Molecular Epidemiology and Drug Resistance of *Mycobacterium tuberculosis* Isolates in the Archangel Prison in Russia: Predominance of the W-Beijing Clone Family

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Prisons play a significant role in the epidemiology of drug-resistant tuberculosis. A total of 114 *Mycobacterium tuberculosis* isolates recovered from patients in the Archangel prison (Archangel, Russia) in 2001 were studied using restriction fragment–length polymorphism analysis and spoligotyping. Drug susceptibility was analyzed by the radiometric broth method (BACTEC; Becton Dickinson Diagnostic Systems). According to genotyping studies, 87 (76.3%) of the isolates belonged to the W-Beijing family. Nearly all (96.6%) W-Beijing isolates were part of a cluster, whereas only 25.9% of the other isolates were clustered (P < .001). The largest cluster comprised 43 patients. Multidrug resistance was high among new (34.0%) and previously treated (55.0%) cases. Resistance to ethambutol (OR, 3.4; 95% CI, 1.0–12.7; P = .03) and streptomycin (OR, 4.2; 95% CI, 1.5–11.6; P = .001) was significantly associated with infection with W-Beijing isolates. Tuberculosis due to drug-resistant W-Beijing isolates is a major problem in the Archangel prison.

Tuberculosis is an emerging problem in penitentiary systems all over the world [1, 2]. Russia is not an exception; it has an enormous prison system with nearly 1 million inmates (~0.7% of the current population) [3]. The high number of imprisoned persons, overcrowded conditions, inadequate ventilation, and poor general health of inmates facilitate the spread of tuberculosis. In 2000, the incidence and mortality associated with tuberculosis in Russian prisons were 3174 cases and 171 deaths per 100,000 prisoners, respectively [4]. The situation becomes worse if the disease is caused by drug-resistant *Mycobacterium tuberculosis* isolates. In prisons in some regions of Russia, the prevalence of resistance to ≥1 drug among new cases was 35%–44% [4], and the prevalence of multidrug resistance (MDR; i.e., resistance to at least rifampin and isoniazid) [5] among new cases varied from 15% to 22% [4].

*M. tuberculosis* strains of the W-Beijing family have been identified as a common cause of tuberculosis [2, 6, 7]. These isolates exhibit closely related restriction fragment–length polymorphism (RFLP) patterns and are found to contain the last 9 of the 43 polymorphic spacer sequences in the chromosomal direct repeat (DR) locus by spoligotyping [8, 9]. Such MDR isolates were identified in the United States in the 1990s and were designated “the W strains” [10]. In 1995, *M. tuberculosis* isolates with similar characteristics were found in Beijing province, China, and were designated “the Beijing genotype” [7, 9]. These genetically related isolates, var-
iously called W or Beijing isolates, are now referred to as “the W-Beijing family” [11].

In the Archangel oblast, located in the northwestern part of Russia (figure 1), the incidence of tuberculosis was decreasing until 1991, when it was 20.0 cases per 100,000 persons among the general population (excluding prisons) [12]. In the following years, the incidence increased each year, and it was 48.0 cases per 100,000 persons in 2000. Epidemiological data from the prison first became available in 1996. When data from the prison system are included, the incidence of tuberculosis increased from 55.6 cases per 100,000 persons to 104.0 cases per 100,000 from 1996 to 2000. This sharp increase in the incidence of tuberculosis is attributed to the high concentration of prisoners in the oblast. The size of imprisoned population in the oblast (1541 prisoners per 100,000 persons) is more than double that in the whole of Russia (700 prisoners per 100,000 persons).

The resurgence of tuberculosis in Archangel has been accompanied by increasing rates of drug resistance and the spread of the W-Beijing isolates in the community [6, 12]. The situation in the Archangel prison has not previously been evaluated. Thus, the objectives of our study were to evaluate the level of active transmission of tuberculosis, to characterize the spread of M. tuberculosis isolates of the W-Beijing family, and to analyze M. tuberculosis resistance to first-line antituberculous drugs in the Archangel prison in 2001.

PATIENTS, MATERIALS, AND METHODS

**Tuberculosis in the Archangel prison.** The Archangel prison consists of 2 entities. This study was performed in the larger entity, which comprises 17,000 inmates from the Archangel oblast and 1500 persons staying in pretrial detention. The medical service has 2 antituberculosis departments with 70 beds each, as well as a colony for isolation of previously treated patients and those with chronic infection. Microbiological diagnostic policy includes examination of 3 sputum specimens by smear microscopy and of 2 specimens by cultivation. All patients who have had tuberculosis diagnosed are registered using standardized categories and receive treatment according to the recommendations of the World Health Organization and the International Union Against Tuberculosis and Lung Diseases [13, 14].

The second entity had only 4000 inmates in 2001. The medical service of this entity did not use smear microscopy and culture methods for diagnosis until the second half of 2001. Thus, patients from this entity were not included in the study.

**Patients and bacterial strains.** In 2001, 616 cases of pulmonary tuberculosis (354 new cases and 262 previously treated cases—i.e., 169 relapses, 13 defaults, and 80 treatment failures) were diagnosed using standardized case categories [13, 14]. Of the 616 registered cases, 360 (58.4%) had positive culture results, 271 (75.3%) of which had positive results of smear microscopy. All prisoners with tuberculosis were routinely tested for HIV infection.

During the first and the fourth quarters of 2001, 115 M. tuberculosis isolates were recovered from patients consecutively diagnosed with culture-positive tuberculosis. The isolates were cultivated on Löwenstein-Jensen medium and forwarded to the Reference Laboratory for Tuberculosis (Norwegian Institute of Public Health, Oslo, Norway) for laboratory analysis.

Data regarding demographic characteristics (age, sex, and geographic origin of the patient), findings of smear microscopy, and length of imprisonment were obtained from the medical records. No significant differences were found in age, sex distribution, and findings of smear microscopy among the 115 patients included in the study and for all 360 culture-positive cases.

**Identification of strains and drug susceptibility testing.** Identification of the isolates was performed using the 16S-rDNA hybridization technique (AccuProbe; GenProbe) and standard microbiological tests (niacin accumulation test and
nitrile reduction test). One strain was excluded from analysis because it did not belong to the *M. tuberculosis* complex. The final number of *M. tuberculosis* isolates analyzed was 114.

Susceptibility testing to antituberculous drugs was performed using the radiometric broth method (BACTEC; Becton Dickinson Diagnostic Systems) [15–17]. The drug susceptibility definitions used were reported elsewhere [12]. Mutations associated with rifampin resistance in the gene encoding the β-subunit of RNA polymerase (*rpoB*) were identified using the Inno-LiPA Rif. TB test (Innogenetics N.V.) [18, 19] for all isolates found to be resistant to rifampin by the BACTEC method. Thirty-seven isoniazid-resistant strains (69.8%) were identified with rifampin resistance in the gene encoding the β-subunit of RNA polymerase (*rpoB*).

**RFLP analyses.** RFLP analysis of *M. tuberculosis* DNA was performed in accordance with the internationally standardized methodology [21–23]. In brief, cells were harvested by centrifugation, and chromosomal DNA was isolated as described by Van Soolingen et al. [23]. DNA was restricted with *Pvu*II (Boehringer Mannheim) in accordance to the manufacturer’s instructions, and fragments were separated by electrophoresis in 0.8% agarose gel. After transfer of the DNA to a GeneScreen Plus membrane (DuPont) by the alkaline-transfer procedure, hybridization was performed with a 245-bp PCR-amplified probe directed against the right arm of IS6110 [23]. The probe was labelled using the digoxigenin-dUTP labelling and detection kit (Boehringer Mannheim). To facilitate the comparison of the IS6110 RFLP patterns, a 1-kb DNA ladder (Gibco BRL) was deposited on the first, middle, and last lines of each gel. The ladder, labelled by using the digoxigenin-dUTP labelling and detection kit, was mixed with the IS6110 probe in hybridization buffer (5× SSC [SSC is 0.15 M NaCl plus 0.015 sodium citrate], 0.1% sarkosyl, and 0.02% sodium dodecyl sulphate) to allow visualization.

The IS6110 RFLP patterns were inspected visually, scanned, and analyzed using GelCompar computer software, version 4.1 (Applied Maths). The unweighted pair-group method of arithmetic averaging with the Dice coefficient as similarity measure was used. Band position tolerance was set to 1.20%, and optimization was 0.50%.

**Spoligotyping.** Spoligotyping was performed using a commercially available spoligotyping kit (Isogen Bioscience BV) in accordance with the instructions supplied by the manufacturer, as described elsewhere [8].

**Definitions.** *M. tuberculosis* isolates of the W-Beijing family were defined as a group of genetically closely related isolates that had the following characteristics: the isolates harbored a high number of IS6110 copies, reaching up to 18 in our study, more than two-thirds of which were present at the same genomic sites; they clustered within 60% similarity; and they had identical spoligotyping results, showing hybridization only with the last 9 of the 43 possible spacers [6, 7, 9]. A cluster was defined as ≥2 *M. tuberculosis* isolates exhibiting 100% identical IS6110 RFLP patterns [9, 24, 25].

**Statistical analysis.** EpilInfo, version 6.04b (Centers for Disease Control and Prevention and World Health Organization), and SPSS for Windows, version 9.0.1 (SPSS), were used for statistical analysis. Associations between categorical variables were assessed using the χ² test and Fisher’s exact test for values of <5. Differences between groups were tested by univariate and multivariate analysis and are expressed as ORs with 95% CIs. Student’s t test was used to test for differences in means of continuous variables. A P value of <.05 was considered statistically significant.

**RESULTS**

**Study population.** The 114 prisoners with tuberculosis tested negative for the HIV infection. All patients were male and were 18–56 years old (mean age, 31.5 years). The patients had been imprisoned for 1–12 years (mean, 2.9 years) before tuberculosis had been diagnosed. Only 3.5% of the prisoners had been living in the Archangelsk oblast before <10 years.

**RFLP analysis and spoligotyping.** RFLP analysis with IS6110 as a probe revealed that 23 (20.2%) of the 114 patients were infected with unique isolates and that 91 (79.8%) were infected with clustered isolates. The number of IS6110 copies varied between 7 and 18, with a mean number of 13.7 copies. Thirty-one distinct RFLP patterns were revealed (figure 2). Of these, 23 patterns (74.2%) were unique, and 8 patterns (25.8%) were represented by 2–43 isolates. Nine RFLP patterns, representing 87 (76.3%) of the 114 isolates, shared the majority of the IS6110 copies and clustered together in the dendrogram. Compared with fingerprints described in the literature [7, 11, 26, 27] and with fingerprints from the Archangelsk oblast [6], these RFLP patterns were found to correspond to the W-Beijing family. All 87 isolates identified as members of the W-Beijing family by their IS6110 patterns showed identical spoligotypes (figure 3), lacking spacers 1–34. Isolates belonging to the W-Beijing family harbored from 14–18 copies of IS6110 (mean, 14.9 copies), and their RFLP patterns clustered together on the dendrogram within 60% similarity (figure 2). The remaining isolates had a significantly lower number of IS6110 copies in their genomes (mean, 10.0 copies; *P* < .001). Sixteen different spoligotypes were identified among 27 non-Beijing isolates (figure 3). Ten spoligotypes were represented by a single isolate, 2 spoligotypes were represented by 6 and 3 isolates each, and 4 spoligotypes comprised 2 isolates. All isolates with identical IS6110 RFLP patterns were found to be identical by spoligotyping.

Of the 87 patients infected with an isolate from the W-Beijing family, 84 (96.6%) were part of 6 clusters. The largest cluster...
Figure 2. Dendrogram showing the genetic similarities of the 31 IS6110 restriction fragment–length polymorphism (RFLP) patterns identified among the 114 Mycobacterium tuberculosis isolates recovered from persons in the Archangel prison in 2001. The RFLP patterns of the W-Beijing isolates are indicated. The number of isolates with each RFLP pattern is also indicated.

Comprised 43 patients; other clusters included 19, 13, 4, 3, and 2 patients each. Of the 27 patients infected with non-Beijing strains, only 7 (25.9%) were part of a cluster. Non-Beijing clusters consisted of 4 and 3 isolates. Significant association was observed between infection by the W-Beijing isolate and being part of a cluster (OR, 80.0; 95% CI, 16.3–463.0; P < .001).

Resistance to antituberculous drugs. The isolates were recovered from 94 new cases (82.5%) and 20 previously treated cases (17.5%). The highest rate of drug resistance among new cases was observed for streptomycin (70.2%); 30.9% and 42.6% of the isolates were resistant to ethambutol and isoniazid, respectively. Equally high rates of resistance to isoniazid (65.0%), streptomycin (65.0%), and ethambutol (35.0%) were observed among previously treated cases. The rates of MDR among new and previously treated cases were 34.0% and 55.0%, respectively. All isolates that were resistant to rifampin were MDR.

Monoresistance to isoniazid or streptomycin was observed among new cases only (table 1), and all of these cases were caused by the W-Beijing isolates. Monoresistance to streptomycin (29.8%) was commonly observed. Resistance to ≥2 drugs was less common among new cases (41.5%) than among previously treated cases (65.0%). Resistance to ≥2 antituberculous drugs was equally common among patients infected with non-Beijing isolates (46.0%) and those infected with W-Beijing isolates (44.4%). The W-Beijing isolates (41.3%) were more often MDR than were the non-Beijing isolates (25.9%), but the observed difference was not significant (P = .15). Of the 43 MDR isolates, 36 (83.7%) were part of 8 clusters. Patients infected with clustered strains had a similar percentage of positive results of smear microscopy compared with those infected with non-clustered strains (67.0% vs. 69.6%, respectively; P = .82).

Mutations in the rpoB gene were identified for 43 rifampin-resistant isolates. Six different mutations were identified (table 2). The 531 TCG→TTG mutation was predominant among both the W-Beijing (83.3%) and non-Beijing (71.4%) isolates. One strain showed both the wild-type M. tuberculosis and the 531 TCG→TTG mutation. Three (7.0%) of the 43 isolates showed pattern representing the 526 CAC→TAC mutation and bands typical of the wild-type M. tuberculosis at the same time; 2 of these isolates belonged to the W-Beijing family. 513 CAA→CTA, 516 GAC→GTC, and 516 GAC→GGC were other mutations identified in the rpoB gene. The observed differences in distribution of mutations between isolates having different genotypes were not significant (P > .05).

Sequencing of the katG gene revealed the 315 AGC→ACC mutation in 34 (91.9%) of the 37 isoniazid-resistant isolates.
examined. The majority (88.2%) of these isolates had MDR. One W-Beijing isolate showed the 315 AGC→ATC mutation, and 2 isolates (1 W-Beijing and 1 non-Beijing) had no mutation in the sequenced region.

The W-Beijing and non-Beijing isolates had different rates of drug resistance. The highest rates of drug resistance among the W-Beijing isolates were observed for streptomycin (77.0%) and isoniazid (47.1%; table 3). The rates of resistance to streptomycin (44.4%) and isoniazid (44.4%) among non-Beijing isolates were equal and were the most commonly observed. Non-Beijing isolates were less likely to be resistant to each drug tested and to have MDR. Logistic regression identified significant association between resistance to ethambutol and streptomycin and infection with the W-Beijing strain. Multivariate analysis showed that resistance to streptomycin (OR, 3.3; 95% CI, 1.2–9.1; *P* = .02) was independently associated with infection with the W-Beijing strain. According to univariate analysis, *M. tuberculosis* resistance to streptomycin was significantly more common among clustered isolates than among nonclustered isolates.

### DISCUSSION

In the Archangel prison, Russia, isolates recovered from 34.0% and 55.0% of prisoners with new and previously treated cases of tuberculosis, respectively, had MDR. Comparison of the situation with that in the community [12] revealed that MDR among new cases was 2.5 times higher in the prison settings;
Table 1. Susceptibility patterns of 114 *Mycobacterium tuberculosis* isolates recovered from patients with tuberculosis in Archangel prison in 2001, according to case category and genotype family.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Case category</th>
<th>Genotype family</th>
<th>No. (%) of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (n = 114)</td>
<td>New cases (n = 94)</td>
<td>Previously treated cases (n = 20)</td>
</tr>
<tr>
<td>Eth INH Rif Str</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R R R R</td>
<td>36 (31.6)</td>
<td>29 (30.9)</td>
<td>7 (35.0)</td>
</tr>
<tr>
<td>S R R R</td>
<td>6 (5.3)</td>
<td>2 (2.1)</td>
<td>4 (20.0)</td>
</tr>
<tr>
<td>S R R S</td>
<td>1 (0.9)</td>
<td>1 (1.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>S R S R</td>
<td>9 (7.9)</td>
<td>7 (7.4)</td>
<td>2 (10.0)</td>
</tr>
<tr>
<td>S R S S</td>
<td>1 (0.9)</td>
<td>1 (1.1)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>S S S R</td>
<td>28 (24.6)</td>
<td>28 (29.8)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>S S S S</td>
<td>33 (28.9)</td>
<td>26 (27.7)</td>
<td>7 (35.0)</td>
</tr>
</tbody>
</table>

**NOTE.** Susceptibility patterns were determined using the radiometric broth method (BACTEC; Becton Dickinson Diagnostic Systems). Eth, ethambutol; INH, isoniazid; R, resistant; Rif, rifampin; S, susceptible; Str, streptomycin.

Table 2. Mutations in the *rpoB* gene in 43 *Mycobacterium tuberculosis* isolates resistant to rifampin, according to genotype family.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>W-Beijing (n = 36)</th>
<th>Non-Beijing (n = 7)</th>
<th>Total (n = 43)</th>
</tr>
</thead>
<tbody>
<tr>
<td>531TCG→TTG</td>
<td>30 (83.3)</td>
<td>5 (71.4)</td>
<td>35 (81.4)</td>
</tr>
<tr>
<td>531TCG→TTG&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1 (2.8)</td>
<td>0 (0)</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>526CAC→TAC&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2 (5.6)</td>
<td>1 (14.3)</td>
<td>3 (7.0)</td>
</tr>
<tr>
<td>513CAA→CTA</td>
<td>2 (5.6)</td>
<td>0 (0)</td>
<td>2 (4.7)</td>
</tr>
<tr>
<td>516GAC→GTC</td>
<td>0 (0)</td>
<td>1 (14.3)</td>
<td>1 (2.3)</td>
</tr>
<tr>
<td>516GAC→GCC</td>
<td>1 (2.8)</td>
<td>0 (0)</td>
<td>1 (2.3)</td>
</tr>
</tbody>
</table>

**NOTE.** Mutations were determined using the Inno-LiPA Rif. TB test (Innogenetics N.V.).

<sup>a</sup> Both the wild-type sequence and the mutant sequence were represented.

The spread of *M. tuberculosis* isolates of the W-Beijing family has been described in many countries of the world, including prisons in the former Soviet Union [2]. The prevalence of the W-Beijing isolates in the prison system in Archangel was similar to that in Azerbaijan [2] and significantly higher than that in the Archangel community (44.5%) [6]. In contrast to other studies [6, 28, 29], we did not find a higher rate of MDR phenotype in the W-Beijing isolates, compared with other *M. tuberculosis* isolates.

The 531 TCG→TTG mutation in the *rpoB* gene was predominant both among the W-Beijing and non-Beijing isolates in the Archangel prison, as in the community [6]. Of the 4 *M. tuberculosis* isolates represented by 2 subpopulations of bacilli (susceptible and resistant), 3 belonged to the W-Beijing family, and 2 were recovered from new cases. These cases might be...
infected with 2 populations of bacilli transmitted either simultaneously or one after the other. The identification of rifampin-susceptible W-Beijing isolates in the Archangel prison suggests that the W-Beijing family strains were susceptible when first introduced in the Archangel prison. Thus, the transmission of both drug-susceptible and drug-resistant bacilli is documented in the Archangel prison.

The high proportion of clustered isolates (79.8%) in the Archangel prison indicates active and recent transmission of the disease. The degree of such transmission is higher in the prison than in the community (59.7%) [6]. Patients infected with the W-Beijing isolates were more likely to be part of a cluster either in the prison (96.6%) or in the community (92.5%) than were patients infected with non-Beijing isolates.

Comparison of individual RFLP patterns from the prison and the community revealed identical RFLPs among both groups. In both studies, the largest clusters were represented by isolates with the same genotype (W-Beijing), but some of the non-Beijing isolates had also identical RFLPs. The W-Beijing family has been detected in Archangel population only since 1997 [6], and transmission of the W-Beijing strains is a recent event.

Patients with infectious tuberculosis who are imprisoned and those who are released from prison before treatment completion may play an important role in the disease epidemiology. Close coordination of tuberculosis programs in the prison and the community is important. Isolation of infectious cases with MDR and implementation of alternative treatment are of great importance. Adequate treatment in settings with high rates of drug resistance is difficult to administer without reliable laboratory diagnosis. Conventional cultivation of *M. tuberculosis* and susceptibility testing usually take 10–12 weeks. Early detection of MDR is therefore crucial. We have shown that resistance to rifampin can serve as a marker of MDR in Archangel [12]. Rifampin has a single target in the 81 bp polymorphic fragment of the *rpoB* gene; 95% of rifampin-resistant isolates have mutations in the *rpoB* gene [30]. The 315 ACC→ACC mutation in the *katG* gene associated with MDR [31] was predominant in our study and was observed in 88.2% of isolates with MDR. Thus, the line probe assay or homemade PCR for detection of MDR may be used as a good solution for early identification of cases of tuberculosis with MDR.

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### References