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Phylogenetic Sequence Variations in Bacterial rRNA Affect Species-Specific Susceptibility to Drugs Targeting Protein Synthesis▽‡

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Antibiotics targeting the bacterial ribosome typically bind to highly conserved rRNA regions with only minor phylogenetic sequence variations. It is unclear whether these sequence variations affect antibiotic susceptibility or resistance development. To address this question, we have investigated the drug binding pockets of aminoglycosides and macrolides/ketolides. The binding site of aminoglycosides is located within helix 44 of the 16S rRNA (A site); macrolides/ketolides bind to domain V of the 23S rRNA (peptidyltransferase center). We have used mutagenesis of rRNA sequences in *Mycobacterium smegmatis* ribosomes to reconstruct the different bacterial drug binding sites and to study the effects of rRNA sequence variations on drug activity. Our results provide a rationale for differences in species-specific drug susceptibility patterns and species-specific resistance phenotypes associated with mutational alterations in the drug binding pocket.

The bacterial ribosome is a target for many antibacterial agents that interfere with protein synthesis, such as aminoglycosides, macrolides, ketolides, oxazolidinones, and lincosamides (23). These compounds target different steps in translation, including decoding, peptide bond formation, and translocation (33, 37, 40). While different classes of antibiotics bind to different ribosomal regions and interfere with different steps in translation, they all interact directly with rRNA nucleotides at or near functionally important sites (38, 39). These rRNA residues typically show high phylogenetic sequence conservation within bacteria. It is largely unclear whether the minor sequence variations present in the bacterial drug binding sites affect antibiotic susceptibility and/or resistance development.

Structures of antibiotics bound to the ribosome have been resolved primarily with extremophiles such as *Thermus thermophilus*, *Deinococcus radiodurans*, or *Haloarcula morismortui* (2, 9, 30). Most genetic data, however, have been generated with *Escherichia coli* and *Mycobacterium smegmatis* (4–6, 10, 14, 15, 20–22, 26, 28, 29, 32). It has still to be established whether the conclusions drawn from diverse model organisms hold true for other bacterial clades as well. To address this question, we investigated rRNA alterations corresponding to phylogenetic sequence variations that are found in bacteria and which are located in two major drug binding sites, the 23S rRNA peptidyltransferase region and the 16S rRNA-decoding region. These two regions are targeted by different classes of antibiotic compounds: macrolides/ketolides (23S rRNA) and aminoglycosides (16S rRNA) (23, 37).

Macrolide/ketolide antibiotics are a diverse class of naturally occurring and synthetic compounds based on a polyketide macrolactone ring substituted with one or more nonnitrogenous and/or amino sugar moieties (27). These compounds exert their inhibitory effect on protein synthesis by binding to the opening of the ribosomal polypeptide exit tunnel to obstruct elongation of the nascent polypeptide chain (30, 34). Aminoglycosides form a large family of water-soluble, polycationic amino sugars (18). Common to all aminoglycosides is the neamine core. Additional sugars are attached to give rise to a variety of compounds categorized as 4,5- or 4,6-aminoglycosides. An important substituent in aminoglycoside specificity is the chemical group at position 6′ of ring I, i.e., 6′-NH_2 or 6′-OH (36). Aminoglycosides target the ribosome by direct interaction with rRNA, and they affect protein synthesis by inducing codon misreading and by inhibiting translocation of the tRNA-mRNA complex (2, 3).

We here used previously described procedures for rRNA mutagenesis in *M. smegmatis* (15) to reconstruct the different bacterial sequence variants as found in the drug binding pockets of both macrolides/ketolides and aminoglycosides. The resulting recombinants were then investigated for drug susceptibility.

MATERIALS AND METHODS

Bacterial strains and DNA techniques. *Mycobacterium smegmatis* rRNA mutants were generated by the following procedures. (i) rRNA gene fragments coding for mutant rRNA were generated by PCR mutagenesis and cloned into an integration-proficient plasmid. RecA-mediated homologous recombination and selective plating were used to introduce point mutations into the single functional rRNA gene operon of *M. smegmatis* Δ*rrnB* (20). DNA sequencing was used to confirm that the point mutation had been introduced and that additional mutations in the area involved in homologous recombination were absent. (ii)

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TABLE 1. Strains used in this study

M. smegmatis strain(s)	Description	Parental strain	*attB*	rRNA mutation(s)[f]	
				16S	23S
SZ379	mc^2155		None	wt	wt
SZ001[d]	mc^2155	SZ379	None	wt	wt
SZ380[e]	Δ*rrnB rrnA*$^+$	SZ379	None	wt	wt
SZ386[d,e]	Δ*rrnA rrnB*	SZ001	None	wt	wt
SZ637[e]	Δ*rrnA* Δ*rrnB attB*: *rrnB*	SZ379	pH 144	wt	wt
SZ558[d]	Δ*rrnA* Δ*rrnB attB*: *rrnB*	SZ386	pH 144	wt	wt
SZ459-SZ461[a,e]	Δ*rrnB rrnA* (A1408G)	SZ380	pH 128	A1408G	wt
SZ463-SZ465[a,e]	Δ*rrnB rrnA* (G1491A)	SZ380	PZ176	G1491A	wt
SZ468-SZ470[a,e]	Δ*rrnB rrnA* (G1491C)	SZ380	PZ178	G1491C	wt
SZ505-SZ507[a,e]	Δ*rrnB rrnA* (G1491U)	SZ380	PZ177	G1491U	wt
SZ605[c,e]	Δ*rrnB rrnA* (C1409G · G1491C)	SZ469	PZ178	C1409G, G1491C	wt
SZ717-SZ720[b,e]	Δ*rrnA* Δ*rrnB attB*: *rrnB* (C1409U · G1491A)	SZ637	pH 297	C1409U, G1491A	wt
SZ721-SZ724[b]	Δ*rrnA* Δ*rrnB attB*: *rrnB* (Eco4)	SZ637	pH 163	G1410A, U1411C, A1489G, C1490U	wt
SZ725-SZ728[b]	Δ*rrnA* Δ*rrnB attB*: *rrnB* (Eco4 A1408G)	SZ637	pH 205	A1408G, G1410A, U1411C, A1489G, C1490U	wt
SZ706-SZ709[b]	Δ*rrnA* Δ*rrnB attB*: *rrnB* (Eco4 G1491A)	SZ637	pH 294	G1410A, U1411C, A1489G, C1490U, G1491A	wt
SZ710-SZ713[b]	Δ*rrnA* Δ*rrnB attB*: *rrnB* (Eco4 G1491C)	SZ637	pH 295	G1410A, U1411C, A1489G, C1490U, G1491C	wt
SZ714-SZ716[b]	Δ*rrnA* Δ*rrnB attB*: *rrnB* (Eco4 G1491U)	SZ637	pH 296	G1410A, U1411C, A1489G, C1490U, G1491U	wt
SZ763-SZ766[b,e]	Δ*rrnA* Δ*rrnB attB*: *rrnB* (Pac2)	SZ637	pH 154	C1409A, G1491U	wt
SZ832-SZ835[b]	Δ*rrnA* Δ*rrnB attB*: *rrnB* (Pac2 A1408G)	SZ637	pH 349	A1408G, C1409A, G1491U	wt
SZ678-SZ680[b,d]	Δ*rrnA* Δ*rrnB attB*: *rrnB*	SZ558	pH 203	wt	wt
SZ674-SZ677[b,d]	Δ*rrnA* Δ*rrnB attB*: *rrnB* (A2058G)	SZ558	pH 191	wt	A2058G
SZ681-SZ684[b,d]	Δ*rrnA* Δ*rrnB attB*: *rrnB* (A2057G · U2611C)	SZ558	pH 219	wt	A2057G, U2611C
SZ685-SZ687[b,d]	Δ*rrnA* Δ*rrnB attB*: *rrnB* (A2058G A2057G · U2611C)	SZ558	pH 221	wt	A2058G, A2057G, U2611C

[a] Mutagenesis by RecA-mediated gene conversion.
[b] Mutagenesis by replacing the wild-type *rrnB*$^+$ plasmid with a mutant *rrnB* plasmid.
[c] Spontaneous C1409G mutation in an G1491C genetic background.
[d] These strains were generated in a *rpsL* K43R background (K43 in *M. smegmatis* is homologous to K42 in *E. coli* [28]).
[e] These strains have been described previously (31).
[f] wt, wild type.

Plasmid exchange mutagenesis was done as described previously (13). In brief, DNA sequences coding for mutant rRNA were generated by PCR mutagenesis and cloned into an integration-proficient plasmid with a fully functional rRNA operon. These plasmids with mutant rRNA genes were then used to replace the single rRNA operon present in *M. smegmatis* Δ*rrn*/pMIG-*rrnB*$^+$-*sacB* by plasmid exchange. Successful gene exchange was controlled by DNA sequence analysis.

For a complete list of strains and plasmids, see Table 1 and Table S1 in the supplemental material, respectively.

Susceptibility testing. Drug susceptibility was assessed by determination of MICs. MICs were determined by broth microdilution assays as described previously (19). In brief, bacterial strains were cultured on Luria-Bertani (LB) agar plates at 37°C. Freshly grown cultures were resuspended in LB broth supplemented with 0.05% Tween 80, diluted to an absorbance at 600 nm of 0.025, and incubated in the presence of 2-fold serial dilutions of antibiotics (Sigma). After incubation at 37°C for 72 h, the MIC was recorded as the lowest concentration of drug inhibiting visible growth. Three to five independent clones were analyzed per mutation. Linezolid was included as unrelated ribosomal inhibitor representative of a different compound class to control for the class specificity of the effects.

RESULTS AND DISCUSSION

Phylogenetic sequence variations in the 23S rRNA peptidyltransferase region. Most rRNA nucleotides in the inner peptidyltransferase region of the large ribosomal subunit are phylogenetically conserved (8). The macrolide/ketolide family of antibiotics binds to a hydrophobic cleft formed by residues 2058, 2059, and 2611 (*E. coli* numbering is used throughout) in domain V of 23S rRNA, with some drugs making additional contacts in domain II (9, 10, 30) (Fig. 1). The adenines at 23S rRNA positions 2058 and 2059 are phylogenetically conserved in bacteria and play an important role in compound binding, emergence of resistance, and drug selectivity (1, 22).

One key interaction appears to be the hydrogen bond between N-1 of A2058 and the 2′-hydroxyl group of the macrolides' desosamine sugar (Fig. 1c) (30, 34). Mutation of A2058 to a guanine (A2058G) greatly impairs the binding of macrolides to ribosomes by both chemical and steric alteration of the binding site (9, 22). Ribosomal susceptibility to macrolides and ketolides is also affected by proper Watson-Crick base pairing between nucleotides at positions 2057 and 2611 (6), which are typically G · C (e.g., in *Proteobacteria*) and A · U (e.g., in *Mycobacteria*) (Fig. 1; for a phylogenetic comparison of the 2057 · 2611 interaction in clinically relevant phyla, see Table S2 in the supplemental material). The composition of the base pair between nucleotides 2057 and 2611 has been shown to affect the resistance phenotype of the A2058G mutation toward ketolides (19). However, the effect of an A2057G-U2611C substitution in the context of a wild-type A2058 has remained elusive. Changing the A2057 · U2611 base pair in *M. smegmatis* to G2057 · C2611, as it is typically

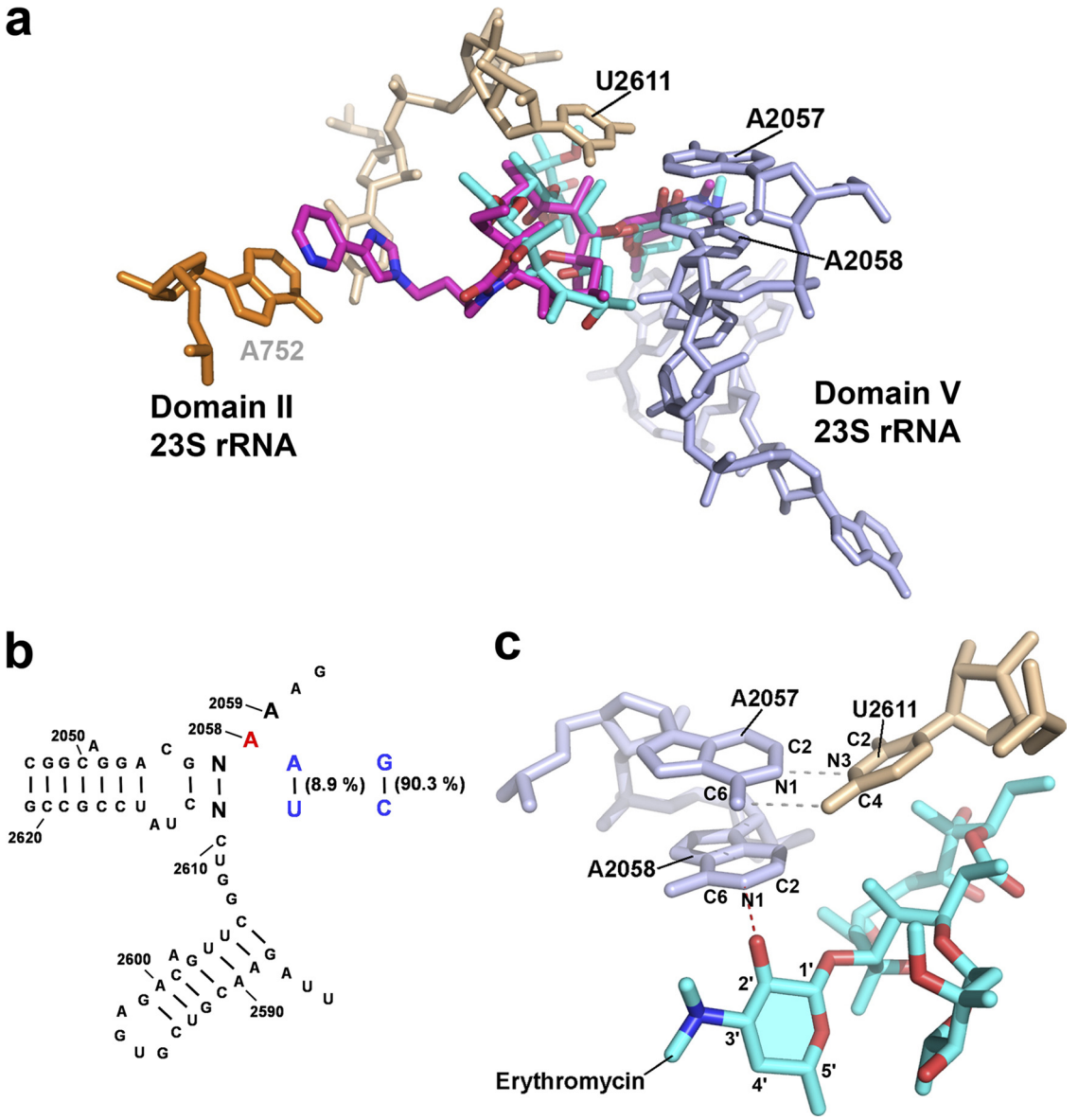

FIG. 1. (a) Structures of erythromycin (blue) (PDB accession code 3OHJ) and telithromycin (pink) (PDB accession code 3OI3) bound to the *Thermus thermophilus* ribosome. Nucleotides investigated in this study are indicated in black. (b) Secondary structure of domain V of the 23S rRNA and sequence conservation in eubacteria. rRNA residues are numbered according to their homologous positions in *E. coli* 23S rRNA. Phylogenetic sequence variations analyzed in this study are highlighted in colors: base pair 2057 · 2611 is represented in blue and the adenine at position 2058 in red. (c) Detailed view of the hydrogen bond interaction between N-1 of A2058 and the 2′-OH moiety of erythromycin's deosamine sugar (the desosamine sugar is highlighted, and the hydrogen bond interaction is shown as a red dotted line). The hydrogen bond interactions between A2057 and U2611 are indicated by gray dotted lines.

found in *Proteobacteria*, had no effect on susceptibility to any of the macrolides or ketolides tested (Table 2). Similarly, the composition of this base pair had no effect on resistance to erythromycin, clarithromycin, azithromycin, spiramycin, tylosin, and josamycin as conferred by the A2058G mutation. However, we confirmed that the ketolide resistance phenotype of A2058G is indeed dependent on the nature of the 2057 · 2611 base pair, as the A2058G mutant is 16-fold more susceptible to telithromycin in the context of a proteobacterial G2057 · C2611 sequence than in the context of a mycobacterial A2057 · U2611 (Table 2).

Phylogenetic sequence variations in 16S rRNA helix 44. Nucleotides of 16S rRNA helix 44 are part of the aminoacyl-tRNA acceptor site (A site) and are highly conserved (Fig. 2) (7). Aminoglycoside antibiotics bind to the A site by direct contacts to helix 44 (2). While aminoglycosides form a number of hydrogen bonds with different nucleotides in helix 44, their interactions with rRNA residues 1408, 1409, and 1491 (*E. coli* numbering) appear to be most critical for drug binding (11, 14, 15, 20) (Fig. 2).

16S rRNA residue 1408 is an adenine in all wild-type bacteria. Among all A-site mutations that confer aminoglycoside

TABLE 2. MICs of various macrolides/ketolides in *M. smegmatis* 23S rRNA variants

Clade homology (mutation)	Strains	Base(s) at 23S rRNA position(s):		MIC (μg/ml)[a]							
		2058	2057 · 2611	ERY	CLR	AZM	SPM	TYL	JSM	TEL	LZ (control)
e.g., *Mycobacteria*	SZ678-SZ680	A	A · U	8	1	4–8	2	2	2	0.25–0.5	1
e.g., *Proteobacteria*	SZ681-SZ684	A	G · C	8–16	1	2–4	1	2	1	0.25–0.5	1
Mycobacterial (A2058G)	SZ674-SZ677	G	A · U	>512	>512	>512	128	8	8	128	1
Proteobacterial (A2058G)	SZ685-SZ687	G	G · C	>512	>512	>512	64	4	8	8	1

[a] ERY, erythromycin; CLR, clarithromycin; AZM, azithromycin; SPM, spiramycin; TYL, tylosin; JSM, josamycin; TEL, telithromycin; LZ, linezolid. The MIC ranges shown are for at least 3 independent clones of the same mutation analyzed in 3 different experiments.

resistance, the 1408 adenine-to-guanine mutation (A1408G) is the predominant alteration in clinical drug-resistant strains (24, 28). This transition mutation alone is sufficient to confer high-level resistance to 6′-NH_2 aminoglycosides by disrupting the interaction between A1408 and the compound's ring 1 amino sugar (21). It is also thought to function as the main specificity determinant of aminoglycosides, because the cytoplasmic ribosomes of higher eukaryotes carry a guanine at this position (1, 25).

Bacterial A-site sequence variations within the aminoglycoside binding pocket involve base pair interactions 1409 · 1491, 1410 · 1490, and 1411 · 1489 (Fig. 2). The 1410 · 1490 pair is always a purine/pyrimidine interaction, either G · C (e.g., in *Actinomycetales*) or A · U (e.g., in *Proteobacteria*). The

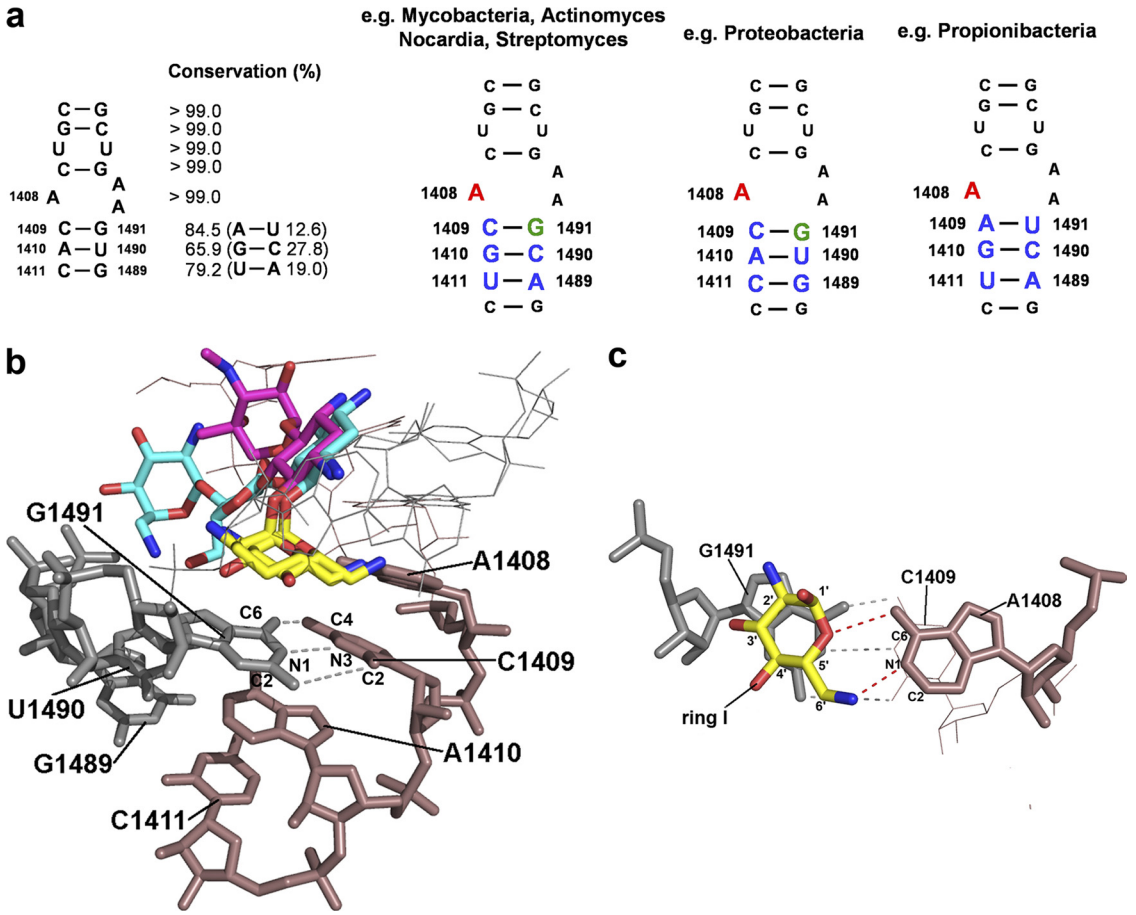

FIG. 2. (a) Secondary structure of 16S rRNA helix 44 decoding site and sequence conservation in eubacteria. rRNA residues are numbered according to their homologous positions in *E. coli* 16S rRNA. Phylogenetic sequence variations analyzed in this study are highlighted in colors: 1409, 1410, 1411, 1489, and 1490 in blue; the adenine at position 1408 in red; and the guanine at 1491 in green. (b) Structures of neomycin (blue) (PDB accession code 2QOY) and gentamicin (pink) (PDB accession code 2QB9) bound to the *Escherichia coli* A site. Ring 1 of the aminoglycosides is highlighted in yellow, nucleotides investigated in this study are numbered in black, and the hydrogen bond interactions between C1409 and G1491 are indicated by gray dotted lines. (c) Detailed view of the hydrogen bond and stacking interactions of neomycin's ring 1 with A1408 (shown as red dotted lines) and G1491, respectively. The gray dotted lines indicate hydrogen bonding between C1409 and G1491.

TABLE 3. MICs of various aminoglycosides in *M. smegmatis* 16S rRNA variants

Clade homology (mutation)	Strain(s)	Base(s) at 16S rRNA position(s):				MIC (μg/ml)[a]						
						4,5-substituted compounds		4,6-substituted compounds				LZ (control)
		1408	1409 · 1491	1410 · 1490	1411 · 1489	6′-OH	6′-NH_2	6′-NH_2				
						PAR	NEO	GEN	TOB	KAN	AMK	
e.g., *Mycobacteria*	SZ380	A	C · G	G · C	U · A	1	0.5	1	1	1	0.5	1
e.g., *Proteobacteria*	SZ721-SZ724	A	C · G	A · U	C · G	1	0.5	0.5–1	0.5–1	0.5	0.5	1–2
e.g., *Propionibacteria*	SZ763-SZ766	A	A · U	G · C	U · A	64	8–16	16	32	16	2	1
Mycobacterial (C1409U · G1491A)	SZ717-SZ720	A	U · A	G · C	U · A	8–16	1–2	1	2	1	0.5	1
Mycobacterial (C1409G · G1491C)	SZ605	A	G · C	G · C	U · A	32	2–4	4	4	2	0.5	0.5–1
Mycobacterial (A1408G)	SZ459-SZ462	G	C · G	G · C	U · A	64	>1,024	>1,024	>1,024	>1,024	>1,024	1
Proteobacterial (A1408G)	SZ725-SZ728	G	C · G	A · U	C · G	64	>1,024	>1,024	1,024	>1,024	>1,024	1
Propionibacterial (A1408G)	SZ832-SZ835	G	A · U	G · C	U · A	>1,024	>1,024	>1,024	>1,024	>1,024	>1,024	1

[a] PAR, paromomycin; NEO, neomycin; GEN, gentamicin; TOB, tobramycin; KAN, kanamycin A; AMK, amikacin; LZ, linezolid. The MIC ranges shown are for at least 3 independent clones of the same mutation analyzed in 3 different experiments.

1411 · 1489 interaction involves a pyrimidine/purine interaction, either U · A (e.g., in *Actinomycetales*) or C · G (e.g., in *Proteobacteria*). The 1409 · 1491 interaction involves a purine-pyrimidine switch: C · G (a pyrimidine/purine interaction) or A · U (a purine/pyrimidine interaction) (7). The majority of eubacteria are characterized by a 1409 pyrimidine · 1491 purine (C · G) interaction, while *Propionibacteria* carry a 1409 purine · 1491 pyrimidine (A · U) base pair (Fig. 2; for a phylogenetic comparison with clinically relevant phyla, see Table S2 in the supplemental material).

Bacterial sequence polymorphism of residues 1410 · 1490 and 1411 · 1489 was found not to affect the susceptibility of the wild-type drug binding pocket to aminoglycoside antibiotics, as the corresponding drug binding sites are highly susceptible to these compounds (Table 3, compare, e.g., *Mycobacteria* with *Proteobacteria*). Likewise, these sequence polymorphisms do not affect the resistance phenotype associated with the predominant mutational resistance alteration A1408G. Independent of the bacterial sequence polymorphism involving residues 1410 · 1490 and 1411 · 1489, an A1408G mutation results in high-level resistance to aminoglycosides with a 6′-NH_2 group, such as gentamicin and neomycin, but only limited resistance to aminoglycosides with a 6′-OH group, such as paromomycin (Table 3).

The sequence polymorphism at residues 1409 · 1491, which involves a purine/pyrimidine switch, was found to significantly affect the aminoglycoside susceptibility of the wild-type drug binding pocket. Replacing the C · G base pair in *M. smegmatis* with a propionibacterial A · U considerably reduced susceptibility to all 4,5- and 4,6-aminoglycosides tested (Table 3). This observation is in agreement with the lower aminoglycoside susceptibility of *Propionibacteria* reported previously (16). Amikacin is the least affected among the aminoglycosides tested, presumably because its L-haba group interacts with additional nucleotides within helix 44 and stabilizes drug binding (17).

To study the contribution of a 1409 · 1491 purine/pyrimidine base pair switch to aminoglycoside susceptibility in more detail, we investigated base pair interactions U · A and G · C. Base pair U1409 · A1491 represents a pyrimidine/purine interaction similar to the C · G found in the majority of eubacteria. Introduction of the U · A base pair decreased susceptibility to paromomycin, a 4,5-substituted aminoglycoside with a 6′-OH group, while it had little if any effect on aminoglycosides with a 6′-NH_2 group (Table 3). Introduction of a G1409 · C1491 purine/pyrimidine interaction similar to the A · U found in *Propionibacteria* resulted in minor but significant changes in susceptibility to both 4,5- and 4,6-aminoglycosides (with the exception of amikacin), with the 4,5-compounds being more affected (Table 3). From these data we infer that in the presence of an A1408 there is a gradient of drug susceptibility for the 1409 · 1491 interaction, indicating that both the purine/pyrimidine interaction and the specific nucleotide are relevant. In line with previous investigations and the different orientation of the aminoglycosides' sugars linked to position 5 or 6 of the neamine core (20) (Fig. 2), our results suggest that, in general, the 4,5-substituted compounds and in particular the 6′-OH paromomycin are more dependent on a proper 1409 · 1491 interaction than the 4,6-substituted compounds.

Combining the propionibacterial A1409 · U1491 pair further with an A1408G alteration resulted in high-level resistance to all aminoglycosides, including paromomycin (Table 3). Typically, binding of paromomycin, which carries a hydroxyl group at the 6′ position of ring I, is only moderately affected by the A1408G mutation, since it can accept a hydrogen bond from the N-1 and N-2 of G1408 (21, 35). Apparently, the high-level resistance to paromomycin is the result of a combined effect of perturbing contacts to both G1491 and A1408, which would be in agreement with previous data demonstrating that alteration of residue 1491 primarily increased resistance toward 6′-OH aminoglycosides such as paromomycin (20).

Given that stacking of aminoglycoside ring I on G1491 is important for binding (Fig. 2), we wished to study whether the bacterial 1410 · 1490 and 1411 · 1489 sequence variations affect the drug susceptibility pattern associated with C1409 · G1491 base pair disruptions. Accordingly, we replaced G1491 with A, C, or U in isogenic hybrid strains carrying the mycobacterial and proteobacterial A-site sequence. Drug sus-

TABLE 4. MICs of various aminoglycosides in *M. smegmatis* 16S rRNA variants with disruption of 1409 · 1491 base pairing

Clade homology (mutation)	Strains	Base(s) at 16S rRNA position(s):				MIC (μg/ml)[a]						
						4,5-substituted compounds		4,6-substituted compounds				
		1408	1409 · 1491	1410 · 1490	1411 · 1489	6′-OH	6′-NH_2	6′-NH_2				LZ (control)
						PAR	NEO	GEN	TOB	KAN	AMK	
Mycobacterial (G1491A)	SZ463-SZ466	A	C · A	G · C	U · A	32–64	2	2	2	1–2	0.5	0.5–1
Proteobacterial (G1491A)	SZ706-SZ709	A	C · A	A · U	C · G	64	2–4	2–4	2	1–2	0.5	1
Mycobacterial (G1491C)	SZ467-SZ470	A	C · C	G · C	U · A	512	16	16–32	16	16–32	4	0.5–1
Proteobacterial (G1491C)	SZ710-SZ713	A	C · C	A · U	C · G	>1,024	8–16	32	16–32	32	8	1
Mycobacterial (G1491U)	SZ505-SZ507	A	C · U	G · C	U · A	512–1,024	8–16	64	64–128	64	16	1
Proteobacterial (G1491U)	SZ714-SZ716	A	C · U	A · U	C · G	512	8	64	64	32	8	1

[a] PAR, paromomycin; NEO, neomycin; GEN, gentamicin; TOB, tobramycin; KAN, kanamycin A; AMK, amikacin; LZ, linezolid. The MIC ranges shown are for at least 3 independent clones of the same mutation analyzed in 3 different experiments.

ceptibility testing of the recombinant mutants revealed that the context of a mycobacterial or proteobacterial A site does not affect the specific drug resistance pattern associated with distinct alterations of residue 1491 (Table 4) (see reference 11 for a structural discussion of resistance patterns conferred by mutational alteration of G1491).

Previously, limitations in genetic manipulation did not allow study of the effect of bacterial A-site polymorphism on aminoglycoside susceptibility in isogenic mutants. Rather, investigations were limited to testing different bacterial species representative of the corresponding sequence polymorphism. In these early studies it was concluded that the C1409 · G1491/A1409 · U1491 polymorphism is not associated with resistance (21). Using more recently developed genetic techniques, we have now been able to refine this statement and to define the role of the 1409 · 1491 base-pairing polymorphism in aminoglycoside susceptibility more precisely. In addition, we have recently observed that the 16S rRNA 1410 · 1490 interaction has a subtle influence on aminoglycoside susceptibility in ribosomes with a non-Watson-Crick 1409 · 1491 interaction, e.g., C1409 · C1491 (12). We extend these findings in our current study, which reveals that in the presence of a 1409 · 1491 base pair interaction, bacterial 1410 · 1490 sequence polymorphisms do not measurably affect aminoglycoside susceptibility.

Conclusions. The ribosome is a target for many different classes of antibiotic compounds (23). We have studied the effects of minor phylogenetic differences in the species-specific compositions of the drug binding site on drug susceptibility and resistance. From our results we conclude that natural sequence variations in the ribosomal peptidyltransferase center of bacteria do not affect macrolide/ketolide susceptibility but do affect the resistance phenotype of the A2058G mutation, in particular resistance to the ketolide telithromycin. In contrast, natural sequence variations in the ribosomal A site of bacteria affect both the aminoglycoside susceptibility of the wild-type drug binding pocket and the resistance phenotype associated with the A1408G alteration.

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