Chapter

The Role of *Pseudomonas aeruginosa* RNA Methyltransferases in Antibiotic Resistance

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Abstract

Methyltransferases play a fundamental role in aminoglycoside resistance of Gram-negative bacteria, and some of its mechanisms were described in the past years, especially in *Escherichia coli*; however, it remains unsolved for other resistant bacteria such as *Pseudomonas aeruginosa*. Despite hurdles to determine resistance acquisition, high-throughput approaches (genomics, transcriptomics, and proteomics) have allowed data mining and analysis in a systemic way. Likewise, bioinformatics modelling of homologous genes or proteins has permitted to elucidate the emerging resistance in this pathogen. *P. aeruginosa* is a bacterial resistance treat since practically all known resistance mechanisms can be described using this model, particularly RNA methyltransferases. The RNA methyltransferases perform methylation or demethylation of ribosomal RNA to allow or restrict the antibiotic resistance development. The Kgm and Kam methyltransferases families are found in P. aeruginosa and confer resistance to several aminoglycosides. Loss of native methylations may also confer a resistant phenotype. The P. aeruginosa RsmG has high structural homology with Thermus aquaticus protein. Today, molecular data will promote a new paradigm on antibiotic therapy for treatment against *P. aeruginosa*. This chapter provides an overview of what role *P. aeruginosa*'s methyltransferases play in antibiotic resistance, induced by methylation or demethylation in the ribosome.

Keywords: *Pseudomonas aeruginosa*, antibiotic resistance, aminoglycoside, methyltransferase, methylation, demethylation, 16S RNA

1. Introduction

Nowadays, aminoglycoside antibiotic regimen remains as a prevailing therapy for the treatment of *Pseudomonas aeruginosa* pathogen, predominantly for respiratory complications in cystic fibrosis patients. However, *P. aeruginosa* strains are emergent multidrug-resistant. Bacteria develop resistance to aminoglycosides by producing aminoglycoside-modifying enzymes such as acetyltransferase, phosphorylase, and adenyltransferase [1]. The bacterial ribosome is a primary antibiotic

target, but bacteria can acquire resistance by modification of drug-binding sites. More than 50 years of studies in *Escherichia coli* have shown that 16S and 23S rRNAs have methylated nucleotides (**Figure 1**). These molecular modifications are performed by methyltransferases (MTases), which take in charge the transfer of a methyl group from a methyl donor S-adenosyl-l-methionine, better known as AdoMet or SAM [2]. These RNA MTases are diverse in posttranscriptional RNA modification, where single RNA nucleosides are chemically transformed. SAM-dependent MTases are involved in biosynthesis, signal transduction, protein repair, chromatin regulation, and gene silencing [3]. More recently, it was shown that aminoglycoside resistance in *E. coli* has its primary target within the decoding

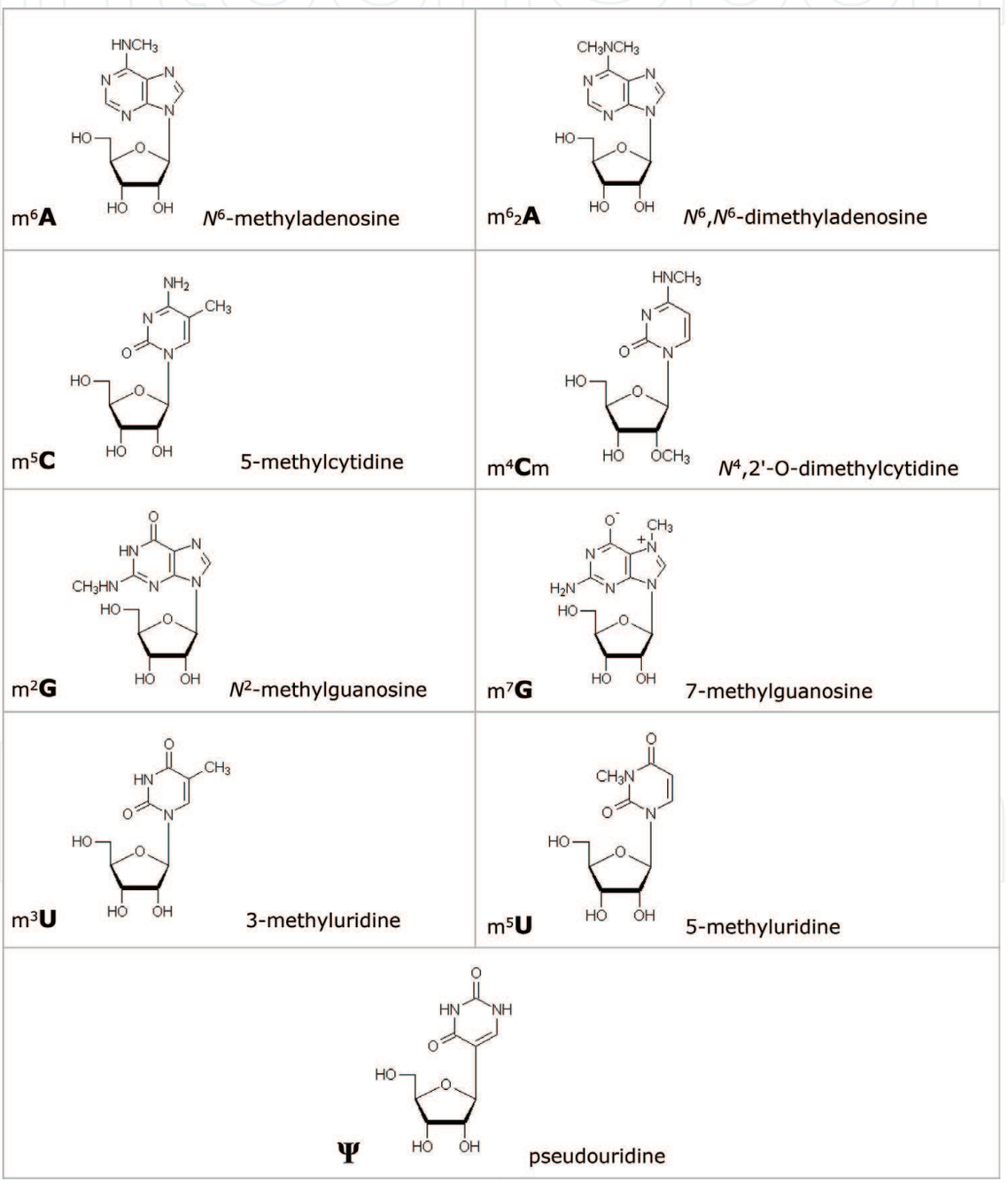

Figure 1.
Ribonucleotides methylated where the methyl moiety is located either in 16S or 23S ribonucleotides: m^6A [66, 67], $m^6{}_2A$ [68], m^5C [67], m^4Cm [69], m^2G [66], m^7G [70], m^3U [67, 71], m^5U [72], and Ψ [68]. Molecules are designed using the figures on the next web page, https://mods.rna.albany.edu/mods/modifications/search/. The structure shows clearly the methyl (CH_3-) but in the last structure bottom does not show this methyl.

region of 16S and 23S rRNAs and it is known to have 10 methylated nucleosides in 16S rRNA and 14 methylated nucleotides in 23S rRNA [4].

Different methylation sites have been identified within the 16S rRNA which yield different aminoglycoside resistance phenotypes [5]. One type group of 16S rRNA methylases is produced by istamycin producer *Streptomyces tenjimariensis*, which methylates m^1A1408 residue. Another group of 16S rRNA methylases is synthesized by gentamicin producer *Micromonospora purpurea* that methylates residue G1405; nonetheless, in *P. aeruginosa* the modification takes place in helix 44, with a secondary target in 23S rRNA helix 69 [6]. They bind specifically to the aminoacyl site (A-site) of 16S rRNA within the prokaryotic 30S ribosomal subunits and interfere with protein synthesis [7].

Probably RNA methylation began prior to DNA methylation in the early forms of life evolved on Earth [8], allowing to hypothesize perhaps that methylases appeared before polymerases. Ribonucleotide can be methylated by methyltransferases or demethylated by demethyltransferases. The structure of the bacterial ribosome has a molecular mass of 2.5 mega Daltons. In *E. coli*, the 50S subunit is composed of 23S rRNA (2904 nt), 5S rRNA (120 nt), and 33 ribosomal proteins, while the 30S subunit is composed of 16S rRNA (1542 nt) and 21 ribosomal proteins (S1–S21) [9].

The 16S rRNA resistance methyltransferases modify only intact 30S subunits, but the molecular details of their target recognition mechanisms are not quite elucidated yet. Such studies are becoming all the more necessary [10]. RNA methylation has been observed in different types of RNA species, viz., mRNA, rRNA, tRNA, snoRNA, snRNA, miRNA, and tmRNAs. Specific RNA methyltransferases are synthesized by cells to label these RNA species according to their needs and prevailing environmental conditions surrounding the cells, and this molecular labeling system is a constituent of epigenetics. New molecular structures provide crucial new insights that may provide a starting point for strategies to suppress these emerging causes of pathogenic bacterial resistance to aminoglycosides [11]. Nonetheless, bacteria develop resistance to aminoglycosides by producing aminoglycoside-modifying enzymes such as acetyltransferase, phosphorylase, and adenyltransferase. These enzymes, however, cannot confer a broad aminoglycoside resistance spectrum due to its substrate specificity [1].

2. Antibiotic resistance

P. aeruginosa has a large genome among *Gamma proteobacteria*, which allows it to improve many resistance mechanisms in a versatile way, for example, by transmissible plasmids or integrons. *P. aeruginosa* derepresses the chromosomal AmpC cephalosporinase [12, 13]; it also acquires genes for AmpC enzymes, class A carbenicillinases or β-lactamases, class D oxacillinases, and class B carbapenem-hydrolyzing enzymes [14], as it occurs in other bacteria like *E. coli* and *K. pneumoniae*. Other mechanisms include modifying the structure of topoisomerases II and IV to become quinolone resistant [15], decreasing outer membrane permeability by the partial or total failure of OprD proteins [12], overexpressing the active efflux systems with broad substrate patterns [16, 17], or synthesizing aminoglycoside-modifying enzymes as adenylyltransferases, acetyltransferases, and phosphoryltransferases [18].

The range of antibiotic resistance in *P. aeruginosa* is wide, and it represents a major difficulty for health care by its unsuccessful treatment, as a consequence of its low intrinsic antibiotic susceptibility, an effect of the interaction between multidrug

efflux pumps, like *mexAB*, *mexXY* [19], *AdeABC*, and *AdeDE* genes [17]. Another factor is its efficient capability of acquiring resistance, developed by transfer of horizontal genes, such as specific gene mutations [20], and finally by the low permeability of the cellular membrane [16, 21].

Protein	Gene	Substrate	Nucleotide methylated	Position of methylation	Ligand	UniProt
RlmB	*rlmB*	23S rRNA	G2251	2'-O-ribose	SAM	Q9HUM8
RlmD	*rlmD*	23S rRNA	U1939	C5	SAM	Q9I525
RlmE	*rlmE*	23S rRNA	U2552	2'-O-ribose	SAM	A6VCK9
RlmF	*rlmF*	23S rRNA	A1618	N6	SAM	A6V0S3
RlmG	*rlmG*	23S rRNA	G1835	N2	SAM	A6VC08
RlmH	*rlmH*	23S rRNA	Ψ1915	N3	SAM	A6V0A6
RlmJ	*rlmJ*	23S rRNA	A2030	N6	SAM	Q9HUF0
RlmK/L	*rlmL*	23S rRNA	G2445	N2	SAM	A6V328
RlmM	*rlmM*	23S rRNA	C2498	2'-O-ribose	SAM	A6V7T6
RlmN	*rlmN*	23S rRNA and tRNA	A2503 in rRNA and A37 in tRNA	C2	Radical SAM	A6V0V7
RsmA	*rsmA*	16S rRNA	A1518 and A1519	N6	SAM	Q9I5U5
RsmB	*rsmB*	16S rRNA	C967	C5	SAM	Q9I7A9
RsmC	*rsmC*	16S rRNA	G1207	N2	SAM	A6VC20
RsmD	*rsmD_2*	16S rRNA	G966	N2	SAM	A0A0F6U8H1
RsmE	*rsmE_2*	16S rRNA	U1498	N3	SAM	A0A0F6U8B3
RsmG	*rsmG*	16S rRNA	G527	N7	SAM	A6VF42
RsmH	*rsmH*	16S rRNA	C1402	N4	SAM	A6VB93
RsmI	*rsmI*	16S rRNA	C1402	2'-O-ribose	SAM	Q9HVZ3
RsmJ	*rsmJ*	16S rRNA	G1516	N2	SAM	Q9HXW0
TrmA	*trmA*	tm/tRNA	U54 in tRNA and U341 in tmRNA	C5	SAM	A6VCH5
TrmB	*trmB*	tRNA	G46	N7	SAM	Q9I6B3
TrmD	*trmD*	tRNA	G37	N1	SAM	Q9HXQ1
TrmH	*trmH*	tRNA(Leu)	Wobble nucleotide	2'-O-ribose	SAM	A0A0H2ZHL8
TrmI	*trmI*	tRNA	A58	N1	SAM	A0A2X4FJT8
TrmJ	*trmJ*	tRNA	C32, U32, and A32	2'-O-ribose	SAM	A0A0H2ZF87
TrmL	*trmL_2*	tRNA(Leu)	Wobble nucleotide	2'-O-ribose	SAM	A0A0G5X8M9

Table 1.
P. aeruginosa's rRNA methyltransferases and their point of modifications [23]. The columns are described as follows: first column, the name protein of RNA methyltransferase; second column, the name of its gene; third column, the substrate either 23S or 16S RNA or tRNA; fourth column, the type of nucleotide methylated; fifth column, the electron in nucleotide methylated; sixth column, the ligand for everyone SAM; and seventh column, the UniProt code. Some interesting proteins such as RsmA, RsmG, RsmH, and RsmI are marked in bold.

Bacterial multidrug resistance (MDR) is an important concern in *P. aeruginosa* since this microorganism is capable of mixing several mechanisms, transposons, plasmids, and chromosomally encoded genes, such as methyltransferases or pumps [22]. Methyltransferase genes are spread in bacterial genome ready to trigger antibiotic resistance. **Table 1** compares the reported methyltransferase proteins worldwide, being annotated for *P. aeruginosa* in UniProt database [23].

One mechanism of adaptation which facilitates natural selection in bacteria is the hypermutation of some genes or chromosomal regions. Previous work in patients with *P. aeruginosa* showed that hypermutation causes a problematic effect during a chronic respiratory infection (CRI) [24], where *P. aeruginosa* was up to 6.5-fold higher in mutator backgrounds. Other elements associated with high antimicrobial resistance are integrons. These elements were found among isolates from Iran patients with *P. aeruginosa*, which the *int1* integron was prevalent [25].

Integrons linked to transposons, plasmids, and chromosome are responsible for bacterial antibiotic resistance [26, 27]. Integrons are composed of three elements: (1) the integrin-associated promoter (Pc), which is required for transcription and expression of gene cassettes (genetic elements that encode antibiotic resistance genes) within the integrin; (2) the *intI* gene, in which coding for the integrase IntI is crucial for site-specific recombination; and (3) the adjacent recombination site *attI*, which is recognized by integrase. On the other hand, *P. aeruginosa* carrying transposon Tn1696 is an element that encodes the *CmlA* gene, an exporter of the major facilitator (MF) superfamily which provides antibiotic resistance, specifically against chloramphenicol [28]. *P. aeruginosa* has a broad spectrum in cephalosporin resistance mechanism, mediated by the extended-spectrum β-lactamases (ESBLs). High prevalence of multidrug resistance in burn patients and production of *oxa-10*, *per-1*, and *veb-1* genes by *P. aeruginosa* isolates confirm the presence of antibiotic-degrading enzymes [29].

The Pathosystems Resource Integration Center (PATRIC) is a massive database that integrates genomic data and analysis tools to support biomedical research on bacterial infectious diseases. The platform provides an interface for biologists to discover data and information and conduct comprehensive comparative genomics and other analyses in a one-step source. PATRIC database provides complete genome information and data regarding susceptibility or resistance [30] to several antibiotics; including aminoglycosides, polymyxin B, colistin, ceftazidime, piperacillin, imipenem, ciprofloxacin, levofloxacin, and meropenem in *P. aeruginosa*. We report

Figure 2.
Resistance and susceptibility profile of P. aeruginosa against a broad spectrum of different types of antibiotics. The data were downloaded from PATRIC database selecting aminoglycosides, beta-lactamases, cephalosporins, licosamides, fluoroquinolones, colistin, doxycycline, ciprofloxacin, nitrofurantoin, and cefazolin. Many strains are resistant to a wide range of antibiotics (red with a larger percentage), and the most strains are susceptible to colistin (green with larger percentage); on the other hand, the overall strains are resistant to ampicillin, cefotaxime, erythromycin, and nitrofurantoin.

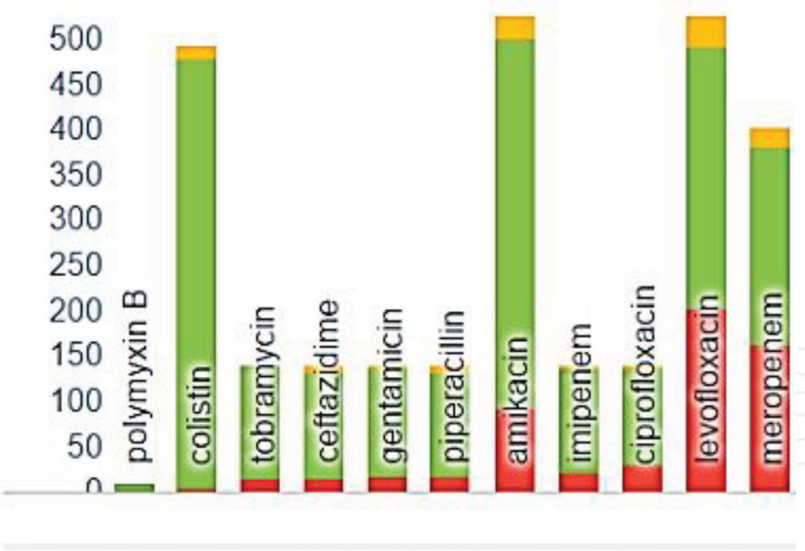

Figure 3.
Strains with P. aeruginosa's genomes showing susceptibility (green) and resistance (red) against some aminoglycosides (amikacin, gentamicin, and tobramycin) and other antipseudomonal antibiotics. Many strains are resistant to a wide range of antibiotics (red with a larger percentage), and most strains are susceptible to doxycycline or colistin (green with larger percentage).

in **Figure 2** antibiotic resistance or susceptibility from different *P. aeruginosa* strains as well as in **Figure 3** with those antibiotics mentioned.

3. Resistance to antibiotics through rRNA methylation

Kgm and Kam families are two different groups of SAM-dependent RNA methyltransferases, which modify nucleotides of 16S rRNAs in the specific drug-binding site to confer self-resistance in aminoglycoside-producing bacteria [31]. The Kgm and Kam families have been distinguished based on their nucleotide targets, G1405 and A1408, respectively. The *kgmB* and *armA* genes (Kgm family kanamycin gentamicin methyltransferase) methylate m^7G1405(N7) position that confers a high level of resistance against gentamicin, kanamycin, and tobramycin. The addition of a methyl group in this position interferes directly with the binding to the antibiotic, inducing a steric hindrance between the modified base and the structure of the antibiotic, causing electrostatic repulsions derived from the positive charge in the modified base [32]. On the other hand, the *kamA* and *npmA* genes (Kam family kanamycin-apramycin methyltransferase) methylate m^1A1408(N1) position conferring a high level of resistance to kanamycin, apramycin, and neomycin [5].

Another interesting non-aminoglycoside resistance related to RNA methylation is the macrolide-lincosamide-streptogramin-B (MLSB) antibiotics, which is strongly associated with the expression of the methyltransferase of ErmC RNA that causes the dimethylation of the N-6 atom of adenine and interacts with the nucleotide 2058 in the 23S rRNA. Such antibiotics bind to overlap sites within the 50S ribosomal subunit tunnel near the peptidyl transferase center, either by inhibiting the catalysis directly at the peptidyl transferase site or by acting as a physical barrier to the extension of the peptide chain inside the tunnel [33]. Many more *erm*-type methyltransferase genes have been identified in a wide range of Gram-positive and Gram-negative bacteria. Among them, the *ermB*, *ermF*, and *ermA* genes are transferred by transposons, and the *ermC* gene transferred by plasmids. The family of Erm methyltransferases that mediate the mono- or dimethylation of A2058 consists of approximately 40 different classes of methylases [34, 35].

4. Resistance or susceptibility to antibiotics through rRNA demethylation

The *rsmG* gene encodes a 16S rRNA mRNA which methylates the N7 of nucleotide G527 within the 530 loop of 16S rRNA; one of the main examples is the loss of native methylations that confers a resistance phenotype to streptomycin. Streptomycin interacts with the rRNA in the adduced region (loop 530), and the loss of methylation correlates with a low level of resistance. Although this resistance is at a low dose of antibiotic, the mutation of *rsmG* apparently has a mutator effect which promotes the appearance of a high number of mutants resistant to high doses of streptomycin [36]. Another interesting aspect is that not only methylation generates resistance; cases have been reported where demethylation also promotes resistance. The first and best characterized example is *ksgA* gene (RsmA protein), which encodes the native methyltransferase KsgA or RsmA, responsible for the N6 dimethylation of A1518 and A1519 in the 3′-terminal fork of the 16S rRNA in the 30S rRNA. It was the first resistance to aminoglycosides (kasugamycin) associated with demethylation in the 16S rRNA [37]. It was found that adenine methylated by MTase is far from the binding site of kasugamycin, so this demethylation should lead to a conformational rearrangement which would be associated with the acquisition of antibiotic resistance [38].

Another research showed that preventing adenine methylation from occurring, resistance to kasugamycin can be induced; the base U793 fills the site usually occupied by the methylated adenines and the adjacent bases, A792 and A794, [39]. The phenomena mentioned above give place to a conformational change, causing the union site of Ksg to be blocked by the U793. Accordingly, it can be assured that this structural change in the helix 24 causes resistance to Ksg [39].

Likewise, it was found that the *tlyA* gene in *Mycobacterium tuberculosis* encodes the MTase 2'-O-ribose TlyA responsible for the C1409 methylations in the 16S rRNA and C1920 in the 23S rRNA. The loss of such methylations confers resistance to capreomycin and viomycin, two antibiotics which bind at the interface of the ribosome subunit and are used to help define their binding site. Another example of the absence of methylations in the 23S rRNA is the lack of methylation in U2584 (*E. coli* numbering), which causes resistance to sparsomycin in 23S rRNA *Halobacterium salinarum* [40].

Recent findings regarding intrinsic resistance refer to the Ψ at position 2504 of the 23S rRNA in *E. coli*, where inactivation of the *rluC* gene confers significant resistance to clindamycin, linezolid, and tiamulin [41]. The *cfr* gene was originally discovered in an isolate of a multiresistant plasmid during a follow-up study of chloramphenicol resistance in *Staphylococcus* spp. isolates. The molecular characterization of the resistance led to the gene encoding a methyltransferase that methylated the nucleotide A2503 in the 23S rRNA. In *E. coli* and *S. aureus*, there is a natural methylation of A2503 mediated by the methyltransferase encoded by the *yfgB* gene (*rlmN*). The lack of natural methylation in A2503 confers a slight increase in susceptibility to tiamulin, hygromycin A, sparsomycin, and linezolid [42].

5. rRNA methyltransferases associated with aminoglycoside resistance in *P. aeruginosa*

Methyltransferases have been intensely studied in *P. aeruginosa*, but this is not the case for RNA methyltransferases, particularly those conferring aminoglycoside resistance. Nowadays, we focus our study in *P. aeruginosa* methyltransferases using

molecular biology, genomics, proteomics, chemistry informatics, and bioinformatics [43–45]. RsmG, RsmH, and RsmI are RNA methyltransferases, and these have been broadly studied. Six crystal structures have been reported in PDB for RsmG, from *Thermus thermophilus* with accession numbers 4NXM, 4NXN, 3G88, 3G89 3G8A, and 3G8B [46, 47] and one from *E. coli*, with number 1JSX [48], and another one from *Bacillus subtilis*, with number 1XDZ. RsmH and RsmI crystal structures from *E. coli* are reported in PDB with numbers 3TKA and 5HW4 [49–51]. Checking these three orthologous genes in the PATRIC database, they are being conserved in *P. aeruginosa*'s pan-genome.

RsmG well known as 16S rRNA (guanine^{527}-N^{7})-methyltransferase methylates guanine^{527} at N^{7} in 16S rRNA [36, 52] (**Table 1**) and catalyzes S-adenosyl-L-methionine + guanine^{527} in 16S rRNA → S-adenosyl-L-homocysteine + N^{7}-methylguanine527 in 16S rRNA (see reaction in UniProt, KEGG, or MetaCyc). Researches in *M. tuberculosis* reveal that *rsmG* mutations confer low-level streptomycin resistance; moreover, it has been reported that combining drug resistance mutations of *rsmG* gene remarkably enhances enzyme production in *Paenibacillus agaridevorans* [53]. Likewise, for *P. aeruginosa*, *rsmG* is conserved in both aminoglycoside-resistant and aminoglycoside-susceptible strains.

RsmH also called S-adenosyl-L-methionine (cytosine^{1402}-N^{4})-methyltransferase methylates the N^{4}-of cytosine^{1402} [54] (**Table 1**). This enzyme catalyzes the following chemical reaction: S-adenosyl-L-methionine + cytosine^{1402} in 16S rRNA → S-adenosyl-L-homocysteine + N^{4}-methylcytosine^{1402} in 16S rRNA (see reaction in UniProt, KEGG, or MetaCyc). Experiments performed with gene knockout of *rsmH* and *rsmI* have shown in *E. coli* BW25113 strain that *ΔrsmH* and *ΔrsmI* increase in doubling times by 15 and 12%, respectively; however, *ΔrsmH/ΔrsmI* increases in doubling time by 29% compared with a wild type cultured at 37°C, indicating that gene knockout caused a slight but significant change in phenotype about cellular growth properties in the absence of both *rsmH* and *rsmI* [54]. As well as *E. coli*, *P. aeruginosa* conserves *rsmH* and *rsmI* genes in both aminoglycoside-resistant and susceptible strains; therefore, it is important to study the mutations also in its strains.

RsmI also named S-adenosyl-L-methionine 16S rRNA (cytidine^{1402}–2'-O)-methyltransferase methylates in cytidine^{1402}–2'-O (**Table 1**). RsmI catalyzes the next chemical reaction: S-adenosyl-L-methionine + cytidine^{1402} in 16S rRNA → S-adenosyl-L-homocysteine + 2'-O-methylcytidine^{1402} (see reaction in UniProt, KEGG, or MetaCyc). RsmI and RsmH react on the same nucleotide, but the first methylates in 2'-O, while the second one in -N^{4} [54]. Such as *rsmG* and *rsmH*, the *rsmI* gene is also conserved in pan-genome. Theoretical modeling of the structure in RsmI protein from *P. aeruginosa* was performed in iTISSER suit [55], and compared with 5HW4 from *E. coli* (**Figure 4**), the homology and the active site in *P. aeruginosa* are apparently well maintained.

Other interesting *P. aeruginosa* methyltransferases associated with aminoglycoside resistance are m^{5}C1404, m^{1}A1408, and m^{7}G1405 [6]. Among the last group mentioned, there are some well-studied methyltransferases, such as ArmA, RmtA, RmtB, RmtC, RmtD, RmtF, and RmtG (**Table 2**). This group is characterized for providing resistance to 4,6-disubstituted 2-deoxystreptamine (2-DOS) aminoglycosides [6]. For example, ArmA was found in *Klebsiella pneumoniae* [56]; as for *P. aeruginosa*, among 100 Korean multidrug-resistant isolates, 14 carried this enzyme [57]. The *armA* gene encodes for 16S RNA methyltransferase that methylates guanine (1405)-N^{7}. The same gene in *P. aeruginosa* (**Table 2**) presents variable occurrence as it is part of the accessory genome. A multiple alignment, using the listed P. aeruginosa ArmA proteins (16S rRNA (guanine (1405)-N(7))- methyltransferase)) revealed identical homology for this marker.

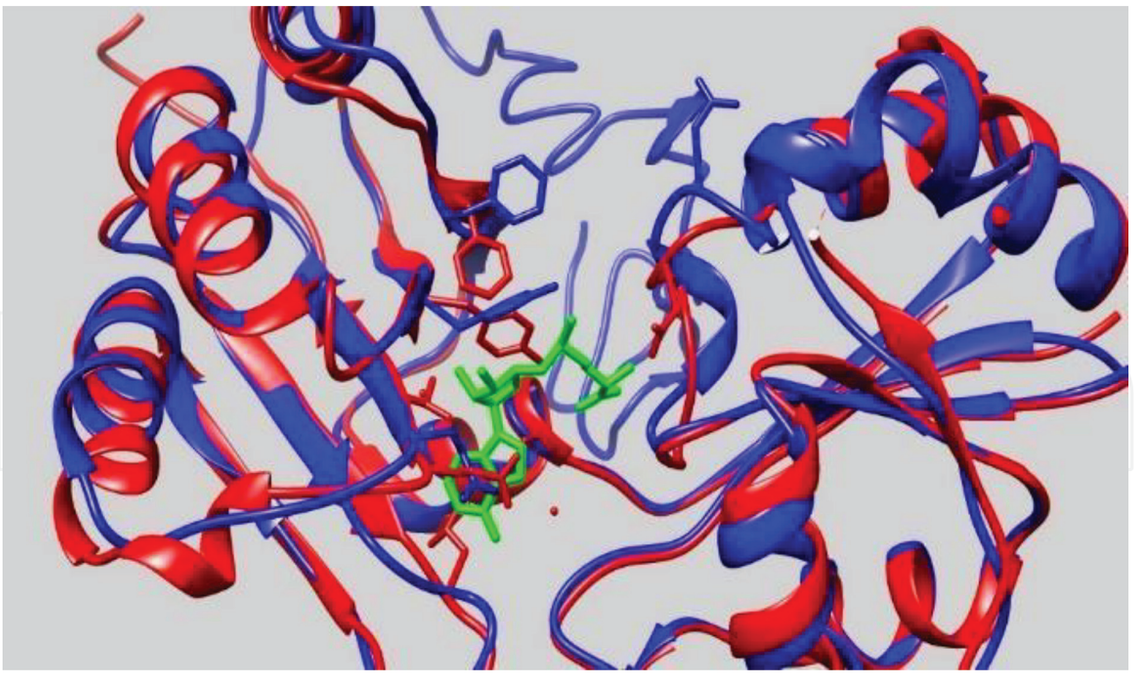

Figure 4.
Structural 3D aligned with chimera 3.1 suit between RsmI proteins from P. aeruginosa (red) and E. coli (blue). Although the alignment displays a structural shifting, the overall topology of the active site is maintained. The protruding residues from each protein depict the active site, harboring SAM (fluorescent green). The structure of E. coli has already been solved by Zhao et al. [49], without obtaining, until now, the crystal structure of RsmI from P. aeruginosa.

Protein	Modification	Strain location	Reference
ArmA	16S rRNA m7G1405	China and Korea	[57, 73]
RmtA	16S rRNA m7G1405	Japan and Korea	[1, 58, 74, 75]
RmtB	16S rRNA m7G1405	China and India	[73, 76]
RmtC	16S rRNA m7G1405	India	[61, 76]
RmtD	16S rRNA m7G1405	Brazil	[77, 78, 79]
RmtF	16S rRNA m7G1405	India	[61, 75]
RmtG	16S rRNA m7G1405	Brazil	[62]

Table 2.
Different 16S rRNA methyltransferases associated with aminoglycoside resistance reported for P. aeruginosa, with classical name, for everyone with the same nucleotide (16S rRNA m7G1405) of isolates from patients who belong to India, Brazil, China, and Korea.

The location of m^7G1405 methyltransferase genes across the prokaryotic genome is variable, as it has been found in several studies mentioned above. The *rmtA* gene in *P. aeruginosa* carries a mobile element Tn5041 [58, 59] identified previously in *Enterobacteriaceae* [56], while the *rmtB* gene identified in *Serratia marcescens* is located in the flanking of Tn3-like region responsible for multiple antimicrobial resistance [59, 60], and both methyltransferases and mobile elements are present in *P. aeruginosa* (**Table 2**). The *rmtC* and *rmtF* genes (**Table 2**) might have been acquired from plasmids as part of mobile genetic elements and finally integrated and stabilized on the chromosome [61]. The *rmtG* gene (**Table 2**) is likely located in the chromosome [62]. The m^1A1408 methyltransferases are present in pan-aminoglycoside-resistant strains, which were identified by Wachino et al. [63] and provide resistance by these classes of methyltransferases to both 4,5-disubstituted 2-DOS and 4,6-disubstituted 2-DOS aminoglycosides as well as NpmA case. These two classes of methyltransferases are

very important for antibiotic resistance, thanks to the similarity of these enzymes with those homologs found in aminoglycoside-producing actinomycetes [6]. Those new genes and proteins will be better studied in expression and structure, to be related to epidemiological data. Looking at **Table 2**, it seems that the expression of methyltransferase might be related to geographical prescription. However, this hypothesis does not seem well founded: microbiological, molecular, and epidemiological understanding of RNA methyltransferases in *P. aeruginosa* will allow the rational use of aminoglycosides and maybe will be not replaced for new antibiotics.

6. Final considerations

Antibiotic resistance is a serious concern for public health and environment. To comprehend the molecular interaction of the methyltransferase in aminoglycoside resistance will be a more efficient way to rationalize its use and consumption. It will be better to clarify the panorama of the rational use of the aminoglycosides to diminish the rapid development of resistance before considering its replacement, since *P. aeruginosa* is still susceptible to them, and, moreover, currently it is known why other *Gammaproteobacteria* are resistant to them. Why are methylation and demethylation a feedback of the antibiotic environmental pressure in bacteria? Bhujbalrao and Anand [64] suggest us some insights using KsgA, exploring the factors which govern the resistance to antibiotics. They observed within loop1 and loop12 of rRNA switched chimera efficiently methylated mini-RNA substrates in vitro, showing that these structural elements suffice for local orientation of the rRNA. In addition, in vivo they notice that the head domain plays a more critical role in leading the enzyme to the select ribosomal region and serves as a sensor of the global environment.

As Kim et al. [65] discuss in letter to editor (Dr. Hur), investigating with P. aeruginosa and aminoglycoside resistance proposes that "less aminoglycoside consumption correlates with less resistance levels"; therefore, we consider that is a requisite for an antibiotic cycling strategy at the global level; also they discussed the rates of amikacin or gentamicin-resistant declining trends, according to the data from KONSAR Korean program in 2011 either for *P. aeruginosa*, *K. pneumoniae*, or *Acinetobacter* spp. [64]. With the knowledge about aminoglycoside resistance molecular mechanisms comparing to the rational prescription cited in Korea, for example, we hypothesize that low methylation rate in the nucleotide substrate of RsmH or RsmI is close to the anchor point of gentamicin in 16S RNA, indicating a possible association with gentamicin or aminoglycosides resistance [5].

RsmG, RsmH, and RsmI methyltransferases belong to the core genome (constitutive genome), while ArmA is part of the accessory genome with identical protein sequences among close species in *Proteobacteria*. Nowadays, the enzymatic activity has been well described; however the antibiotic resistance remains unsolved, perhaps as a consequence of broad usage of aminoglycoside in hospital environment, allowing the development of resistant bacteria. In the future, probably the treatment of *P. aeruginosa* will take into account the genetic trait of each isolate, strain, or species with the set of resistance genes, and surely methyltransferases will be included routinely in clinical care and high throughput or genomic medicine therapies.

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