


### RESEARCH NOTE

**Molecular, epidemiological and infectivity characterisation of a *Mycobacterium tuberculosis* strain prevalent in Madrid**


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**ABSTRACT**

The most prevalent strain of *Mycobacterium tuberculosis* in Madrid, Spain (strain 5) was recovered from 45 cases between 1997 and 2004 and showed a highly homogeneous genetic composition. This strain was not exclusive to Spain, and its spoligotyping signature (ST20) was found in entries from different countries in the SITVIT1 database. Patients infected with strain 5 were more frequently positive for human immunodeficiency virus and autochthonous, and had been in prison more frequently, but strain 5 did not show increased infectivity in an in-vitro model of infection.

**Keywords** Epidemiology, infectivity, *Mycobacterium tuberculosis*, spoligotyping, ST20

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Molecular tools allow different *Mycobacterium tuberculosis* (MTB) strains circulating in a population to be distinguished, with fingerprint data obtained in molecular epidemiology programmes [1] making it possible to identify prevalent strains or genetic families that are over-represented in certain settings [2,3]. In Spain, there has been a sharp increase in the number of cases of tuberculosis among immigrants [4]. It is assumed that some of these patients import MTB strains from their countries of origin, and that such strains could have an impact on the profile of strains circulating in the host population, considering the high rate of transmission between the autochthonous and immigrant populations in this area [4].

Molecular fingerprinting tools have been used in Madrid, Spain since 1997 [5]. The MTB isolates from nine urban districts (1 459 232 inhabitants) in Madrid were genotyped. Isolates from all nine districts were genotyped during 2002–2004, and isolates from five districts during 1997–2001. In total, 1207 MTB isolates were analysed by IS6110 restriction fragment length polymorphism [6], yielding 867 different genotypes, with 455 (37.7%) isolates grouped in 115 clusters; 51.3% of the clusters included two isolates, and only 9.6% of the clusters included more than six isolates. Nevertheless, some large clusters were detected, with the four largest clusters corresponding to strains 5 (45 cases), 2, 8 and 38 (20 cases each). Strain 5 was
isolated from the highest number of cases (3.7% of the total) and was therefore selected for in-depth characterisation. This strain showed an eight-band restriction fragment length polymorphism pattern (Fig. 1) and was isolated in all but one of the years of the study. Of 45 cases infected with strain 5, 37 were males, 18 were positive for human immunodeficiency virus, two were foreign-born, 12 were diagnosed while in prison, and ten were intravenous drug users.

Strain 5 was detected previously during 1993–1994, at which time it was predominant among three strains responsible for the highest number of cases in a prison population [7]. In the present study, strain 5 still persisted after 13 years, while the two other strains had declined in importance, suggesting that strain 5 is favoured over other strains.

Additional spoligotyping [8] and mycobacterial interspersed repetitive unit variable number tandem repeats (MIRU-VNTR) analysis [9] revealed a highly robust and homogeneous genetic profile, with all but one of the patients infected by isolates sharing identical genotypes (ST20: 67777607760771, which is a feature of the LAM-superfamily), and all isolates sharing identical MIRU-VNTR types (MIT25: 224226153321; Fig. 1). In one of the patients, MIRU-VNTR analysis detected a co-infection with two clonal variants, differing by one and three repeats in two loci, but indistinguishable by restriction fragment length polymorphism or spoligotyping (Fig. 1b). This genotypic homogeneity also suggests that strain 5 is somehow favoured, which may ensure its genetic homogeneity, or that this strain was only introduced to Spain recently and has not yet diverged genetically. In contrast, isolates of the three other prevalent strains (strains 2, 8 and 38) frequently showed differences among their spoligotypes (data not shown).

Features linked to clustering in Madrid are an age of <35 years and intravenous drug use [4,5,7]. Compared with cases infected by other strains, Spanish birth, human immunodeficiency virus infection and prison stay were associated significantly with infection by strain 5 (p <0.05). Twelve cases infected with strain 5 were from the same district, but epidemiological links were found for only three pairs of brothers. Evidence of strain 5 infecting immigrants was detected, suggesting that immigrants could become infected after their arrival by strains that are prevalent in the host country.

In order to establish whether strain 5 was specific to Spain, the SITVIT1 database (http://www.pasteur-quadeloupe.fr/SITVIT) was searched for strains sharing the same spoligotype (ST20). Unlike the Madrid 1 and 2 strains [10], the spoligotype

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Fig. 1. Molecular genotyping patterns of: (a) the four strains of Mycobacterium tuberculosis (MTB) prevalent in Madrid; (b) the 45 strain 5 isolates of MTB. N, number of cases infected by the strains; spoligotypes and mycobacterial interspersed repetitive unit variable number tandem repeats (MIRU-VNTR) types are indicated with their international designations according to the SITVIT1 database (http://www.pasteur-quadeloupe.fr/SITVIT). RFLP, restriction fragment length polymorphism. *One case was co-infected by two variants with different MIRU-VNTR types.
signature of strain 5 was not specific to Spain, with 378 cases matching ST20 reported from 37 countries other than Spain. Of these, strain 5 was over-represented in the database entries from Brazil (7%), Portugal (12%), Venezuela (6%), Haiti (6%) and Namibia (15%).

The infectivity of a representative of strain 5 was assayed in comparison with the virulent reference strain H37Rv, and with a representative ‘orphan’ strain (isolated from only one patient during the same study period), by measuring its replication leading to a high prevalence of specific strains have been observed in other settings. For example, in Manitoba, Canada, the prevalent ‘type 1’ strain seems to have epidemiological links with the native population that could favour its prevalence, although bacterial factors also seem to be involved [13,14]. The present study showed that strain 5 could have higher infectivity compared with other less frequent clinical strains, but that it is not a highly infective clone. More refined genomic or transcriptomic-based approaches may identify specific bacterial features that give a theoretical advantage to this strain, but the in-vitro data currently available suggest that epidemiological factors are mainly responsible for the prevalence of this strain.

Identification of other prevalent strains could help in monitoring changes in the profile of circulating MTB strains, and also in monitoring the epidemiological features associated with ongoing transmission. A panel of marker strains would be extremely useful in countries such as Spain, where the rise in the number of tuberculosis cases in immigrants is currently causing a transformation in the epidemiology of tuberculosis.

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**RESEARCH NOTE**

Spread of the *Streptococcus pneumoniae* Taiwan<sup>19F</sup>-14 clone among children in Greece

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**ABSTRACT**

Serotype 19F pneumococci were a leading cause of infections among children in Athens, Greece during 2001–2006. In total, 143 19F isolates were typed by pulsed-field gel electrophoresis (PFGE), and 38 isolates representing the main PFGE types were also characterised by multilocus sequence typing. A diversity of distinct strains belonging to sequence types 236, 1035, 274, 172 and 319 were identified, but multidrug-resistant isolates related to the Taiwan<sup>19F</sup>-14 clone (ST236) constituted 76.9% of the isolates. Spread of the Taiwan<sup>19F</sup>-14 clone explains, in part, the high incidence of antibiotic resistance observed among pneumococci reported recently from Athens.

**Keywords** Children, epidemiology, Greece, pneumococci, *Streptococcus pneumoniae*, Taiwan<sup>19F</sup>-14

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Treatment of pneumococcal infections can be complex because of the dissemination of clones

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