Supplemental Materials

Figure S1. Generation of *rplD* erythromycin-resistance mutations. Pairwise alignment of wild-type *R. marinus rplD* gene and uL4 protein sequences with the corresponding sequences from each of the three erythromycin-resistant mutants SOP57 (*rplD1*), SOP25 (*rplD2*), and SOP16 (*rplD3*). The sequence deleted and mutated in *rplD1* is highlighted in grey, as are the direct repeat sequences involved in homologous recombination to produce the *rplD2* and *rplD3* alleles. Sequences were aligned using Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) [54].

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WT
AAGAACCGGGCCGAAAACGCCCACAGCACCCGCAAGCTGTACCGCCAGAAAGGTACGGGTTACGCCCGGGCCGGCGATGCCAAGTCGCCCATTCGTCGGGGGGGTGGTACGGCCCACGGG
 K  N  R  A  E  N  A  H  S  T  R  K  L  Y  R  Q  K  G  T  G  Y  A  R  A  G  D  A  K  S  P  I  R  R  G  G  G  T  A  H  G
      50                         58 59 60                         70                              80

rplD1 (K58N,ΔA50-R69)
AAGAACCGGGCCGAAAACGCCCACAGCACCCGCAACCGCCAGAAAGGTACGGGTTACGCCCGGGCCGGCGATGCCAAGTCGCCCATTCGTCGGGGGGGTGGTACGGCCCACGGG
 K  N  R  A  E  N  A  H  S  T  R  N  R  Q  K  G  T  G  Y  A  R  A  G  D  A  K  S  P  I  R  R  G  G  G  T  A  H  G
      50                        K58N,ΔL59-Y60              70                              80


WT
AAGAACCGGGCCGAAAACGCCCACAGCACCCGCAAGCTGTACCGCCAGAAAGGTACGGGTTACGCCCGGGCCGGCGATGCCAAGTCGCCCATTCGTCGGGGGGGTGGTACGGCCCACGGG
 K  N  R  A  E  N  A  H  S  T  R  K  L  Y  R  Q  K  G  T  G  Y  A  R  A  G  D  A  K  S  P  I  R  R  G  G  G  T  A  H  G
      50                            60                    70                              80

rplD2 (ΔA50-R69)
AAGAACCGGGCCGGCGATGCCAAGTCGCCCATTCGTCGGGGGGGTGGTACGGCCCACGGG
 K  N  R  A  G  D  A  K  S  P  I  R  R  G  G  G  T  A  H  G
      50 71                        80


WT
AAGAACCGGGCCGAAAACGCCCACAGCACCCGCAAGCTGTACCGCCAGAAAGGTACGGGTTACGCCCGGGCCGGCGATGCCAAGTCGCCCATTCGTCGGGGGGGTGGTACGGCCCACGGG
 K  N  R  A  E  N  A  H  S  T  R  K  L  Y  R  Q  K  G  T  G  Y  A  R  A  G  D  A  K  S  P  I  R  R  G  G  G  T  A  H  G
      50                            60       64           70                              80          84

rplD3 (ΔT65-G82)
AAGAACCGGGCCGAAAACGCCCACAGCACCCGCAAGCTGTACCGCCAGAAAGGTACGGCCCACGGG
 K  N  R  A  E  N  A  H  S  T  R  K  L  Y  R  Q  K  G  T  A  H  G
      50                            60       64    84
```

Table S1. Oligonucleotides used in this study. Locus tags and genome coordinates are taken from the accession number NC_013501.1 [17].

Name	Sequence (5'-3')	Gene (Gene Product)	Locus Tag	Genome Coordinates
Rma_rrnA_f1	GCGAATCTGGCCGGAACCGAACAGGC	*rrsA* (16S rRNA)	RMAR_RS00885	186847-186872
Rma_rrnA_f2	CATGCGGCTGAAAGGAACCCGGCCG	*rrsA* (16S rRNA)	RMAR_RS00885	186751-186775
Rma_rrnA_f3	CGACGGTTGATGTAAATTGCCGGTCC	*rrsA* (16S rRNA)	RMAR_RS00885	187033-187058
Rma_rrnA_f4	GTAAACCGAGCAAAGCAAGGAG	*rrlA* (23S rRNA)	RMAR_RS00900	189144-189165
Rma_rrnA_f5	TAGCCCTTAATTATAAGGTATCGG	*pyrR* (PyrR)	RMAR_RS00870	185941-185964
Rma_rrnA_f6	GGAGCATGTGGCTTAATTCGATGC	*rrsA* (16S rRNA)	RMAR_RS00885	188156-188179
Rma_rrnA_f7	GACGCGAAACCGGGTGAG	*rrlA* (23S rRNA)	RMAR_RS00900	189932-189949
Rma_rrnA_f8	GGTCCTAAGGTAGCGAAATTCC	*rrlA* (23S rRNA)	RMAR_RS00900	191195-191216
Rma_rrnA_r1	GAACGCACAAGGCGCGCGATCTATCC	*rrfA* (5S rRNA)	RMAR_RS00905	192478-192503
Rma_rrnA_r2	TCGTGGAGCAGCGTTTCGGCCATAGG	*purL* (PRFG synthase)	RMAR_RO00910	192532-192557
Rma_rrnA_r3	TCGACGATCCAGCCGTATTCTTCGTC	*purL* (PRFG synthase)	RMAR_RO00910	192601-192626
Rma_rrnA_r4	CTGTCGTTCCGCGAGTAGGCGGAG	*rrsA* (16S rRNA)	RMAR_RS00885	188770-188793
Rma_rrnA_r5	CCGAAGCTTCGGCGCCAGGCTTG	*rrlA* (23S rRNA)	RMAR_RS00900	190406-190428
Rma_rrnA_r6	GCACAACTGGTACACTAGAGG	*rrlA* (23S rRNA)	RMAR_RS00900	191954-191974
Rma_rrnA_r7	TTGCACTCTACGCACGATTACC	*rrlA* (23S rRNA)	RMAR_RS00900	191581-191602
Rma_rrnA_r8	CCTGATATCTACGCATTTCACC	*rrsA* (16S rRNA)	RMAR_RS00885	187903-187924
Rma_rplD_f1	CTGTAAACGGCATTGTCGAATTG	*rplD* (r-protein uL4)	RMAR_RS04205	965925-965947
Rma_rplD_r1	CGTCCATCTGCTGCGAGAGC	*rplD* (r-protein uL4)	RMAR_RS04205	966705-966724
Rma_rplV_f1	GGTCTATATCACGGAGAACATGGTC	*rplV* (r-protein uL22)	RMAR_RS04225	968074-968098
Rma_rplV_r1	CGCCCAGGCGAAATCCTATCGG	*rplV* (r-protein uL22)	RMAR_RS04225	968617-968638
Rma_rplK_f1	ACAGAAGCATTCGAGAGGCGC	*rplK* (r-protein uL11)	RMAR_RS05505	1275101-1275121
Rma_rplK_r1	TCTCAAGCGCCTGGCGATAGCG	*rplK* (r-protein uL11)	RMAR_RS05505	1275704-1275725
Rma_rpoB_f1	CTCTATCCAATTTGCAGGCG	*rpoB* (RNAP β-subunit)	RMAR_RS05525	1277636-1277655
Rma_rpoB_f2	CGCATGGCCCGTACCATC	*rpoB* (RNAP β-subunit)	RMAR_RS05525	1279042-1279059
Rma_rpoB_r1	TCTTCCCTTGCGGCATAGCG	*rpoB* (RNAP β-subunit)	RMAR_RS05525	1281600-1281619
Rma_rpoB_r2	CTTGACGAACTCGTTGAGG	*rpoB* (RNAP β-subunit)	RMAR_RS05525	1279521-1279539

Table S2. Antibiotic zones of inhibition. Discs were 6 mm in diameter.

Antibiotic Class (Target)	Antibiotic[1]	Zone (mm)
14-atom macrolide (ribosome, 50S)	erythromycin	41
	oleandomycin	26
	roxithromycin	46
	clarithromycin	53
15-atom macrolide (ribosome, 50S)	azithromycin	31
16-atom macrolide (ribosome, 50S)	spiramycin	35
	chalcomycin	18
	tylosin	59
	carbomycin	53
lincosamide (ribosome, 50S)	lincomycin	64
	clindamycin	71
streptogramin B (ribosome, 50S)	pristinamycin	51
pleuromutilin (ribosome, 50S)	tiamulin	44
amphenicol (ribosome, 50S)	chloramphenicol	19
thiopeptide (ribosome, 50S)	thiostrepton	43
elfamycin (EF-Tu)	kirromycin	38
fusidane (EF-G)	fusidic acid	36
rifamycin (RNA polymerase)	rifampicin	43
fluoroquinolone (DNA gyrase)	ofloxacin	41
	ciprofloxacin	46

[1] Antibiotics not listed produced no zones of inhibition.

References

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