*Staphylococcus aureus* (*S. aureus*) is one of the most common human pathogens. *S. aureus* is responsible for many diseases, such as skin and soft tissue infections, endocarditis, osteomyelitis, and bacteremia. It is estimated that about 30% of the world's population are carriers of this species, mainly in the nasal mucosa. In some groups of people, such as those suffering from atopic dermatitis (AD), *S. aureus* has been detected on the skin of up to 90% of those tested. Atopic dermatitis is an extremely troublesome disease that primarily affects children (5-20% worldwide) and, less commonly, adults (1-3% of the world's population). The disease severely limits the quality of life of patients and their families due to its clinical manifestations and symptoms. AD is a chronic, relapsing, and inflammatory disease characterized by pruritus. The disease results from the interaction of genetic and environmental factors. Treatment with topical corticosteroids is the first-line therapy for AD. Treatment also includes calcineurin inhibitors, ultraviolet light phototherapy (not recommended in children), and, if secondary bacterial infections occur, antibiotics. The problem, however, is that bacteria become resistant to antibiotics very easily, so dermatologists do not recommend the use of antibiotics in the treatment of infected atopic skin.

Another problem in the elimination of *S. aureus* is that it can penetrate human cells, e.g., skin cells such as keratinocytes. Antibiotics do not penetrate human cells effectively, so intracellular bacteria are difficult to kill. In addition, once *S. aureus* enters a human cell, it undergoes a type of transformation. This transformation involves a decrease in metabolism and a change in the production of virulence factors. For this reason, methods are being sought that can complement and, in some cases, even replace the action of antibiotics.

In the project presented here, we will address the problem of fighting intracellular *S. aureus* by photodynamic inactivation of bacteria (PDI). In the first step of our work, we will focus on the analysis of the process of intracellular persistence of *S. aureus* in keratinocytes. We will analyze a large population of bacteria isolated from patients with AD. We will investigate how they interact with skin cells and to what extent virulence factors (enterotoxins) are involved in this process. In the next step, we will develop a method to kill the intracellular staphylococci and inactivate the virulence factors they produce. For this purpose, we will use PDI. PDI involves the use of compounds activated by light and visible light, whose combined action in the presence of oxygen leads to the production of cytotoxic reactive oxygen species responsible for the destruction of important bacterial biomolecules. Unlike UV phototherapy, which does not penetrate deep into the skin and has mutagenic properties, PDI uses light from the visible spectrum. It is safe for human tissues and penetrates the deeper layers of the skin. In our research to date, we have shown that we can use FIB not only to kill the bacterial cells themselves, but also to destroy the virulence factors produced by the bacteria. Importantly, this can be done without affecting human tissue.

In the Laboratory of Photobiology and Molecular Diagnostics, we have developed a model of infected skin cells that will allow us to follow the fate of *S. aureus* under conditions similar to infected atopic human skin. To track the fate of bacteria in such a complex system, we will use immunofluorescence methods in which bacterial cells can be readily identified using fluorescent probes. We will also track the growth and proliferation dynamics of the infected keratinocytes in real time. In our collection, thanks to collaboration with physicians from Gdansk Medical University, we have collected numerous clinical isolates from patients with AD, which we will analyze using this approach. Based on the results, we will be able to answer the question of whether there are staphylococci that penetrate skin cells more effectively than others and whether they can be efficiently inactivated by using PDI.

The research results from this project will contribute on the one hand to the understanding of important aspects of the interaction of staphylococci with host cells and on the other hand to the development of a method that can control atopic skin infections. The application of the PDI method, which is also effective against multidrug-resistant bacteria, including MRSA, will make it possible to reduce the use of antibiotics and, in the long term, help to slow down the growing trend of microbial resistance.

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