AGA, the crystal structure also reveals the presence of two pentacoordinated Mg^{2+} ions (Figure 1.3c). Two tyrosine residues form stacking interactions with the antibiotic. The authors hypothesize that the pocket is adequate in size to accommodate a nucleoside triphosphate. While *Mycobacterium tuberculosis* and *Mycobacterium abscessus* natively express AMEs, most pathogenic bacteria produce analogous enzymes that originate from horizontal gene transfer (Garneau-Tsodikova and Labby 2016), and this promiscuity is a major concern. This topic is addressed in Section 1.4.2. Furthermore, different activities can coexist in the same macromolecule, which then behaves as a bifunctional enzyme. An example of characterized bifunctional AME found in clinically relevant *Staphylococcus aureus* strains is the AAC(6′)-Ie/APH(2″)-Ia, which confers resistance to gentamycin kanamycin, and tobramycin (Smith et al. 2014).

1.2.2 Mutation or Modification of Ribosomal Target Sequences

As the main target for the action of aminoglycosides, bacterial ribosomes have a fundamental role in the mechanisms of resistance. These can take place either from specific mutations of the ribosome, mainly at the A-site, or from enzymatic modifications, performed by the 16S ribosomal RNA methyltransferases (RMTases). The latter mechanism is probably a resistance strategy of the aminoglycoside-producing bacteria, as observed for actinomycetes that produce RMTases to methylate their own 16S rRNA (Garneau-Tsodikova and Labby 2016).

In the bacterial ribosome, adenine 1408 and guanine 1491 (*Escherichia coli* numbering) of h44, located at the 16S RNA of the 30S subunit, are two of the residues directly involved in the inhibitory action of aminoglycosides (Carter et al. 2000). As in the human ribosomes these residues are replaced by guanine and adenine, respectively, AGAs cannot bind in the A-site and could be envisaged as ideal antibiotics. However, as a consequence of the probable prokaryotic origin of the mitochondrial ribosomes, residues adenine 1408 and guanine 1491 are present in mitochondrial ribosomes, making them susceptible to the inhibitory action of aminoglycosides (Han et al. 2005). This seems to be the cause for aminoglycoside toxicity in humans (Hobbie et al. 2008). In a different mechanism, translation is still enabled but, flipping of h44 helix (induced by binding of the aminoglycoside), results in mistranslated proteins (McCoy et al. 2011). Although uncommon (because usually lethal), mutations of the *rss* gene, which codes for this part of the rRNA, may confer resistance to aminoglycosides. Examples of mutations in h44 helix were found in resistant strains of *M. tuberculosis* (Georghiou et al. 2012) and *M. abscessus* (Nessar et al. 2011), where key hydrogen bonding interactions of the aminoglycosides with the rRNA are disrupted.

Bacterial resistance mechanisms that rely on modification of ribosomes by 16S RMTases are strongly related to plasmid transference among bacterial