Abstract

In clinical practice, infections caused by persistent bacteria have become a worldwide problem. We are talking about a subpopulation of cells that are able to withstand lethal doses of antibiotics and after their elimination are capable of resuscitation and re-induction of the disease. The opportunistic pathogen *Staphylococcus aureus* is one of this bacteria and causes various serious chronic infections. During the long-term persistence in patients, persistent bacteria acquire adaptive mutations.

The aim of this diploma thesis was to monitor the degree of persistence in selected clinical isolates, and at the same time to demonstrate the effect of adaptive mutations on the degree of persistence as well as to molecularly characterize the persistent state by gene expression. I had chronological isolates of *S. aureus* at my disposal, the initial one being the primoisolate, an isolate taken at the diagnostics of cystic fibrosis before the start of antibiotic treatment. Another was taken at a distance of one year and the last with a half-year interval from the previous one. Following whole genome sequencing, genes in which adaptive mutations occurred were identified.

The first method determines the degree of persistence by calculating CFU (Colony Forming Units) after antibiotic treatment. I found that this ability depends mainly on the adaptation of the isolate and the adaptation mutations obtained. I have determined that a mutation in the gene *sigB* may increase the rate of persistence. By flow cytometry and dual dying with $DiOC_2(3)$ and TO-PRO-3, I determined the subpopulation of cells with membrane potential, subpopulation of cells without potential and dead cells. The division into subpopulations was different for the selected antibiotics. The loss of membrane potential after ciprofloxacin was not as severe as after oxacillin and vancomycin treatment. Next, I monitored the redox potential of the cells with fluorescent dyes CTC and DAPI and found that the cells lost their redox potential over time after treatment with all three antibiotics. I determined the expression of genes that are associated with persistence (*sigB, mazEF, agrA, rnaIII*). It has been shown that the expression of the genes *agrA* and RNAIII has been suppressed in most cases, which correlates with the fact that these genes also have lower activity during chronic infection.

To create a deletion of the *agrA* gene, I created a construct using the CRISPR - Cas9 system to modify the *S. aureus* genome. However, I was unable to transform the plasmid into clinical isolates and isolate the *agrA* gene deletion mutant.

Key words: *Staphylococcus aureus*, persistence, Agr system, SigB, adaptive mutations, antibiotic treatment