

MISS RIE DYBBOE (Orcid ID : 0000-0002-0110-6320)

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The Role of the Skin Microbiome in Atopic Dermatitis: A Systematic Review

R. Dybboe¹; J. Bandier²; L. Skov²; L. Engstrand³ and J. D. Johansen¹

Authors affiliations:

¹: National Allergy Research Centre, Herlev & Gentofte Hospital, University of Copenhagen, Hellerup, Denmark

²: Department of Dermatology and Allergy, Herlev & Gentofte Hospital, University of Copenhagen, Hellerup, Denmark

³: Department of Microbiology, Tumor and Cell Biology and Center for Translational Microbiome Research, Karolinska Institutet & Science for Life Laboratory, Stockholm, Sweden

Corresponding Author: Rie Dybboe, MSc, PhD-student. National Allergy Research Centre, Herlev & Gentofte Hospital, Kildegårdsvej 28, 2900 Hellerup, Denmark. E-mail address: rie.dybboe@regionh.dk. Telephone: +45 38677302

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What's already known about this topic?

- Dysbiosis is a hallmark of atopic dermatitis: *Staphylococcus aureus* colonisation is frequent and affects disease severity adversely.

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- Recent availability of culture-independent methods to profile microorganisms has enabled studies of whole microbial communities and their role in dermatitis.

What does this study add?

- The atopic dermatitis skin has low bacterial diversity, high non-*Malassezia* fungal diversity, high abundance of *Staphylococcus aureus* and *Staphylococcus epidermidis* and reduced abundances of other genera.
- An animal study indicates that dysbiosis is a driving factor in eczema.
- More data are warranted for better characterization of the role of the microbiome in atopic dermatitis and the influence of methodological approaches needs to be resolved.

Summary

Background: Dysbiosis is a hallmark of atopic dermatitis. The composition of skin microbiome communities and the causality of dysbiosis in eczema have not been well established.

Objective: To describe the skin microbiome profile in atopic dermatitis and address if there is a causal relationship between dysbiosis and atopic dermatitis.

Methods: The protocol is registered in PROSPERO (CRD42016035813). We searched PubMed, Embase, Scopus and ClinicalTrials.gov for primary research studies applying culture-independent analysis on the microbiome on atopic dermatitis skin of humans and animal models. Two authors independently full-text screened studies for eligibility and assessed risk of bias. Because of heterogeneity no quantitative synthesis is made.

Findings: Of 5735 texts, 32 met the inclusion criteria and 17 of these are published; 11 human and 6 animal studies. The studies varied in quality and applied different methodology. The skin in atopic dermatitis had low bacterial diversity (lowest at dermatitis involved sites) and 3 studies showed depletion of *Malassezia* species and high non-*Malassezia* fungal diversity. The relative abundance of *Staphylococcus aureus* and *Staphylococcus epidermidis* were elevated and other genera were reduced, incl. *Propionibacterium*. A mouse study indicated that dysbiosis is a driving factor in eczema pathogenesis.

Conclusion: The data is not sufficiently robust for good characterisation; however, dysbiosis in atopic dermatitis does not only implicate *Staphylococcus* species, but also microbes such as

Propionibacterium and *Malassezia*. A causal role of dysbiosis in eczema in mice encourages future studies to investigate if this applies in humans too. Other important aspects are temporal dynamics and the influence of methodology on microbiome data.

Introduction

Recent availability of culture-independent methods to profile microorganisms and study microbial communities has increased our understanding of the microbiome and its impact in health and disease. Much research has focused on the gut microbiome where findings demonstrate associations between dysbiosis and diseases such as diabetes and asthma ¹. The number of skin microbiome studies is rising. The skin is composed of a variety of niches selecting for colonization by specific microorganisms ². Host factors, e.g. sex ³, age ⁴, and environmental exposures ^{3,5,6}, also affect the niches and microbiome communities and it is becoming increasingly apparent that the skin microbiome in turn influences vital functions in the host such as immunity and colonisation by pathogenic microorganisms ⁵.

Atopic dermatitis (AD) is a chronic skin disease affecting up to 20% children, with lesser prevalence in adults ⁷. It manifests with dry, itchy skin, relapsing eczema at sites depending on age: The cheeks on infants are typically the first place to be affected, extensor aspects of joints in toddlers, flexures in older children and a various presentation in adults. AD is characterised by immune dysregulation predisposing to IgE production ⁸. Conventional culture-based work has established that dysbiosis is a hallmark too ⁹, 70% of lesional and 39% non-lesional skin sites are colonised by *S. aureus* ¹⁰, which adversely affects disease severity. Not only bacteria but also the fungi are implicated ¹¹. Conventional culturing fails to grow about 80% of bacterial species ¹². By applying culture-independent molecular methodology dysbiosis is broadly described and the relative amount of present microbes becomes evident, which is also true for microbes not present. Though skin dysbiosis and the microbiome are anticipated an important role in development of treatments, there has been no systematic review on the skin microbiome profile in AD. AD is a multifactorial disease, but the gene-environment interactions leading to development of AD are not fully understood. Controversy remains as to

distinguishing between primary events leading to AD and secondary events resulting from AD¹³.

Whether the skin microbiome is a primary factor in AD pathogenesis is uncertain.

This systematic review provides an overview of the AD skin microbiome profile. It is questioned if causal relationships between the skin microbiota and disease exist. To elaborate on this question animal studies are also included and the effect of treatment of AD on the microbiome is evaluated. A discussion of future directions in AD microbiome research is included.

Methods

Complete methods of the literature search, risk of bias and data extraction were specified in advance and documented in a protocol registered in PROSPERO (CRD42016035813).

A systematic literature search was conducted October 21 2016 in PubMed, Embase, Scopus and ClinicalTrials.gov using search terms from the categories: Skin, microbiome and AD – without language and date limitations. After an initial screen of title and abstracts, two authors independently screened full texts for eligibility. Primary research studies (observational and interventional) were included if they applied culture-independent methods and whole community analyses to characterise the AD skin microbiome of humans and animal models. Studies were excluded if they did not present data, included a wrong study population, investigated the microbiome of other body sites or investigated selected microbial taxonomic units. Duplicate studies were excluded. Disagreements on eligibility were resolved by contacting the authors of the original studies.

Two authors assessed risk of bias. For the human studies we used the Cochrane Collaboration's tool for randomised controlled trials (RCT's) and adjusted Newcastle-Ottawa Scales for analytical non-randomized case-control studies and cohort studies without a control group. For the animal studies we used adjusted versions of the Systematic Review Centre for Laboratory Animal Experimentation's (SYRCLE) risk of bias tool¹⁴. Highest-quality evidence received greatest emphasis.

Two authors collected study characteristics and relative abundances of microbial taxonomic units (when >1% and significant differences were found compared to a reference).

The criteria for study inclusion allow for heterogeneity in study population, design and methods. Therefore no quantitative synthesis was carried out.

Results

5735 studies were identified (fig. 1). After review of title and abstract, 90 were full text screened. Based on selection criteria, 32 records were included – 17 were published (table 1) and 15 were ongoing (supplementary table 1). The published studies were examined in most detail.

Human studies

Study description

Eleven human studies were identified (table 1): 2 RCTs, 7 case-control studies and 2 cohort studies. The studies included 355 AD patients. Oh et al.¹⁵ included also patients with primary immunodeficiencies (N=41), characterised with AD-like eczema. Their data is regarded as from an AD population. The age of the participants spanned from 2 months to 62 years and both sexes were included in 10/11 studies¹⁵⁻²². AD was clinically characterised by SCORing of Atopic Dermatitis (SCORAD) in 8/11 studies^{15-19, 21-23}, Eczema Area and Severity Index in 1/11 studies²⁴ and Rajka-Langeland in 1/11 studies⁴. Patients with mild AD were included in 2 studies^{16, 20}, moderate in 9 studies^{4, 15-20, 23, 24} and severe in 7 studies^{4, 15-17, 19, 20, 24, 25}. Only one study²⁰ distinguished microbiome compositions according to disease severity. The skin microbiome of anatomically defined skin area(s) were investigated in 9/11 studies^{4, 15, 17, 19-21, 23, 25}. Other compared affected and non-affected skin sites^{4, 16, 18, 23, 24}. Well defined criteria for treatment allowed before and during the studies were provided in 10/11 studies^{4, 15, 16, 18-25}.

Methodology

The primary sampling technique of skin was swabs. No biopsies were taken. Different protocols were used for DNA extraction. One study applied a metagenomic sequencing approach profiling all microbes²⁵. For bacterial microbiome analyses 10/11 studies used 16S rRNA sequencing^{4, 15-19, 21-24} applying either broad-range 16S gene primers^{17, 19} or targeting hypervariable region 1-3 (V1-V3)^{4, 15, 21, 22}, V3¹⁶, V4²⁴, V1-V2¹⁸ or V2-V3²³. The fungal microbiome was characterised in 2 studies^{15, 20}, using either the Internal Transcriber Spacer sequence and 18S rDNA as targets for amplification¹⁵ or

the D1/D2 hypervariable region of the 28S rDNA gene²⁰. The amount of PCR cycles differed from 30^{16, 20, 23} to 35^{18, 24} to 40¹⁷. Five studies did not provide information on the number of PCR cycles^{4, 15, 19, 21, 22}. Relative abundances of microbial taxonomic units were provided in percentages in 7/11 studies^{4, 15-17, 19, 20, 25}, in 5 studies estimations were made from readings of figures^{18, 21-24}. Taxonomic classification was performed either on genus-^{15, 17-19, 21, 24}, family-^{22, 23} or species-level^{4, 17, 20, 25}. More studies included additional species-level identification of *Staphylococcus*^{15, 18, 19, 22} or only *S. aureus*²¹. The study by Bourrain¹⁶ only identified *S. aureus* versus diversified microbiota.

Risk of bias

The quality of the human studies varied (table 2). We rated them as very good (total score=9 and one RCT rated low in risk of bias; N=4), good (total score=7-8; N=4), fair (total score=5; N=2) and poor (a poorly reported RCT; N=1). The main reason for downgrading the quality of the “fair” studies was either lack of information on cases and controls²⁰ or missing a proper non-exposed control¹⁸.

AD skin microbiome profile

S. aureus was abundant on AD skin compared to control^{4, 15, 19} and correlated positively to disease severity¹⁵ (table 3). Affected skin sites were more *S. aureus* dominated than unaffected^{4, 15, 16, 26}, especially inflamed areas (compared to xerotic)¹⁶ – and during a flare the abundance increased dramatically in untreated patients¹⁹. Besides *S. aureus*, other species from the genus *Staphylococcus* increased on involved sites²⁴. These included *S. epidermidis*^{15, 19, 26} and *S. haemolyticus*¹⁵.

The bacterial diversity on AD skin was low compared to control^{15, 19} and reduced during a flare¹⁹. Reductions in species from the genera *Streptococcus*, *Propionibacterium*⁴, *Acinetobacter*, *Corynebacterium* and *Prevotella* were found – not solely attributed to *S. aureus* increase¹⁹. *Propionibacterium acnes* was also found less frequent on facial AD skin compared to control¹⁷ and was inversely correlated to disease severity¹⁵. Interestingly, though *Corynebacterium* decreased during AD flares¹⁹, it was increased in the antecubital flexure of primary immunodeficiency patients¹⁵. After a flare, the species that were reduced increased in relative abundance¹⁹.

The fungal microbiome showed that AD patients had overall depleted *Malassezia* family members²⁵, however enrichment of *M. dermatis*²⁵ and more diverse non-*Malassezia* species compared to healthy controls^{15, 20}. These included *Aspergillus*¹⁵, *Candida albicans* and *Cryptococcus diffluens*²⁰.

Effect of treatment on the skin microbiome in AD

Compared to no treatment, intermittent-treatment decreased *S. aureus* predominance and loss of bacterial diversity during a flare¹⁹ (table 3) – with no improvement in SCORAD (data in original paper by Kong). In contrast, Oh et al. found no such treatment-associated shifts in bacterial community diversity¹⁵.

One study evaluated the effect of dilute bleach baths and found that 10 days of baths improved SCORAD and number of lesional sites colonised by *S. aureus*¹⁶. Another study evaluated the effect of topical corticosteroid treatment alone or in combination with dilute bleach baths. Both treatments improved the clinical eczema representation and suppressed *Staphylococcus* on both lesional and non-lesional sites – concluding no effect of the additional dilute bleach baths²⁴.

Emollient usage improved SCORAD and resulted in minor changes in the microbiome: 28 days of emollient usage did not induce changes in genus-level microflora at unknown skin site(s) but *S. aureus* increased in the non-emollient control group only²¹. 84 days of emollient usage on affected and unaffected sites improved SCORAD in 26 individuals out of 36²⁶. These 26 individuals had less relative abundance of *Staphylococcus* spp. and significantly more *Stenotrophomonas* – which also was inversely correlated to disease severity¹⁵. However, *Stenotrophomonas maltophilia* was found in facial skin in AD patients (not in controls)¹⁷.

Association between dysbiosis and AD

Two months old infants who later were diagnosed with AD and had affected skin at the age of 12 months had demonstrated significantly lower number of commensal *Staphylococcus* species in their antecubital fossae than children with unaffected skin at the age of 12 months²². These data suggest that cutaneous dysbiosis might play a role in initiation of AD and further that exposure to commensal staphylococci during early infancy might be important.

Animal studies

We included 6 animal studies (table 1) either with AD dogs or mouse models; 4 non-interventional and 2 interventional. Four studies sampled the skin by swabbing²⁷⁻³⁰ and 2 by biopsies^{31, 32}. Different DNA extraction protocols were used. The bacterial microbiome was analysed in 5/6 studies by 16S

rRNA sequencing^{27, 29-32}. The fungal microbiome was characterized in one study²⁸. No information on the amount of PCR amplification cycles were given in 4/6 studies²⁷⁻³⁰.

Relative abundances of microbial taxonomic units was provided in percentages in 3/6 studies^{27, 28, 32} and estimated from readings of figures in 3 studies²⁹⁻³¹. Taxonomic classification was performed either on family-level^{29, 32} with additional analysis of *Staphylococcus* species in one study²⁹, genus-^{27, 28, 31} or phylum- with species-level identifications of *Staphylococcus* and *Corynebacterium*³⁰.

The animal studies were mostly unclear in risk of bias (table 2) due to poor reporting, which is common for animal studies¹⁴.

Animal AD skin microbiome profile and effect of treatment

Like humans, AD dogs^{27, 29} and Adam17-deficient mice³³ had decreased bacterial diversity, increased abundance of *Staphylococcus* species²⁹ and *S. aureus* at the onset of eczematous inflammation³³ (table 4). *Corynebacterium* species were also increased^{29, 31, 33}.

Antimicrobial treatment of dogs presenting AD lesions²⁹ decreased the clinical eczema score and transepidermal water loss. No difference was found in skin pH. Furthermore, bacterial diversity normalised with decreased relative abundance of *Staphylococcus* species.

Causality between dysbiosis and AD

In Adam17-deficient mice³³ a prescreening of microbial composition was used to target systemic antimicrobial therapy. Therapy resulted in decreased clinical scores and transepidermal water loss along with decreased relative abundance of the targeted species, *S. aureus* and *C. bovis*, and increased bacterial diversity. Withdrawal of treatment dissipated the improvements in diversity. Eczema and dysbiosis re-appeared after 2 weeks, as shown in a cross over design, where systemic antibiotics was shown to protect the Adam17-deficient mice from developing eczema and losing microbiome diversity. These data suggest a causal relationship between dysbiosis and AD in an animal model.

Discussion

In this systematic review we demonstrated that AD skin in humans is characterised by low bacterial diversity and high non-*Malassezia* fungal diversity. On involved skin the bacterial diversity was even

lower. The relative abundance of both *S. aureus* and *S. epidermidis* was elevated and the abundance of *Propionibacterium* was reduced, along with other genera; *Streptococcus*, *Acinetobacter*, *Corynebacterium* and *Prevotella*. A birth cohort study indicated that absence of early colonization with commensal staphylococci might precede AD presentation and an animal study indicated that dysbiosis was a driving factor in pathogenesis of eczema. In interpreting this data synthesis, it should be emphasised that the data was drawn from few studies with substantial heterogeneity and varied quality. Many of the included studies (15 of 17) analysed the microbiome using 16S rRNA sequencing, and even though the 16S rRNA gene is widely accepted as a biological fingerprint for bacterial species, there are some limitations. Some bacterial species have multiple copies of 16S rRNA genes, which may lead to an artificial overrepresentation in data ³⁴. In addition, technical aspects may introduce uncertainty too; these include sampling technique ³⁵, DNA extraction ³⁶ and sequencing protocol ³⁷. For instance classification accuracy varies with the specific regions of the 16S rRNA gene chosen to be sequenced ³⁷. The limitations to 16S surveys have made the newer approach whole metagenome shotgun sequencing attractive. This method allows for analysis of the entire gene content of the microbial population, catch most species and may sequence deep enough to identify strains ³⁷. This is crucial when it comes to understanding the physiological implications of a modified microbiome. Only 1/17 studies applied this method. Sequences obtained have short read lengths and many have no representative within databases. Therefore the different methodology applied in the included studies likely affect outcome in microbiome composition and underline the importance of transparency in methodological approach. Not all studies included provided enough information on each methodological step. This shows a need for a guideline for good reporting on microbiome studies.

A common criticism using DNA-based technology to identify microbial communities is that DNA from dead and viable microorganisms are not distinguished. In the future, attempts to reduce DNA from dead microorganisms or performing RNA (cDNA) based community analysis may help minimizing detection of dead microorganisms. Such approaches would also contribute to enlighten potential interplays and communication between host and microbiome, e.g. in processes such as eczematous inflammation. Studies are moving from describing the microbiome to focus on

interactions by implicating also RNA, protein and/or metabolite data. A study by Fyhrquist showed a positive correlation between the relative abundance of skin *Acinetobacter* species and expression of anti-inflammatory molecules among healthy subjects, which was not present in atopic individuals³⁸. In the study by Kong¹⁹, the relative abundance of *Acinetobacter* increased post flare – which supports a potential anti-inflammatory role of *Acinetobacter* in AD.

Ongoing studies investigate the effect of age and treatment of AD (supplementary table 1). The findings of *Corynebacterium* being reduced in AD flares, but increased in the antecubital flexure of primary immunodeficiency patients suggest that underlying genetics may affect the microbiome. Mutations in the gene encoding the protein filament aggregating protein, filaggrin, leading to a functional absence of the protein predisposes individuals to develop atopic eczema³⁹, increase stratum corneum pH⁴⁰ and increase susceptibility to recurrent bacterial skin infection among patients with AD⁴¹. Filaggrin deficiency in ichthyosis vulgaris is associated with a low abundance of proteolytic Gram-positive anaerobic cocci, which are shown better at inducing expression of antimicrobial peptides in cultured keratinocytes⁴². This could be a mechanism favoring growth of *S. aureus* or infection. A trend for lower bacterial diversity in one control and two AD filaggrin-null mutation subjects was seen in the study by Chng²⁵. However, the role of filaggrin on the skin microbiome in AD is not known and none of the studies included in this review could elaborate thoroughly on this.

In line with the *S. aureus* data in this review, a recent meta-analysis showed that patients with AD were more likely to be colonized with *S. aureus* than healthy controls, with higher odds ratios on lesional skin (19.74, 95% CI: 10.88-35.81) compared to non-lesional (7.77, 95% CI: 3.82-15.82)¹⁰. With *S. aureus* being more abundant on non-lesional skin suggests that the skin is susceptible to pathogen colonization and in risk to progress toward diseased state. This indicates that anti-staphylococcal treatment could be beneficial. However, a systematic review by Bath-Hextall⁴³ showed that reducing the numbers of *S. aureus* in people with uninfected eczema, did not result in reduced disease activity. Targeting specific *S. aureus* strains could potentially improve the outcome of anti-staphylococcal treatment. This is supported by the finding of a single nucleotide polymorphism in a staphylococcal lipase gene being preferentially hosted in AD²⁵. However, targeting other bacteria

might also be beneficial. An idea of a critical window early in life where exposure to certain microbes are important for development of the immune system and allergic diseases has arisen and is supported by studies showing reduced microbial diversity in the gut before atopy development^{1, 44, 45}. Further, tolerance to the skin commensal *S. epidermidis* is preferentially established in neonatal life in mice⁴⁶. Current data²² is limited and it is difficult to evaluate whether the cutaneous microbiome play a role in initiation of AD. Hypothesising that dysbiosis precede AD flares and severity, studies are currently investigating prevention and treatment targeting dysbiosis. Moisturisers are key in AD management to restore and preserve skin barrier integrity. A RCT showed that emollient therapy from birth in high risk AD babies enhanced the skin barrier and reduced the relative risk of AD incidence with 50% after 6 months⁴⁷. An ongoing study by Glatz (supplementary table 1) investigates if shifts in the skin microbiome are associated with this improvement. Preliminary data show that preventative emollient usage lowers pH, does not change transepidermal water loss and increases the number of bacterial taxonomic units and *Streptococcus* spp.⁴⁸. Since *Streptococcus* was reduced during flares¹⁹ but increased in the absence of commensal staphylococci in infants before AD presentation²² future studies should investigate the role of *Streptococci* species in AD. *Stenotrophomonas* spp. may also have an important role with restoration of the skin microbiome¹⁸.

Another approach to manipulate the skin microbiome is adding beneficial bacteria to moisturisers. A RCT showed that cream containing 5% lysate of the nonpathogenic Proteobacteria *Vitreoscilla filiformis* significantly improved SCORAD, transepidermal water loss, the AD patient's assessment of itch and loss of sleep compared to placebo⁴⁹. Ongoing studies by Gallo and colleagues apply the same principle: In attempt to decrease *S. aureus* colonisation in AD skin, they isolate beneficial *Staphylococcal* species from the patients themselves and place them in a moisturiser, applied to the subjects own arms (supplementary table 1).

To utilize the microbiome in prevention and treatment strategies of AD, more data from human studies are needed on the skin microbiome dynamics related to clinical measures, temporal resolution and how different factors modify the microbial abundances to be able to predict responses in the

microbiome to perturbations. Good speciation and strain-level identification in combination with RNA, protein and metabolite data would strengthen such data and provide valuable insight.

Conclusion

While the microbiome draw increasingly attention as target in prevention and treatment of AD, new methodological approaches have not yet brought us far in understanding the impact of dysbiosis in AD. Staphylococcal species are key players in worsening of AD, and may also be important in the establishment of the disease. Other microbes such as *Propionibacterium*, *Streptococcus*, *Acinetobacter* and *Malassezia* have been found to be implicated in AD dysbiosis. However, robust data are missing on the influence of methodological procedures, characteristics on the microbiome structure related to temporal dynamics, clinical measures and factors altering the microbiome.

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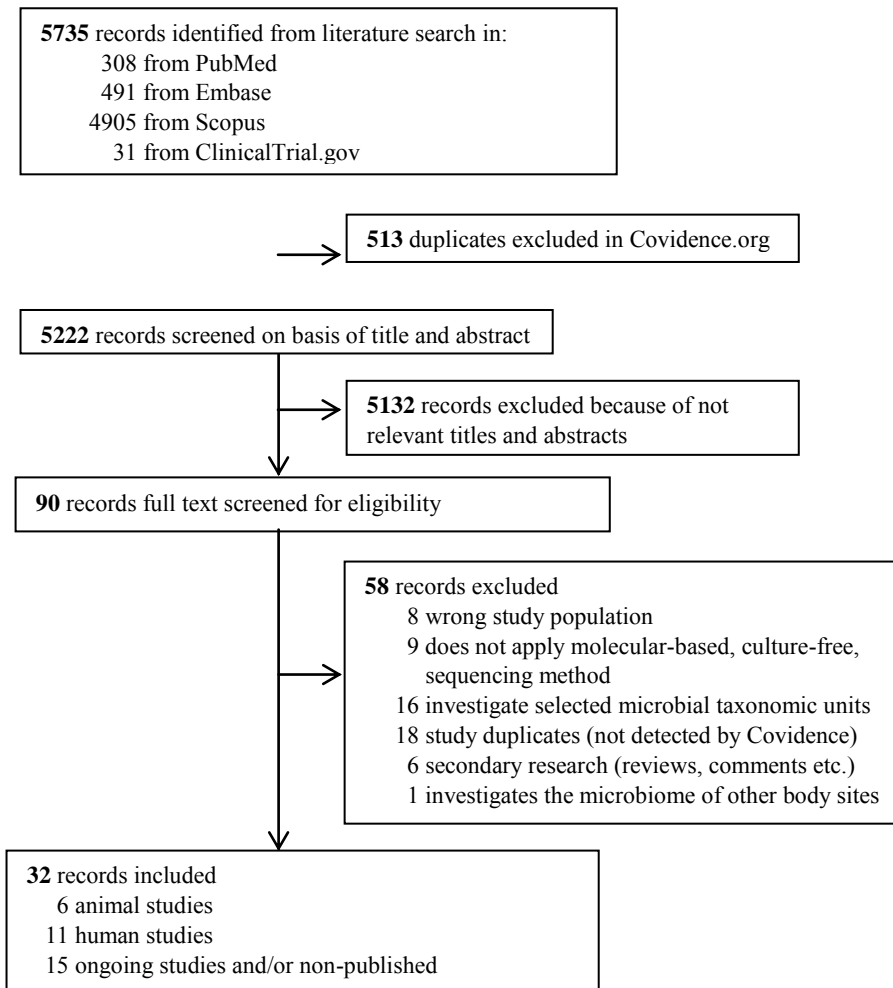
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Figure 1: Flow diagram of study selection



First author	Sampling	Area sampled	Setting	Study population	Treatment	N	Study type	Sample #	Method Extraction and sequencing	Physiological and clinical measures
Bourrain ¹⁶	Swab	5 cm ² Inflam., Non-lesional and Xerotic sites Body site: NA (dry, moist, seb.)	France	Mild-severe AD 18-40 years Mixed sex	Before and during the study: 1 wk: No use of top. steroids. 2 wk: No use of top. or oral immunomodulators, antibiotics, antiseptics or antifungal	25	Prospective cohort study: 18 days of hydrotherapy	4	DNeasy Blood and Tissue kit (Qiagen) 16S rRNA (V3), 30 PCR cycles Diversified microflora or <i>S. aureus</i> abundant	SCORAD
Dekio ¹⁷	Swab-scrub	4.9 cm ² facial skin	Japan	Mod.-severe AD, Healthy ctrl 19-54 years Mixed sex	Not specified	13 AD 10 Ctrl	Case-control	1	Extraction buffer, glass beads 16S rRNA (V1-V9), 40 PCR cycles, Terminal Restriction Fragment Length Polymorphism	SCORAD
Flores ¹⁸	Swab	1 cm ² of affected and nearby unaffected skin Body site: Diverse	France, Slovakia and USA, California and Colorado	Mod. AD 3-39 years Mixed sex	During the study: Instructed not to use other emollients or drugs, incl. corticotherapy and antibiotherapy	49	Prospective cohort study: 84 days emollient treatment	2	MoBio PowerSoil DNA isolation kit 16S rRNA (V1-V2), 35 PCR cycles, 454-pyrosequencing	SCORAD, erythema, dryness, desquamation
Kong ¹⁹	Swab	Antecubital and popliteal creases, volar forearms, nares	USA, Maryland	Mod.-severe AD Healthy ctrl 2-15 years Mixed sex	“No”: No top. for 1 wk, no oral antibiotics for 4 wk prior to sampling. “Intermittent”: Top. in the prev. 1 wk and/or oral antibiotics in the prev. 4 wk	12 AD 11 Ctrl	Case-control: Baseline-flare-post flare Treatment (No N=7, intermittent N=5)	3	Lysis buffer and lysozyme, bead-beated, Invitrogen PureLink Genomic DNA kit 16S rRNA (V1-V8), Sanger sequencing	SCORAD
Oh ¹⁵	Swab and scrape	4 cm ² . Nares, retroauricular crease, antecubital fossa, volar forearm	USA, Maryland	PID patients (2-37 y) with 1) Hyper IgE, 2) Wiskott-Aldrich, 3) DOCK8 deficiency. Mod.-severe AD (2-17 y). Healthy ctrl (2-40 y). Mixed sex	Only data on PID patients: 22/25 H patients got antifungals and/or antibiotics. 6/10 W patients got antibiotics. 4/6 D patients got antibiotics	41 PID: 25H, 10W, 6D. 13 AD 49 Ctrl	Case-control	1	Lysis buffer and lysozyme, bead-beated, Invitrogen PureLink Genomic DNA kit 16S rRNA (V1-V3) 18SF and ITS1 (Only H patients) Sanger and 454 Sequencing	SCORAD
Zhang ²⁰	Strip (x3)	63 cm ² facial skin	Japan	Mild, mod., severe AD Healthy ctrl Mixed sex	Intermittent medium/strong top. steroids. No systemic or top. antibiotics or antifungals	3+3+3 AD 10 Ctrl	Case-control	1	Lysing solution, ethanol precipitation 28S rDNA (D1/D2), 30 PCR cycles, Sanger (3730x)	
Bianchi ²¹	Scratch	Right antecubital fossa (unaffected)	France, Italy	Children with mild AD 1-4 years Mixed sex	No immunosuppressant's a month before. Systemic antibiotics, probiotics or anti-inflammatory treatment 2 wk before, local top. a wk and no cream 48 h before	55	RCT: 28 days of 1) hygiene product or 2) hygiene product + emollient	2	QIAamp DNA Investigator Kit 16S rRNA (V1-V3), 454-pyrosequencing	SCORAD TEWL
Drago ²³	Scrape	Behind the ear (lesional + non-lesional)	Italy	3 first cousins: mod. AD, mod. psoriasis, healthy ctrl 50 y Males	No pharmacological therapy or probiotics 1 month before sampling. Restricted on lifestyle, diet, sexual activity, personal care	1 AD 1 Ctrl	Case-control	1	Geneaid Genomic DNA Mini Kit (tissue) 16S rRNA (V2-V3), 30 PCR cycles Torrent PGM	SCORAD
Kennedy ²²	Swab	Antecubital and popliteal fossae, nasal tip, cheek	Ireland USA, Maryland	AD and healthy ctrl infants from the Cork BASELINE Birth Study Mixed sex	Emollient usage in 6/10 AD infants and 2/10 healthy. No differences in bathing frequency or antibiotic usage	10 AD (4 affected at 12 months of age) 10 Ctrl	Case-control from a prospective birth cohort study. Swabbed at months 2, 6 and 12 and also clinical assessed at 24 months of age	3	Epicentre MasterPure Kit, bead-beated, Invitrogen PureLink Genomic DNA Kit, 16S rRNA (V1-V3), 454-pyrosequencing (GS FLX)	SCORAD (month 24). Filaggrin genotype (no mutations)

	Chng ²⁵	Tape-strip	Antecubital fossae	Singapore	Singaporean Chinese population, non-flare AD > 18 years Mixed sex	Only restricted from using antibiotics	19 AD 15 Ctrl	Case-control	1	Qiagen EZ1 DNA Tissue Kit, Shotgun whole-metagenome sequencing	Filaggrin genotype (mutations in 2 AD, 1 Ctrl) TEWL, pH
	Gonzalez ²⁴	Swab	3 lesional (2 representative + the worst) and 1 contralateral or adjacent non-lesional site. Ctrl at 4 sites with AD predilection	USA, New York	Mod.-severe AD Healthy ctrl 3 months – 5 years Mixed sex	Excluded if overt infection, concurrent chronic skin disorders or use of antibiotics, systemic or top. corticosteroids or calcineurin inhibitors in the prior 2 wk	21 AD 14 Ctrl	RCT: 4 wk treatment of 1) top. corticosteroid (plus water baths) or 2) top. corticosteroid plus bleach baths	2	MoBio PowerSoil DNA Isolation kit 16S rRNA (V4), 35 PCR cycles, Illumina MiSeq	Hanifin and Rajka, EASI
	Shi ⁴	Swab	25 cm ² lesional and adjacent non-lesional skin on volar forearm	USA, California	Mod-severe AD Healthy ctrl 2-12, 13-17 and 18-62 years Mixed sex	Excluded if temp > 38.5 Prior sampling: 20 days: No phototherapy or immunosuppressant's 1 wk: No antibiotics, topicals, bleach baths 24 h: No creams/lotions, bathes.	128 AD 68 Ctrl	Case-control: Comparison among age groups	1	QIAamp DNA micro kit incl. bead beating. 16S rRNA (V1-V3) Illumina MiSeq	Rajka-Langeland
Animal studies	Rodrigues Hoffmann ²⁷	Swab	Axilla, groin, nasal, skin in-between digits	USA, Texas	Allergic dogs (6, 5 with AD) Healthy ctrl dogs	No syst. antibiotics 30 d prior to sampling. 3 got glucocorticoids or cyclosporine, 3 got allergen-specific immunotherapy	6 A 12 Ctrl	Case-control	1	MoBio Power Soil DNA isolation kit 16S rRNA (V1-V3), 454-pyrosequencing	
	Meason-Smith ²⁸	Swab	Axilla, groin, nasal, skin in-between digits, ear canal, lumbar	USA, Texas	Allergic dogs (8, 6 with AD) Healthy ctrl dogs 1.5-11 y Mixed sex	No syst. antibiotics or antifungals in the allergic dogs 1 month prior sampling (6 in the healthy). Top. allowed	8 A 10 Ctrl	Case-control	1	MoBio Power Soil DNA Isolation kit Internal Transcribed Spacer region (1F and 4R), Illumina MiSeq	
	Bradley ²⁹	Swab	Axilla, groin, pinna, mouth	USA, Pennsylvania	AD dogs with active lesions Healthy control dogs	4 used antibiotics within 45 d before. Targeted antimicrobial therapy in the interventional period	14 AD 16 Ctrl	Prospective cohort study: Flare-post therapy-post conclusion	3	Lysozyme, bead-beating, protein precipitation, Genomic DNA Isolation Kit (Life Tech) 16S rRNA (V1-V3) Illumina MiSeq	Clinical scoring, TEWL, pH
	Kobayashi ³³	Swab	Cheek	JapanUS A, Maryland and Minnesota	Disintegrin and metalloproteinase 17 deficient mice in Sox9-tissue, incl. epidermidis (AD17 ^{fl/fl} Sox9-Cre).		A) 3 WT 3 AD17 B) 8 WT 8 AD17 C) 12 AD17	A) Tanner stage B) Antibiotics targeting <i>S. aureus</i> and <i>C. bovis</i> C) Crossover D) Characteristics	A) 7 B) 3 C) 2 D) 1	Incubated in lysis buffer and lysozyme, (maybe bead-beated), Invitrogen PureLink Genomic DNA kit. 16S rRNA (V1-V3), 454-pyrosequencing	TEWL
	Kubica ³²	Punch biopsy (4 mm)	Ear	Belgium	Caspase-14 (involved in filaggrin degradation) knock out hairless mice		5 WT 4 KO	Animal study, case-control	1	QIAamp DNA Stool Mini Kit, 16S rRNA (V3-V5), 25 PCR cycles, 454-pyrosequencing	
	Schar-schmidt ³¹	Biopsy	Ear flexure	USA, Maryland	Ichthyotic model: Matripase (degrades profilaggrin) deficient Mice, 1% of WT levels (St14 ^{hypoc})		3 WT + 3 St14 ^{hypoc}	Animal study, case-control	1	DNAeasy kit (Qiagen), protocol for Gram-pos. bacteria (incl. bead-beating) 16S rRNA (V1-V8), 23 PCR cycles, Sanger	

Table 1: Characteristics of included published studies. Inflam.: Inflammatory. NA: Not available. Seb.: Sebaceous. NIH: National Institutes of Health. AD: Atopic dermatitis. Mod.: Moderate. Wk: Week. Top.: Topical. Prev.: Previous. PID: Primary Immunodeficiency. H: Hyper-IgE. W: Wiskott-Aldrich. D: DOCK8 deficiency. Syst.: Systemic. H: Hours. D: Days. EGFR: Epidermal Growth Factor Receptor. AD17: AD17^{fl/fl}Sox9-Cre (AD17=ADAM17, a metalloproteinase involved in epidermal barrier integrity). WT: Wild Type. KO: Knock Out. St14^{hypoc}: Mice with one null and one hypomorphic allele of "Suppressor of tumorigenicity 14", matriptase = a serine protease. Ctrl: Control. A: Allergic. AD: Atopic dermatitis. RCT: Randomised Controlled Trial. *S. aureus*: *Staphylococcus aureus*. *C. bovis*: *Corynebacterium bovis*. PCR: Polymerase Chain Reaction. rRNA: ribosomal RNA. ITS1: Internal Transcribed Spacer region 1. V3: Variable region 3 of the 16S rRNA gene. Gram-pos.: Gram-positive. SCORAD: SCORing Atopic Dermatitis. TEWL: TransEpidermal Water Loss. EASI: Eczema Area and Severity Index.

(a): Cochrane Collaboration's tool for assessing risk of bias in randomised controlled trials			
Study	Domain	Review authors judgement	Support for judgement
Bianchi ²¹	Random sequence generation	Unclear	No information
	Allocation concealment	Unclear	No information
	Blinding of participants and personnel	Unclear	Patients not blinded, but no information on personnel.
	Blinding of outcome assessment	Unclear	No information
	Incomplete outcome data	Low risk	Reason given for one exclusion
	Selective reporting	Unclear	No study protocol available
	Other sources of bias	Unclear	Insufficient rationale: No sample size calculation. Objective is given but no clear hypothesis. No specified setting
Gonzalez ²⁴	Random sequence generation	Low risk	Shuffling envelopes
	Allocation concealment	Low risk	Numbered containers
	Blinding of participants and personnel	Low risk	Participants (incl. parents) and clinical personnel blinded
	Blinding of outcome assessment	Low risk	Investigators, data analysts, and sequences blinded to treatment until unblinding was necessary for comparative data analysis after ended experiment
	Incomplete outcome data	Low risk	Reasons for missing outcome data and balanced across intervention groups
	Selective reporting	Unclear	No study protocol available
	Other sources of bias	Low risk	The study appears to be free of other sources of bias

(b): Newcastle-Ottawa Scale for assessing quality of case-control studies				
Studies	Selection Definition and selection of cases and controls (max=4*)	Comparability of cases and controls (max=2*)	Exposure Blinding, same method, rel. abundances as outcome, complete data (max=4*)	Total (max=10*)
Dekio ¹⁷	***	**	**	7
Kong ¹⁹	***(*) (4/11 healthy children have fam. history of AD)	**	***	9
Oh ¹⁵	***	**	***	8
Zhang ²⁰	*	*	***	5
Drago ²³	****	**	**	8
Kennedy ²²	****	**	***	9
Chng ²⁵	***	**	***	8
Shi ⁴	****	**	***	9

(c): Newcastle-Ottawa Scale for assessing quality of cohort studies				
Studies	Selection True and/or somewhat representatives of AD, ascertainment of exposure, outcome at baseline (max=4*)	Comparability +/- treatment of matched skin areas, controlling for additional factors (max=2*)	Outcome Blinding, time to follow-up, complete follow-up, bias due to missing follow-ups (max=4*)	Total (max=10*)
Bourrain ¹⁶	***	*	****	9
Flores ²⁶	**	*	**	5

(d): Adjusted SYRCLE's tool for assessing risk of bias in animal studies							
Type of bias	Domain	Scharschmidt ³¹	Kubica ³²	Rodrigues Hoffmann ²⁷	Meason-Smith ²⁸	Kobayashi ³³	Bradley ²⁹
Selection bias	Group similarity (sex, age)	Low risk	Low risk	High risk	High risk	Low risk	High risk
Performance bias	Random housing	Unclear	Low risk	Low risk	Unclear	Unclear	Unclear
Detection bias	Blinding	High risk	Unclear	Unclear	Unclear	Unclear	High risk
Detection bias	Blinding of outcome assessor	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Attrition bias	Incomplete outcome data	Unclear	Unclear	Low risk	Low risk	Unclear	Low risk
Reporting bias	Selective outcome reporting	Unclear	Unclear	Unclear	Unclear	Unclear	Unclear
Biases associated with interventional studies	Allocation					Unclear	Low risk
	Baseline characteristics					B) Unclear C) Low risk	Low risk

Table 2: Review authors scores of risk of bias of included studies using the Cochrane Collaboration's risk of bias tool (a), an adjusted Newcastle-Ottawa Scale for case-control studies (b) and cohort studies (c) where points (*) are assigned for no biases and an adjusted SYRCLE's tool for non-interventional (6 entries) and interventional (8 entries) animal studies (d).

Accepted Article

Phylum	Family	Genus or species	Bourrain ¹⁶ # Baseline/D1 D10 D18 X I N	De- kio ¹⁷ # C AD	Flores ²⁶		Kong ¹⁹			Oh ¹⁵			Zhang ²⁰			Bianchi ²¹ #	Drago ²³			Kennedy ²²	Chng ²⁵	Gonzalez ²⁴			Shi ⁴				
					Pre-tr U A	Post-tr Resp Non U+A U+A	C	B	F	P	C	PID	AD	AD	C	M	Mo	S	D0 D28 C E	C	AD	U A	Antecub M2 U A M12	C	Tr+bl L N	Tr-bl L N	C AD L/N Ch Teen-Adu		
Firmicutes	Staphylococca- ceae																												
		<i>Staphylococcus</i>		75 54	17	33*	15	52	16	35	31	90	20	11	28* 6	7 18*	35* 11	47* 8*				9 7	12	62	31	58	34	6	9
		<i>S. aureus</i>	36 48 24	52/56* 16 44 24 20		1	8		1	17	15	65*	6	0	10*	1	8										0	1	
		<i>S. capitis</i>							2	2	6	1	3									3	0						
		<i>S. epidermidis</i>				11	19		9	7	7	20*	8	5	13*	2	7*										1	4	
		<i>S. hominis</i>							3	5	2	2	2													2	2		
		<i>S. haemolyticus</i>												0	1*														
		<i>S. cohnii</i>																				19	1						
	Alicyclobacilla- ceae				5	4	6	4																					
	Streptococca- ceae																												
		<i>Streptococcus</i>		80 54	8	7	6	5	15*				2	14*								1 3*	16	9	8	7	9	26	14
		<i>S. mitis</i>																				1 2*	20	13	8	13	8	20/26*	10/11
	Neisseriaceae	<i>Neisseria</i>																									2	1	
	Veillonellaceae	<i>Veillonella</i>																									2	2	
																											1.5/2*	1/1	
Proteo- bacteria	Rhodobactera- ceae																												
	Moraxellaceae	<i>Acinetobacter</i>			2	1.2	2	1	1				0	3*													7	3	
	Xanthomonada- ceae	<i>Stenotropho- monas</i>						7	1.5*																				
		<i>S. maltophilia</i>		0* 38																									
	Alcaligenaceae	<i>Alcaligenes xylooxidans</i>		0 7																									
	Enterobactera- ceae	<i>Serratia marcescens</i>												0	8*	3	0	0											
									0	8*	3	0	0																
Actino- bacteria	Dietziaceae	<i>Dietzia maris</i>		80* 15																									
	Propionibacteria -ceae																												
		<i>Propioni- bacterium</i>			11	8	11	5	6			1	12*	24	11	26	9	7										13	28
																						56 67						5/5	11/14

		<i>P. acnes</i>		75* 35										13 4/5	28 10/13
	Corynebacteria- ceae									3 2 1	2 4				
		<i>Coryne- bacterium</i>			4 3 8 3	9* 1 9*	9 17* 14 15 5 8 18 9 17 5					6 2		3 8 3/4* 8/11*	
	Dermacocca- ceae											1 0.5*			
	Actinomycetace ae	<i>Rothia</i>												2 2 2/2 1/1	
Bacte- roidetes		<i>Prevotella</i>			1 2	1 0 1								3 3 2/2 2/2	
Asco- mycota	Trichocomaceae	<i>Aspergillus</i>					1 4* 0 7*								
	Saccharomyce- tales	<i>Candida</i>					0 0.4* 0 0.3*								
		<i>C. albicans</i>						1 2 3 0							
	Davidiellaceae	<i>Cladosporium</i>						5 5 6 3							
	Capnodiales	<i>Toxicoclado- sporium irritans</i>						2 2 1 0							
Basidio- mycota	Malasseziaceae											2 1*			
		<i>Malassezia</i>					96* 71 96* 67	69(all) 79							
		<i>M. restricta</i>						48 49 34 59							
		<i>M. globosa</i>						15 16 27 14				19 10*			
		<i>M. dermatis</i>										2 8*			
	Tremellaceae	<i>Cryptococcus diffluens</i>						2 1 3 1							
Shannon diversity (- <i>S. aureus</i>)					A=6.0; U=6.3 Pre-post: ΔU=0.2 ΔA=0.08	3.4 2.8 2.7 0.7* 3.4 2.8 2.5 2.5 1.5* 2.8	2.8 2.4 2.7 2.3 2.1* 2.8 2.7 2.9 2.8 2.7 No treatment effect in P at any site		2 2 2 2		NS	1.7 1.4	Improved with no differences between tr-groups. Inversely corr. to EASI		
SCORAD			Reduction at D18 (30.8±7.2 to 20.0±10.2)	36	Δ = -12. 78% sites had ↓severity	NA 21.8 42.1 18.1	0 22 6 11 28		10 12 8* 6*	32	Not relevant				
EASI														Improved with no differences between tr-groups	
Spearman corr.							To SCORAD, inverse: <i>Stenot.</i> , <i>P. acnes</i> , <i>Neisseria</i> , <i>Streptococcus</i> . Pos.: <i>S. aureus</i>					Inverse: <i>P. acnes</i> and <i>S. epidermidis</i> . <i>Dermacoccus</i> and <i>S. aureus</i>			
TEWL (g/m ² /hour)									D28: No change in C, 34% ↓ in E			No diff			
Skin pH												No diff			

Table 3: Summary of relative abundance (in percent) of microorganisms found on skin, clinical and physiological outcomes, human studies. Taxonomic units with % relative abundance ≤ 1 are not included in this table.

#: Percentage of total amount of samples dominated by *S. aureus* (vs. diversified microbiota)

□: Percentage of individuals in the study population with a specific microorganism (in percent)

X: Xerotic. I: Inflammatory. N: Non-lesional. L: Lesional site. .D1: Day 1. M2: Month 2. AD: Atopic Dermatitis. Antecub: Antecubital Fossa. Popl: Popliteal region. C: Control. Tr: Treatment. U: Unaffected. A: Affected. Resp: Responders. B: Before. F: Flare. I: Intermittent (treatment). N: No (treatment). P: Post flare. NA: Not available. PID: Primary Immunodeficiency. H: Hyper-IgE. W: Wiskott-Aldrich. D: DOCK8 deficiency. M: Mild. Mo: Moderate. S: Severe. E: Emollient (group). Ch: Children. Teenagers-Adults. SCORAD: SCORing Atopic Dermatitis. EASI: Eczema Area and Severity Index. TEWL: TransEpidermal Water Loss. * Indicate statistical differences found in the original papers.

Phylum	Family	Genus or species	Meason-Smith ²⁸	Rodriguez Hoffmann ²⁷	Kobayashi ³³												Bradley ²⁹			Kubi- ca ³²	Schar- schmidt ³¹	
			$\frac{C}{A}$	$\frac{C}{A}$	Time (wk) after birth				Antibiotic treatment				Crossover, AD17				Mechanism	$\frac{C}{AD}$			$\frac{WT}{KO}$	$\frac{WT}{St14^{hypo/-}}$
			# Diff. between C and A, all sites															Flare Post TR Followup				
			Ax G I N E L	Ax G I N	2 4 6 8 10 12 14	2 4 8 2 4 8	-AB → +AB	+AB → -AB	10 13 10 13	Ax G P Ax G P Ax G P												
Firmicutes																			2 9*			
		Other than <i>Streptococcus</i> + <i>Staphylococcus</i>			82 53 76 60 46 41 36	82 13 0 86 41 49	0 33 38 0															
	Class: Bacilli				83 15 10 3 1 0 7	94 35 48 92 50 48													5 2*			
	Order: Bacillales																		2 8			
	Bacillaceae	<i>Bacillus</i>		3 1 1 0 4 2 0* 0																		
	Staphylococcaceae																		92 77*			
	Staphylococcaceae	<i>Staphylococcus</i>		1 1 1 0 0 3 1 0															50 52			
		<i>S. aureus</i>			0 0 0 0 0 0 0 0	0 16 39 0 0 0	23 5 5 17												Across all skin sites: Flare: 10 Post TR: 1 P: 11 10 7 5			
		<i>S. lugdunensis</i>																	Across all skin sites: Flare: 20 Post TR: 18 P: 20 2 10 12			
		<i>S. pseudintermedius</i>																	Across all skin sites: Flare: 52 Post TR: 62 P: 52 82 68 68			
		<i>S. lentus</i>			3 5 2 2 0 0 0 0	0 1 0 0 0 0	0 4 3 1															
		Other than <i>aureus</i> and <i>lentus</i>			4 1 0 0 0 0 0 0	0 1 0 0 0 0	0 2 6 3															
					0 0 0 0 1 2 4 0 0 1 0	0 0 0 0 4 0																
	Alicyclobacillaceae	<i>Alicyclobacillus</i>		0 0 0 0 1 1 2 0																		
	Streptococcaceae	Streptococcus			0 1 0 16 3 10 11	1 2 0 0 10 5	1 1 5 2												0 6*			
					0 4 0 2 1 1 0	1 1 0 0 1 0																
					4 16 3 3 8 3 3	3 4 0 3 14 9	0 12 16 0												97 75*			
					1 5 2 1 0 0 0	3 19 2 3 6 5																
	Class: Beta-proteobacteria	Dominated by <i>Janthinobacterium</i>																	35 31			
	Neisseriaceae	<i>Conchiformibius</i>																				
	Class: Gamma-proteobacteria	Dominated by <i>pseudomonas</i>																	48 33			
	Pasteurellaceae																					
	Rhodobacteraceae	<i>Rubellimicrobium</i>		1 0 0 0 1 1 0*0																		
	Ralstoniaceae	<i>Ralstonia</i>		4 2 7 17 0 0*0*0*0*																		
	Sphingomonadaceae	<i>Sphingomonas</i>		0 1 0 0 3 2 0 0																		
	Xanthomonadaceae																		1 3*			
		<i>Stenotrophomonas</i>																	0			

									4*			2 13*
Actinobacteria												
	Propionibacteriaceae	<i>Propionibacterium</i>										
	Corynebacteriaceae	<i>Corynebacterium</i>										
		<i>C. bovis</i>										
		<i>C. mastitidis</i>										
		Other than <i>bovis</i> , <i>mast.</i> , <i>jeikeium</i> , <i>tuberculoosteicum</i>										
Bacteroidetes												
	Porphyromonadaceae	<i>Porphyromonas</i>										
	Flavobacteriaceae											
Tenericutes	Mycoplasmataceae	<i>Mycoplasma</i>				0 0 0 0 2*0 0 0						
Ascomycota	Pleosporaceae	<i>Alternaria</i>	30 28 22 33 23 32 20 20 26 30 11 22									
		<i>Epicoccum</i>	5 5 9 11 7 16 10 9 10 10 14 15									
	Davidiellaceae	<i>Cladosporium</i>	33 31 37 16 17 16 35 37 30 16 36 22									
	Saccharomycetales	<i>Candida</i>	#0 1 0 0 0 0 0 4 0 0 0 0									
	Clavicipitaceae	<i>Claviceps</i>	#1 1 0 0 0 1 0 4 0 0 0 0									
	Nectriaceae	<i>Fusarium</i>	1 2 3 0 2 0 2 5 2 0 0 2									
Basidiomycota	Malasseziales	<i>Malassezia</i>	1 1 0 5 1 1 1 2 2 0 2 0									
	Wallemiaceae	<i>Wallemia</i>	#0 0 4 14 9 1 0 0 0 12 22 5									
	OTHER		#2 4 5 0 7 4 5 1 2 5 12 2									
Shannon Diversity			All sites: $\frac{19}{1.7}$. Only diff in ear (C>A)	AGI: N: 6 2.9 5.4 2			2.3 1.3*0.8* 2 3.5 3.3 2.2 3 2.5 1.9 3 2.5					
Chao1 richness				432 100 168* 40*								
Struc. similarity (θ)												WT \neq KO
TEWL							5 6 8 NA 8 NA 9 7 19 NA 30 13*10 7*23*27*NA 34*NA 40* 7 12*NA 37 9 18					
Clinical score							NA 20 37 NA 12*8*	38 15* 5 43				
Antimicrobial activity												KO> WT

Table 4: Summary of relative abundance (in percent) of microorganisms found on skin, clinical and physiological outcomes, animal studies. Taxonomic units with % relative abundance ≤ 1 are not included in this table. C: Control. A: Allergic. Ax: Axilla. G: Groin. I: In-between digits. N: Nasal. E: Ear Canal. L:lumbar. Wk: week. WT: Wild Type. AD: Atopic dermatitis. AD17: AD17^{fl/fl}Sox9-^{Cre}. AB: Antibiotics. EGFR: Epidermal Growth Factor Receptor. P: Pinna. KO: Knock Out. St14^{hyp0/-}: Mice with one null and one hypomorphic allele of “Suppressor of tumorigenicity 14”, matriptase = a serine protease. * Indicate statistical differences found in the original paper.