Key mutations alter the activity and substrate specificity of enzymes; they constitute no more than 10% of the total number of mutating residues. Enzymes with mutations of residues E104, R164, A237, G238, and E240 correspond to the phenotype 2be; enzymes with mutations of residues M69, S130, R244, R275, and N276 correspond to the phenotype 2br; enzymes with a combination of mutations of these groups of residues correspond to the phenotype 2ber.

All residues, which mutations are the key, are located near the catalytic site in the tertiary structure of the protein. Their location in the secondary structure of the protein is shown in Figure 6.1. The residues, whose mutations lead to the expansion of the substrate specificity (2be), are located in functionally significant loops (E104, R164, E240, and G238) and on S3 β-strand (A237). The residues, whose mutations affect the resistance to β-lactam inhibitors (2br), are located on conservative elements with a regular structure: on H2 and H11 α-helices (M69, R275, N276) and on S4 β-strand (R244). The residue S130 is located in the SDN loop. Key mutations lead, as a rule, to destabilization of the protein globule.

Most of the residues whose mutations are attributed to the secondary ones are located in loops far from the active site (Abriata et al. 2012). The role of most secondary mutations remains unclear. For some of them, it has been established that they can compensate the destabilizing effects of the key mutations (see Section 6.4).

6.3 Effect of the Key Mutations on Activity of TEM-Type β-Lactamases

To date, 84 TEM-type β-lactamases isolated from clinical bacterial strains have an ESBL phenotype (2be) and contain from one to four key mutations. The analysis of the distribution of combinations of these mutations in TEM-type β-lactamases was carried out relying on information from http://www.lahey.org/studies as of 1 November 2018. Figure 6.4 shows the classification of mutated residues of TEM-type β-lactamases into the key and secondary and their localization in the secondary structure and the frequency of the key mutations occurred in single and double combinations, as well as in multiple combinations with the other key and secondary mutations. Figure 6.5 presents the data on the frequency of occurrence of the key single mutations and their combinations in the enzymes with different phenotypes. To analyze the effect of combinations of mutations on the catalytic properties of β-lactamases, a parameter of catalytic efficiency (k_{cat}/K_M) was used as it is more consistent and less dependent on experimental conditions (Palzkill 2018) (Figure 6.6).