of *S. aureus* colonization was associated with clinical statistically significant improvement of the treated AD lesions (using local objective SCORAD). Of note, patients who "responded" best to treatment were those with higher *S. aureus* counts at the time the lotion was first applied (Day 8). Indeed, a level of 2.89 Log₁₀ CFU/cm² in the AD target lesion appeared to be the minimum *S. aureus* load required to observe a response to HT La1 lotion, suggesting that this lotion may be useful for patients with particularly

high levels of *S. aureus* colonization. Incidentally, in a recent systematic review, the prevalence of *S. aureus* colonization was found to increase with disease severity.⁹ Although we still need to elucidate the mechanism underlying this effect of the HT La1 lotion, another study has shown that the *Lactobacillus reuteri* ATCC 55730 strain can compete with *S. aureus* for $\alpha 5\beta 1$ integrin binding sites on the keratinocyte cell surface.²⁵ However, this effect was only observed with viable *L. reuteri* cells. In this study, we have shown that a lotion containing heat-treated HT La1 can reduce *S. aureus* load in AD lesions and improve the severity of disease. Whether, HT La1 lotion improves AD by solely reducing the *S. aureus* load or by combining different modes of action (i.e., enhancement of the production of certain anti-microbial peptides) needs to be further investigated.

In our study, the in vivo effects on *S. aureus* were observed with a heat-treated nonreplicating probiotic strain, which is particularly encouraging and would support the development of a more user-friendly product that is easier to store and can be delivered in a standardized dose.

Although interpretation of the results presented in this study is limited by the small number of patients who were treated with HT La1 lotion, the limited study duration, effective control of *S. aureus* loads, and the associated improvement in local objective SCORAD warrant further investigation of topical treatments containing heat-inactivated beneficial bacteria.

Conclusion

The results of this study show that the HT La1 lotion is well tolerated and induces a clinical improvement of AD lesions in association with a control of SA colonization.

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Disclosure

SB-R and VB are employees of Galderma R&D. PA is a former employee of Galderma R&D. AM is an employee of the Nestlé Research Center, Nestec Ltd. The authors report no other conflicts of interest in this work.

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