species. A list of known exogenously acquired 16S RMTases has been compiled and can be found in Garneau-Tsodikova and Labby (2016). Depending on the methylation position (which prevents the binding of the aminoglycoside), RMTases are classified into two families: (i) the methyl group is added in the N7 position of nucleotide G1405 (enzymes ArmA, RmtA, RmtB, RmtC, RmtD1, RmtD2 RmtE, RmtF, RmtG, and RmtH, which confer resistance to 4,6-disubstituted 2-DOS aminoglycosides), and (ii) the methyl group is added in the N1 position of A1408 (enzyme NpmA, which confers resistance to both 4,5- and 4,6-disubstituted 2-DOS aminoglycosides and to apramycin) (Garneau-Tsodikova and Labby 2016).

1.2.3 Changes in Uptake and Efflux

Other relevant causes of resistance to AGAs are related to the crossing of the bacterial cell wall, required for the action of the AGAs. The accumulation of the AGAs in the cell is against a concentration gradient, so it is predicted to be an energy-requiring process. However, this process has only been studied for two AGAs, streptomycin and gentamycin (Hancock 1981; Hancock et al. 1991). The mechanism of uptake of AGAs by the bacterial cell remains elusive, but several hypotheses have been suggested. It is thought that positively charged AGAs enter the cell by electrostatically interacting with the negatively charged lipopolysaccharides distributed in the outer bacterial membrane, a process denominated as self-promoted uptake (Taber et al. 1987; Hancock et al. 1991). These hypotheses comprise changes in membrane composition, overexpression of efflux pumps, and changes in membrane permeability. Mechanisms of resistance to this crossing involve modification of lipopolysaccharides of the outer membrane or downregulation of porins. A less negative outer membrane will have decreased affinity for aminoglycosides, and this occurs, most commonly, by incorporation of positively charged 4-amino-4-deoxy-L-arabinose in the lipopolysaccharide layer (Macfarlane et al. 2000; Kwon and Lu 2006; Fernandez et al. 2010), but incorporation of phosphoethanolamine was also reported (Nowicki et al. 2015). The presence of diacyl phosphatidylinositol dimannoside in mycobacteria is also proposed to decrease the membrane fluidity and, consequently, decrease its permeability (Bansal-Mutalik and Nikaido 2014).

Concerning porins, some mycobacteria have approximately 50-fold less MspA-like porins than other Gram-negative bacteria, which may account for a low permeability of the mycobacterial outer membrane (Jarlier 1994; Niederweis 2003). Mutations that lead to a lower number of porins may be part of a resistance strategy, but more studies are needed to support this.

In a different perspective, an alternative resistance mechanism is the removal of AGAs from the interior of bacteria by efflux pumps. The AcrAD-TolC type is a multidrug transporter and the main AGA efflux pump present in several