Abstract

Staphylococcus aureus is a opportunistic pathogen that can cause severe and chronic infections. The reason of the infections relapse is often the persistence. It is about adapting to stressful conditions by inducing a dormant state, which would allow bacteria to survive exposure to antibiotics and grow again after their elimination. Bacteria that persist in the patient acquire various adaptive mutations, which are transmited creating subpopulations that have a better ability to persist.

The aim of this diploma thesis was to compare individual methods of persistent study that could be used in clinical practice in the future, and at the same time to try a closer molecular characterization of the persistent state with using methods for calculating gene expression. I had chronological isolates of *Staphylococcus 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 three-quarters of a 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 confirmed that the major mutations that lead to increased persistence are mutations in *agr* genes. I also found other mutations leading to increased persistence, especially in the *hyp* gene, which encodes an unknown protein that has not been linked to persistence yet. By flow cytometry using fluorescent labels DiOC2 (3) and TO-PRO-3, I determined the proportion of cells with membrane potential, the proportion 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. By continuous cultivation in a microtiter plate with regular measurement of optical density, I observed the growth from a persistent state. This growth was slower than normal growth without the antibiotic and there was a prolongation of the lag phase, especially after vancomycin. The results also showed that the density of the bacterial suspension had a significant effect on the growth from persistence.

I determined the expression of genes in which the adapted isolates have the acquired mutation (*hyp*, *agrA*, *glmU*, *rnaIII*). The results show that the *hyp* and *glmU* genes evince higher expression in the longest harvested isolate after all antibiotics. Furthermore, I detected low expression of *agrA* and *rnaIII* genes, which have previously been shown to have lower activity in chronic infections.

Keywords: persistence, antibiotic treatment, *agr* operon, adaptive mutations, *Staphylococcus aureus*