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Supplementary Materials for

Antimicrobials from human skin commensal bacteria protect against *Staphylococcus aureus* and are deficient in atopic dermatitis

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
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SUPPLEMENTARY MATERIALS:

ONLINE METHODS

Human Subjects

Adults with AD and age-matched non-AD subjects were recruited from the University of California San Diego (UCSD), San Diego, CA and National Jewish Health (NJH), Denver, CO (demographic data are shown in table S1). All experiments involving human subjects were carried out according to the IRB protocols approved by UCSD (Project#071032) and NJH (HS-2581). Informed consent was obtained from all subjects. All subjects avoided any treatments and therapies that potentially affect skin microbiome prior to sample collection (refer to Supplementary information for sampling collection criteria). Collection of surface bacteria was done from a pre-measured area (15-30 cm^2) of lesional skin on the antecubital fossa, and nonlesional skin of the upper arm at least 2 cm separated from the lesional site. Similar collections were obtained from non-AD subjects at identical skin sites.

Sample collection criteria

Swab sample collection was performed on a subset of participants at the University of California San Diego, San Diego, CA and National Jewish Health, Denver, CO, under the consistent sample collection criteria established by Rho Federal Systems Division, Inc., Chapel Hill, NC. A subject who met the following criteria was avoided or rescheduled; (1) did not have active lesions on upper extremities, (2) had a fever $\geq$38.5 ^{o}C (101.3^{o}F), (3) had used oral antibiotics within the last 7 days, (4) had taken systemic immunosuppressive drugs including cyclosporine or oral steroids within the last 20

days, (5) had received total body phototherapy (e.g., ultraviolet light B, psoralen plus ultraviolet light A, tanning beds) within the past 20 days, (6) had used topical prescription medications including (but not limited to) Elidel, Protopic, topical corticosteroids, or topical antibiotics at the sites where the swabs will be collected within the last 7 days, (7) had used a bleach bath within the past 7 days, (8) had used creams/lotions at the sites where the swabs will be collected within 24 hours of the day of sampling, (9) had bathed or showered after midnight before the day of sampling.

Randomization of subjects

To randomly select subjects from non-AD and AD groups, an online randomizer (www.randomization.com) was used. Subjects who did not have enough number of CoNS colonies (< 25 colonies/ swab) were excluded.

Screening for antimicrobial activity

Up to 84 individual isolated colonies of CoNS from each skin site were randomly picked and transferred to individual wells containing TSB in a 96-well cluster tube. Each plate also contained wells with a non-antimicrobial strain of *S. epidermidis* (ATCC1457) as negative control, a potent antimicrobial strain of *Staphylococcus hominis* (A9 strain) as positive control, and blank wells without bacteria. CoNS were cultured at 37 ^{o}C overnight with shaking. Growth was evaluated by OD_{600}. Bacteria were removed by centrifugation followed by sterile filtration with a 0.22μm membrane. The antimicrobial activity released from each CoNS clone was evaluated by mixing with 1×10^{4} colony-forming units (CFU) of *S. aureus* (ATCC35556). Antimicrobial strains were defined as those that suppressed *S. aureus* growth after 22 hrs to less than 50% (I_{50}) of growth seen in negative controls. Insufficient CoNS colonies (<25) were grown from the entire target area of some subjects recruited,

therefore, data are reported for 29 non-AD subjects and 41 nonlesional and 40 lesional sites of AD subjects. All CoNS isolates were stored frozen for species identification. Full-length 16S rRNA genes were amplified from 48 representative colonies with universal 16S primers, 27-F and 1525-R (table S4). Amplicons were sequenced from both ends by Sanger method.

Microbiome sequencing

Bacterial DNA was collected from, pre-measured areas similar to those used for bacterial culture collection, but were rubbed with a swab pre-moistened with Tris-EDTA buffer containing 0.1% TritonX-100 and 0.05% Tween-20 (w/v). Bacterial cells were enzymatically lysed by proteinase K, followed by purified achromopeptidase (Wako Chemical) and Ready-Lyse® (Epicenter Inc.), followed by heating at 100^{o}C. Total genomic DNA was purified with QIAamp DNA micro kit (Qiagen) and eluted with 50µL of elution buffer.

V1-V3 16S rRNA was amplified with Phusion High-Fidelity polymerase (Thermo Scientific, Waltham, MA) using dual-indexed coded primers, normalized, pooled and paired-end sequenced (2 × 300bp) on an Illumina MiSeq (Illumina) in the University of Rochester Genomics Research Center (*45*). Each sequencing run included: (1) positive controls consisting of a 1:5 mixture of *Staphylococcus aureus*, *Lactococcus lactis*, *Porphyromonas gingivalis*, *Streptococcus mutans*, and *Escherichia coli*; and (2) negative controls consisting of sterile saline.

16S rRNA sequencing and analysis

The 16S rRNA bacterial sequence reads were assessed for quality and analyzed using phylogenetic and Operational Taxonomic Unit (OTU) methods using the Quantitative Insights into Microbial Ecology (QIIME) software, version 1.9.1 (*46*). Read pairs were assembled using fastq-join from the ea-utils package (http://code.google.com/p/ea-utils), requiring at least 20 bases of overlap and allowing a maximum of 10% mismatched bases. OTUs were picked using the reference-based USEARCH (version 5.2) (*47*) pipeline in QIIME, using the May 2013 release of the GreenGenes 99% OTU database (*48*). Chimera detection and removal was performed *de novo* using UCHIME (*49*). OTU clusters with less than four sequences were removed, and representative sequences used to make taxonomic assignments for each cluster were selected on the basis of abundance. The RDP Naïve Bayesian Classifier was used for taxonomic classification with the GreenGenes reference database, using a minimum confidence threshold of 0.85 and otherwise default parameters (*50*).

OTU table analysis

For the purposes of alpha and beta diversity calculations, twenty iterations of rarefaction at an even depth of 8,000 reads per sample were performed. Samples 148 and 153 did not meet the 8000 read threshold and were therefore excluded from further analysis. The mean alpha diversity over all rarefactions was computed for each sample using QIIME's PD Whole Tree metric, the raw count of OTUs observed, the Shannon diversity index, and Strong's dominance index. For the purposes of OTU relative abundance analysis, the raw OTU table was normalized using the cumulative sum stabilization method from the metagenomicSeq R package (*51*). Weighted and unweighted Unifrac distances between all samples were computed using QIIME, and were used to perform principal coordinate analysis (PCoA). PCoA visualization was done using the Emperor package within QIIME.

Quantification of live Staphylococcus

Patients with AD or non-AD subjects were instructed to avoid any active treatments for at least 2 weeks, and body wash for 24 hrs prior to sample collection. Collection of surface bacteria was done from a pre-measured area (15-30 cm^2) of lesional skin on the antecubital fossa, and nonlesional skin of the upper arm at least 2 cm separated from the lesional site. Similar collections were obtained from non-AD subjects at identical skin sites. The entire target area of the skin was rubbed 50 times with consistent firm pressure with Catch-ALL™ sample collection swabs (Epicenter Inc.) pre-moistened with tryptic soy broth (TSB). Samples were then suspended in 1.5mL TSB containing 15% (v/v) glycerol and inoculated on a mannitol salt agar with egg yolk for selective growth of Staphylococcus species (*52, 53*). *S. aureus* was distinguished from CoNS according to mannitol metabolism and the egg yolk reaction (*39*). The samples were stored at -80^{o}C for further analyses.

Quantification of Staphylococcus DNA

The abundance of bacterial DNA in the elution was determined by quantitative real-time PCR (qPCR) with species- or genus-specific primers (table S4). To determine relative CFU (rCFU) of *Staphylococcus* spp. DNA, a standard curve was generated with genomic DNA extracted from known CFU of *S. epidermidis* (ATCC12228). The specificity of all primer pairs was confirmed by melting curve analysis and comparison with standard curves.

Screening for CoNS antimicrobial activity

Up to 84 individual colonies of CoNS isolated from one skin site were randomly picked from a mannitol salt agar with egg yolk and transferred to TSB (400 µL) in a 96-well cluster tube. Each plate contained internal controls of a non-antimicrobial strain of *S. epidermidis* (ATCC1457) as negative

control (6 wells), a known antimicrobial strain of *Staphylococcus hominis* producing *Sh*-lantibiotics as positive control (2 wells), and blank wells without bacteria (2 wells). CoNS were cultured at 37 ^{o}C overnight with shaking at 250 rpm. Bacterial growth was evaluated by measuring OD_{600} and only CoNS grown over late stationary phase (OD_{600}>6.0) were used for subsequent analysis. Bacteria were removed by centrifugation followed by sterile filtration by a 96-well filter plate with 0.22 μm PVDF membrane (Corning Inc). The antimicrobial activity in each sterile filtered media (100μL) was evaluated by mixing with fresh TSB (10μL) containing 1×10^4 colony-forming unit (CFU) of *S. aureus* (ATCC35556). Antimicrobial CoNS strains were defined as those that suppressed *S. aureus* growth after 22 hrs to less than 50% (I_{50}) of average growth seen in negative controls. The frequency of antimicrobial CoNS was determined to total CoNS numbers subjected to the assay. Insufficient CoNS colonies (<25) were grown from some of the subjects recruited. Therefore data are reported for 29 non-AD subjects and 41 nonlesional and 40 lesional sites of AD subjects. All CoNS isolates were stored frozen for species identification by sequencing full-length 16S rRNA gene. Full-length 16S rRNA genes were amplified from 48 representative colonies with universal 16S primers, 27-F and 1525-R. Amplicons were sequenced from both ends by Sanger method. Obtained 16S rRNA gene sequence was searched against Ribosomal Database Project (http://rdp.cme.msu.edu/) to assign operational taxonomic units at species level (*54*).

Purification of antimicrobials produced by CoNS strains

Sterile conditioned media from a representative antimicrobial *S. hominis* strain A9 isolated from a healthy subject was used to further identify molecules with antimicrobial activity on normal skin that were in low abundance on AD. Activity was precipitated by ammonium sulfate (70% saturation), dissolved in H_2O and applied on a Sep-Pak cartridge (Waters Co.). The cartridge was washed by 20%

acetonitrile in H_2O, and active fractions were eluted with 30% acetonitrile in H_2O. The elution was lyophilized, reconstituted in 25mM phosphate butter (pH 7.2), subjected to HiTrap® SP (GE Healthcare Life Sciences) separation, and then activity eluted at 0.125mM NaCl. Third step HPLC purification was done with CapCel Pak C8 (5 µm, 300 Å, 4.6 × 250mm) (Shiseido Co.) with a linear gradient of acetonitrile from 5% to 50% in 0·1% (v/v) TFA at 0.8 mL/min. Antimicrobial activity of each fraction was determined by radial-diffusion assay against *S. aureus* (ATCC35556).

Mass spectrometry

Mass spectra of HPLC-purified AMPs from *S. hominis* A9 were recorded using a MALDI-TOF/TOF Bruker Autoflex Speed instrument (Bruker Daltonics) controlled by the flexcontrol software (Bruker Daltonics). Mass spectrometric analyses were performed in positive ion reflectron mode using cyano-4-hydroxycinnamic acid as a matrix (CHCA) 10 mg/mL (Sigma-Aldrich) dissolved in 50% acetonitrile (ACN) and 0.1% trifluoroacetic acid (TFA). Full scan mass spectra were acquired in positive ion reflectron mode for mass range 1000-4000 m/z. Each mass spectrum is the result of 750 averaged laser shots with the laser intensity set around 65% of full laser intensity and a detector gain enhanced at 8x 4GS/s (as selected within the Bruker Flex Control software). MALDI-MS/MS spectra of manually selected ion m/z 3547, with a window range of 5 Da, were acquired using TOF/TOF collision-induced dissociation. Each MS/MS spectrum is the result of 1000 averaged laser shots with the laser intensity set around 60% selected within the software and a detector gain enhanced at 10x 4GS/s. Resulting mass spectra were analyzed using flex analysis software (Bruker Daltonics). Spectra were calibrated to PepMix internal standard solutions.

N-terminal protein sequencing

The N-terminal amino acid sequence of *Sh*-lantibiotic-α (Fraction 30, Fig. 5A) was analyzed by 15 cycles of Edman degradation on Procise® 494HT Protein Sequence system (Applied Biosystems). A gene corresponding to the obtained amino acid sequence was found in the whole genome of the *S. hominis* strain A9 when sequenced (Fig. 5, B and C), but not in that of a potential pathogenic strain with multiple drug resistance, which was previously sequenced (*32*). A mature form of *Sh*-lantibiotic-α was predicted from the genome sequence, with the calculated molecular weight of a hypothetical mature form [3152.52 (M+H)], identical to the observed molecular masses [m/z 3152.22 (M+H)].

Protein sequencing by MALDI-TOF/TOF

Because N-terminus of *Sh*-lantibiotic-β (Fraction 32, Fig. 5, A and B) contains a modified amino acid, sequence could not be obtained by Edman degradation. Whole protein sequence of this AMP was therefore obtained based using genome guided MALDI-TOF/TOF analysis as described previously (*55*). Analysis of the genome sequence of *S. hominis* A9 was performed on antibiotics and secondary metabolite analysis shell - AntiSMASH platform in order to identify secondary metabolites biosynthesis gene clusters (*56*). AntiSMASH results provided one gene cluster for lantipeptides with potential candidate loci at 2050~ 2250 and 26354~26554 (Fig. 5C). NCBI BlastP analyses of genes involved in lantipeptide synthesis and modification show high degree of identity to the class 2 lantibiotic peptide. A Core peptide with sequence ATPTITTSSATCGGIIVAASAAQCPTLACSSRCGKRKK cleaved from leader peptide with a GG cleavage site, common for type-2 lantibiotics (*57*) was identified. Combining genome mining and MS/MS fragmentation predicted a mature form of *Sh*-lantibiotic-β. A mature form of *Sh*-lantibiotic-β was predicted from the genome sequence of *S. hominis* A9, as the calculated

molecular weights of a hypothetical mature form [3548.04 (M+H)] was identical to the observed molecular masses [m/z 3547.71 (M+H)].

Genome sequencing

Because the protein sequences of AMPs from *S. hominis* A9 did not match to any molecules found in the existing genome database, we obtained whole genome sequence of this antimicrobial strain of *S. hominis*. Genomic DNA was purified using UltraClean® Microbial DNA Isolation kit (MO Bio). Whole genome DNA sequencing libraries were constructed using the Nextera-XT DNA Sample Prep Kit (Illumina) following the vendor's protocol. The final library was sequenced paired-end (300×300 bp) on an Illumina MiSeq. Sequenced reads were *de novo* assembled using SPAdes 2.5.1 with k-mers of lengths 21, 33, 55, 77, and 127 and the flag for 'careful' turned on. On all of the produced scaffolds, a six-frame translation was performed using translate Whole Genome Multi Chromosome.pl (http://proteomics.ucsd.edu/Downloads/) (*58*). This output was then queried for a match to the peptide fragment identified via mass spectrometry.

In vitro antimicrobial assays

Radial diffusion assay was performed using *S. aureus* (ATCC35556) strain to test antimicrobial activity of purified fractions as previously described (*59*). Briefly, melted TSB agar (10 mL) was mixed with *S. aureus* (1×10^6 CFU) and poured in a 10 cm petri dish. Two to four µL of test samples was applied in a small well (1 mm in diameter) punched on the agar plate. Plates were incubated at 37 ^{o}C overnight to allow visible growth of bacteria. Antibacterial activity was indicated by the clear zone (no bacterial growth) around the well.

Dose-dependent activity of isolated lantibiotic peptides were determined by incubating 1×10^5 CFU/mL *S. aureus* (ATCC35556) with 2-fold serial dilutions of lantibiotics in half strength Muller-Hinton broth (MHB) in PBS at 37 ^{o}C for 24 hrs. After incubation, the number of viable bacteria was measured by counting CFU after spreading 10-fold serial dilutions of bacteria on suitable agar plates. Minimal bactericidal concentration (MBC) was determined as a 3-log reduction (99.9 %) in viable bacteria after 24 hour incubation.

Colony PCR for Sh-lantibiotic-α

To determine frequency of CoNS isolates that have gene encoding *Sh*-lantibioic-α, colony PCR was conducted. 632, 842 and 872 CoNS colonies were picked up from skin swabs of 9 non-AD, nonlesional and lesional sites of 11 AD subjects, respectively. Picked CoNS bacteria were suspended in TE buffer containing 0.1% Triton X-100 and 0.05% Tween-20. Bacteria were lysed by purified achromopeptidase (Wako Chemical) and Ready-Lyse® (Epicenter Inc.), followed by heating at 100^{o}C. Lysed bacteria was subjected to PCR using gene specific primers (table S4) and BAC-DIRECT™ PCR Kit (LAMDA Biotech).

Western blotting for Sh-lantibiotic-α

Female Balb/c mice (8 weeks) were used for development of antibodies against *Sh*-lantibiotic-α. Linear unmodified peptide of *Sh*-lantibiotic-α (KCSWWNASCHLGNNGKICTVSHECAAGCNL) was synthesized by GeneMed Synthesis Inc.. The peptide was dissolved in PBS and mixed with an equal volume of complete or incomplete Freund's adjuvant. For the first vaccination, 50 μg of peptide in complete Freund's adjuvant was injected subcutaneously into the dorsal skin. Two and 4 weeks later, 50 μg of recombinant protein in incomplete Freund's adjuvant was intraperitoneally injected for second and

third boosts. Two weeks after the third boot, serum containing immunoglobulin G (IgG) antibody was harvested. IgG fraction purified with NAb™ Protein A/G skin kit (Thermo Scientific) was used for Western blotting. Total proteins were extracted from skin swab samples from upper arm of non-AD subjects, nonlesional (upper arm) or lesional skin (antecubital fossa) of AD patients by bead-beating in PBS containing 0.1% Triton-X100 and 0.05% Tween-20. Twenty μg of total proteins was subjected to SDS-PAGE for detection of *Sh*-lantibiotic-α by Western blotting. Immunoreactivity of *Sh*-lantibiotic on PVDF membrane was detected by mouse anti-*Sh*-lantibiotic polyclonal IgG (5 μg/mL), followed by Donkey anti-mouse IgG-IRDye®800CW (1:2500) (LI-COR). The membrane was incubated in NewBlot® PVDF stripping buffer (LI-COR) to remove antibodies and re-stained with anti-cytokeratin-10 IgG_1 (DE-K10 monoclonal, Thermo Fisher Scientific) (0.5 μg/mL), followed by Donkey anti-mouse IgG-IRDye®680CW (1:5000) (LI-COR).

Transplantation of antimicrobial CoNS on pigskin

Fresh-frozen pig skin sheets were obtained from Loretta Tomlin Animal Technologies (Livermore, CA) and sanitized by surgical brush with 3% chloroxylenol. The skin sheet was cut into 2.5cm × 2.5cm and rinsed with sterile PBS more than 20 times to remove chloroxylenol residue. *S. aureus* (ATCC35556) (1×10^5 CFU/cm^2) were epicutaneously challenged on the pig skin (2.5 × 2.5 cm) for 1 hr. *S. hominis* A9 strain isolated from a normal subject, that produces *Sh*-lantibiotics-α and -β, or *S. hominis* strains isolated from lesional skin of AD subject, which did not produce antimicrobial activity in culture, were formulated at 4×10^7 CFU/g in a skin moisturizer (Cetaphil™, Galderma) which was confirmed not to have a short term effect on bacterial viability. Either *S. hominis* A9 strain with anti-*S. aureus* activity, UV-killed *S. hominis* A9, inactive strains of *S. hominis* C4, C5 and C6 (1×10^5 CFU/cm^2), or vehicle (2.5 mg/cm^2) were subsequently applied on the surface of pigskin for 20 hrs. Purified

lantibiotic (0.5nanomol), conditioned media of *S. hominis* A9 (50μL) were applied to the surface of sanitized pig skin. Pig skin was incubated at 30^{o}C in a 6-well plate.

Live bacteria were harvested with a Catch-All Swab pre-wetted with TSB from the skin surface as described above. Bacteria were suspended by vortex swab head vigorously in 1mL TSB. Ten-fold serial dilution of the bacteria suspension was spread on a Baird-Parker agar with egg yolk tellurite for selective count of *S. aureus*. *S. aureus* (a large black colony with halo) were distinguished from *S. hominis* (a small gray colony without halo) on the selective agar plate.

Transplantation of antimicrobial CoNS on mouse

Back skin of C57BL6 female, 6 week-old mice that were randomly selected was shaved, treated with depilatory cream and rinsed with water 24 hrs before bacteria application. The shaved skin was cleaned with and alcohol swab twice to remove originally colonized bacteria. *S. aureus* (ATCC35556) (4×10^5 CFU/5 μL of PBS) were epicutaneously challenged on the dorsal skin of mice (2×2 cm) and was dried. *S. hominis* A9 strain or *S. hominis* strains, which did not produce antimicrobial activity in culture, were formulated at 4×10^7 CFU/g in Cetaphil™ lotion as described above. One hour after *S. aureus* challenge, *S. hominis* (1×10^5 CFU/cm^2), or an equal amount of vehicle (2.5 mg/cm^2) were subsequently applied on the surface of mouse dorsal skin (2×2 cm) and was dried. Mice treated with the same strain of *S. hominis* were co-caged for 20 hrs (2-3 mice/ cage) under a specific-pathogen free condition. Live bacteria were harvested from the dorsal skin and *S. aureus* CFU was counted as described above.

For long term/ multiple applications, each *S. hominis* strain was formulated in Cetaphil™ lotion as describe above, aliquoted and stored at -80^{o}C. *S. hominis* A9 or inactive control strain (1×10^5 CFU/cm^2), or an equal amount of vehicle (2.5 mg/cm^2) were subsequently applied on the surface of mouse dorsal skin (2×2 cm) twice a day for 7 days. Mice treated with the same strain of *S. hominis* were

co-caged (2 mice/ cage) under a specific-pathogen free condition and the cage was exchanged with a new one every day. After 3 and 7 days, *S. aureus* CFU on the mouse dorsal skin was counted as described above.

Supplementary Figures

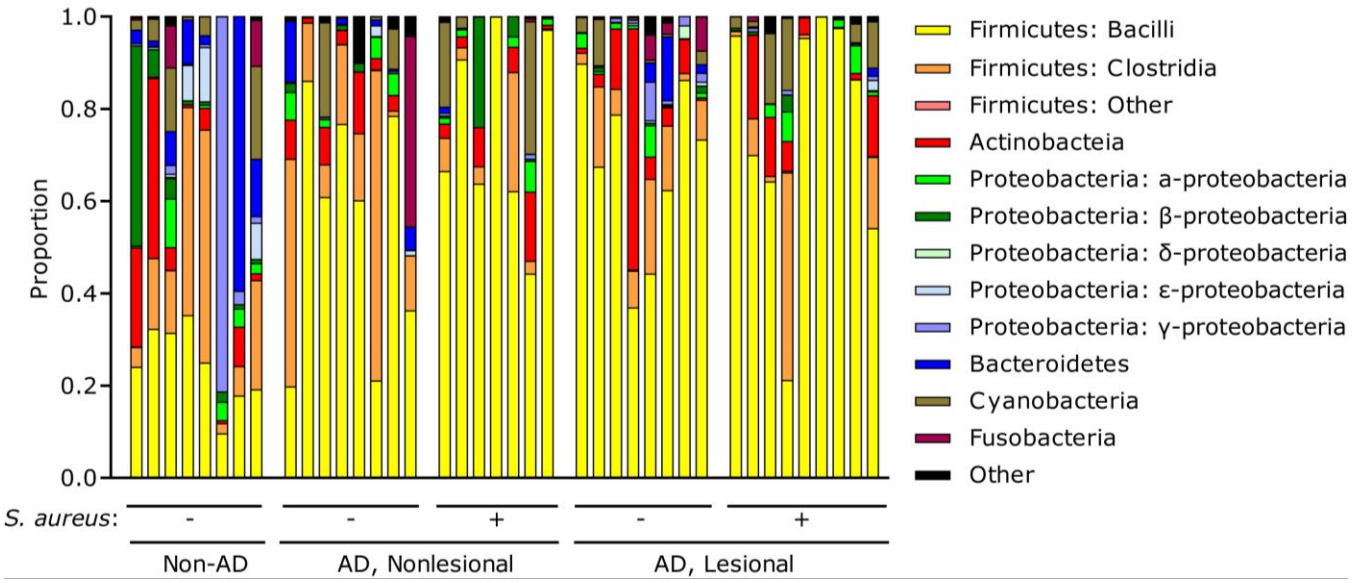


Fig. S1. Analysis of the skin bacterial community by 16*S* rRNA gene sequencing and its relationship to the capacity to culture *S. aureus* from the skin.

Relative abundance of bacterial taxa associated with each skin site is shown for each skin sample. The AD cohort was divided into *S. aureus*-culture positive (+) and negative (-) groups.

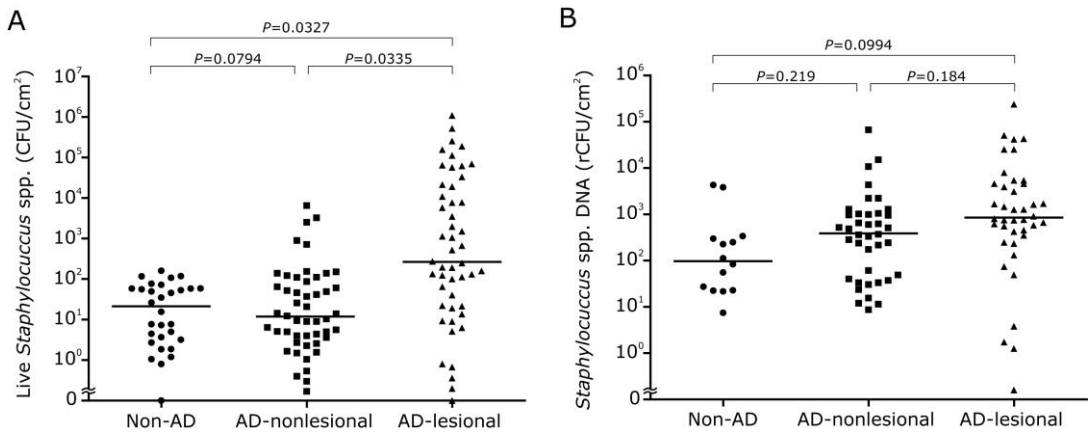


Fig. S2. Abundance of live *Staphylococcus* and *Staphylococcus*-specific DNA on the skin of non-AD and AD subjects.

(A) Live *Staphylococcus* spp. was measured from subjects with AD and subjects without AD by determining colony forming units (CFU) on selective egg yolk mannitol salt agar.

(B) DNA abundance for total *Staphylococcus* spp. was determined by qPCR on DNA extracted from swabs collected in parallel to collection of swabs used for CFU analysis in panel (A). Relative CFU (rCFU) of DNA abundance was determined by comparison to a standard DNA of known CFUs of *S. epidermidis* (ATCC12228). Horizontal bar represents the Median. *P*-values were calculated by two-tailed paired *t*-test for lesional vs nonlesional samples or two-tailed independent *t*-test for non-AD vs AD groups.

A

Gene matched to insert	Number of clones
Staphylococcus 16S rRNA	22
Total	22

B

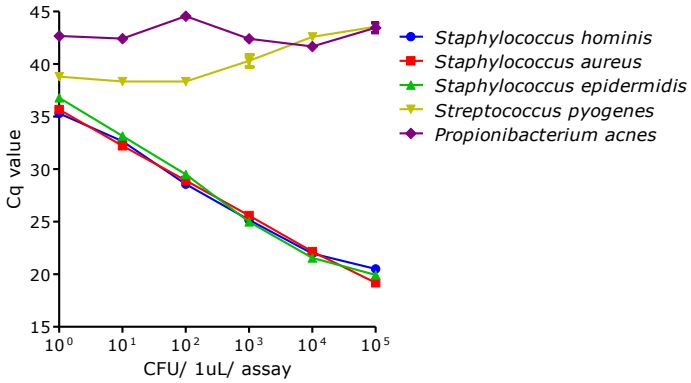


Fig. S3. Specificity of *Staphylococcus* genus–specific primers for real-time qPCR.

(A) To evaluate the specificity of Staphylococcus-specific primers used for real-time qPCR [g-staph F and R (table S4)], qPCR products amplified from total DNA extracted from non-AD skin swabs were cloned into pGEM-T vector and transformed into *E. coli* competent cells. Resulting bacteria colonies harboring a plasmid with insert (22 colonies) were picked, plasmids were purified, and insert DNA was sequenced with SP6 primer. Obtained sequence was analyzed by BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The genus nearest matched to the insert sequence (>99% identity) is shown.

(B) To further evaluate the specificity of Staphylococcus-specific primers used for real-time qPCR, standard curves were generated from DNA extracted from known CFU of following laboratory strains of bacteria; *Staphylococcus hominis* (ATCC27847), *Staphylococcus aureus* (ATCC35556), *Staphylococcus epidermidis* (ATCC12228), *Streptococcus pyogenes* (NZ131), *Propionibacterium acnes* (ATCC6919) . Data represents mean ± s.e.m. of duplicate determinations.

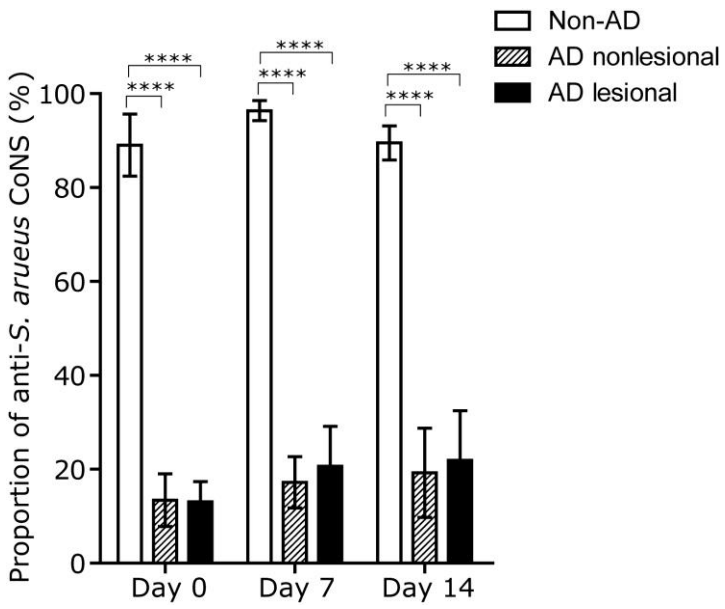


Fig. S4. Anti–*S. aureus* activity in CoNS is stable over time.

The proportion of CoNS with anti–*S. aureus* activity was repeatedly measured in the same subjects at day 0, day 7 and day 14. ****$P<0.0001$. 11 AD and 11 non-AD subjects were randomly selected for this analysis. The data represent mean ± s.e.m.. *P*-values were calculated by two-tailed paired *t*-test.

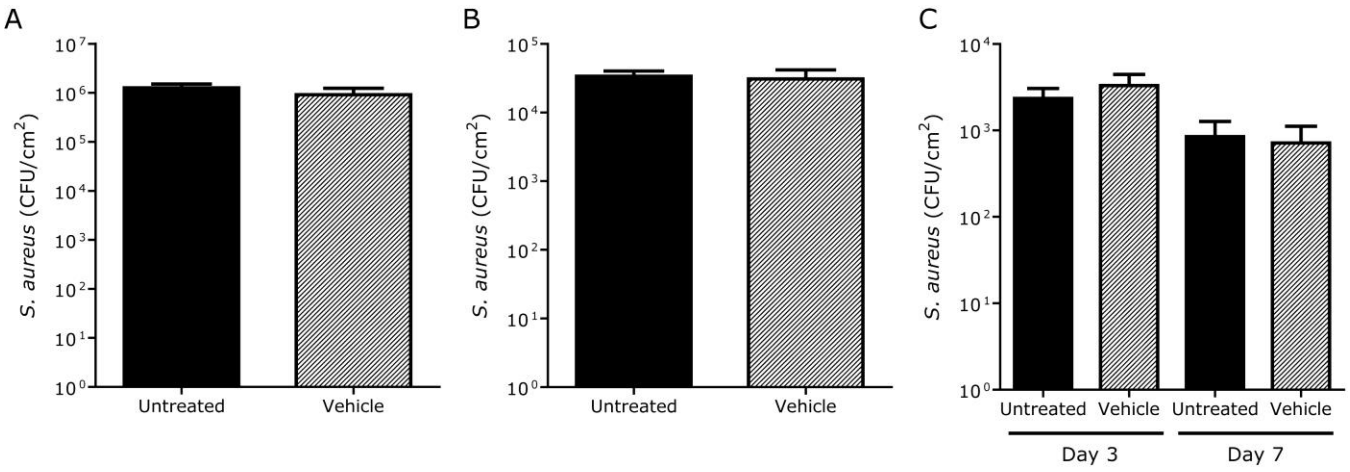


Fig. S5. Effect of vehicle treatment on the survival of *S. aureus* on ex situ pigskin or live mouse skin.

(A-B) *S. aureus* was first applied to on *ex-situ* pigskin (A) or live mouse skin (B) as described in methods. The skin colonized by *S. aureus* was treated with single application of vehicle. The action of vehicle treatment was compared to untreated control. Data represent mean ± s.e.m. of 7 (A) or 10 (B) independent applications.

(C) Effect of multiple applications of vehicle on survival of *S. aureus* on mouse skin. Vehicle was applied twice a day to mouse back skin colonized by *S. aureus* over the indicated periods. The action of vehicle treatment was compared to untreated control. Data represent mean ± s.e.m. of 5 independent animals.

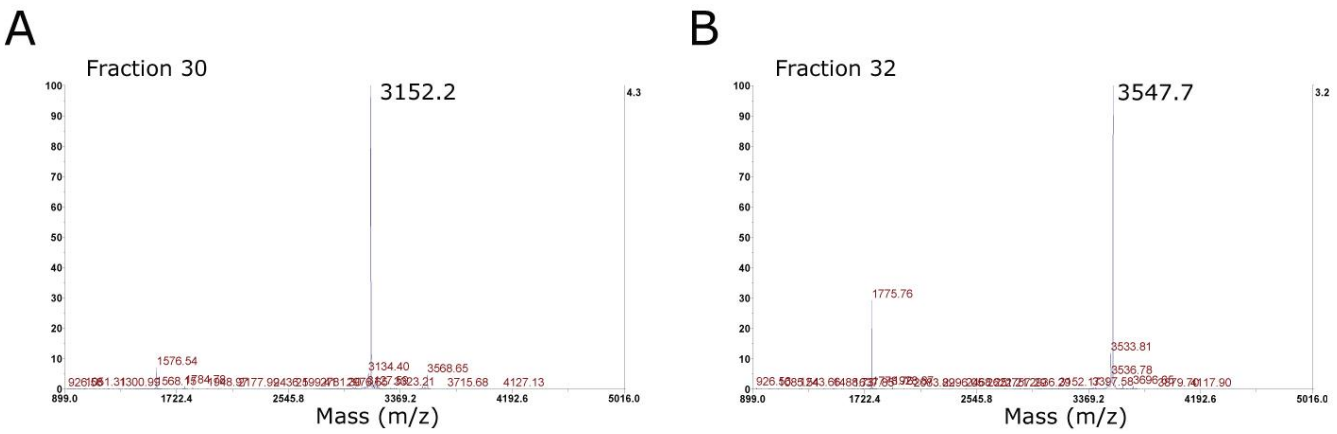


Fig. S6. MALDI-TOF MS analysis for two AMPs purified from *S. hominis*.

Fractions 30 and 32 with anti-*S. aureus* activity separated by HPLC (Fig. 5A) were analyzed by MALDI-TOF-MS to estimate molecular size of AMPs.

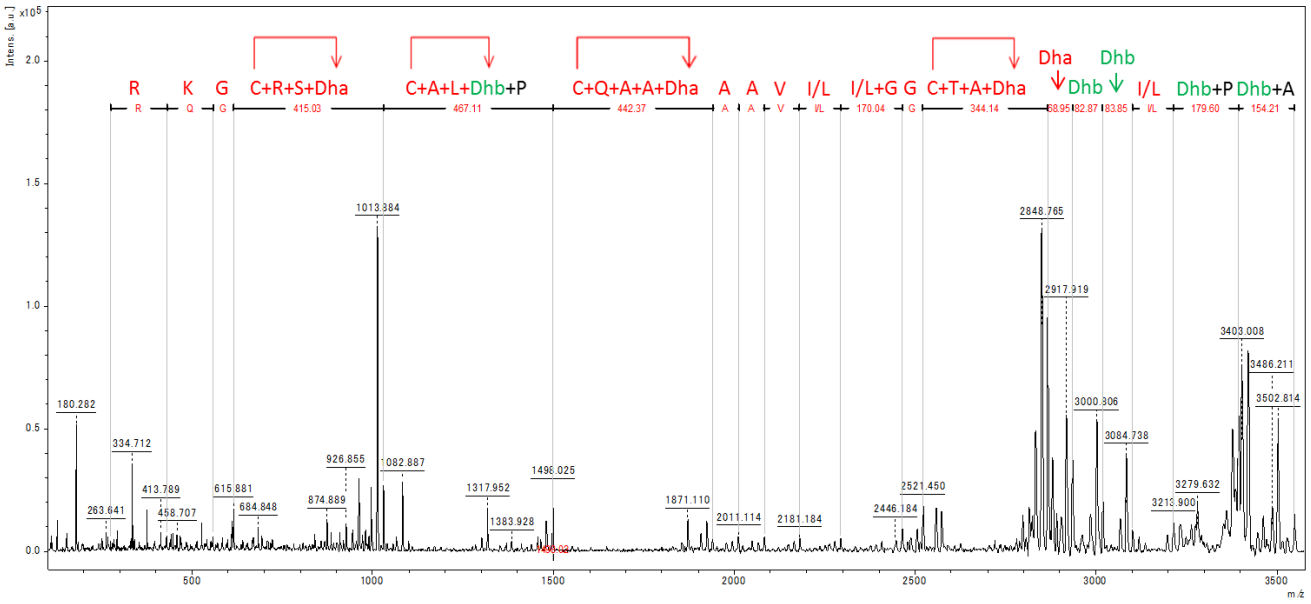


Fig. S7. Representation of amino acid losses in genome-guided MALDI-TOF/TOF analysis for *Sh*-lantibiotic-β.

Amino acid sequence of purified *Sh*-hogocidin-β was obtained from amino acid losses in MS/MS fragmentation spectrum of precursor mass 3547.7 m/z. Dha, 2,3-didehydroalanine; Dhb, (Z)-2,3-didehydrobutyrine

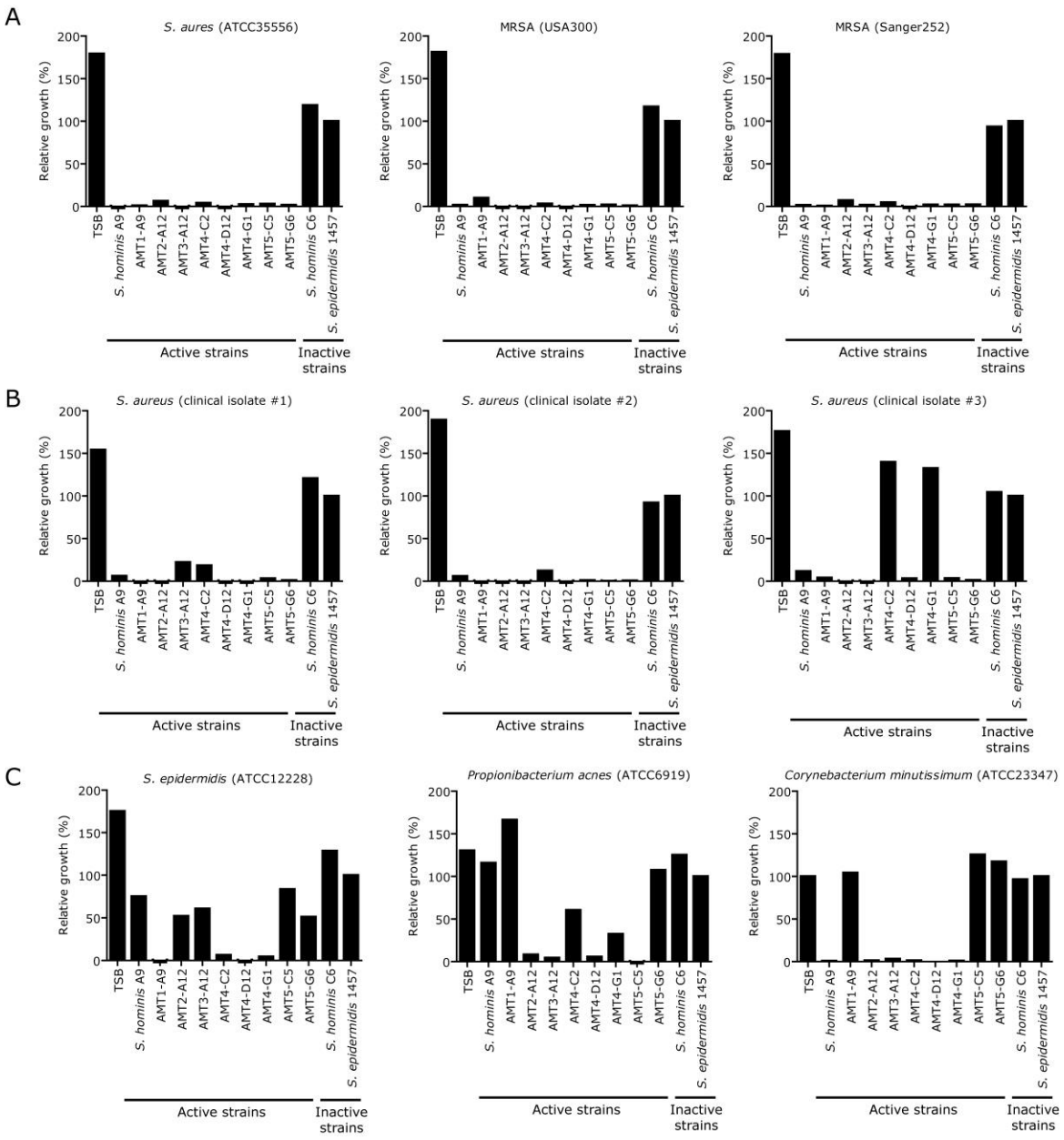


Fig. S8. Skin isolate strains of CoNS exert selective antimicrobial activity against *S. aureus*.

(A-C) Indicated strains of CoNS were cultured at 37 ºC overnight with shaking. Bacteria were removed by centrifugation followed by sterile filtration with a 0.22μm membrane. The antimicrobial activity of each sterile conditioned media (100 μL) was evaluated by mixing with 1×10^4 CFU of laboratory strains of *S. aureus* (A), clinical isolates of *S. aureus* from patients with AD (B), and skin commensal bacteria, including *S. epidermidis*, *Propionibacterium acnes* and *Corynebacterium minutissimum*, in 10 μL fresh

TSB on a 96-well microplate. Clinical strains of *S. aureus* (#1-3) were isolated from lesional site (antecubital fossa) of 3 independent subjects with AD. *S. aureus* and *S. epidermidis* were aerobically cultured at 37^{o}C for 22 hrs. *C. minutissimum* was aerobically cultured at 37^{o}C for 72 hrs. *P. acnes* was anaerobically cultured at 37^{o}C for 72 hrs. Bacterial growth was monitored by measuring OD_{600} and relatively represented as (%) growth index which bacteria growth in *S. epidermidis* ATCC1457 conditioned media set as 100%.

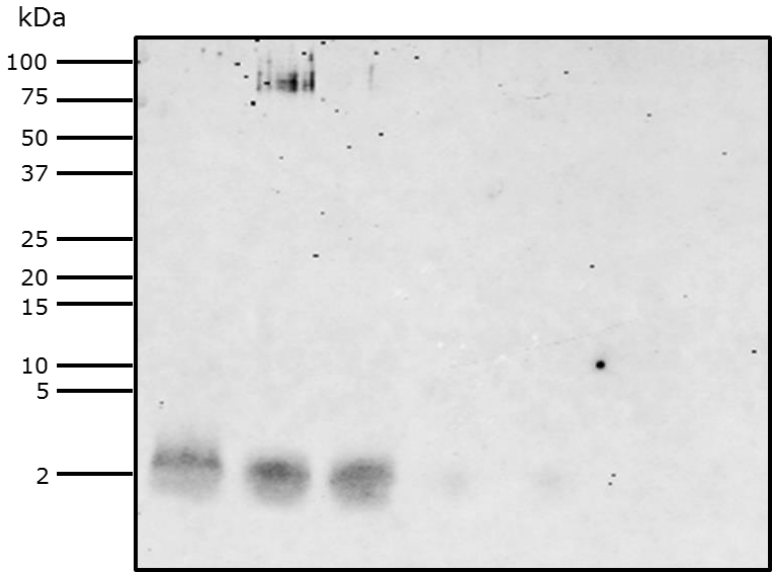
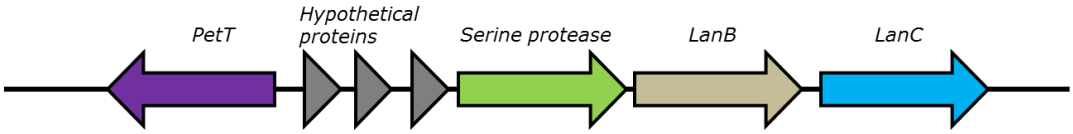


Fig. S9. Detection of *Sh*-lantibiotic-α peptide by Western blotting from extracts of skin swabs taken from two non-AD subjects and two AD subjects.

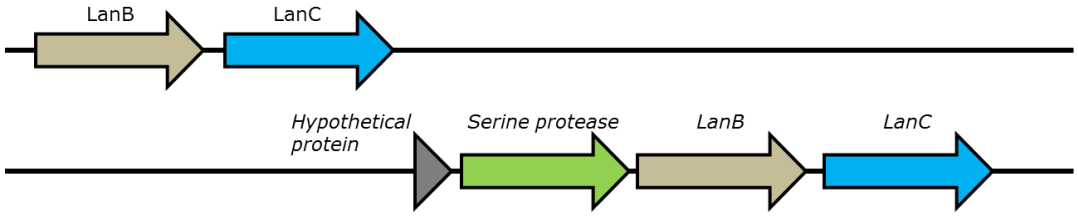
Non-AD subjects were colonized by bacteria possessing the *Sh*-lantibiotic-α gene and AD subjects that were PCR negative for *Sh*-lantibiotic-α gene. *S. hominis* culture supernatant was loaded as a positive control. 20 μg total protein was loaded in each lane. Full range of image is shown.

***S. epidermidis* AMT1-A9**



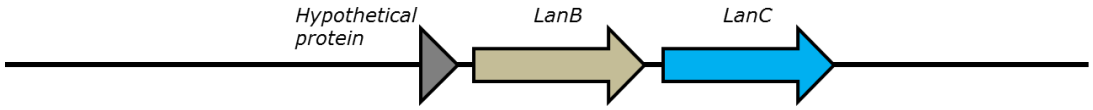
Gene	Gene locus	Putative functions
S8 serine protease	11761~12618	Peptidase for proteolytic maturation of lantibiotics
LanB	12605~15508	Lantibiotic modifying enzyme
LanC	15505~16701	Lantibiotic modifying enzyme
PetT	10806~9283	Lantibiotic resistance

***S. hominis* AMT2-A12**



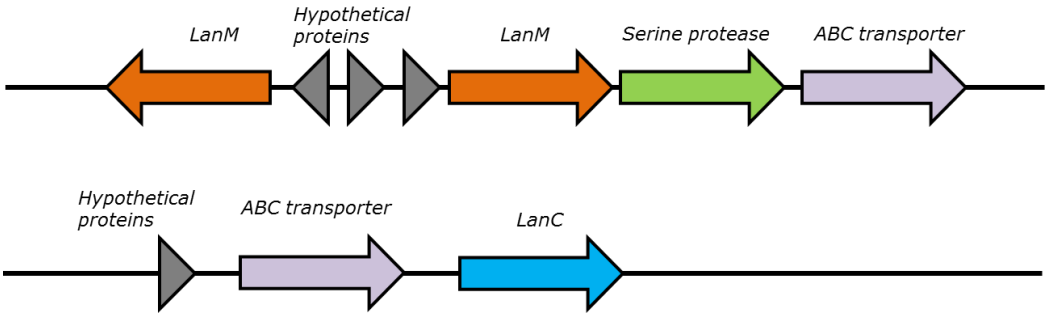
Gene	Gene locus	Putative functions
LanB	9336~6403 425641~428622	Lantibiotic modifying enzyme
LanC	6418~5225 428615~429859	Lantibiotic modifying enzyme
S8 Serine protease	10225~9323	Peptidase for proteolytic maturation of lantibiotics

***S. hominis* AMT3-A12**



Gene	Gene locus	Putative functions
LanB	828325~825344	Lantibiotic modifying enzyme
LanC	825351~824107	Lantibiotic modifying enzyme

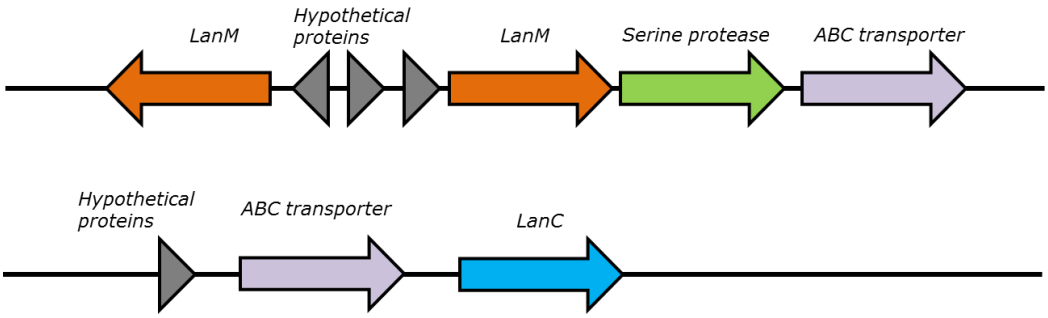
***S. hominis* AMT4-C2**



Gene	Gene locus	Putative functions
LanM	11394~14336 10610~8001	Lantibiotic modifying enzyme
S8 serine protease	7989~6682	Peptidase for proteolytic maturation of lantibiotics
ABC transporter	6669~4942	Lantibiotic export
LanC	24262~22973	Lantibiotic modifying enzyme

* AMT4-C2 and AMT4-G1 had the same genome sequence.

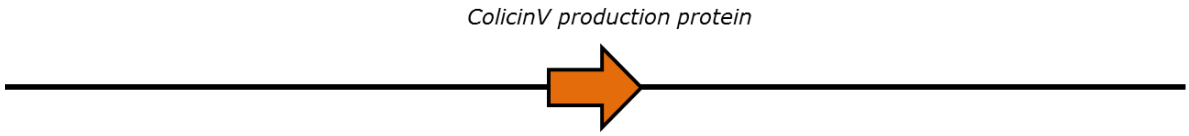
***S. hominis* AMT4-G1**



Gene	Gene locus	Putative functions
LanM	11394~14336 10610~8001	Lantibiotic modifying enzyme
S8 serine protease	7989~6682	Peptidase for proteolytic maturation of lantibiotics
ABC transporter	6669~4942	Lantibiotic export
LanC	24262~22973	Lantibiotic modifying enzyme

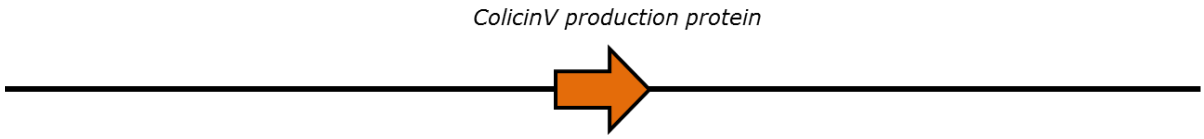
* AMT4-C2 and AMT4-G1 had the same genome sequence.

***S. hominis* AMT4-D12**



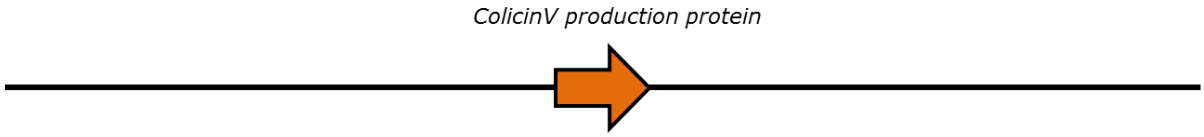
Gene	Gene locus	Putative functions
Colicin V	474249~474770	Bacteriocin

***S. epidermidis* AMT5-C5**



Gene	Gene locus	Putative functions
Colicin V	27664~28185	Bacteriocin

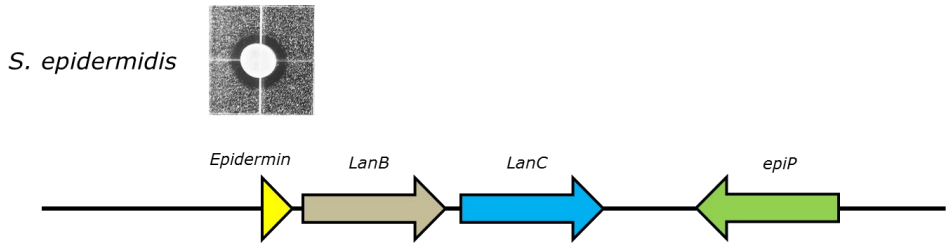
***S. epidermidis* AMT5-G6**



Gene	Gene locus	Putative functions
Colicin V	27664~28185	Bacteriocin

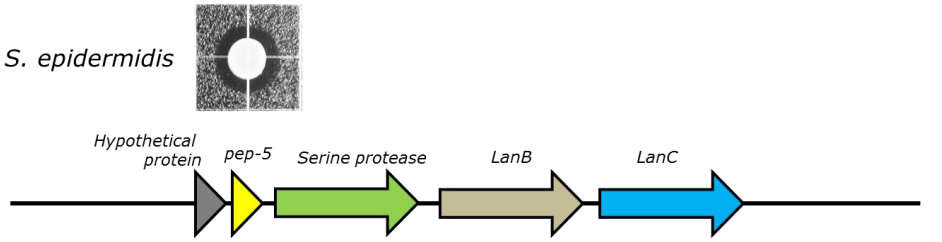
Fig. S10. List of hypothetical antimicrobial genes identified in anti–*S. aureus* CoNS clones used for AMT.

Whole genome sequence of active CoNS clones were obtained by miSeq and analyzed on the RAST Server (rast.nmpdr.org) to identify antimicrobial class.




```
Putative lantibiotic: MEAVKEKNDLFNLDVKVNAKESNDSGAEPRIASKFICTPGCAKTGSFNSYCC (100% identity)
                      **************************************************
          Epidermin: MEAVKEKNDLFNLDVKVNAKESNDSGAEPRIASKFICTPGCAKTGSFNSYCC
```

Gene	Gene locus	Putative functions
Epidermin	44160~44002	Lantibiotic precursor
LanB	43936~40976	Lantibiotic modifying enzyme
LanC	40983~39773	Lantibiotic modifying enzyme
epiP	37162~38547	Epidermin leader peptide processing serine protease




```
Putative lantibiotic: MKNNKNLFDLEIKKETSQNTDELEPQTAGPAIRASVKQCQKTLKATRLFTVSCKGKNGCK (100% identity)
                      ************************************************************
          Pep-5: MKNNKNLFDLEIKKETSQNTDELEPQTAGPAIRASVKQCQKTLKATRLFTVSCKGKNGCK  60
```

Gene	Gene locus	Putative functions
pep5	10428~10610	Lantibiotic precursor
S8 serine protease	10672~11526	Peptidase for proteolytic maturation of lantibiotics
LanB	11516~14419	Lantibiotic modifying enzyme
LanC	11416~15612	Lantibiotic modifying enzyme

Fig. S11. List of previously known lantibiotic genes identified in antimicrobial *S. epidermidis* clones isolated from non-AD skin.

Whole genome sequence of active CoNS clones were obtained by miSeq and analyzed on the RAST Server (rast.nmpdr.org) to identify antimicrobial class. Radial diffusion assay was conducted for antimicrobial activity against *S. aureus* ATCC35556 strain.

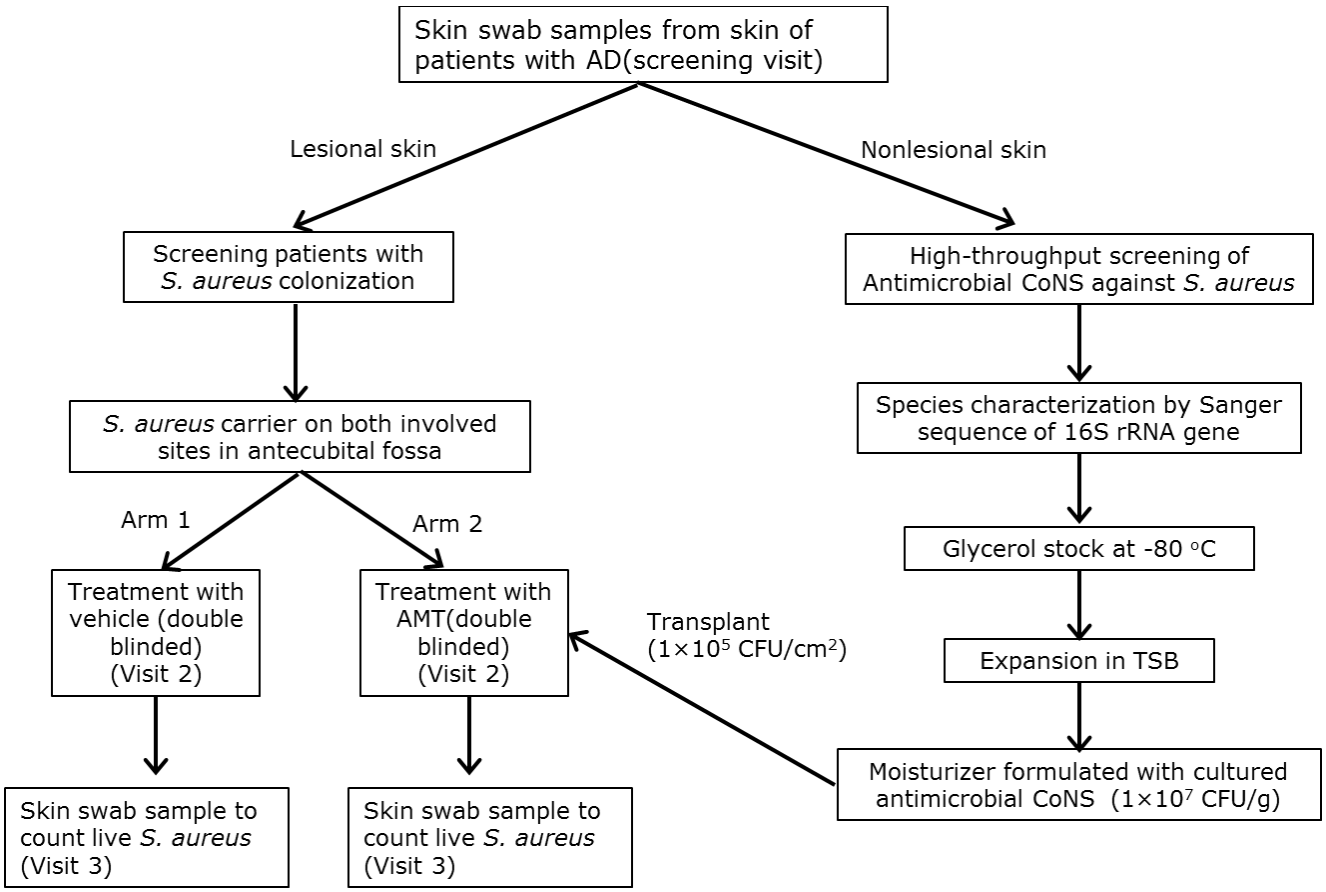


Fig. S12. Study design of AMT therapy for patients with AD.

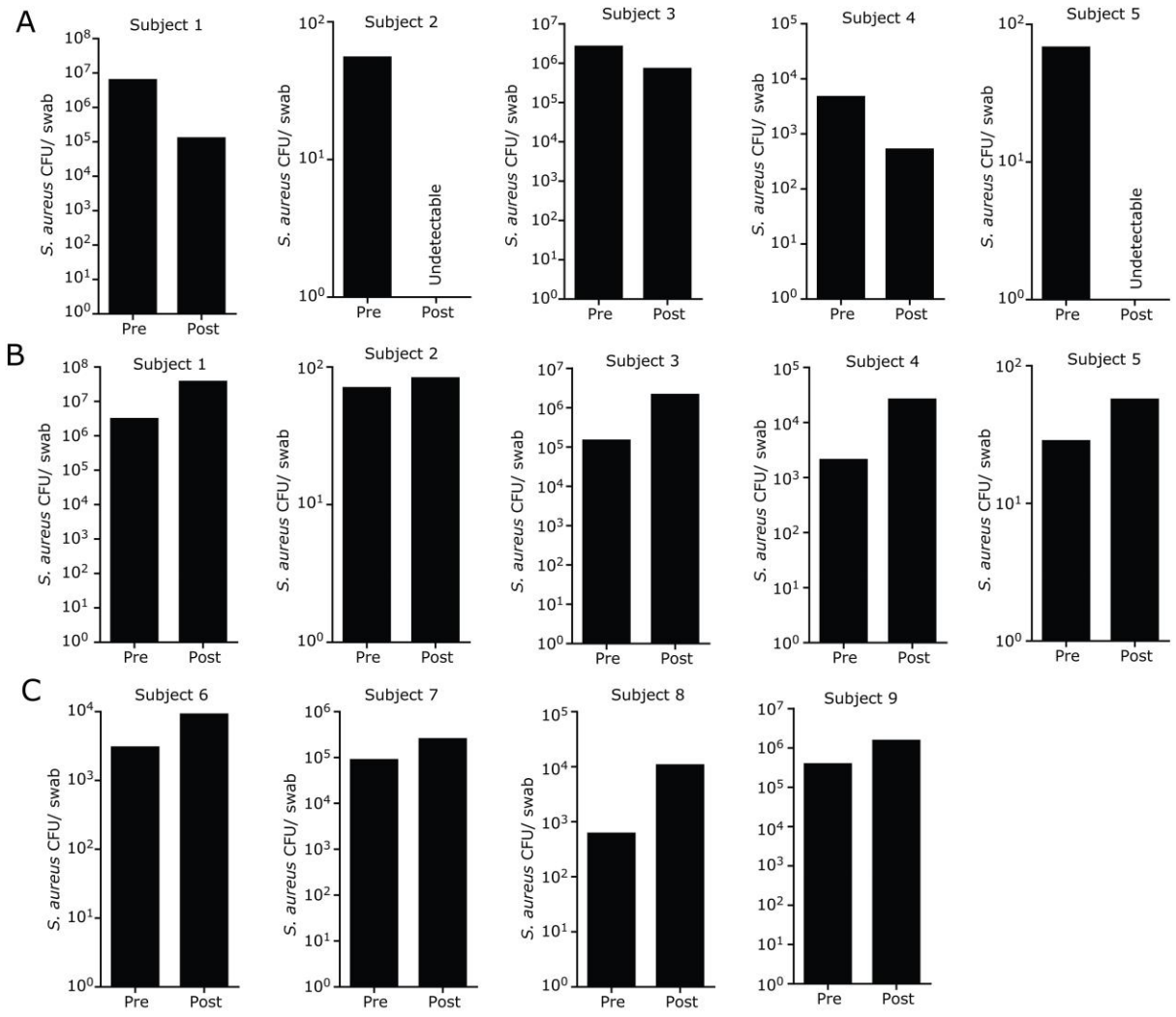


Fig. S13. Effect of transplantation of antimicrobial CoNS or vehicle on the survival of *S. aureus* on the skin of five subjects with AD.

(A-B) *S. aureus* survival was measured by colony counting of swabs taken before transplant (Pre) and 24 hrs after treatment with CoNS with anti-S. aureus activity (A) or vehicle (B) (Post). Application and analysis was done in a blinded fashion and samples are from the contralateral arm of subjects treated with the identical vehicle containing antimicrobial bacteria or vehicle only.

(C) Data of autologous microbiome transplant (AMT) or placebo treatment (vehicle) are compared with data from 4 control untreated subjects. *S. aureus* survival was measured in swabs taken at 0 hr (Pre) and 24 hr (Post).

Table S1. Clinical characteristics of AD and non-AD subjects

		AD subjects (N= 49)*	Non-AD subjects (N= 30)
Facility [N (%)]	UCSD (San Diego)	27 (55.0)	19 (63.3)
	NJH (Denver)	22 (45.0)	11 (36.7)
Age	Mean±SD	33.4±14.1	33.9±18.2
BMI	Mean±SD	25.0 ±9.3	23.4± 3.8
	Not recorded (%)	30.6	63.3
Gender [N (%)]	Male	21 (42.9)	17 (56.7)
	Female	28 (57.1)	13 (43.3)
Race [N (%)]	Caucasian	24 (49)	18 (60)
	Asian	16 (32.7)	10 (33.3)
	Hispanic	3 (6.1)	2 (6.7)
	African-American	4 (8.2)	0
	Other	2 (4.1)	0
EASI score [N (%)]	<=6	17 (34.7)	N/A
	>6	32 (65.3)	N/A

*No live *Staphylococcus* and *Staphylococcus* DNA were detected in 1 subject with AD out of 50 patients recruited. Therefore the data are reported for 49 AD subjects.

BMI, body mass index; EASI, eczema area and severity index; NJH, National Jewish Health; SD, standard deviation; UCSD, University of California, San Diego

Table S2. Proportion of CoNS species identified with antimicrobial and nonantimicrobial activity.

A. CoNS species identified from non-AD subjects

Subject ID	Skin site	*S. aureus* culture[1]	Anti-*S. aureus* CoNS (%)	Anti-*S. aureus* acitivty[2]	Total CoNS analyzed[3]	Species[4]	Colony number	Proportion (%)
Non-AD-1	Non-AD	Negative	100	+	24	*Staphylococcus epidermidis*	23	95.83
						Staphylococcus captis	1	4.17
						Total	24	100
Non-AD-2	Non-AD	Negative	98.7	+	24	*Staphylococcus epidermidis*	22	91.67
						Staphylococcus hominis	2	8.33
						Total	24	100
Non-AD-3 UCSD-	Non-AD	Negative	100	+	24	*Staphylococcus hominis*	24	100
						Total	24	100
Non-AD-4	Non-AD	Negative	100	+	23	*Staphylococcus epidermidis*	22	95.65
						Staphylococcus pasteuri	1	4.35
						Total	23	100
Non-AD-5	Non-AD	Negative	100	+	24	*Staphylococcus epidermidis*	24	100
						Total	24	100

B. CoNS species identified from a subject with AD.

Subject ID	Skin site	*S. aureus* culture[1]	Anti-*S. aureus* CoNS (%)	Anti-*S. aureus* acitivity[2]	Total CoNS analyzed[3]	Species[4]	Colony number	Proportion (%)
AD-1	AD-lesional	Negative	20.7	+	18	*Staphylococcus pasteuri*	16	88.89
						Staphylococcus haemolytics	2	11.11
						Total	18	100
				-	23	*Staphylococcus haemolytics*	19	82.61
						Staphylococcus epidermidis	3	13.04
						Staphylococcus pasteuri	1	4.35
						Total	23	100
	AD-nonlesional	Negative	2.8	+	9*	*Staphylococcus pasteuri*	7	77.78
						Staphylococcus warneri	2	22.22
						Total	9	100
				-	24	*Staphylococcus haemolytics*	16	66.67
						Staphylococcus epidermidis	7	29.17
						Staphylococcus hominis	1	4.17
						Total	24	100
AD-2	AD-lesional	Positive	30.2	+	16	*Staphylococcus epidermidis*	13	81.25
						Staphylococcus capitis	3	18.75
						Total	16	100
				-	22	*Staphylococcus lundunensis*	13	59.09
						Staphylococcus epidermidis	8	36.36
						Staphylococcus capitis	1	4.55
						Total	22	100
	AD-nonlesional	Positive	21.6	+	24	*Staphylococcus epidermidis*	22	91.67
						Staphylococcus capitis	2	8.33
						Total	24	100
				-	24	*Staphylococcus epidermidis*	21	87.50
						Staphylococcus lundunensis	3	12.50
						Total	24	100

AD-3	AD-lesional	Negative	19.23	+	15	*Staphylococcus epidermidis*	9	60
						Staphylococcus capitis	6	40
						Total	15	100
				-	24	*Staphylococcus epidermidis*	19	79.17
						Staphylococcus capitis	4	16.67
						Staphylococcus hominis	1	4.17
						Total	24	100
	AD-nonlesional	Negative	19.23	+	15	*Staphylococcus epidermidis*	13	86.67
						Staphylococcus capitis	2	13.33
						Total	15	100
				-	24	*Staphylococcus epidermidis*	23	95.83
						Staphylococcus capitis	1	4.17
						Total	24	100
AD-4	AD-lesional	Positive	30.23	+	13	*Staphylococcus epidermidis*	9	69.23
						Staphylococcus capitis	3	23.08
						Staphylococcus warneri	1	7.69
						Total	13	100
				-	22	*Staphylococcus epidermidis*	22	100.00
						Total	22	100
	AD-nonlesional	Positive	21.67	+	13	*Staphylococcus epidermidis*	13	100.00
						Total	13	100
				-	22	*Staphylococcus epidermidis*	21	95.45
						Staphylococcus lugdunensis	1	4.55
						Total	22	100

AD-5	AD-lesional	Negative	100	+	22	*Staphylococcus epidermidis*	10	45.45
						Staphylococcus hominis	9	40.91
						Staphylococcus lugdunensis	2	9.09
						Staphylococcus haemolytics	1	4.55
						Total	22	100
				-		Undetectable	0	Undetectable
	AD-nonlesional	Negative	100	+	24	*Staphylococcus epidermidis*	12	50.00
						Staphylococcus hominis	11	45.83
						Staphylococcus capitis	1	4.17
						Total	24	100
				-	0	Undetectable	0	Undetectable
AD-6	AD-lesional	Negative	2.9	+	2*	*Staphylococcus hominis*	2	100
						Total	2	100
				-	23	*Staphylococcus hominis*	18	78.26
						Staphylococcus epidermidis	5	21.74
						Total	23	100
	AD-nonlesional	Negative	6.5	+	4	*Staphylococcus hominis*	3	75.00
						Staphylococcus epidermidis	1	25.00
						Total	4	100
				-	23	*Staphylococcus hominis*	12	52.17
						Staphylococcus epidermidis	11	47.83
						Total	23	100
AD-7	AD-lesional	Positive	2.6	+	2*	*Staphylococcus epidermidis*	2	100
						Total	2	100
				-	24	*Staphylococcus epidermidis*	23	95.83
						Staphylococcus capitis	1	4.17
						Total	24	100
	AD-nonlesional	Negative	64.1	+	22	*Staphylococcus hominis*	22	100
						Total	22	100
				-	23	*Staphylococcus hominis*	11	47.83
						Staphylococcus epidermidis	11	47.83
						Staphylococcus devriesei	1	4.35
						Total	23	100

[1]*S. aureus* CFU was counted on a mannitol salt agar with egg yolk. Positive is defined as > 1CFU/cm^2 and negative as < 1CFU/cm^2.

[2]Strains with anti-*S. aureus* activity (+), inactive strains (-).

[3]CoNS colonies were randomly picked up to identify species by sequencing full-length 16S rRNA gene.

[4]Operational taxonomic units were assigned by searching against Ribosomal Database Project (http://rdp.cme.msu.edu/) (*54*).

*Less CoNS colonies were obtained because of low frequency of anti-*S. aureus* CoNS.

Table S3. Clinical characteristics of AD subjects receiving AMT.

Patient Demographics	**Treated AD subjects (N = 9)**
Age (mean ± SD)	28.89 ± 13.61
Gender	
Male [N (%)]	2 (22.22%)
Female [N (%)]	7 (77.78%)
Race	
Caucasian [N (%)]	4 (44.44%)
Asian [N (%)]	5 (55.56%)
Body Mass Index (mean ± SD)	24.79 ± 4.36
Baseline EASI (mean ± SD)	8.21 ± 6.00
Baseline SCORAD (mean ± SD)	35.00 ± 14.80

Table S4. Sequences of PCR primers.

Primer name	Sequence (5' – 3')	Target gene	References or accession #
S. aureus-femA-2F	AACTGTTGGCCACTATGAGT	*S. aureus*-specific sequence	Reference (*60*)
S. aureus-femA-2R	CCAGCATTACCTGTAATCTCG		
S.epidermidis-sodA-F	TCAGCAGTTGAAGGGACAGAT	*S. epidermidis*-specific sequence	Reference (*61*)
S.epidermidis-sodA-R	CCAGAACAATGAATGGTTAAGG		
g-Staph-F	TTTGGGCTACACACGTGCTACAATGGACAA	*Staphylococcus*-genus specific 16S sequence	Reference (*62*)
g-Staph-R	AACAACTTTATGGGATTTGCWTGA		
Univ16S-27-F	AGAGTTTGGATCMTGGCTCAG	Universal sequence of bacterial 16S rRNA	Reference (*63*)
Univ16S-1525-R	AAGGAGGTGWTCCARCC		