Advanced Survey of Tuberculosis Transmission in a Complex Socioepidemiologic Scenario with a High Proportion of Cases in Immigrants

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Background. An increase in the incidence of tuberculosis (TB) in immigrants has changed the socioepidemiologic scenario in Spain. It is generally assumed that TB in immigrants is the result of importation of infection, but the role of recent transmission is rarely considered. Standard contact tracing is not suitable for the survey of transmission in this complex scenario.

Methods. During the study period (2003–2006), we genotyped 356 (90.4%) of 394 isolates from patients with microbiologically confirmed TB in Almería, the province with the highest percentage of TB cases among immigrants in Spain. The epidemiologic survey of TB transmission was performed by active data collection using standardized interviews of the patients with TB and subsequent interviews of the clustered patients (who were clustered on the basis of the restriction fragment–length polymorphism types of their isolates) to identify transmission locations (supported by nominal and/or photographic recognition by the clustered patients).

Results. Of all 356 genotyped isolates, 131 (36.8%) were clustered, suggesting recent transmission. The difference between the clustering rate for immigrants (32.8%) and that for native patients (41.6%) was not statistically significant ($P = .087$); of the 45 clusters, 15 (33.3%) involved only immigrants, 17 (37.8%) involved only autochthonous patients, and 13 (28.9%) involved both immigrants and autochthonous patients. The advanced system to investigate the clustered patients succeeded in detecting links in 10 of the 12 clusters that involved >4 patients, whereas the conventional approach, based on contact tracing, could detect links in only 2 clusters.

Conclusions. Recent transmission among immigrants and transmission permeability between the immigrant and autochthonous populations were found. Epidemiologic strategies that combine universal genotyping and refined surveys of the clustered patients are needed to investigate transmission patterns in complex scenarios.

There has been a sharp increase in immigration in Spain in recent years, which has led to a marked increase in the number of cases of tuberculosis (TB) in immigrants [1, 2]. One of the provinces with the highest rates of TB among immigrants is Almería, where a progressive increase in the incidence of TB has been registered (21 cases per 100,000 inhabitants per year in 1997 to 26 cases per 100,000 inhabitants per year in 2006). During this period, the percentage of cases of TB in immigrants in Almería has increased continuously, from 19% in 1997 to 48.9% in 2006. One of the districts in Almería, Poniente, has registered even higher values, with 67.1% of cases occurring in immigrants in 2006. These immigrants (mainly from North and sub-Saharan Africa) are highly mobile, and many live in substandard conditions outside the main cities, rendering access to the health care system difficult.

In general, TB in immigrants is assumed to result from reactivation of infection acquired in their countries of origin [3–9]; therefore, recent transmission after arrival in the host country is rarely taken into account. Our study had the 2 following major aims: (1) to analyze whether recent transmission has a role in TB in

immigrants in a highly complex socioepidemiologic context, such as that in Almería, and if so, to dissect the patterns and dynamics of transmission in the host country and (2) to evaluate new strategies to optimize the tracking of TB transmission in such complex socioepidemiologic circumstances.

Therefore, we investigated transmission of TB by not only using standard epidemiologic approaches but also using a more advanced strategy. This strategy involved (1) universal genotyping of *Mycobacterium tuberculosis* isolates, (2) active recruitment of patient data by standardized interviews, (3) use of a newly developed computer application to manage patient data, and (4) subsequent detailed interview of the clustered patients (who were clustered on the basis of the restriction fragment–length polymorphism types of their isolates) to identify the transmission locations (supported by nominal and/or photographic recognition by the clustered patients).

METHODS

Sample

The population covered by the study centers—health care centers of the Servicio Andaluz de Salud (Regional Health Service) and the public network of mycobacteriology laboratories (Hospital de Poniente, Complejo Hospitalario Torrecárdenas, and Hospital La Inmaculada; Almería, Spain)—ranged from 565,310 in 2003 to 635,850 in 2006 (mean population, 598,388). Our study sample was composed of all patients who had received a microbiological diagnosis of TB during the period 2003–2006. Incidence rates for the different nationalities were estimated by considering the number of registered residents from each country.

Microbiological Procedures

Clinical specimens were processed according to standard methods. Susceptibility testing was performed using the BacT/ALERT 3D system (bioMérieux).

Molecular Analysis

M. tuberculosis isolates were genotyped by IS6110 restriction fragment–length polymorphism [10]; spoligotyping [11] was used as a second-line genotyping method. Genotypes were analyzed using Bionumerics 4.6 (Applied Maths). Clusters were defined when the isolates shared identical restriction fragment–length polymorphism types. Identical spoligotypes were also required when the isolates showed <6 IS6110 copies. The Beijing genotype was identified by observation of its specific spoligotypes.

Epidemiologic Survey

Transmission of TB was investigated using the 2 following information sources: data obtained using the standard approach (based on conventional contact tracing) and data obtained by applying the advanced survey (based on 2 interviews). In the advanced survey, the objective of the first standardized interview was to collect complete data and photographs. During the second interview, the objective was to compile new data for the patients with clustered isolates identified by genotyping and to search for potential epidemiologic links on the basis of nominal and/or photographic recognition among these patients.

Standard Contact Tracing

Standard contact tracing applied the "stone on the pond" principle [12]. Each patient was contacted to be interviewed by his or her family physician after the diagnosis of TB to create a list of contacts, applying the concept of concentric circles, as follows: close contacts in the home (first circle), the most frequent contacts outside the home (second circle), and sporadic contacts (third circle).

Advanced Epidemiologic Survey

First interview. When the first microbiological evidence became available, a standardized first interview was performed to collect complete socioepidemiologic information from all of the patients. A photograph of each patient was taken at this first interview. The information was used to complete 2 forms, adapted from the study by van Deutekom et al. [13] that compiled information on sociocultural aspects, current TB episode, country and province of origin, chronology of the residence(s), date of arrival in Spain, and information on where, how, and with whom the patient spent the infectivity period. The information sources used were the clinical records from the diagnostic and follow-up periods, the contact-tracing clinical record, and the data obtained from the patient interview. Informed consent was obtained for interviews, use of photographic images, and inclusion of data in the computerized register.

Second interview. A second standardized interview was performed only with the clustered patients to identify epidemiologic links. These patients were interviewed by a multidisciplinary team (a physician, a nurse, a social worker, and cultural mediators) to find epidemiologic links among them.

Each of the clustered patients was interviewed again to obtain more-detailed data that was not recorded at the first interview (e.g., data regarding jobs, educational and social activities, and leisure time) and more information about known contacts with TB. Finally, the patient was asked whether he or she recognized some or all of the patients clustered with him or her. For this, the GenContacTB application (described below) generated a list of the clustered patients' names and an automatic, randomly generated, equivalent number of names of patients who were unclustered, to rule out bias. The names were presented as a list of names from the general public, and the patients were never referred to as persons with TB. If the patient could not

Table 1. Tuberculosis (TB) cases in Almería during the period 2003–2006, distributed by geographic regions of origin.

Area of origin	Mean no. of registered residents	No. of patients with culture-confirmed TB cases	Ratio of patients with TB to registered residents	Mean annual incidence rate of TB, no. of cases per 100,000 population	Countries with high TB rates (no. of cases)	Ratio of clustered to orphan isolates	Percentage of clustered isolates
Spain	516,845	183	1:2825	8.9	...	67:94	41.6
Immigrants							
All	81,543	211	1:386	64.7	...	64:131	32.8
Africa	32,547	131	1:248	100.6	Morocco (84), Senegal (16), Mali (14)	42:80	34.4
Europe	31,055	46	1:675	37.0	Romania (33), Lithuania (4)	15:26	36.6
Ecuador and Bolivia	16,571	32	1:518	48.3	Ecuador (18), Bolivia (7)	7:23	23.3
Asia	1349	2	1:674	37.0	...	0:2	0
Oceania	20	0	...	...	...	...	...
Total	598,388	394	1:1519	16.5	...	131:225	36.8

identify the names, the application offered the matrix of photographs corresponding to the list of names.

When a patient recognized a patient clustered with him or her, we considered a potential epidemiologic link. To support this finding, we used the GenContacTB system to analyze all of the data about the time, place, and period of potential contact and to estimate the infectivity periods to define the transmission contexts that involved the clustered patients.

Management of patient data.   The coordinator distributed the names of the new patients to the interviewers, who were members of the research group and who performed the first and second interviews. Personal data were entered into the database by the coordinator, and all of the remaining data were compiled on the forms by the interviewers. These data were finally entered into the database (GenContacTB) by experts hired for this purpose. GenContacTB is a newly developed computer application to manage all microbiological, clinical, epidemiologic, and molecular data and photographs of patients. The program was written in Borland Builder C++ for Windows and used a local database and a remote database (MySQL) in a UNIX server, which distributed and updated the information. All legal requirements to ensure data protection and security measures for automatized databases (laws L.O.15/1999 and R.D.994/1999) were fulfilled. These measures required registration of the database in the General Register of the Spanish Data Protection Agency and application of high-level security measures (including all measures of inferior level plus the dissociation-of-identification data, coded telecommunications, register of accesses, coded distribution of supports, and backup copies, which were stored separately from the support equipment). The ethics committee of the institutions approved this modus operandi.

RESULTS

During the study period, 394 TB cases were microbiologically diagnosed in Almería (68.6% of the total 695 TB cases diagnosed in Almería); 281 cases (71.3%) occurred in men, and the mean age of the patients was 36 years (mean age of the immigrant patients, 30 years; mean age of the autochthonous patients, 43 years; $P < .001$). Ninety-two percent of the patients had pulmonary TB. The distribution of confirmed cases among the 3 districts in Almería was 194 (49.2%) in Poniente, 125 (31.7%) in the central region, and 65 (16.5%) in the Levante region. Ten patients (2.5%) with TB lived outside Almería and were included, because their TB was diagnosed in the study area.

Two hundred eleven (53.6%) of the 394 microbiologically confirmed TB cases occurred in immigrants. The evolution of the percentage of confirmed cases that occurred in immigrants during the study period was as follows: 51.2% in 2003, 54.4% in 2004, 58% in 2005, and 50.0% in 2006. The immigrant patients were from 22 different countries, and 131 (62%) of the 211 immigrant patients were from Africa (table 1). The highest numbers of patients with TB were from Morocco (84 patients; 39.8%), Romania (33; 15.6%), Senegal (16; 7.6%), and Mali (14; 6.6%). The incidence rates for the different geographic regions (table 1) ranged from 37 cases per 100,000 population (Europe) to 100.6 cases per 100,000 population (Africa). The median time between arrival in Spain and development of TB was 36.6 months.

Susceptibility testing was performed for 384 (97.5%) of 394 patients, and the results revealed that, of 193 previously untreated immigrant patients, 18 (9.3%) had TB that was resistant to isoniazid, 0 had TB that was resistant to rifampicin, and 2

(1.0%) had multidrug-resistant TB. For the 157 previously untreated patients in the autochthonous population, these values were 8 (5.1%), 0, and 5 (3.2%), respectively.

Genotyping analysis. Genotyping was performed for isolates from 356 (90.4%) of the 394 patients with microbiologically confirmed TB. Four isolates were found to belong to the Beijing family (these isolates were from 1 patient from Ecuador, 1 patient from Russia, and 2 patients from Spain).

In total, 131 (36.8%) of the 356 patients were grouped in 45 clusters (with 2–7 patients each) (figure 1 and table 1); 64 (32.8%) of 195 immigrants and 67 (41.6%) of 161 autochthonous patients were clustered ($P = .087$). Only 1 cluster (with 4 patients) had <6 IS6110 copies; therefore, this cluster was subtyped by spoligotyping, and only 2 patients were finally

clustered (cluster 553). A more detailed analysis revealed that, of the 45 clusters, 15 (33.3%) clusters involved only immigrants, 17 (37.8%) involved only autochthonous patients, and 13 (28.9%) involved both immigrant and autochthonous patients (i.e., mixed clusters) (figure 1). In summary, 28 (62.2%) of the 45 clusters involved at least 1 immigrant patient.

The highest percentages of clustered patients were from Morocco (33%), Romania (39%), Senegal (27%), and Mali (43%). Among the immigrant clusters, 16% were multinational, involving 2 different nationalities (figure 1).

Epidemiologic analysis of clusters. We initially tried to find the transmission locations associated with the clusters. Standard epidemiologic approaches based on contact tracing revealed links in only 15% of the clusters.
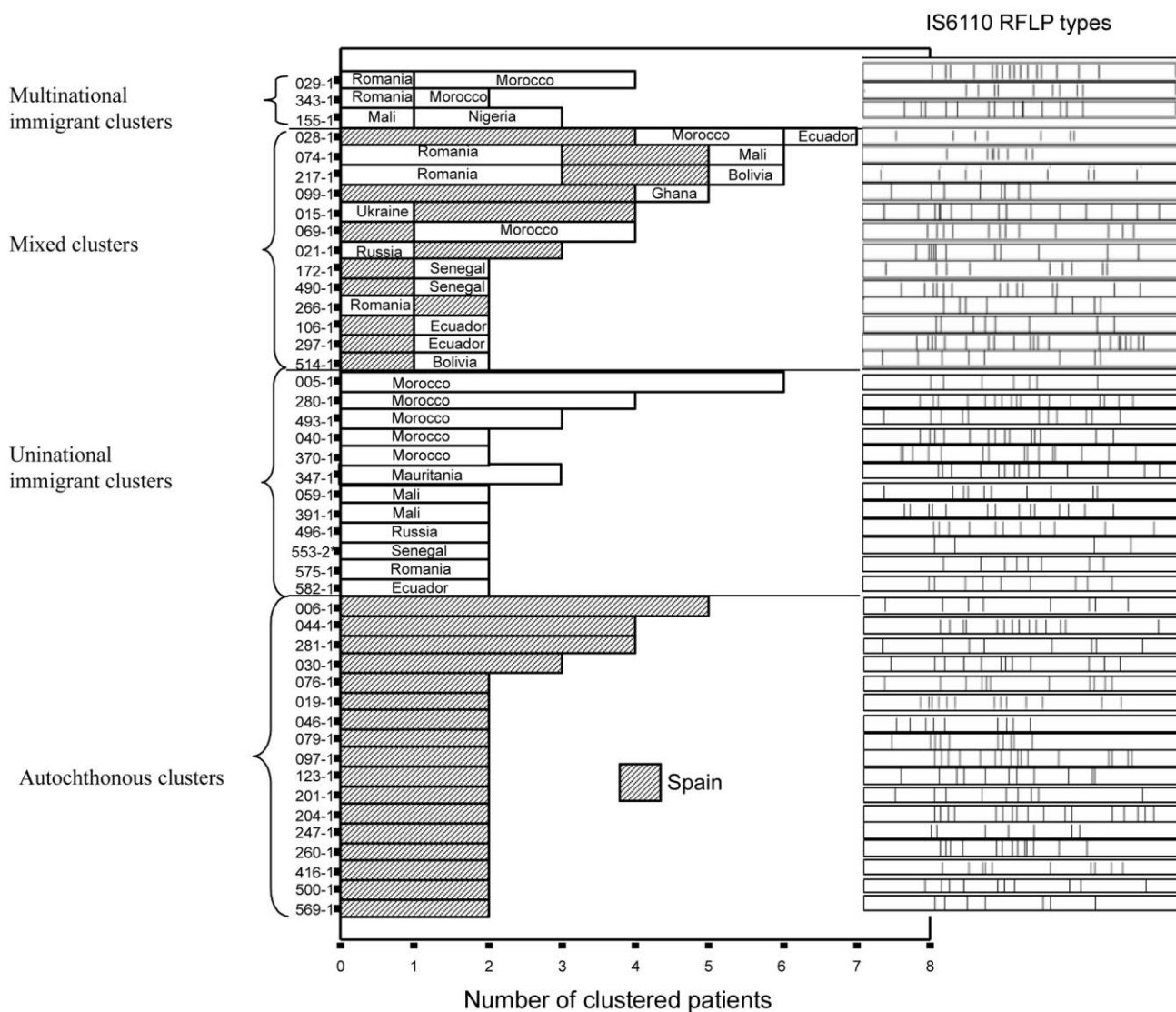
Figure 1. Clustered isolates from patients in Almería, grouped by nationality of the patients. Each bar represents 1 cluster. RFLP, restriction fragment–length polymorphism.

We then used a more advanced strategy based on the application of the GenContacTB tool and standardized interviews of the clustered patients to optimize the identification of links in the clusters. Therefore, we selected those clusters that involved a higher number of patients (>4 patients), because they represented the most active transmission chains, and we considered that the possibility of finding links would be higher because of the higher number of patients involved. We analyzed 61 patients in 12 clusters (with 4–7 patients each). The standard epidemiologic approaches were able to find links in only 2 clusters (both were clusters of autochthonous patients who had contact with infected family members) but failed to identify transmission links in the remaining 10 clusters. On the contrary, the standardized interviews in the GenContacTB application approach succeeded in finding links in 10 clusters (figure 2). Patients in a cluster never identified names or photographs of unclustered patients or of patients grouped in other clusters. Apart from the 2 clusters revealed by standard approaches, the remainder consisted of 2 clusters of Moroccan patients, 5 clusters with both immigrant and autochthonous patients (i.e., mixed clusters), and 1 multinational cluster. Some of the transmission contexts that had not been revealed by standard epidemiology were sporadic sharing of substandard housing, occasional contact at temporary jobs, consuming alcohol and smoking drugs together, and attending the same bar or gymnasium (figure 2).

DISCUSSION

There has been a sharp increase in the number of TB cases in immigrants in Spain [1, 2], and several molecular epidemiology studies that are being performed in several Spanish cities are attempting to analyze the dynamics of TB. Similar studies have also been performed in other countries with a longer history of immigration than that of Spain [3–9, 14, 15].

Our study focused on Almería, because this area has the highest incidence of TB among immigrants in Spain. In addition, by focusing the study in Almería, we were able to study patients from areas such as North and sub-Saharan Africa, which are not as well represented in other provinces. Furthermore, many of the immigrants are highly mobile and live in substandard conditions in rural areas, which makes it difficult for them to gain access to the health care system.

Our study had the 2 following major aims: (1) to analyze whether recent transmission has a role in TB in immigrants in this complex scenario, and if so, to dissect the patterns and dynamics of transmission in the host country and (2) to evaluate new strategies to optimize the tracking of TB transmission in such complex socioepidemiologic circumstances.

TB in immigrants is generally assumed to occur as a result of reactivation of imported infection. However, the 4-year universal genotyping of the isolates from patients in Almería allowed us to identify a proportion of isolates that were clustered, and these were generally assumed to indicate recent transmission events. Remarkably, this proportion and the rates of clustering found among the autochthonous population did not differ significantly, contrary to findings of most studies from other countries [3–9]. Moreover, the percentage of clusters constituted of only immigrants was equivalent to that of clusters that involved only autochthonous patients, and most of the clusters identified (62%) involved at least 1 immigrant patient. These data suggest that recent transmission of TB has a role in the immigrant population.

Recent transmission among immigrants in Almería was not restricted to patients from specific countries—we detected cases in patients from 11 different countries that were involved in clusters—or to the immigrant collective, as indicated by the frequent finding of mixed clusters that involved both immigrant and autochthonous patients. However, immigrant clusters that involved different nationalities were found less frequently in Almería than in other areas [2].

RFLP Cluster	RFLP Type	Type of cluster	Countries of origin (No. of cases)	SEI	GenContacTB interview	Epidemiologic links
029	‖ │ ‖‖‖│ │ ‖	Immigrant	Morocco (3), Romania (1)	No	Yes	Alcohol abuse. Sporadic sharing of housing
005	‖ │ │ ‖ │	Immigrant	Morocco (6)	No	Yes	Drug abuse: hashish
280	‖│ │‖‖‖ ‖ │ ‖‖	Immigrant	Morocco (6)	No	Yes	Sporadic sharing of housing
028	│ │‖ │ ‖	Mixed	Morocco (2), Spain (4) Ecuador (1)	No	No	Unknown
074	│ ‖ ‖	Mixed	Romania (3), Spain (2) Mali (1)	No	Yes	Bar. Gymnasium
217	│ │‖ │ │ ‖	Mixed	Romania (3), Spain (2), Bolivia (1)	No	Yes	Sporadic sharing of housing
099	│ ‖ │‖	Mixed	Spain (4), Ghana (1)	No	Yes	Home. Health-care centre
015	│ ‖‖ ‖│ │‖‖	Mixed	Spain (3), Ukraine (1)	No	Yes	Friends. Drug abuse: hashish
069	‖│ ‖‖ │ │‖	Mixed	Morocco (3), Spain (1)	No	Yes	Neighbours, Sporadic sharing of housing
006	│ │‖ │ ‖	Autochthonous	Spain (5)	Yes	Yes	Family
044	‖│ ‖‖‖‖ │	Autochthonous	Spain (4)	Yes	Yes	Family
281	│ │‖ │ ‖ │	Autochthonous	Spain (4)	No	No	Unknown

Figure 2. Efficiency of standard epidemiological investigation (SEI) and GenContact tuberculosis (TB) interview. "No" indicates that there was no link between clustered patients, and "yes" indicates that there were links between clustered patients. RFLP, restriction fragment–length polymorphism.

Although clusters are generally accepted to be markers of recent transmission, they could also be considered to be coincidental groupings of patients infected with *M. tuberculosis* strains that are prevalent in the countries of origin. The frequent finding of multinational clusters sharing the same *M. tuberculosis* strain weakens this hypothesis. However, some of the immigrant clusters that involved a single nationality could also be considered to have resulted from contacts before the patients' arrival in the host country. Unfortunately, refined epidemiologic data from the countries of origin were not available. To compensate for this, interviewed clustered patients were asked for information about contacts with TB in the country of origin to rule out the possibility of recent transmission before arrival in Spain. However, in addition to the links found in Spain, we were not able to rule out this possibility in only 1 cluster (005), composed of Moroccans (from the same village).

The definitive documentation of recent transmission requires proof of the existence of epidemiologic links among patients belonging to the same cluster. Unfortunately, when different studies have attempted to establish such proof, they have found few links [2, 9, 14, 16]. This also occurred in Almería, where only 15% of the clusters were supported by epidemiologic evidence obtained through conventional contact tracing. The weak points of conventional contact tracing and the value of standardizing approaches to detect epidemiologic links have been highlighted elsewhere [17, 18]. Thus, our second objective was not only to evaluate the improved efficiency of contact tracing by applying standardized approaches but also to fully redefine epidemiologic strategies to optimize the identification of the transmission contexts suggested by the clusters.

The strategy used a working model to track TB transmission in complex socioepidemiologic situations, such as that experienced in Almería. To take full advantage of the molecular analysis, this strategy needed to guarantee the availability of complete epidemiologic information from patients and to investigate links among clustered patients efficiently.

Our strategy used a multidisciplinary approach that involved a physician, a nurse, a social worker, and cultural mediators to actively compile complete epidemiologic data through 2 interviews (the first was for all patients, and the second was for the clustered patients only). A new computer application, GenContacTB, was also developed for the global management of all data. Finally, we implemented a system of interviewing clustered patients to find potential links based on detailed epidemiologic data and/or nominal or photographic recognition, using methods from the study by Weis et al. [19]. Similar to other studies in which refined epidemiologic surveys were applied [13, 20–22], we detected epidemiologic links in a high number of clusters. Of the 12 clusters studied (involving >4 patients), standard contact tracing could detect links in only 2 clusters, which grouped autochthonous patients who had contact with a family member with TB, but failed to find links in the other, more-complex clusters (immigrant clusters or clusters with both immigrant and autochthonous patients). By contrast, our advanced strategy succeeded in finding links in 10 of the 12 clusters. Nevertheless, our approach was limited in that it did not include patients with culture-negative TB cases, which might have helped to reveal new links. In addition, to disclose the limitations of our strategy, it would have been useful to monitor the clusters that included <4 individuals.

The transmission contexts revealed by our strategy corresponded to scenarios that are not usually considered by standard approaches. These scenarios indicated the relevance of temporary and leisure contacts; such relevance has also been found in other studies [21, 23, 24]. These findings validated clusters in Almería as indicators of recent transmission and alerted us to the need to apply refined approaches to reveal transmission patterns in complex contexts.

Although it would be interesting to replicate our study in similar locations, the combination of factors and resources may not be as readily available. Nevertheless, our findings demonstrate the usefulness of cluster analysis when performing universal genotyping in complete populations, even if advanced epidemiologic strategies are not available to verify the significance of these clusters.

During this study, new challenges were identified. Genotyping by restriction fragment–length polymorphism is slow, and our approach focused mainly on intervention, which requires real-time genotyping. A parallel study in Almería has been evaluating the usefulness of the recently developed Mycobacterial interspersed repetitive unit variable number tandem repeat method [25–27] to identify clusters early enough to allow for intervention.

In summary, our experience in Almería showed that recent transmission in addition to importation should be considered in the management of TB in immigrants. Transmission between nationalities and between the immigrant and autochthonous populations was also found. Novel epidemiologic strategies combining molecular analysis and collection of refined epidemiologic data with advanced analysis of clusters are required to optimize the identification of transmission in complex situations. Our findings also suggest that the contexts in which transmission is suspected should be carefully reconsidered to optimize our often limited epidemiologic resources.

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