Supplementary Table S1. Sequencing metrics by samples.

Sample ID	Sequencing Quality	Composite reference coverage	Median depth coverage	Min coverage	Max coverage
1	2	100%	1405x	192x gyrA	3961x eis
2	2	100%	798x	92x rrs2	6248x eis
3	2	100%	2533x	156x rrs2	3458x eis
4	2	100%	1981x	212x rrs2	4041x inhA
5	2	100%	3483x	173x rrs2	3469x inhA
6	2	100%	1784x	135x rrs2	6060x eis
7	2	100%	3737x	78x rrs2	21058x eis
8	2	100%	1106x	92x rrs2	2660x eis
9	2	100%	2263x	148x rrs2	11042x eis
10	3	100%	2287x	80x rrs2	6687x eis
11	2	100%	2823x	71x rrs2	24093x eis
12	2	100%	2874x	298x rrs2	13530x eis
13	2	100%	3240x	359x rrs2	15185x eis
14	2	100%	2011x	144x rrs2	7653x eis
15	3	100%	30235x	1076x rrs2	37582x eis
16	1	100%	1899x	156x rrs2	8372x eis
17	2	100%	2306x	66x rrs2	4030x eis
18	2	100%	5071x	269x rrs2	16628x eis
19	3	100%	32724x	503x rrs2	71049x eis
20	2	100%	3769x	53x rrs2	5445x eis
21	3	100%	3835x	260x rrs2	5181x embB
22	2	100%	6165x	133x rrs2	9653x eis
23	2	100%	3406x	153x rrs2	13289x eis
24	3	100%	11024x	314x rrs2	9975x inhA
25	3	100%	5214x	201x rrs2	10580x eis

26	2	100%	5263x	213x rrs2	7505x eis
27	3	100%	13181x	496x rrs2	22753x eis
28	3	100%	9769x	638x rrs2	13089x rpoB2
29	3	100%	5086x	341x rrs2	11578x eis
30	3	100%	6628x	531x rrs2	19388x eis
31	3	100%	7924x	322x rrs2	14913x eis
32	3	100%	4516x	302x rrs2	7514x eis
33	3	100%	4449x	262x rrs2	15215x eis
34	3	100%	11209x	270x rrs2	9022x rpoB1
35	3	100%	8504x	431x rrs2	34742x eis
36	2	100%	2786x	160x rrs2	13109x eis
37	2	100%	5357x	268x rss2	10495x eis
38	3	100%	7081x	301x gyrA	11564x rrl
39	3	100%	8105x	248x rrs2	9582x eis
40	2	100%	2170x	73x gyrA	1837x embB
41	2	100%	3663x	27x rrs2	4828x eis
42	1	100%	1833x	42x rrs2	1856x eis
43	2	100%	2052x	94x rrs2	1670x eis
44	NTM	62,20%	12x	0x	5499x
45	3	100%	6147x	204x rrs2	4751x rpoB2
46	2	100%	1322x	55x gyrA	1852x inhA
47	2	100%	854x	55x rrs2	5563x eis
48	2	100%	1187x	115x rrs2	2922x rrs1
49	ND	100%	38x	0x	637x rrs1
50	1	99.9%	875x	79x rrs2	3869x rrs1
51	ND	89,50%	0x	0x	20x eis
52	2	100%	849x	56x rrs2	1970x rrs1
53	2	99,90%	604x	55x rrs2	898x rrl
54	2	100%	1832x	126x rrs2	4080x eis
55	2	100%	2272x	221x rrs2	11997x eis

56	1	99.90%	383x	23x rrs2	1207x eis
57	2	100%	1382x	113x rrs2	2338x rrs1
58	2	99.90%	831x	51x gyrA	1617x rrs1
59	1	99.90%	617x	29x rrs2	2043x eis
60	1	99,80%	267x	19x rrs2	446x rrl
61	2	100%	847x	40x rrs2	1449x eis
62	1	99.90%	713x	51x rrs2	7564x eis
63	1	100%	885x	60x rrs2	1478x rrl
64	2	100%	1013x	105x rrs2	2576x eis
65	1	100%	1859x	32x rrs2	2057x eis
66	2	100%	1508x	159x rrs2	3434x eis
67	2	99.90%	782x	98x rrs2	1615x rrl
68	2	100%	1122x	115x rrs2	4352x eis
69	2	100%	1669x	100x rrs2	4974x eis
70	3	93,30%	2298x	0x rrs1	10692x rrl
71	1	99,90%	760x	16x rrs2	1148 gyrA
Average		99.21%	**3950,4x**		
Median			**2263x**		

1: All resistance-associated positions in database with enough data to identify mutations from 80-100%; **2:** All resistance-associated positions in database with enough data to identify mutations from 10-100%; **3:** All resistance-associated positions in database with enough data to identify mutations from 3-100%; **ND:** Mycobacteria not detected; **NTM:** Nontuberculous mycobacteria detected; **Composite reference coverage**: coverage breadth over the concatenated reference sequences associated with drug resistance; **Median depth of coverage**: median of average read depths among reference sequences associated with drug resistance; **(min x, max x)**: minimal/maximal average coverage depth among the targets.

Supplementary Table S2. Median depth of coverage per gene targeted by the Deeplex-MycTB assay across samples.

Sample ID	Median depth coverage	*rpob1*	*rpoB2*	*katG*	*fabG1*	*ahpC*	*inhA*	*pncA*	*embB*	*gidB*	*rpsL*	*gyrA*	*gyrB*	*ethA*	*eis*	*rrs2*	*rrs1*	*tlyA*	*rplC*	*rrl*	*Rv0678*
1	1405x	2776	2007	1044	1523	1106	1333	1093	696	533	1567	192	1140	522	3961	271	1082	288	1120	1241	673
2	798x	4699	2672	621	2081	1790	3167	1089	852	570	1908	427	691	257	6248	92	202	179	1164	606	556
3	2533x	3413	3282	1336	2009	1192	2977	1651	1352	873	1434	667	2071	718	3457	156	738	387	820	1230	975
4	1981x	4040	3991	1745	2842	2240	3821	2482	1849	1201	2946	644	1533	690	3936	211	769	542	1418	1471	1574
5	3483x	3197	3289	1313	1519	1584	3463	2548	2648	1156	1293	451	2686	724	2693	172	1064	355	646	1997	1172
6	1784x	3365	3012	1149	2298	1398	2236	2029	1363	734	1969	744	1459	650	6065	134	596	306	1335	1431	1118
7	3737x	10362	5959	1106	4917	2432	4132	2147	1489	523	3908	1630	2822	515	21057	78	376	50	3882	1911	1164
8	1106x	1214	2659	1744	834	758	2096	1413	1504	746	538	113	987	820	922	92	1187	562	264	1249	900
9	2263x	7595	4645	1521	3482	1748	3982	2916	1940	966	2784	1177	1755	669	11042	148	1150	443	2403	1805	1330
10	2287x	6106	5027	2593	2695	1761	5190	3226	3629	1342	2225	594	1909	1111	6687	80	373	616	1432	911	1651
11	2823x	16537	7683	1760	7298	2345	6024	3802	2909	928	5297	2853	2151	992	24093	71	50	50	5559	855	2227
12	2874x	10598	6343	2792	4714	2641	5399	3819	3017	1529	4144	1843	2166	1082	13529	288	1555	642	3287	3714	1954
13	3240x	13750	7331	2056	5001	2966	5492	3620	2619	1188	4925	2170	2572	950	15184	358	2051	447	3737	5927	1818
14	2011x	5954	4257	1648	2555	1292	3098	2167	1720	822	1882	883	1558	776	7653	144	1680	367	1538	2788	1087
15	30235x	32270	22477	7923	15722	7531	13911	11203	8465	4262	13361	5707	8301	3624	37582	1075	9740	1671	10495	18630	6705
16	1899x	7408	4759	893	3071	1827	3071	1923	1361	450	2447	1112	1523	541	8372	156	862	180	2064	3180	1169
17	2306x	3186	2866	918	1654	1334	1502	1384	1045	381	1180	695	1926	630	4030	66	297	150	980	780	806
18	5071x	12810	6030	2120	5114	2998	4429	3329	2861	1086	4447	2388	3848	1059	16627	269	1794	470	4284	3777	2282
19	32724x	52541	25184	6032	22824	8755	18514	11911	9493	2962	18369	9754	7676	3520	71049	503	2615	1291	16484	8377	7250
20	3769x	4426	3018	805	2143	936	1835	1513	1380	408	1361	798	738	579	5444	53	256	243	1369	824	758
21	3835x	3571	3104	3044	1630	1498	2672	3516	5181	2079	1405	637	2963	1491	3215	206	1321	1030	958	2260	1430
22	6165x	6683	4871	2009	2993	1848	4103	3352	2093	1281	2326	1378	2428	942	9653	133	829	510	1920	1430	1432
23	3406x	9018	6056	2368	4982	2505	4856	4430	3291	1729	4273	2153	2585	1041	13289	153	815	768	3774	1662	2231
24	11024x	7161	8411	4802	4761	4931	9975	7075	6174	4489	4053	1266	5143	2814	8116	314	2413	1845	2307	3628	3123
25	5214x	8784	7961	3466	4305	2609	7183	5932	4936	2246	3397	1524	3934	1506	10579	201	1767	998	2439	2624	2521

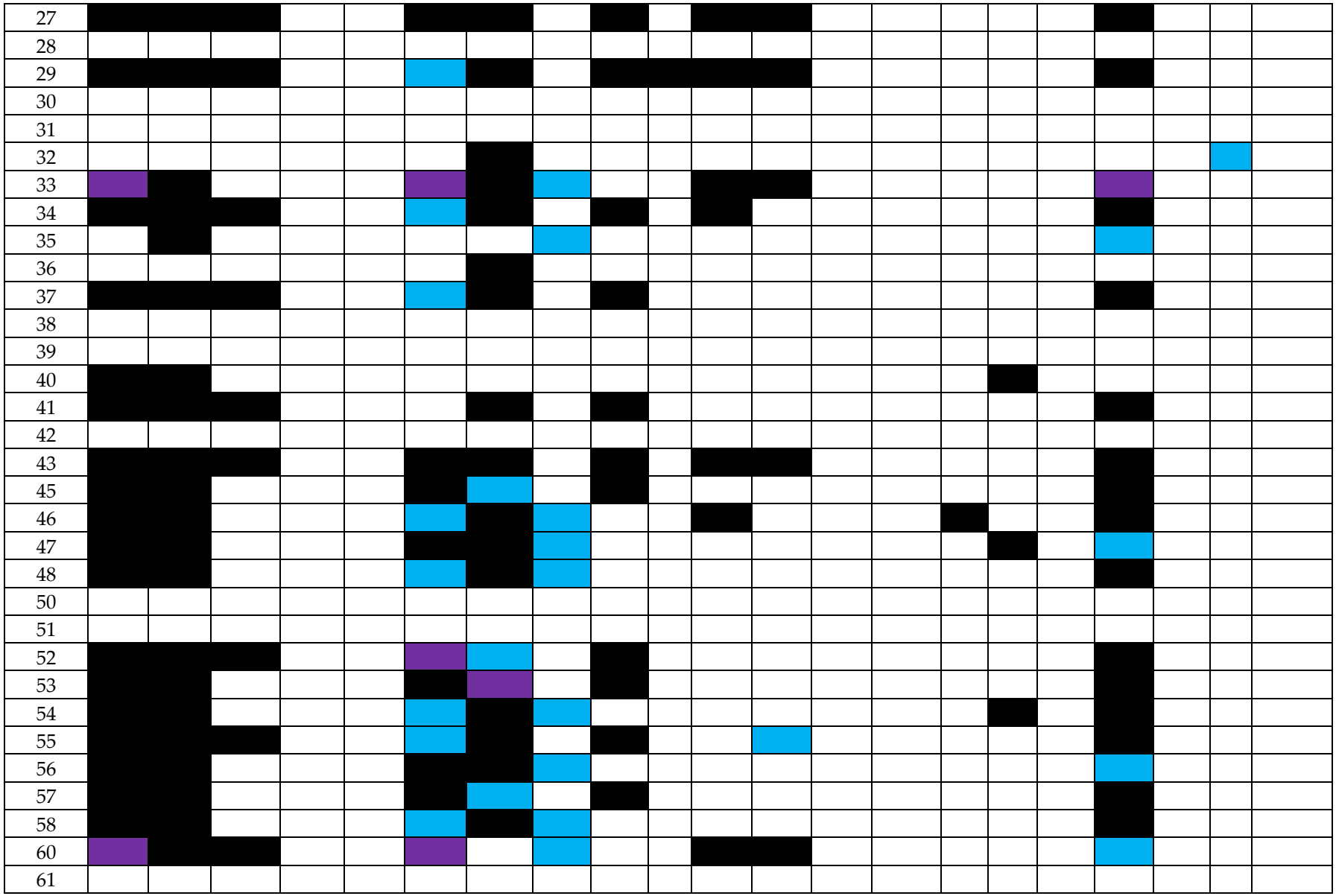
26	5263x	6655	4165	1386	3215	2017	3297	2701	1909	1087	3623	1570	2052	735	7504	213	826	405	2412	1756	1716
27	13181x	17172	9987	4337	9658	5545	7924	7295	5667	3324	10553	3797	4435	2197	22753	496	2145	1549	7299	3795	3908
28	9769x	10544	13089	5824	3968	4467	11763	11635	6477	3938	4437	1341	7594	2100	7619	638	4565	1145	2091	9715	4214
29	5086x	10308	8132	4278	5107	2385	5705	4087	4603	2477	4027	1686	3929	2228	11577	341	2947	1169	2932	3763	2691
30	6628x	15770	10483	4311	7921	4150	7964	6778	5283	2949	6612	3331	5042	1854	19388	531	2758	1243	5297	5138	3630
31	7924x	11715	6018	2277	6230	3805	5234	4068	3116	1932	6098	2124	2532	1202	14913	332	1120	731	4446	2763	2255
32	4516x	7063	6731	3407	3591	2245	5090	4547	4117	2259	2836	1192	3547	1602	7514	302	2606	1071	1994	4228	2336
33	4449x	12724	9256	3269	7091	3331	6382	6654	5861	2654	5205	2640	3368	1745	15214	262	1590	1078	4574	3246	3755
34	11209x	9022	8729	3134	4186	2825	6303	6016	5219	2109	3375	1250	4096	1449	7943	270	3004	735	2142	6340	3185
35	8504x	25190	14643	5116	10912	5320	11133	9636	7429	3552	9228	5154	6386	2497	34742	431	2857	1503	7896	5019	4721
36	2786x	10528	6076	2371	5196	2389	5147	4085	2950	1219	4821	2441	2118	947	13108	160	664	515	3622	1346	2144
37	5357x	7838	4040	1792	3198	1505	3495	2600	2326	1045	2226	1441	1678	660	10494	267	2657	545	2249	4316	1385
38	7081x	4933	5549	4179	1434	1617	5182	4058	5260	2690	1033	300	3456	2107	3694	351	7454	1501	599	11564	1494
39	8105x	7599	6178	3100	3945	1901	4522	3916	3145	1812	2503	1176	2676	1277	9582	249	3500	994	2265	3782	1837
40	2170x	1088	1656	1608	496	554	1374	1263	1837	1114	323	73	954	1275	781	102	1697	1018	158	1545	570
41	3663x	4286	2876	816	1362	726	2153	1252	1221	527	1226	568	1168	659	4828	77	768	247	952	900	635
42	1833x	1007	1426	843	901	574	665	909	857	414	465	204	771	552	1855	42	571	240	416	707	514
43	2052x	1337	1558	1325	860	851	1319	1174	1352	958	973	270	784	1102	1670	93	706	642	453	550	579
44	12x																				
45	6147x	2889	4751	2501	1464	1562	2975	3284	2884	1425	1234	408	3076	1496	2502	204	2331	871	696	3491	1489
46	1322x	1030	1669	1523	848	1517	1851	1333	1666	1417	687	55	1127	1275	1163	132	1330	625	344	1772	915
47	854x	2410	1510	769	1269	436	1434	1023	930	419	499	475	671	450	5563	55	1477	250	939	1289	472
48	1187x	1168	1590	1017	758	525	1151	1038	848	605	528	187	1029	659	2368	115	2922	426	418	2185	447
49	38x																				
50	875x	503	595	394	238	144	882	361	405	157	112	228	748	308	1137	79	3869	110	177	2197	168
51	0x																				
52	849x	825	1076	1142	373	321	865	797	895	750	197	73	728	880	859	56	1970	597	164	1557	337
53	604x	646	817	626	464	445	777	860	829	521	342	66	439	482	702	55	739	316	202	898	432
54	1832x	2345	2397	1593	1640	991	1836	1532	1982	646	1524	541	1562	917	4079	125	1922	406	1168	2740	1418
55	2272x	8676	4188	2332	3358	1538	3494	2979	2867	1330	2340	1430	1729	840	11996	211	1792	793	2762	3667	1499

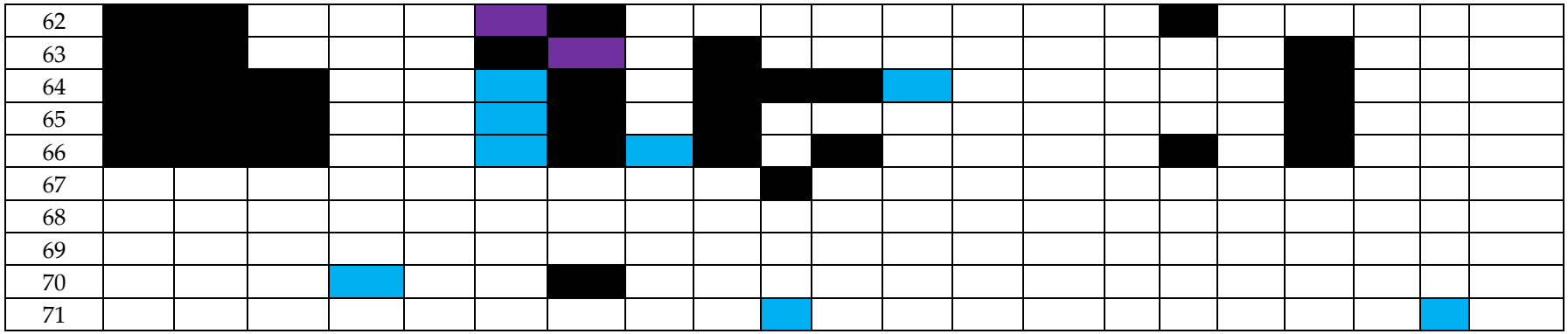
56	383x	569	559	325	372	219	355	409	344	205	264	158	312	177	1207	23	636	161	233	639	192
57	1382x	651	1015	975	384	493	803	949	1723	809	346	131	1192	848	927	113	2337	635	226	1964	412
58	831x	407	840	793	185	425	836	1073	991	721	217	51	677	455	520	68	1616	501	125	1601	348
59	617x	1436	960	560	586	308	791	611	627	358	370	228	476	196	2043	29	466	228	397	478	301
60	267x	266	297	216	137	111	211	246	276	168	133	48	221	150	299	19	362	106	84	445	146
61	847x	1232	1303	1207	529	414	1030	1063	1407	800	309	112	733	755	1448	40	501	1002	294	846	535
62	713x	3816	1880	330	1340	598	1396	703	400	193	846	427	585	197	7563	51	485	85	1059	1150	416
63	885x	912	1183	1107	358	348	688	752	912	679	242	65	709	763	765	59	726	390	216	1477	480
64	1013x	1487	1569	687	827	557	1177	998	848	367	597	324	856	445	2576	105	1886	303	651	2052	622
65	1859x	1642	1535	392	1011	758	1516	910	688	245	820	228	530	251	2057	32	257	184	541	535	520
66	1508x	2022	2292	1062	1120	801	1576	1436	1311	571	777	477	1301	693	3434	159	2976	455	848	3140	959
67	782x	991	907	1154	427	344	736	780	898	902	328	130	683	870	1225	97	1580	791	262	1615	456
68	1122x	1817	1849	599	1117	650	1325	1171	682	366	808	475	932	436	4352	114	607	267	863	1697	510
69	1669x	2769	3358	1378	1770	1257	2591	1889	1928	644	914	443	1355	876	4973	100	1294	593	976	2157	937
70	2298x	2239	4183	0	1269	1206	3010	3233	3680	1716	870	259	2113	1603	2733	400	7968	963	594	10691	1232
71	760x	572	714	410	404	275	592	767	506	286	297	1148	606	227	1118	16	456	202	298	338	322

Median depth of coverage: median of average read depths among reference sequences associated with drug resistance; **(min x, max x)**: minimal/maximal average coverage depth among the target

Supplementary Table S3. Graphical summary of resistance prediction by the Deeplex -MycTB assay for 71 sequenced clinical samples.

Sample ID	*rpoB*	*katG*	*fabG1*	*ahpC*	*inhA*	*pncA*	*embB*	*gidB*	*rpsL*	*rrs*	*gyrA*	*gyrB*	*inhA*	*fabG1*	*Eis*	*Rrs*	*tlyA*	*ethA*	*rplC*	*rrl*	*Rv0678*
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Black frame: Drug resistance associated variant/s; **Blue frame**: Uncharacterized variant/s; **Purple frame**: Uncharacterized variant/s in addition to drug resistance-associated variant/s.

Supplementary Table S4. Detailed phylogenetic analysis of 71 clinical samples by the Deeplex®-MycTB assay

Sample ID	hsp-65 based species identification				Spoligotype		SNP phylogenetic lineage	Mixed infection
	Av.coverage depth	Consensus length	Identity	Best match	Av.coverage depth	Clade	Lineage	Detected
1	2665x	400	100%	*MTBC*	1751x	LAM1	L4.3	No
2	4285x	400	100%	*MTBC*	690x	n/a	L4.3	No
3	1556x	400	100%	*MTBC*	809x	UNKW	Other than Hrv37	No
4	3274x	400	100%	*MTBC*	613x	LAM5	L4.3	No
5	1174x	400	100%	*MTBC*	613x	T1	Other than Hrv37	No
6	2185x	400	100%	*MTBC*	323x	n/a	Other than Hrv37	No
7	6990x	400	100%	*MTBC*	1004x	H3	Other than Hrv37	No
8	410x	400	100%	*MTBC*	307x	LAM9	L4.3	No
9	3727x	400	100%	*MTBC*	323x	T1	Other than Hrv37	No
10	2355x	400	100%	*MTBC*	884x	LAM9	L4.3	No
11	8312x	400	100%	*MTBC*	495x	LAM9	L4.3	No
12	4885x	400	100%	*MTBC*	1320x	LAM9	L4.3	No
13	6518x	400	100%	*MTBC*	736x	T1	L4.3	Yes
14	2281x	400	100%	*MTBC*	305x	T1	Other than Hrv37	No
15	16042x	400	100%	*MTBC*	1419x	T1	L4.3	No
16	3500x	400	100%	*MTBC*	258x	S	Other than Hrv37	No
17	1634x	400	100%	*MTBC*	499x	Beijing	L2	No
18	6165x	400	100%	*MTBC*	983x	Manu2	Other than Hrv37	Yes
19	28096x	400	100%	*MTBC*	2249x	LAM9	L4.3	No
20	2563x	400	100%	*MTBC*	194x	T1	Other than Hrv37	No
21	806x	400	100%	*MTBC*	3286x	UNKW	L2	No
22	2721x	400	100%	*MTBC*	412x	LAM9	L4.3	No
23	4943x	400	100%	*MTBC*	780x	H3	Other than Hrv37	No
24	4425x	400	100%	*MTBC*	855x	T1	L4.3	No

25	3020x	400	100%	*MTBC*	718x	T1	Other than Hrv37	Yes
26	4252x	400	100%	*MTBC*	308x	T1	L4.3	Yes
27	11955x	400	100%	*MTBC*	1333x	T1	Other than Hrv37	Yes
28	2851x	400	100%	*MTBC*	766x	LAM4	L4.3	No
29	3983x	400	100%	*MTBC*	780x	LAM9	L4.3	No
30	7244x	400	100%	*MTBC*	1609x	UNKW	Other than Hrv37	No
31	8544x	400	100%	*MTBC*	884x	S	Other than Hrv37	No
32	2859x	400	100%	*MTBC*	576x	T1	L4.3	Yes
33	5801x	400	100%	*MTBC*	429x	T1	Other than Hrv37	Yes
34	2855x	400	100%	*MTBC*	301x	T1	L4.3	No
35	10840x	400	100%	*MTBC*	805x	T1	L4.3	Yes
36	4789x	400	100%	*MTBC*	485x	T1	Other than Hrv37	No
37	2993x	400	100%	*MTBC*	529x	LAM9	L4.3	No
38	886x	400	100%	*MTBC*	1012x	LAM9	L4.3	No
39	2401x	400	100%	*MTBC*	750x	LAM9	L4.3	No
40	244x	400	100%	*MTBC*	820x	LAM9	L4.3	No
41	2116x	400	100%	*MTBC*	362x	LAM9	L4.3	No
42	535x	400	100%	*MTBC*	160x	S	Other than Hrv37	No
43	1270x	400	100%	*MTBC*	590x	H1	Other than Hrv37	No
44	1653x	400		M IntraCellular	-	-	-	-
45	823x	400	100%	*MTBC*	1322x	Beijing	L2	No
46	696x	400	100%	*MTBC*	353x	S	Other than Hrv37	No
47	705x	399	100%	*MTBC*	517x	T1	Other than Hrv37	No
48	493x	399	100%	*MTBC*	221x	S	Other than Hrv37	No
49	14x	396	100%	*MTBC*	89x	LAM9	Not detected	**
50	86x	397	100%	*MTBC*	404x	T1	Other than Hrv37	No
51	5x	394	99,30%	*MTBC*	14x	n/a	ND	**
52	167x	399	100%	*MTBC*	379x	Manu2	L2	Yes
53	337x	399	100%	*MTBC*	1272x	Beijing	L2	No
54	1873x	400	100%	*MTBC*	494x	S	Other than Hrv37	No

55	2953x	400	100%	*MTBC*	6596x	T1	L4.3	No
56	209x	400	100%	*MTBC*	1086x	T1	Other than Hrv37	No
57	204x	395	100%	*MTBC*	1250x	Beijing	L2	No
58	133x	399	100%	*MTBC*	133x	S	Other than Hrv37	No
59	460x	400	100%	*MTBC*	1265x	n/a	L4.3	No
60	103x	397	100%	*MTBC*	65x	n/a	Other than Hrv37	Yes
61	347x	400	100%	*MTBC*	832x	X1	Other than Hrv37	No
62	2122x	400	100%	*MTBC*	546x	LAM9	L4.3	Yes
63	179x	397	100%	*MTBC*	2828x	Beijing	L2	No
64	514x	400	100%	*MTBC*	280x	LAM9	L4.3	No
65	1288x	400	100%	*MTBC*	251x	LAM9	L4.3	No
66	706x	400	100%	*MTBC*	322x	LAM9	L4.3	No
67	277x	400	100%	*MTBC*	2105x	n/a	Other than Hrv37	No
68	785x	400	100%	*MTBC*	528x	LAM9	L4.3	No
69	1248x	400	100%	*MTBC*	291x	n/a	Other than Hrv37	No
70	645x	400	100%	*MTBC*	1766x	H1	Other than Hrv37	No
71	233x	400	100%	*MTBC*	163x	T-H37Rv	No specific lineage	No

n/a: No statistically significant threshold reached/additional data required; **ND:** Mycobacteria not detected.