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Abstract of the doctoral dissertation titled:

"Mechanisms of *Staphylococcus aureus* adaptation to oxidative stress induced with antimicrobial photoinactivation".

Staphylococcus aureus is a Gram-positive, round-shaped bacterium, colonizing the nasopharyngeal cavity and human skin, and due to the ability to produce a number of virulence factors, including enzymes and toxins, it is a clinically significant etiological agent of many infections. *S. aureus*, especially methicillin-resistant strains (MRSA), can cause a variety of diseases such as skin, soft tissue, and systemic infections, food poisoning, and toxic shock syndrome. MRSA strains are characterized by multi-drug resistance due to the acquisition of a number of drug resistance mechanisms, which makes *S. aureus* hospital-acquired infections extremely difficult to treat. Considering the increasing drug resistance and the lack of new drugs, there is an urgent need for new, alternative therapy against multi-drug resistant pathogens. One alternative is the antimicrobial photodynamic inactivation (aPDI), which is an innovative method for combating microbes, based on the use of a chemical compound called photosensitizer (PS), visible light of the appropriate wavelength and molecular oxygen. The photodynamic reaction may occur in accordance with two mechanisms that depend on the oxygen concentration in the environment and PS structure. In both mechanisms, the photon is absorbed through the PS molecule, which leads to its excitation. The excited photosensitizer reacts with biomolecules such as proteins, nucleic acids or membrane lipids, which then react with molecular oxygen, forming oxygen radicals and other reactive oxygen species (mechanism I) or transfer energy directly to molecular oxygen, resulting in the formation of highly reactive singlet oxygen (mechanism II). Reactive oxygen species can cause DNA damage, inactivate proteins, and cause lipid peroxidation of cell membranes that can lead to bacterial cell death. Antimicrobial blue light therapy (aBL), unlike aPDI, is not based on the use of the exogenous photosensitizers, but endogenous photosensitizing compounds, e.g. intracellular porphyrins. Our preliminary research has shown that the microbial response to photoinactivation is strain-dependent and it leads to damage to genetic material in still living microbial cells, which can prompt the microbial adaptation to light-induced oxidative stress. Therefore, one of the goals of this thesis was to identify genetic markers of *S. aureus*' response to photoinactivation. In all experiments, a total of 750 strains were used, isolated between 2002-2012 at the Provincial Hospital in Gdańsk and at the Provincial Hospital in Koszalin. Depending on the response of the strains to aPDI,

S. aureus isolates were classified as aPDI susceptible, intermediate sensitive, or aPDI tolerant. When comparing the response to aPDI in the MRSA and MSSA populations, the percentage distribution of strains characterized by a higher tolerance to aPDI was higher in the MRSA population, which allowed concluding that MRSA strains are less sensitive to this type of therapy. In order to determine whether the observed differences in response to aPDI between MRSA and MSSA populations result from differences in level and drug resistance mechanisms of these strains, antimicrobial susceptibility testing was performed including routinely used antibiotics, and identification of drug resistance mechanisms was conducted. Obtained results revealed no correlation between the level and mechanism of drug resistance and the response to aPDI, which suggests that other mechanisms than those associated with drug resistance may determine the observed differences between MRSA and MSSA population. Next, MRSA strains were characterized in terms of epidemiological features like *agr* group, SCC*mec* cassette type, clonal complex, and *spa* type. The characterization performed allowed to observe that the strains lacking the functional *agr* operon display increased sensitivity to photodynamic inactivation. In addition, classifying *S. aureus* into one of the four major polymorphic groups of the *agr* operon revealed that there were significant differences in the response to aPDI between the *S. aureus* from different *agr* groups, while the presence of the different SCC*mec* cassette did not affect the response to aPDI. Additionally, grouping isolates into clonal complexes and *spa* types have enabled the identification of *S. aureus* lines sensitive to photodynamic inactivation. Studies have revealed that a specific genetic background may be a predisposing factor to increased tolerance to photodynamic inactivation.

The second goal of the current thesis was to determine the probability of development of *S. aureus* tolerance to photodynamic inactivation (aPDI/aBL) and to determine the mechanism of the observed adaptation to oxidative stress induced by photoinactivation. It has been investigated whether multiple treatments of *S. aureus* cultures with sub-lethal doses of aPDI or aBL can lead to the development of tolerance to both therapies. The obtained results show that the sub-lethal treatment of aPDI and aBL led to the development of tolerance in *S. aureus*, however, the observed phenomenon did not translate into other types of the photosensitizers. The observed tolerance is characterized by phenotypic stability, which suggests that it is the result of genetic changes. In addition, an increased frequency of mutations leading to rifampicin resistance and increased expression of the gene coding for error prone polymerase (*umuC*) have been observed, suggesting a possible mechanism for the development of tolerance. Additionally, in microbial cultures repeatedly treated with sub-

lethal doses of aBL, increased susceptibility to gentamicin and doxycycline were observed, which confirms the assumption that the observed phenomenon of tolerance results from the genetic changes.