

Supplementary Table S1: Mutation rate, calculated as stated in the text, of serial isolates of *Mycobacterium abscessus*.

Patient number	Subspecies	Dates of isolation (dd/mm/yy)	Interval time (from the initial isolate)	SNP differences between successive pairs of isolates	Mutation rate calculated since the initial isolate SNP/genome size (5Mb)/year ($\times 10^{-6}$)	Type of infection
P1.1	<i>massiliense</i>	28/4/14	-			Persistent
P1.2	<i>massiliense</i>	13/5/14	15	6	29.200	Persistent
P1.3	<i>massiliense</i>	9/6/14	42	8	13.905	Persistent
P1.4	<i>massiliense</i>	7/7/14	70	9	9.386	Persistent
P1.5	<i>massiliense</i>	16/7/14	79	8	7.392	Persistent
P1.6	<i>massiliense</i>	16/10/14	171	6	2.561	Persistent
P1.7	<i>massiliense</i>	19/10/14	174	11	4.615	Persistent
P1.8	<i>massiliense</i>	22/10/14	177	8	3.299	Persistent
P1.9	<i>massiliense</i>	30/11/14	216	8	2.704	Persistent
P3.1	<i>abscessus</i>	23/11/15	-			Colonization
P3.2	<i>abscessus</i>	15/2/16	84	1	0.869	Colonization
P4.1	<i>massiliense</i>	9/7/15	-			Persistent
P4.2	<i>massiliense</i>	17/7/15	8	4	36.500	Persistent
P6.1	<i>abscessus</i>	12/6/13	-			Persistent
P6.2	<i>abscessus</i>	20/6/13	8	6	54.750	Persistent
P7.1	<i>massiliense</i>	6/3/14	-			Persistent
P7.2	<i>massiliense</i>	19/5/14	74	14	13.811	Persistent
P8.1	<i>massiliense</i>	18/6/13	-			Re-infection
P8.2	<i>massiliense</i>	10/9/13	84	140	121.667	Re-infection
P9.1	<i>massiliense</i>	2/7/16	-			Persistent
P9.2	<i>massiliense</i>	26/7/16	24	9	27.375	Persistent
P9.3	<i>massiliense</i>	4/10/16	94	14	10.872	Persistent

Supplementary Table S1: Mutation rate, calculated as stated in the text, of serial isolates of *Mycobacterium abscessus*. (Cont.)

Patient number	Subspecies	Dates of isolation (dd/mm/yy)	Interval time (from the initial isolate)	SNP differences between successive pairs of isolates	Mutation rate calculated since the initial isolate SNP/genome size (5Mb)/year ($\times 10^{-6}$)	Type of infection
P10.1	<i>abscessus</i>	14/11/12	-			Persistent
P10.2	<i>abscessus</i>	9/4/13	146	5	2.500	Persistent
P10.3	<i>abscessus</i>	25/6/13	223	6	1.964	Persistent
P11.1	<i>massiliense</i>	8/7/15	-			Persistent
P11.2	<i>massiliense</i>	3/3/16	239	12	3.665	Persistent
P12.1	<i>massiliense</i>	23/1/12	-			Persistent
P12.2	<i>massiliense</i>	11/5/12	109	8	5.358	Persistent
P13.1	<i>massiliense</i>	12/2/15	-			Persistent
P13.2	<i>massiliense</i>	17/8/15	186	11	4.317	Persistent
P13.3	<i>massiliense</i>	14/1/16	336	8	1.738	Persistent
P14.1	<i>abscessus</i>	9/1/13	-			Persistent
P14.2	<i>abscessus</i>	2013/1/11	2	1	36.500	Persistent
P14.3	<i>abscessus</i>	19/7/13	191	2	0.764	Persistent
P15.1	<i>massiliense</i>	17/8/15	-			Persistent
P15.2	<i>massiliense</i>	23/5/16	280	12	3.129	Persistent
P15.3	<i>massiliense</i>	1/8/16	350	102	21.274	Re-infection
P16.1	<i>massiliense</i>	28/6/13	-			Persistent
P16.2	<i>massiliense</i>	8/8/14	406	10	1.798	Persistent
P17.1	<i>abscessus</i>	16/2/15	-			Persistent
P17.2	<i>abscessus</i>	30/3/15	42	3	5.214	Persistent
P17.3	<i>abscessus</i>	12/5/15	85	6	5.153	Persistent
P18.1	<i>massiliense</i>	30/7/13	-			Colonization
P18.2	<i>massiliense</i>	29/8/14	395	5	0.924	Colonization
P19.1	<i>abscessus</i>	22/8/12	-			Persistent
P19.2	<i>abscessus</i>	12/6/14	659	5	0.554	Persistent

Supplementary Table S1: Mutation rate, calculated as stated in the text, of serial isolates of *Mycobacterium abscessus*. (Cont.)

Patient number	Subspecies	Dates of isolation (dd/mm/yy)	Interval time (from the initial isolate)	SNP differences between successive pairs of isolates	Mutation rate calculated since the initial isolate SNP/genome size (5Mb)/year ($\times 10^{-6}$)	Type of infection
P20.1	<i>abscessus</i>	24/6/14	-			Colonization
P20.2	<i>abscessus</i>	21/10/14	119	5	3.067	Colonization
P21.1	<i>massiliense</i>	28/9/12	-			Persistent
P21.2	<i>massiliense</i>	4/10/12	6	11	133.833	Persistent
P22.1	<i>massiliense</i>	9/10/14	-			Persistent
P22.2	<i>massiliense</i>	29/1/15	112	8	5.214	Persistent
P23.1	<i>abscessus</i>	6/12/12	-			Persistent
P23.2	<i>abscessus</i>	7/12/12	1	0	0.000	Persistent
P24.1	<i>abscessus</i>	5/10/14	-			Colonization
P24.2	<i>abscessus</i>	7/10/14	2	4	146.000	Colonization
P24.3	<i>abscessus</i>	8/10/14	3	2	48.667	Colonization
P24.4	<i>abscessus</i>	10/10/14	5	5	73.000	Colonization
P25.1	<i>abscessus</i>	30/1/12	-			Persistent
P25.2	<i>abscessus</i>	31/1/12	1	3	219.000	Persistent
P26.1	<i>abscessus</i>	23/3/15	-			Persistent
P26.2	<i>abscessus</i>	27/3/15	4	6	109.500	Persistent
P27.1	<i>abscessus</i>	25/4/16	-			Persistent
P27.2	<i>abscessus</i>	1/6/16	37	6	11.838	Persistent
P27.3	<i>abscessus</i>	26/6/17	427	0	0.000	Persistent
P28.1	<i>massiliense</i>	30/6/17	-			Persistent
P28.2	<i>massiliense</i>	23/7/17	23	12	38.087	Persistent
P28.3	<i>massiliense</i>	30/7/17	30	8	19.467	Persistent

Supplementary Table S2: Estimation of *Mycobacterium abscessus* mutation rates at different time intervals relative to the initial isolate.

the initial isolate.

Subsp.	No. of SNPs	Interval time (days)	Mutation rate ($\times 10^{-6}$)	Mutation rate ($\times 10^{-6}$), Mean (SD)
≤ 7 days				
<i>abscessus</i>	1	2	36.50	127.75 (164.59)
<i>abscessus</i>	0	1	0.00	
<i>abscessus</i>	5	1	365.00	
<i>abscessus</i>	6	4	109.50	
<i>massiliense</i>	11	6	133.83	133.83 (0.00)
8–30 days				
<i>abscessus</i>	6	8	54.75	54.75
<i>massiliense</i>	6	15	29.20	30.13 (7.52)
<i>massiliense</i>	4	8	36.50	
<i>massiliense</i>	9	24	27.38	
<i>massiliense</i>	12	23	38.09	
<i>massiliense</i>	8	30	19.47	
31–180 days				
<i>abscessus</i>	5	146	2.50	6.18 (3.98)
<i>abscessus</i>	3	42	5.21	
<i>abscessus</i>	6	85	5.15	
<i>abscessus</i>	6	37	11.84	
<i>massiliense</i>	8	42	13.90	7.64 (4.16)
<i>massiliense</i>	9	70	9.39	
<i>massiliense</i>	8	79	7.39	
<i>massiliense</i>	6	171	2.56	
<i>massiliense</i>	11	174	4.61	
<i>massiliense</i>	8	177	3.30	
<i>massiliense</i>	14	74	13.81	
<i>massiliense</i>	14	94	10.87	
<i>massiliense</i>	8	109	5.36	
<i>massiliense</i>	8	112	5.21	

Supplementary Table S2: Estimation of *Mycobacterium abscessus* mutation rates at different time intervals relative to the initial isolate. (Cont.)

Subsp.	No. of SNPs	Interval time (days)	Mutation rate ($\times 10^{-6}$)	Mutation rate ($\times 10^{-6}$), Mean (SD)
> 180 days				
<i>abscessus</i>	6	223	1.96	0.82 (0.83)
<i>abscessus</i>	2	191	0.76	
<i>abscessus</i>	5	659	0.55	
<i>abscessus</i>	0	427	0.00	
<i>massiliense</i>	8	216	2.70	2.89 (1.02)
<i>massiliense</i>	12	239	3.67	
<i>massiliense</i>	11	186	4.32	
<i>massiliense</i>	8	336	1.74	
<i>massiliense</i>	12	280	3.13	
<i>massiliense</i>	10	406	1.80	

Note: mutation rate was calculated based on SNP/genome size (5Mb)/year

Confirmatory analysis in Patient 1 with 8 serially isolated strains

No. of SNPs	Interval time (days)	Mutation rate ($\times 10^{-6}$)
6	15	29.20
8	42	13.90
9	70	9.39
8	79	7.39
6	171	2.56
11	174	4.61
8	177	3.30
8	216	2.70

Supplementary Table S3. Frequency and MIC-distribution of isolates with drug resistance-conferring mutations.

Drug	Mutations	MIC (µg/mL) distribution for relevant isolate										No. of isolates
Clarithromycin		≤0.06	0.12	0.25	0.5	1	2	4	8	16	>16	
(CC = 0.25 µg/ml)	No known CLA mutations	1	2	5	6	6	3	3		2		28
	<i>erm(41)</i> T28								3	19	10	32
	<i>rrl</i> A2058G										6	6
	<i>rrl</i> A2059C									2		2
	<i>rrl</i> A2059G										1	1
Amikacin		≤1	2	4	8	16	32	64	>64			
	No known AMK mutations		2	18	32	9	5	3				69
Ciprofloxacin		≤0.12	0.25	0.5	1	2	4	>4				
(CC = 4 µg/ml)	No known CIP mutations						28	3				31
	<i>gyrB</i> C-41G						8	4				12
	<i>gyrB</i> A-78G						20	6				26
Linezolid		≤1	2	4	8	16	32	>32				
(CC = 2 µg/ml)	No known LZD mutations			3	3	7	18					31
	<i>rrl</i> C742T						3					3
	<i>rrl</i> A1717G			3	3	6	16					28
	<i>rrl</i> C742T & <i>rrl</i> A1717G		2	2								4
	<i>rrl</i> C3042T				1	1	1					3

Supplementary Table S4 Literature of Mutation associated *M. abscessus* drug resistance

Antibiotics	Genes	<i>E.coli</i> numbering	<i>M.abscessus</i> numbering	Remark	Ref
Amikacin	<i>rrs</i>		A-33G		[1]
		T1406A	T1372A		[2]
		A1408G	A1374G		[1,2]
		C1409T	C1375T		[2]
		G1491T	G1455T		[2]
		C1496T	C1460T		[3]
		T1498A	T1462A		[3]
Clarithromycin	<i>erm(41)</i>		A-31T		[1]
			C28T		[4-6]
	<i>rrl</i>		G795A		[1]
			T371C		[1]
			T1401C		[1]
			A1932G		[1]
			A2039C		[1]
		A2057G	A2269G		[1]
		A2058G	A2270G		[7,8]
		A2058C	A2270C		[7,8]
		A2058T	A2270T		[7,8]
		A2059T	A2271T		[7,8]
		G2067A	G2279A		[1]

Antibiotics	Genes	<i>E.coli</i> numbering	<i>M.abscessus</i> numbering	Remark	Ref
Clarithromycin	<i>rrl</i>	A2069A	G2281A		[1]
		A2082C	A2293C		[1]
Ciprofloxacin	<i>gyrA</i>	C275T	Ala92Val		[9]
		G286A	Asp96Asn		[9]
		T2287C	Ser763Pro		[1]
Ciprofloxacin	<i>gyrB</i>		C-41G		[1]
			A-78G		[1]
			C1474T	Arg492Cys	[9]
			C1727G	Thr576Ser	[1]
Linezolid	<i>rplC</i> (L3)		T54A		[10]
Linezolid	<i>rplD</i> (L4)		G142A		[10]
			A175P		[10]
Linezolid	<i>rrl</i> (23S rRNA)		G15A	Only R strain	[10]
			T328C	Only R strain	[10]
			G348A	Only R strain	[10]
			C1445T	Only R strain	[10]
			C1582A	Only R strain	[10]
			T2138C	Only R strain	[10]
			A2271C	Only R strain	[10]
			C2432T	Only R strain	[10]
			G3048A	Only R strain	[10]

Antibiotics	Genes	<i>E.coli</i> numbering	<i>M.abscessus</i> numbering	Remark	Ref
Linezolid	<i>rrl</i> (23S rRNA)		T54A	R+S strain	[10]
			C109T	R+S strain	[10]
			G399A	R+S strain	[10]
			A437G	R+S strain	[10]
			C633T	R+S strain	[10]
			C742T	R+S strain	[10]
			A1717G	R+S strain	[10]
			A2270C	R+S strain	[10]
			T3001C	R+S strain	[10]
			C3042T	R+S strain	[10]

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