**Supplementary Methods**

**IRB**. This study was approved by the NYULMC Institutional Review Board (protocol #10-01622); all parents were informed the study protocol in detail provided written consent for their child's participation.

**Patients and study design.** All patients who presented for evaluation and treatment of moderate to severe atopic dermatitis at the Charles C. Harris Skin and Cancer Unit (SCU) and NYU Dermatologic Associates, both at NYU Langone Medical Center, and at Bellevue Hospital Center (BHC) who met the inclusion criteria were asked to participate. At the baseline visit, a detailed baseline questionnaire was administered to all parents of study participants. Parents were instructed to use the fluticasone propionate cream twice daily to areas of eczema and could continue using their own moisturizer. In addition, patients were instructed to bathe twice a week with the solution with which they were provided at the concentrations they were given. Since the main endpoint was to determine changes in bacterial load and microbiota composition within a particular microenvironment over time, the same skin site was sampled at the 4-week follow-up visit.

**EASI score.** A single investigator (ME Gonzalez) performed the EASI score at the baseline visit and at the follow up visit.

**Bleach baths.** Parents were provided with an instruction handout on how to prepare the bleach baths. They were instructed to add a ¼ cup of household bleach (6.15% sodium hypochlorite) in a half-full bathtub, or a ½ cup in a full bathtub (~40 gallons), or 0.5 teaspoons per gallon of water if they were using a baby tub. These dilutions achieve a concentration of approximately 0.005% sodium hypochlorite, the typical concentration used in clinical practice. At follow up,

each family was asked if the treatments were used as prescribed. No patient had used systemic (or topical) antibiotics in the interval 4 weeks.

**Quantitative PCR.** TaqMan quantitative PCR (qPCR) was performed using genus-specific specific primers and probes for the 16S rRNA sequences of Bacteria, Propionibacterium, Staphylococcus, Streptococcus, and Corynebacterium. The standards were cloned PCR products from the 16S rRNA genes of specified genera and *nuc* for *S. aureus*; qPCRs were performed as described.[1] PCRs were performed using 3.5mM MgCl2, 0.4 ng/µl bovine serum albumin, 0.2mM of each deoxynucleoside triphosphate, 10 pmol of each primer, 5 pmol of each probe, 0.625U Taq DNA polymerase (Qiagen), and 2 µl extracted DNA in a final 25 µl volume. PCR conditions were 5 min at 94 C, 45 cycles of 10 s at 94C, 45 s at 54 C [Primers: 8F/Eub361R] or 56 C [Primers: Eub519R/U785R] and 60 s at 72 C. Assays were run in duplicate, using the Roche 480 Lightcycler (Indianapolis IN).

**High throughput DNA Sequencing.** For each sample, the V4 region of bacterial 16S rRNA genes was amplified in triplicate reactions using the universal bacterial primer set 515F/806R, which amplifies bacterial and archaeal 16S genes near universally. PCR reactions contained 11 µl Molecular biology grade water (Corning cellgro), 10 µl 5 Prime Hot Master Mix (5 Primer, Inc., MD, USA), 1.0 µl each of the forward and reverse primers (5 µM final concentration per each primer), and 2.0 µl genomic DNA. Reactions were held at 94°C for 3 min to denature the DNA, run for 35 cycles of amplification at 94°C for 45 s, 50°C for 60 s, and then 72°C for 90 s, and completed with a final extension step of 10 min at 72°C. Amplicon from each sample was quantified using PicoGreen dsDNA Assay Kit (Invitrogen). Equal amounts of DNA from each sample were pooled, followed by PCR purification (Qiagen). DNA concentration in these sub-pools were quantified with the Qubit high sensitivity dsDNA Assay (Invitrogen), and combined

at equal concentration. DNA sequencing was performed with Illumina MiSeq instrument located in Genome Technology Center, NYU. sPaired-end reads of the amplicons were joined with FastqJoin script from EA-utils,[2] and only reads perfectly matching in the overlapping region were kept. QIIME version 1.7.0[3] was used for further processing and the Green Genes version 13.8 database release was used for reference.[4] Representative sequences were further aligned using PyNAST[5] with the Greengenes core-set alignment template. We used the alignment to reconstruct an approximate phylogenetic tree using FASTTREE.[6]

**Blinding.** Investigators, data analysts and sequencers were blinded to treatment until unblinding was necessary for comparative data analysis after the experiment ended.

**Statistical analyses of the sequencing data.** Statistical analyses of the sequencing data set were performed in the R statistical programming environment.[7] The abundance table was equally sampled 20 times at 1,000 sequence depth per sample to analyze α-diversity, and the significance of differences determined by Mann-Whitney test. The significance of the correlation between α-diversity and EASI was determined using the linear Pearson correlation coefficient. We used the ade4 package[8] in R to perform Principal Coordinates Analysis (PCoA) on Unifrac distances. To avoid negative eigenvalues, we used the Cailliez method[9] to convert the distance matrices into closest corresponding matrices with Euclidean properties, which was further used for PCoA. Statistical significance of the PCoA observations were determined with the ADONIS test at 9999 permutations[10] using the R vegan package. Univariate testing of operational taxonomic unit (OTU) relative abundances was performed according to the PESAME (Predictive Effect Size Analysis in Multivariate Ensembles) protocol (A. V. Alekseyenko: unpublished), which assesses the significance of multilevel differences using Kruskal Wallis ANOVA controlled for multiple testing by FDR (false discovery rate);[11] pairwise differences are assessed using the Mann-

Whitney test and predictive effect sizes estimated by converting the U-statistics to area under ROC (receiver-operator characteristic) curve (AUC). Confidence intervals on the AUC metrics were estimated by normal approximation.[12]

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[1] Redel H, Gao Z, Li H, *et al* (2013). Quantitation and composition of cutaneous microbiota in diabetic and nondiabetic men. *J Infect Dis* 207:1105-14.
[2] Aronesty, E. (2013). Comparison of Sequencing Utility Programs. *Open Bioinformatics Journal* 7:1-8
[3] Caporaso JG, Kuczynski J, Stombaugh J, *et al* (2010). QIIME allows analysis of high-throughput community sequencing data. *Nat Methods* 7:335-36.
[4] McDonald, D., Price, MN., Goodrich, J.*et al* (2011). An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. *ISME J* 6:610-18.
[5] Caporaso JG, Bittinger K, Bushman FD, *et al* (2010). PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics* 26:266-67.
[6] Price MN, Dehal PS, Arkin AP, *et al*. (2010). "FastTree 2--approximately maximum-likelihood trees for large alignments." *PLoS One* 5:e9490.
[7] R Development Core Team (2012). R: A Language and Environment for Statistical Computing. Vienna, Austria, R Foundation for Statistical Computing.
[8] Dray S, Dufour AB. (2007). The ade4 package: implementing the duality diagram for ecologists. *Journal of Statistical Software* 22:1-20.
[9] Cailliez, F. (1983). The analytical solution of the additive constant problem. *Psychometrika* 48: 305-10.
[10] Anderson, MJ. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26: 32-46.
[11] Benjamini Y, Hochberg Y (1995). Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society Series B-Methodological* 57: 289-300.
[12] Newson R. (2006). Confidence intervals for rank statistics: Somers' D and extensions. *Stata Journal* 6:309-34.

**Supplemental Figure Legends.**

**Supplemental Figure 1. Clinical outcome over time.** All 18 AD subjects had significant improvements in IGA, total EASI and local EASI scores over time, with no significant differences between the groups. a) Change in IGA; b) Change in total EASI; c) Change in local EASI at worst lesional site.

**Supplemental Figure 2. Density of bacterial species in relation to lesion status.** Baseline densities determined by specific qPCR assays at moist, dry, and sebaceous sites. a) Total bacteria. b) *Staphylococcus* species; c) *Streptococcus* species; d) *Corynebacterium* species; e) *Propionibacterium* species. At baseline for dry skin sites, total bacterial counts on lesional skin were significantly higher than for controls. At sebaceous sites, *S. aureus* density on lesional skin was significantly higher than control skin although there were no significant differences in genus *Staphylococcus*. *Corynebacterium* was greater on control dry sites than dry sites from lesional AD skin. Bacterial densities were similar for control and nonlesional AD skin at dry and sebaceous sites.

**Supplemental Figure 3. Correlations of overall EASI score with bacterial densities and Shannon index.** Mean lesional total bacterial density (a) and mean lesional *S. aureus* density (b) each were moderately correlated with overall EASI scores. Bacterial densities determined by specific qPCR assays. (c) Relationship of baseline EASI score to mean Shannon diversity index (SDI). SDI for all in microbial communities at all lesional sites was inversely correlated with overall EASI score at baseline. (d) Follow-up after 4 weeks of treatment, both treatments TCS + bleach (blue) amd TCS alone (red) resulted in decreased overall EASI scores, and higher diversity scores. Using linear regression, the follow-up EASI scores by treatment group were not

significantly associated (p>0.05) with Shannon and richness diversities; thus correlation of disease severity with diversity was equally lost with both successful treatments.

**Supplemental Figure 4. Diversity scores for control, lesional, and nonlesional sites in moist, dry, and sebaceous microenviroments.** a) Community richness at baseline and after treatment; b) Mean Shannon diversity index (SDI) at baseline and after treatment. There were significant increases in bacterial community richness and SDI after both treatments in each cutaneous microenvironment. Symbols: C, control; NL, nonlesional site; L, lesional site.

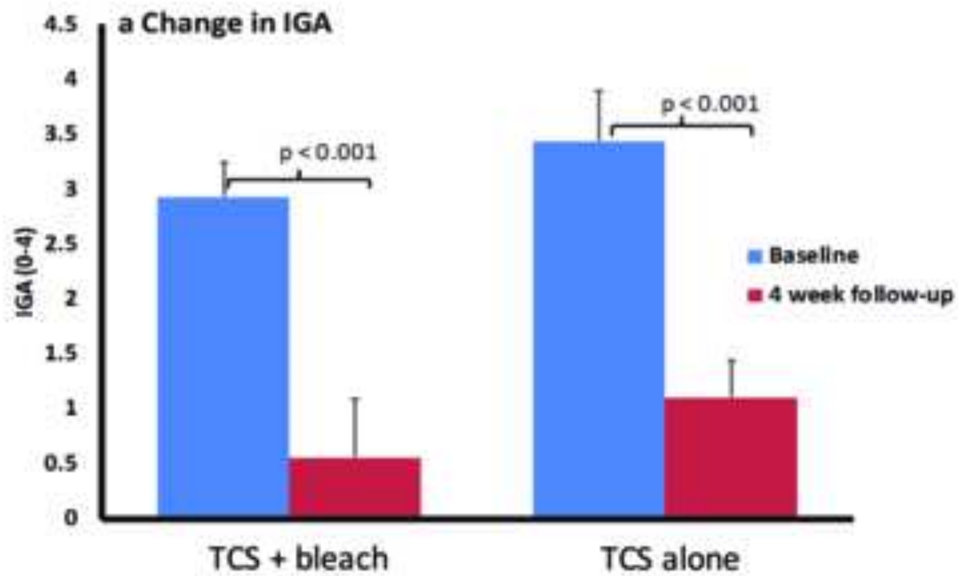
**Supplemental Figure 5. Relative abundance of major genera in controls and AD lesional and nonlesional sites at baseline and after treatment.** The minimal sequence depth for each of the 249 specimens was >4,800. Times were: **A-E,** baseline; and **F-J,** after 4 weeks of treatment. Groups were: **E,J** for Control subjects. TCS +bleach group: lesional sites: **A,F**; nonlesional sites; **B,G**; TCS alone group: lesional sites: **C,H**; nonlesional sites: **D,I**. In most controls at Baseline, 13 major genera comprised < 50% of all reads**,** with Staphylococci generally < 25%, indicating high diversity in the lower abundance genera. In contrast, the median representation of Staphylococci in lesional skin was 60-70%, and nonlesional skin showed intermediate patterns. At the 4-week follow up, there was little change in controls **(Panel J),** but both lesional and the nonlesional AD samples normalized **(Panels F-I).** On nonlesional skin, bleach baths lead to a greater suppression of *Staphylococcus* species compared to the TCS group (**Panel G**).

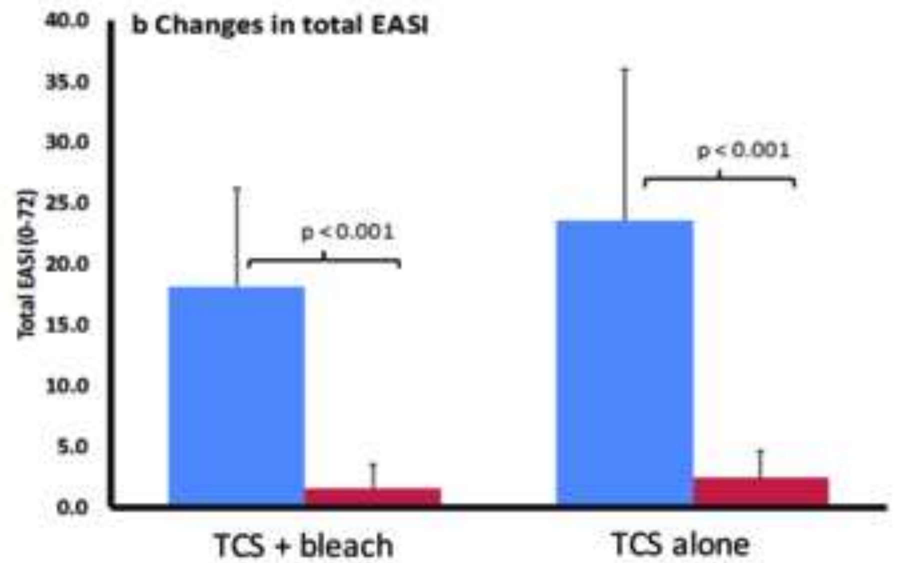
## Supplemental Tables

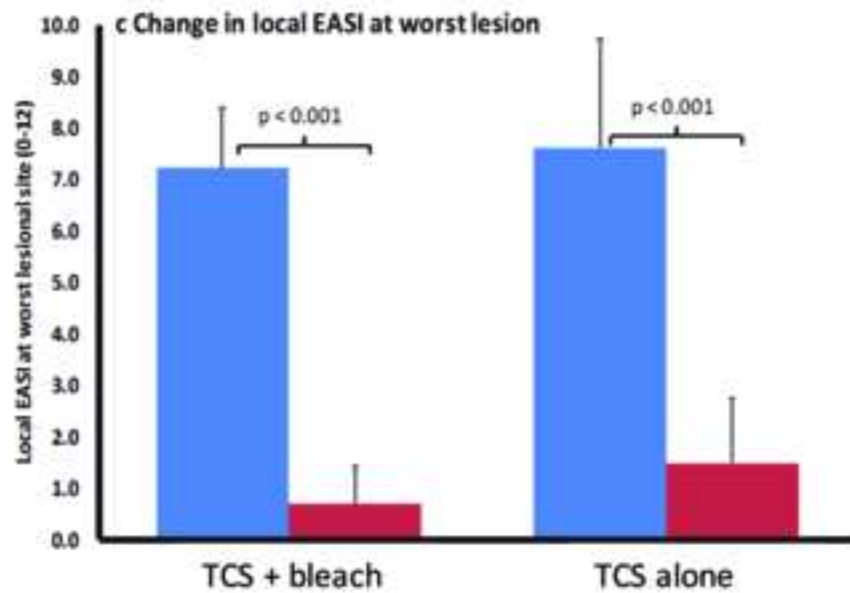
### Supplemental Table 1. Clinical characteristics of subjects completing the study.

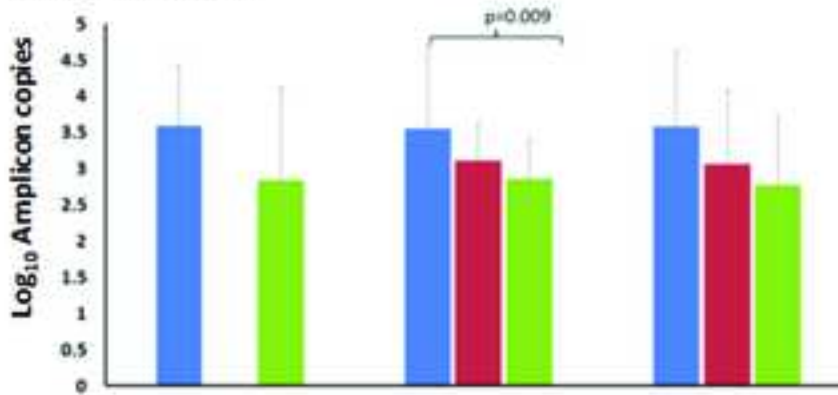
| | Bleach bath group | Placebo group | B vs P[e] | Control | B vs C[e] | P vs C[e] |
|---|---|---|---|---|---|---|
| **Total number of subjects** | 9 | 9 | | 11 | | |
| **% Male** | 44 | 78 | | 45 | | |
| **Age, months, Median** | 11 | 4 | | 21 | | |
| **Age, months, Mean ± SD (range)** | 22 ± 21 (4.5 – 60) | 5.4 ± 3.6 (3 - 14) | p=0.01 | 21 ± 17 (3.5 – 47) | | p=0.02 |
| **Race** | C: 44%; H: 22%<br>A: 22%; AA: 11% | C: 33%; H: 44%<br>A: 11%; AA: 11% | | C: 54%; H: 9%<br>A: 27%; AA: 9% | | |
| **IGA[a], Mean ± SD (range)** | 2.94 ± 0.3 (2.5 – 3.5) | 3.4 ± 0.5 (3 – 4) | | NA | | |
| **Total EASI[b], Mean ± SD (range)** | 18.2 ± 8.0 (6.8-29) | 23.6 ± 12.4 (7- 45.6) | | NA | | |
| **Local EASI[b], Worst affected, Mean ± SD (range)** | 7.2 ± 1.2 (5 – 8.5) | 7.6 ± 2.1 (5.5 – 11) | | NA | | |
| **Parent severity score[d], Mean ± SD (range)** | 7.8 ± 2.0 (5 – 10) | 7.2 ± 2.5 (2 -10) | | NA | | |
| **% delivered vaginal** | 78 | 78 | | 64 | | |
| **% of patients bathing <1/day** | 33 | 56 | | 18 | | |
| **Hours since last bath, Mean ± SD (range)** | 17.6 ± 13.7 (4-48) | 14.1 ± 4 (5-18) | | 14.5 ± 7 (3-23) | | |
| **Hours since last non-medicated cream, Mean ± SD (range)** | 20.7 ± 29.2 (1-96) | 16.9 ±18.2 (4-48) | | 42.8 ± 46 (3 – 168) | | |
| **Family Hx/Atopic Dermatitis (%)** | 67 | 44 | | 0 | p=0.002 | p=0.03 |
| **Family Hx/Asthma (%)** | 33 | 11 | | 9 | | |
| **Family Hx/Hayfever (%)** | 44 | 44 | | 64 | | |

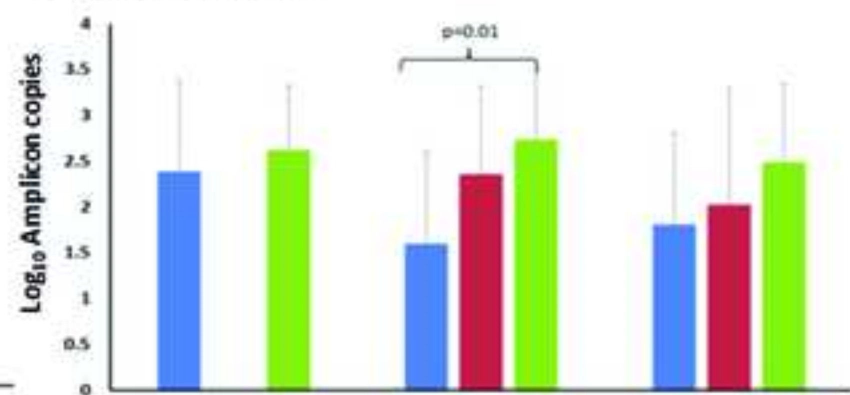
NA: not applicable, C: Caucasian,H: Hispanic, A: Asian, AA: African-American;
[a] IGA, Investigator Global Assessment, score range 0-4; [b] EASI Eczema Area and Severity Index; score range 0-72; [c] Score range 0-12; [d] Score range 0-10. [e] Statistics: Kruskal-Wallis Test, Fisher exact test.

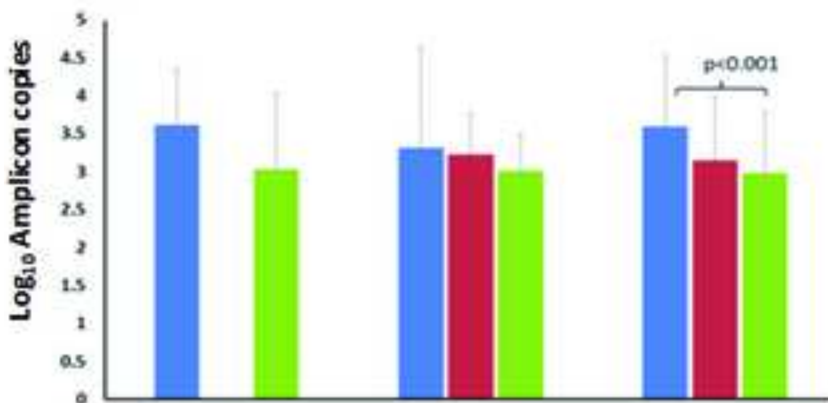

a Change in IGA
IGA (0-4)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
p < 0.001
p < 0.001
Baseline
4 week follow-up
TCS + bleach
TCS alone

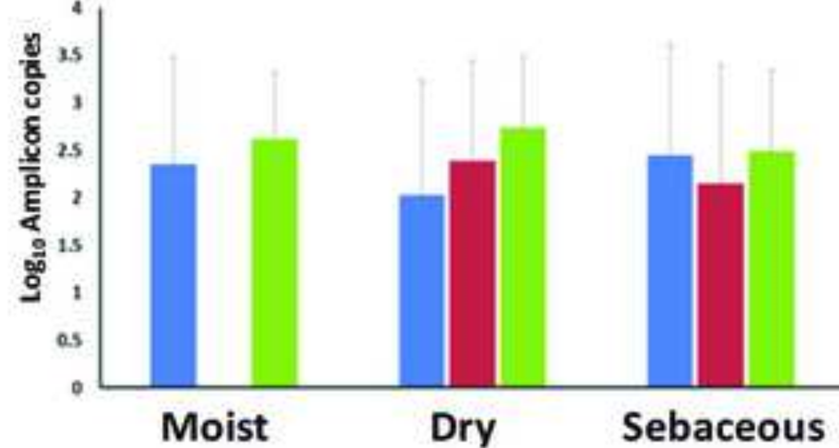

b Changes in total EASI
Total EASI (0-72)
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
p < 0.001
p < 0.001
TCS + bleach
TCS alone

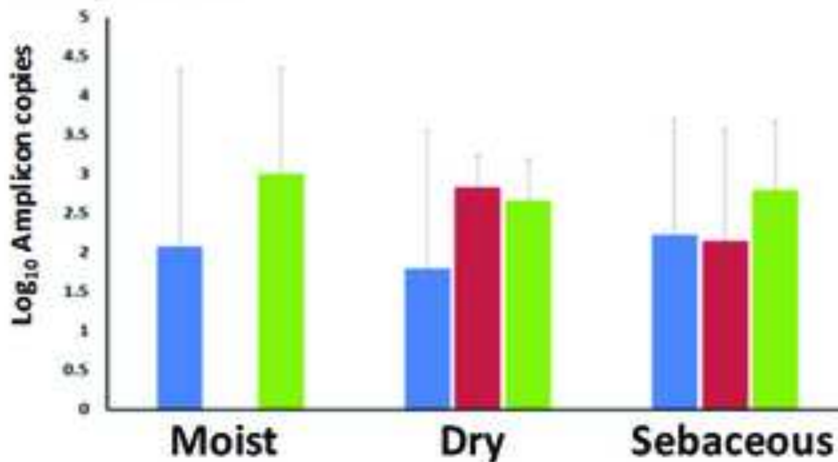

c Change in local EASI at worst lesion
Local EASI at worst lesional site (0-12)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
p < 0.001
p < 0.001
TCS + bleach
TCS alone



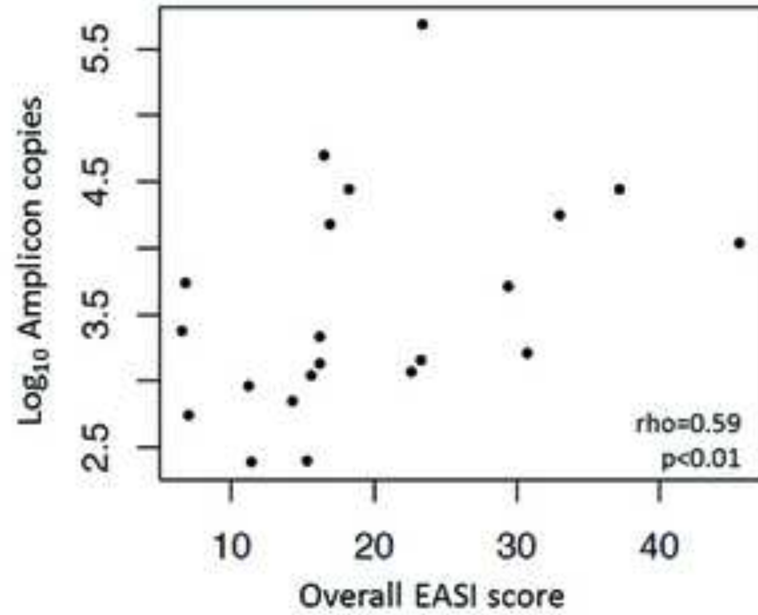



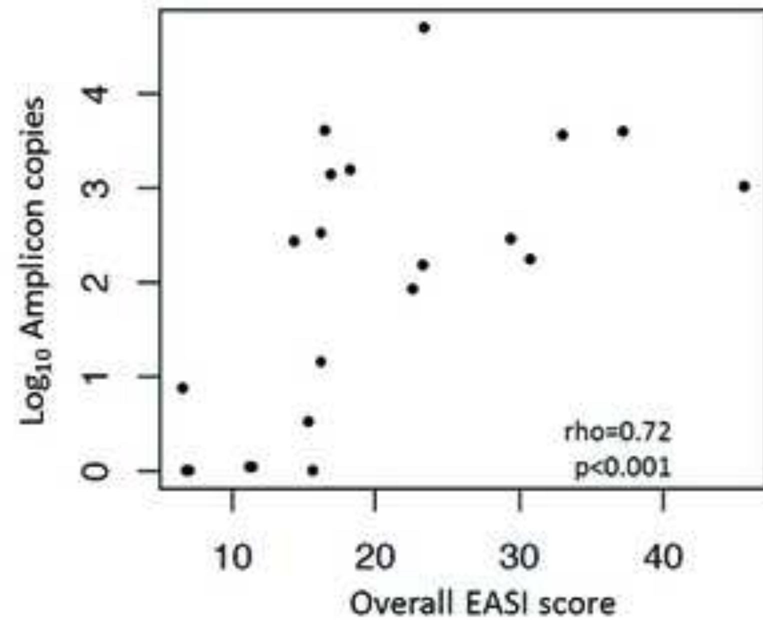



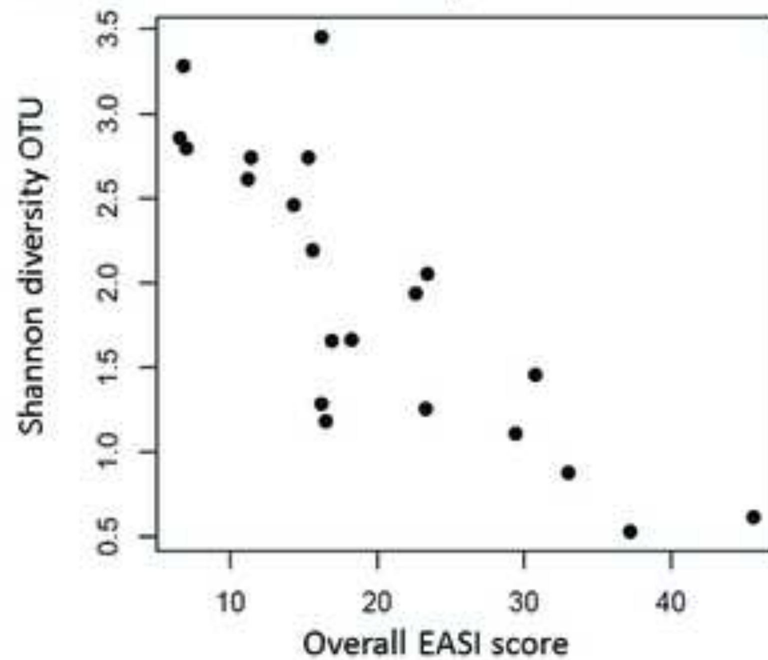



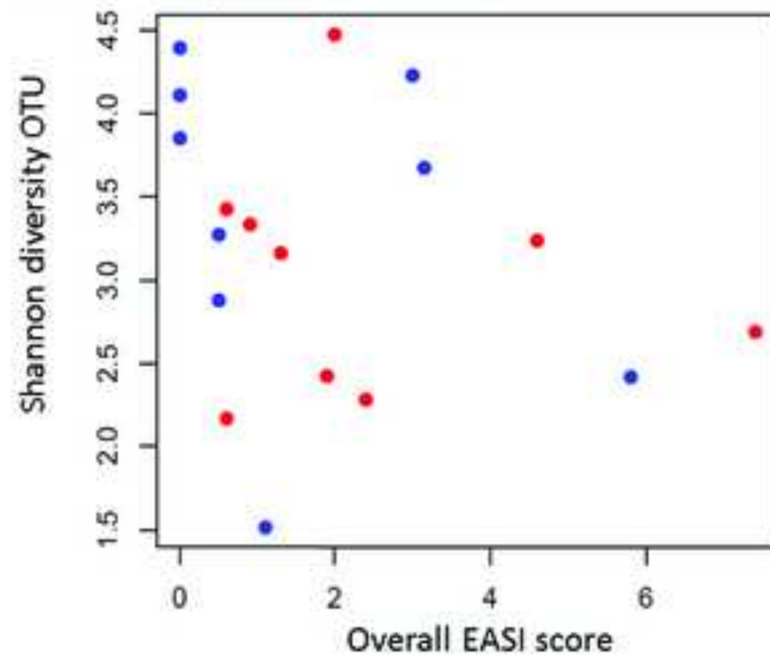



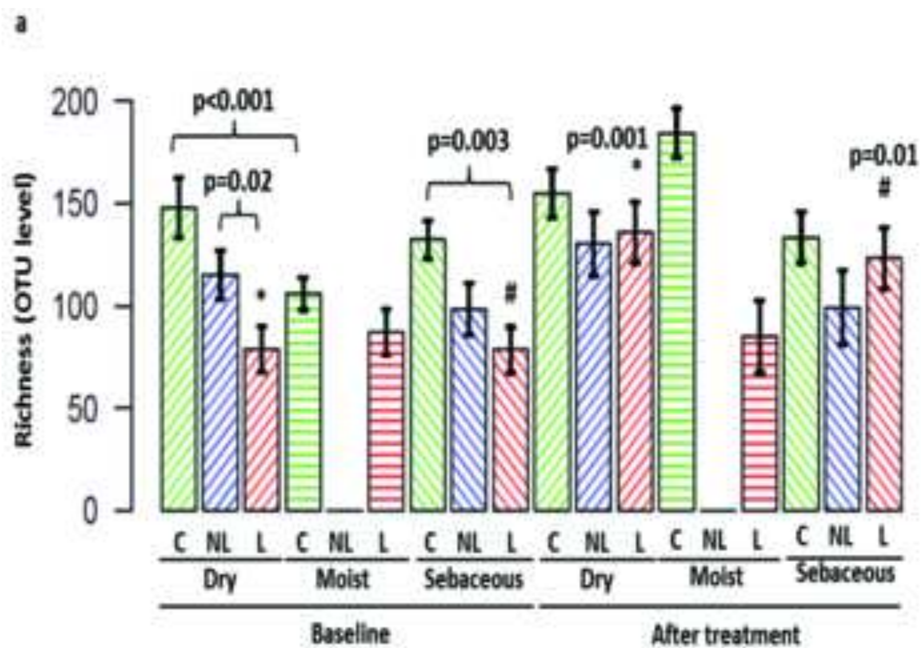

| Moist | Dry | Sebaceous | |
|---|---|---|---|
| 12 | 34 | 17 | Lesional |
| - | 9 | 12 | Nonlesional |
| 4 | 29 | 23 | Control |

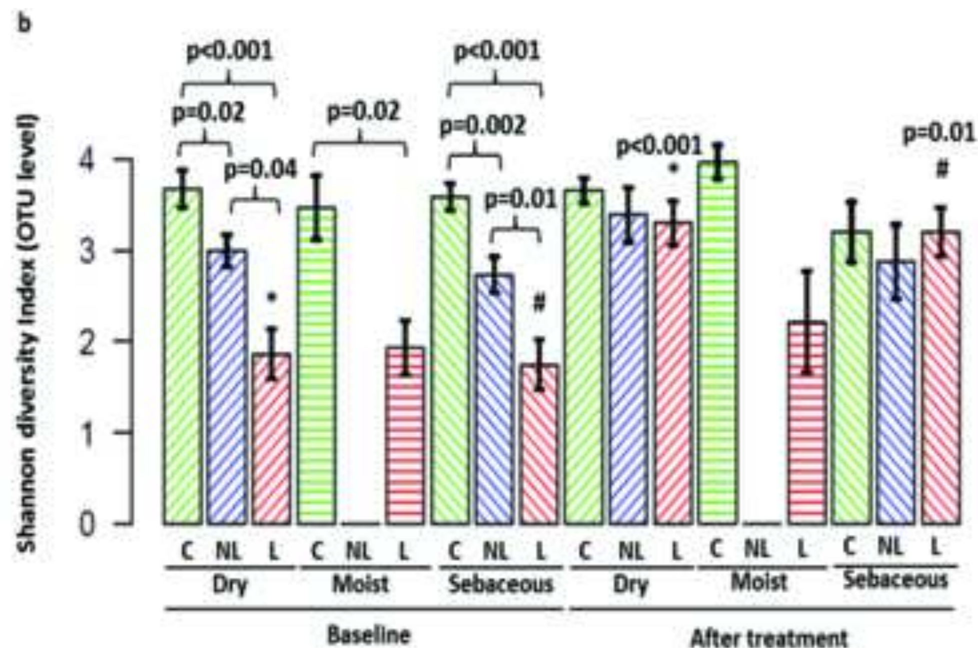

a Total bacteria
Log10 Amplicon copies
5.5
4.5
3.5
2.5
rho=0.59
p<0.01
10
20
30
40
Overall EASI score


b S. aureus
Log10 Amplicon copies
4
3
2
1
0
rho=0.72
p<0.001
10
20
30
40
Overall EASI score


c Mean Shannon diversity index
Shannon diversity OTU
3.5
3.0
2.5
2.0
1.5
1.0
0.5
10
20
30
40
Overall EASI score


d Follow-up with treatment
Shannon diversity OTU
4.5
4.0
3.5
3.0
2.5
2.0
1.5
0
2
4
6
Overall EASI score


a
Richness (OTU level)
200
150
100
50
0
p<0.001
p=0.02
p=0.003
p=0.001
p=0.01
*
#
C NL L C NL L C NL L C NL L C NL L C NL L
Dry
Moist
Sebaceous
Dry
Moist
Sebaceous
Baseline
After treatment


b
Shannon diversity Index (OTU level)
4
3
2
1
0
p<0.001
p=0.02
p=0.04
p=0.02
p<0.001
p=0.002
p=0.01
p<0.001
p=0.01
*
#
C NL L C NL L C NL L C NL L C NL L C NL L
Dry
Moist
Sebaceous
Dry
Moist
Sebaceous
Baseline
After treatment

TCS + bleach

TCS alone

Lesion

Nonlesional

Lesion

Nonlesional

Control

Baseline

Follow-up

value

Sample

A B C D E F G H I J

1.00 0.75 0.50 0.25 0.00

0 5 10 15

1

2

variable

Staphylococcus

Lactobacillus

Streptococcus

Veillonella

Acinetobacter

Enhydrobacter

Haemophilus

Escherichia

Neisseria

Corynebacterium

Bifidobacterium

Rothia

Prevotella