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EPIDEMIOLOGY

Transmission of multi-drug resistant tuberculosis in Mongolia is driven by Beijing strains of *Mycobacterium tuberculosis* resistant to all first-line drugs

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SUMMARY

Background: Mongolia has high and rising rates of multi-drug resistant tuberculosis (MDR-TB). Spatio-temporal and programmatic evidence suggests a major contribution from MDR-TB transmission, but genotypic evidence has not been assessed.

Methods: All MDR-TB cases identified during 2012 were examined. Demographic and bacteriological data were obtained from the National Tuberculosis Reference Laboratory. Isolates of *Mycobacterium tuberculosis* from culture-confirmed category 1 treatment failures were genotyped using 24-loci mycobacterium interspersed repetitive unit (MIRU-24) analysis.

Results: Of the 210 MDR-TB cases identified, 115 (54.8%) were treatment failures (34.8% category 1; 20.0% category 2). Streptomycin resistance was present in 156 (74.3%) cases; including 55/73 (75.3%) category 1 treatment failures who had never been exposed to streptomycin. Among category 1 treatment failures, Beijing lineage strains predominated (88.0%; 59/67 of genotyped isolates). MIRU-24 clustering was documented in 62.7% (42/67) of strains; 55.2% (37/67) remained clustered when drug susceptibility test results were considered. In total 59.5% (25/42) of clustered strains were Beijing lineage and demonstrated in-vitro resistance to all first-line drugs tested.

Conclusion: The MDR-TB epidemic in Mongolia appears to be driven by primary transmission of Beijing lineage strains resistant to all first-line drugs. Enhanced infection control strategies together with early MDR-TB case detection and appropriate treatment are necessary to limit escalation of the MDR-TB epidemic.

1. Introduction

The global rise of drug-resistant tuberculosis (TB) presents a major public health challenge [1,2]. The World Health Organization (WHO) estimated that 9.6 million people developed TB in 2014, of whom 480,000 (5%) had multidrug-resistant (MDR; resistant to isoniazid and rifampicin) disease [3]. The highest MDR-TB case-loads were found in the Indian subcontinent, China and the Russian Federation [3]. Mongolia is situated between the northwestern provinces of China and the Irkutsk Region of the Russian Federation, with emerging evidence of cross-border MDR-TB spread along the Trans-Siberian Railway line [4]. Despite relatively low absolute numbers, Mongolia has one of the highest MDR-TB incidence rates within the Western Pacific Region [5]. In Mongolia, few MDR-TB cases were diagnosed before programmatic management of MDR-TB was introduced in 2006. From 2006 to 2012, 1106 MDR-TB cases were detected at an annualized rate of 5.9 cases per 100,000 [4]. The proportion of new TB patients with MDR-TB increased from zero in 2006 to 17% in 2012 (Mongolian National

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TB Program, unpublished data), which reflects enhanced MDR-TB case detection, but also suggests significant MDR-TB transmission within the community.

A retrospective review of all new TB cases started on treatment during 2010–2011 in Mongolia found that 35% (54/136) of patients that failed first-line treatment had MDR-TB [6]. The fact that 59% (32/54) of these treatment failure cases displayed resistance against all first-line drugs tested, including streptomycin to which they have not been exposed, suggested high levels of transmitted MDR-TB missed at initial diagnosis. Spatio-temporal analysis of MDR-TB cases diagnosed from 2006 to 2012 identified three MDR-TB hotspots; all in close proximity to the Trans-Siberian Railway line [4]. The capital city Ulaanbataar is a major hub along the line. It is also the coldest capital city in the world with high rates of overcrowding among people living in poorly ventilated traditional housing, together with high rates of indoor and outdoor air pollution [4]. Despite spatio-temporal and programmatic indications of MDR-TB transmission in Mongolia, no previous study assessed genotypic evidence that might support this assumption.

Mongolia does not perform routine drug susceptibility testing (DST) or genotyping of *Mycobacterium tuberculosis* isolates. The contribution of primary MDR-TB transmission among first-line treatment failures has not been explored using genotypic approaches. We reviewed all MDR-TB cases and used 24-loci mycobacterial interspersed repetitive unit (MIRU-24) analysis to assess the evidence for MDR-TB transmission among TB patients with category 1 (first-line) treatment failure.

2. Materials and methods

2.1. Study population

We conducted a retrospective laboratory-based study of all MDR-TB patients diagnosed in Mongolia between January and December 2012. Demographic data including age, gender and area of residence were acquired from the National Tuberculosis Reference Laboratory (NTRL) database. It is the only laboratory in the country that performs *M. tuberculosis* DST. DST included the first-line drugs isoniazid (H), rifampicin (R) and ethambutol (E), as well as the second-line drug streptomycin (S), using methods described previously [5]. Pyrazinamide (Z) susceptibility was not tested. According to Ministry of Health guidelines at the time, culture was performed on TB cases. However DST was performed on patients that 1) failed first-line treatment (category 1) or retreatment (category 2), 2) defaulted on treatment, 3) had a TB recurrence (relapse), 4) was in close contact with a known MDR-TB patient or 5) tested positive for human immunodeficiency virus (HIV) infection.

2.2. M. tuberculosis genotyping

Genotyping of *M. tuberculosis* isolates was focussed on all MDR-TB cases classified as category 1 (HRZE) treatment failures. These patients remained sputum smear-positive after 3 months of supervised first-line treatment and have not had previous exposure to streptomycin. Isolates from these patients were retrieved from the freezer, where they had been stored at minus 20 °C, and recovered on solid culture (Löwenstein-Jensen medium). DNA was extracted using a Wizard Genomic DNA purification kit (Promega, Madison, USA) and subsequently genotyped using standard procedures for MIRU-24 typing [7]. MIRU-12 was used to identify MIRU international type using the online SITVIT v.2 database [8] and MIRU-24 was employed to assign strain lineage using the MIRU-VNTRplus open source platform (http://www.miru-vntrplus.org) [9].

2.3. Statistical analysis

Descriptive statistics were performed using SPSS 23.0 (IBM, New York, USA); a p-value of less than 0.05 was considered statistically significant. The rate of recent transmission was calculated as follows [(number of clustered isolates − number of clusters)/total number of cultured isolates] [10]. A MIRU-24 minimum spanning tree (MST) was created using BioNumerics 5.0 (Applied Maths, Sint-Martens-Latem, Belgium); two isolates sharing identical MIRU-24 patterns were considered to be a minimum spanning tree node or genotype cluster. Ethics approval was obtained from the Institutional Review Board of the Mongolian National University of Medical Sciences (reference number 14-11/1A).

3. Results

3.1. MDR-TB epidemiology

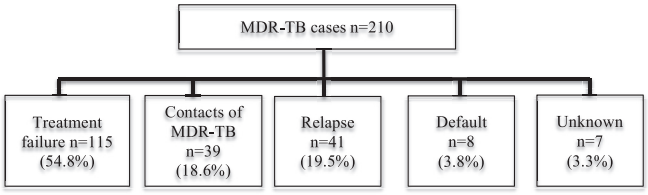
Overall, 3944 TB cases were notified in 2012 with 905 (22.9%) meeting criteria to perform culture and DST. Of the isolates tested, 23.2% (210/905) were found to be MDR-TB; representing 5.3% (210/3944) of all TB cases notified in Mongolia during 2012. Treatment failure accounted for 54.8% (115/210) of the drug-resistant cases identified; 34.8% were category 1 (HRZE) and 20.0% category 2 (HRZES) failures. Figure 1 provides an overview of all MDR-TB cases identified; 18.6% (39/210) were detected among close contacts of MDR-TB cases and none among HIV-infected patients.

3.2. Population demography and drug susceptibility test profile

Table 1 summarizes the characteristics and drug susceptibility profiles of all MDR-TB patients identified. Among MDR-TB patients, the demographic features of those identified as treatment failures and MDR-TB contacts did not differ, except that the mean age of MDR-TB contacts was significantly lower than treatment failure patients (28 vs. 37 years, $p = 0.002$). Streptomycin resistance was found in 74.3% (156/210) of all cases; including 55/73 (75.3%) category 1 treatment failures who had never been exposed to streptomycin. Pan-resistance against all first-line drugs tested (HRES) was present in 58.6% of patients. The overall mortality rate was high (9.5%; 20/210), especially among category 1 treatment failures (12.3%, 9/73); seven (77.8%) of whom had resistance to all first-line drugs.

3.3. Population structure of MDR-TB isolates

Mycobacteria isolated from 67/73 (91.8%) MDR-TB patients who failed category 1 treatment were genotyped. Six isolates could not be revived or were contaminated. Beijing lineage strains were identified in the majority (88.0%, 59/67) of cases. The rest included Latin American Mediterranean (LAM; 7.5%; 5/67), Haarlem (3.0%; 2/67) and NEW1 (1.5%; 1/67) lineages. In total, 62.7% (42/67) of



MDR-TB – multi-drug resistant tuberculosis

Figure 1. Breakdown of all multidrug-resistant tuberculosis cases identified in Mongolia in 2012.