thermal stability of the enzyme. Later it was confirmed experimentally that the mutant is characterized by a T_m value higher by 1.6 °C (Brown et al. 2010). The effect of the V84I mutation on increasing the kinetic stability of β-lactamase TEM-171 has also been demonstrated recently (Grigorenko et al. 2018a). This mutation is rarely found in β-lactamases from clinical strains.

The Q39K substitution has long been thought to be an example of a neutral secondary mutation in TEM-type β-lactamases. It is located on the surface of a protein globule on H1 α-helix and is quite frequently observed in different β-lactamase phenotypes both as a single mutation and in combination with the key substitutions as well. It was shown to increase slightly the minimal inhibitory concentration (MIC) for cephaloridine, ceftazidime, and aztreonam (Blazquez et al. 1995). It was recently shown that a combination of Q39K with the key substitutions related to ESBL (E104K, R164S) and IRT (M69V) phenotypes results in decreased K_M and k_{cat} values, and a thermal stability of mutants with Q39K substitution was also reduced (Grigorenko et al. 2018b). In this case, the weakening of the protein structure occurs due to the exposure of hydrophilic side chains to the solvent. Thus, depending on the location of the mutations, the type of amino acid substitution, and combination with other mutations, the mechanisms of their influence can be oppositely directed.

6.5 Conclusions

The resistance of pathogens to β-lactam antibiotics is a unique mechanism of bacterial biological protection against foreign toxins due to production of enzymes – β-lactamases. The peculiarity of serine β-lactamases of molecular class A is their universal three-domain structure. Each domain has its own conservative fold, consisting of α-helices or β-strands connected by flexible loops. The domains are structured by a network of ionic and hydrogen bonds, and the active site is located in a compact core scaffold. TEM-type β-lactamases isolated from clinical strains are characterized by high mutability. Substitutions of amino acid residues occur mainly in the loops. Key mutations provide a broad hydrolytic specificity of β-lactamases to different groups of β-lactam antibiotics. They contribute to an increase in active site size and improve its availability for antibiotics with bulky substituents like the third- and fourth-generation cephalosporins. On the other hand, these changes lead to a decrease in the stability of the mutant enzyme. Secondary mutations occur, as a rule, in peripheral regions of the protein globule and may regulate the protein structure. That is why some of them (for example, M182T mutation) with increasing β-lactamase stability are called "the global suppressors." Combinations of different mutations increase the plasticity of protein structure and keep the activity–stability compromise.