





Article

The Effect of an Oral Probiotic Mixture on Clinical Evolution and the Gut and Skin Microbiome in Patients with Alopecia Areata: A Randomized Clinical Trial

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Abstract: (1) Background: Given the autoimmune nature of Alopecia Areata (AA) and the immunomodulatory properties of probiotics, this trial was conducted to evaluate the efficacy of a probiotic mixture, consisting of *Lactobacillus rhamnosus* and *Bifidobacterium longum* strains, as an adjuvant treatment in a group of AA patients. (2) Method: This study was a 24-week, randomized, double-blind, placebo-controlled clinical trial. Twenty-six patients with AA were included in this study, and their clinical progression, along with changes in gut and skin microbiota, were analyzed. (3) Results: A higher proportion of AA patients treated with the probiotic formula showed improvement compared to the placebo group, based on both the reduction in the number of AA plaques (56% vs. 30%) and the affected scalp surface area (45% vs. 20%). For “activity”, “inactivity”, and “regrowth”, an improvement in 55%, 67%, and 55% of patients was, respectively, observed in the probiotic group, compared to 50%, 40%, and 30% in the placebo group. No changes were observed in the gut microbiota during the intervention period. Regarding skin microbiota, changes were detected in the probiotic group, with reductions in characteristic genera during the study. (4) Conclusions: To our knowledge, this is the first clinical trial assessing the efficacy of a probiotic product in patients with AA. This probiotic mixture in a routine clinical practice setting appears to improve the course of patients. In addition, the skin microbiota of scalp lesions was modified using the probiotic treatment.

Keywords: alopecia areata; probiotics; *Lactobacillus rhamnosus*; *Bifidobacterium longum*; gut microbiota; skin microbiota; scalp microbiota; hair follicle microbiota; randomized controlled trial



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1. Introduction

Alopecia Areata (AA) is an autoimmune disorder influenced by a genetic predisposition along with other relevant factors [1–4]. The most common manifestation is the appearance of patches of hair loss on the scalp or body without signs of inflammation. The recommended treatments include topical or intralesional corticosteroids in mild cases, and systemic corticosteroids, immunosuppressive agents, and Janus Kinase (JAK) inhibitors, among others, in moderate and severe cases [5].

Multiple studies have highlighted a bidirectional communication between the gut microbiota and skin homeostasis, mainly through the modulation of the immune system [6,7]. Indeed, there is clear evidence of an association between gut microbiota and inflammatory skin diseases such as acne vulgaris, atopic dermatitis, or psoriasis [8–10]. Therefore, a connection between the development of a gut dysbiosis and an imbalance in skin homeostasis has been reported. In this sense, a limited number of previous investigations have analyzed both gut [11–14] and skin microbiota [15–17] in AA patients, evidencing the differences

in their composition when compared with healthy subjects, and proposing not only their involvement in the origin [11,13], but also their usefulness as prognostic biomarkers of the disease [12,17].

AA patients present an increased risk of developing other autoimmune diseases, and these pathologies could share common pathogenic mechanisms in which the gut microbiota could play a key role [18]. In this context, the beneficial effect of some oral probiotic mixtures has been proven in clinical trials in several inflammatory skin diseases such as atopic dermatitis [19], psoriasis [20], acne [21], and rosacea [22]. The disruption of gut integrity, imbalance within microbial communities, and systemic and local inflammation are some of the mechanisms related to the pathogenesis of these diseases that oral probiotics can contribute to balance [23].

Although the success of the Fecal Microbiota Transplantation (FMT) in patients with AA has been documented in some case reports [24,25], to date and to our knowledge, no clinical trials with an intervention with FMT nor oral probiotics have been published. Previous studies report the beneficial effects of different *Lactobacillus rhamnosus* and *Bifidobacterium longum* strains, improving intestinal permeability [26], gut dysbiosis [27], the appropriate balance between Th17 and Treg lymphocytes [28], and decreased proinflammatory cytokines [29].

Therefore, considering the autoimmune nature of AA [2,3] and the immunomodulatory and anti-inflammatory properties of probiotics [29], this clinical trial was proposed to evaluate the clinical efficacy and safety of an oral probiotic treatment composed of *Lactobacillus rhamnosus* and *Bifidobacterium longum* strains in patients with AA and its effect on gut and skin microbiota.

2. Materials and Methods

2.1. Study Design and Ethics

This study was a 24-week randomized, double-blind, two-arms, placebo-controlled, pilot clinical trial. The study received approval from the Ethics Committee of the University Hospital Vinalopó (Elche, Spain) and was carried out at the Centro Dermatológico Estético (Alicante, Spain). This clinical trial was registered in the American Registry of Clinical Trials (Clinicaltrials.gov identifier: NCT05599607).

The study protocol established 6 face-to-face visits—a baseline visit and at weeks 4, 8, 12, 16, and 24. Patients received the intralesional corticosteroid treatment along with the probiotic or placebo, and a dermatologist took photographs of affected scalp areas in every visit to assess the severity and evolution of the lesions.

2.2. Study Population

For inclusion in the trial, patients had to comply with the following defined criteria: male or female ≥ 18 years with a diagnosis of AA presenting at least two signs of AA activity visualized using trichoscopy, as assessed by a dermatologist. Pregnant or breastfeeding women and subjects who had required topical or systemic administration of antifungals and antibiotics in the previous two weeks or had consumed probiotics in the two months prior to the beginning of the study were excluded. Signed informed consent was obtained from all participants prior to enrollment in the study.

2.3. Randomization and Intervention

Participants were initially randomized to each of the two intervention groups (probiotic and placebo) in a 1:1 ratio (50% probability in both groups) following a list previously prepared by blinded staff.

Patients allocated to the probiotic group received a daily capsule containing freeze-dried *Lactobacillus rhamnosus* Bths-08 (CECT 30580) and *Bifidobacterium longum* Bths-06 (CECT 30616) strains in a 1:1 ratio at concentrations of 10^9 colony-forming units per dose, with maltodextrin as a carrier. Participants of the placebo group received a daily capsule containing only maltodextrin in an identical and indistinguishable format to the probiotic

product. Moreover, all patients received the habitual AA pharmacological treatment based on intralesional corticosteroids (triamcinolone acetonide 12 mg/mL) every 4 weeks during the intervention period [5].

The probiotic product evaluated in this clinical trial was obtained from the promoter collection of probiotic strains and was selected using data previously communicated in the patent document PCT/EP2023/063439.

2.4. Outcomes

Primary variables were as follows: (i) percentage of patients with a reduction in AA plaques, and (ii) percentage of patients with a reduction in the affected scalp surface area, assessed according to the Severity of Alopecia Tool (SALT) scale [30]. This scale categorizes patients as S0 (0% scalp affected), S1 (1% to 24%), S2 (25 to 49%), S3 (50% to 74%), S4 (75% to 99%), and S5 (100%).

Secondary variables were as follows: (i) percentage of patients with “activity” signs of improvement and the mean number of signs of “activity” (such as black dots, exclamation mark hairs, broken hair, tapered hairs, and pseudomonilethrix), (ii) percentage of patients with “inactivity” signs of improvement and the mean number of signs of “inactivity” (such as yellow dots, vellus hairs, and empty follicular openings), and (iii) percentage of patients with “regrowth” signs of improvement and the mean number of signs of “regrowth” (such as upright regrowing hairs, vellus hairs, and pigtail hairs). These clinical secondary variables were assessed using trichoscopy [31] at baseline and at week 24. Another secondary variable was (iv) the characterization of the skin and gut microbiota composition (changes from baseline to the end of the study were analyzed).

Finally, to assess the safety of the intervention, the total number of adverse events were registered during the follow-up period from baseline to week 24 in both groups of treatment.

The scoring interval for the “activity” variable, based on the mean number of signs observed in the plaque set, was 0 to 5 for each plaque. To assess the difference in the “activity” variable between baseline and at the 24-week follow-up, the formula takes into account not only the total number of signs in all plaques, but also the resolved plaques at the end of the 24-week follow-up. These resolved plaques were scored with the maximum value of 5, as appears in the following formula: “Activity” = [(Activity Score_{24w} × AA Plaques_{24w}) − (Activity Score_{0w} × AA Plaques_{0w})] − (5 × AA Resolved Plaques_{24w}). It is important to note that the “activity” variable is inversely proportional to the “inactivity” and “regrowth” variables.

The scoring interval for the “inactivity” variable, based on the mean number of signs observed in the plaque set, was 0 to 3 for each plaque. To assess the difference in the “inactivity” variable between baseline and at the 24-week follow-up, the formula takes into account not only the total number of signs in all plaques, but also the resolved plaques at the end of the 24-week follow-up. These resolved plaques were scored with the maximum value of 3, as appears in the following formula: “Inactivity” = [(Inactivity Score_{24w} ÷ AA Plaques_{24w}) − (Inactivity Score_{0w} ÷ AA Plaques_{0w})] + (3 × AA Resolved Plaques_{24w}).

The scoring interval for the “regrowth” variable, based on the mean number of signs observed in the plaque set, was 0 to 3 for each plaque. To assess the difference in the “regrowth” variable between baseline and at the 24-week follow-up, the formula takes into account not only the total number of signs in all plaques, but also the resolved plaques at the end of the 24-week follow-up. These resolved plaques were scored with the maximum value of 3, as appears in the following formula: “Regrowth” = [(Regrowth Score_{24w} ÷ AA Plaques_{24w}) − (Regrowth Score_{0w} ÷ AA Plaques_{0w})] + (3 × AA Resolved Plaques_{24w}).

2.5. Statistical Analysis

Descriptive statistics for quantitative variables were presented as mean and 95% confidence interval, while categorical variables were presented as total number and proportion of cases.

Data analysis was carried out on an intention-to-treat basis. Analysis of the continuous variables “activity”, “inactivity”, and “regrowth” was performed as the quantitative change in the score (see Section 2.4. Outcomes) and by conversion to a respective qualitative variable (expressed in number and percentage) as “patients who improve” at week 24 compared to baseline. The same strategy was used for the number of AA plaques. The effectiveness was considered as a reduction in the number of plaques (1 at least) at the 24-week follow-up compared with baseline, and by conversion to a qualitative variable as “patients who improve”. The affected scalp surface area was assessed according to the SALT scale and the percentage of “patients who improve” (defined as the change in at least 1 category of less extension) between baseline and the 24-week follow-up was compared between both study groups.

For categorical and continuous variables, Pearson’s chi-squared test and Student’s *t*-test were, respectively, used for the analysis of statistically significant differences between the study groups. Statistical significance was defined as $p < 0.05$. Previously, the condition of the normality of the continuous variables was evaluated using the Shapiro–Wilk test. However, the “inactivity” and “regrowth” variables did not present normality using this test, but when examining their Q-Q plots, it was observed that their distributions resembled normality and were treated as such, but including a second analysis with a non-parametric test, due to the fact that there is not a large sample size, to check the results revealed that these do not change substantially.

All statistical analysis was performed with SPSS Statistics for Windows v27.0 (IBM Corporation, Armonk, NY, USA).

2.6. Gut and Skin Microbiota Study

The swabbing procedure of scalp surfaces of AA lesions was used to obtain skin samples. Stool and skin samples were introduced in sterile tubes with the nucleic acid stabilizing solution RNAlater (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) and were conserved at $-20\text{ }^{\circ}\text{C}$ until analysis. For the characterization of the microbiota, it was performed as a high-throughput sequencing of the *16S rRNA* bacterial gene.

In summary, this microbiota study was conducted as follows: In the first step, DNA extraction from the samples was performed. This DNA was also purified and tested for quality control. The second step was the preparation of the amplicon libraries. The genetic material obtained was amplified using a two-step PCR using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA, USA) following the “16S Metagenomic Sequencing Library Illumina 15044223 B protocol”, which allows for the capture and amplification of the V3-V4 hypervariable region of the *16S rRNA* gene. Then, 16S-based libraries were quantified using fluorimetry using the Quant-iT™ PicoGreen™ dsDNA Assay Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA). Prior to sequencing, 16S libraries were pooled and the size and quantity of the pool were assessed on the Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA) and with the KAPA Library Quantification Kit (Kapa Biosystems, Wilmington, MA, USA), respectively. These sequencing libraries were loaded onto the platform MiSeq (Illumina, San Diego, CA, USA) following a 300 bp \times 2 paired end design. The last step was the bioinformatic analysis, which included the identification and elimination of low quality or chimeric sequences, the generation of the Amplicon Sequence Variants (ASVs), and the taxonomic assignment.

3. Results

From March to October 2021, 26 AA patients who met all the inclusion criteria and none of the exclusion criteria were enrolled in the study (13 were allocated to the probiotic group and 13 to the placebo group). In the CONSORT diagram (Figure 1), patients assessed for eligibility, included, randomized, lost to follow-up, and analyzed in both groups are reported.

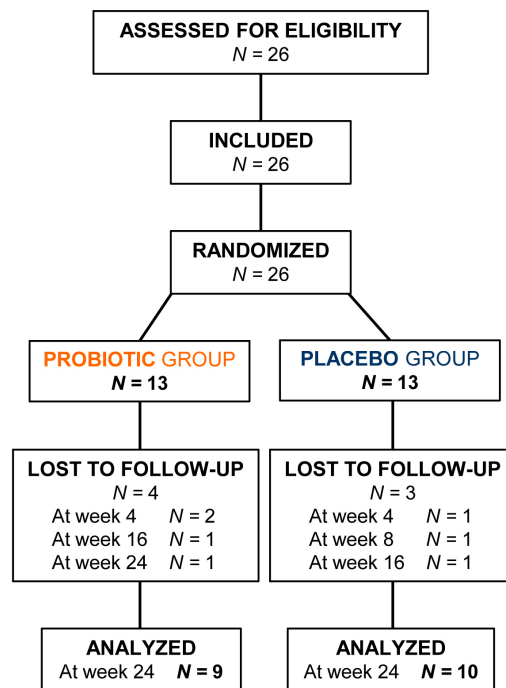


Figure 1. CONSORT diagram.

3.1. Baseline Descriptive Data

The patients' data included in this study at baseline appear in Table 1. A homogeneity analysis of this baseline data in both study groups revealed that neither important variable was unbalanced after randomization.

Table 1. Baseline demographic and clinical data in both study groups.

	Placebo	Probiotic
Sex (woman) *	11 (85%)	7 (54%)
Age (years) †	43.4 [34.7–52.2]	35.5 [29.9–41.1]
Allergies and intolerances *	3 (23%)	2 (15%)
Pre-existing diseases *	6 (46%)	6 (46%)
TRICHOSCOPY		
Activity Score _{w0} × AA plaques _{w0} †	10.4 [5.8–14.9]	10.2 [6.4–14.0]
Inactivity Score _{w0} /AA plaques _{w0} †	0.7 [0.4–1.0]	0.4 [0.2–0.6]
Regrowth Score _{w0} /AA plaques _{w0} †	0.8 [0.4–1.1]	0.4 [0.2–0.6]
AA plaques _{w0} †	3.3 [1.8–4.8]	4.0 [2.7–5.1]
SALT scale		
S1 *	8 (61.5%)	6 (46.1%)
S2 *	4 (30.8%)	7 (53.8%)
S3 *	0 (0.0%)	0 (0.0%)
S4 *	1 (7.7%)	0 (0.0%)

* Total number (percentage); † mean [95% confidence interval].

3.2. Number of AA Plaques

A higher proportion of patients in the probiotic group had a reduced AA plaque count after 24 weeks, being 5 of 9 (55%) compared to 3 of 10 (30%) in the placebo group ($p = 0.10$). Notably, 2 of the 8 patients with an improved AA plaque count at the end of the study (therefore decreasing this count) resolved the plaques completely (disappearing); these belonged to the probiotic group.

3.3. Affected Scalp Surface Area (SALT Scale)

Regarding the SALT scale, an improvement in 4 of 9 (45%) patients receiving the probiotic product was observed after 24 weeks compared to 2 of 10 (20%) patients in the placebo group ($p = 0.51$).

3.4. Activity, Inactivity, and Regrowth Scores

AA patients who received the probiotic product showed average changes in the “activity,” “inactivity,” and “regrowth” scores of -5.8 , 4.6 , and 4.4 , respectively, compared to changes of -3.0 , 2.3 , and 2.0 in patients treated with a placebo after 24 weeks (Table 2).

Table 2. Differences in AA “activity”, “inactivity”, and “regrowth” scores at the end of the study.

	Activity	Inactivity	Regrowth
Probiotic	-5.8 [(-19.2)–7.7]	4.6 [0.38–8.8]	4.4 [0.1–8.7]
Placebo	-3.0 [(-8.6)–2.6]	2.2 [(-0.8)–5.3]	2.0 [(-1.0)–5.1]
p -value [†]	0.67	0.31	0.31

Differences from baseline are represented as average [95% confidence interval].[†] Student’s t -test.

Moreover, the percentage of patients with an improvement at 24 weeks of 55%, 67%, and 55% was, respectively, observed among those with probiotic treatment, compared to 50%, 40%, and 30% found in the placebo group (Figure 2).

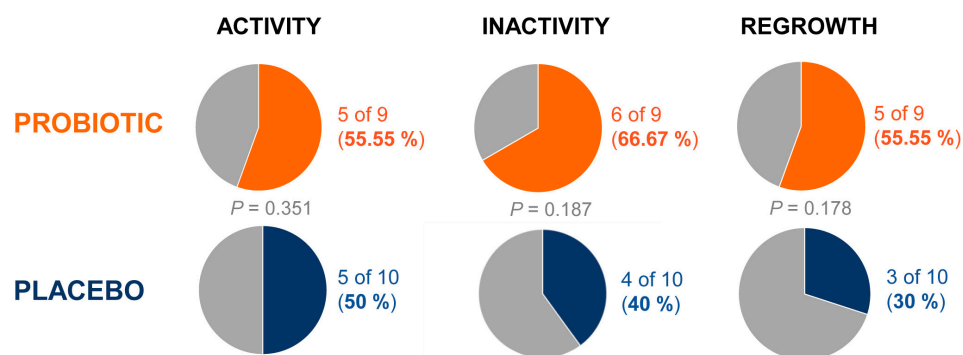


Figure 2. Patients who improved in the variables activity, inactivity, and regrowth.

3.5. Microbiota Analysis

3.5.1. Gut Microbiota

A significant change in α -diversity according to the Shannon index was not evidenced between study groups or after 24 weeks of intervention (Figure S1 in Supplementary Material). There was no obvious clustering of stool samples using Principal Coordinate Analysis (PCoA) between study groups or between baseline and after the follow-up period.

Firmicutes, Bacteroidetes, and Actinobacteria were the phylum exhibiting the highest relative abundances in all stool samples (Figure S2 in Supplementary Material). The families with the highest relative abundance in all stool samples were Lachnospiraceae, Bacteroidaceae, Oscillospiraceae, and Prevotellaceae (Figure S3 in Supplementary Material). Bacterial genera bar plots of fecal samples from the study groups at baseline and after 24 weeks of treatment are illustrated in Figure 3. The genera with the highest relative abundances were *Phocaeicola*, *Blautia*, and *Faecalibacterium*.

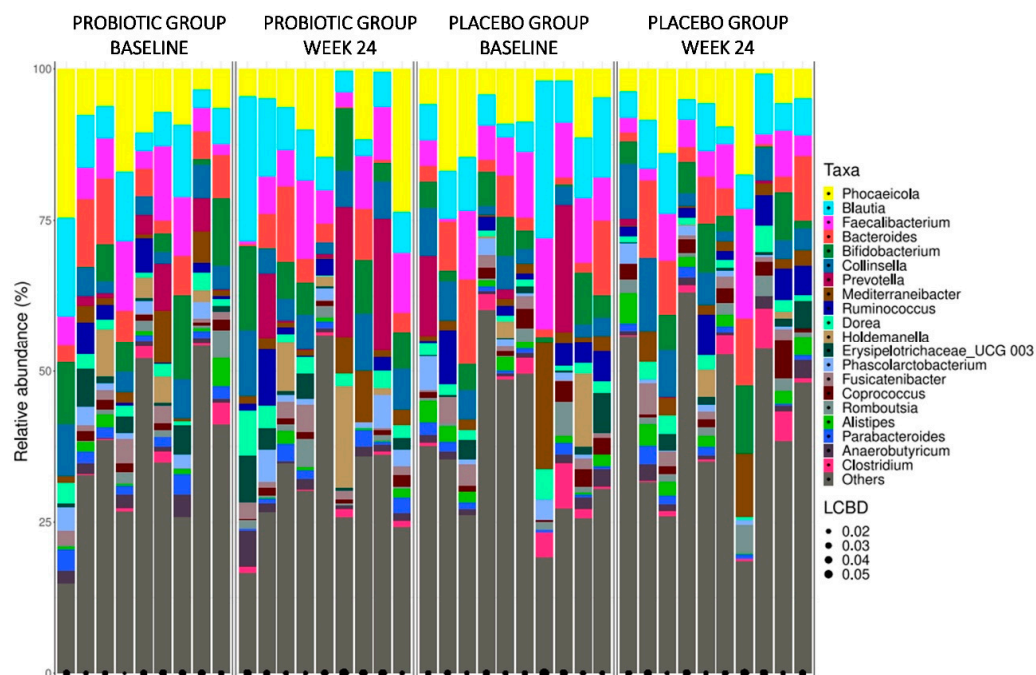


Figure 3. Bacterial genera profiles of fecal samples at the genus level. (LCBD: Local contribution to beta-diversity).

After the intervention period with probiotic or placebo, no differentially abundant taxonomic markers from phylum to genus were detected between the groups compared to baseline data.

3.5.2. Skin Microbiota

As with gut microbiota, there were no observed significant changes in α -diversity according to the Shannon index between study groups or after 24 weeks of intervention (Figure S4 in Supplementary Material). In addition, no obvious clustering of skin samples using PCoA between study groups or after 24 weeks was observed.

The phylum with the highest relative abundance in all skin samples were Actinobacteria, Firmicutes, and Proteobacteria (Figure S5 in Supplementary Material). The families with the highest relative abundance were *Propionibacteriaceae*, *Staphylococcaceae*, *Bifidobacteriaceae*, and *Moraxellaceae* (Figure S6 in Supplementary Material). In Figure 4 are illustrated the bacterial genera bar plots of skin samples from the study groups at baseline and after 24 weeks of treatment. The genera with the highest relative abundance regarding all skin samples were *Cutibacterium*, *Staphylococcus*, and *Bifidobacterium*.

Notably, when comparing baseline versus week 24, a significant reduction in Bifidobacteriaceae, Erysipelotrichaceae, and Nocardiaceae families was detected in the probiotic group at the end of the follow-up period. Among these families, a reduction in the *Bifidobacterium*, *Erysipelothrix*, and *Rhodococcus* genera was also, respectively, significant. All bacteria that were characteristically reduced in the probiotic group at the end of the study are shown in Figure 5.

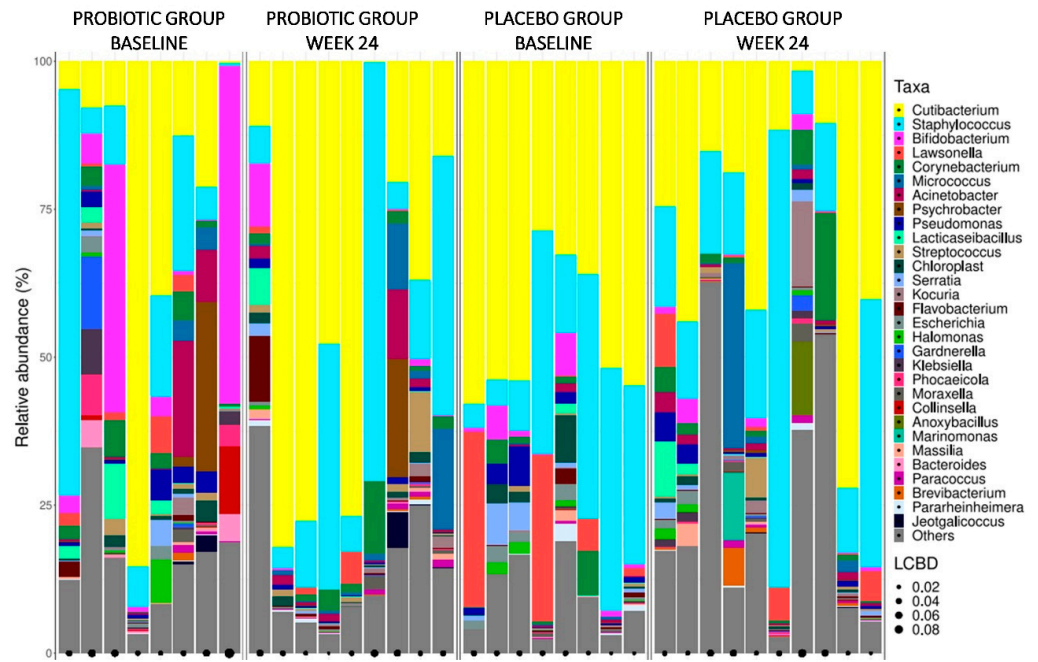


Figure 4. Bacterial genera profiles of skin samples at the genus level. (LCBD: Local contribution to beta-diversity).

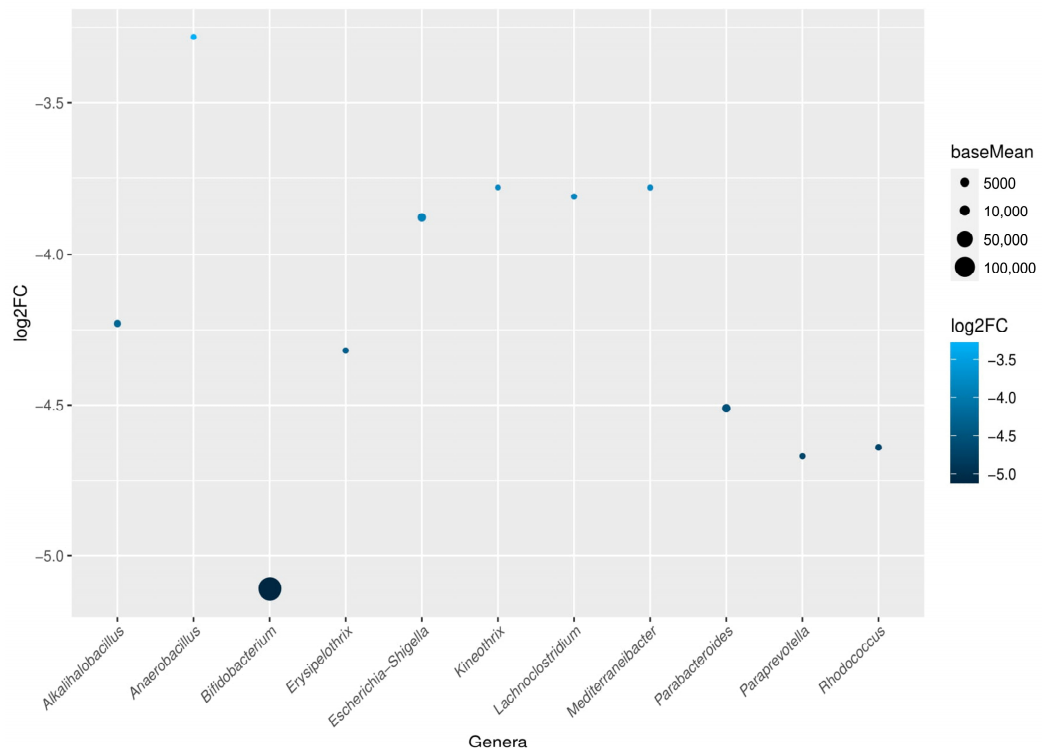


Figure 5. Heatmap (limma model) of biomarker bacterial genera decreasing in skin samples of the probiotic group at the end of the study. (BaseMean: Mean of normalized counts belonging to a bacterial genus in a study population; Log2FC: “Log Fold Change”).

3.6. Safety

Overall, five adverse events were reported—one in the probiotic group and four in the placebo group. Of the five adverse events reported, three of them were potentially attributable to the treatment received by the patients; all of them were in the placebo group (abdominal pain, constipation, and diarrhea).

4. Discussion

To date, no clinical studies with oral probiotics targeting microbiome modulation have been published. Notably, this is the first clinical trial assessing the efficacy and safety of a probiotic product in patients with AA in a conventional clinical practice setting.

The results obtained from this proof-of-concept study indicate the beneficial effect of the probiotic preparation on the signs of “activity”, “inactivity”, and “regrowth”, with a decrease in the number of AA plaques and a reduced affected skin surface area. Due to the type of study proposed, with a limited sample size, the changes between groups throughout the study did not reach statistical significance. However, analyzing the overall data, we consider the effects obtained with the probiotic as being clinically relevant, since they are consistent with the same trend in beneficial effects on the evolution of AA. To confirm these results, a clinical trial with a larger sample size should be conducted.

Any significant change in gut microbiota was not observed between AA patients belonging to either of the two study groups and after 24 weeks of treatment. This involves the fact that the administration of the probiotic formula does not appear to modify the composition and diversity of gut microbiota in AA patients. It is also unknown whether the probiotic strains have colonized the gut microbiota or whether its effects have been transient. To verify this issue, it is necessary to perform a quantitative PCR (qPCR) specific to the probiotic strains used in the present study. On the other hand, only three studies have compared the gut microbiota of AA patients with that of healthy controls (an adult population, as in the present study) and although no changes in α - and β -diversity are evident, there are characteristic differences in relation to the composition. In the study of Moreno-Arrones et al., an increased relative abundance of *Holdemania filiformis*, *Parabacteroides johnsonii*, *Clostridiales vadin BB60* group, *Bacteroides eggerthii*, and *Parabacteroides distasonis*, as well as the Eggerthellaceae, Erysipelotrichaceae, and Lachnospiraceae families, was reported in AA patients [12]. Lu et al. found increased levels in *Blautia*, *Phyllobacterium*, *Dorea*, *Anaerostipes*, *Megasphaera*, *Collinsella*, *Sphingomonas*, and *Pseudomonas* [13]. In the study of Rangu S et al. there were no evidenced significant differences regarding α - and β -diversity between siblings with and without AA, but a linear mixed model revealed that *Ruminococcus bicirculans* exhibited a lower relative abundance in children with AA [14]. These results, although discordant, could indicate the presence of a dysbiosis in patients with AA. It is necessary to emphasize that in the present study, no comparison with healthy controls was performed and only a microbiota analysis between groups and after the follow-up period has been assessed. Therefore, it cannot be concluded that these patients with AA exhibited a gut dysbiotic state.

In relation to the skin microbiota, some studies have reported differences in the scalp microbiota of AA lesions compared to healthy controls. The study of Pinto et al. describes an increase in *Propionibacterium* and a decrease in *Staphylococcus* in AA subjects [15]. Juhasz et al. also communicated a significant decrease in class *Clostridia* in AA patients [16]. In another study, an increased α -diversity in AA patients was described, with decreased levels in the *Staphylococcaceae* and *Burkholderiaceae* families. This study also describes how the *Cutibacterium/Staphylococcus caprae* ratio was increased compared to healthy controls [17]. In our study, skin microbiota was similar in both groups at baseline. After 24 weeks of treatment, several changes were detected in patients of the probiotic group, with reductions in characteristic families and genera, but not observed in patients that received the placebo. These findings show that the skin microbiota in this group has been modified by the administration of the probiotic strains. In relation to the pathophysiology, it suggests a reduced penetration of immunogenic material of bacterial origin into the hair follicle and, therefore, a reduction in peribulbar inflammation, which is a key mechanism in the pathogenesis of AA [1,32]. In addition, results reported in patients receiving the probiotic product evidence a potential usefulness of measuring the levels of some of these genera (for example *Bifidobacterium*, which showed the greatest reduction), as a potential diagnostic and prognostic skin biomarker in patients with AA.

Importantly, throughout this clinical trial, the probiotic formula was used as an adjuvant treatment to intralesional corticosteroids. Intralesional corticosteroids are a first-line treatment mainly in mild-to-moderate cases such as those included in this study [33]. Because the dose of corticosteroid is important for its efficacy in these AA cases, one hypothesis we propose is that probiotic treatment could reduce the dose and treatment time required and, therefore, could lead to a lower frequency of adverse effects, such as skin atrophy [34]. Future studies would be necessary to verify this promising hypothesis.

The main limitation of the study was sample size, which has not allowed statistically significant differences to be reached in relation to the primary and secondary endpoints. Nevertheless, all findings consistently support the enhanced efficacy of the probiotic when added to standard intralesional corticosteroid therapy compared to the placebo. This is ultimately the purpose of the pilot study—to obtain preliminary data on the efficacy of the experimental treatment compared to a placebo—although these results could be neither conclusive nor definitive. As a strength, we emphasize that this is the first clinical trial involving probiotics for patients with AA published to date, and that expert dermatologists specializing in trichology collected all variables analyzed for the established endpoints.

As conclusions, this study investigated the probiotic formulation as an adjuvant treatment in a routine clinical practice setting, demonstrating improvements in the clinical course of AA patients. It increased the proportion of patients who reduced plaque counts and AA-affected scalp surface area, and improved scores related to signs of “activity”, “inactivity”, and “regrowth”. Additionally, probiotic treatment led to a modification in the skin microbiota. As this is a preliminary study, this trend needs to be confirmed in larger clinical trials.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/cosmetics11040119/s1>, Figure S1: Boxplot of α -diversity in stool samples at the ASV level according to the Shannon index; Figure S2: Bacterial profiles of fecal samples from study groups at the phylum level; Figure S3: Bacterial profiles of fecal samples from study groups at the family level; Figure S4: Boxplot of α -diversity in skin samples at the ASV level according to the Shannon index; Figure S5: Bacterial profiles of skin samples from study groups at the phylum level; Figure S6: Bacterial profiles of skin samples from study groups at the family level.

Author Contributions: Conceptualization, V.N.-L. and M.R.N.-B.; Methodology, V.N.-L. and M.R.N.-B.; Software, P.S.-P., E.N.-D., L.N.-M. and V.N.-L.; Validation, M.R.N.-B., Á.A.-G., P.S.-P., E.N.-D., L.N.-M., M.M.-V., A.G.-N. and V.N.-L.; Formal analysis, Á.A.-G., P.S.-P., E.N.-D., L.N.-M. and V.N.-L.; Investigation, M.R.N.-B. and A.G.-N.; Resources, M.R.N.-B. and V.N.-L.; Data curation, P.S.-P., E.N.-D., L.N.-M. and V.N.-L.; Writing—Original draft preparation, P.S.-P., E.N.-D. and V.N.-L.; Writing—Review and editing, P.S.-P., Á.A.-G., E.N.-D., M.M.-V. and V.N.-L.; Visualization, M.R.N.-B., Á.A.-G., P.S.-P., E.N.-D., L.N.-M., M.M.-V., A.G.-N. and V.N.-L.; Supervision, V.N.-L.; Project administration, V.N.-L.; Funding acquisition, V.N.-L. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: This study was conducted in accordance with the Declaration of Helsinki and was approved on 24 February 2021 by the Research Ethic Committee of University Hospital Vinalopó (Elche, Spain), protocol code ALO.PRO.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

Conflicts of Interest: P.S.-P., E.N.-D. and L.N.-M. belong to the scientific staff of Bioithas. V.N.-L. is a shareholder of Bionou Research and Bioithas. The other authors declare no conflicts of interest.

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