Supplementary material

Supplementary laboratory methods

Phenotypic DST (pDST)

Phenotypic drug susceptibility testing (pDST) was performed using the mycobacterial growth incubator tube (MGIT) culture system (Becton, Dickinson and Company (BD), Franklin Lakes, NJ, USA)(1,2), with a MIC (minimum inhibitory concentration) method on Middlebrook 7H10 agar plates, or for two strains with the proportional method (2-4) for the drugs and concentrations or resistance limits described in supplementary table 1. The primary cultures were enriched on solid media before performing the DST.

Supplementary table 1. The DST method, drugs tested, critical concentrations or minimum inhibitory concentrations required for resistant result for the drug, and the number of isolates tested with the method.

Method	Drug	CC/R limit (µg/ml)	n
MGIT	rifampicin (RIF)	1.0	45
MGIT	isoniazid (INH)	0.1	45
MGIT	streptomycin (STR)	1.0	45
MGIT	ethambutol (EMB)	5.0	45[a]
MGIT	pyrazinamide (PZA)	0.1	44[b]
MGIT	amikacin (AMK)	1.0	29
MGIT	capreomycin (CAP)	2.5	29
MGIT	clofazimine (CFZ)	1.0	8
MGIT	ethionamide (ETH)	5.0	10
MGIT	kanamycin (KAN)	2.5	29
MGIT	linezolid (LZD)	1.0	14
MGIT	moxifloxacin (MFX)	0.25	13
MGIT	ofloxacin (OFX)	2.0	29
MIC	amikacin (AMK)	>6	16
MIC	cycloserin (CYC)	>40	22
MIC	ethionamide (ETH)	>4	20
MIC	ofloxacin (OFX)	>4	16
MIC	para-aminosalicylic acid (PAS)	>2	22

[a] The number includes two isolates analyzed by the proportional method with the same critical concentrations for the drugs as the MGIT method.
[b] A result for PZA could not be obtained for one isolate.

NAA test for the detection of resistance for rifampicin and isoniazid was performed for the isolates from 35 cases. For 13 isolates/cases, pyrosequencing was used to determine the resistance for RIF and INH by sequencing the RRDR region of the rpoB gene and the katG gene position 315, respectively. In addition, the promoter region of the *inhA* gene was included for 11 cases. The Hain MTBDR plus test (Hain Lifescience GmbH, Nehren, Germany) was used for 11 isolates to determine RIF and INH resistance and the MTBDRsl test to determine resistance for second line drugs from 17 cases.

Spoligotyping and MIRU-VNTR analyses were performed as described earlier (5-7), and isolates belonging to the same MIRU-VNTR cluster and having the same spoligotype were considered to form a traditional genotyping cluster.

For this study, the DNA of one isolate from each case was isolated using Magattract DNA extraction kit (Qiagen, Hilden, Germany) and sequenced using Illumina (San Diego, CA, USA) platform at THL or using sequencing service of LGC Genomics GmbH, Berlin, Germany. Ridom SeqSphere+ (Ridom GmbH, Münster, Germany) was used to map the sequencing reads to the reference and for the MTBc cgMLST v2 analysis (2891 loci). Isolates with allelic distance ≤12 were assigned to the same complex type (CT) by the software (8,9). An epidemiologic link could however be suspected for isolates having allelic distance of ≤5 loci (10,11). For the isolates that were clustered based on the cgMLST CT analysis, the SNP distances within the 2891 loci included in the cgMLST analysis were determined by the SeqSphere+ software. Resistance mutations (gDST) were determined by the automatic settings of PhyResSE (12). However, for the determination of genomic PZA-resistance, all *pncA* mutations found by PhyResSE and leading to an amino acid substitution known to cause PZA-resistance (13), a frameshift, or an otherwise abnormal PncA protein were considered as resistance mutations. The mutations found by PhyResSE in the rpoB gene were inspected similarly for the phenotypically rifampicin resistant isolates that were not reported resistant by the automatic settings of PhyResSE (n=2). The *M. tuberculosis H37Rv* sequence NC_000962.3 was used as the reference in all analyses.

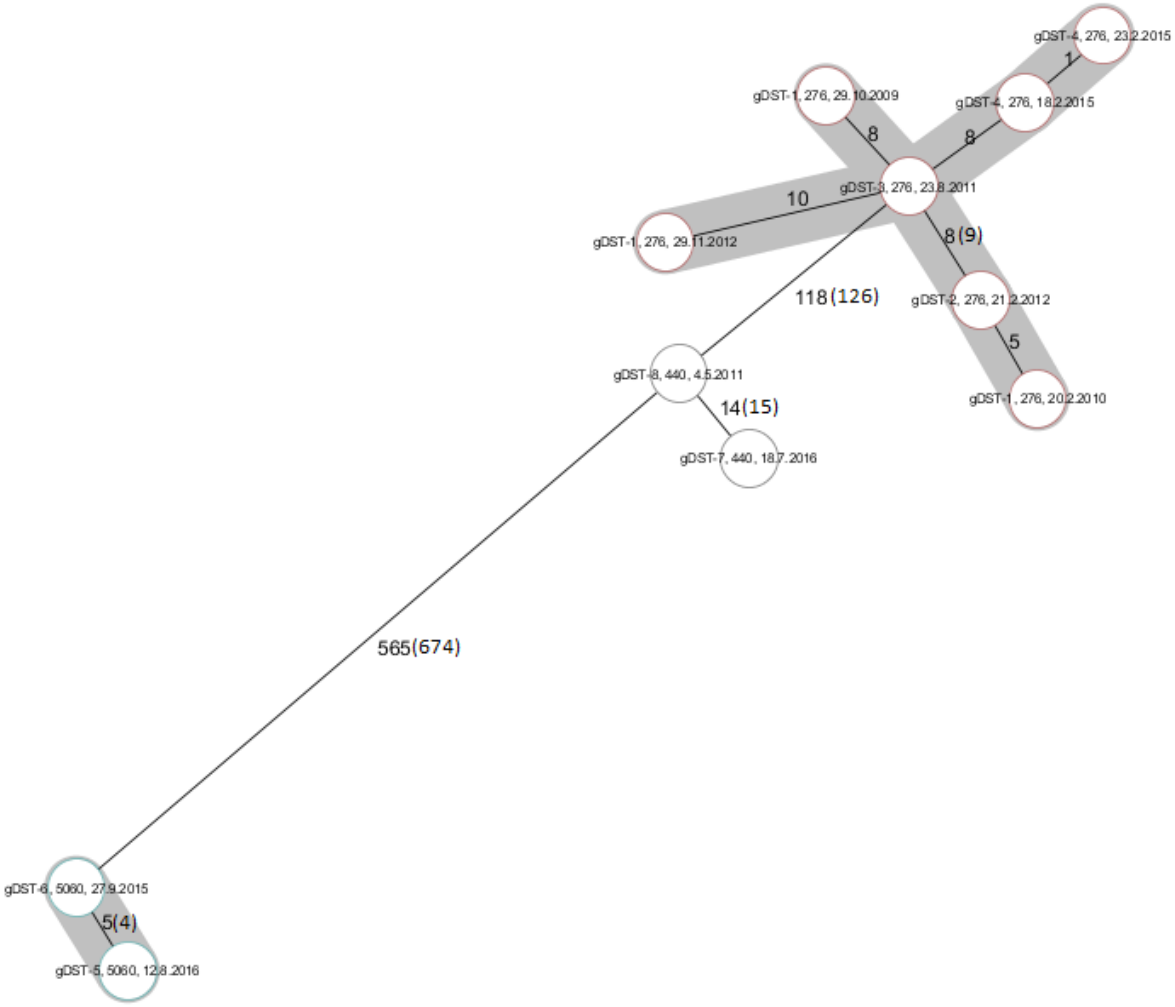
Supplementary laboratory results

The results of the RIF and INH NAATs were in agreement with the whole genome sequencing results except for three isolates having resistance mutations that were not included in the rapid tests (inhA and ndH coding regions, fabG1 promoter region, and the rpoB mutation ins433Phe).

Rifampicin resistance mutations were not found by the automatic settings of PhyResSE in the sequences of two isolates. One of these was found to contain an insertion (ins433Phe) in the rpoB gene. The mutation has earlier been found in highly rifampicin resistant isolates (14,15). The other isolate had wild-type RpoB sequence, and any rifampicin resistance mutations described in the mutation catalog published by WHO (16) were not found in the isolate.

Based on the clustering analysis by the cgMLST CT method, 11 (24.4%) of the isolates were clustered. The 11 isolates were found to contain eight different resistance mutation combinations. Six isolates, three isolate pairs, were within 5 loci from each other and could be suspected to be epidemiologically linked based on WGS. Of these, one pair was a mother-daughter pair with almost identical isolates (1 allele/SNP difference) and with identical resistance mutations. The two other pairs had an allelic distance of five and had different resistance mutations. One of these contained foreign-born cases that probably had been infected before their arrival in Finland. The other contained isolates from two Finnish -born cases which may have an epidemiological link. (Supplementary figure1).

a



b

gDST profile	RIF	INH	STR	EMB	PZA	KAN	FQ
1	rpoB Ser450Leu*	katG Ser315Thr*	rpsL Lys43Arg*	embB Met306Val*		eis c-10t	
2	rpoB Ser450Leu*	katG Ser315Thr*	rpsL Lys43Arg*	embB Met306Val*		eis g-10a	
3	rpoB Ser450Leu*	katG Ser315Thr*	rpsL Lys43Arg*	embB Met306Val*	pncA Asp136Asn	eis c-10t	gyrA Ala90Val*
4	rpoB Ser450Leu*	katG Ser315Thr*	rpsL Lys43Arg*	embB Met306Val*	pncA_186_ins1_c_ct**	eis c-10t	gyrA Ala90Val*
5	rpoB Ser450Leu*	katG Ser315Thr*	rpsL Lys43Arg*	embB Met306Val*	pncA Asp8Gly		
6	rpoB Ser450Leu*	katG Ser315Thr*	rpsL Lys43Arg*	embB Met306Val*	pncA Thr76Pro		
7	rpoB Ser450Leu*	katG Ser315Thr*		embB Met306Val*	pncA Phe94Cys*		
8	rpoB Ser450Leu*	katG Ser315Thr*			pncA Phe94Cys*		
	* High confidence mutation						
	** not included in the PhyResSe resistance mutation database						

Supplementary figure 1.

a) Minimum spanning tree of the isolates that were clustered based on the cgMLST complex type analysis. The isolates with a distance ≤12 loci are shaded. SNP distance detected within the cgMLST loci of is indicated in parentheses in case it differs from the respective allelic distance. Each isolate is marked with the gDST profile (see below), cluster type, and sampling date. In one isolate-pair, the SNP distance is bigger than the allelic distance due to insertions (in the CRISPR-associated protein Cas10) that were not included in the SNP analysis.

b) The genotypic DST profiles or resistance mutation combinations found in the isolates that were clustered by the cgMLST cluster type analysis.

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