select for cross-resistance to tigecycline as a result of RamA-mediated AcrAB upregulation (Hornsey et al. 2010).

Thus, the increase of minimum inhibitory concentrations (MICs) toward tigecycline in clinical isolates is mainly associated with the increased nonspecific efflux of the drug, largely due to the overexpression of the RND family of efflux pumps, which, in turn, most likely due to mutations in the regulatory regions that drive their expression (Peleg et al. 2007). Low expression or the lack of expression of global regulators such as *ramR*, *marR*, or *soxR* also contributes to the elevated expression of the RND family of efflux pumps, resulting in the resistance to many drugs including tigecycline (Hentschke et al. 2010; Li et al. 2015a). These efflux pumps are structurally complex and chromosomally located and are subjected to complex regulation (Piddock 2006), which make these mechanisms as unlikely candidates for the acquired tigecycline resistance. Thus, the probability of horizontal dissemination of these mechanisms of tigecycline resistance via HGT remains low, although the clonal dissemination cannot be ruled out. Besides, mutants with the overexpression of nonspecific drug efflux pumps may be co-selected by structurally unrelated antibiotics and thus confer cross-resistance to multiple antibiotics, which may compromise antibiotic therapy. The worrying trend, however, is that despite being reserved as a drug of last resort, the use of tigecycline is steadily increasing (Huttner et al. 2012). This will lead, expectedly, to the higher occurrence of tigecycline-resistant clones in clinical settings. Moreover, the initial low-level resistance conferred by the overexpression of less specific drug efflux pumps may precede and facilitate the development of high-level drug resistance mediated by other mechanisms (Singh et al. 2012).

23.10 Other Potential Resistance Mechanisms Toward Third-Generation Tetracyclines

Because of the extremely broad use of tetracyclines in the past and present, the corresponding resistance genes are widespread in many ecological compartments including the environment (Chee-Sanford et al. 2001; Aminov and Mackie 2007; Chee-Sanford et al. 2009). And many of these tetracycline resistance genes are located on MGEs (Aminov 2011, 2012). Thus, there are preexisting conditions with the tetracycline resistance gene pool located on MGEs that may serve as a starting point for the emergence of strains with a lesser susceptibility to tigecycline. For example, during the early stages of drug development, it has been discovered that mutations in the interdomain loop region of *tet*A(A) may increase the efflux of minocycline and glycylcyclines (Tuckman et al. 2000). More recently, it has been shown that the increased expression of two tetracycline resistance genes, *tet*(L) (encoding efflux of the drug) and *tet*(M) (encoding ribosomal protection protein), confers tigecycline resistance