„*Escherichia coli* and *Klebsiella pneumoniae* persisters formed under stressful conditions"

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The problem of antibiotic resistance is one of the most serious threats to public health. Many studies indicate that the main cause, apart from antibiotic resistance, is persistent bacteria that tolerate high concentrations of antibiotics. Persistence is a phenotypic variant usually exhibited by a small number of bacteria that often are non- or slow-growing. Persisters, unlike typical antibiotic-resistant bacteria, do not divide in the presence of antibiotics and are genetically identical to antibiotic-sensitive cells. Persisters can cause recurrent infections because they can resume growth after antibiotic treatment. They usually represent a small percentage of the population; therefore, they are difficult to detect. Over time, persisters can enter the VBNC (Viable But Non-Culturable) state, which is characteristic of cells that are unable to divide in standard microbiological/culture media. Persisters and VBNC can emerge stochastically in the population, but their formation can also be induced by a number of stress factors (e.g., starvation, oxidative stress, and pH change). Literature data indicate that aggregation of endogenous proteins can slow down metabolism and allow bacteria to enter a persistent state. It is also known that the formation of aggregates can result from water loss in the bacterial cell and protein glycation.

The aim of this work was to determine whether protein aggregates formed during *Escherichia coli* desiccation and rehydration contain glycated proteins, and whether in *Klebsiella pneumoniae* cultures, similar to *E. coli*, there is a correlation between protein aggregation and the appearance of persisters. In the case of *K. pneumoniae*, most experiments were conducted using macrocolonies, a form of biofilm on a solid media exposed to gradual water loss. It turned out that such conditions were particularly contributive to the formation of persister bacteria. The results obtained in this work also showed that during *E. coli* desiccation/rehydration, most glycation products remained in the soluble fraction and were not trapped in the aggregates. Analysis of the level of aggregates and glycated proteins in *E. coli* subpopulations with different content of live-dividing, VBNC and dead cells allowed us to conclude that protein aggregates might play a protective role during stress. As expected, in the case of *E. coli*, protein aggregation during desiccation and rehydration was associated with an increase in the level of persistent bacteria. Due to difficulties in isolating protein aggregates from *K. pneumoniae*, I focused on other aspects related to persister bacteria and the diversity of *K. pneumoniae* macrocolonies. Several clinical isolates formed two subpopulations in macrocolonies: mucoid center and non-mucoid ring. The center subpopulation was characterized by a higher level of persister, VBNC, and colistin-heteroresistant cells and produced an increased level of glycation products compared to the ring subpopulation. In addition, proteome analysis by SWATH-mass spectrometry revealed that the center

contains reduced levels of ribosomal proteins and a higher level of the ribosome hibernation-promoting factor (YhbH/Hpf), which might be responsible for the formation of antibiotic-tolerant bacteria.

Due to the fact that persisters are the cause of antibiotic therapy failure, in recent years increased number of studies have focused on the search for compounds with *anti-persister* properties. As part of my work, I initially examined the effect of sulforaphane and the deep eutectic solvent (reline) on the formation and eradication of persister bacteria. Depending on the *K. pneumoniae* isolates, the concentration of sulforaphane or reline used and the type of sample used (whole bacterial cultures or isolated persister bacteria), different results were obtained. Both of these compounds were effective in killing persister bacteria isolated from *K. pneumoniae* macrocolonies after incubation with antibiotic (meropenem). However, adding sulforaphane or reline to the culture together with meropenem partly or entirely abolished the effect of the antibiotic, causing an increase in the level of persistent bacteria.

The presented results expand our knowledge of the mechanisms that protect bacteria from stress and determine various forms of tolerance or resistance to antibiotics. In the future, they can be used to develop new strategies to eradicate persistent bacteria.