

antigen-presenting cells acquiring antigen from targeted LCs, we FACS-sorted antigen-presenting cells subsets from LNs of α -huLang-2W1S-immunized huLangerin mice, and co-cultured them in vitro with carboxyfluorescein succinimidyl ester-labeled OT-II T cells. Only migratory CD11b⁺ cDC2 and double-negative dDCs induced potent OT-II T-cell proliferation, indicating that they can acquire antigen from LCs (Figure 2d). A similar, but less robust, result was obtained from these DC subsets in huLang LC^{MHC II} mice (Figure 2e). These results demonstrate that CD11b⁺ dDCs and double-negative dDCs can acquire antigen from LCs in vivo.

In summary, these data have demonstrated that in vivo LC can acquire MHC II from other cells and that CD11b⁺ dDCs and double-negative dDCs can acquire antigen from LCs in the steady state. This finding reveals, to our knowledge, a previously unreported mechanism of DC cooperation in vivo and provides important insights on the design of DC vaccines aiming to target specific DC subtypes. These mechanisms of antigen and MHC II transfer may also occur in the contexts

of skin infection, vaccination, and/or autoimmune disease, although this remains to be elucidated.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at <https://doi.org/10.1016/j.jid.2018.02.005>.

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Methicillin-Resistant *Staphylococcus aureus* Colonization Is Associated with Decreased Skin Commensal Bacteria in Atopic Dermatitis



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TO THE EDITOR

Skin micro-organisms play an important role in the disease progression of atopic dermatitis (AD). The skin pathogen, *Staphylococcus aureus*, in particular, methicillin-resistant *S. aureus* (MRSA), contributes to AD by producing virulence factors that enhance immune activation and skin barrier dysfunction (Schlievert et al., 2010). While it is known that the relative abundance of *S. aureus* generally increases in AD

lesions (Kong et al., 2012; Shi et al., 2016), it is not clear whether the skin microbiome composition in MRSA-colonized skin differs from methicillin-sensitive *S. aureus* (MSSA)—colonized skin. It is important to understand the potential differences in the composition of skin commensal bacteria, as they play a critical role in reducing the growth of pathogenic *S. aureus* and thus the risk of AD (Kennedy et al., 2017; Nakatsuji et al., 2017).

In this study, we screened for the presence of MRSA and MSSA strains on the skin in a cohort of 339 AD patients, including 169 young children (2–12 years) and 170 teenagers (13–17 years) and adults (18–74 years). This study was approved by the Institutional Review Boards at National Jewish Health in Denver and University of California, Los Angeles. Written informed consent was obtained from all participants. As the skin microbiome of teenagers is closer to adults than young children (Shi et al., 2016), we combined teenagers and adults into one group in the analysis. We collected skin swabs from lesional skin and adjacent normal-appearing non-lesional skin of

Abbreviations: AD, atopic dermatitis; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*

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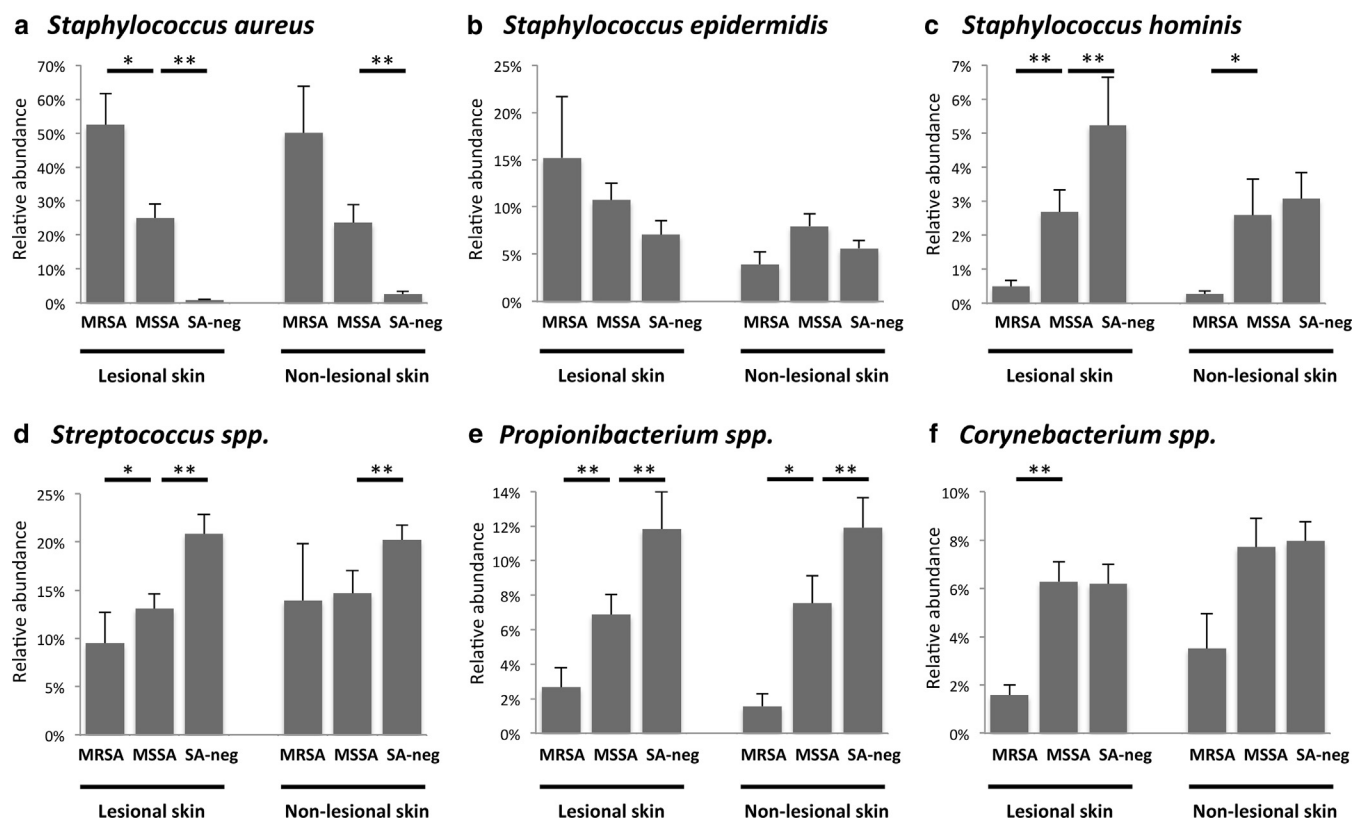


Figure 1. Methicillin-resistant *Staphylococcus aureus* (MRSA) colonization is associated with changes in the skin bacterial composition on atopic dermatitis (AD) skin. The AD skin samples are grouped based on MRSA ($n = 19$), methicillin-sensitive *Staphylococcus aureus* (MSSA) ($n = 70$), no *S. aureus* ($n = 39$) on the lesional site, and MRSA ($n = 9$), MSSA ($n = 43$), no *S. aureus* ($n = 76$) on the non-lesional site detected in the culture. The microbiome composition was determined using the culture-independent 16S ribosomal RNA gene analysis. The relative abundance of AD pathogen *S. aureus* (a) was significantly greater on MRSA-colonized lesional skin than MSSA-colonized lesional skin. MRSA colonization was associated with significantly lower relative abundances of skin commensals *Staphylococcus hominis* (c), *Streptococcus* (d), *Propionibacterium* (e), and *Corynebacterium* (f), but not *S. epidermidis* (b). Data are presented as mean $\pm$ standard error of mean. Wilcoxon rank-sum test with two-tailed distribution was performed. * $P < 0.05$ and ** $P < 0.01$. AD samples with no *S. aureus* detected in culture are labeled as "SA-neg."

the volar forearm from each AD patient. Using a culture-based assay (Supplementary Material online), MSSA was detected on the lesional skin of 199 (58.7%) AD patients (95 young children and 104 teenagers/adults), while MRSA was found in 23 (6.8%) AD patients (15 young children and 8 teenagers/adults). The detection frequency of MSSA and MRSA strains in pediatric patients of our cohort is consistent with previous studies (Suh et al., 2008). *S. aureus* was not detected at the lesional site of the remaining 117 (34.5%) AD patients. Among the 23 AD patients carrying MRSA on the lesional skin, 11 were also carrying MRSA at the adjacent nonlesional site.

In addition to the culture-based assay, we compared the microbiome of MRSA-versus MSSA-colonized AD lesional skin in a subgroup of these subjects. The microbiome composition of 128 of the 339 AD patients (Supplementary Table S1 online), including 19 MRSA

carriers, 70 MSSA carriers, and 39 patients without *S. aureus* detected in the culture-based assay, was profiled using the culture-independent 16S ribosomal RNA gene sequencing analysis (Supplementary Figure S1). The negative *S. aureus* culture results of 39 patients were corroborated by 16S ribosomal RNA gene sequencing analysis, which showed a significantly low relative abundance ($<1\%$) of *S. aureus* in the microbiome of these subjects. We found that the microbial diversity (alpha diversity) was significantly decreased in MRSA-colonized than MSSA-colonized lesional skin ($P = 0.001$) (Supplementary Figure S2 online). The relative abundance of *S. aureus* was significantly higher on MRSA-colonized ($52.5\% \pm 9.3\%$) than MSSA-colonized ($25.0\% \pm 4.1\%$) lesional skin ($P = 0.017$) (Figure 1a), suggesting that MRSA plays a more significant role in reducing the skin microbiome diversity than MSSA.

Our previous study demonstrated that the prevalent skin commensals, *Streptococcus*, *Propionibacterium*, and *Corynebacterium*, were significantly decreased in relative abundance on AD skin compared to healthy skin (Shi et al., 2016). In this study, we found that the relative abundances of *Streptococcus* and *Propionibacterium* were significantly decreased on MSSA-colonized lesional skin (both $P < 1E^{-3}$) when compared to the skin with no *S. aureus* detected in culture (Figures 1d, 1e). We found a significant further reduction in *Streptococcus* and *Propionibacterium* on MRSA-colonized lesional skin compared to MSSA-colonized lesional skin ($P = 0.023$ and $P = 0.007$, respectively). The relative abundance of *Corynebacterium* was significantly decreased on MRSA-colonized lesional skin as well ($P < 1E^{-3}$) when compared to MSSA-colonized lesional skin (Figure 1f). *Staphylococcus*

epidermidis has been considered a skin commensal, however, its role in AD is unclear. Previous studies reported an increase in abundance of *S. epidermidis* on lesional skin of pediatric AD, which may suggest a compensatory mechanism of *S. epidermidis* to control pathogens (Byrd et al., 2017; Kong et al., 2012). In the current study, we found that the relative abundance of *S. epidermidis* was not significantly changed in MRSA colonization (Figure 1b). Other commensal *Staphylococcus* species, including coagulase-negative *Staphylococcus* with antimicrobial activity, such as *Staphylococcus hominis*, are commonly found on healthy skin but rarely on AD skin (Nakatsuji et al., 2017). In this study, we found that the relative abundance of *S. hominis* was significantly decreased on MSSA-colonized lesional skin compared to non-*S. aureus* skin ($P < 0.004$) and further decreased on MRSA-colonized lesional skin ($P < 0.004$) (Figure 1c), with the same trend as skin commensals *Streptococcus* and *Propionibacterium*.

We also compared the microbiome between MRSA and MSSA colonization at the non-lesional site. Nine of the 19 MRSA carriers had MRSA detected not only on the lesional skin but also on the adjacent nonlesional site. On the non-lesional site, we found that compared to MSSA colonization, MRSA colonization was not associated with a significant increase of *S. aureus* ($P = 0.25$). The relative abundances of *Propionibacterium* ($P = 0.018$) and *S. hominis* ($P = 0.013$) decreased significantly, but other commensals did not change significantly (Figures 1a–1f). Overall, our data suggest that compared to MSSA, MRSA colonization is associated with a significantly greater decrease in relative abundance of skin commensal bacteria.

Previous studies have shown that the skin microbiome varies by age in both healthy individuals (Oh et al., 2012) and AD patients (Shi et al., 2016). In our cohort of 128 AD patients, 59 (46%) were young children and 69 (54%) are teenagers/adults. Among the 19 AD patients carrying MRSA at the lesional site (Supplementary Table S2 online), we found that MRSA colonization shifted

the microbiome composition in both age groups with an increased relative abundance of *S. aureus* and decreased relative abundances of skin commensals except for *S. epidermidis* (Supplementary Figure S3 online).

To identify correlated interactions among the bacteria in the AD skin microbiome, we calculated the Pearson correlation coefficients among the prevalent skin commensals and *S. aureus* based on their relative abundances on the lesional skin. To avoid false positives, correlations were not counted in samples that had no *S. aureus* detected in the culture. We found that the relative abundances of the prevalent skin commensals mentioned were negatively correlated with *S. aureus*, suggesting an antagonistic relationship between the skin commensals and this AD pathogen. We compared the correlations between each skin commensal and *S. aureus* on MRSA- versus MSSA-colonized lesional skin. We found that in teenager/adult patients, the skin commensals, *Streptococcus*, *Propionibacterium*, *Corynebacterium*, *S. hominis*, and *S. epidermidis*, had greater inverse correlations with *S. aureus* on MRSA-colonized skin compared to MSSA-colonized skin. In young children, only *S. epidermidis* and *S. hominis* showed greater inverse correlations with *S. aureus* on MRSA-colonized skin (Supplementary Figure S3). Previous studies have identified the skin commensals *Streptococcus*, *Propionibacterium*, and *Corynebacterium* with inhibitory activities against *S. aureus* in vitro (Bessesen et al., 2015; Shu et al., 2013). *S. epidermidis* is able to protect the skin from *S. aureus* infection through microbe–microbe interactions, as well as microbe–host interactions by enhancing immune responses to inhibit the growth of *S. aureus* (Iwase et al., 2010; Lai et al., 2010). The role of skin commensals in AD, in particular when MRSA colonizes the skin, needs future investigations.

We also investigated the influences of other confounding factors. In our cohort, disease severity was not significantly associated with MRSA colonization (Supplementary Table S2). Within the patients with severe AD ($n = 71$), we found that the microbial diversity was

lower in MRSA-colonized than MSSA-colonized lesional skin ($P = 0.002$, Supplementary Figure S4 online). The number of patients with moderate AD and MRSA colonization ($n = 2$) in our cohort was too low to allow us to perform a similar analysis. Additionally, to exclude the influence of medications on the skin microbiome, AD patients were excluded if they received medications, including antibiotic treatment within 7 days of sampling (more details in Supplementary Material).

In summary, our study suggests that MRSA colonization on AD skin correlates with a more profound change in the composition of commensal bacteria than MSSA colonization. The prevalent commensal bacteria had significant reductions in relative abundance in MSSA-colonized lesional skin compared to no *S. aureus* colonization and an even more significant decrease on MRSA-colonized lesional skin (Figures 1b–1f). Future studies are needed to better understand the mechanisms of potential microbial competition between skin commensals and MRSA in AD pathogenesis. Importantly, the maturation stage of host immune responses is an important factor, given the observation of differences in the prevalence of MRSA colonization between age groups in our cohort (Supplementary Table S2). Our current findings determined by 16S ribosomal RNA sequencing data may limit the representation of the absolute cell counts of microbial species and the taxonomic composition at the strain level. Additionally, our skin samples were collected from volar forearm of AD patients. Future studies are needed to investigate the influence of MRSA colonization on the skin microbiome at other body sites. Our findings suggest, however, that antibiotic treatment in AD patients with MRSA infection may not have long-term benefit due to its antimicrobial against of beneficial bacteria and, therefore, lacks a therapeutic strategy to promote commensal bacteria recolonization of the skin. This highlights the need to develop new approaches, such as probiotics or prebiotics, that replenish skin commensals as a means of combating skin infection and preventing MRSA relapse (Nakatsuji et al., 2017).

CONFLICT OF INTEREST

The authors state no conflict of interest.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at <https://doi.org/10.1016/j.jid.2018.01.022>.

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Highly Sensitive Virome Capture Sequencing Technique VirCapSeq-VERT Identifies Partial Noncoding Sequences but no Active Viral Infection in Cutaneous T-Cell Lymphoma



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TO THE EDITOR

The potential role of viruses as oncogenic triggers in cutaneous T-cell lymphoma (CTCL) pathogenesis is a subject of ongoing investigation. CTCL occurs with an increased incidence in immunosuppressed patients (Nikolaou et al., 2015; Pomerantz et al., 2010; Wilkins et al., 2006). Spectratyping studies have shown depleted T-cell receptor diversity in CTCL patients similar to that

seen in patients with advanced HIV, and Ingenuity Pathway Analysis (Qiagen, Hilden, Germany) shows increased expression of genes critical to host viral response (Yawalkar et al., 2003). However, no consistent association between a viral pathogen and CTCL has been established (Mirvish et al., 2013).

The development of high-throughput sequencing (HTS) has provided

powerful new tools for pathogen discovery, yet HTS studies have not identified viral sequences in CTCL (Dereure et al., 2013; Dulmage et al., 2015; Lee et al., 2012). One explanation for failure is that viral burden in samples may be below the threshold for detection in complex backgrounds of host nucleic acid. To address this possibility, we used a positive selection method for HTS with enhanced sensitivity (Briese et al., 2015).

The Virome Capture Sequencing Platform for Vertebrate Viruses (VirCapSeq-VERT; Roche, Pleasanton, CA) is a positive-selection probe-based method that targets all 207 known vertebrate viruses, allowing for a 100- to 10,000-fold increase in the number of viral

Abbreviations: contig, contiguous sequence; CTCL, cutaneous T-cell lymphoma; HERV, human endogenous retrovirus; HHV, human herpesvirus; HTLV-1, human T-lymphotropic virus-1; HTS, high-throughput sequencing; NCBI, National Center for Biotechnology Information; TNA, total nucleic acid; VirCapSeq-VERT, virome capture sequencing of vertebrate viruses

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1 **Supplementary Materials**

2 **Study Participants**

3 This study was approved by the Institutional Review Boards at National Jewish Health in Denver
4 and UCLA in Los Angeles. Written informed consent was obtained from all participants (parents
5 or guardians of participating children). All study participants underwent a detailed history survey
6 and physical examination. Exclusion criteria included having a fever ≥ 38.5 °C; having bathed or
7 showered after midnight before the day of sampling; using creams/lotions at the sites where the
8 specimens would be taken within 24 hours of sampling; receiving oral antibiotics, a bleach bath,
9 topical prescription medications (including but not limited to Elidel, Protopic, topical
10 corticosteroids, or topical antibiotics) at the sites where the swabs would be collected within
11 seven days of skin sampling; systemic immunosuppressive drugs (including cyclosporine or oral
12 steroids), total body phototherapy (e.g., ultraviolet light B, psoralen plus ultraviolet light A,
13 tanning beds) within 20 days prior to sampling. Atopic dermatitis (AD) was diagnosed with
14 standard criteria based on the American Academy of Dermatology and the NIH/NIAID Atopic
15 Dermatitis Research Network (Beck et al., 2009; Eichenfield et al., 2003). Disease severity was
16 assessed by using the Rajka-Langeland scoring system (Rajka et al., 1989).

17

18 **Sample Collection and MRSA Identification**

19 Two swab samples, one from lesional skin and one from non-lesional skin, were collected from
20 the volar forearm of each AD patient. A non-lesional site was defined as a clinically
21 normal-appearing area that is approximately 5 cm away from the edge of the lesional site that is
22 being swabbed. All samples were collected from a 5cm X 5cm skin area. The swabs were placed
23 into the sterile culturette tubes, stored at 4 °C and transported to the laboratory within 48 hours of

24 collection.

25

26 All swab samples were simultaneously tested for *S. aureus* and MRSA. Briefly, skin swabs were
27 inoculated directly onto a blood agar plate (Blood agar, 5% Sheep blood, Remel Inc.) and an
28 MRSA agar plate (Remel Spectra MRSA Screen plate, Remel Inc.). Blood agar plates were
29 incubated in a 5% CO₂ incubator at 37 °C, and MRSA plates were incubated at 35 °C in a
30 non-CO₂ incubator. After 24 hours, both plates were examined for the presence of *S. aureus*.
31 Bacterial colonies that appeared to be *S. aureus* had a slide coagulase and catalase test performed.
32 The colonies were identified as *S. aureus* if the two tests were positive. If no growth was seen on
33 the blood plate after 24 hours, the plate was reincubated for another 24 hours to check for visible
34 bacterial growth. Colonies growing on the MRSA plate after 24 hours incubation were identified
35 as MRSA strains.

36

37 **The Microbiome Analysis**

38 The microbiome composition was determined based on the sequencing analysis of the
39 hypervariable regions V1-V3 of the 16S rRNA genes (Shi et al., 2016). A data cleaning process
40 was applied to all sequence data prior to analysis. Briefly, low-quality bases with a Phred quality
41 value lower than 20 were trimmed off the read ends. The read pairs were removed if any of the
42 two reads was trimmed to shorter than 200 bp or had $\geq 3\%$ uncertain bases. The 16S rRNA
43 sequences with paired reads were mapped against Greengenes (v13.5, non-redundant
44 precalculated OTU references, 97_otus from PICRUSt) (DeSantis et al., 2006; Langille et al.,
45 2013). The alignments were performed using Bowtie2 to search sequences with the best hits to
46 the references (Langmead et al., 2012). We required that the paired-end reads align to the same

reference sequence in different strand directions and within 600 bp from each other. The annotation of 16S rRNA references was refined at the species level by cross-referencing the SILVA rDNA database (Quast et al., 2013).

50

The microbial diversity (alpha diversity) was measured by Shannon index (Shi et al., 2016), and was calculated using QIIME (Caporaso et al., 2010).

53

54 **Statistical Analysis**

Wilcoxon rank-sum test was performed in statistical testing of differences in microorganisms between samples from different groups.

57

58 **Data Availability**

The 16S rRNA sequence data from this study have been deposited to NCBI BioProject accession number 268694.

61

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84 different in pediatric versus adult atopic dermatitis. J Allergy Clin Immunol 2016;
85 138:1233-6.

86

87 **Supplementary Figure Legends**

88 **Supplementary Figure S1. The microbiome composition in AD patients.**

89 The AD skin samples are grouped based on MRSA, MSSA, no *S. aureus* on the lesional site
90 detected in the culture. AD samples with no *S. aureus* detected in culture are labeled as "SA-neg".

91 The microbiome composition was determined using the 16S rRNA gene analysis. Twenty
92 prevalent genera from five bacterial phyla were identified in the AD skin microbiome. The
93 microbiome compositions of AD lesional (upper panel) and non-lesional (lower panel) skin are
94 shown at the phylum level, the genus level, and *Staphylococcus* species level. Samples collected
95 from the same patients are shown in the same order in both panels. AD patients carrying MRSA
96 on both the lesional and the adjacent non-lesional site are labeled with asterisks at the bottom of
97 the figure.

98

99 **Supplementary Figure S2. The microbial diversity was significantly decreased in MRSA**

100 **colonized than MSSA colonized lesional skin.**

101 Data are represented as mean $\pm$ s.e.m. Wilcoxon rank-sum test with two-tailed distribution was
102 performed. The overall microbial diversity was measured by Shannon index. AD samples with
103 no *S. aureus* detected in culture was labeled as "SA-neg".

104

105 **Supplementary Figure S3. MRSA colonization affected the skin bacterial composition in**
106 **pediatric and adult AD patients.**

107 MRSA colonization influenced the relative abundances of skin commensals and their correlations with
108 *S. aureus* on the lesional skin of pediatric and adult AD patients. The relative abundances of *S. aureus*
109 (i) and the skin prevalent commensals (ii-vi) were represented as mean $\pm$ s.e.m from the lesional skin
110 of 13 MRSA colonized and 26 MSSA colonized young children and 6 MRSA colonized and 44 MSSA
111 colonized teenagers-adults patients. The correlations between skin commensals and *S. aureus* were
112 calculated using Pearson correlation coefficients for both MRSA colonized and MSSA colonized AD
113 skin (vii).

114

115 **Supplementary Figure S4. The microbial diversity was decreased in MRSA colonized than**
116 **MSSA colonized lesional skin in severe AD patients.**

117 Data are represented as mean $\pm$ s.e.m. Wilcoxon rank-sum test with two-tailed distribution was
118 performed. The overall microbial diversity was measured by Shannon index.

119

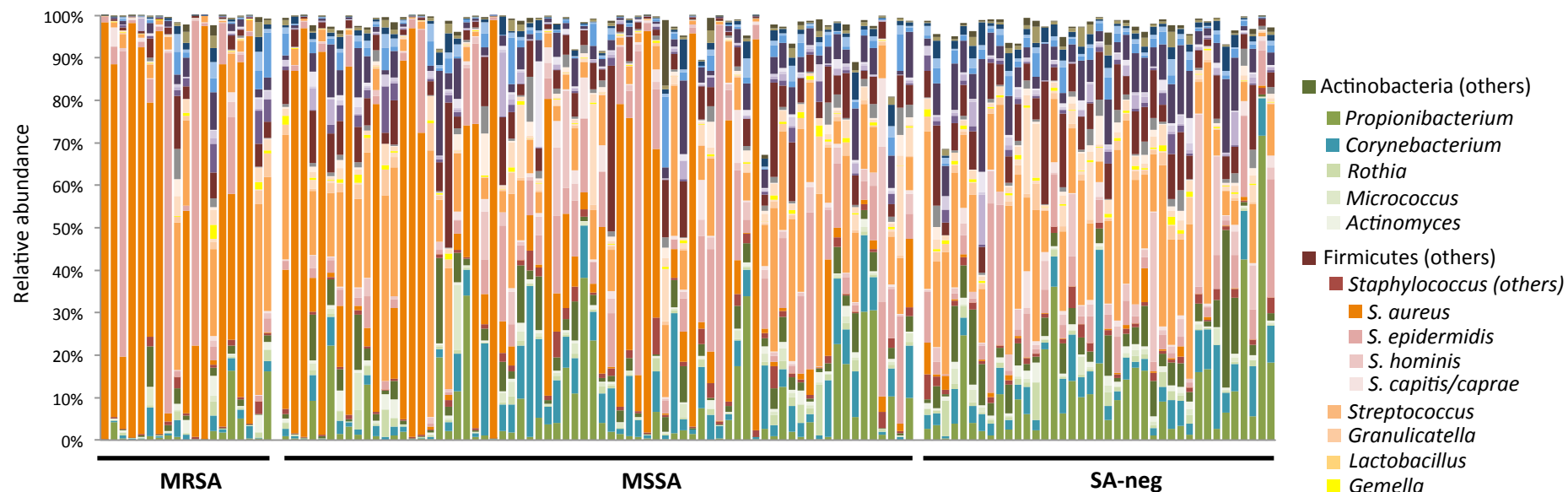
120 **Supplementary Tables**

121 **Table S1.** Demographics of the study participants in the microbiome analysis

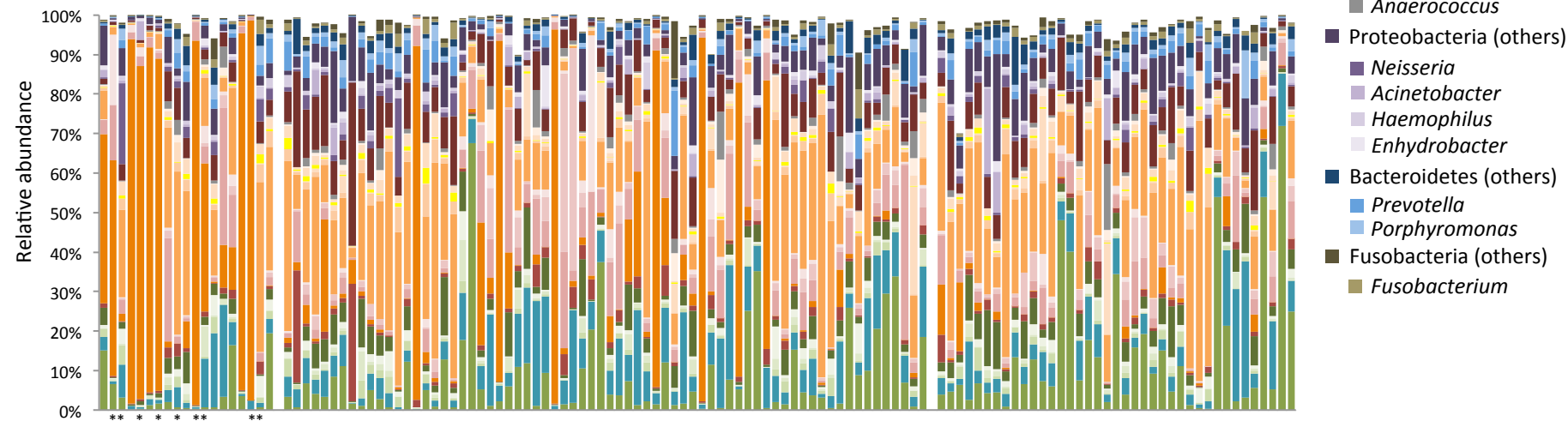
122 **Table S2.** Demographics of the study participants with MRSA or MSSA colonization

Supplementary Figure S1

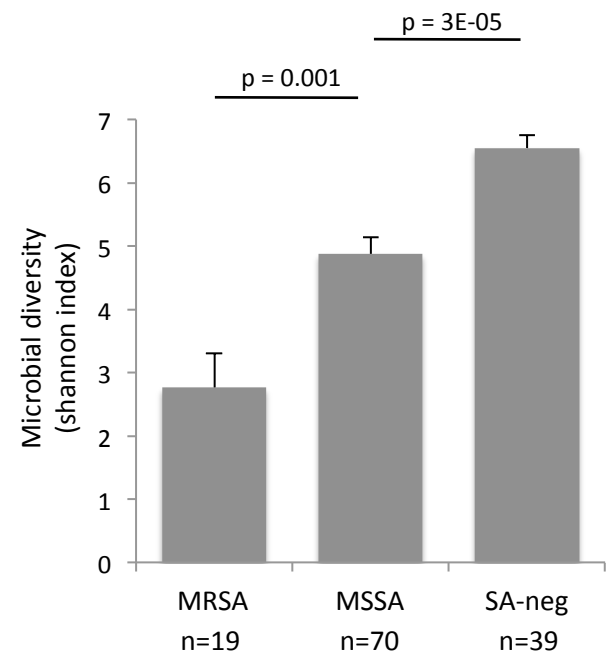
Lesional skin



Non-lesional skin

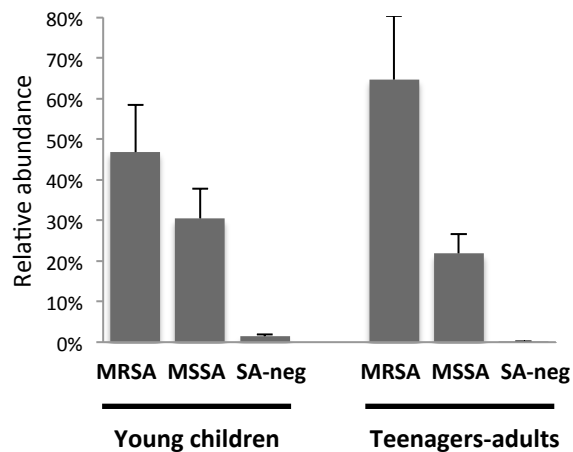


Supplementary Figure S2

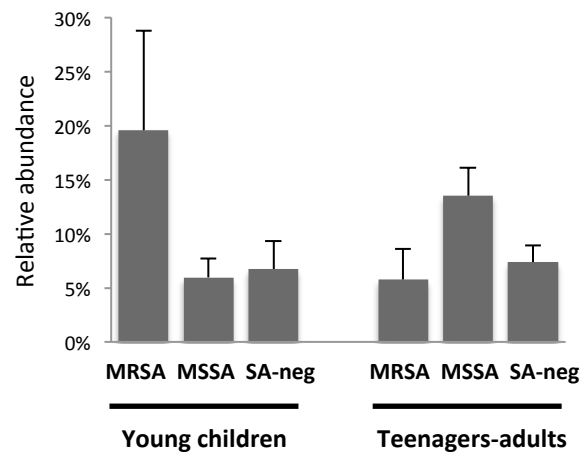


Supplementary Figure S3

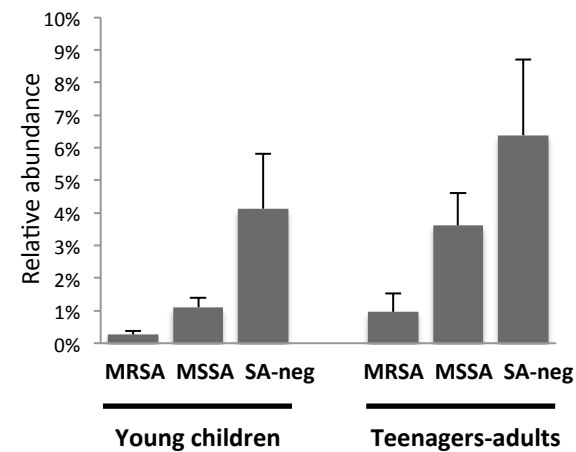
i) *Staphylococcus aureus*



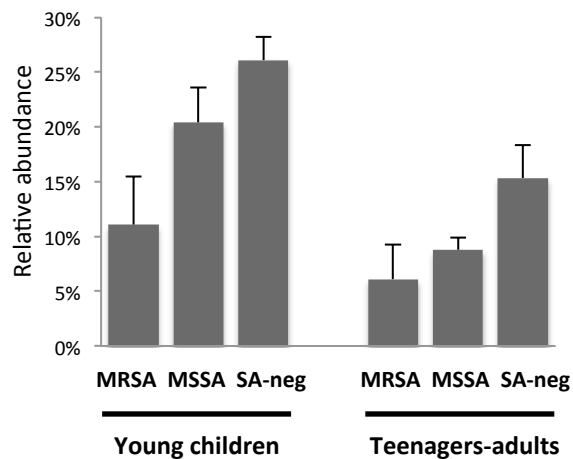
ii) *Staphylococcus epidermidis*



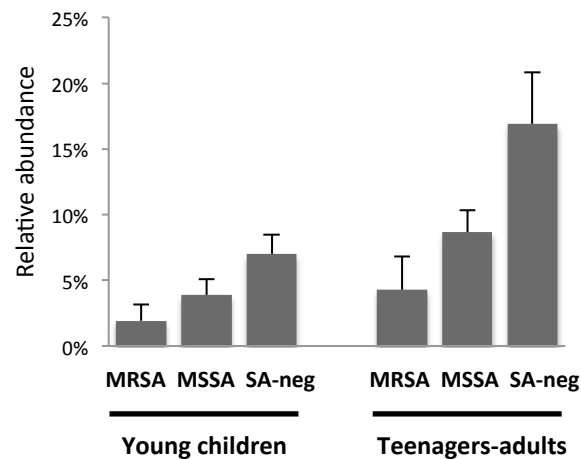
iii) *Staphylococcus hominis*



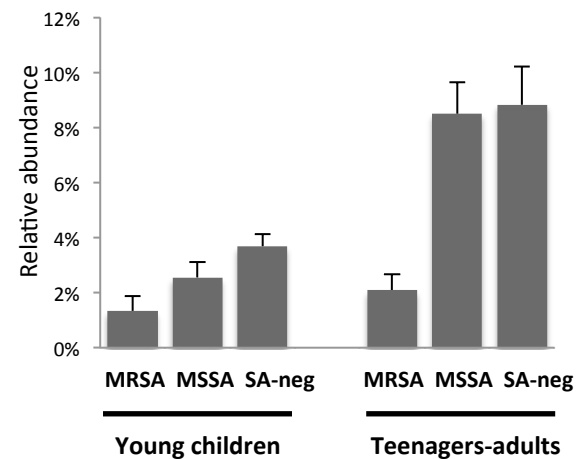
iv) *Streptococcus spp.*



v) *Propionibacterium spp.*

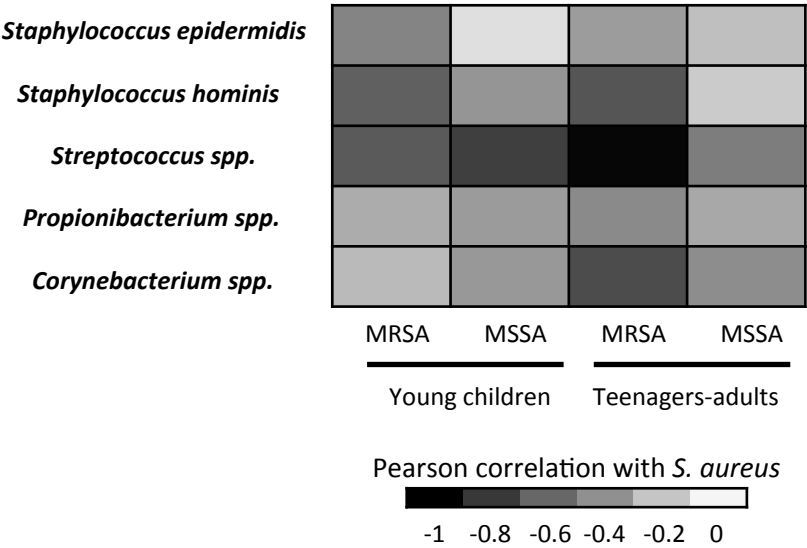


vi) *Corynebacterium spp.*



Supplementary Figure S3

vii)



Supplementary Figure S4

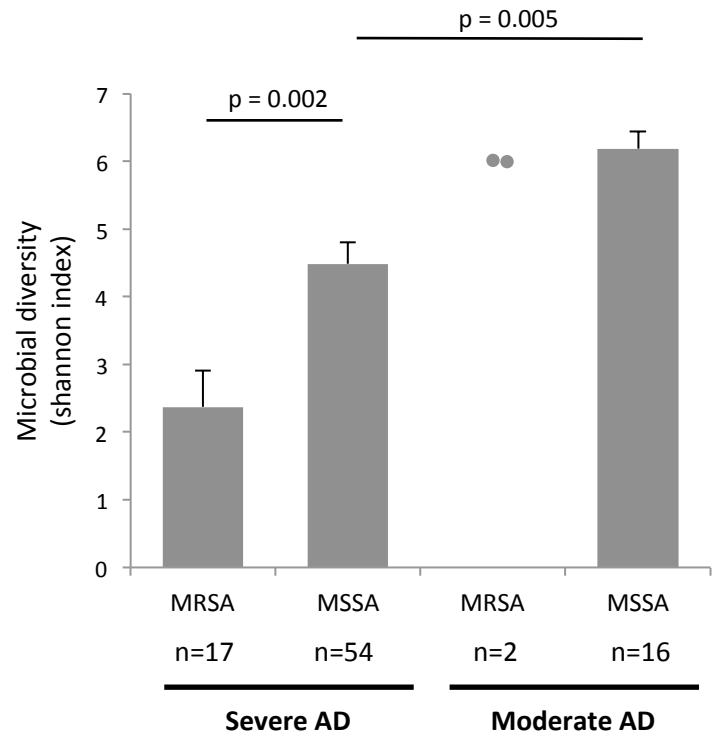


Table S1. Demographics of the study participants in the microbiome analysis (n=128)

Characteristics		Children n=59	Teens and Adults n=69
Age median (Q1, Q3)*		8 (6,10)	26 (20,37)
EASI median (Q1, Q3)*		25 (14,35)	20 (14,33)
Rajka-Langeland Total Score median (Q1, Q3)*		9 (7,9)	8 (7,9)
Gender (%)	Female	27 (46%)	38 (55%)
	Male	32 (54%)	31 (45%)
Race (%)	American Indian or Alaska Native	1 (2%)	1 (1%)
	Black or African American	15 (25%)	9 (13%)
	Caucasian	42 (71%)	58 (84%)
	Multi-racial	0 (0%)	1 (1%)
	Other	1 (2%)	0 (0%)

*Median (with interquartile range)

Table S2. Demographics of the study participants with MRSA or MSSA colonization (n =89)

Characteristics		MRSA carriers n=19 (%)	MSSA carriers n=70 (%)	p-value *
Age group	Young children (2 - 12 yrs)	13 (68.4%)	26 (37.1%)	0.02
	Teens and Adults (13 - 74 yrs)	6 (31.6%)	44 (62.9%)	
Gender	Female	9 (47.4%)	31 (44.3%)	1.0
	Male	10 (52.6%)	39 (55.7%)	
Disease severity	Moderate	2 (10.5%)	16 (22.9%)	0.34
	Severe	17 (89.5%)	54 (77.1%)	

*two-tailed Fisher's Exact test