Primary Multidrug-Resistant Mycobacterium tuberculosis in 2 Regions, Eastern Siberia, Russian Federation

Svetlana Zhdanova,¹ Scott K. Heysell,¹ Oleg Ogarkov, Galina Boyarinaova, Galina Alexeeva, Suporn Pholwat, Elena Zorkaltseva, Eric R. Houpt, and Eugeniy Savilov

Of 235 Mycobacterium tuberculosis isolates from patients who had not received tuberculosis treatment in the Irkutsk oblast and the Sakha Republic (Yakutia), eastern Siberia, 61 (26%) were multidrug resistant. A novel strain, S256, clustered among these isolates and carried eis-related kanamycin resistance, indicating a need for locally informed diagnosis and treatment strategies.

In 2010, tuberculosis (TB) prevalence in the Russian Federation was 136 cases per 100,000 population; the estimated proportion of multidrug resistance, defined as resistance to isoniazid and rifampin in the absence of prior treatment (primary MDR TB), was 18% (†). However, at the subnational level, primary MDR TB might be highly variable; in oblasts or republics with continuous surveillance data, drug resistance varies from 5.4% to 28.3% (2). These data are predominantly from the western half of the country and do not include eastern Siberia.

In 2009, in the Irkutsk oblast in eastern Siberia, TB prevalence was 373 cases per 100,000 population and HIV prevalence was among the highest in the Russian Federation (3,4). In contrast, in the sparsely populated neighboring Sakha Republic (Yakutia), TB prevalence was lower (188 cases/100,000 population) and HIV was thought to be scarce (4). Molecular typing has found that more than half of the Mycobacterium tuberculosis isolates from the Russian Federation are the Beijing genotype, a pandemic lineage associated with MDR phenotype and characteristic drug-resistance mutations; prevalence of this genotype in Irkutsk is high (5,6). However, such investigation has not been performed in Yakutia. Given the distinct sociocultural patterns between Irkutsk and Yakutia, we hypothesized that the molecular epidemiology and drug-resistance patterns of M. tuberculosis from patients with primary MDR TB would be regionally distinct.

The Study

From November 2008 through May 2010, M. tuberculosis isolates were cultured during routine care of adults ≥18 years of age with primary TB and no history of treatment. The patients were from 2 regional referral centers, the Irkutsk Regional TB-Prevention Dispensary and the Research Practice Center for Phthisiatry (Yakutia); the study was approved by the institutional review boards at the University of Virginia and Irkutsk State Medical University.

Initial pretreatment isolates were grown on Lowenstein-Jensen agar slants and identified to species in accordance with World Health Organization recommendations. Drug susceptibility was tested by absolute concentration method on agar slants; drugs tested were rifampin (critical concentration 40 µg/mL), isoniazid (1 µg/mL and 10 µg/mL), ethambutol (2 µg/mL), streptomycin (10 µg/mL), ethionamide (30 µg/mL), and kanamycin (30 µg/mL). Susceptibility to a fluoroquinolone and pyrazinamide was not routinely tested. DNA extraction was performed on all isolates, followed by 12-loci mycobacterial interspersed repetitive unit–variable number tandem repeat (MIRU-VNTR) analysis (7) and further lineage definition by region of difference deletions, or for Ural strains as described (5). Phylogenetic tree construction was based on the MIRUVNTRplus database (8), and VNTR international type numbers were confirmed on the SITVIT database (9). DNA from MDR isolates was amplified and sequenced for the known drug-resistance determining regions katG, inhA, rpoB, embB, gyrA, rrs, and eis by using methods described by the Centers for Disease Control and Prevention (10). For pncA, the entire open reading frame and upstream promoter region were amplified. Sequences were compared with published sequences for M. tuberculosis H37Rv by using GeneDoc version 2.7.0.

Among 235 patients with primary TB (130 from Yakutia, 105 from Irkutsk), isoniazid mono-resistance was found in isolates from 16 (12%) from Yakutia and 19 (18%) from

Author affiliations: Russian Academy of Medical Sciences, Irkutsk, Russian Federation (S. Zhdanova, O. Ogarkov, E. Savilov); University of Virginia, Charlottesville, Virginia, USA (S.K. Heysell, G. Boyarinaova, S. Pholwat, E.R. Houpt); Regional TB-Prevention Dispensary, Irkutsk (O. Ogarkov, E. Zorkaltseva); Research Practice Center for Phthisiatry, Yakutsk, Russian Federation (G. Alexeeva); and State Medical Continuing Education Academy, Irkutsk (E. Zorkaltseva, E. Savilov)

DOI: http://dx.doi.org/10.3201/eid1910.121108

¹These authors contributed equally to this article.
Irkutsk (p = 0.27). Multidrug resistance was found for 61 patients (36 [28%] from Yakutia and 25 [24%] from Irkutsk) (p = 0.55). Mean age (± SD) for these 61 patients was 33 (± 12) years, 40 (66%) were male, and these characteristics did not differ significantly between patients from Irkutsk and from Yakutia. However, no HIV-infected patients were identified from Yakutia compared with 11 (44%) with MDR TB from Irkutsk (p<0.001). Twelve MDR TB patients from Irkutsk died (outcome unknown for the other 13 patients), including all with HIV, compared with 4 (11%) from Yakutia who died (p = 0.002). Follow-up varied and was limited mostly to inpatients.

Among all 235 patients with primary TB, strains of the Beijing family were significantly more common among those from Irkutsk (70 [67%]) than from Yakutia (40 [31%]) (p<0.001). However, strains found in Yakutia (S 256 [11%], T 8 [7%], and Ural 171 [5%]) were not found in Irkutsk (Table 1). The cluster of S 256 (MIRU profile 233325153325) was the most common among primary MDR TB isolates from Yakutia and was fully 86% MDR (Table 1; online Technical Appendix, wwwnc.cdc.gov/EID/article/19/10/13-1108-Techapp1.pdf). Among isolates from patients with primary MDR TB, 51 (84%) were available for DNA sequencing: 27 from Yakutia and 24 from Irkutsk (Table 2; an expanded version of this table is available at wwwnc.cdc.gov/EID/article/19/10/13-1108-T2.htm). Among isoniazid-resistant isolates, the mutation in codon 315 of katG was present in 91%. Among rifampin-resistant isolates, mutations in the resistance-determining region of rpoB (codons 511–533) were present in only 79%. The pncA mutation was common across genotypes from both sites, occurring in 62% of isolates amplified. Notably, both isolates with mutation in eis from Yakutia occurred in MDR strains with the S 256 genotype and without rrs mutation.

Conclusions

In eastern Siberia, >25% of primary TB was MDR, equivalent to the highest proportion reported from the Russian Federation (2). However, regionally specific genotypic patterns and resistance mutations were identified. As expected, in Irkutsk primary MDR TB was driven by strains of Beijing lineage (5,6). Yet in the more geographically isolated population of Yakutia, a strain previously unidentified in the Russian Federation, S 256, had a MIRU profile recently found among Canadian Aboriginal populations (11). In Yakutia, S 256 was highly drug resistant and was the most common genotype among patients with primary MDR TB.

Although rpoB mutations were found in only 79% of rifampin-resistant isolates, these findings are consistent with those in a recent report from Novosibirsk oblast, which similarly included non-Beijing and S-family strains and found a sensitivity of only 63% for the rpoB mutation (12). Lack of phenotypic correlation can result from alternate mechanisms of resistance or imperfect conventional susceptibilities in Lowenstein-Jensen medium or from use of old drug stock. Such discrepancy necessitates urgent clarification because substitution of conventional susceptibility testing with molecular probe–based methods such as GeneXpert MTB/RIF (Cepheid, CA, USA) has been strongly advocated but would lead to dramatically different results and treatment regimens (13). Of note, isolates of the S 256 strain accounted for a proportion of the cases in which mutation in the promoter region of eis was associated with kanamycin resistance, but rrs was wild type. Commercial assays have focused on the rrs locus, which has greater sensitivity for amikacin, as the sole target for the class of injectable agents (14), yet in Eastern Siberia, the injectable agent available is kanamycin. Furthermore, we found a range of reported and unreported mutations across the entire pncA gene; most were point mutations resulting in amino acid substitution, but some strains had mutations that resulted in deletion or frameshift. Phenotypic methods and assays of functional pyrazinamidase activity should be performed in this region because results might have major implications for novel MDR TB drugs that work best with pyrazinamide (15).

Study limitations include selection bias of isolates from passive surveillance. We were unable to obtain detailed clinical information about all patients with primary TB, thus preventing adequate comparison of nongenotypic risk factors for MDR TB or establishment of definitive

| MIRU-VNTR 12 | Family/ MIT | No. (%) total, n = 105 | No. (%) MDR, n = 25 | p value | No. (%) total, n = 130 | No. (%) MDR, n = 36 | p value |
|--------------|-------------|------------------------|---------------------|---------|------------------------|---------------------|---------|
| 223325153533 | Beijing 16  | 32 (31)                | 7 (28)              | <0.001  | 12 (9)                 | 1 (3)              | 0.006   |
| 223325173535 | Beijing 17  | 13 (12)                | 6 (24)              | 0.27    | 10 (8)                 | 7 (19)             | 0.76    |
| 233325153325 | S 256       | 0                      | 0                   | <0.001  | 14 (11)                | 12 (33)            | 0.001   |
| 223125153324 | T 8         | 0                      | 0                   | NA      | 9 (7)                  | 0                  | 0.005   |
| 227225113223 | Ural 171    | 0                      | 0                   | NA      | 6 (5)                  | 0                  | 0.03    |
| 223325153433 | Beijing 592 | 1 (1)                  | 0                   | NA      | 4 (3)                  | 0                  | 0.38    |

* MIRU-VNTR, mycobacterial interspersed repetitive unit–variable number tandem repeat (original 12-loci profile). Included genotypes found in ≥5 isolates only; MIT, MIRU-VNTR international type; MDR, multidrug-resistant tuberculosis (conventional resistance to isoniazid and rifampin); NA, not applicable. Significance determined by χ² analysis with Yates correction or Fisher exact test when appropriate.
emerging epidemiologic links among clustered isolates. Furthermore, lack of conventional fluoroquinolone or pyrazinamide susceptibility testing limited comparison with gyrA and pncA mutations, respectively. Despite these limitations, this work characterizes severe isoniazid monoresistant and MDR TB in eastern Siberia among patients with no history of TB treatment. The regionally distinct phylogenetic patterns and certain drug-resistance mutations necessitate careful application of novel diagnostics and empiric therapeutic strategies.

S.K.H. was supported by National Institutes of Health grant K23AI099019 and an award from the Burroughs Wellcome Fund/American Society of Tropical Medicine and Hygiene. E.R.H.

Table 2. Resistance mutations in Mycobacterium tuberculosis from 51 patients from Irkutsk and Yakutia, Russian Federation*

| Drug, locus | Amino acid change | Nucleotide change | Drug resistance, no. (% with mutation) |
|-------------|-------------------|-------------------|----------------------------------------|
| Rifampin, rpoB | Ser531Leu 19 (37) | Gln513Lys 2 (4) | 15 (79) |
| | Ser513Leu/Thr481Ala 1 (2) | Leu533Pro 1 (2) | 0 |
| | Ser531Leu/Thr480Ile 2 (4) | His516Tyr 1 (2) | 2 (100) |
| | Ser531Tryp/Val456Gly 1 (2) | Leu511Pro 1 (2) | 1 (100) |
| | No mutation 22 | No amplification 1 (2) | 6 (27) |

Fluoroquinolones, gyrA

| Drug | Amino acid change | Nucleotide change | Drug resistance, no. (% with mutation) |
|------|-------------------|-------------------|----------------------------------------|
| Ser95Thr | Asp94Gly 1 (2) | Gly90Ala 1 (2) | 1 (33) |
| | Asp94Ala 1 (2) | No amplification 2 (4) | 0 |

Ethambutol, embB

| Drug | Amino acid change | Nucleotide change | Drug resistance, no. (% with mutation) |
|------|-------------------|-------------------|----------------------------------------|
| Asp354Ala 3 (6) | Asp354Ala 1 (2) | Met306Val 3 (6) | 1 (33) |
| | Met306Ile 3 (6) | Gly406Ser 3 (6) | 3 (100) |
| | Gly406Ala 2 (4) | Gly406Cyct 1 (2) | 2 (67) |
| | No mutation 25 (49) | No amplification 10 (20) | 0 |

Pyrazinamide, pncA‡

| Drug | Amino acid change | Nucleotide change | Drug resistance, no. (% with mutation) |
|------|-------------------|-------------------|----------------------------------------|
| Gly113Phe | Gly113Phe 3 (6)§ | G338T and C96T | 4 (57) |
| | Leu19Arg 2 (4)§ | G338T and C96T/G362T | 3 (75) |
| | Arg121Gly 2 (2)§ | G362T | 1 (33) |
| | Gln10Pro 1 (2) | A29C | 3 (100) |
| | Val7Gly 1 (2) | T20G/G481C | 3 (100) |
| | Ala161Pro/Val155Ala 1 (2)§ | G203A | 0 |
| | His137Asp/Frame1 1 (2)§ | T464C/insertion C480 | 1 (100) |
| | Tryp68Stop 1 (2)§ | C409G | 9 (36) |
| | Frameshift 1 (2)§ | deletionG5 | 3 (30) |
| | No mutation 8 (14) | Not performed |
| | No amplification 30 (59) |

Kanamycin

| Drug | Amino acid change | Nucleotide change | Drug resistance, no. (% with mutation) |
|------|-------------------|-------------------|----------------------------------------|
| rrs | A1401G 4 (57) | C1443G 3 (43) | 3 (75) |
| | No mutation 35 | 12 (34) |
| | No amplification 9 | 3 (33) |
| eis | G(−10)A 4 (44) | C(−14)T 1 (11) | 2 (50)¶ |
| | C(−14)G 2 (22) | C(−14)G 1 (11) | 0 |
| | C(−12)T 1 (11) | C(−12)T 1 (11) | 0 |
| | No mutation 36 | No amplification 6 | 11 |
| | 3 (50) |

*Sequencing for inhA and katG and correlation with isoniazid resistance available in expanded online version of Table 2 (wwwnc.cdc.gov/EID/article/19/10/12-1108-T2.htm).
†Previously demonstrated not to be associated with phenotypic resistance.
‡Excluding 25 silent pncA mutations (Ser32Ser most common, n = 14).
§Mutations in pncA not previously reported. Conventional susceptibility testing was unavailable for pyrazinamide and the fluoroquinolones.
¶For all 3 mutations of eis associated with kanamycin resistance, rrs was wild type.
was supported in part by National Institutes of Health grant R01AI093358.

Dr Zhdanova is a senior researcher and epidemiologist at the Scientific Centre of the Family Health and Reproductive Problems (Siberian Branch, Russian Academy of Medical Sciences) in Irkutsk. Her primary research interest is the molecular epidemiology of M. tuberculosis.

References

1. World Health Organization. Global tuberculosis control report. Geneva: The Organization; 2011.
2. World Health Organization. Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response. Geneva: The Organization; 2010.
3. Bobkov A, Kazennova E, Khanina T, Bobkova M, Selimova L, Kravchenko A, et al. An HIV type 1 subtype A strain of low genetic diversity continues to spread among injecting drug users in Russia: a study of the new local outbreaks in Moscow and Irkutsk. AIDS Res Hum Retroviruses. 2001;17:257–61. http://dx.doi.org/10.1089/088922201750063188
4. Central Research Institute of Health Organization and Information. Key indicators of tuberculosis in Russia in 2009 [in Russian] [cited 2012 Apr]. http://tbpolicy.ru/statistic/national/
5. Ogarkov OB, Medvedeva TV, Zozio T, Pogorelov VI, Nekipelov OM, Gunikova MY, et al. Molecular typing of Mycobacterium tuberculosis strains in Irkutsk (East Siberia) in 2000–2005 [in Russian]. Mol Med. 2007;2:33–8.
6. Mokrousov I, Narvskaya O, Vyazovaya A, Millet J, Otten T, Vishnevsky B, et al. Mycobacterium tuberculosis Beijing genotype in Russia: in search of informative variable-number tandem-repeat loci. J Clin Microbiol. 2008;46:3576–84. http://dx.doi.org/10.1128/JCM.00414-08
7. Gagneux S, DeRiemer K, Van T, Kato-Maeda M, de Jong BC, Narayanan S, et al. Variable host-pathogen compatibility in Mycobacterium tuberculosis. Proc Natl Acad Sci U S A. 2006;103:2869–73. http://dx.doi.org/10.1073/pnas.0511240103
8. Weniger T, Krawczyk J, Supply P, Niemann S, Harmsen D. MIRU-VNTRplus: a web tool for polyphasic genotyping of Mycobacterium tuberculosis complex bacteria. Nucleic Acids Res. 2010;38(Suppl):W326–31. http://dx.doi.org/10.1093/nar/gkq351
9. Demay C, Liens B, Burguière T, Hill V, Couvin D, Millet J, et al. SITVITWEB—a publicly available international multimarker database for studying Mycobacterium tuberculosis genetic diversity and molecular epidemiology. Infect Genet Evol. 2012;12:755–66. http://dx.doi.org/10.1016/j.meegid.2012.02.004
10. Campbell PJ, Morlock GP, Sikes RD, Dalton TL, Metchock B, Starks AM, et al. Molecular detection of mutations associated with first and second-line drug resistance compared with conventional drug susceptibility testing in M. tuberculosis. Antimicrob Agents Chemother. 2011;55:2032–41. http://dx.doi.org/10.1128/AAC.01550-10
11. Pepperell CS, Granka JM, Alexander DC, Behr MA, Chui L, Gordon J, et al. Dispersal of Mycobacterium tuberculosis via the Canadian fur trade. Proc Natl Acad Sci U S A. 2011;108:6526–31. http://dx.doi.org/10.1073/pnas.1016708108
12. Dymova MA, Kinsht VN, Cherednichenko AG, Khrapov EA, Svistelnik AV, Filipenko ML. Highest prevalence of Mycobacterium Beijing genotype isolates in patients newly diagnosed with tuberculosis in the Novosibirsk oblast, Russian Federation. J Med Microbiol. 2011;60:1003–9. http://dx.doi.org/10.1099/jmm.0.027995-0
13. Huygens LF, Huisman TV, van de Waterbeemd H. The future of molecular diagnostics for drug-resistant tuberculosis. Expert Rev Mol Diagn. 2012;12:395–405. http://dx.doi.org/10.1586/erm.12.25
14. Hilleman D, Rüscher-Gerdes S, Richter E. Feasibility of the GenoType MTBDRsl assay for fluoroquinolone, amikacin-capreomycin, and ethambutol resistance testing of Mycobacterium tuberculosis strains and clinical specimens. J Clin Microbiol. 2009;47:1767–72. http://dx.doi.org/10.1128/JCM.00081-09
15. Williams K, Minkowski A, Amoabeng O, Peloquin CA, Taylor D, Andries K, et al. Sterilizing activity of novel combinations lacking first- and second-line drugs in a murine model of tuberculosis. Antimicrob Agents Chemother. 2012;56:3114–20. http://dx.doi.org/10.1128/AAC.00384-12

Address for correspondence: Scott K. Heysell, PO Box 801337, Charlottesville, VA 22908, USA; email skh8r@virginia.edu
Primary Multidrug-Resistant *Mycobacterium tuberculosis* in 2 Regions, Eastern Siberia

Technical Appendix

Technical Appendix Figure 1. Phylogenetic tree of *Mycobacterium tuberculosis* from 130 patients with primary tuberculosis, Yakutia, Russian Federation, determined by UPGMA (unweighted pair group method using arithmetic averages) tree by 12-loci mycobacterial interspersed repetitive unit–variable number tandem repeats (MIRU-VNTR). Yellow squares indicate drug resistance; H, isoniazid; S,
streptomycin; E, ethambutol; R, rifampin; K, kanamycin; C, capreomycin; susc, pansusceptible. Strain numbers are followed by lineage, VNTR international type number (if existing), and MIRU profile.

Technical Appendix Figure 2. Phylogenetic tree of *Mycobacterium tuberculosis* from 105 patients with primary tuberculosis, Irkutsk, Russian Federation, determined by UPGMA (unweighted pair group method using arithmetic averages) tree by 12-loci mycobacterial interspersed repetitive unit–variable number tandem repeats (MIRU-VNTR). Yellow squares indicate drug resistance; H, isoniazid; S, streptomycin; E, ethambutol; R, rifampin; K, kanamycin; C, capreomycin; susc, pansusceptible. Strain numbers are followed by lineage, VNTR international type number (if existing), and MIRU profile.