Strains of the Beijing genotype family of Mycobacterium tuberculosis have been associated with outbreaks and multidrug resistance. We performed a retrospective thirteen-year surveillance study (1993–2005) on the occurrence of this strain in Elche, Spain. Only one of the available isolates from 332 cases of tuberculosis tested positive for Beijing strain. The case, detected in 2001, was that of an immigrant patient from Senegal with pulmonary tuberculosis. The strain was not drug resistant and besides six close contact persons that were infected no secondary cases of this strain were detected. In the Elche area, the incidence of Beijing strains is very low and there is no evidence of transmission or higher virulence.

Introduction
Molecular epidemiology studies have revealed a genotype of M. tuberculosis strains that seem to possess selective advantages compared with other strains, have increased virulence and are sometimes associated with multidrug resistance [1,2]. This genotype has been called ‘Beijing’ or ‘Beijing/W’ family [1,2] and it is widespread around the world. The fact that this family of strains is widespread and, in some situations, associated with multidrug resistance has led to concern that these strains may be spreading and may have a predilection for acquiring drug resistance [2].

The detection of the Beijing genotype in a particular region and the study of trends over time is of great interest. However, reports from Western Europe (countries with low incidence of this strain) are very scarce. We performed a 13-year retrospective surveillance population-based study in our area in order to detect any possible isolation of M. tuberculosis Beijing genotype and to analyse its clinical and epidemiological characteristics.

Methods
Population and data collection
Elche Health District is a region in the southeast of Spain with a population of about 265,000 people during the study period. All microbiological investigations were performed at the regional hospital microbiology laboratory, which is the only laboratory that performs culture-based tuberculosis diagnosis in our region. A search for Beijing/W family strains was performed among 332 M. tuberculosis isolates obtained from tuberculosis patients from beginning 1993 to end of 2005, which represent 73% of isolations during this period, and clinical and epidemiological data for each isolate was obtained. The remaining 27% were not available for analysis due to problems during their frozen conservation.

Identification of Beijing Strains
The identification of Beijing strains was performed as proposed by Warren et al. [3]. To identify a Beijing strain, the DNA was subjected to four amplifications with different primers using a polymerase chain reaction (PCR) method based on comparative genomic data. A positive amplification product of 393 base pairs (bp) and 239 bp, respectively, indicated the presence of an IS6110 insertion in Rv2820 that is unique to the Beijing evolutionary lineage. A positive amplification product of 569 and 308 bp, respectively, indicated the presence of M. tuberculosis strain(s) belonging to non-Beijing evolutionary lineages. All tests were performed in duplicate. As a control we used a strain of the Beijing family that was provided by the Mycobacteria Laboratory of Zaragoza University, Spain.

Results
In the study period a total of 455 cases of TB were laboratory diagnosed in the Elche region and isolates for 332 patients were available for further investigation. Of those 332 patients 72% were men, and the mean age was 42 (standard deviation 24). Two hundred and seventy-five patients were of Spanish origin and two were from Western Europe. The origin of 55 immigrants with tuberculosis was Africa for 26, South-America for 17, East-Europe for 10 and Asia for two patients. All 27% of isolates that were unavailable for analysis were obtained from patients of Spanish origin.

We performed two PCR assays in each isolate allowing us to classify the strains into two lineages: Beijing and non-Beijing. Only one isolate of the M. tuberculosis Beijing family was obtained, in 2001. The patient was a 24-year old, HIV-negative male from Senegal with pulmonary tuberculosis with cavitations in both lungs. Bacilli were obtained in three sputum samples. The isolate was susceptible to the five antituberculous drugs tested. The patient had close contact with six individuals: three from Senegal and Gambia at the patient’s home, and two from Senegal and one Spaniard at work. On investigation all showed a tuberculin skin test higher than 20 mm. Chemoprophylaxis with isoniazid was prescribed for all six contact persons and none of them developed tuberculosis during the follow-up.

Discussion
In this 13-year population-based study in the southeast of Spain, we found a very low occurrence of the Beijing strain of M. tuberculosis. Only one case of this strain was found in 332 patients...
with laboratory-confirmed tuberculosis. However, it should be noted that 27% of the strains in our area were unavailable for analysis. Interestingly, although all six close contact persons examined were infected, we found no secondary cases. This finding is important because in another report from Spain (Gran Canaria island) an immigrant patient from Africa with laryngeal tuberculosis was the origin of a dissemination of this strain that only five years later was responsible for more than 20 percent of all cases of tuberculosis in the island [4]. The experience in our region is very different to that reported in other regions. Although the study does not report a high number of infections due to the Beijing type strain, we feel that is important to present our findings because they show the differences in the distribution of this strain. In a recent publication that includes patients from 49 studies in 35 countries, the authors describe four patterns for Beijing genotype tuberculosis:

- endemic not associated with drug resistance,
- epidemic associated with drug resistance,
- epidemic but drug sensitive, and
- very low level or absent [5].

Our population can be included in the fourth group, which is the most common in Europe.

Studies on time trends of this genotype are scarce. It has been reported that all Western European sites analysed except London showed a slight increase in Beijing strains over time. In St Petersburg, Okayama, Buenos Aires, Sao Paulo and San Francisco, no significant change occurred over time, but the studies only covered a few years [5]. In Cape Town and Malawi, significant increases occurred over time and were unchanged after adjusting for age [5].

In Western Europe, the Beijing genotype is more common among immigrant patients than in indigenous patients [5]. Reports from Spain are scarce but show that infections with the Beijing strain are almost exclusively found in immigrant patients [4,6,7]. In our region, the proportion of recent transmission is high (established by molecular epidemiology) [8,9], and immigration has markedly increased during this period [10]. For this reason, we expected to find more cases of tuberculosis belonging to the Beijing family in recent years. However, no cases have been detected after 2001. We can assume that the immigrant population in our area, which is now an important origin of newly diagnosed cases of tuberculosis, is not infected by \textit{M. tuberculosis} Beijing strain, probably because they come mainly from regions with no predominance of this strain.

Our study shows a very low occurrence of Beijing genotype of \textit{M tuberculosis} in the Elche region, without evidence of secondary cases. Continuous control of the possible presence and characteristics of this strain will provide further information on the true epidemiological situation of the Beijing genotype.

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\section*{References}


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