

ARE subfamily proteins belonging to ABC transporters confers a different degree of resistance to macrolides, linkosamides and streptogramins antibiotics. Among the most clinically ARE subfamily proteins in staphylococci is Vga(A) protein lead to the award resistance to streptogramins A. In 2006, discovered the new variant called the Vga(A)_{LC}, which in addition to streptogramins A resistance also confers linkosamides. Vga(A) and Vga(A)_{LC} differ in only 7 amino acids, yet confer different resistance phenotypes. In previous experiments it was found that the central role in determining substrate specificity play a 4 amino acid differences that accumulate in the section of 15 amino acids within the linker connecting the two ABC domains (positions 212, 219, 220 and 226). The combination of amino acids LGAG Vga(A) increases resistance to streptogramins A while present in combination SVTS Vga(A)_{LC} increased resistance to linkosamides. Although in this subfamily includes a large number of resistance proteins, the mechanism of resistance has not yet been established with certainty.

The aim was to create a new Vga(A) variants that contain specific combinations of amino acids for Vga(A) and Vga(A)_{LC} protein at positions 212, 219, 220 and 226 and compared their ability to grant resistance to linkosamides. We also studied the effect of mutations in the same variable linker, which occur in the new versions vga (A) whose sequences are available in the GenBank database. Of the 14 possible combinations of amino acids specific for Vga(A) and Vga(A)_{LC}, we managed to prepare 8 of which 6 (SGTG, SVTG, LGTS, SGTS, SVAS and LVTS) gave a similar resistance as Vga(A)_{LC}. Mutated proteins carrying these combinations of amino acids promulgated higher resistance to linkosamides than streptogramins A. In addition, only one mutation K218T in protein Vga(A) was responsible for the phenotype change of Vga(A) to Vga(A)_{LC}. It seems, therefore, that Vga(A) proteins generally prefer to lincosamides before streptogramins, which should be taken into account when assessing resistance in clinical practice. As part of this thesis was also preparing fusion Vga(A) proteins, which would allow immunodetection Vga(A) in cell fractions. Incorporation of FLAG or HIS anchors, however, is likely to inactivate the Vga(A) protein.