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

TO THE EDITOR

Skin micro-organisms play an important role in the disease progression of atopic dermatitis (AD). The skin pathogen, Staphylococcus aureus, in particular, methicillin-resistant S. aureus (MRSA), contributes to AD by producing virulence factors that enhance immune activation and skin barrier dysfunction (Schlievert et al., 2010). While it is known that the relative abundance of S. aureus generally increases in AD lesions (Kong et al., 2012; Shi et al., 2016), it is not clear whether the skin microbiome composition in MRSA-colonized skin differs from methicillin-sensitive S. aureus (MSSA)—colonized skin. It is important to understand the potential differences in the composition of skin commensal bacteria, as they play a critical role in reducing the growth of pathogenic S. aureus and thus the risk of AD (Kennedy et al., 2017; Nakatsuji et al., 2017).

In this study, we screened for the presence of MRSA and MSSA strains on the skin in a cohort of 339 AD patients, including 169 young children (2–12 years) and 170 teenagers (13–17 years) and adults (18–74 years). This study was approved by the Institutional Review Boards at National Jewish Health in Denver and University of California, Los Angeles. Written informed consent was obtained from all participants. As the skin microbiome of teenagers is closer to adults than young children (Shi et al., 2016), we combined teenagers and adults into one group in the analysis. We collected skin swabs from lesional skin and adjacent normal-appearing non-lesional skin of...
the volar forearm from each AD patient. Using a culture-based assay (Supplementary Material online), MSSA was detected on the lesional skin of 199 (58.7%) AD patients (95 young children and 104 teenagers/adults), while MRSA was found in 23 (6.8%) AD patients (15 young children and 8 teenagers/adults). The detection frequency of MSSA and MRSA strains in pediatric patients of our cohort is consistent with previous studies (Suh et al., 2008).

S. aureus was not detected at the lesional site of the remaining 117 (34.5%) AD patients. Among the 23 AD patients carrying MRSA on the lesional skin, 11 were also carrying MRSA at the adjacent nonlesional site.

In addition to the culture-based assay, we compared the microbiome of MRSA-versus MSSA-colonized AD lesional skin in a subgroup of these subjects. The microbiome composition of 128 of the 339 AD patients (Supplementary Table S1 online), including 19 MRSA carriers, 70 MSSA carriers, and 39 patients without S. aureus detected in the culture-based assay, was profiled using the culture-independent 16S ribosomal RNA gene sequencing analysis (Supplementary Figure S1). The negative S. aureus culture results of 39 patients were corroborated by 16S ribosomal RNA gene sequencing, which showed a significantly low relative abundance (<1%) of S. aureus in the microbiome of these subjects. We found that the microbial diversity (alpha diversity) was significantly decreased in MRSA-colonized than MSSA-colonized lesional skin (P = 0.001) (Supplementary Figure S2 online). The relative abundance of S. aureus was significantly higher on MRSA-colonized (52.5% ± 9.3%) than MSSA-colonized (25.0% ± 4.1%) lesional skin (P = 0.017) (Figure 1a), suggesting that MRSA plays a more significant role in reducing the skin microbiome diversity than MSSA.

We found a significant further reduction in Streptococcus and Propionibacterium on MRSA-colonized lesional skin compared to MSSA-colonized lesional skin (P = 0.023 and P = 0.007, respectively). The relative abundance of Corynebacterium was significantly decreased on MRSA-colonized lesional skin as well (P < 1E-3) when compared to MSSA-colonized lesional skin (Figure 1f). Staphylococcus
epidermidis has been considered a skin commensal, however, its role in AD is unclear. Previous studies reported an increase in abundance of *S. epidermidis* on lesional skin of pediatric AD, which may suggest a compensatory mechanism of *S. epidermidis* to control pathogens (Byrd et al., 2017; Kong et al., 2012). In the current study, we found that the relative abundance of *S. epidermidis* was not significantly changed in MRSA colonization (Figure 1b). Other commensal *Staphylococcus* species, including coagulase-negative *Staphylococcus* with antimicrobial activity, such as *Staphylococcus hominis*, are commonly found on healthy skin but rarely on AD skin (Nakatsuji et al., 2017). In this study, we found that the relative abundance of *S. hominis* was significantly decreased on MSSA-colonized lesional skin compared to non-*S. aureus* skin (*P* < 0.004) and further decreased on MRSA-colonized lesional skin (*P* < 0.004) (Figure 1c), with the same trend as skin commensals *Streptococcus* and *Propionibacterium*.

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

Previous studies have shown that the skin microbiome varies by age in both healthy individuals (Oh et al., 2012) and AD patients (Shi et al., 2016). In our cohort of 128 AD patients, 59 (46%) were young children and 69 (54%) are teenagers/adults. Among the 19 AD patients carrying MRSA at the lesional site (Supplementary Table S2 online), we found that MRSA colonization shifted the microbiome composition in both age groups with an increased relative abundance of *S. aureus* and decreased relative abundances of skin commensals except for *S. epidermidis* (Supplementary Figure S3 online).

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

We also investigated the influences of other confounding factors. In our cohort, disease severity was not significantly associated with MRSA colonization (Supplementary Table S2). Within the patients with severe AD (*n* = 71), we found that the microbial diversity was lower in MRSA-colonized than MSSA-colonized lesional skin (*P* = 0.002, Supplementary Figure S4 online). The number of patients with moderate AD and MRSA colonization (*n* = 2) in our cohort was too low to allow us to perform a similar analysis. Additionally, to exclude the influence of medications on the skin microbiome, AD patients were excluded if they received medications, including antibiotic treatment within 7 days of sampling (more details in Supplementary Material).

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

TO THE EDITOR

The potential role of viruses as oncogenic triggers in cutaneous T-cell lymphoma (CTCL) pathogenesis is a subject of ongoing investigation. CTCL occurs with an increased incidence in immunosuppressed patients (Nikolaou et al., 2015; Pomerantz et al., 2010; Wilkins et al., 2006). Spectratyping studies have shown depleted T-cell receptor diversity in CTCL patients similar to that seen in patients with advanced HIV, and Ingenuity Pathway Analysis (Qiangen, Hilden, Germany) shows increased expression of genes critical to host viral response (Yawalkar et al., 2003). However, no consistent association between a viral pathogen and CTCL has been established (Mirvish et al., 2013).

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

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

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

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VirCapSeq-VERT Analysis of CTCL


