Multidrug-resistant *Mycobacterium tuberculosis* Beijing/W genotype in Venezuela

Mycobacterium tuberculosis Beijing/W family, described in 1995 (van Soolingen *et al.*, 1995), is an emerging pathogen in several areas of the world, and a predominant endemic strain in others; it is frequently associated with drug resistance (European Concerted Action on New Generation Genetic Markers and Techniques for the Epidemiology and Control of Tuberculosis, 2006). However, in Latin America little information about the prevalence of this genotype is available. Cuba and Argentina have reported Beijing genotype-infected patients, but only in Cuba has an association been found with drug resistance (Díaz *et al.*, 1998; Morcillo *et al.*, 2005).

In Venezuela, a country of moderate tuberculosis (TB) incidence (25 per 100 000 population), the first countrywide study of drug resistance was carried out during 1998–1999. The majority (77 %) of the strains isolated in this survey were genotyped by spoligotyping and the Beijing/W genotype was not found (Aristimuño *et al.*, 2006).

In this study we investigated the genetic profiles of a collection of 36 multidrug-resistant (MDR) *M. tuberculosis* isolates, cultured from smear-positive sputum of 36 TB cases diagnosed by the Venezuelan National Tuberculosis Programme (VNTP), during the period 2000–2004. The isolates were identified as *M. tuberculosis* complex using biochemical tests, and drug susceptibility testing (DST) was carried out on Lowenstein–Jensen medium by the proportion method

(Canetti *et al.*, 1969) and the BACTEC 460 radiometric method (Siddiqi *et al.*, 1981). Extraction of genomic DNA, spoligotyping and IS6110-RFLP were performed by standard methods (Ausubel *et al.*, 1988; Kamerbeek *et al.*, 1997; van Embden *et al.*, 1993). The comparison of spoligotypes with the international spoligotype database [SpolDB4 (<http://www.pasteur-guadeloupe.fr:8081/SITVITDemo/index.jsp>)] showed that the isolates belonged to the following genomic families of *M. tuberculosis*: LAM (23, 64 %), T (8, 22 %), Haarlem (3, 8 %) and Beijing/W (2, 6 %). The two Beijing/W isolates (VEN 3748, VEN 4145) presented identical DNA fingerprinting with 17 copies of IS6110, and the same mutation conferring resistance to isoniazid (INH) (Ser315Thr in *katG*) and rifampicin (RIF) (Ser531Leu in *rpoB*). The comparison of the IS6110-RFLP patterns with a set of 19 Beijing/W reference patterns representative of the Beijing genotype (Kremer *et al.*, 2004) and with a set of 22 MDR Beijing/W genotype isolates at UNIZAR database (Zaragoza, Spain) revealed, respectively, 85 % similarity to the Beijing/W reference pattern NLA009500592 and >90 % similarity to the Beijing/W strains isolated in 1999 from two Peruvian immigrant patients (P1, P2) resident in Spain (Codina *et al.*, 1999) (Fig. 1).

The 3748 Beijing/W strain was isolated from a 37-year-old Peruvian, human immunodeficiency virus (HIV)-negative male, resident in Caracas, Venezuela, who used to travel to and from Lima, Peru. He had been diagnosed with pulmonary TB

and received non-supervised treatment. He emigrated to Venezuela with respiratory symptoms, in 2002, and received standard retreatment (VNTP); however, he was not cured and died 2 years later.

The 4145 Beijing/W strain was isolated from a 17-year-old Venezuelan, HIV-negative male, diagnosed with pulmonary TB in 2002, and resident in Caracas, who declared no travel abroad. He had not previously received anti-TB treatment and received the standard treatment for new TB cases, according to VNTP. However, his sputum was not cleared. He was then treated with a regimen that included amikacin, pyrazinamide, cycloserine, ethionamide and ofloxacin, but again the sputum was not cleared.

The VNTP prescribes for all forms of TB, with or without culture confirmation, a standardized 6 month treatment for all new TB cases, consisting of a 2 month initial phase of INH, RIF, pyrazinamide and ethambutol, followed by a phase of 4 months with INH and RIF three times a week. If a patient is not cured with this regimen the VNTP indicates culture and DST. The regimen with second line drugs is prescribed in accordance with the results of the DST.

In summary, the findings in this study document for what is believed to be the first time a cluster of two TB patients infected by Beijing/W genotype MDR strains in Venezuela. The high similarity observed with the fingerprints of the isolates from Peruvian immigrant patients resident in Spain suggest that the first

Fig. 1. Dendrogram of IS6110-RFLP and spoligotyping of the clinical isolates from Venezuelan (VEN 3748, VEN 4145) and Peruvian patients (P1, P2).

strain isolated could have been imported from Peru, and that further local transmission as MDR TB occurred. Our data confirm the importance of the molecular typing of MDR strains in order to monitor movement, change and expansion of emerging genotypes.

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