

Factors Associated with Differences between Conventional Contact Tracing and Molecular Epidemiology in Study of Tuberculosis Transmission and Analysis in the City of Barcelona, Spain[∇]

Sònia Borrell,¹ Montserrat Español,² Àngels Orcau,³ Griselda Tudó,¹ Francesca March,² Joan A. Caylà,³ Josep Maria Jansà,³ Fernando Alcaide,⁴ Núria Martín-Casabona,⁵ Margarita Salvadó,⁶ José Antonio Martínez,⁷ Rafael Vidal,⁸ Francesca Sánchez,⁹ Neus Altet,¹⁰ Pere Coll,¹¹ and Julià González-Martín^{1*}

Servei de Microbiologia, CDB, H. Clínic de Barcelona-IDIBAPS, Universitat de Barcelona,¹ Servei de Microbiologia, H. U. Sant Pau (HSCSP),² Servei d'Epidemiologia, Agència de Salut Pública de Barcelona,³ Servei de Microbiologia, H. U. Bellvitge-IDIBELL, L'Hospitalet de Llobregat,⁴ Servei de Microbiologia, Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona,⁵ Laboratori de Referència de Catalunya, El Prat de Llobregat,⁶ Servei de Malalties Infeccioses, ICMID, Hospital Clínic-IDIBAPS,⁷ Servei de Pneumologia, Hospital Universitari Vall d'Hebron,⁸ Servei de Malalties Infeccioses, Hospital del Mar,⁹ Unitat de Prevenció i Control de la Tuberculosi,¹⁰ and Servei de Microbiologia, Hospital de la Santa Creu i Sant Pau, Departament de Genètica i Microbiologia, Universitat Autònoma de Barcelona,¹¹ Barcelona, Spain

Received 14 March 2008/Returned for modification 6 July 2008/Accepted 11 November 2008

The aim of this study was to analyze the factors associated with conventional contact tracing (CCT) and molecular epidemiology (ME) methods in assessing tuberculosis (TB) transmission, comparing the populations studied and the epidemiological links established by both methods. Data were obtained from TB case and CCT registries, and ME was performed using IS6110-based restriction fragment length polymorphism (RFLP) analysis and mycobacterial interspersed repetitive unit 12 (MIRU12) typing as a secondary typing method. During two years (2003 and 2004), 892 cases of TB were reported, of which 687 (77%) were confirmed by culture. RFLP analysis was performed with 463 (67.4%) of the 687 isolated strains, and MIRU12 types in 75 strains were evaluated; 280 strains (60.5%) had a unique RFLP pattern, and 183 (39.5%) shared patterns, grouping into 65 clusters. CCT of 613 (68.7%) of 892 cases detected 44 clusters involving 101 patients. The results of both CCT and ME methods yielded 96 clusters involving 255 patients. The household link was the one most frequently identified by CCT (corresponding to 80.7% of the cases clustered by this method), whereas nonhousehold and unknown links were associated with 94.1% of the strains clustered by ME. When both methods were used in 351 cases (39.3%), they showed the same results in 214 cases (61%). Of the remainder, 106 (30.2%) were clustered only by ME, 19 (5.5%) were clustered only by CCT, and 12 (3.4%) were clustered by both methods but into different clusters. Patients with factors potentially associated with social problems were less frequently studied by CCT ($P = 0.002$), whereas patients of <15 years of age, most with negative cultures, were less frequently studied by ME ($P = 0.005$). Significant differences in the populations studied by ME versus CCT were observed, possibly explaining the scarce correlation found between the results of these methods. Moreover, ME allowed the detection of nonhousehold contact relationships, whereas CCT was more useful for tracing transmission chains involving patients of <15 years of age. In conclusion, the two methods are complementary, suggesting the need to improve the methodology of contact study protocols.

Tuberculosis (TB) continues to be one of the infectious diseases of greatest incidence in the world. In 2005, 8,811,000 cases were reported, with 1.6 million deaths (13). Although most cases occur in poor countries, in recent years an increase in cases in industrialized countries, favored by migratory movements, has been observed (14).

TB is an airborne disease with a subacute or chronic clinical course. Among the control measures currently in use, the detection of new infections and secondary cases by conventional contact tracing (CCT) is fundamental (39). During the last 15 years, molecular epidemiology (ME) techniques have been demonstrated to be helpful in the study of TB transmission,

and they have been applied to population studies (2, 5, 11, 12, 19, 30), such as those of defined risk groups (22, 34) and analyses of outbreaks (6, 20, 21).

Attempts to correlate the results of the two methods have already been reported in the literature, with most of the studies showing a low level of correlation ranging from 5 to 40% (2, 8, 19, 24, 28, 30, 36, 37); however, very few studies have analyzed the causes of this poor correlation in depth (24, 28). The CCT method detects secondary cases and subjects most likely to undergo treatment for latent TB infection, mainly in the household and employment settings. The results obtained by ME techniques, which require positive cultures from TB patients, suggest the importance of the leisure environment and casual contacts in the investigation of secondary cases and in the overall study of TB transmission (8, 24, 28). Moreover, the results of the two methods are usually correlated several months after the onset of the cases (5), making it difficult to recover complementary information and additional isolates.

* Corresponding author. Mailing address: Servei de Microbiologia, Hospital Clínic de Barcelona, c/ Villarroel 170, 08036 Barcelona, Spain. Phone: 34932275522. Fax: 34932279372. E-mail: gonzalez@clinic.ub.es.

[∇] Published ahead of print on 19 November 2008.

In Barcelona, Spain, TB continues to be a public health care problem, with an incidence rate of 27.7 cases per 10⁵ inhabitants (25), thereby requiring a good TB control program adapted to the constant changes in the dynamics of the transmission of this disease.

Therefore, the main objectives of the present study were to analyze and compare the factors and characteristics associated with the populations studied by ME and CCT in the dynamics of TB transmission in Barcelona and to determine their influence on the low correlation between the results of the two methods to thereby improve the TB control program.

MATERIALS AND METHODS

Setting and patients. The study was carried out in the city of Barcelona, Spain (1,580,642 inhabitants), and included all cases reported to the TB program in the city from 1 January 2003 to 31 December 2004. TB cases were defined as those of patients clinically diagnosed with TB who initiated and completed antituberculous treatment or died during the treatment, with or without the isolation of *Mycobacterium tuberculosis*.

ME. The first isolate from each patient identified as *M. tuberculosis* by standardized methods (26) was used for the different ME study techniques. The isolates were frozen until analysis. The molecular study was performed in two of the six participating centers (Hospital Clínic de Barcelona and Hospital de la Santa Creu i Sant Pau).

Extraction of the mycobacterial DNA and the IS6110-based restriction fragment length polymorphism (RFLP) technique were performed using standardized protocols (38). The IS6110 fingerprint patterns were analyzed with whole-band analyzer software (version 3.2.2; BioImage, Inc., Ann Arbor, MI) by using the unweighted-pair group method with arithmetic means and the Dice coefficient. Isolates were grouped into the same RFLP cluster when they showed identical RFLP patterns (equal numbers of IS6110 bands at identical positions). All isolates with ≤ 6 IS6110 bands belonging to an RFLP cluster underwent mycobacterial interspersed repetitive unit 12 (MIRU12) typing to provide a second molecular marker (32, 33), as did those isolates with ≥ 6 IS6110 bands that differed in a unique band.

A molecular cluster was defined as two or more isolates with RFLP patterns containing >6 IS6110 bands at the same positions, ≤ 6 IS6110 bands at the same positions but identical MIRU12 types, or a unique band difference in the IS6110 patterns and identical MIRU12 types.

CCT. For each TB case, the TB control program of Barcelona performed a census of possible household and nonhousehold contacts according to "the stone in the pond principle" (39) to identify secondary cases associated with the case or the true source case, as well as the subjects most likely to undergo treatment for latent TB infection, by following standard protocols (3).

A CCT cluster was defined as a group of two or more TB cases with an epidemiological link established using CCT.

Definition of index case. The index case of a molecular cluster or a CCT cluster was defined as that of the patient who first manifested symptoms with pulmonary localization. When these data were not available or the patients were asymptomatic, the index case was considered to be that of the patient who initiated treatment earliest.

Definition of secondary case. Secondary cases of a molecular cluster or a CCT cluster were the cluster-associated cases of patients who showed symptoms later than the patient with the index case.

Epidemiological links. In the CCT cluster and the molecular cluster studies, the epidemiological links between the index case and the secondary cases were categorized as household contacts and nonhousehold contacts. The latter group included contacts through employment, neighborhood (living in the same city block), and leisure (attending the same social activity sites). For cases not reclassified into the two main defined groups, we created a new category named unknown link. The records of the epidemiological interviews in the cases that ME revealed to be molecular cluster cases with an unknown link were reviewed to obtain supplementary information about the epidemiological relationship.

When CCT and ME techniques were performed simultaneously, discrepancies were solved through ME results after the exclusion of laboratory cross contamination, which was investigated when samples included in the same molecular cluster were processed for culture in the same laboratory on the same day.

Additionally, taking into account that CCT is focused mainly on household and employment or school contacts, we carried out, as described previously (8, 24, 28), a new analysis considering traditional and nontraditional transmission

settings: traditional settings were defined as household and employment settings, with all other settings in which transmission may occur being considered non-traditional.

Definition of epidemiologically recent transmission. Isolates clustered by ME reflected the proportion of TB disease due to recent transmission and defined the index of recent transmission (29).

Index of secondary cases. The rate of secondary cases associated with an index case was calculated as the difference between the total number of cases included in clusters and the number of index cases, divided by the number of clusters (applicable to molecular clusters and CCT clusters).

Statistical analyses of the databases. Demographic, epidemiological, clinical, and microbiological data were obtained from the databases of the TB control program of Barcelona and the microbiology departments of the participating health care centers and hospitals. The following data were recorded: TB localization, smear positivity, age distribution over three categories (under 15 years, 15 to 65 years, and over 65 years), sex, use of illegal drugs, alcohol abuse, country of origin, diagnostic delay after the initiation of symptoms (0 to 17, 18 to 42, 43 to 88, and more than 88 days), human immunodeficiency virus infection, homelessness, smear resistance, and residence in the old city district, which includes housing with the lowest-income rents and the most crowded living conditions in the city.

Univariate analysis was performed. The chi-square test with the Yates correction and the Fisher exact test were used for qualitative variables and analysis of variance, and the nonparametric Mann-Whitney U test was used for quantitative variables.

The odds ratio (OR) with the 95% confidence interval (95% CI) was calculated as a measure of association. Logistic regression was used for multivariate analyses, and variables with a *P* value of ≤ 0.1 were introduced. The goodness of fit was verified using the Hosmer-Lemeshow test. Significance was considered to correspond to *P* values of ≤ 0.05 .

The analysis was performed using statistical packet software version 13.0 (SPSS Inc., Chicago, IL) and EpiInfo version 6.04d.

RESULTS

Study population. During the study period, 892 TB cases were reported to the TB control program of Barcelona: 463 cases in 2003 and 429 cases in 2004, corresponding to an incidence of 31.8 cases/100,000 inhabitants in 2003 and 25.9 cases/100,000 inhabitants in 2004. Of the 892 cases declared, 687 (77%) were confirmed by culture. The localization of TB was pulmonary in 636 (71.3%) of the cases, with 398 (62.6%) of these being smear positive. Of the overall number of cases, 305 (34.2%) corresponded to foreign-born patients.

ME. Analysis by ME was possible in 463 (51.9%) of 892 cases and was not performed in 429 (48.1%) of 892 cases for the following reasons: negative culture results in 205 (23%) of 892 cases, lack of cultures in 71 cases (8%), and lack of recovery of isolates in 153 cases (17.1%). Moreover, 112 (24%) of the 463 cases studied by ME were not studied by CCT.

Twenty-nine isolates included in RFLP clusters with RFLP patterns of ≤ 6 bands were analyzed with the MIRU12 technique, as were 46 isolates with RFLP patterns differentiated by only 1 band. With this method, 13 (44.8%) of 29 isolates with ≤ 6 bands remained clustered, as did 9 (19.6%) of the 46 isolates with differences in 1 band. In total, ME showed 280 (60.4%) isolates having a unique pattern and 183 (39.6%) sharing matching patterns, grouped into 65 molecular clusters. The sizes of the molecular clusters ranged from two to eight isolates; those with two and three members (37 and 15 clusters, respectively) predominated.

The index of recent transmission was 25.5%, and that of secondary cases detected by ME was 1.8.

CCT. The CCT method was carried out in 613 (68.7%) of the 892 cases reported. In these 613 cases, a total of 5,087 interviews (mean number of contacts studied per case, 8.3)

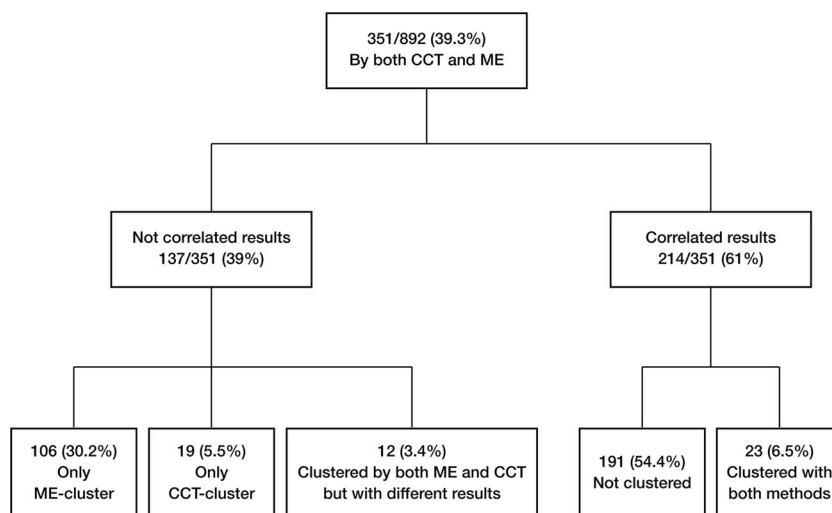


FIG. 1. Summary of the total number of cases studied by both ME and CCT.

were undertaken, with 30.6% being with household contacts and 69.4% being with nonhousehold contacts. The CCT was not performed in 279 cases (31.2%) for the following reasons: lack of consent in 31 (3.5%) of 892 cases, logistic difficulties in 40 cases (4.5%), the patient's status of living alone in 104 cases (11.6%), and lack of indication of the study by the patient's physician in 104 cases (11.6%). In addition, 262 (42.7%) of 613 cases studied by CCT were not included in the ME study.

A total of 44 CCT clusters involving 101 (16.5%) of 613 patients were identified, most including two or three cases (34 and 8 clusters, respectively). Fifty-seven secondary cases were detected, representing an index of 1.22 secondary cases per CCT cluster. These secondary cases were detected in 2.6% of the household contacts and in 0.8% ($P < 0.05$) of the non-household contacts. Treatment of latent TB infection was indicated for 18.2% of contacts living in the same household and for 7.3% ($P < 0.05$) of contacts not living in the same household.

Cases studied by both CCT and ME. Both methods were performed in 351 (39.3%) of 892 cases, with correlation between the results of the methods being found in 214 (61%) of 351 cases (Fig. 1). No correlation between the results for the remaining 137 (39%) of 351 cases was observed: 106 (30.2%) of 351 were clustered only by ME, 19 (5.5%) of 351 cases were clustered only by CCT, and 12 (3.4%) of 351 cases were clustered by both methods, but into different clusters. Moreover, upon analyzing the population studied by each technique, 112 (24%) of the 463 cases studied by ME were found not to be studied by CCT, and 262 (42.7%) of the 613 cases studied by CCT were found not to be included in the ME analysis.

Figure 2 shows the comparison of the results of the two methods for the clustered cases. CCT was not done in 22.4% of the cases clustered by ME, and in 46.6% of cases clustered by CCT, ME was not performed.

Epidemiological link with both techniques. Forty-four CCT clusters and 65 ME clusters were identified, representing 109 index and 175 secondary cases. Upon discarding the cases linked by both methods and the CCT-established links ruled out by ME results, a total of 96 clusters including 96 index

cases and 159 secondary cases (255 total cases) were identified. Table 1 shows the links established between index and secondary cases for each method.

The household link was the most frequent in CCT clusters (accounting for 80.7% of CCT links), with the mother-son relationship predominating (20 [43.5%] of 46 links). It is of

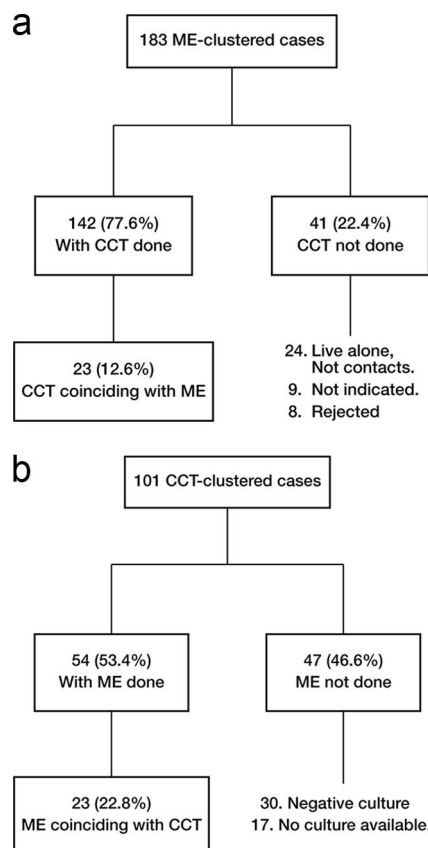


FIG. 2. Comparative results of both ME and CCT techniques for the clustered cases. (a) Clustered by ME. (b) Clustered by CCT.

TABLE 1. Links established between secondary and index cases according to CCT, ME, and the combination of both methods

Type of link	No. (%) of secondary cases ^a linked by:			P value
	CCT and ME (n = 159*)	CCT (n = 57†)	ME (n = 118‡)	
Household	42 (26.4) ^b	46 (80.7)	7 (5.9)	<0.0001
Nonhousehold				
Neighborhood	11 (6.9)	1 (1.8)	10 (8.5)	0.08
Employment	9 (5.7) ^c	9 (15.7)	4 (3.4)	0.003
Leisure	12 (7.6) ^d	1 (1.8)	12 (10.2)	0.035
Unknown	85 (53.4)	0	85 (72)	<0.0001

^a The total numbers of secondary cases in clusters linked by either method (*), by CCT only (†), and by ME only (‡) are indicated.

^b Four of the 46 secondary cases linked by CCT were discarded by ME, and 7 were linked by both methods.

^c Four cases were linked by both methods.

^d One case was linked by both methods.

note that in 12 of the 20 mother-son CCT clusters, the son had a negative culture. On the other hand, in the ME clusters, nonhousehold links were more frequent than the household link, with those isolates having an unknown epidemiological relationship (Table 1) predominating.

Risk factors associated with the population studied by ME or CCT. Of the 892 cases reported during the study period, 463 (51.9%) were studied by the ME method, while 613 (68.7%) were studied by CCT.

Upon the study of several factors and characteristics, differences between the population studied and that not studied by either of the two methods were observed. Patients with pulmonary localization and smear positivity were more frequent in the population studied than in the population not studied (Table 2). Moreover, the CCT-studied population, included a greater proportion of patients of <15 years of age than the population not studied by CCT, but CCT failed to satisfactorily trace male subjects, inhabitants of the old city district, and intravenous drug users (Table 2).

In comparing the populations studied by each method, a univariate analysis showed that the population considered by ME included significantly more intravenous drug users, human immunodeficiency virus-infected patients, homeless persons, smear-positive patients, and individuals with drug-resistant TB than the population studied by CCT, while the CCT study population included significantly more patients of <15 years of age, 49 (92.5%) of the 53 patients in this age category, compared to the 14 such patients (26.4%) studied by ME.

Risk factors associated with clustering. The subjects less clustered by the ME technique were those >65 years old and foreign-born patients. In the CCT-studied population, the patients of >65 years of age and those with a history of alcohol abuse were less associated with being in clusters while subjects of <15 years of age were grouped more often (Table 2).

Upon multivariate analysis of the clustered cases, regardless of the method of cluster detection (ME or CCT), an age of >65 years and foreign birth remained significantly associated with not being in a cluster.

The index case patients were more frequently smear positive (Table 2) than the secondary-case patients, and a higher pro-

portion of index case patients than secondary-case patients (37 versus 18.1%) experienced diagnostic delay.

Risk factors associated with the type of epidemiological link established. Analysis of the clustered cases according to the epidemiological links established showed that the nonhousehold link (employment, leisure, neighborhood, or unknown) was more frequent for cases in adults, males, and smear-positive subjects than for those in nonadults, females, and smear-negative subjects, while the household link was more frequent for cases in subjects of <15 years of age (Table 2). The significant risk factors associated with the traditional and nontraditional settings of transmission (8, 24, 28) were the same.

DISCUSSION

The relationship between TB cases in the transmission chains in the city of Barcelona over a 2-year period was studied using ME and CCT. Analyses of the results allowed the identification of the factors related to the low level of correlation between the data provided by the two methods.

During the last 15 years, ME has been broadly used in the study of TB transmission, representing an important improvement in the detection and description of TB clusters, (6, 20, 21, 22, 30, 34) as well as in studies of TB transmission at a population level (2, 5, 11, 12, 19). Since the beginning of its use, several studies have shown a proportion of cases with no correlation between the results of the two methods. Various reasons have been mentioned in the literature to explain this scarce correlation (8, 11, 27, 28, 36). The main differences are the time points in the pathogenesis of the disease at which the two methods are used. CCT seeks secondary cases around the index case and subjects recently infected, most likely to undergo treatment for latent TB infection. The ME technique seeks a relationship between different TB cases of disease diagnosed during the study period, independently of the time transmission occurred. Finally, ME cannot be performed in culture-negative cases, as can be observed in a high proportion of pediatric cases.

To our knowledge, no study has analyzed in depth the populations sampled by both methods. For that reason, after analyzing the population sampled by each method, we especially focused on the correlation of the results for the patients studied by the two methods.

Upon analyzing the results of this study by taking into account the population studied by each method, some differences were observed. First of all, an important proportion of cases studied by one method were not studied by the other. Moreover, as shown in Table 2, the characteristics of the populations studied by the two methods were not the same. CCT included almost all of the patients under 15 years, in contrast to the ME technique, which included less than one-fourth of these patients, probably explained by the fact that sputum samples for culture are often not available from children (31, 42). On the other hand, ME included significantly more patients with factors associated with precarious economic conditions and social difficulties, with whom interviews for CCT are often difficult (30, 36). In addition, a detailed analysis of the cases studied by each technique showed that ME was not performed because the culture was not done or was negative or the isolates were not recovered for ME study. On the contrary, CCT was not

TABLE 2. Multivariate analysis expressed as significant ORs and CIs for risk factors associated with several characteristics of each population studied by ME and CCT techniques

Comparison	OR (95% CI) for characteristic of:											
	Presence of PTB ^a	Smear positivity	Age of <15 yrs	Age of 15 to 65 yrs	Age of >65 yrs	Male sex	Residence in old city district ^b	IVDU ^c	Alcohol abuse	Spanish birth	Foreign birth	Diagnostic delay ^d
Population screened by ME vs population not screened by ME	1.6 (1.1–2.3)	2.7 (2.3–4.2)	0.3 (0.15–0.6)									
Population screened by CCT vs population not screened by CCT	6.1 (4.2–9.0)	2 (1.4–2.9)	4.9 (1.6–14.5)			0.45 (0.3–0.6)	0.55 (0.4–0.8)	0.25 (0.1–0.5)				
Individuals clustered by CCT vs individuals not clustered by CCT			3.7 (1.9–6.9)		0.1 (0.4–0.4)			0.4 (0.2–0.8)				
Individuals clustered by ME vs individuals not clustered by ME					0.4 (0.2–0.7)				2.1 (1.3–3.4)			
Individuals grouped by techniques vs individuals not grouped					0.4 (0.2–0.7)						0.4 (0.3–0.7)	
Index case individuals of CCT clusters vs secondary case individuals of CCT clusters												4.6 (1.5–13.5)
Index case individuals of ME clusters vs secondary case individuals of ME clusters	45.1 (10.3–197)											
Individuals associated by household links vs individuals associated with nonhousehold links				9.3 (2.2–38.4)	28.2 (4.2–188)	4.2 (1.5–11.5)						

^a PTB, pulmonary tuberculosis.^b The old city district includes a larger proportion of immigrants than the rest of the city and conditions of poverty and overcrowding.^c IVDU, intravenous drug use.^d Diagnostic delay of more than 88 days.

done for several reasons such as the lack of consent, the patient's living alone, logistic difficulties, or the study's not being indicated by physicians.

Concerning the results obtained in 39.3% of the TB cases declared during the study period, in which both methods were applied, differences were also remarkable. Although the results of both methods coincided in 61% of cases studied (Fig. 1), this correlation was due mainly to the results of nonclustered cases, with only 6.5% of the cases clustered by both methods belonging to the same cluster. This proportion is similar to those in other reports (2, 8, 19, 30, 35) and is clearly unsatisfactory for methods that should be complementary in transmission studies.

Analyses of cases with results that did not coincide showed discordant results for cases clustered by both methods and for cases clustered only by CCT (Fig. 1). This probably indicates that true links established between cases are not always as evident as they seem. This idea is demonstrated by four family cases clustered by CCT, which were supposed to involve the same strain but were not clustered by ME, thereby invalidating CCT results (4, 9, 23, 28). What is evident, however, is that we detected a higher proportion of clusters with ME than with CCT, since, as reported in the literature, CCT detects mainly the links among household and professional contacts (40). As can be observed in the results of this study, ME allowed several links not detected by CCT to be established, such as those found between leisure activities and neighbors. These two links, together with those related to employment, represented almost the same proportion of clusters as the households links described by CCT (Table 1). Other studies have previously reported the relevance of the environmental links considered nontraditional by CCT, suggesting the importance of extending the scope of this method (7, 8, 23, 24, 28). On the other hand, this study found 85 relationships (53.4%) indicated only by ME to have an unknown link. In the literature, these clusters have been attributed mainly to unsuspected recent transmission (2, 36, 37), emphasizing the potentiality of sporadic contacts and indicating that CCT procedures should be directed to improve the search for these cases. However, as has been suggested previously (11), a proportion of these clusters may be related to TB reactivation caused by prevalent strains. In this sense, previous reports have indicated that cluster investigation with exhaustive reinterviews may markedly reduce the proportion of isolates of unknown epidemiologic origins clustered by ME (18, 28, 36). However, this investigation is often limited by the difficulty of reinterviewing patient contacts weeks or months after the possible contact occurred (19) and the lack of information to guide the search in the context of nonhousehold transmission (36), which has also been observed in the results of this study.

Nonetheless, some other aspects should be taken into account with regard to the information provided by ME. First of all, our ME results probably underestimate the true extent of recent transmission because they focused on cases reported during a 2-year period in a specific area (41). Moreover, since the beginning of the use of the IS6110 marker, it has been accepted that the biological clock that regulates the significant changes in the distribution of the copies of IS6110 in a certain isolate does not interfere with the creation of transmission chains (2, 10, 15, 29). However, as several authors have re-

ported previously, the IS6110 marker does not always indicate recent transmission (1, 11, 16, 17, 24). This finding is especially important regarding clustered isolates with an unknown link. Hence, there is a growing belief that several markers should be used simultaneously in the study of clusters. With respect to ME, using analyses of two different markers, spoligotyping and mycobacterial interspersed repetitive unit-variable-number tandem repeat typing, in addition to RFLP analysis, van Deutekom et al. (37) recently found that only 28.6% of the strains in RFLP clusters with an unknown link had the same patterns for the three markers. These data support the previously postulated hypothesis (16, 32) about the use of two or more markers when the epidemiological link is not clearly established. From this standpoint, since the analysis of a second marker was not systematically applied to all the strains in this study, we are aware that a possible limitation of this work is that some of the clusters categorized as having an unknown link may correspond to epidemiologically unrelated isolates. Nevertheless, despite this possibility, an important number of clusters in this study may correspond to links undetected by traditional methods.

In conclusion, the populations studied by the two methods presented differences which may explain the scarce correlation of the results. Although the combination of the two methods provides more information than the use of only one, each method acts as a quality control for the other and, consequently, clarifies points in which both could be improved. Therefore, more efforts should be made to extend the culture of *M. tuberculosis* to all TB cases, particularly in children, by using serial samples whenever possible and to extend the CCT, especially to people living in precarious conditions, to thereby eliminate any bias in the population analyzed. On the other hand, the links demonstrated by each method indicate that despite the importance of the household relationship, other links, such as neighborhood and leisure settings, are also relevant, and in an important percentage of cases, the link was unknown. These data indicate the necessity of designing new strategies that allow the extension of the spectra and environments of the CCT studies and the need to include the use of at least one marker in addition to RFLP in ME studies.

ACKNOWLEDGMENTS

We thank Darío García de Viedma and his group, from the Department of Microbiology of the Gregorio Marañón Hospital in Madrid, for their excellent technical assistance.

This study was financed by grants from Fondo de Investigaciones Sanitarias (no. 02/1489, 02/0348, and 04/2381) and a grant from la Fundació La Marató de TV3 and was supported by Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III; the Spanish Network for the Research in Infectious Diseases (grant no. REIPI RD06/0008); and CIBER Enfermedades Respiratorias, Instituto de Salud Carlos III. S.B. received a grant of Formació de Recerca i Docència (BRD) from the University of Barcelona.

REFERENCES

1. Alito, A., N. Morcillo, S. Scipioni, A. Dolmann, M. I. Romano, A. Cataldi, and D. van Soolingen. 1999. The IS6110 restriction fragment length polymorphism in particular multidrug-resistant *Mycobacterium tuberculosis* strains may evolve too fast for reliable use in outbreak investigation. *J. Clin. Microbiol.* 37:788-791.
2. Alland, D., G. E. Kalkut, A. R. Moss, R. A. McAdam, J. A. Hahn, W. Bosworth, E. Drucker, and B. R. Bloom. 1994. Transmission of tuberculosis in New York City. An analysis by DNA fingerprinting and conventional epidemiologic methods. *N. Engl. J. Med.* 330:1710-1716.

3. Anonymous. 1999. A consensus document on the study of the contacts of tuberculosis patients. *Med. Clin. (Barcelona)* **112**:151–156. (In Spanish.)
4. Bennett, D. E., I. M. Onorato, B. A. Ellis, J. T. Crawford, B. Schable, R. Byers, J. S. Kammerer, and C. R. Braden. 2002. DNA fingerprinting of *Mycobacterium tuberculosis* isolates from epidemiologically linked case pairs. *Emerg. Infect. Dis.* **8**:1224–1229.
5. Blackwood, K. S., J. N. Wolfe, and A. M. Kabani. 2004. Application of mycobacterial interspersed repetitive unit typing to Manitoba tuberculosis cases: can restriction fragment length polymorphism be forgotten? *J. Clin. Microbiol.* **42**:5001–5006.
6. Caminero, J. A., M. J. Pena, M. I. Campos-Herrero, J. C. Rodriguez, I. Garcia, P. Cabrera, C. Lazo, S. Samper, H. Takiff, O. Afonso, J. M. Pavon, M. J. Torres, D. van Soolingen, D. A. Enarson, and C. Martin. 2001. Epidemiological evidence of the spread of a *Mycobacterium tuberculosis* strain of the Beijing genotype on Gran Canaria Island. *Am. J. Respir. Crit. Care Med.* **164**:1165–1170.
7. Classen, C. N., R. Warren, M. Richardson, J. H. Hauman, R. P. Gie, J. H. Ellis, P. D. van Helden, and N. Beyers. 1999. Impact of social interactions in the community on the transmission of tuberculosis in a high incidence area. *Thorax* **54**:136–140.
8. Cronin, W. A., J. E. Golub, M. J. Lathan, L. N. Mukasa, N. Hooper, J. H. Razeq, N. G. Baruch, D. Mulcahy, W. H. Benjamin, L. S. Magder, G. T. Strickland, and W. R. Bishai. 2002. Molecular epidemiology of tuberculosis in a low- to moderate-incidence state: are contact investigations enough? *Emerg. Infect. Dis.* **8**:1271–1279.
9. Dahle, U. R., S. Nordtvedt, B. A. Winje, T. Mannsaaker, E. Heldal, P. Sandven, H. M. Grewal, and D. A. Caugant. 2005. Tuberculosis in contacts need not indicate disease transmission. *Thorax* **60**:136–137.
10. de Boer, A. S., M. W. Borgdorff, P. E. de Haas, N. J. Nagelkerke, J. D. van Embden, and D. van Soolingen. 1999. Analysis of rate of change of IS6110 RFLP patterns of *Mycobacterium tuberculosis* based on serial patient isolates. *J. Infect. Dis.* **180**:1238–1244.
11. Diel, R., S. Schneider, K. Meywald-Walter, C. M. Ruf, S. Rusch-Gerdes, and S. Niemann. 2002. Epidemiology of tuberculosis in Hamburg, Germany: long-term population-based analysis applying classical and molecular epidemiological techniques. *J. Clin. Microbiol.* **40**:532–539.
12. Driver, C. R., B. Kreiswirth, M. Macarraig, C. Clark, S. S. Munsiff, J. Driscoll, and B. Zhao. 2007. Molecular epidemiology of tuberculosis after declining incidence, New York City, 2001–2003. *Epidemiol. Infect.* **135**:634–643.
13. Dye, C., D. Maher, D. Weil, M. Espinal, and M. Raviglione. 2006. Targets for global tuberculosis control. *Int. J. Tuberc. Lung Dis.* **10**:460–462.
14. Falzon, D., and F. Ait-Belghiti. 2007. What is tuberculosis surveillance in the European Union telling us? *Clin. Infect. Dis.* **44**:1261–1267.
15. Genewein, A., A. Telenti, C. Bernasconi, C. Mordasini, S. Weiss, A. M. Maurer, H. L. Rieder, K. Schopfer, and T. Bodmer. 1993. Molecular approach to identifying route of transmission of tuberculosis in the community. *Lancet* **342**:841–844.
16. Gillespie, S. H., A. Dickens, and T. D. McHugh. 2000. False molecular clusters due to nonrandom association of IS6110 with *Mycobacterium tuberculosis*. *J. Clin. Microbiol.* **38**:2081–2086.
17. Hawkey, P. M., E. G. Smith, J. T. Evans, P. Monk, G. Bryan, H. H. Mohamed, M. Bardhan, and R. N. Pugh. 2003. Mycobacterial interspersed repetitive unit typing of *Mycobacterium tuberculosis* compared to IS6110-based restriction fragment length polymorphism analysis for investigation of apparently clustered cases of tuberculosis. *J. Clin. Microbiol.* **41**:3514–3520.
18. Ijaz, K., Z. Yang, H. S. Matthews, J. H. Bates, and M. D. Cave. 2002. *Mycobacterium tuberculosis* transmission between cluster members with similar fingerprint patterns. *Emerg. Infect. Dis.* **8**:1257–1259.
19. Inigo, J., A. Arce, J. M. Martin-Moreno, R. Herruzo, E. Palenque, and F. Chaves. 2003. Recent transmission of tuberculosis in Madrid: application of capture-recapture analysis to conventional and molecular epidemiology. *Int. J. Epidemiol.* **32**:763–769.
20. Lillebaek, T., A. B. Andersen, A. Dirksen, J. R. Glynn, and K. Kremer. 2003. *Mycobacterium tuberculosis* Beijing genotype. *Emerg. Infect. Dis.* **9**:1553–1557.
21. Macarraig, M., T. Agerton, C. R. Driver, S. S. Munsiff, J. Abdelwahab, J. Park, B. Kreiswirth, J. Driscoll, and B. Zhao. 2006. Strain-specific differences in two large *Mycobacterium tuberculosis* genotype clusters in isolates collected from homeless patients in New York City from 2001 to 2004. *J. Clin. Microbiol.* **44**:2890–2896.
22. March, F., P. Coll, R. A. Guerrero, E. Busquets, J. A. Cayla, and G. Prats. 2000. Predictors of tuberculosis transmission in prisons: an analysis using conventional and molecular methods. *AIDS* **14**:525–535.
23. McNabb, S. J., C. R. Braden, and T. R. Navin. 2002. DNA fingerprinting of *Mycobacterium tuberculosis*: lessons learned and implications for the future. *Emerg. Infect. Dis.* **8**:1314–1319.
24. McNabb, S. J., J. S. Kammerer, A. C. Hickey, C. R. Braden, N. Shang, L. S. Rosenblum, and T. R. Navin. 2004. Added epidemiologic value to tuberculosis prevention and control of the investigation of clustered genotypes of *Mycobacterium tuberculosis* isolates. *Am. J. Epidemiol.* **160**:589–597.
25. Orcau, A., P. Garcia de Olalla, and J. A. Cayla. 2007. La tuberculosis en Barcelona, informe 2006. Programa de Prevenció y Control de la Tuberculosis en Barcelona. Agencia de Salud Pública de Barcelona, Barcelona, Spain.
26. Pfyffer, G. E., B. A. Brown-Elliott, and R. J. Wallace, Jr. 2003. *Mycobacterium*: general characteristics, isolation, and staining procedures, p. 532–560. In P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Tenover (ed.), *Manual of clinical microbiology*, 8th ed. ASM Press, Washington, DC.
27. Proding, W. M. 2007. Molecular epidemiology of tuberculosis: toy or tool? A review of the literature and examples from Central Europe. *Wien. Klin. Wochenschr.* **119**:80–89.
28. Sintchenko, V., and G. L. Gilbert. 2007. Utility of genotyping of *Mycobacterium tuberculosis* in the contact investigation: a decision analysis. *Tuberculosis (Edinburgh)* **87**:176–184.
29. Small, P. M., P. C. Hopewell, S. P. Singh, A. Paz, J. Parsonnet, D. C. Ruston, G. F. Schecter, C. L. Daley, and G. K. Schoolnik. 1994. The epidemiology of tuberculosis in San Francisco. A population-based study using conventional and molecular methods. *N. Engl. J. Med.* **330**:1703–1709.
30. Solsona, J., J. A. Cayla, E. Verdu, M. P. Estrada, S. Garcia, D. Roca, B. Miquel, P. Coll, F. March, and Cooperative Group for Contact Study of Tuberculosis Patients in Ciutat Vella. 2001. Molecular and conventional epidemiology of tuberculosis in an inner city district. *Int. J. Tuberc. Lung Dis.* **5**:724–731.
31. Sun, S. J., D. E. Bennett, J. Flood, A. M. Loeffler, S. Kammerer, and B. A. Ellis. 2002. Identifying the sources of tuberculosis in young children: a multistate investigation. *Emerg. Infect. Dis.* **8**:1216–1223.
32. Supply, P., C. Allix, S. Lesjean, M. Cardoso-Oelemann, S. Rusch-Gerdes, E. Willery, E. Savine, P. de Haas, H. van Deutekom, S. Roring, P. Bifani, N. Kurepina, B. Kreiswirth, C. Sola, N. Rastogi, V. Vatin, M. C. Gutierrez, M. Fauville, S. Niemann, R. Skuce, K. Kremer, C. Locht, and D. van Soolingen. 2006. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of *Mycobacterium tuberculosis*. *J. Clin. Microbiol.* **44**:4498–4510.
33. Supply, P., E. Mazars, S. Lesjean, V. Vincent, B. Gicquel, and C. Locht. 2000. Variable human minisatellite-like regions in the *Mycobacterium tuberculosis* genome. *Mol. Microbiol.* **36**:762–771.
34. Tudo, G., J. Gonzalez, J. M. Gatell, J. A. Cayla, E. Martinez, A. Garcia, M. Navarro, E. Soriano, M. T. Jimenez de Anta, and Tuberculosis Investigation Unit of Barcelona, Spain. 2001. Detection of unsuspected cases of nosocomial transmission of tuberculosis by use of a molecular typing method. *Clin. Infect. Dis.* **33**:453–459.
35. van Deutekom, H., J. J. Gerritsen, D. van Soolingen, E. J. van Ameijden, J. D. van Embden, and R. A. Coutinho. 1997. A molecular epidemiological approach to studying the transmission of tuberculosis in Amsterdam. *Clin. Infect. Dis.* **25**:1071–1077.
36. van Deutekom, H., S. P. Hoijing, P. E. de Haas, M. W. Langendam, A. Horsman, D. van Soolingen, and R. A. Coutinho. 2004. Clustered tuberculosis cases: do they represent recent transmission and can they be detected earlier? *Am. J. Respir. Crit. Care Med.* **169**:806–810.
37. van Deutekom, H., P. Supply, P. E. de Haas, E. Willery, S. P. Hoijing, C. Locht, R. A. Coutinho, and D. van Soolingen. 2005. Molecular typing of *Mycobacterium tuberculosis* by mycobacterial interspersed repetitive unit-variable-number tandem repeat analysis, a more accurate method for identifying epidemiological links between patients with tuberculosis. *J. Clin. Microbiol.* **43**:4473–4479.
38. van Embden, J. D., M. D. Cave, J. T. Crawford, J. W. Dale, K. D. Eisenach, B. Gicquel, P. Hermans, C. Martin, R. McAdam, and T. M. Shinnick. 1993. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. *J. Clin. Microbiol.* **31**:406–409.
39. Veen, J. 1992. Microepidemics of tuberculosis: the stone-in-the-pond principle. *Tuber. Lung Dis.* **73**:73–76.
40. Verver, S., R. M. Warren, Z. Munch, M. Richardson, G. D. van der Spuy, M. W. Borgdorff, M. A. Behr, N. Beyers, and P. D. van Helden. 2004. Proportion of tuberculosis transmission that takes place in households in a high-incidence area. *Lancet* **363**:212–214.
41. Vynnycky, E., N. Nagelkerke, M. W. Borgdorff, D. van Soolingen, J. D. van Embden, and P. E. Fine. 2001. The effect of age and study duration on the relationship between ‘clustering’ of DNA fingerprint patterns and the proportion of tuberculosis disease attributable to recent transmission. *Epidemiol. Infect.* **126**:43–62.
42. Wootton, S. H., B. E. Gonzalez, R. Pawlak, L. D. Teeter, K. C. Smith, J. M. Musser, J. R. Starke, and E. A. Graviss. 2005. Epidemiology of pediatric tuberculosis using traditional and molecular techniques: Houston, Texas. *Pediatrics* **116**:1141–1147.