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A study on the efficacy of *Staphylococcus aureus* biofilm formation in atopic dermatitis severity

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Abstract--The chronic, recurrent, inflammatory skin condition known as atopic dermatitis (AD) is a significant contributor to the global burden of disease in terms of public health. *Staphylococcus aureus* and *Staphylococcus epidermidis* are two types of bacteria that frequently inhabit AD lesions. Recent research has shown that clinical isolates of *Staphylococcus* spp. that colonise AD skin are frequently biofilm-positive. The tendency of *Staphylococcus* spp. to form biofilms, which are colonies that attach themselves to surfaces and become highly resistant to antibiotics and immune responses, is an important characteristic of this genus of bacteria. The production of biofilms leads to the development of intricate bacterial communities, each of which has its own distinct impact on the keratinocytes and the immune system of the host. In this paper, we investigate the function that staphylococcal biofilms play in atopic dermatitis as well as the implications for treatment.

Keywords---atopic dermatitis, *staphylococcus aureus*, biofilms.

Introduction

Patients who suffer from atopic dermatitis (AD; often referred to as "atopieczema") have a higher likelihood of experiencing recurrent skin infections.(1-6) Even while beta-haemolytic streptococci may potentially be implicated, the most prevalent infecting organism is *staphylococcus aureus*.(7) In more severe situations, a cutaneous bacterial infection may develop abscesses, fever, and lymphadenopathy. This is especially the case when the infection is caused by methicillin-resistant *S. aureus* (MRSA).(8) The prevalent correlation

with a disease flare is a factor that can complicate the process of detecting infection in AD.(9)

It is possible for the symptoms of a flare-up of AD, such as increased erythema, oedema, papulation, seeping, and excoriation, to disguise or appear to be the symptoms of an infection. (10) The mechanisms that underlie bacterial infection in AD are multifaceted and include components from both the host and the bacterium. Scratching causes trauma that raises the risk of skin infection, which also contributes to the decreased skin barrier, anomalies in the cutaneous innate and adaptive immune systems, and the increased risk of skin infection. Bacterial virulence factors, such as super antigens, proteases, and cytolytic phenol soluble modulins (PSMs), which are secreted by *S. aureus*, may also contribute to bacterial persistence and/or epithelial penetration and infection. This is because bacterial virulence factors cause skin inflammation. (11)

Recent research has pointed to the possibility that biofilms play a significant part in the development of a number of dermatological illnesses, including AD. (12) It has been discovered that *S. aureus* biofilms infiltrate the eccrine ducts of AD skin. These biofilms have been shown to impact the release of keratinocyte cytokines as well as cause the differentiation and apoptosis of keratinocytes. These actions have the potential to interfere with the function of the barrier and to promote the development of disease as well as allergen sensitivity. The formation of biofilm is an effective technique that protects the bacteria from the environmental hazard, antibiotics, and phagocytosis, which enables prolonged persistence in the host. Staphylococcal biofilm communities are common on the skin of patients who have AD, and an increasing percentage of *S. aureus* skin isolates are resistant to standard treatments. (13) The colonisation of the skin by staphylococci has an effect on the function of the skin barrier and plays numerous essential functions in the pathogenesis of AD.(14). Because there has never been a study conducted in India that specifically focused on the bacterial colonisation of atopic skin, this research was deemed necessary in order to identify the *S. aureus* and biofilms that are present in eczematous lesions as well as healthy areas on the skin of patients who have atopic dermatitis. (15)

Materials and Methods

Sample Collection and Processing

For the purpose of conducting bacteriologic research, 75 samples were taken from patients with AD who were seen at the Dermatology department.(16) The prescribed sterile swabs that were made for travel were used to collect the samples. Patients ranged in age from 8 months to 75 years and included 47 males and 23 females. According to the presentation of the AD, samples were collected from lesions in various sites, such as the antecubitals in flexural AD and the face in facial-extensor AD. These lesions were clinically non-impetiginized at the time of sample collection. The preparation of the microbial cultures followed the standard protocols.(17) In addition to the specimens taken from individuals with lesions, we also obtained and processed samples from 25 control patients. These consisted of 13 samples taken from inflamed skin (pityriasis rosea and other conditions) and 12 samples taken from skin that was not inflamed and was not

atopic. The normal operating procedure was followed in the processing of skin scrapings and tissue biopsy specimens taken from areas of AD lesional and nonlesional skin.(18) After being stained with Gram's stain, the scrapings were looked at using bright-field microscopy.(19)

Identification and Speciation of Isolates

Using the Staphaurex test kit, the isolates, which would often be dismissed as "normal flora," were broadly identified as coagulase-negative staphylococci.(20) This classification was based on the staphylococci's resistance to coagulase (Thermo Fisher Scientific). Inoculations were made on Mannitol Salt Agar plates, and the API Staph (bioMerieux SA) phenotypic system was utilised in order to perform additional identification and speciation procedures. The API system was utilised in accordance with the directions provided by the manufacturer and in the manner that was detailed elsewhere. Genotyping the isolates with a well-established technology called colony-direct species-specific polymerase chain reaction revealed that they belonged to the genus *Staphylococcus* as well.

Antibiotic Susceptibility Testing

Following the procedure provided by the manufacturer, the plate assay was used to determine the minimum inhibitory concentrations of the isolates that were tested for antimicrobial resistance. The Clinical and Laboratory Standards Institute was consulted in order to determine how to categorise the values for the minimal inhibitory concentration. From these pieces of information, an antibiogram was constructed.

Detection of biofilm production

For the purpose of determining whether or not the collected bacterial isolates were capable of producing biofilm, two separate tests were carried out on the isolates:

Tube method

After that, loopfuls of bacteria were poured into trypticase soy broth that had been added with 1 percent glucose (TSBglu), and the mixture was then cultured at 37 degrees Celsius for 24 hours. This is how the bacterial isolates were obtained. After decanting the contents of the tubes and rinsing them with PBS, the tubes were then dried (pH 7.3). Crystal violet was utilised so that the dried tubes could not be identified (0.1 percent). The surplus pigment was eliminated, and the tubes were washed with water that had been deionized before being sterilised. After that, the tubes were turned upside down to dry while being observed for the development of biofilm. It was determined that the production of a biofilm had been successful if there was a film that could be seen on the inside of the tube as well as on the bottom. The formation of rings at the interface between liquid and solid did not provide any evidence that biofilm was being formed. According to the magnitude of the colour that was produced, they were given the labels of moderately positive and strongly positive, respectively. We used

a strain that has already been demonstrated to produce biofilm in the laboratory so that we could use it as a positive control.

Biofilm formation in 96-well microtiter plates

In order to analyse the formation of biofilm, the semi-quantitative approach of detecting the amount of biofilm that formed in 96-well flat bottom plates was used. As was said previously, this method was used to conduct the research. To summarise, fresh bacterial suspensions were produced in either TSB or LB from overnight cultures and adjusted to an OD₆₀₀ of 0.1 (more than 10⁷ CFU/mL). These suspensions were then used to test the viability of the bacteria. These suspensions were produced by the microorganisms and can be found here. Following that, individual wells of a 96-well polystyrene plate with a flat bottom were inoculated with 100 μ L aliquots of bacterial suspension, and the plate was subsequently placed in an incubator at 37 °C for a period of 48 hours. After an overnight incubation, the plates were carefully cleaned with 1X phosphate buffered saline (PBS; pH 7.4) and then dyed with 100 μ L of 0.1 percent Crystal Violet (Sigma-Aldrich, St. Louis, MO) for 30 minutes while the plates were kept at room temperature. After the dyeing process, the plates were stored at room temperature. After the crystal violet was solubilized in ethanol at a concentration of 95 percent, the excess crystal violet was removed by washing, and the amount of biofilm was determined by measuring the corresponding optical density (OD)_{570 nm} of the supernatant. This was done after the crystal violet had been solubilized in ethanol. Following the completion of biofilm studies that were carried out in triplicate for each clinical strain that was evaluated, the average biofilm absorbance value was determined using the data obtained. These strains were considered successful at biofilm formation if they produced biofilms with an optical density at 570 nanometers (OD₅₇₀) that was higher than the one produced by the positive control. On the other hand, those strains whose values were lower than the control were regarded as being strains that did not generate a significant amount of biofilm.

Antibiotic Susceptibility Testing

Plate assays were carried out in accordance with the protocol that was supplied by the manufacturer in order to calculate the minimum inhibitory concentrations of the isolates that were put through antimicrobial resistance testing. In order to decide how to classify the different values for the minimal inhibitory concentration, the Clinical and Laboratory Standards Institute was consulted. An antibiogram was built using the pieces of information provided here.

Statistical Analysis

Chi-Square test and ANOVA test were carried by using computer program, SPSS. ver.11.

Results

Staphylococci were plentiful in virtually pure cultures when produced on blood agar plates, and the API Staph identification system revealed that 75 percent of

the patients were in fact staphylococci. The isolates were found to be staphylococci after they were subjected to standard Gram staining and biochemical testing. 70 percent of them were identified as staphylococci in the same way. The Gram staining of all one hundred percent of the testedlesional skin samples revealed staphylococci both free and entangled in the tissue, with many organisms being confined within biofilms. Gram staining revealed no evidence of biofilms in any of the 25 control samples taken from skin that was free of lesions. According to the results of the speciation study, the most common species were *Staphylococcus aureus* (75.0% of the total) and *Staphylococcus epidermidis* (17.0% of the total). Other staphylococcal species that are normally present in the flora of normal skin have been identified. The results obtained with the Staphaurex kit, which was used to identify the coagulase-negative and coagulase-positive isolates, matched the species identification one hundred and ten percent of the time. *Staphylococcus aureus* was detected in 23.0 percent of the control samples, while *Staphylococcus epidermidis* was found in 27.0 percent of the control samples. The remaining samples exhibited additional staphylococci that were comparable to those found in the lesional skin. We carried out minimum inhibitory concentration tests in order to ascertain whether or not the isolates were susceptible to antibiotics. According to the findings, the majority of the isolates displayed resistance to multiple drugs. Antibiotics that have the highest level of percentage of isolates that showed resistance were erythromycin (90 %), clindamycin (67.0%), and levofloxacin (45%). Tigecycline was the most effective among the antibiotics tested, with only 32 % of the isolates resistant to it. Methicillin resistance was observed in 24 of 40 isolates (30.0%) and in 7 of 20 control samples (35.0%). Biofilm formation was detected using the crystal assay. The isolates were classified according to the literature as strong, moderate, or weak biofilm producers. The results indicated that 95.0% of the isolates were strong biofilm producers, which included both *S aureus* (98.0% of the isolates) and *S epidermidis* (95.0% of the isolates).

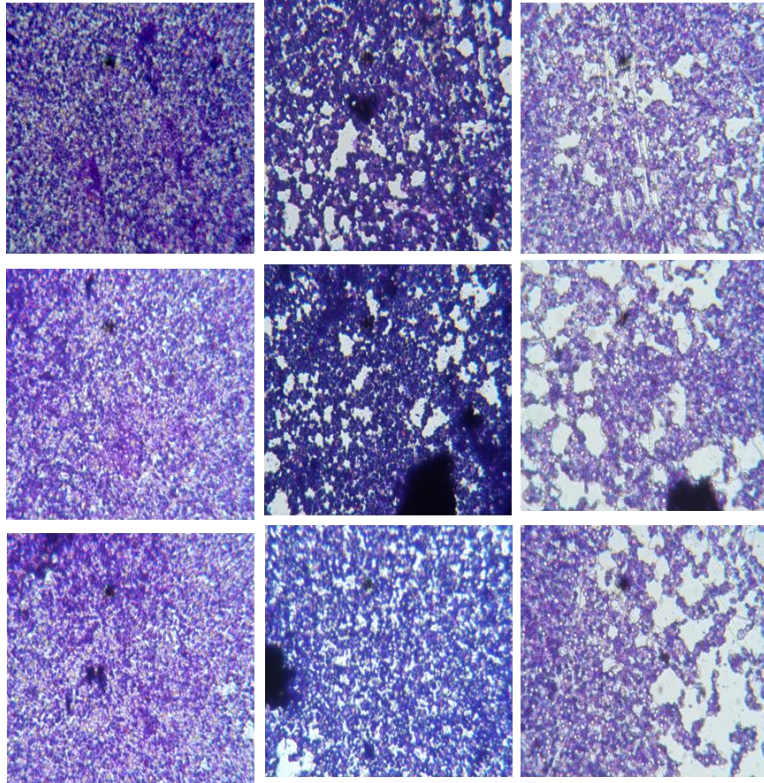


Figure 1. Biofilms formed by *S. aureus*

Phenotypic testing with Congo red agar revealed that all of the staphylococci isolates were either moderately or significantly positive for extracellular polysaccharide (biomass). Samples that displayed a less intense staining using the CV assay were found to be positive using either the polymerase chain reaction or the Congo red agar test, or both. The organisms were found in skin that had active lesions as well as skin that had lesions that had disappeared. Only the skin that had lesions was shown to have biofilms.

Conclusion

The findings of this investigation might point to the existence of a connection between the formation of biofilm by *S. aureus* strains that have colonised the anterior nares and the progression of atopic dermatitis. It would appear that the formation of biofilm is essential for the pathogen's ability to colonise broad portions of the skin and to do so persistently. Destruction of the biofilm produced by *S. aureus* could have a beneficial effect on the progression of atopic dermatitis.

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