Table S1. List of bacterial strains used in this study.

Name	Description/genotype	Marker	Reference
Strains			
M. abscessus Smooth (S)	*M. abscessus sensu stricto*, strain CIP104536^T, S morphotype	_	Laboratoire de Référence des Mycobactéries
M. abscessus Rough (R)	*M. abscessus sensu stricto*, strain CIP104536 ^{T}, R morphotype	_	Laboratoire de Référence des Mycobactéries
M. massiliense (R)	*M. abscessus massiliense*, strain CIP108297^T, R morphotype	_	Laboratoire de Référence des Mycobactéries
M. bolletii (S)	*M. abscessus bolletii*, strain CIP108541^T, S morphotype	_	Laboratoire de Référence des Mycobactéries
M. abscessus S 1298 (S)	*M. abscessus sensu stricto,* clinical isolate from a cystic fibrosis (CF) patient, S morphotype	_	(1)
M. abscessus S 2069 (S)	*M. abscessus sensu stricto,* clinical isolate from a non-CF patient, S morphotype	_	(1)
M. abscessus R 2648 (R)	*M. abscessus sensu stricto,* clinical isolate from a CF patient, R morphotype	_	(1)
M. abscessus R 3022 (R)	*M. abscessus sensu stricto,* clinical isolate from a non-CF patient, R morphotype	_	(1)
M. abscessus S - Δ*erm(41)*	*erm(41)* unmarked deletion mutant in the S variant *of* CIP104536^T	-	This study
E. coli XL1-Blue	*recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac* [F´ *proAB lacI*q*Z*Δ*M15* Tn*10* (Tetr)].	Tet	Stratagene

Table S2. PCR primers used in this study. Fow and Rev stand for forward and reverse, respectively.

Primers	5' to 3' sequence
Cloning in pMV261_*lacZ* derivatives	
P_{123} Fow	GGTATATCTAGAGCGCACGTTCTGACGAAAGAA (XbaI)
P_{123} Rev	GGTATAGGATCCGACGCAGCACCATCATGAATA (BamHI)
P_{92} Fow	GGTATATCTAGAATGGCGACCGGGGCCTTCTTCGTG (XbaI)
P_{61} Fow	GGTATATCTAGAGAAACCAGTTGCATGCCCCGATAT (XbaI)
P_{38} Fow	CTAGATCTTTGGAGCATGGGCATATTCATGATGGTGCTGCGTCG (XbaI)
P_{38} Rev	GATCCGACGCAGCACCATCATGAATATGCCCATGCTCCAAAGAT (BamHI)
$P_{Mut(B7)}$ Fow	CTAGAG**GGGG**CAGTTGCATGCCCCGATATCTTTGGAGCATGGGCATATTCATGATGGTGCTGCGTCG (XbaI)
$P_{Mut(B7)}$ Rev	GATCCGACGCAGCACCATCATGAATATGCCCATGCTCCAAAGATATCGGGGCATGCAACTG**CCCC**CT (BamHI)
$P_{Mut(-35)}$ Fow	CTAGAGAAACCAG**CCATGC**GCCCCGATATCTTTGGAGCATGGGCATATTCATGATGGTGCTGCGTCG (XbaI)
$P_{Mut(-35)}$ Rev	GATCCGACGCAGCACCATCATGAATATGCCCATGCTCCAAAGATATCGGGGC**GCATGG**CTGGTTTCT (BamHI)
$P_{Mut(B7/-35)}$ Fow	CTAGAG**GGGG**CAG**CCATGC**GCCCCGATATCTTTGGAGCATGGGCATATTCATGATGGTGCTGCGTCG (XbaI)
$P_{Mut(B7/-35)}$ Rev	GATCCGACGCAGCACCATCATGAATATGCCCATGCTCCAAAGATATCGGGGC**GCATGG**CTG**CCCC**CT (BamHI)
Cloning in pMV361-ApraR	
361-*whiB7* Fow	ACTTCGCAATGATGACCGTTGAAGTGGAG
361-*whiB7* Rev	CTAAGCGTAATCTGGAACATCGTATGGGTATGCCGCGGCGGTGTCGGCGTC
Cloning in pMV261_P_{123}_*tdTomato*	
261-td*Tomato* Fow	GAGAGAGGATCCGTGAGCAAGGGCGAGGAG (BamHI)
261-td*Tomato* Rev	GAGAGAAAGCTTCTACTTGTACAGCTCGTC (HindIII)
Cloning in pUX1-*katG*	
*erm(41)*KO_U_F	GAGAGACAATTGCGCGATCTGCAGCCGTATATC (MfeI)
*erm(41)*KO_U_R	CCGTTGGCCGGACACGAC
*erm(41)*KO_D_F	TGGTGCTGCGTCGTGTCCGGCCAACGGGTGCTGGTGATCAGGCGGCGCTGA
*erm(41)*KO_D_R	GAGAGAGCTAGCTGCACCAGAACGGCGCGT (XbaI)
Sequencing	
pMV5' Ext	CGCCCGGCCAGCGTAAGTAGC
l*acZ* intern Rev	GATACAGCGCGTCGTGATTA
erm(41) Fow	ACGCCGAGGCCGAGCGCCGTCACA
erm(41) Rev	CGCAGTATCGTTTCTCCAAAGGCC

[a]Restriction sites are underlined and specified inside brackets.

[b]Mutagenized bases are shown in bold.

Tables S3. List of the plasmids used in this study.

Plasmids			
pTEC27	Multicopy *E. coli*/mycobacterial shuttle vector to express *tdTomato* under the control of a strong mycobacterial promoter	Hyg	Addgene (plasmid 30182)
pMV261	Multicopy *E. coli*/mycobacterial shuttle vector	Kan	(2)
pMV261_P_{hsp60}_*lacZ*	The *hsp60* promoter region is cloned upstream of *lacZ* into pMV261.	Kan	(3)
pMV261_P_{123}_*lacZ*	The full intergenic region of *erm(41)* of 123 bp is cloned upstream of *lacZ* into pMV261.	Kan	This study
pMV261_P_{92}_*lacZ*	A truncated version of 92 bp of the *erm(41)* intergenic region is cloned upstream of *lacZ* into pMV261.	Kan	This study
pMV261_P_{61}_*lacZ*	A truncated version of 61 bp of the *erm(41)* intergenic region is cloned upstream of *lacZ* into pMV261.	Kan	This study
pMV261_P_{38}_*lacZ*	A truncated version of 38 bp of the *erm(41)* intergenic region is cloned upstream of *lacZ* into pMV261.	Kan	This study
pMV261_ $P_{61_Mut(B7)}$_*lacZ*	A truncated version of 61 bp of the *erm(41)* intergenic region containing mutations in the *whiB7* binding site is cloned upstream of *lacZ* into pMV261.	Kan	This study
pMV261_ $P_{61_Mut(-35)}$_*lacZ*	A truncated version of 61 bp of the *erm(41)* intergenic region containing mutation in the putative *erm(41)* -35 box is cloned upstream of *lacZ* into pMV261.	Kan	This study
pMV261_ $P_{61_Mut(B7/-35)}$_*lacZ*	A truncated version of 61 bp of the *erm(41)* intergenic region containing mutation into the *whiB7* binding site and in the putative *erm(41)* -35 box is cloned upstream of *lacZ* into pMV261.	Kan	This study
pMV261_*lacZ*	The *lacZ* reporter gene is cloned into a promoter-less pMV261.	Kan	(3)
pMV261_P_{MAB_4384}_*lacZ*	pMV261_*lacZ* carrying the promoter region of *MAB_4384* cloned upstream of *lacZ*	Kan	(3)
pMV261_ P_{123}_*tdTomato*	The red fluorescent marker td*Tomato* is cloned into the pMV261 under the control of the full/123bp *erm(41)* intergenic region.	Kan	This study
pMV361_*whiB7*	*whiB7* cloned into the integrative vector pMV361 under the control of the strong and constitutive *hsp60* promoter.	Apra	This study

Hyg, hygromycin; Kan, kanamycin; Apra, apramycin.

References

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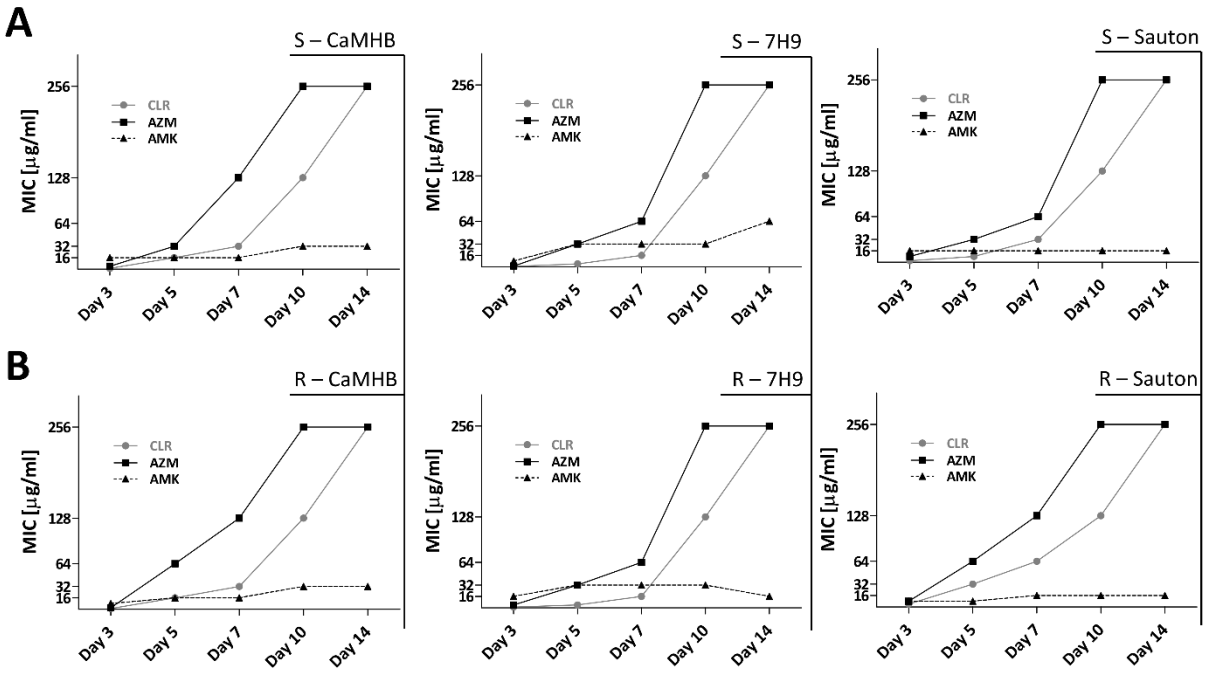

Figure S1. Macrolide-induced resistance profile of *M. abscessus* CIP104536^T smooth (A) and rough (B) variants in different broth media. MICs of the two morphotypes were assessed over a period of 14 days in CaMHB, Sauton's medium and Middlebrook 7H9-Glycerol broth. AMK was included as a non-related control drug.

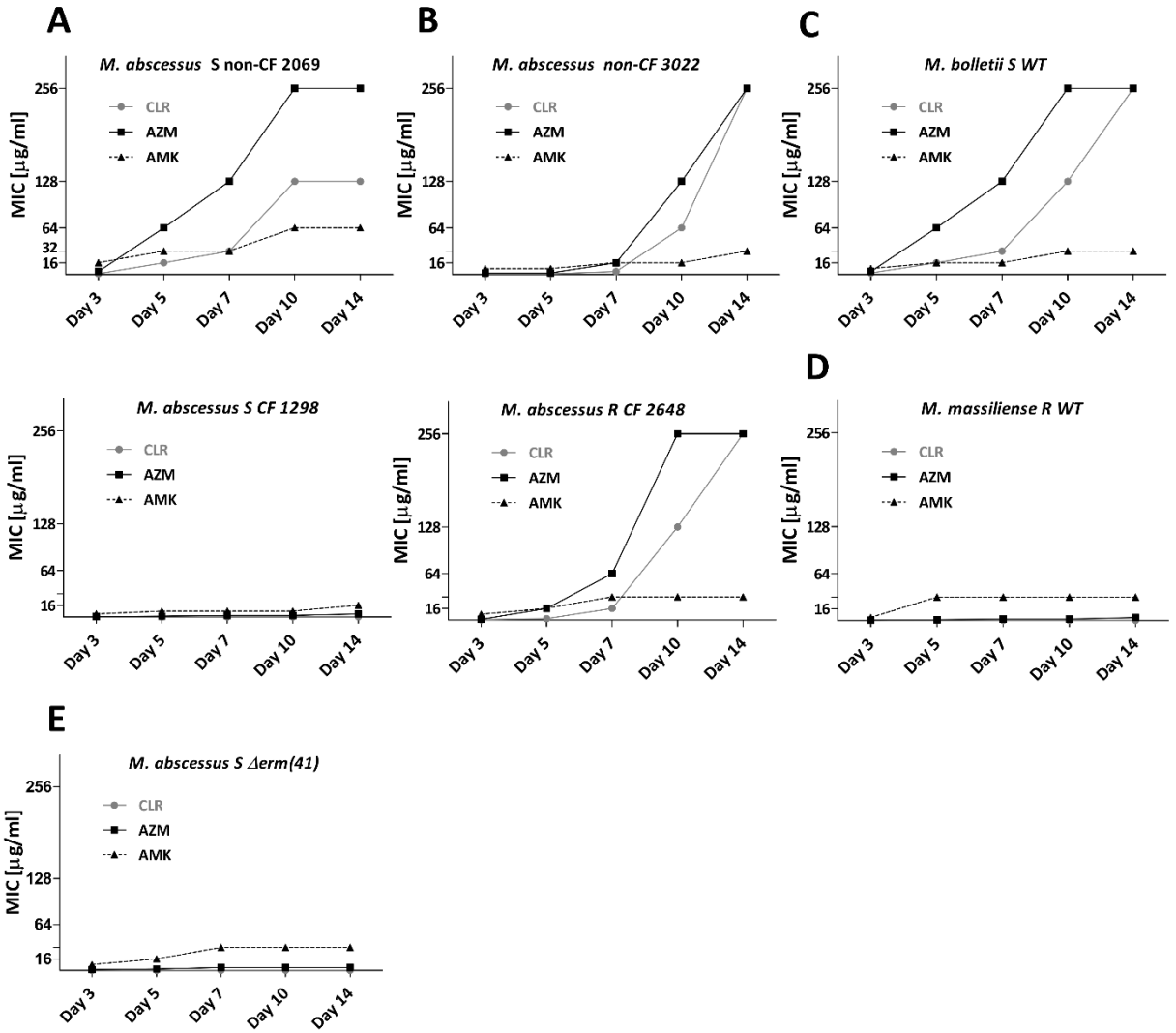

Figure S2. Macrolide-induced resistance profile of clinical isolates in CaMHB. (A) Smooth *M. abscessus* clinical isolates. **(B)** Rough *M. abscessus* clinical isolates. **(C)** Smooth *M. bolletii* CIP108541^{T}. **(D)** Rough *M. massiliense* CIP108297^{T}. **(E)** Smooth *M. abscessus* CIP104536^{T} in which the *erm(41)* gene has been deleted by double homologous recombination using the suicide-vector pUX1-*katG*. AMK was included as a non-related control drug.

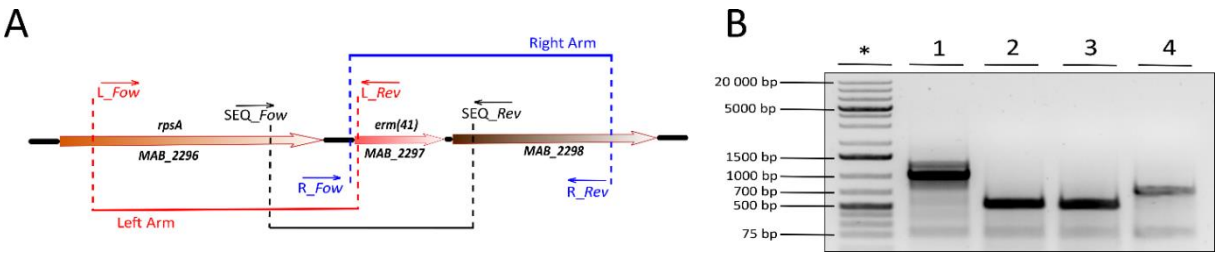

Figure S3. Unmarked deletion of the *erm(41)* gene. (A) Genomic environment of *erm(41)*. The L_*Fow*/L_*Rev* and the R_*Fow*/R_*Rev* primer sets were used to produce left and right arms, respectively, which were subsequently used to generate pUX1_*katG-erm(41)* to delete *erm(41)* by double homologous recombination. **(B)** 1 % agarose gel of the amplicons using the SEQ_Fow an SEQ_Rev primers. The expected sizes are: *M. abscessus* WT (1034 bp; lane 1), *M. abscessus* Δ*erm(41*) Clone 1 (509 bp; lane 2), *M. abscessus* Δ*erm(41)* Clone 2 (509 bp; lane 3) and *M. massiliense* WT (758 bp; lane 4). (*) GeneRuler^{TM} 1 kb Plus DNA Ladder.