

Drug Resistance and Population Structure of *Mycobacterium tuberculosis* Beijing Strains Isolated in Poland

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Submitted 11 March 2015, revised 15 April 2015, accepted 21 April 2015

Abstract

In total, 1095 *Mycobacterium tuberculosis* clinical isolates from 282 patients with drug-resistant and 813 with drug-sensitive tuberculosis (TB) in Poland during 2007–2011 were analysed. Seventy-one (6.5%) patients were found to have strains of Beijing genotype as defined by spoligotyping. The majority of patients were Polish-born; among foreign-born a large proportion came from Chechnya and Vietnam. Analysis showed strong associations between Beijing genotype infection and MDR, pre-XDR and XDR resistance, with a considerable relative risk among new patients, suggesting that this is due to increased spread of drug-resistant strains rather than acquisition of resistance during treatment.

Key words: Beijing family, drug resistance, genotype, spoligotyping

This is the first study on the genetic diversity, drug resistance and population structure of Beijing-TB in Poland. The study included a total of 1095 (~4% all bacteriologically confirmed cases) *Mycobacterium tuberculosis* isolates collected in 2007–2011 in the National TB Reference Laboratory (NTRL) at the National Tuberculosis and Lung Diseases Research Institute in Warsaw. Primary isolation, species identification, and drug susceptibility testing (DST) were done at regional mycobacteriology laboratories. The isolates were subcultured and sent to NTRL, where confirmatory identification and DST were performed. Available demographic data was collected for all patients, such as age, gender, and country of origin. Genomic DNA was obtained from *M. tuberculosis* colonies on L-J slants by the cetyltrimethyl-ammonium bromide (CTAB) method (van Embden *et al.*, 1993).

All isolates were characterized by spoligotyping with a commercially available kit (Isogen Bioscience BV, Maarssen, The Netherlands) according to the instructions provided by the manufacturer and as described previously (Kamerbeek *et al.*, 1997).

The patterns obtained by spoligotyping were compared by visual examination and by sorting the results in BioNumerics software version 5.10 (Applied Maths, Kortrijk, Belgium). The spoligotypes were also compared with those contained in the international database SpolDB4 (Brudey *et al.*, 2006). The isolates of the

Beijing genotype were defined by showing hybridization to eight or nine (absent the 37 spacer) spacers between spacers 35 and 43, and showing the absence of hybridization to spacers 1–34 (Kremer *et al.*, 2004; Mokrousov *et al.*, 2002).

Of the 71 (6.5%) strains that were defined by spoligotyping to be of the Beijing genotype, 61 (86%) had all the characteristic spacers from 35 to 43, corresponding to the shared type ST1, and 10 (14%) isolates lacked spacer 37, corresponding to ST265.

Of the 1024 patients with non-Beijing strains, 1018 (99.4%) were born in Poland and 6 were (0.6%) born abroad. Two-hundred twenty-four (21.9%) were infected with drug-resistant (DR) (222 Polish-born, 2 foreigners) and 800 (78.1%) with drug-sensitive (DS) strains (796 Polish-born, 4 foreigners). Of the 71 patients with Beijing strains, 42 (59.2%) were born in Poland. Five of them (11.9%) were infected with DS and 37 (88.1%) were infected with DR strains. Among 29 (40.8%) Beijing strains isolated from foreigners, 8 (27.6%) were DS and 21 (72.4%) were DR strains. Among Polish-born, the majority (57.1%) of patients with Beijing strains were in the 45–64 age group; among foreigners the majority were in the 15–34 age group (58.6%) (Table I).

Of the 29 patients with Beijing strains who were foreign-born, a large proportion (93%) came from Europe and Asia; the majority of these patients were

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Table I
Key data on patients with Beijing – TB

	Polish-born	Foreign-born % (no. of patients)	Total
Age group			
0–14	2.4 (1)	10.3 (3)	5.6 (4)
	2.4 (1)	24.1 (7)	11.3 (8)
	7.1 (3)	34.5 (10)	18.3 (13)
15–24	14.3 (6)	13.8 (4)	14.1 (10)
	33.3 (14)	10.3 (3)	23.9 (17)
	23.8 (10)	3.5 (1)	15.5 (11)
25–34	16.7 (7)	3.5 (1)	11.3 (8)
35–44			
45–54			
55–64			
65+			
Sex			
Male	88.1 (37)	79.3 (23)	84.5 (60)
Female	11.9 (5)	20.7 (6)	15.5 (11)
TB treatment history*			
New patient	57.1 (24)	51.7 (15)	55.0 (39)
Previously treated	31.0 (13)	10.3 (3)	22.5 (16)

* Data missing for 11 foreign-born and 5 Polish-born patients.

from Chechnya (38%) and Vietnam (24%), where the Beijing genotype has been reported to be most prevalent (Merker *et al.*, 2015). The others were from Georgia, Russia, Tibet, India, Ukraine, Slovakia, one from Africa (Nigeria) and one from North America (USA).

Altogether 282 Drug Resistant (DR) and 813 Drug Sensitive (DS) isolates from 1095 patients were identified. The results of drug resistance testing are shown in Table II. While 27 (38%) of the Beijing strains were MDR, 15 (21.1%) pre-XDR and 4 (5.4%) XDR, only 59 (5.8%) of the 1024 non-Beijing strains were MDR, 16 (1.6%) pre-XDR and 14 (1.4%) were XDR during the same period.

Several studies have suggested an association between young age and the Beijing genotype family (Buu *et al.*, 2009; Zanini *et al.*, 2014). Although the Beijing genotype is more common among young patients, we did not find such a correlation in the group of Polish-born patients (9.5%), but observed it among foreign-born males (58.6%) ($P < 0.001$). This suggests that the primary reservoir of Beijing family strains in Poland is young immigrants who came to Poland from the East. It is possible that some of the Beijing strain infections were in fact acquired among Polish-born patients during transmission from young and middle-aged immigrants crossing the Polish border and seeking employment.

An additional argument for the transmission of tuberculosis is the observation that the majority of patients with Beijing-TB belonged to the group of newly diagnosed patients, both among immigrants and Polish-born population.

Numerous molecular epidemiological studies, carried out in various geographical settings, have suggested an association between drug resistance, specifically MDR-TB, and the Beijing genotype strain (Filliol *et al.*, 2002; Toungousova *et al.*, 2004; Drobniowski *et al.*, 2005; Kubica *et al.*, 2005; Park *et al.*, 2005; Caws *et al.*, 2006; Hasan *et al.*, 2006; Victor *et al.*, 2007; Dymova *et al.*, 2014; Maeda *et al.*, 2014). Review of molecular epidemiological data from XDR-TB strains has shown an association between XDR-TB and the Beijing genotype in isolates from South Africa, Estonia, China, Japan and Russia (Iwamoto, 2009; Dheda *et al.*, 2010; Casali *et al.*, 2012).

In this study the prevalence of drug-resistance was significantly higher ($P < 0.001$) in Beijing strains than in non-Beijing isolates (81.7% vs 21.9%). We found strong associations between Beijing genotype infection and MDR, pre-XDR and XDR resistance, with a considerable relative risk among new patients ($P = 0.02$), suggesting that it is increased spread of MDR-TB strains

Table II
Proportion of patients with drug-resistant strains

	Total	% drug resistant (no. of patients with drug resistance)			
		Any drug	MDR	Pre-XDR	XDR
Polish-born					
Beijing	42	88.1 (37)	50 (21)	28.6 (12)	7.1 (3)
Non-Beijing	1018	21.8 (222)	5.8 (59)	1.6 (16)	1.4 (14)
Immigrants					
Beijing	29	72.4 (21)	20.7 (6)	10.3 (3)	3.4 (1)
Non-Beijing	6	33.3 (2)	0 (0)	0 (0)	0 (0)
Overall					
Beijing	71	81.7 (58)	38 (27)	21.1 (15)	5.4 (4)
Non-Beijing	1024	21.9 (224)	5.8 (59)	1.6 (16)	1.4 (14)

rather than acquisition of MDR-TB during treatment that defines this association.

This is the first study on Beijing-TB in the patient population in Poland. Further molecular and epidemiological analyses are required to determine the genetic relatedness between strains and analyse possible transmission of tuberculosis between patients.

Literature

- Brudey K., J.R. Driscoll, L. Rigouts, W.M. Prodinge, A. Gori, S.A. Al-Hajj, C. Allix, L. Aristimuño, J. Arora, V. Baumanis and others. 2006. *Mycobacterium tuberculosis* complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. *BMC Microbiol.* 6: 6–23.
- Buu T.N., M.N. Huyen, N.T. Lan, H.T. Quy, N.V. Hen, M. Zignol, M.W. Borgdorff, F.G. Cobelens and D. van Soolingen. 2009. The Beijing genotype is associated with young age and multidrug-resistant tuberculosis in rural Vietnam. *Int. J. Tuberc. Lung Dis.* 13: 900–906.
- Casali N., V. Nikolayevskyy, Y. Balabanova, O. Ignatyeva, I. Kontsevaya, S.R. Harris, S.D. Bentley, J. Parkhill, S. Nejentsev, S.E. Hoffner and others. 2012. Microevolution of extensively drug-resistant tuberculosis in Russia. *Genome Res.* 22: 735–745.
- Caws M., G. Thwaites, K. Stepniewska, T.N. Nguyen, T.H. Nguyen, T.P. Nguyen, N.T. Mai, M.D. Phan, H.L. Tran, T.H. Tran and others. 2006. Beijing genotype of *Mycobacterium tuberculosis* is significantly associated with human immunodeficiency virus infection and multidrug resistance in cases of tuberculous meningitis. *J. Clin. Microbiol.* 44: 3934–3939.
- Dheda K., R.M. Warren, A. Zumla and M.P. Grobusch. 2010. Extensively drug-resistant tuberculosis: epidemiology and management challenges. *Infect. Dis. Clin. North Am.* 24: 705–725.
- Drobniewski F., Y. Balabanova, V. Nikolayevsky, M. Ruddy, S. Kuznetsov, S. Zakharova, A. Melentyev and I. Fedorin. 2005. Drug-resistant tuberculosis, clinical virulence, and the dominance of the Beijing strain family in Russia. *JAMA* 293: 2726–2731.
- Dymova M. A., A.G. Cherednichenko, O.I. Alkhovik, E.A. Khrapov, T.I. Petrenko and M.L. Filipenko. 2014. Characterization of extensively drug-resistant *Mycobacterium tuberculosis* isolates circulating in Siberia. *BMC Infect. Dis.* 14: 478.
- Filliol I., J.R. Driscoll, D. van Soolingen, B.N. Kreiswirth, K. Kremer, G. Valétudie, D.D. Anh, R. Barlow, D. Banerjee, P.J. Bifani and others. 2002. Global distribution of *Mycobacterium tuberculosis* spoligotypes. *Emerg. Infect. Dis.* 8: 1347–1349.
- Hasan Z., M. Tanveer, A. Kanji, Q. Hasan, S. Ghebremichael and R. Hasan. 2006. Spoligotyping of *Mycobacterium tuberculosis* isolates from Pakistan reveals predominance of Central Asian Strain 1 and Beijing isolates. *J. Clin. Microbiol.* 44: 1763–1768.
- Iwamoto T. 2009. Population structure analysis of *Mycobacterium tuberculosis* Beijing family in Japan. *Kekkaku* 84: 755–759.
- Kamerbeek J., L. Schouls, A. Kolk, M. van Agterveld, D. van Soolingen, S. Kuijper, A. Bunschoten, H. Molhuizen, R. Shaw, M. Goyal and others. 1997. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. *J. Clin. Microbiol.* 35: 907–914.
- Kremer K., J.R. Glynn, T. Lillebaek, S. Niemann, N.E. Kurepina, B.N. Kreiswirth, P.J. Bifani and D. van Soolingen. 2004. Definition of the Beijing/W lineage of *Mycobacterium tuberculosis* on the basis of genetic markers. *J. Clin. Microbiol.* 42: 4040–4049.
- Kubica T., R. Agzamova, A. Wright, M.A. Aziz, G. Rakishev, V. Bismilda, E. Richter, S. Rüscher-Gerdes and S. Niemann. 2005. The Beijing genotype is a major cause of drug-resistant tuberculosis in Kazakhstan. *Int. J. Tuberc. Lung Dis.* 9: 646–653.
- Maeda S., N.T. Hang, L.T. Lien, P.H. Thuong, N.V. Hung, N.P. Hoang, V.C. Cuong, M. Hijikata, S. Sakurada and N. Keicho. 2014. *Mycobacterium tuberculosis* strains spreading in Hanoi, Vietnam: Beijing sublineages, genotypes, drug susceptibility patterns, and host factors. *Tuberculosis* 94: 649–656.
- Merker M., C. Blin, S. Mona, N. Duforet-Frebouge, S. Lecher, E. Willery, M. G. Blum, S. Rüscher-Gerdes, I. Mokrousov, E. Aleksic and others. 2015. Evolutionary history and global spread of the *Mycobacterium tuberculosis* Beijing lineage. *Nat. Genet.* 47: 242–249.
- Mokrousov I., O. Narvskaya, E. Limeschenko, T. Otten and B. Vyshnevskiy. 2002. Novel IS6110 insertion sites in the direct repeat locus of *Mycobacterium tuberculosis* clinical strains from the St. Petersburg area of Russia and evolutionary and epidemiological considerations. *J. Clin. Microbiol.* 40: 1504–1507.
- Park Y.K., S. Shin, S. Ryu, S.N. Cho, W.J. Koh, O.J. Kwon, Y.S. Shim, W.J. Lew and G.H. Bai. 2005. Comparison of drug resistance genotypes between Beijing and non-Beijing family strains of *Mycobacterium tuberculosis* in Korea. *J. Microbiol. Methods* 63: 165–172.
- Toungoussova O.S., D.A. Caugant, P. Sandven, A.O. Mariandyshev and G. Bjune. 2004. Impact of drug resistance on fitness of *Mycobacterium tuberculosis* strains of the W-Beijing genotype. *FEMS Immunol. Med. Microbiol.* 42: 281–290.
- van Embden J.D.A., M.D. Cave, J.T. Crawford, J.W. Dale, K.D. Eisenach, B. Gicquel, P. Hermans, C. Martin, R. McAdam, T.M. Shinnick and others. 1993. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. *J. Clin. Microbiol.* 31: 406–409.
- Victor T.C., E. M. Streicher, C. Kewley, J.M. Jordaan, G.D. van der Spuy, M. Bosman, H. Louw, M. Murray, D. Young, P.D. van Helden and others. 2007. Spread of an emerging *Mycobacterium tuberculosis* drug-resistant strain in the western Cape of South Africa. *Int. J. Tuberc. Lung Dis.* 11: 195–201.
- Zanini F., M. Carugati, C. Schioli, G. Lapadula, A. Lombardi, L. Codecasa, A. Gori and F. Franzetti. 2014. *Mycobacterium tuberculosis* Beijing family: analysis of the epidemiological and clinical factors associated with an emerging lineage in the urban area of Milan. *Infect. Genet. Evol.* 25: 14–19.

