COMPARATIVE ANALYSIS OF MYCOBACTERIUM TUBERCULOSIS GENOTYPES IN THE REPUBLIC OF YAKUTIA AND IRKUTSK REGION

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In 2010, the Russian Federation had a tuberculosis (TB) prevalence of 136 per 100,000 population and the estimated proportion of cases of multidrug-resistant (MDR)-TB, defined as resistance to isoniazid and rifampin, among new cases (primary TB) was 18% [2]. Yet primary MDR-TB may be highly variable at the subnational level, with proportions varying from 5.4-28.3% in oblasts or republics with continuous surveillance data on drug-resistance as reported to the World Health Organization (WHO) [1].

In 2009 Irkutsk had a TB prevalence of 373 per 100,000 population, and is burdened by a significant HIV epidemic, timed initially with the dissolution of the Soviet Union and availability of injected heroin [3, 23]. Irkutsk carries one of the highest rates of HIV in the Russian Federation, with more than 21,000 people registered with HIV in the oblast and the majority reported from Irkutsk city [5]. In contrast, the Sakha Republic (Yakutia), is sparsely populated and 49% of the population are indigenous Yakuts [6]. TB prevalence was lower at 188 per 100,000 population and HIV is thought scarce [9].

Molecular typing from surveillance studies have found the Beijing genotype, a pandemic lineage, in over half of *Mycobacterium tuberculosis* from the Russian Federation, with one of the highest prevalence in Irkutsk [4, 5, 24]. Comparative genotyping has not been performed from Yakutia. The Beijing genotype has previously been associated with the MDR phenotype and characteristic resistance mutations, as well as increased transmissibility [11]. Given the distinct sociocultural patterns between Irkutsk and Yakutia, we hypothesized that unique genotypic characteristics of primary MDR-TB would be found in each region. Thus the following study sought to characterize the molecular epidemiology among cases of primary TB from Irkutsk and Yakutia.

METHODS

Study population

M. tuberculosis isolates were cultured during routine care of adults, ≥ 18 years of age, with primary TB from November 2008 to May 2010 at two regional referral centers, the Irkutsk Regional

TB-Prevention Dispensary in Irkutsk city, and the Research Practice Center for Phthisiatry in Yakutsk city (Yakutia). Primary TB was defined as a culture positive specimen in a subject without prior history of TB treatment.

Molecular typing

The initial cultured specimen from each subject was used following mycobacterial growth on Lowenstein-Jensen agar slants. Isolation and species identification was performed per WHO recommendations at each site [21]. Drug susceptibility testing was carried out by the absolute concentration method on agar slants for 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). Flouroquinolone and pyrazinamide susceptibility testing was not routinely performed.

DNA extraction was performed on all isolates at the Institute of Epidemiology and Microbiology, SB RAMS, in Irkutsk city, using cetyltrimethylammonium bromide-NaCl according to prior protocol [18]. All DNA underwent typing by original 12-loci mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) analysis [19]. Region of difference (RD) deletions were used to define major strain lineages (e.g. RD105 and RD207 for Beijing) [10, 16]. Further lineage-definition was carried out using oligonucleotide primers for Ural strains as previously described [5]. The public database MIRUVNTR*plus* (http://www.miru-vntrplus.org/MIRU/index.faces) and online instruments [20] were used for phylogenetic tree construction by the unweighted pair group method using arithmetic averages. VNTR international type numbers were further confirmed with the online database SITVIT (Institute Pasteur de Guadeloupe; http://www.pasteur-guadeloupe.fr:8081/SITVITDemo/outilsConsultation.jsp) [6]. Comparison of genotype frequency between sites was by chi-square analysis with Yates correction or Fisher's exact test as appropriate.

Primary MDR-TB outcomes

Those subjects identified as having primary MDR-TB were retrospectively traced for treatment outcome. Basic demographics and clinical characteristics were abstracted from registry databases including age at diagnosis, gender, means of referral (symptoms or screening fluorography), prior imprisonment and HIV status. Typically, patients were treated for TB as an inpatient for the intensive phase of chemotherapy (≥ 2 months), then discharged to complete a continuation phase. Resources for directly observed therapy following discharge were not routinely available or programmatically enforced. Chemotherapeutic regimens for MDR-TB were variable but generally included a flouroquinolone and an injectable agent (kanamycin or capreomycin), at

times continued despite reported in vitro resistance. For selected patients, thoracic surgery for pulmonary TB was performed and data regarding this outcome was abstracted from the registry.

Subjects with documented culture conversion to negative and having completed the prescribed course of inpatient chemotherapy were categorized as intensive phase complete. Subjects having completed intensive phase and the prescribed continuation phase without symptom recurrence were categorized as treatment complete. Default occurred if a subject failed to complete the intensive phase of treatment. Death was attributed to TB, HIV and TB, or other cause as documented in the registry. Comparisons of pre-treatment characteristics and outcomes between sites were made by chi-square analysis, t-test for continuous variables or when appropriate, the Mann-Whitney U test for nonparametric data.

RESULTS AND DISCUSSION

During the study period, 235 consecutive isolates from subjects with primary TB were available; 130 were from Yakutia and 105 from Irkutsk. Isoniazid monoresistance occurred in 16 (12%) from Yakutia and 19 (18%) from Irkutsk (p=0.27). Multidrug resistance occurred in 36 (28%) from Yakutia and 25 (24%) from Irkutsk (p=0.55). Of the subjects with primary MDR-TB, there were no significant differences in age, gender distribution or reason for referral between those in Irkutsk or Yakutia [Table 1]. Of critical difference, there were no HIV infected patients identified from Yakutia compared to 11 (44%) of subjects from Irkutsk (p<0.001).

Molecular typing

Strains of the Beijing family were the predominant cause of primary TB at both sites but significantly more common in Irkutsk, n= 70 (67%), than Yakutia, n= 40 (31%) (p<0.001). Furthermore, a substantial amount of S 256 (11%), T 8 (7%) and Ural 171 (5%) were observed in Yakutia that were not found in Irkutsk [Table 2]. Importantly, the cluster of S 256 (MIRU profile 233325153325), previously unreported as an epidemic strain in the Russian Federation, was the most common among primary MDR-TB isolates from Yakutia and was fully 86% MDR [Table 2]. The S 256 strain was found to be resistant to streptomycin in all cases and kanamycin in 4 (29%), differing significantly from the other Yakutia isolates where kanamycin resistance was reported in only 9 (8%) (p=0.035). In Irkutsk a previously described Beijing strain predominated among those with primary MDR-TB [9,10].

Primary MDR-TB outcomes

All HIV infected patients died during the inpatient phase of therapy and accounted for 92% of all deaths from Irkutsk [Table 3]. Individual use of antiretroviral therapy was not known, but all

died within the intensive phase. Of those that died in Irkutsk, kanamycin resistance was found in 4 (33%) and was no more common than in those that survived, 4 (31%). All of those with HIV that died in Irkutsk had an isolate of Beijing family. In comparison, death was less common in Yakutia, occurring in only 4 (11%) of those with primary MDR-TB (p=0.002), 3 of whom died of causes other than TB. Of the deaths in Yakutia, two occurred in subjects with the Beijing 17 strain (one of which was kanamycin resistant), one in a subject with a kanamycin susceptible S 256, and the other with a kanamycin susceptible strain of orphan lineage. Due to the proportion that died, significantly fewer subjects in Irkutsk completed intensive phase, but of those that completed, only 1 (13%) from Irkutsk went on to complete the full course of treatment, compared to 19 (71%) from Yakutia (p=0.01). Overall surgery was well tolerated and performed in a similar proportion of subjects at both sites. All subjects receiving surgery were HIV negative. One death was reported in a subject from Yakutia unrelated to TB, while the remainder, 90%, completed the total treatment course. Four subjects from Irkutsk completed intensive phase, but 2 (33%) defaulted after surgery and were lost to follow-up.

We found that more than 25% of primary TB was MDR, among the highest proportion reported from the Russian Federation [1]. Importantly however, regionally specific genotypic patterns and resistance mutations were identified. Expectedly, primary MDR-TB was driven by strains of Beijing lineage in Irkutsk oblast [5, 13]. The dominance of Beijing strains has been described for over a decade in both civilian and prison populations from other oblasts, in greater association with drug-resistance secondary to common mutations at codon 531 of *rpoB* and 315 of *katG*, and in patients of younger age suggestive of more recent transmission [7, 12, 17]. Yet in the more geographically isolated population of Yakutia, a strain previously unidentified in the Russian Federation, S 256, had a MIRU profile recently described among Canadian Aboriginal populations [15]. In Yakutia, S 256 was highly drug-resistant and the most common genotype among cases of primary MDR-TB. Yet our findings are consistent with a recent report from Novosibirsk oblast that similarly included non-Beijing and S-family strains [17].

Conclusion. The phylogenetic patterns, certain drug-resistance mutations and treatment outcomes were regionally distinct. In Yakutia, a newly described genotype, S 256, appeared to drive the MDR-TB epidemic and may be more common among indigenous Yakuts. To contrast in Irkutsk, MDR-TB was found commonly in strains of Beijing lineage but was associated with higher mortality and HIV infection.

Table 1

Characteristics of patients with primary multidrug-resistant tuberculosis by region

Characteristic	Irkutsk N= 25	Yakutia N= 36	P value
Age, mean years (±SD)	34 (13)	32 (12)	P=0.76
Gender, male (% N)	17 (68)	23 (64)	P=0.79
HIV infected (% total reported)	11 (44)	0[a]	P<0.001
Prior imprisonment (% N)	0 (0)	4 (11)	P=0.14
Symptomatic at referral (% total reported)	17 (68)	12 (50)[a]	P=0.25

Significance determined by t-test for age and chi-square or Fisher's exact for categorical variables, excluding those with missing values.

[a]In Yakutia HIV status was not recorded in 10 (28%) and referral symptoms in 12 (33%).

Table 2

Genotype by region

MIRU-VNTR 12[a]	Family/ VIT[b]	Irkutsk total =105, MDR = 25 N (% total) N (% MDR)	Yakutia total = 130, MDR = 36 N (% total) N (% MDR)	P value
223325153533	Beijing 16	32 (31) 7 (28)	12 (9) 1 (3)	P<0.001 P=0.006
223325173533	Beijing 17	13 (12) 6 (24)	10 (8) 7 (19)	P=0.27 P=0.76
233325153325	S 256	0	14 (11) 12 (33)	P<0.001 P=0.001
223125153324	T 8	0	9 (7) 0	P=0.005
227225113223	Ural 171	0	6 (5) 0	P=0.03
223325153433	Beijing 592	1 (1) 0	4 (3) 0	P=0.38

[a]MIRU-VNTR= mycobacterial interspersed repetitive unit-variable number tandem repeat (original 12 loci profile). Included genotypes found in 5 or more isolates only.

[b]VIT= VNTR international type. MDR = multidrug-resistant TB (conventional resistance to isoniazid and rifampin). Significance determined by chi-square analysis with Yates correction or Fisher's exact test when appropriate.

Table 3

Multidrug-resistant tuberculosis treatment outcomes by region

Outcome	Irkutsk N= 24[a]	Yakutia N = 36	P value
Death (% N)	12 (50)	4 (11)	P=0.002
HIV/TB related (% death)	11 (92)	0 (0)	
TB related (% death)	0	1 (25)	
Other cause (% death)	1 (8)	3 (75)[b]	
Intensive phase complete (% N)	8 (33)	27 (75)	P=0.003
Total treatment complete (% intensive phase)[c]	1 (13)	19 (71)	P=0.01
Surgery (% N)[c]	6 (25)	10 (28)	P=0.78
Intensive phase complete (% surgery)	4 (67)[d]	9 (90)	

[a]Excludes one patient from Irkutsk with unknown outcome.

[b]One patient died following surgery but unrelated to procedure.

[c]Excludes subjects that did not complete intensive phase due to death or default.

[c]All patients receiving surgery at both sites were HIV negative.

[d]Other 2 surgical patients from Irkutsk had defaulted.

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