

# Tuberculosis transmission patterns among Spanish-born and foreign-born populations in the city of Barcelona

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## Abstract

During a 2-year period (2003–2004), tuberculosis (TB) transmission in Barcelona and the factors related to transmission among the Spanish- and foreign-born populations were studied by molecular epidemiology. Data were obtained from TB cases and Conventional Contact Tracing registries and genotyping was performed using restriction fragment length polymorphism (RFLP)-IS6110 and MIRU12 as a secondary typing method. Of the 892 TB cases reported, 583 (65.3%) corresponded to Spanish-born and 309 (34.6%) to foreign-born. Six hundred and eighty-seven cases (77%) were confirmed by culture. RFLP typing of 463/687 (67.4%) isolates was performed, revealing 280 (60.5%) unique and 183 (39.5%) shared patterns, which were grouped into 65 clusters. Spanish-born individuals were significantly more clustered than foreign-born individuals (44.6% vs. 28.8%;  $p$  0.016). Clustering in foreign-born individuals was associated with HIV ( $p$  0.051, odds ratio = 3.1, 95% confidence interval 1–10.9) and alcohol abuse ( $p$  0.022), whereas, in the Spanish-born individuals, clustering was associated with age in the range 21–50 years, ( $p$  0.024). Of the total clusters, 36/65 (55.3%) included only Spanish-born patients, whereas 22/65 (33.8%) included individuals from both populations. In mixed clusters, the index case was Spanish-born in 53% and foreign-born in 47%. Among the foreign-born, 2.8% were ill on arrival, 30% developed TB within the first year and 50.3% developed TB within the first 2 years; 58.3% were from South America. In conclusion, half of the foreign-born TB patients developed the disease during the first 2 years after arrival, which, in most cases, was the result of endogenous reactivation. Recent TB transmission among Spanish-born and foreign-born populations, as well as bidirectional transmission between communities, contributed significantly to the burden of TB in Barcelona, suggesting the need to improve Public Health interventions in both populations.

**Keywords:** Immigration, molecular epidemiology, native-born, transmission, tuberculosis

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## Introduction

Tuberculosis (TB) continues to be one of the infectious diseases of greatest incidence in the world. In 2006, 9 200 000 cases were declared, with 1.7 million deaths [1].

HIV infection and drug resistance have contributed to the re-emergence of TB in Europe and North America. On the other hand, the increased numbers of migrants during the last 10 years and the growing number of international travellers are both factors that could influence the epidemiology of TB. Thus, at present, one-third of the cases of TB in the European Union are found in foreign-born subjects [2], involving up to 60% of the cases in Holland, Sweden and Denmark.

Several European studies [3–9] have analyzed the changes in patterns of TB transmission, particularly within the context of the growing proportion of TB cases in foreign-born

subjects. Molecular characterization strategies have demonstrated that, in some cases, the impact of TB in foreign-born subjects on the host-born population is low [10,11], most likely because of social and cultural barriers between the two populations. Other studies have detected higher recent transmission rates after arrival in the country [4,8] or up to 50% of mixed clusters between foreign-born and native-born patients [7].

During recent years, the foreign-born population in Barcelona (1 580 642 inhabitants) has risen to represent 17.9% of the total population in the study period [12]. Although, subsequent to 1996, the overall incidence of TB in Barcelona has decreased to a mean of 6.3% annually, reaching values of 28.5/100 000 inhabitants during the study period (2003–2004), a progressive increase has been observed in the proportion of TB cases developing in foreign-born patients in the study period, rising from 5.6% in 1996 to 34.7% [13].

Because immigration features may not be shared in all study contexts and no previous study has analyzed the contribution of the foreign-born populations to TB transmission in Barcelona, the main objective of the present study was to analyse the transmission patterns of the TB patients diagnosed in the specific setting of Barcelona during 2003–2004 and to describe the factors that influence transmission among populations, using molecular analysis and epidemiological data.

## Materials and Methods

### Setting and patients

This study was carried out in Barcelona and included all cases reported to the TB program from 1 January 2003 to 31 December 2004. TB cases were defined as patients clinically diagnosed with TB, who initiated and completed antituberculous treatment or died during the treatment with or without isolation of *Mycobacterium tuberculosis*.

### Microbiology

Patient samples were processed in the city hospitals and healthcare centres. Positive culture identification and drug susceptibility tests were performed in accordance with standardized methods [14]. The definition of TB drug resistance followed the WHO and International Union Against Tuberculosis and Lung Disease guidelines [15].

### Molecular analysis

Extraction of the mycobacterial DNA, and restriction fragment length polymorphism (RFLP)-*IS6110* techniques were performed using standardized protocols [16]. The *IS6110* fingerprint patterns were analyzed with Bioimage software,

version 3.2.2 (Bio Image, Inc; Ann Arbor, MI, USA). A molecular-cluster was defined as two or more patients with: RFLP patterns containing more than six *IS6110* bands at the same position, RFLP patterns containing up to six *IS6110*-bands at the same position but with an identical MIRU12 type [17,18], or an *IS6110* pattern with more than six *IS6110*-bands that differed in a unique band and with an identical MIRU12 type. The isolates showing a RFLP pattern likely to belong to the Beijing group [19] were analyzed using standardized spoligotyping [20].

### Epidemiological investigation

Epidemiological, clinical, microbiological and demographic data were obtained from the TB control program databases of Barcelona and the Departments of Microbiology in the participating healthcare centres and hospitals. All foreign-born patients were classified into six geographic origins: South America, Western Europe/North America, Asia, Eastern Europe, the Maghreb and Sub-Saharan Africa.

The index case of a cluster was defined as the earliest case to initiate symptoms with pulmonary localization. When such data were not available or the patients were asymptomatic, the index case was considered the patient who initiated treatment earliest.

### Statistical analysis

Univariate analysis was performed by chi squared or Fisher's exact test with Yates correction. Logistic regression was realized. Goodness-of-fit was verified using the Hosmer—Lemeshow test. The OR and 95% CI was calculated. The analysis was performed using SPSS, version 13.0 (SPSS Inc., Chicago, IL, USA).

## Results

### Study population

A total of 892 cases of TB were reported during the 2-year study period: 583 (65.3%) were Spanish-born and 309 (34.6%) were foreign-born patients. The mean incidence was 21.6/10<sup>5</sup> in Spanish-born inhabitants and 64.12/10<sup>5</sup> in foreign-born inhabitants (Table 1).

The localization was pulmonary in 636 (71.3%) of the cases included (Table 2). Of the total cases, 77% (687/892) were culture-positive and smear positivity was observed in 53.6% (341/636) of pulmonary cases, in 22.3% (57/256) of extra-pulmonary cases and was not performed in 5.2% (47/892). Significant differences ( $p < 0.001$ ) were observed in patients of Asian origin, with 51.6% (49/95) presenting extra-pulmonary diseases.

**TABLE 1.** Distribution of tuberculosis (TB) cases in the city of Barcelona during the period 2003–2004 according to geographical origin

Geographic origin	Population <sup>a</sup>	TB cases	Mean TB Incidence ( $\times 10^5$ ) <sup>b</sup>	Drug resistance <sup>c</sup>	Pulmonary smear-positive cases
Spain	1 348 613	583	21.6	6.1	232 (39.8%)
Other than Spain	240 947	309	64.1	15.8	109 (35.3%)
South America	131 873	126	47.8	20.3	53 (42%)
Western Europe/North America	36 459	15	20.6	12.5	8 (53.3%)
Asia	34 435	95	137.9	13.5	23 (24.2%)
Eastern Europe	15 486	21	67.8	30	11 (52.4%)
Maghreb	18 135	38	104.8	0	11 (28.9%)
Sub-Saharan Africa	4559	14	153.5	12.5	3 (21.4%)
Total population	1 589 560	892	28.05	9.3	341 (38.2%)

<sup>a</sup>Number of inhabitants of each geographic origin living in Barcelona.

<sup>b</sup>The mean incidence of the patients per geographical origin is calculated by dividing the mean number of cases during the study period by the mean number of habitants of each specific geographical origin at 2 years.

<sup>c</sup>Drug-resistance among new cases (%) ( $n = 517$  drug sensitivity tests of 682 positive cultures).

**TABLE 2.** Analysis of demographic characteristics and risk factors associated with Spanish-born and foreign-born patients

Characteristics	Spanish-born patients ( $n = 583$ )	Foreign-born patients ( $n = 309$ )	OR crude	OR adjusted
Male sex	356 (61.1%)	198 (64%)	1.1 (0.8–1.5)	
Age				
0–20 years	50 (8.6%)	34 (11%)	1	1
21–50 years	277 (47.5%)	252 (81.5%)	1.3 (0.8–2.1)	1.6 (0.9–2.7)
>50 years	256 (43.9%)	23 (7.5%)	0.1 (0.07–0.2)	0.3 (0.07–0.36)
Residence in old city district	83 (14.2%)	114 (36.9%)	3.5 (2.5–4.9)	3.5 (2.4–5.2)
HIV	66 (11.3%)	38 (12.3%)	1.05 (0.7–1.6)	
Intravenous drug use	47 (8%)	16 (5.2%)	0.6 (0.3–1.03)	0.2 (0.1–0.4)
Homelessness	30 (5.1%)	27 (8.7%)	1.76 (1.03–3)	2.2 (1.04–4.6)
Pulmonary localization	432 (74%)	204 (66%)	0.7 (0.5–0.9)	0.6 (0.36–0.77)
Pulmonary smear positivity ( $n = 432$ and 204)	232 (53.7%)	109 (53.4%)	1.12 (0.8–1.55)	
Diabetes	48 (8.2%)	2 (0.6%)	0.07 (0.02–0.3)	0.2 (0.05–0.8)
Alcohol abuse	151 (25.9%)	51 (16.5%)	0.6 (0.4–0.8)	0.5 (0.4–0.9)
Resistance to any drug ( $n = 517$ )	25 (7.2%)	27 (16%)	3 (1.7–5.5)	3.2 (1.7–6.7)

The analysis of demographic characteristics and risk factors defined different population patterns (Table 2). Thus, pulmonary forms, diabetes, age >50 years, use of illegal drugs and alcohol abuse were associated with Spanish-born patients, whereas TB drug resistance, homelessness, residence in the old city district and age <50 years were factors associated with foreign-born patients. Moreover, male gender, extra pulmonary forms and negative smear were observed more frequent ( $p < 0.05$ ) in Asian subjects.

Information on TB drug resistance was available in 517/687 (75.2%) of the culture-positive TB cases: 348 Spanish-born and 169 foreign-born patients. TB drug resistance in new cases was 6.1% (20/326) in Spanish-born and 15.8% (25/158) in foreign-born patients ( $p = 0.002$ ). In Spanish patients, mono-resistance to isoniazid was observed in 2.8%, to streptomycin in 2.1% and to pyrazinamide in 0.9%. In foreign-born patients, mono-resistance to isoniazid was found in 3.8% and to streptomycin in 6.3%, whereas resistance to ethambutol and pyrazinamide accounted for 0.6% in both cases. On the other hand, drug resistance in previously-treated cases was 22.7% (5/22) and 18.2% (2/11) in Spanish-born and

foreign-born patients, respectively. Multi-drug resistance was observed in 2.5% of foreign-born and in 0.3% of Spanish-born new cases ( $p = 0.02$ ). In previously-treated cases, multi-drug resistance was found in 18.2% of the foreign-born cases, whereas no case was observed among the Spanish-born population. On analyzing the burden of resistant isolates in Barcelona, independently of the treatment history, isoniazid resistance was present in 4.2% of the cases, streptomycin in 4.8%, rifampicin in 1.35%, pyrazinamide in 1.2% and ethambutol in 0.1%.

#### Molecular study and associated risk factors

Analysis by molecular genotyping was possible in 67.4% (463/687) of the culture-positive cases. Of the patients studied, 148 (32%) were foreign-born individuals from 32 countries in the geographical areas defined in Table 1.

Genotyping showed 280 isolates (60.5%) with a unique pattern and 183 (39.5%) isolates sharing patterns grouped into 65 clusters. These clusters included 140 (76.5%) Spanish-born and 43 (23.5%) foreign-born subjects (OR 2.15, 95% CI 1.3–3.4). The size of the clusters was in the range of

**TABLE 3.** Factors associated with clustering in foreign-born patients

Characteristics	N° clustered	Total (n = 148)	% Clustered	OR crude	OR adjusted
Male	29	88	33%	1	
Female	14	60	23.3%	1.6 (0.7–3.4)	
Age					
0–20 years	4	13	30.8%	1	
20–50 years	35	125	28%	0.9 (0.25–3)	
>50 years	4	10	40%	1.5 (0.3–8.4)	
Residence in old city district	8	42	19%	0.5 (0.2–1.2)	
HIV	10	17	58.8%	4.2 (1.5–11.9)	4.3 (1.4–12.6)
Intravenous drug use	5	8	62.5%	4.5 (1–19.6)	
Pulmonary TB	37	115	32.2%	1.7 (0.6–4.8)	
Smear positivity	24	78	30.8%	1.02 (0.5–2.2)	
Diabetes	2	2	100%	–	
Homelessness	4	12	33.3%	1.25 (0.35–4.4)	
Alcohol abuse	14	27	51.8%	3.4 (1.4–8.1)	3.0 (1.2–7.5)

TB, tuberculosis.

two–eight patients, with those comprising two and three patients predominating (37 and 15 clusters, respectively).

On analyzing risk factors and patient characteristics, clustering was associated with HIV infection and alcohol abuse in foreign-born subjects (Table 3), whereas clustering was associated with age in the range 21–50 years (OR 3.9, 95% CI 1.6–9.1) in the Spanish-born. Regarding geographical origin, being Asian was less associated with clustering (OR 0.4, 95% CI 0.0–0.4). Antibiotic resistance was not significantly associated with clustering (data not shown).

Of the total number of clusters, 36/65 (55.3%) only included Spanish-born patients, 7/65 (10.9%) included only foreign-born patients and 22/65 (33.8%) were mixed. No differences were observed in the size of the three types of clusters.

Among Spanish-born patients, 13.9% participated in mixed clusters and 30.4% in clusters including only Spanish-born people. Concerning foreign-born patients, 17.5% were in mixed clusters and 11.5% in clusters comprising only foreign-born individuals.

We characterized 13/463 (2.8%) isolates as Beijing strains, with seven participating in three clusters, including three Georgians, one Colombian, one Ecuadorian and two Spanish-born. Five Asian patients and one South American were infected with a Beijing family isolate with a unique pattern. All the Beijing strains showed susceptibility to all the antibiotics.

Comparison between foreign-born included in mixed clusters and the remaining foreign-born subjects with TB showed that those in mixed clusters had a higher proportion of HIV-infected subjects (OR 3.1, 95% CI 1–10.9) and alcohol abusers (OR 3.5, 95% CI 1.2–10.6).

We established a relationship between the date of arrival to Spain and the development of TB in 143/148 (96.6%) of the foreign-born subjects analyzed by genotyping. Four (2.8%) were ill on arrival, 16 (11.2%) developed the disease

within the first 6 months of arrival, 43 (30%) during the first year and 25 (17.5%) in the second year. In summary, 50.3% presented TB in the first 2 years of residence in Spain.

The seven clusters of foreign-born individuals included 17 patients, 65% of them from South America. In all cases, the members of each cluster had the same geographical origin. An epidemiological association was established in 3/7 clusters (42.9%), with a relationship between friends in one case and members of the same family in two (Fig. 1).

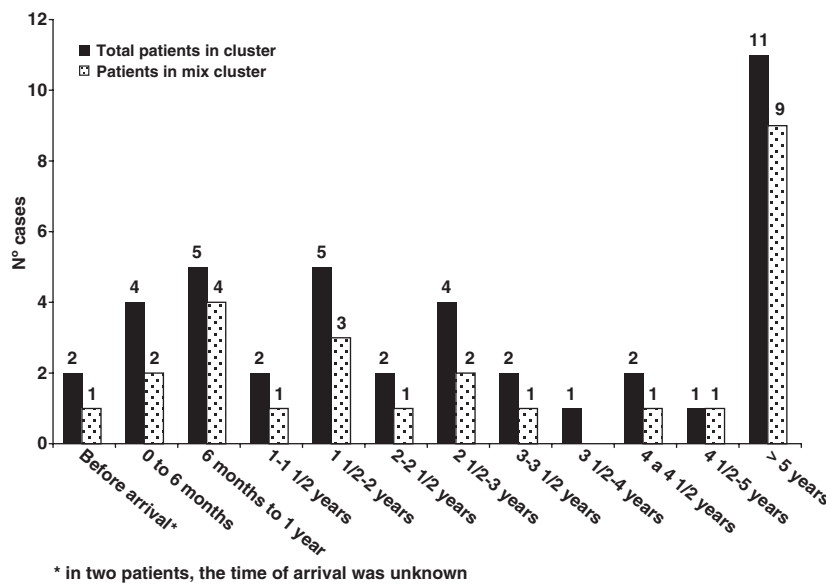
The 36 clusters of Spanish-born individuals included 96 patients. An epidemiological association was observed in 11 (30.6%) clusters: four being family- and seven neighbourhood-associated.

The 22 mixed clusters (including Spanish-born and South-American patients in ten clusters, Western Europeans/North Americans in six, patients from Maghreb in five and patients from Asia in one cluster) were made up of 70 patients, 44 Spanish-born and 26 foreign-born individuals (Fig. 1). An epidemiological association was found in seven (32%) of the clusters, with four made up of professionally-related individuals, two of individuals from the same neighbourhood and the remaining cluster made up of members of one family. In the 22 mixed clusters, the index case was a foreign-born patient in ten (45.5%) and a Spanish-born patient in 12 (54.5%).

Analyzing the percentage of recent transmission among foreign-born patients showed that 17 were clustered as index cases and 105 had a unique molecular pattern, resulting in 122/148 (82.4%) cases related to endogenous reactivation and 26/148 (17.6%) to recent transmission.

## Discussion

The proportion of foreign-born patients among TB cases in Barcelona has recently increased, reaching 34.6% during the



**FIG. 1.** Initiation of symptoms of foreign-born patients participating in mixed clusters according to time of arrival.

study period, and reflecting the growing proportion of foreign-born individuals arriving in the city [13]. This phenomenon has already been reported in other Spanish cities and in other parts of Europe, in the range 28–79% [7,9,21,22].

The results of the present study show the factors and characteristics associated with TB in both foreign- and Spanish-born populations and the TB transmission between them. The largest foreign-born group with TB in Barcelona is from South America, possibly for linguistic reasons and, together with the Asian-born population, they account for more than 70% of the foreign-born TB patients. This population composition is not seen in other cities in Spain; in Madrid, the Asian-born population is less represented [7,23] and, in Almeria (Southern Spain), patients from the Maghreb predominate [8]. This implies several differences in the results, which should be considered separately for every country or city.

As in previous studies [2,9], factors associated with the development of TB in Barcelona revealed that foreign-born patients were younger than Spanish-born, lived in the poorest district of the city, and showed higher drug-resistance rates. These factors may be explained by the young age of the immigrants, their economic difficulties on arrival and, on becoming ill, the strains being more resistant, probably reflecting the drug-resistance rates of their country.

Only small proportions (2.8%) of foreign-born TB patients were ill on arrival. We also found that 50.3% developed the disease within the first 2 years after arrival to Spain and that mixed transmission was present from the beginning of their stay. However, 82.4% of the foreign-born cases were not included in clusters and most could be attributed to reactivation of previous latent TB. This is probably facilitated by the stressful living conditions encountered on arrival because

20% do not know the language and 20% are without correct documentation [13].

Special mention should be made regarding the Asian cases. Isolates of 39 Asian patients were included in the molecular analysis and only one (0.025%) was a member of a cluster. Although 51.6% of the Asian cases were extrapulmonary [24], this could not explain this low frequency of clustering. Neither did culture nor language appear to justify this low frequency because most of the Asian-born patients (46%) were from Pakistan and a higher frequency of recent transmission should be expected among cases sharing the same nationality.

During the study period, 39.5% of the cases analyzed were in clusters, in accordance with several previous European studies [6,7,22,23]. Mixed population clusters, reflecting transmission between Spanish- and foreign-born patients, represented 33.8% of the total clusters. Analysis of the epidemiological link between cases included in mixed clusters demonstrated that transmission occurred in both directions because index cases were found practically equally among Spanish- and foreign-born patients. Compared with nonclustered foreign-born, the foreign-born patients in mixed clusters with Spanish-born were significantly associated with HIV infection and alcoholism, indicating that, in this setting of poor social conditions and where immune suppression favours higher rates of progression to active TB, contact may be closer, thereby facilitating transmission [22]. On the other hand, most of the foreign-born patients included in mixed cases were from South America, suggesting that the use of the same language and cultural proximity could be important factors in breaking down the barriers to inter-population transmission.

Several studies in Europe have been taken into consideration when comparing the percentages of mixed clusters, ranging from the low percentages of 11.8% and 25.7% observed in Norway [10] and Denmark [25], respectively, to the higher percentages reported in Madrid (50.7%) [7] and Milan (53%) [21]. A moderate percentage was observed in Almeria (28.9%) [8], Hamburg (29.4%) [6] and the present study (33.8%). The predominant foreign-born patients in Madrid and Barcelona are from South America, being culturally closer to the native-born population than the Somalian-born are to the Norwegian-born patients [10] or the Maghreb-born to the Spanish-born in Almeria [8]. These results suggest that the native TB incidence, together with socially-related factors, such as cultural and language similarities, influences the proportion of mixed clusters, revealing the TB transmission relationships established between the two populations.

Nevertheless, some factors should be taken into account when interpreting the results of the present study. First, our molecular results may underestimate the true extent of recent transmission because they focus on cases reported during a 2-year period in a specific area. Second, not all the cases were studied mainly as a result of a negative TB culture or uncultured cases, or some other difficulties in recovering positive cultures because of the magnitude of the study.

In conclusion, although half of the foreign-born TB patients developed the disease during the first 2 years after arrival, and most were the result of endogenous reactivation, recent transmission in Spanish-born, and foreign-born and bidirectionally between populations contributes significantly to the burden of TB in Barcelona.

Additionally, the present study not only demonstrates the mixed TB transmission observed in Barcelona, but also that this transmission occurs after arrival, being more marked after 5 years. All these results and the different pattern of transmission observed in other study settings, even within the same country, suggest the need to improve and adapt Public Health interventions depending on the setting.

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