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Extremely high prevalence of multidrug resistant tuberculosis in Murmansk, Russia: a population-based study

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Abstract Drug resistance and molecular epidemiology of tuberculosis (TB) in the Murmansk region was investigated in a 2-year, population-based surveillance of the civilian population. During 2003 and 2004, isolates from all culture-positive cases were collected ($n=1,226$). Prevalence of multi-drug resistance (MDR) was extremely high, as 114 out of 439 new cases (26.0%), and 574 out of 787 previously treated cases (72.9%) were resistant to at least isoniazid (INH) and rifampin (RIF). Spoligotyping of the primary MDR-TB isolates revealed that most isolates grouped to the Beijing SIT1 genotype ($n=91$, 79.8%). Isolates of this genotype were further analyzed by IS6110 RFLP. Sequencing of gene targets associated with INH and RIF resistance further showed that the MDR-TB strains are highly homogeneous as 78% of the MDR, SIT1 strains had the same resistance-conferring mutations. The genetic homogeneity of the MDR-TB strains indicates that they are actively transmitted in Murmansk.

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Introduction

Tuberculosis (TB) remains the world's deadliest curable infectious disease. In 2007 alone, there were an estimated 9.27 million new reported cases and 1.77 million deaths [1]. The problem is further exacerbated by the growing number of cases of multidrug-resistant TB (MDR-TB), accounting for 511,000 reported cases in 2007. MDR-TB is caused by *Mycobacterium tuberculosis* isolates resistant to at least isoniazid (INH) and rifampin (RIF) [2]. The acute spread of TB and MDR-TB in the Russian Federation has led the WHO to designate it the 11th highest TB burden country in the world; although recently the rising levels of MDR-TB have been reversed in the Russian oblast of Orel and Tomsk [1]. The case notification data in parts of Russia is increasingly accompanied by the phenomenon of drug resistance [3], making successful treatment and control of the disease difficult. The situation has become even more critical with the emergence of extensively drug-resistant tuberculosis (XDR-TB), defined as MDR strains that are additionally resistant to the second-line fluoroquinolones and at least one injectable drug (amikacin, kanamycin or capreomycin) [4]. Since this study was carried out in 2003–2004, "MDR-TB" comprises both MDR and XDR-TB isolates.

Since the implementation of *M. tuberculosis* genotyping, one group of clinical isolates, the "Beijing/W family," has received considerable attention [5]. These strains have been

shown to be highly prevalent throughout East Asia and the countries of the former Soviet Union, and have often been associated with community outbreaks, global transmission, and drug resistance [5, 6]. The Beijing strains have also been associated with TB treatment failures and relapse cases [7]. It has been further proposed that Beijing strains have some selective advantage over others [8].

Since 1997, a collaborative agreement on TB control has been established between Finland and the bordering regional government of Murmansk, the Russian Federation. The aim of the project "Improvement of prevention and treatment of tuberculosis in the Murmansk Region," implemented by the Finnish Lung Health Association (FILHA) and the Murmansk TB Dispensary, was to assist the regional tuberculosis program to combat the growing TB epidemic, drug-resistant TB cases, and reduce the number of cases of tuberculosis-associated mortality and morbidity. The project strategy was based on the international recommendations of the WHO and the International Union Against Tuberculosis and Lung Disease (IUATLD). This study is a part of the project.

The Murmansk region is situated in north-western Russia by the Arctic sea. The total population of the region is 885,000 inhabitants, of which 370,000 live in the city of Murmansk. A steady increase in TB notification rates was observed in the Murmansk region, as for the rest of the Russian Federation, since 1991. The peak of TB notification was registered in 2000 (72.4 cases per 100,000 inhabitants) followed by a decrease to 56.6 and 62.6 per 100,000 people in 2001 and 2002 respectively. During this period the reported primary (untreated patients) MDR-TB cases were 11 and 13 accounting for 6.5 and 7.3% of all new cases reported in 2000 and 2001 [9, 10]. During the years 1999–2002, treatment failure rates of new cases and relapses increased from 2.8% to 16.5% and from 9.1% to 36.4% respectively. Overall mortality due to TB per 100,000 inhabitants increased from 5.6 in 1999 to 11.5 in 2002. Nonetheless, the rate remained lower than in other parts of the Russian Federation including north western Russia. The DOTS program (Direct Observed Short Course Therapy) was introduced gradually to the region, reaching full geographical coverage in 2002. In 2003 and 2004, the tuberculosis notification rates in the Murmansk oblast comprised 396 and 451 tuberculosis cases, corresponding to incidences of 51.7 and 43.1 per 100,000 people respectively. The prevalence of human immunodeficiency virus (HIV) infection in the region in 2003 and 2004 was 147 and 137 cases (www.epinorth.org) and 9 new HIV-TB co-infection cases were found both in 2003 and 2004 (personal communication, Rauni Ruohonen, FILHA). Nonetheless, it has been suspected that these numbers are under-estimates given the limited resources, infrastructure, inadequate laboratory facilities, standardization, quality

control, and detection available. In addition, the increased treatment failure and mortality rates suggest that the actual number of MDR cases is considerably higher than reported.

The aims of this 2-year population-based study were to properly determine the frequency and characteristics of MDR-TB in the Murmansk region of the Russian Federation using molecular epidemiology techniques. The present study on the prevalence of drug-resistant tuberculosis was carried out in the certified new TB facilities in Murmansk following the recommendation of the WHO [11], and the performance of the new Murmansk TB Dispensary was also evaluated. Our results identify an extremely elevated number of cases of clustered MDR-TB in the region.

Materials and methods

Bacterial isolates and drug susceptibility testing

A total of 1,226 *M. tuberculosis* isolates, representing all culture-positive cases, were collected from civilian TB patients during the years 2003 and 2004 by the regional TB dispensary in Murmansk. Over one third (35.8%) of the patients ($n=439$) had never been treated for TB (new cases) while 64.2% ($n=787$) had been previously treated for TB (Table 1). Drug susceptibility testing (DST) was conducted for the four first-line drugs INH, RIF, streptomycin (STR), and ethambutol (EMB) by the proportion method on Löwenstein–Jensen media according to the WHO guidelines [12]. The performance of the DST was evaluated with a parallel analysis of a blind subset of isolates ($n=149$) in the Finnish Mycobacterial Reference Laboratory using the same methods as in the newly certified facilities in Murmansk. Furthermore, isolates that gave discrepant results between phenotypic DST and known genotypic markers associated with drug resistance were subjected to further DST analysis by BD BACTEC MGIT 960 SIRE (BD Biosciences, Sparks, MD, USA) for confirmation.

Table 1 Drug susceptibility test results in Murmansk

	2003 (%)	2004 (%)	Total (%)
New cases			
Total tested	197	242	439
Total susceptible	125 (63.5)	147 (60.7)	272 (62.0)
Any resistance	72 (36.5)	95 (39.3)	167 (38.0)
Multi-drug resistance	47 (23.9)	67 (27.7)	114 (26.0)
Previously treated cases			
Total tested	399	388	787
Total susceptible	69 (17.3)	78 (20.1)	147 (18.7)
Any resistance	330 (82.7)	310 (79.9)	640 (81.3)
Multi-drug resistance	296 (74.2)	278 (71.6)	574 (72.9)

Spoligotyping and IS*6110* RFLP

Mycobacterium tuberculosis isolates from new cases (*n*=439) were subjected to spoligotyping according to standard recommendations [13]. The spoligotyping results were entered in binary format (1 and 0) according to the hybridization results (positive or negative) as Excel (Microsoft) spreadsheets. The spoligotype SIT (spoligo-international type number) was determined by comparing the patterns with those in the international spoligotype database of the Institute Pasteur of Guadeloupe (the SITVIT database, http://www.pasteur-guadeloupe.fr:8081/SITVITDemo/index.jsp).

The primary MDR-TB isolates that could not be discriminated by spoligotyping (the Beijing SIT1 isolates, *n*=91) were further genotyped by IS6110 RFLP [14]. The RFLP data were analyzed using the BioNumerics v.4.01 software (Applied Maths, Sint-Martens-Latem, Belgium) and the unweighted pair group (UPGMA) method of arithmetic averaging with the Dice coefficient and 1% position tolerance settings for cluster analysis. The UPGMA method clusters the pair of isolates that are most similar to each other first, and then clusters the next-most similar isolates, until all isolates are analyzed. A cluster was defined as two or more isolates that were indistinguishable by IS6110 RFLP and spoligotype patterns.

Molecular characterization of drug resistance

The *rpoB* and *katG* genes of phenotypically MDR isolates from new cases (*n*=114) were sequenced as previously described [14–16]. The complete *rpoB* gene and flanking 100 bp were sequenced for phenotypically MDR isolates encoding a wild-type rifampin resistance-determining region (RRDR, 81 bp). All samples were sequenced in the forward and reverse directions.

Results

This 2-year population-based study revealed an extremely high prevalence of MDR-TB in the Murmansk region (Table 1). In total, 688 (56.1%) of all isolates were resistant to at least INH and RIF. Primary MDR-TB was encountered in 114 of the 439 new cases (26.0%) and in 574 of the 787 (72.9%) previously treated patients. Only 419 isolates (34.2%) were fully susceptible, while 119 (9.7%) displayed drug resistance other than MDR. An integral part of the project was the quality control of the DST results obtained in Murmansk. Parallel analysis of 149 *M. tuberculosis* isolates showed that the overall concordance during the study period was 94.0%. The accuracy of individual drugs was 93.4% for INH, 98.7% for RIF, 96.0% for EMB, and 87.8% for STR.

Mycobacterium tuberculosis isolates from new cases were subjected to genotyping. As such, 437 (99.5%) of the 439 isolates were successfully spoligotyped to 82 different spoligotypes, of which 48 (389 isolates) matched known SITs, while 34 were new spoligopatterns not previously deposited in the international spoligotype database of the Institute Pasteur of Guadeloupe. Furthermore, 387 isolates (88.6%) grouped into 34 different spoligoclusters with between 2 and 184 isolates per cluster, while 50 isolates had a unique spoligotype. Of the clustered isolates, 367 (94.8%) belonged to a spoligotype with a matching spoligotype profile in the SITVIT database and the remaining 20 isolates (5.2%) belonged to eight spoligotypes that were not retrievable from the spoligotype database (Table 2). Among the 34 clusters, 12 contained five or more isolates and were defined as major clusters, which comprised 86.8% (*n*=336) of the clustered isolates. The largest cluster, the SIT1 spoligotype of the Beijing family, comprised 184 *M. tuberculosis* isolates (41.9% of all new cases). Accordingly, the majority of the primary MDR-TB isolates (108 out of 114, 94.7%) displayed a spoligopattern present in the SITVIT database and 92 (80.7%) MDR-TB isolates belonged to the Beijing family spoligotypes SIT1 or SIT255 (Table 2). The MDR Beijing SIT1 isolates (*n*=91) were further sub-grouped using IS6110 RFLP. Twelve distinct clusters involving 73 isolates (80%) were recognized. The largest IS6110 cluster had 29 isolates (32%), and the remaining 11 clusters included 2 to 9 isolates each (Fig. 1). The IS6110 patterns of 18 MDR Beijing isolates were unique in this study. The number of IS6110 copies per isolate varied from 12 to 21.

The *katG* and *rpoB* genes of the primary MDR isolates (*n*=114) were sequenced in order to identify the mutations associated with the INH and RIF resistance respectively. Ser315Thr (AGC→ACC) mutation in *katG* occurred in 97.4% (*n*=111) of the sequenced isolates (Table 3). All the Beijing SIT1 and SIT255 isolates (*n*=92) contained *katG* Arg463Leu (CGG→CTG) single nucleotide polymorphism (SNP). This SNP is characteristic of isolates belonging to the principal genetic grouping 1 (PPG1) [17]. Two phenotypically isoniazid-resistant isolates encoded a wild-type *katG* gene and one Beijing isolate had only the Arg463Leu SNP in the *katG*. One Beijing SIT1 isolate contained an additional silent GGC→GGA mutation on codon 451Gly.

The *rpoB* Ser531Leu (TCG→TTG) mutation was found in 87 (76.3%) of the primary MDR isolates, including 74 Beijing SIT1 isolates (Table 3). The second most commonly found SNP was on codon *rpoB*526, in which four different mutations were found in 13 isolates (11%) representing various spoligotypes. Five of the *rpoB*526 mutants had additional *rpoB* mutations within the RRDR. Three isolates shared dual Leu511Pro and His526 mutations. In 2 Beijing

Table 2 Prevalence of *M. tuberculosis* spoligotype clusters (*n*=34) found in Murmansk

SIT[a]	Family[b]	*n*[e]	Percentage	*n* (MDR)[f]
1	Beijing	184	47.5	91
255	Beijing	3	0.8	1
260	Beijing	2	0.5	0
269	Beijing-like	4	1.0	0
262	H4	28	7.2	3
35	H4	21	5.4	0
762	H4	2	0.5	0
1134	H4	2	0.5	1
50	H3	7	1.8	2
47	H1	10	2.6	2
531	H1	8	2.1	1
1155	H1	3	0.8	0
42	LAM9	16	4.1	5
53	T1	15	3.9	0
264	T1	6	1.6	0
205	T1	2	0.5	0
498	T1	2	0.5	0
766	T1	2	0.5	0
280	T1-RUS	3	0.8	0
1173	T1-RUS2	3	0.8	0
52	T2	26	6.7	0
560	U	10	2.6	0
1562	U	2	0.5	0
254	T5-RUS1	2	0.5	0
40	T4	2	0.5	0
482	BCG[c]	2	0.5	0
577763777720771	ND[d]	2	0.5	0
747637777760771	ND	2	0.5	0
777467607760771	ND	2	0.5	2
777763774020771	ND	5	1.3	0
777770774020771	ND	2	0.5	2
777777347760411	ND	2	0.5	0
777777763760771	ND	2	0.5	0
777741001760771	ND	3	0.8	0
Total		387	100	110

[a] SIT, designation of the spoligotype in the international database (http://www.pasteur-guadeloupe.fr:8081/SITVITDemo/trouverSIT.jsp). If an SIT designation was not found in the database, the spoligotype is described by its octacode

[b] Spoligotype family designated in SPOLDB4 [8]

[c] *M. bovis* BCG

[d] ND, spoligotype not designated in SpolDB4 [8]

[e] Number of isolates with a certain spoligotype

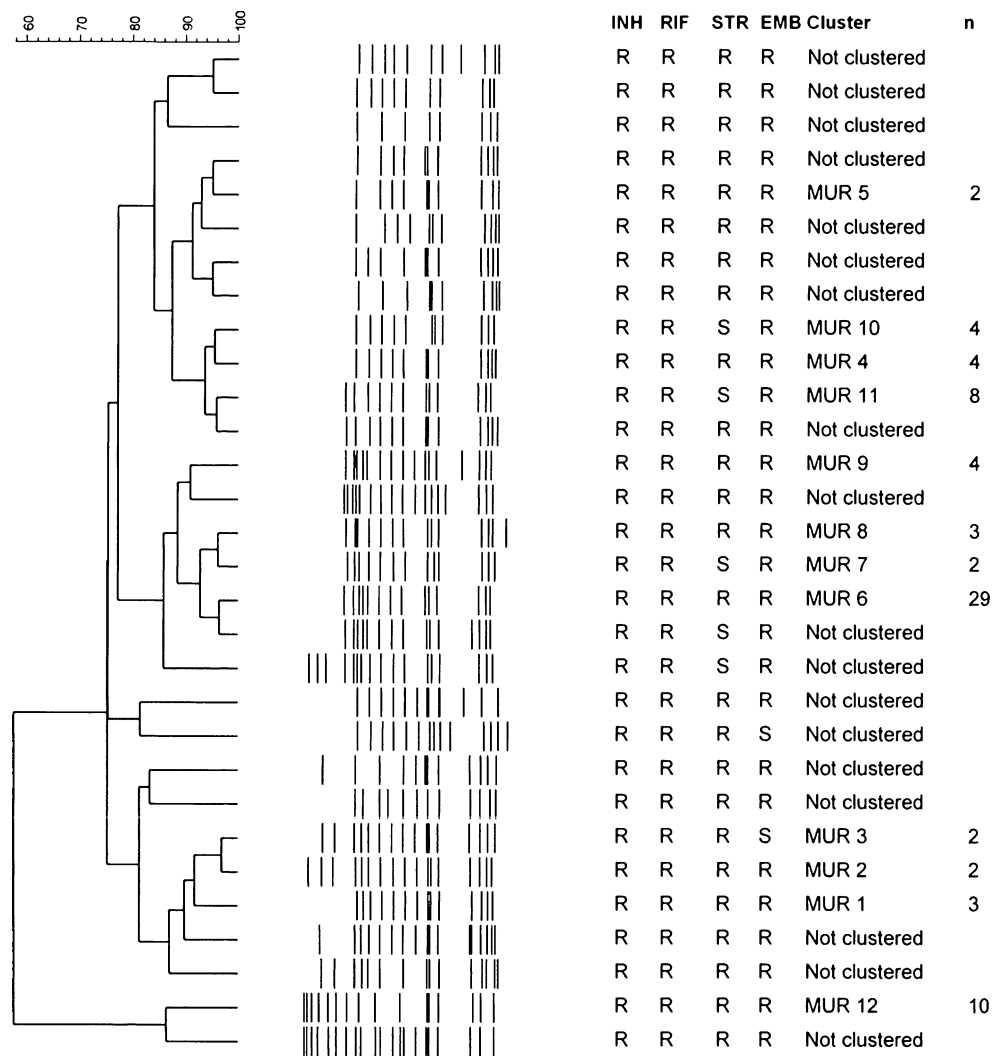[f] Number of multi-drug resistant isolates with a certain spoligotype

SIT1 isolates, multiple *rpoB* mutations were found, 1 isolate encoded His526Leu and an in-frame deletion of codons 508–509 and the other had 4 point mutations on codons 512, 525, 526, and 527 (Table 3). These isolates were clustered by IS*6110* RFLP (clusters 6 and 12, Fig. 1). Based on the IS*6110* fingerprinting and spoligotyping data, no indication of double infection was found. Original samples of all isolates encoding multiple SNPs within the *rpoB* were re-sequenced from fresh isolates in order to rule out sequencing errors or selection of mutants from the sub-culture. Finally, 4 other isolates had mutations on codon *rpoB*516 and 10 (8.8%) rifampin-resistant isolates encoded a wild-type RRDR in the *rpoB*. Correlation of the rifampin-resistant but wild-type RRDR isolates with spoligopatterns showed that 9 out of 10 grouped to the Beijing SIT1 family, and 1 to SIT262. Judging by IS*6110* RFLP, only 1 of the 9 Beijing SIT1 family members was clustered (cluster 16, Fig. 1), and the remaining 8 isolates represented unique IS*6110* fingerprints. Interestingly, the SIT262 wild-type *rpoB* isolate also had wild-type *kat*G. The entire *rpoB* gene and 100 nucleotides upstream were sequenced for the 10 rifampin-resistant isolates encoding a wild-type RRDR fragment. Seven of the 9 Beijing-type isolates were found to encode an SNP on codon Ser450Leu (TCG→TTG) in addition to the silent mutation on codon 1075 (GCG→GCT).

Discussion

This is the first population-based report on the prevalence of *M. tuberculosis* drug resistance in Murmansk. Our drug resistance surveillance results during 2003 to 2004 revealed that a quarter (26.0%) of all new TB cases and three quarters (72.9%) of the re-treatment cases comprised MDR-TB, resulting in a rapid overall increase in the cumulative number of MDR cases. This is the highest rate of MDR prevalence among both new and re-treatment cases in a civilian population ever reported [3, 18, 19] to our knowledge, although the situation is very similar in Azerbaijan, where nearly a quarter of all new TB cases (22.3%) are MDR [20]. The high mortality (15% and 22% in years 2003 and 2004) and treatment failure (11% and 17% in 2003 and 2004 respectively) rates in the Murmansk Region could most likely be attributed to the elevated number of MDR cases. The high level of MDR tuberculosis in these regions may partly be due to the difficulties in the drug logistics that occurred in Russia after the collapse of the Soviet Union. Because of these difficulties, TB patients were treated with separate drugs instead of the recommended four-drug regimen, which may have contributed to the development of antibiotic resistance.

Control of TB in the Murmansk region has remained difficult and the problems with treatment adherence and

Fig. 1 IS6110 RFLP dendro-gram of Beijing (SIT1) MDR-TB strains. *n*=number of isolates in defined RFLP cluster

	INH	RIF	STR	EMB	Cluster	n
	R	R	R	R	Not clustered	
	R	R	R	R	Not clustered	
	R	R	R	R	Not clustered	
	R	R	R	R	Not clustered	
	R	R	R	R	MUR 5	2
	R	R	R	R	Not clustered	
	R	R	R	R	Not clustered	
	R	R	R	R	Not clustered	
	R	R	S	R	MUR 10	4
	R	R	R	R	MUR 4	4
	R	R	S	R	MUR 11	8
	R	R	R	R	Not clustered	
	R	R	R	R	MUR 9	4
	R	R	R	R	Not clustered	
	R	R	R	R	MUR 8	3
	R	R	S	R	MUR 7	2
	R	R	R	R	MUR 6	29
	R	R	S	R	Not clustered	
	R	R	S	R	Not clustered	
	R	R	R	R	Not clustered	
	R	R	R	S	Not clustered	
	R	R	R	R	Not clustered	
	R	R	R	R	Not clustered	
	R	R	R	S	MUR 3	2
	R	R	R	R	MUR 2	2
	R	R	R	R	MUR 1	3
	R	R	R	R	Not clustered	
	R	R	R	R	Not clustered	
	R	R	R	R	MUR 12	10
	R	R	R	R	Not clustered	

DOTS are persistent while working with a socially marginalized population. The problem is exacerbated with the elevated numbers of MDR and HIV co-infection. A well-functioning DOTS program, coupled with accurate timely diagnosis and laboratory identification, is a prerequisite for effective management of MDR-TB. This is only possible while laboratory diagnostics and DST are reliable and include appropriate quality control. Timely detection of drug-resistant TB, and adequate treatment regimens administered early in the course of the disease, are essential in the prevention of primary transmission [21]. The results of this study confirm the accuracy of DST results obtained by the central TB laboratory of the Murmansk region. It also shows the extremely high proportion of MDR among newly detected cases, reflecting the ongoing transmission of MDR in society.

The use of a rapid test for RIF resistance, a surrogate marker for MDR, is an excellent method for identifying MDR-TB strains. Patients infected with RIF-resistant or MDR strains that are rapidly identified can be properly isolated and placed on alternative anti-microbial treatment regimens [21]. In this study, 91.2% of the MDR-TB isolates could have been identified by screening the RRDR region of the *rpoB* gene. The profiles of mutations associated with drug resistance observed in this study coupled with those reported in the literature allows existing molecular markers to be utilized and new ones to be designed that target specific SNPs to accelerate the identification process of RIF-resistant isolates. For example, SNP-specific primers targeting *rpoB* mutations 531 and 526 [22] may be used upfront while susceptibility tests are still under evaluation gaining valuable time and allowing for adequate treatment and isolation procedures.

All isolates obtained from new cases were characterized by genotyping (spoligotyping) and the MDR-TB isolates of new TB patients were further analyzed by sequencing of the *kat*G and *rpoB* genes. In addition, MDR isolates belonging to the SIT1 genotype were further genotyped by IS*6110* RFLP. Comparison of spoligotypes from Murmansk with the international spoligotyping database

Table 3 Characterization of MDR-TB isolates by spoligotyping and *rpo*B and *kat*G gene sequencing

SIT[a]	Clade[b]	katG	rpoB	n	Percentage
1	Beijing	315 ACC, 463 CTG	531 TTG	71	62.3
		315 ACC, 463 CTG	526 GAC	6	5.3
		315 ACC, 463 CTG	wt	9	7.9
		315 ACC, 463 CTG	Deletion codons 508, 509; 526 CTC	1	0.9
		315 ACC, 463 CTG	512 AGA, 525 ACG, 526 TCC, 527 CAG	1	0.9
		315 ACC, 451 GGA, 463 CTG	531 TTG	1	0.9
		463 CTG	531 TTG	1	0.9
		wt	531 TTG	1	0.9
42	LAM-9	315 ACC	526 GAC	2	1.8
		315 ACC	511 CCG, 526 AAG	3	2.6
47	H-1	315 AGA	531 TTG	2	1.8
50	H-3	315 ACC	531 TTG	2	1.8
237	U	315 ACC	516 TTC	1	0.9
255	Beijing	315 ACC, 463 CTG	531 TTG	1	0.9
262	H-4	315 ACC	516 GTC	1	0.9
		315 ACC	531 TTG	1	0.9
		wt	wt	1	0.9
531	H-1	315 ACC	531 TTG	1	0.9
746	H-3	315 ACC	531 TTG	1	0.9
1134	H-4	315 ACC	516 GTC	1	0.9
					0.0
777467607760771		315 ACC	516 GTC	1	0.9
		315 ACC	531 TTG	1	0.9
777770774020771		315 ACC	531 TTG	2	1.8
777771774020771		315 ACC	531 TTG	1	0.9
777777607760031		315 ACC	531 TTG	1	0.9
			Total	114	100.0

[a] SIT, designation of the spoligotype in the international database (http://www.pasteur-guadeloupe.fr:8081/SITVITDemo/trouverSIT.jsp). If a SIT designation was not found in the database, the spoligotype was described by its octacode

[b] Clade, defined as described in [8]: LAM: Latin American and Mediterranean, H: Haarlem, U: undefined

SpolDB4 [8], revealed that the majority (389 out of 437, 89.0%) of the isolates belonged to known SIT patterns. Several families of *M. tuberculosis* strains have been recognized based on spoligotypes, including Beijing, T, Haarlem (H), and LAM. In this study, most samples were grouped with the Beijing strain family (44%, *n*=193; SIT1, SIT255, SIT260, and SIT269). This is similar to the findings from the Archangel oblast (44.5%) [23] and to the results of a study containing 36 TB isolates from the Murmansk region, of which 47.2% represented Beijing spoligotypes [24]. The most prevalent spoligotype of this study, SIT1, included 41.9% of the isolates and 79.8% of the MDR isolates. The SIT1 spoligotype is characteristic of the Beijing/W family, which is known for its frequent association with MDR outbreaks and high emergence worldwide. The prevalence of Beijing isolates in Russia, both in prisons and in the community, and the high rate of

MDR typical for this group have been recognized in a number of studies [5, 6, 23, 25–27]. Our results are consistent with this observation and demonstrate that the Beijing genotype plays an important role in the epidemiology of MDR-TB in the civilian population of this high incidence region of the north western Russia.

Clustering of IS*6110* fingerprints has been used to estimate the amount of recent transmission in a population [28]. The high proportion (80%, *n*=73) of IS*6110* clustered Beijing isolates, as well as the fact that one quarter of new TB cases are MDR-TB, indicates that these MDR-TB strains are actively transmitted throughout the community. The rate of IS*6110* clustered MDR cases (80%) is similar to that observed in Hungary (75%) [29], but lower than in reports from Estonia (95.8%) [23, 30].

The most common resistance mutation found in primary MDR isolates was the *katG* Ser315Thr mutation

AGC→ACC ($n=109$, 95.6%). This is consistent with the observation that an unusually high proportion of Ser315Thr mutation is characteristic of INH-resistant strains in Russia [31–33]. As previously reported, mutations in codons 531, 526, and 516 of *rpoB* are responsible for 86–100% of RIF-resistant TB cases in Russia, in agreement with other global reports originating from regions with high rates of MDR-TB [34]. This was also seen in Murmansk, where mutations in codon rpoB531 accounted for 91.2% ($n=104$) of the RIF resistance among the primary MDR isolates. The *rpoB*531 mutation was more prevalent among the Beijing than the non-Beijing MDR strains (81.5% and 54.5%), whereas the Ser315Thr in *kat*G was highly prevalent independent of the genotype. There are conflicting reports concerning the correlation of prevalence of mutations and spoligotypes [35–37], suggesting that the association of particular mutations and the Beijing genotype could be a regional evolutionary phenomenon. The biased presentation of resistance mutations in the Beijing family strains observed in some former Soviet Union countries could be due to the frequent spread of particular resistant and virulent clones of the Beijing genotype.

No SNPs within the 81bp RRDR were found in 10 rifampin-resistant isolates (8.8%). Complete sequencing of the *rpoB* identified mutation Ser450Leu (TCG>TTG) in 7 of these isolates. However, correlation between this newly reported SNP and rifampin resistance has not been confirmed. Likewise, this mutation is has not been reported in the TB Drug Resistance Database (http://www.tbdreamdb.com/RIF_Rv0667_AllMutations.html). The absence of SNPs within the RRDR has been reported in approximately 10% of MDR cases in other studies [33, 38] indicating that other resistance mechanisms are also involved and should be studied. Two isolates presented with multiple mutations in *rpoB*. Based on the fingerprinting data, no indication of dual infection was found in these isolates. Dual or multiple mutations in the *rpoB* have been reported in Kazakhstan [37], Turkey [39] Belarus [40], and South Korea [41] to mention a few.

Conclusions

An extremely high prevalence of MDR-TB combined with an ongoing active transmission of the Beijing genotype was observed in Murmansk. Early case finding is crucial for prevention of the spread of MDR-TB. Since INH and RIF resistance in Murmansk were caused by "common" mutations, molecular assays targeting these mutations can be used for rapid detection of MDR-TB in most cases. Thus, introduction of molecular methods suitable for the rapid detection of MDR-TB could improve disease management and decrease the transmission rate in this area.

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