side chains, is 10 times lower. R164 has the highest variability of substitutions, most often changing with serine or histidine and more rarely with cysteine (Figure 6.5). These mutants differ in the efficiency of hydrolysis of various cephalosporins.

The residue G238 is replaced by serine only in six enzymes. It is located in the loop 238–243 near S3 β-strand, and the substitution leads to significant displacement of the loop, which results in the widening of the active site and promotes an accommodation of third-generation cephalosporins with larger oxyimino side chains (Orencia et al. 2001). G238S mutant is active toward both cefotaxime and ceftazidime; the value of k_{cat}/K_M increases by 2–3 orders of magnitude (Figure 6.6). Catalytic efficiency toward penicillins becomes 5–10-fold less.

The mutation E104K was found as a single substitution in six β-lactamases. The residue 104 is located in the 101-loop, near the catalytically important H2 α-helix, on which catalytic S70 is located. As a result of the replacement, the residue 104 changes its orientation, and the volume of the enzyme active site expands. When the oxyimino cephalosporins bind, a new ionic bond is formed between the negatively charged carboxylic group of antibiotic and positive charge of K104. This leads to an increase in k_{cat}/K_M values for ceftazidime by 2–3 orders of magnitude (Palzkill 2018). Effect of E104K on hydrolysis of penicillins and first-generation cephalosporins is slightly negative.

The substitution E240K is rarely found as a single mutation ($n = 1$), but it is often found in combinations with other mutations (Figures 6.4 and 6.5). The residue is located in the loop 238–242, near the catalytically significant S3 β-strand. The effect of this substitution consists in the formation of an additional electrostatic bond between K240 and side chain of the cephalosporins (Knox 1995).

The residue A237 precedes the key residues G238 and E240 on the S3 β-strand, which forms a wall of the active site. There is no structural information available on a mutant with a replacement of alanine with threonine. It is assumed that the substitution A237T improves the interaction of the residue side chain with a carbonyl group of the antibiotic molecule, which improves coordination of cephalosporins and enhances slightly cefotaxime hydrolysis (1.3-fold increase in k_{cat}/K_M) (Cantu et al. 1997). In addition, A237G has been shown to increase resistance to aztreonam (Cantu et al. 1996).

6.3.2 Combinations of Key Mutations in TEM-Type ESBLs (2be)

More than half of ESBLs ($n = 59$) contain combinations of two or more key mutations related to this phenotype (Figure 6.4). It should be noted that only certain combinations of mutations occur in β-lactamases isolated from clinical strains (104/164, 104/238, 164/240, and 238/240). Combinations of mutations of residues 164/238 and 104/240 occur only in triple mutants (104/164/238 and 104/164/240).