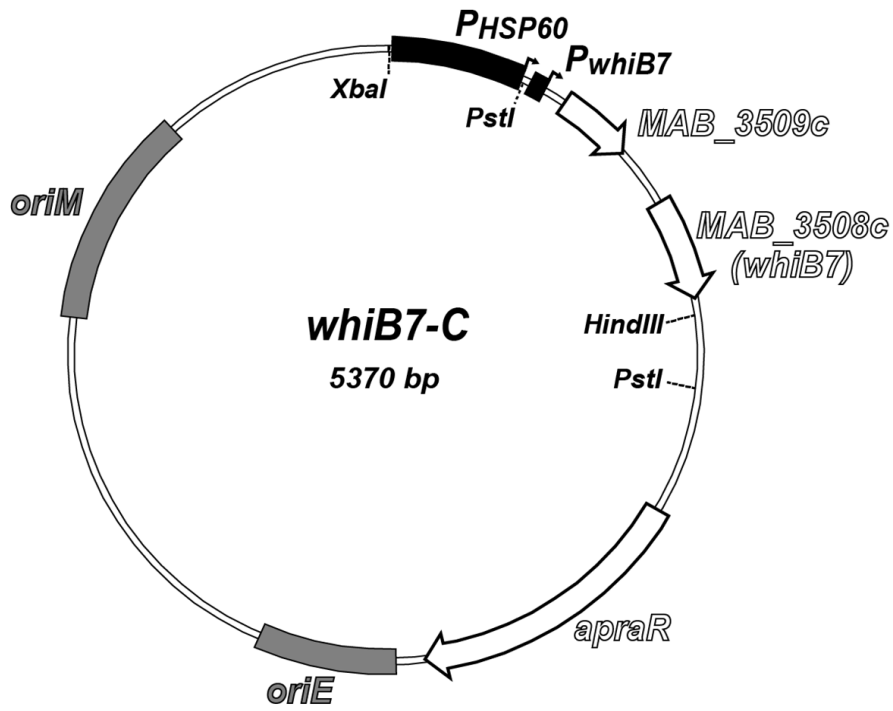


1 Supplemental Figure S1.

2 Map of pWhiB7-C



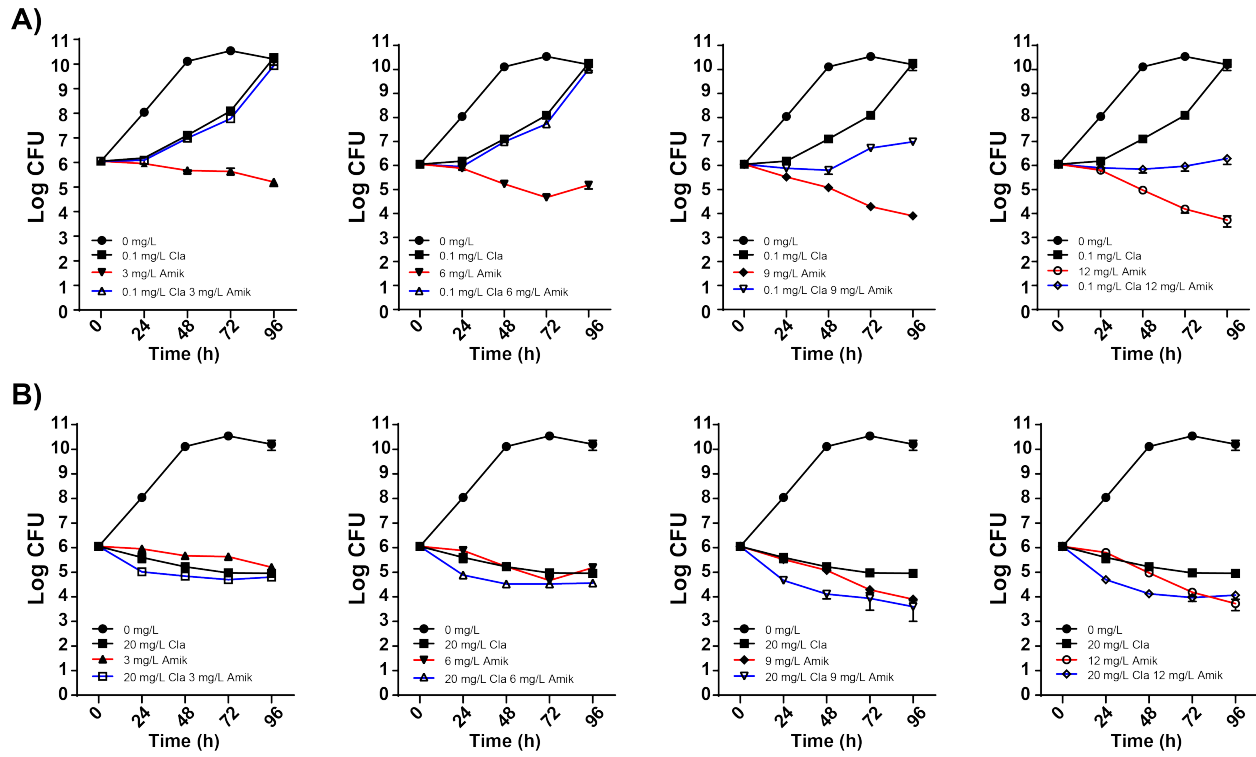
- 3
- 4
- 5
- 6
- 7
- 8
- 9
- 10
- 11
- 12
- 13
- 14
- 15
- 16
- 17
- 18
- 19
- 20
- 21
- 22
- 23
- 24
- 25

26 **Supplemental Figure S1. *whiB7* (MAB_3508c) sequence.**

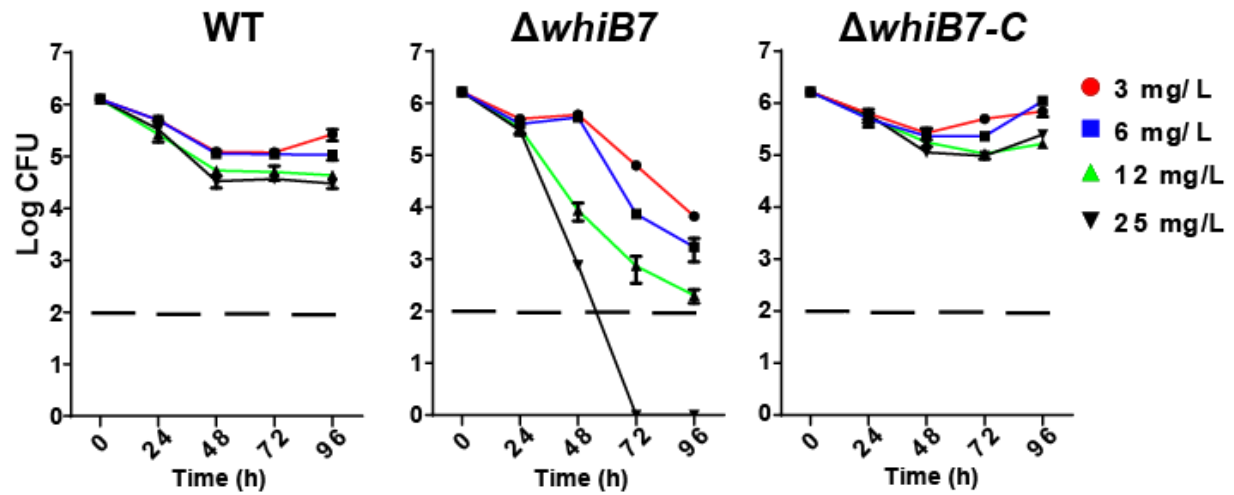
27	ID	whiB7-C	standard; circular DNA;	; 5370 BP.
28	FH	Key	Location/Qualifiers	
29	FH			
30	FT	CDS	508..720	
31	FT		/vntifkey="4"	
32	FT		/label=MAB_3509c	
33	FT	CDS	890..1174	
34	FT		/vntifkey="4"	
35	FT		/label=MAB_3508c\ (whiB7)	
36	FT	rep_origin	4134..4734	
37	FT		/vntifkey="33"	
38	FT		/label=oriM	
39	FT	promoter	7..377	
40	FT		/vntifkey="30"	
41	FT		/label=PHSP60	
42	FT		/note="HSP60 promoter region"	
43	FT	CDS	1790..2574	
44	FT		/vntifkey="4"	
45	FT		/label=apraR	
46	FT	rep_origin	2654..3034	
47	FT		/vntifkey="33"	
48	FT		/label=oriE	
49	FT	promoter	399..445	
50	FT		/vntifkey="30"	
51	FT		/label=PwhiB7	
52	SQ	Sequence	5370 BP; 1107 A; 1560 C; 1642 G; 1061 t;	

53							
54	ctagacggtg	accacaacgc	gccgcgtttg	atcggggacg	tctgcggccg	accatttacg	60
55	ggtcttggtg	tcggttggcg	tcattgggccc	aacataactca	cccggatcgg	agggccgagg	120
56	acaaggtcga	acgaggggca	tgaccgggtg	cggggcttct	tgcactcggc	ataggcgagt	180
57	gctaagaata	acgttggcac	tcgcgaccgg	tgagtgctag	gtcgggacgg	tgaggccagg	240
58	cccgtcgctg	cagcgagtgg	cagcgaggac	aacttgagcc	gtccgctcgg	ggcactgcgc	300
59	ccggccagcg	taagtagcgg	ggttgccgtc	accgggtgac	ccccgtttca	tccccgatcc	360
60	ggaggaatca	cttcgcactg	caggtatcgc	cgcaggtcaa	aaataacttg	tcaaccgata	420
61	ggtgaccttt	tactctgggc	ttcaggaatt	cgcgaggatt	ccaaaccaag	acctgttcac	480
62	gtgcgagtag	acggaaggag	gagcgaaatg	aacacaccca	ccaattacgg	agacaccttg	540
63	tggcgtgatg	cccattggtg	ctttgtcgtg	ttgccgatcg	ctccgcccgc	gctccagacg	600
64	cgtgccgccc	ccaagccggc	ggtgactgcc	gtggtgatgg	ccggctcggc	ttcccgatatt	660
65	aagcaccccg	ccgcccgtcg	agccgcgaat	gtcgcgtccg	tgaatagggg	atacatctag	720
66	gtatccccac	aaccgcctcc	caggccacgg	accggacacc	accgggatcc	gtggccacat	780
67	ttgtttgagc	cccaaacctc	aagcgtggtt	ccggatctcg	atcgataaga	caccgatacg	840
68	agaccagcag	ttttacgagg	aaccgaagag	gaagcaggtg	aacggacaca	tgatgaccgt	900
69	tgaagtggag	gcccgaagac	tcgcgctgcc	gtgccacgtc	gcgagcggcg	acctgtgggt	960
70	cgcggaagc	ccgcccgacc	tggagcgggc	gaaggcgtg	tgcgcccgact	gcccgatccg	1020
71	gtcgcagtgc	ctggccgctg	cgctggaccg	cgagagccg	tggggagtgt	ggggcggatga	1080
72	gattcttgag	caggggacca	ttgtggcggc	caagcggccg	cgtggacgcc	cgcgcaagaa	1140
73	cccactgccc	gacgcccaca	ccgcccgggc	atgacgccgc	cgcccaagaa	ggttgtaggg	1200
74	cctgaagctt	ttaaggttta	acggttgggg	acaacaagcc	agggatgtaa	cgcactgaga	1260
75	agcccttaga	gcctctcaaa	gcaattttga	gtgacacagg	aacacttaac	ggctgacatg	1320
76	ggaattcttg	aagacgaaaag	ggcctcgtga	tacgcctatt	tttatagggt	aatgtcatga	1380
77	taataatggt	ttcttagacg	tcaggtggca	cttttcgggg	ttaaagggct	gcaggaattc	1440
78	gatagcttgc	tgacgcccgt	ggatacacca	aggaaagtct	acacgaaccc	tttggcaaaa	1500
79	tcctgtatat	cgtgcgaaaa	aggatggata	taccgaaaaa	atcgctataa	tgaccccgaa	1560
80	gcaggggtat	gcagcggaaa	atgcagctca	cggtaactga	tgccgtatgt	gcagtaccag	1620
81	cgtacggccc	acagaatgat	gtcacgctga	aaatgccggc	ctttgatggg	gttcatgtgc	1680
82	agctccataa	gcaaaagggg	atgataagtt	tatcaccacc	gactatttgc	aacagtgccg	1740
83	ttgatcgtgc	tatgatcgac	tgatgtcatc	agcgggtggag	tgcaatgtcg	tgcaatacga	1800
84	atggcgaaaa	gccagactca	ctcgtcagct	tctcaacctt	ggggttacc	ccggcgggtg	1860
85	gctgctggtc	cacagctcct	tccgtagcgt	ccggcccctc	gaagatgggc	cacttggact	1920
86	gatcgaggcc	ctgcgctgct	cgctgggtcc	gggagggacg	ctcgtcatgc	cctcgtggtc	1980
87	aggtctggac	gacgagccgt	tcgatcctgc	cacgtcgccc	gttacaccgg	accttggagt	2040

tgtctctgac	acattctggc	gcttgcacaa	tgtaaagcgc	agcgcceatc	catttgccctt	2100
cgcgccagcg	gggccacagg	cagagcagat	catctctgat	ccattgcccc	tgccacctca	2160
ctcgccctgca	agcccggctc	cccgtgtcca	tgaactcgat	gggcaggtag	ttctcctcgg	2220
cgtgggacac	gatgccaaaca	cgacgctgca	tcttgccgag	ttgatggcaa	aggttcccta	2280
tggggtgccg	agacactgca	ccattcttca	tgatggcaag	ttggtagcgc	tcgattatct	2340
cgagaatgac	cactgctgtg	agcgctttgc	cttggcggac	aggtggctca	aggagaagag	2400
ccttcagaag	gaaggtccag	tcggtcatgc	ctttgctcgg	ttgatccgct	cccgcgacat	2460
tgtggcgaca	gccctgggtc	aactgggccc	agatccgttg	atcttctctg	atccgccaga	2520
ggcgggatgc	gaagaatgcg	atgcccgtcg	ccagtcgatt	ggctgagctc	ataagttcct	2580
attccgaagt	tcctattctc	tagtcgaggg	ggggcccag	cttagctggc	tagcgagtca	2640
tgaggtcacc	atgctagtct	cactgagcgt	cagaccccgt	agaaaagatc	aaaggatctt	2700
cttgagatcc	ttttttctg	cgctaatct	gctgcttca	acaaaaaaaa	ccaccgctac	2760
cagcgggtgt	ttgtttgccg	gatcaagagc	taccaactct	ttttccgaag	gtaactggct	2820
tcagcagtcg	gcagataccc	aatactgtcc	ttctagtga	ccgtagtta	ggccaccact	2880
tcaagaactc	gttagcaccg	cctacatacc	tcgctctgct	aatcctgtta	ccagtggtcg	2940
ctgccagtgg	cgataagtcg	tgtcttaccg	ggttggactc	aagacgatag	ttaccggata	3000
aggcgcagcg	gtcgggctga	acggggggtt	cgtgcacaca	gccagcttg	gagcgaacga	3060
cctacaccga	actgagatac	ctacagcgtg	agcattgaga	aagcgcacag	cttcccgaag	3120
ggagaaaggc	ggacaggtat	ccgtaagcg	gcagggctcg	aacaggagag	cgcacagagg	3180
agcttccagg	gggaaacgcg	tggtatcttt	atagtcctgt	cgggtttcgc	cacctctgac	3240
ttgacgctcg	atthttgtga	tgctcgctcag	gggggcggag	cctatggaaa	aacgccagca	3300
acgcggcctt	tttacggttc	ctggcctttt	gctggccttt	tgctcacatg	ttctttcctg	3360
cgttatcccc	tgattctgtg	gataaccgta	ttaccgcctt	tgagtgagct	gataccgctc	3420
gccgcagccg	aacgaccgag	cgcaacgcgt	gagcccacca	gctccgtaag	ttcgggtgct	3480
gtgtggctcg	taccgcgca	ttcaggcggc	agggggtcta	acgggtctaa	ggcggcgtgt	3540
acggccgcca	cagcggctct	tagcggcccg	gaaacgtcct	cgaaacgacg	catgtgttcc	3600
tcctggttgg	tacaggtggt	tgggggtgct	cggctgtcgc	tggtgtttca	tcacagggc	3660
tcgacgggag	agcgggggag	tgtgcagttg	tgggggtggc	cctcagcgaa	atatctgact	3720
tggagctcgt	gtcggaccat	acaccgggtga	ttaatcgtgg	tttattatca	agcgtgagcc	3780
acgtcgcgga	cgaatthgag	cagctctggc	tgccgtactg	gtccctggca	agcgacgatc	3840
tgctcgaggg	gatctaccgc	caaagccgcg	cgctcggcct	aggccgcgg	tacatcgagg	3900
cgaaccacac	agcgtctggc	aacctgctgg	tcgtggacgt	agaccatcca	gacgcagcgc	3960
tccgagcgtc	cagcgcccgc	gggtcccac	cgctgcacaa	cgcgatcgtg	ggcaatcgcg	4020
ccaacggcca	cgcacacgca	gtgtgggcac	tcaacgcccc	tgttccacgc	accgaatac	4080
cgcgcgtaaa	gccgctcgca	tacatggcgg	cgctgcgccg	agcccttcgg	cgcgccgtcg	4140
atggcgaccg	cagttactca	ggcctcatga	ccaaaaaccc	cgccacatc	gcctgggaaa	4200
cggaatggct	ccactcagat	ctctacacac	tcagccacat	cgaggccgag	ctcggcgcga	4260
acatgccacc	gcgcgctg	cgtcagcaga	ccacgtacaa	agcggctccg	acgccgctag	4320
ggcggaattg	cgcactgttc	gattccgtca	ggttgtgggc	ctatcttccc	gccctcatgc	4380
ggatctacct	gccgaccggg	aacgtggacg	gactcggccg	cgcgatctat	gccgagtgcc	4440
acgcgcgaaa	cgccgaatth	ccgtgcaacg	acgtgtgtcc	cggaccgcta	ccggacagcg	4500
aggctccgcg	catcgccaac	agcatttggc	ggttgatcac	aaccaagtcg	cgcatttggg	4560
cggacgggat	cgtggtctac	gaggccacac	tcagtgcgcg	ccatgcccgc	atctcgcgga	4620
agggcgcagc	agcgcgcacg	gcggcgagca	cagttgcgcg	gcgcgcaaa	tccgcgtcag	4680
ccatggaggc	attgctatga	gcgacggcta	cagcgacggc	tacagcgacg	gctacaactg	4740
gcagccgact	gtccgcaaaa	agcggcgcgt	gaccgcgcc	gaaggcgcct	gaatcaccgg	4800
actatccgaa	cgccacgtcg	tccggctcgt	ggcgcaggaa	cgacgcgagt	ggttcgccga	4860
gcaggctgca	cgccgcgaac	gcattccgcg	ctatcacgac	gacgagggcc	actcttggcc	4920
gcaaacggcc	aaacatttct	ggctgcatct	ggacaccgtt	aagcgactcg	gctatcgggc	4980
gagaaagag	cgtgcggcag	aacaggaagc	ggctcaaaa	gcccacaacg	aagccgacaa	5040
tccaccgctg	ttctaacgca	attggggagc	gggtgtcgcg	ggggttccgt	ggggggttcc	5100
gttgcaacgg	gtcggacag	taaaagtct	ggttagacgt	agttttctgg	tttgggcat	5160
gcctgtctcg	ttgctgttth	cgttgcgtcc	gttttgaata	ccagccagac	gagacggggt	5220
tctacgaaac	ttggtcgata	ccaagccatt	tccgctgaat	atcgtggagc	tcaccgccag	5280
aatcgtgggt	tgtgggtgat	tacgtggcga	actccgttgt	agtgccttgt	gtggcatccg	5340
tggcgcggcc	gcggtaccag	atctttaa				5370



Supplemental Figure S2. Simultaneous clarithromycin and amikacin treatment of *MAB*. *MAB* was incubated with clarithromycin at **A)** 1/2 MIC (0.1 mg/L) or **B)** 100X MIC (20 mg/L) followed by immediate addition of a range of amikacin concentrations (3-12 mg/L). CFUs were monitored at 24 h intervals. Data points are the means of three replicates with error bars indicating standard deviation.



Supplemental Figure S3: Effect of clinically-relevant clarithromycin concentrations on *MAB* wild type, $\Delta whiB7$ and $\Delta whiB7-C$ derivatives. Strains were incubated with clarithromycin concentrations found in the lung. CFUs were monitored at 24 h intervals. Dashed lines represent the limit of detection. Data points are the mean of 3 replicates with standard deviation given as error bars. The data represents 3 independent experiments.

Supplemental Table S1. Primers used in this study

Primer	Sequence (5' to 3')
whiB7-FW	GAAATGAACACACCCACCAATTACG
whiB7-RV	GATCAGGTGATGGATTACATCAGAC
aac(2)-FW	GATCCACGGAAACTGGATAGTC
aac(2)-RV	GTTGTCTGACGAGGCGAC
tap-FW	GATTCGCCGACTATCAATCAGGTGAAT
tap-RV	GTTGGTTCCTTGTCGGGCTG
WhiB7-iPCR-RV	GCATAGGCCTCTTCCGCGAACCCACAGGTC
WhiB7-iPCR-FW	GCATAAGCTTGTGCGAGTGCCTGGC
aac(2)-iPCR-RV	GCATAGGCCTATGAGTATGCCGTGGTACCAG
aac(2)-iPCR-FW	GCATAAGCTTCGTGGAACGGACTTGGTAC
tap-iPCR-RV	CGATAAGCTTCTTCGTCGTGTCGATCATCG
tap-iPCR-FW	GCATACTAGTGTGACCCACATGGTGTGAC
whiB7-OS-FW	GGTAGCTGCCGTGTTGAT
whiB7-OS-RV	GTCGACCGACCTGTTGGAC
aac(2)-OS-FW	CCGATCCCGATGCCTTG
aac(2)-OS-RV	CGTGTTCTACATCGGTAGTTGTCTG
tap-OS-FW	GATCCCGATCTGGAGGC
tap-OS-RV	CCCGTGATCAGTGCGAC
whiB7-C-FW	CGATCTGCAGGTATCGCCGAGGTCAAAAATAAC
whiB7-C-RV	GCATAAGCTTCAGGCCCTACAACCTTCTTG
whiB7-qPCR-FW	ACCTCAAGCGTGGTTCCG
whiB7-qPCR-RV	CCTCCACTTCAACGGTCATC
aac(2)-qPCR-FW	CTGTGTCCAATATGCCAACC
aac(2)-qPCR-RV	GAATGCCTCAATGAGTAATTCAC
erm-qPCR-FW	GGAGTTCGTTGTGGATCTGG
erm-qPCR-RV	AAACCGTGAACGAAGGTGTC
eis2-qPCR-FW	GAGGTCAACCGCAAATTCAC
eis2-qPCR-RV	CACGACATGACGGCTGAAC
sigA-qPCR-FW	CACAAAGGGTTACAAGTTCTCG
sigA-qPCR-RV	GCTTGTTGATGACCTCGACC

Supplemental Table S2. The effect of *whiB7* deletion on the resistance profile of *MAB*.

Drug	MIC (mg/L)	
	WT	$\Delta whiB7$
Imipenem	6.3	6.3
Cefoxitin	50	50
Doxycycline	>100	>100
Tetracycline	>100	>100
Clindamycin	12.5	25
Levofloxacin	3.1	3.1
Moxifloxacin	1.6	1.6
Rifabutin	6.3	6.3
Rifamycin	>100	>100
Rifamycin SV	25	25
Isoniazid	>100	>100
Ethambutol	>100	>100

Supplemental Table S3: Roles of *tap* and *eis* in aminoglycoside resistance.

Drug	Amikacin MIC (mg/L)					
	0 mg/L Clarithromycin			$\frac{1}{2}$ MIC Clarithromycin		
	WT	$\Delta aac(2')$	Δtap	WT	$\Delta aac(2')$	Δtap
Amikacin	3.1	3.1	3.1	12.5	12.5	12.5
Tobramycin	6.3	0.4	6.3	25	0.4	25
Gentamicin	6.3	3.1	3.1	25	6.3	12.5
Sisomicin	3.1	0.8	3.1	12.5	0.8	12.5

Values are the medians of 3 experiments.