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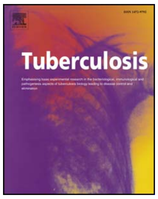

Epidemiology

Major genotype families and epidemic clones of *Mycobacterium tuberculosis* in Omsk region, Western Siberia, Russia, marked by a high burden of tuberculosis-HIV coinfection

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ABSTRACT

This population-based study characterized *Mycobacterium tuberculosis* isolates from HIV-positive and HIV-negative tuberculosis (TB) patients in the Omsk region in Western Siberia, Russia. We sought to gain insight into the major genotype families and epidemic and endemic clones of *M. tuberculosis* in the area with a high burden and adverse trend of TB/HIV coinfection. The study collection included *M. tuberculosis* isolates from 207 newly-diagnosed patients with pulmonary TB; 55 (26.5%) of patients were HIV-infected. The *M. tuberculosis* isolates were subjected to drug susceptibility testing and molecular typing based on spoligotyping and analysis of the robust genotype and cluster-specific markers. Patients with disseminated TB disease were more prevalent in the HIV-positive (34.5%) than in the HIV-negative group (4.6%) ($P < .001$). The Beijing genotype was predominant (62.3% of isolates), and its major subtypes were 94-32-cluster (Central Asian/Russian strain, $n = 80$) and B0/W148-cluster (successful Russian strain, $n = 28$). The main non-Beijing families were represented by Latin-American Mediterranean (14.5%), T family (11.1%), Ural (5.8%), and Haarlem (3.9%). Under multivariate logistic regression analysis, MDR was associated with Beijing genotype and not associated with HIV coinfection status ($P < .001$). Beijing genotype isolates were found more frequently in TB/HIV patients than in TB HIV-negative patients (74.5% versus 57.9%, respectively; $P = .031$). The non-Beijing genotypes were mainly drug susceptible except for the drug-resistant Ural SIT262 isolates. To summarize, the alarming situation in the Omsk region in Siberia regarding TB/HIV coinfection is seriously influenced by the active circulation of *M. tuberculosis* isolates of MDR-associated Beijing genotype. Among the non-Beijing families, emergence of the drug-resistant Ural family strains of spoligotype SIT262 warrants attention.

1. Introduction

Tuberculosis (TB) and HIV co-epidemic has been recognized as a global health concern reaching alarming levels in some parts of the world [1]. The increase in the circulation of multi-drug resistant (MDR) *Mycobacterium tuberculosis* strains along with epidemic of the HIV infection present a major to control TB in many regions across Russia [2]. The TB incidence is unequally distributed across the country, and Siberian Federal District is characterized by a high prevalence of TB infection, morbidity and mortality [3,4]. The TB/HIV incidence in 2015 in Siberia was the highest among Russian Federal Districts (20/100,000) and 2.5 higher than national average (Supplementary Fig. S1) [3]. The city of Omsk has the population of 1.14 million people and it is the second most populated city in Siberia and seventh in Russia. TB incidence decreased in the Omsk region to 82.4 per 100,000 in 2015 (compared to 115.2 in 2010). However, the prevalence of MDR-TB increased from 29.6 in 2005 to 37.9 per 100,000 in 2015 and the incidence of MDR-TB was 8.8 per 100,000 in 2015 [5]. A particularly adverse feature of Omsk is the very high rate of TB/HIV coinfection. Its incidence increased from 0.3 in 2006 to 12.7 per 100,000 in 2015, and prevalence increased from 1.4 to 21.2 per 100,000 population [6] (see insert in Supplementary Fig. S1).

The impact of HIV co-infection on the emergence of resistance and transmission of *M. tuberculosis* is not straightforward. Studies show both

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an increase of the probability of infection of HIV-infected individuals with *M. tuberculosis* isolates of certain genetic lineages [7–10] and a lack of such correlations [11–13]. This discrepancy may be due in part to different ethnic backgrounds (the above cited articles were from Latin America, China, Central and South Africa) as well as differences in the local *M. tuberculosis* populations.

In the present study, we genotypically characterized *M. tuberculosis* isolates from TB patients with and without HIV co-infection in the Omsk region in Western Siberia, Russia. We sought to gain insight into the major families and significant epidemic clones of *M. tuberculosis* in the area with high burden and adverse trend of TB/HIV coinfection.

2. Materials and methods

2.1. Study population

This was a prospective study based on all consecutive patients enrolled during 1 year and newly diagnosed with TB, i.e. patients who have never had treatment for TB, or have taken anti-TB drugs for less than 1 month (https://www.ncbi.nlm.nih.gov/books/NBK138741/). According to the records at Omsk Clinical Tuberculosis Dispensary, a total of 1597 patients of all ages (1519 adults, > 18 years old) with pulmonary tuberculosis were detected in the Omsk region from March 1, 2015 to March 1, 2016. Of 1519 adult patients, 1473 had pulmonary TB. Of them, 542 were *M. tuberculosis* culture-positive. Further, isolates from temporary migrants were excluded from analysis. In addition, certain number of cases/isolates were excluded due to poor growth of bacterial culture, patient's death, or a patient's loss to follow-up. As a result, isolates from 419 patients were available, and a first isolate from each second patient was enrolled in the study. The strategy of including every second isolate was based solely on resource availability. This was a limitation to the study but no additional selection criteria were applied and selection bias is unlikely to occur as inclusion was done in a consecutive manner. The groups of included (n = 207) and non-included (n = 212) cases did not differ significantly in either patient-related characteristic, such as gender, residence, age, diagnosis, HIV-coinfection (Supplementary Table S1). Therefore we firmly believe the sample of 207 patients/isolates was sufficient and representative of the entire sample of eligible cases.

This study was approved by the Ethical Committee of the Omsk State Medical University (protocol #66 of January 30, 2015). All patients gave informed written consent to participate in this study.

M. tuberculosis drug susceptibility testing for the 1st and 2nd line drugs (streptomycin, isoniazid, rifampicin, ethambutol, pyrazinamide, ofloxacin, kanamycin, capreomycin, cycloserine; *para*-aminosalicylic acid) was carried out for all strains using the method of absolute concentrations on the solid Loewenstein-Jensen medium according to Order #109 of Ministry of Health of the Russian Federation and using Bactec MGIT 960 system (Becton Dickinson, Sparks, Md.) according to the manufacturer instructions. The laboratory is externally quality assured by the System for External Quality Assessment “Center for External Quality Control of Clinical Laboratory Research” (Moscow, Russia).

2.2. Genotyping

DNA was extracted from *M. tuberculosis* cultures using the recommended method [14]. Beijing genotype was detected by PCR analysis of the *dnaA-dnaN*::IS*6110* insertion [15]. Beijing B0/W148 cluster was identified by PCR analysis of the *Rv2664-Rv2665*::IS*6110* insertion [16]. Beijing 94-32-cluster was detected by PCR-RFLP assay to detect a specific SNP in the *sigE* gene [17].

All non-Beijing strains were subjected to spoligotyping following a macroarray-based standard procedure [18]. The spoligotyping profiles were entered into Excel spreadsheets and compared with entries in SITVIT2, an international spoligotype database in Institut Pasteur de Guadeloupe, which is the most recent update of the published SITVIT_WEB database (http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE/). Spoligotyping profiles were assigned to the particular families/clades through comparison with SITVIT_WEB database with additional correction for Ural and LAM (Latin-American Mediterranean) families. Ural family was defined by the specific spoligotype signature [13,19]. LAM family was identified by PCR-RFLP analysis of the specific *Rv0129c* SNP [20].

2.3. Statistical analysis

A chi-square test was used to detect any significant difference between the two groups. Yates corrected $\chi 2$ and *P*-values were calculated with 95% confidence interval (CI) around the mean at http://www.medcalc.org/calc/odds_ratio.php online resource. The significance threshold was set at $P = .05$.

A multivariate logistic regression model was calculated to test if there are associations between MDR, as the outcome variable, and TB/HIV-coinfection and Beijing genotype, as the two predicator variables. Each variable was dummy encoded as 1 and 0 for presence and absence, respectively [21]. This data were analyzed using STATISTICA 6.0 (StatSoft Inc., USA).

3. Results

This study included 207 *M. tuberculosis* isolates recovered from 207 newly-diagnosed patients with pulmonary tuberculosis; 55 (26.5%) of them were HIV-infected. One hundred forty-two patients were male (68.5%, 19–76 years old, mean age 41.5 years), and 65 were female (31.5%, 22–84 years, mean age 42.9 years) (Supplementary Table S2). The proportion of women was slightly higher in HIV-infected patients compared to the HIV-negative groups: 36.4% vs 29.6%. The patients in the TB/HIV group were significantly younger, ages 25–44 years old compared to the HIV-non-infected group ($P < .001$; OR 4.60 [2.1–10.1]). Among TB/HIV patients, 32.7% were drug-users ($P < .001$; OR 36.5 [8.1–164.2]), 72.2% were male.

The infiltrative TB disease was the most frequent diagnosis (152/207; 73.4%), followed by disseminated TB (26; 12.5%) and fibrous-cavernous TB (11; 5.4%) (Supplementary Table S2). Disseminated TB prevailed in the HIV-positive compared to the HIV-negative patients: 34.5% versus 4.6%, respectively ($P < .001$; OR 10.9 [4.2–28.0]).

Monoresistance was found in 6.3%, multidrug resistance in 38.2%, and extensively drug resistance (XDR) in 3.8% of strains (Supplementary Table S3). MDR and XDR *M. tuberculosis* strains were more frequently detected in the HIV-infected compared to the HIV-negative patients: 47.3% vs 34.9% for MDR strains and 7.3% vs 2.6% for XDR strains, although at a non-significant level ($P = .106$ and $P = .142$, respectively).

The Beijing genotype was identified in 129 (62.3%) isolates. Spoligotyping of the 78 non-Beijing isolates assigned them to the genotype families and shared international types (SIT). The main non-Beijing families were represented by LAM (14.5%, mainly SIT42, SIT254, and SIT1451), “ill-defined” T family (11.1%), Ural (5.8%), and Haarlem (3.9%) (Table 1).

Beijing genotype strains were more frequently found among HIV-infected than HIV-negative TB patients (74.5% versus 57.9%; $P = .031$; OR 2.13 [1.07–4.23]) (Table 2). The MDR rate among the Beijing genotype strains in both groups was similar and accounted for 53.7% and 48.9%, respectively. Eight XDR strains were identified in this study and all belonged to the Beijing genotype. Results from the multivariate logistic regression analysis found that multidrug resistance was associated with the Beijing genotype (OR = 4.36; 95% CI 2.21–8.63) and was not associated with HIV-coinfection status (OR = 1.28; 95% CI 0.66–2.48) (Supplementary Table S4).

Subtyping within the Beijing genotype revealed that Beijing 94-32-cluster (“Central Asian/Russian strain”) was the most predominant