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Review Article

Advances in Genomics and Bioinformatics Methods for Analyzing Gene Expression in *Staphylococcus Aureus* Associated with Atopic Dermatitis

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Abstract

This comprehensive review explores the latest advancements in genomics and bioinformatics methods for studying how *Staphylococcus aureus* behaves in Atopic Dermatitis (AD). It focuses on crucial aspects to understand how this bacterium acts in AD. Firstly, it examines how genes in *S. aureus* are controlled and how they affect AD. It also looks at the less explored area of how *S. aureus'* genes are influenced by factors beyond their DNA sequence. The review also investigates how *S. aureus* settles on the skin in AD and how it manages to stay there. This sheds light on possible ways to intervene and stop its presence. Additionally, it tackles the problem of *S. aureus* becoming resistant to drugs used to treat AD related infections. It mines into the reasons behind this resistance and how it impacts potential treatments. Moreover, the review emphasizes the crucial role of computer-based tools in studying *S. aureus'* genes. It evaluates different methods used to understand the complex genetic information linked to AD related *S. aureus*. By combining different areas like genetics, how the bacterium lives on the skin, drug resistance, and the tools scientists use, this review offers a complete understanding of *S. aureus* in AD.

Keywords: *Staphylococcus aureus, Atopic dermatitis, Gene expression, Epigenetics*

Introduction

Atopic dermatitis (AD), a persistent inflammatory skin ailment, showcases intense itching and recurring eczema-like lesions, impacting approximately 15-20% of children and 2-5% of adults in industrialized nations (Sybilski *et al.*, 2015; Hadi *et al.*, 2021). Many investigations indicate that more than 90% of individuals suffering from atopic dermatitis exhibit colonization of their skin by *Staphylococcus aureus* (Bieber, 2008; Kong *et al.*, 2012; Totté *et al.*, 2016; Meylan *et al.*, 2017; Altunbulakli *et al.*, 2018). AD arises from multiple factors, encompassing genetic defects in the skin barrier caused, in part, by inherited mutations in keratinocyte proteins like filaggrin and loricrin (Kim *et al.*, 2008; Howell *et al.*, 2009; Callewaert *et al.*, 2020), an imbalance towards a T-helper-2 (Th2) immune response characterized by significant cytokines IL-4, IL-5, and IL-13 (Homey *et al.*, 2006; Brandt and Sivaprasad, 2011), and microbial dysbiosis, all contributing to increased susceptibility to AD (Bjerre *et al.*, 2017). Histological examinations also revealed that an accumulation of immune cells is known to play an essential role in AD development, including macrophages, dendritic cells, T cells, eosinophils, and group 2 innate lymphoid cells (ILC2s) (Chieosilapatham *et al.*, 2021). Th2 cytokines are commonly thought to be the primary cause of AD. Nevertheless, when the illness worsens, the penetration of certain T cell subsets, such as Th1, Th17, and Th22 cells, might jeopardize skin integrity even more and fuel persistent inflammation (Kim *et al.*, 2016).

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Various virulence factors, notably *staphylococcal* enterotoxin B in allergic inflammation induced by ovalbumin, enhance epithelial-derived cytokine production in *S. aureus* infections of epidermal and mucosal barriers; additionally, *S. aureus* utilizes phenol- soluble modulins (PSMs), a specialized protein family with multifaceted functions including colony spread, biofilm formation, and immune cell death, crucial for successful host colonization (Nakagawa *et al.*, 2017). Patients with AD experience a decrease in microbial diversity in their skin because of a higher percentage of *S. aureus* in their skin microbiome (Kong *et al.*, 2012). *Staphylococci* thrive in skin environments where there's less filaggrin, irregular corneocyte structure, elevated skin pH levels, and a lowered production of antimicrobial peptides, making colonization more favorable for these pathogens (Paller *et al.*, 2019). *S. aureus* colonization disrupts skin balance, aggravating skin inflammation through its range of toxins, known to worsen the progression of atopic dermatitis (AD); exposure to *S. aureus* on the skin triggers increased release of epithelial cell-derived cytokines TSLP and IL-33, stimulating Th2 cytokine production from basophils, eosinophils, mast cells, ILC2, and Th2 cells, leading to elevated IgE levels (Chung *et al.*, 2022).

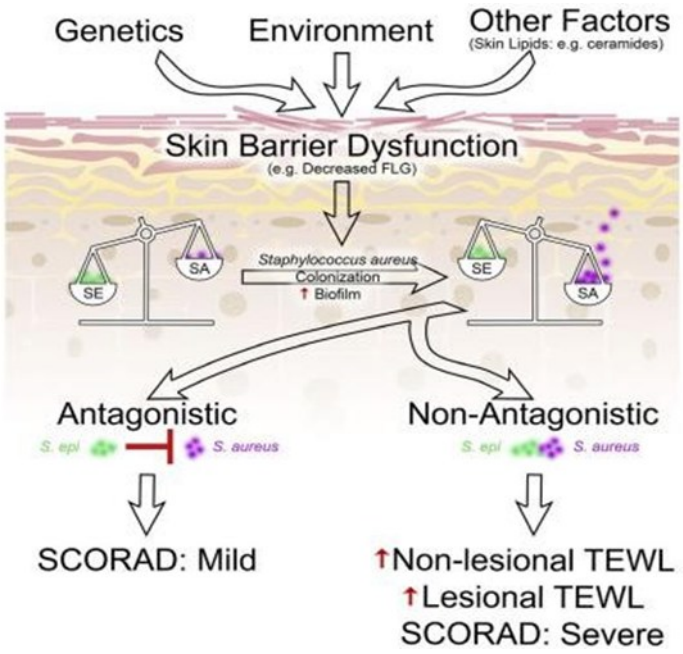

Figure 1: *A proposed paradigm that links clinical outcomes in AD, staphylococcal colonization and biofilm formation, and failure of the skin barrier (Gonzalez et al., 2021).*

Prior research has centered on understanding how the host's innate immune system identifies *S. aureus*, specifically by triggering the inflammasome via Toll-like receptors (TLRs), leading to the generation of cytokines like IL-1b (Askarian *et al.*, 2018). *S. aureus* strains have developed resistance to nearly all antimicrobial medications utilized for treatment, particularly demonstrating resilience against commonly employed drugs for gram-positive infections, including beta-lactams, glycopeptides, and oxazolidinones (Mlynarczyk-Bonikowska *et al.*, 2022).

Resistance to beta-lactams in MRSA strains and numerous MRS-CN is linked to the presence of transferable genomic islands (GI) known as SCCmec (staphylococcal chromosomal cassette mec) within the bacterial genome, housing the mec gene responsible for methicillin resistance; these islands exhibit rapid evolution and harbor various mobile genomic elements. Different SCCmec types may carry mecA or mecC genes and resistance genes against other antibiotic groups like aminoglycosides, macrolides, lincosamides, streptogramins B, and tetracyclines (MLS-B) (Mlynarczyk *et al.*, 2009). *S. aureus* expresses an array of secreted virulence factors, notably phenol-soluble modulins (PSMs) encompassing PMSa, PMSb, and d-toxin, contributing to epidermal barrier disruption, cytotoxic effects on diverse cells like keratinocytes, facilitation of biofilm development essential for *staphylococcal* colonization and persistence, and initiation of cutaneous inflammation upon epidermal colonization (Peschel and Otto, 2013; Nakagawa, 2017).

Biofilm-encased *S. aureus* cells exhibit altered traits compared to their planktonic form, displaying heightened antibiotic resistance, differences in cell size, growth behavior, gene expression, and protein synthesis (Otto, 2018). The primary constituent of the EPS in *S. aureus* biofilm, the polysaccharide intercellular adhesin (PIA) (Reffuveille *et al.*, 2017), functions in such an intercellular adhesion among bacterial cells, along with poly-β (1-6)-N-acetylglucosamine (PNAG), possesses a cationic nature owing to its chemical structure, which significantly contributes to colonization, the formation of biofilms, infections associated with biofilms, immune evasion, antimicrobial resistance, and defense against phagocytosis (Nguyen *et al.*, 2020). Additionally, certain studies indicated that 55% of individuals with AD consistently harbored *S. aureus* (Alsterholm *et al.*, 2017). Nevertheless, its contribution to the development of AD is not thoroughly comprehended.

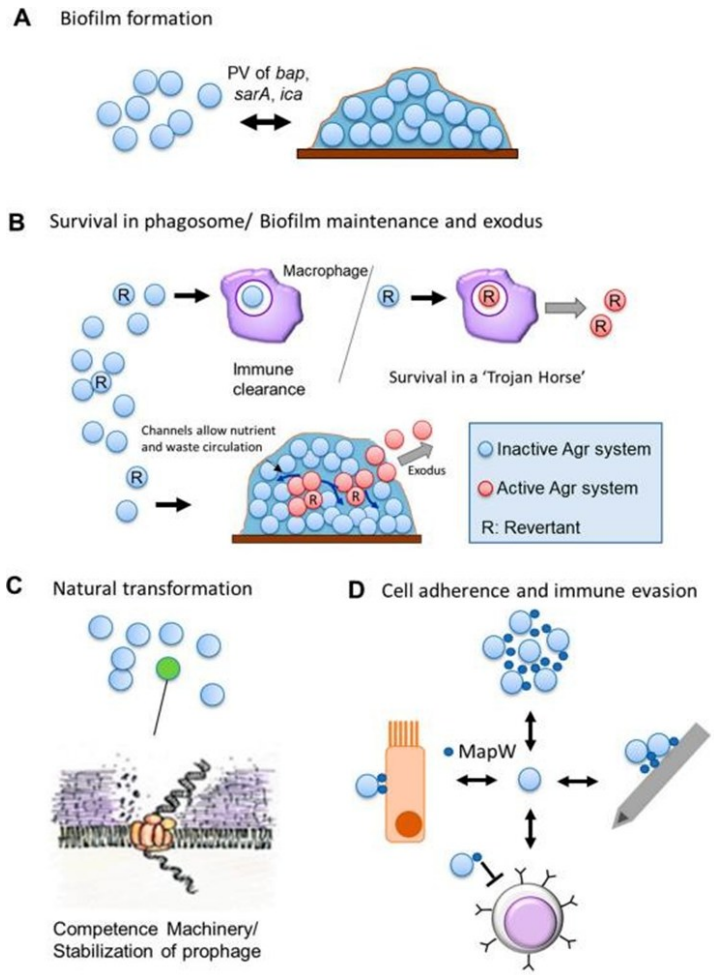

Figure 2: *Phase variation in S. aureus in AD patients (Gor et al., 2021). An animation illustrating the roles of known PV in S. aureus.*

The research gap mainly relies on the evolutionary genetic expression of the *S. aureus* that subsequently associates with atopic dermatitis. Ogonowska and Nakonieczna (2020) sought to identify the most reliable reference genes in *S. aureus* that remained stable despite undergoing aPDI treatment with the objective to ascertain suitable reference genes for studying the expression of the SEB gene. As of now, there have been no publications or studies released on this topic. This review aims to uncover and comprehend the specific gene expression profiles of *S. aureus* within Atopic Dermatitis conditions; to elucidate the underlying mechanisms *of S. aureus* pathogenesis in Atopic Dermatitis by analyzing the differential gene expression; potentially uncovering novel avenues for treatment or management of AD associated with *S. aureus* and to contribute insights into personalized treatment strategies by understanding the gene expression patterns.

Modulation of gene expression in *S. aureus*

The study by Ogonowska and Nakonieczna (2020) determined stable reference genes (fsZ, gmk) for *S. aureus* gene expression under green light photodynamic treatment and (fsZ, proC, fabD) under red light, suggesting fsZ as a universal reference gene which revealed significant downregulation of SEB, a virulence factor, after sublethal aPDI treatment, indicating potential modulation of *S. aureus* gene expression by photodynamic therapy, offering insights into therapeutic impacts on bacterial virulence. The accuracy of the chosen primers was confirmed in the study (Ogonowska and Nakonieczna, 2020) through real-time PCR, melting curve analysis, and electrophoresis on a 2% agarose gel (utilizing Mupid-One, Eurogentec, USA). The visualization of the gel was performed under UV light using a ChemiDoc Imaging System (Bio-Rad, USA).

S. aureus can adopt the small-colony variant (SCV) phenotype, evading host defenses, persisting within the host for extended periods, causing persistent infections despite antibiotic use, attributed to bacterial persisters surviving antibiotic treatments and leading to recurrent infections (Tuchscherr *et al.*, 2020). SCVs consistently display lower RNAIII levels, a product of the agrBDCA locus that regulates a quorum-sensing system and controls the expression of multiple virulence genes. The current understanding is that the genes responsible for Hla (hla) and TSST-1 (tst) are controlled by the accessory gene regulatory Agr (agr) operon (agrBDCA) through the action of RNAIII, as described by Novick *et al.* in 1993 and Proctor in 2006. Proctor, 2019 suggested that the downregulation or absence of Agr expression was found to be an enhancer of *staphylococcal* survival within the host for extended periods.

Tuchscherr *et al.* (2020) concluded that mutations in electron transport system genes, while linked to decreased virulence factor expression in SCVs, aren't consistently found in dynamic SCVs, suggesting other influences on the Agr system. The downregulation of RNAIII and virulence factors, governed by changes in gene regulation, is crucial. The Agr system plays a key role in *S. aureus*' adaptation for survival within host cells, immune evasion, and antimicrobial resistance. SCVs typically display reduced membrane potential, lowered virulence due to Agr changes, extended survival in host cells, high resistance to specific antimicrobials, and effective evasion of the host immune response. These combined traits pose challenges in treating chronic *staphylococcal* infections.

Schluepen *et al.* (2013) assessed the threshold of gene similarity necessary for precise identification by incorporating a set of closely related *S. aureus* genes. These genes encode conserved *staphylococcal* antigens known as Csa proteins, capable of triggering cross-protective immunity. To encompass diverse *staphylococcal* isolates, Haag *et al.* (2023) examined different versions of individual genes, found in five distinct *staphylococcal* strains representing various clonal complexes (Newman, USA400 MW2, USA300 FPR3757, and USA100 Mu50 and N315). Subsequently, Haag *et al.* (2023) developed specialized probes targeting conserved segments within each gene. These probes were intended for utilization within a high-throughput microfluidics qRT-PCR (HT- qRT-PCR) system. In this study, the qRT-PCR analysis was conducted employing Platinum SYBR Green qPCR SuperMix-UDG from Invitrogen-Life Technologies, with ROX serving as an internal control. This was executed on a STRATAGEN Mx3000P qPCR system, following specific cycling conditions: an initial step at 95°C for 10 minutes, succeeded by 45 cycles comprising 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. Subsequently, a final step involved maintaining temperatures at 95°C for 1 minute, 55°C for 30 seconds, and a concluding step at 95°C for 30 seconds. The quantification of bacterial RNA in individual samples was achieved by utilizing specific 16S rRNA primers (Sa_16s_+332_F and Sa_16s_+437_R) and establishing a correlation between each sample and a calibration curve (Bacconi *et al.*, 2017).

TaqMan qRT-PCR assays were developed targeting distinct and conserved segments within the target genes of various strains, namely Newman, FPR3757, Mu50, MW2, N315, and COL. These assays were designed to specifically detect and quantify RNA sequences from these strains using TaqMan technology (Haag *et al.*, 2023). The manu- facturer's instructions were followed to the letter for both the chip loading and qPCR pro- cesses. The procedure was to start at 50° C for two minutes, then increase the tempera- ture to 95°C for ten minutes, and then run 40 cycles of 95°C for 15 seconds and 60°C for one minute. Genex software was used to analyze the final data, using interpolation cali- bration with a control sample. The ΔΔCT method was utilized to determine the relative expression values compared to the reference culture after the expression levels of gyrB were employed as a reference to standardize the samples.

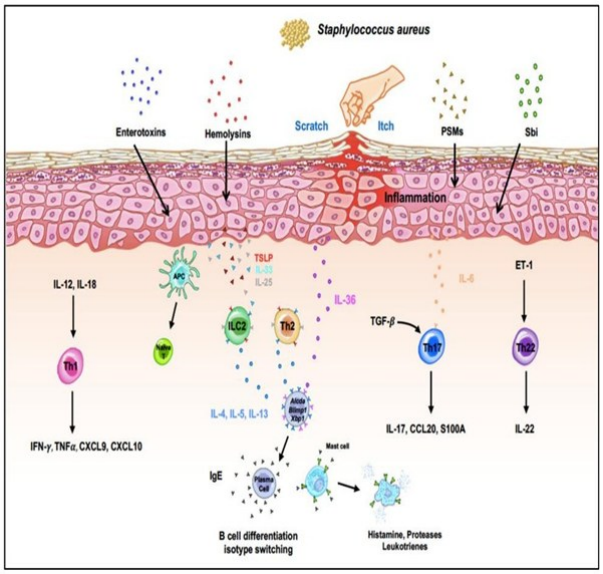

Figure 3: *Staphylococcus aureus triggers cytokine production, leading to diverse responses (Th1, Th2, Th17, and Th22) from keratinocytes and immune cells infiltrating the skin (Chung et al., 2022).*

Drug resistance of *S. aureus*

In several bacterial species, including *S. aureus*, the survival of bacteria has shown an inverse relationship with ATP levels. Bacteria that are metabolically active but not actively dividing were observed to be more vulnerable to antibiotics compared to actively dividing bacteria (Yamaguchi *et al.*, 2003; Wang *et al.*, 2018; Pu *et al.*, 2019). A genetic mutation in cold shock protein B (cspB) leads to the creation of a small-colony variant (SCV) with reduced pigmentation and heightened resistance to aminoglycosides and trimethoprim- sulfamethoxazole. However, this mutation results in increased vulnerability to daptomycin, teicoplanin, and methicillin (Duval *et al.*, 2010).

These bacteria are notable for swiftly propagating clones that exhibit heightened virulence and resistance to numerous antibiotics. Among the most significant occurrences fostering resistance in *S. aureus* is the emergence of methicillin-resistant strains, notably characterized by the incorporation of the mec cassette within the genome. The dissemination of strains harboring the Van operon, originating from enterococci, poses a substantial concern. While reports exist of MRSA containing the VanA operon in literature, fortunately, their spread has, thus far, been restricted.

Bioinformatics tools used in the genomic study of *S. aureus*

The Pfaffl model was used in this investigation because different PCR efficiencies of the target gene and reference genes were observed (Pfaffl, 2001). The expression of a target gene is represented as a ratio (R) in the Pfaf model, which may be defined using the following equation:

$$R = \frac{\left(E_{target}\right)^{\Delta Cp_{target}\left(control-sample\right)}}{\left(E_{ref}\right)^{\Delta Cp_{ref}\left(control-sample\right)}}$$

Statistical analysis utilized the GraphPad Prism 8 software (GraphPad Software, Inc., CA, USA), employing one-way analysis of variance (ANOVA) alongside Dunnett's multiple comparisons test. A significance threshold was set at $p < 0.05$ to identify notable differences within the data.

In Gonzalez's *et al.*, research, they used the Kruskal-Wallis's test to investigate differences in distribution among four levels of bacterial colonization concerning clinical outcomes (SCORAD and TEWL) and gene expression data. To delve deeper into potential distinctions, they conducted Wilcoxon rank-sum tests to investigate the relationship between two-level variables (such as *S. aureus* colonization and *S. aureus* biofilm propensity) and both clinical outcomes (SCORAD and TEWL) and gene expression.

Before conducting the analyses, the researchers assessed the distributional characteristics of the data. They found that SCORAD, TEWL, and gene expression data displayed skewed distributions, leading them to use nonparametric statistical methods. Categorical variables were described using frequencies, while continuous variables were summarized using the median and the interquartile range (IQR). Initially, they examined the relationship between a four-level bacterial colonization variable (group 1: positive for both *S. aureus* and *S. epidermidis*; group 2: positive for *S. aureus* but not *S. epidermidis*; group 3: positive for *S. epidermidis* but not *S. aureus*; and group 4: negative for both *S. aureus* and *S. epidermidis*) by employing contingency tables (focused on Black race and sex). Additionally, they used linear regression to explore associations with age.

The research utilized the Human Inflammation Array (MWG Biotech, Ebersberg, Germany) in their experiments, which includes validated oligonucleotide probes targeting 110 inflammatory genes, as previously detailed (Holzberg *et al.*, 2003). Breuer *et al.* (2005) employed microarray analysis to investigate the potential proinflammatory impacts of a toxin. The findings revealed that Interferon-gamma (IFN-g) was stimulated in both CD4+ T cells and peripheral blood mononuclear cells (PBMC). Notably, the up-regulation of IFN-g was significantly more pronounced compared to other genes, which showed only slight increases in expression when contrasted with the elevation seen in IFN-g. To validate these results, the induction of IFN-g was further confirmed at the messenger RNA (mRNA) level through quantitative real-time PCR (polymerase chain reaction).

In a study (Wang *et al.*, 2020), Prism software (version 8 by GraphPad Software, San Diego, CA, USA) facilitated linear regression analysis to assess gene expression across experimental groups. The RT-qPCR data underwent Student's t-test to identify significant differences between the groups, enabling a robust examination of gene expression levels in the study.

Challenges and future directions

Challenges involve the complexity of microbial communities on AD skin, necessitating precise isolation and analysis of *S. aureus* transcripts amidst diverse microbial RNA. Additionally, the dynamic nature of gene expression in *S. aureus* during AD exacerbations and remissions poses hurdles in capturing temporal changes accurately. Another challenge lies in integrating multi-omics data (transcriptomics, genomics, and metagenomics) to comprehend the interplay between host and microbe in AD pathogenesis comprehensively.

Future directions in this field involve refining sequencing techniques for single-cell or single-bacterium RNA sequencing to delineate *S. aureus* gene expression within AD lesions at a higher resolution. Advancements in machine learning algorithms tailored for microbiome data analysis will be pivotal in deciphering complex microbial interactions and predicting disease outcomes in AD. Furthermore, the development of standardized pipelines for data integration and interpretation across multi-omics platforms will enable a more holistic understanding of the intricate host-microbe interplay in AD.

Efforts to address these challenges and pursue these future directions will facilitate the elucidation of *S. aureus* gene expression in AD, offering insights into disease mechanisms and potential therapeutic targets for precision management strategies.

Abbreviations

AD – Atopic Dermatitis

DNA – Deoxy ribonucleic acid Th2 – T- helper 2

IL – Interleukin

T cells – Thymus cells

ILC2s – Innate lymphoid cells Th – T-helper

PSMs – Phenol-soluble modulins pH – Percentage of hydrogen

TSLP – Thymic stromal lymphopoietin IgE – Immunoglobulin E

TLRs – Toll like receptors IL-1b – Interleukin 1 beta

MRSA - Methicillin resistant Staphylococcus aureus

GI – Genomic islands

SCCmec – Staphylococcal chromosomal cassette mec MLS-B – Macrolides, lincosamides, streptogramins B

EPS – Extracellular polymeric substances PIA – Polysaccharide intercellular adhesin

PNAG – Poly-beta (1-6)-N-acetylglucosamine

aPDI treatment - antimicrobial photodynamic inactivation

proC – Protein C

fabD – Fatty acid biosynthesis PCR – Polymerase chain reaction SCV – Small colony variant

AgrBDCA -Accessory gene regulatory Hla – Human leukocyte antigen

TSST-1 – Toxic shock syndrome toxic Csa – Conserved staphylococcal antigens

qRT-PCR – Quantitative real-time polymerase chain reaction srrAB – Staphylococcal respiratory response regulator AB ATP – Adenosine triphosphate

SucA/B – Succinyl-AB

EV – Extracellular vesicle cspB – Cold shock protein B IFN- g – Interferon-gamma

PBMC – Peripheral blood mononuclear cells IFN-G – Interferon gamma

mRNA – Messenger ribonucleic acid

Conflict of Interest

The author declare no conflict of interest.

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