



Diversity of *Mycobacterium tuberculosis* Isolates in an Immigrant Population: Evidence against a Founder Effect

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Received for publication July 1, 2003; accepted for publication October 3, 2003.

Population-based studies have used DNA typing of *Mycobacterium tuberculosis* organisms to estimate the extent of ongoing tuberculosis transmission in various communities and to characterize associated risk factors. The finding of matched DNA “fingerprints” among isolates from an immigrant subgroup may reflect transmission in the adopted country but could also reflect limited diversity among *M. tuberculosis* organisms within that immigrant community. The authors sought to determine which hypothesis is more likely to explain the high frequency of matched isolates among Haitian-born tuberculosis patients in Montreal, Quebec, Canada. The authors determined the number of different bacterial genotypes in this community as compared with other foreign-born tuberculosis patients and applied a recently described measure of genetic similarity between *M. tuberculosis* organisms (“genetic distance”). Among 76 Haitian-born tuberculosis patients diagnosed during 1996–1998, the authors identified 47 distinct genotypes on the basis of standard IS6110 DNA typing and categorical analysis. In genetic distance analysis, these 47 genotypes showed as great a genetic diversity as that observed among the 191 distinct genotypes identified in 216 other foreign-born tuberculosis patients. A mycobacterial “founder effect” is unlikely to account for the high proportion of shared isolates among Haitian-born Montrealers. Recent transmission remains the most likely explanation.

disease transmission; DNA fingerprinting; emigration and immigration; tuberculosis; variation (genetics)

Abbreviations: CI, confidence interval; IS, insertion sequence; RFLP, restriction fragment length polymorphism.

For over a decade, researchers have exploited variability in the chromosomal locations of the DNA insertion element IS6110 in order to type and differentiate strains of *Mycobacterium tuberculosis* (1). In population-based studies (2–5), patients whose *M. tuberculosis* isolates share identical or highly similar patterns of these insertion elements (visualized as DNA “fingerprints”) are considered “clustered”; they are assumed to have been involved in outbreaks, whether previously recognized or not. Patients whose *M. tuberculosis* isolates have unique patterns (i.e., unmatched) are assumed to have reactivation disease. In these studies, epidemiologic

predictors of cluster membership have identified subgroups at highest risk for ongoing transmission, thus allowing public health authorities to plan targeted tuberculosis control interventions.

In low-incidence contexts, tuberculosis is consistently concentrated among persons born in countries where the disease remains endemic. In the United States, foreign-born patients most often have unique *M. tuberculosis* isolates and thus appear to be less likely than US-born residents to be implicated in outbreaks (5, 6). However, researchers in Amsterdam, the Netherlands, documented a high frequency

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of clustering among tuberculosis patients born in Surinam and the Netherlands Antilles, which they related to ongoing transmission (7). In another study in Norway, identical fingerprints among East African-born residents were, according to clear epidemiologic links, the result of local transmission (8).

The presence of *M. tuberculosis* isolates with matching DNA fingerprints can only imply a high probability of recent transmission when these isolates are observed against a background of isolates with sufficient genetic diversity. This reduces the likelihood of detecting false clusters, since isolates *not* truly linked by new transmission are likely to be distinct. For example, investigators in Copenhagen, Denmark, attributed the high frequency of clustering among Somalia-born tuberculosis patients partly to limited mycobacterial diversity within Somalia and time spent in refugee camps prior to departure, rather than exclusively to ongoing transmission after the patients' arrival in Denmark. The finding of additional matching isolates in the same subgroup in the Netherlands provided additional support for this hypothesis (9).

In Montreal, Quebec, Canada, the overall incidence of tuberculosis has been stable at approximately 10 per 100,000 population annually (10), and 80 percent of tuberculosis patients are foreign-born. Their most frequent country of birth is Haiti. Haitian-born persons accounted for 18 percent of all tuberculosis cases observed in Montreal during 1996–1998, though they represent less than 3 percent of the population. The estimated incidence of tuberculosis is 54 per 100,000 population among the 50,800 Montrealers born in Haiti (based on the 1991 census (11)). Moreover, Haitian-born immigrants are more likely than other Montreal immigrants to share isolates by IS6110 typing (12).

The seeming genetic similarity of *M. tuberculosis* isolates among Haitian-born Montrealers could reflect a lack of diversity among strains endemic to Haiti or, alternatively, a restricted diversity in strains circulating in the communities from which emigrants to Canada originate. With either scenario, a limited diversity of strains would be imported by Haitian-born persons into Canada, potentially resulting in a bacterial founder effect in Montreal. We wished to determine whether frequent clustering among the Haitian-born was due to limited genetic diversity of *M. tuberculosis* organisms or recent transmission.

We supplemented the categorical approach to classifying DNA fingerprints (identical/near-identical vs. unique) with a quantitative descriptor of genetic similarity between isolates, called the "genetic distance" (13). Using these two techniques, we investigated the genetic diversity of IS6110 isolates in the Montreal Haitian population.

MATERIALS AND METHODS

Study population

All cases of active tuberculosis among Montreal residents for which *M. tuberculosis* isolates were sent to the provincial public health laboratory for drug susceptibility testing between January 1, 1996, and December 31, 1998, were eligible for inclusion in this study. Procedures used for

restriction fragment length polymorphism (RFLP) typing and epidemiologic data collection, described briefly below, are presented in greater depth elsewhere (12, 14).

We obtained clinical and epidemiologic information from a review of public health records, supplemented by the public health reporting database. As part of mandatory case reporting in Canada, country of birth is recorded for virtually all patients; however, no information on ancestral origin is recorded for Canadian-born patients. We selected Haitian-born and other foreign-born tuberculosis patients and compared their demographic, clinical, and fingerprint patterns produced in RFLP analysis.

A unique identifier, the public health reporting number, was used to link individual demographic and clinical data with laboratory results for each patient. All personal identifiers were removed prior to use of reporting data for this study. This study was approved by the research ethics committees of the Montreal General Hospital, the Quebec Public Health Laboratory, and the regional public health board.

DNA fingerprinting

We typed all culture-positive *M. tuberculosis* isolates using standard insertion sequence (IS) 6110-based RFLP analysis (15). Only isolates with five or more copies of IS6110 bands (termed adequate copy isolates) were included in the analysis. We set the minimum number of copies at five because of the previously reported poor specificity of low-copy-number fingerprints (13).

Matching versus unique isolates

We used a categorical classification scheme to identify matched and unique isolates: Two or more isolates for which gel electrophoresis demonstrated identical numbers of IS6110 bands of matching molecular weights, as well as those for which there was no more than a single band difference (band addition, band deletion, or band shift), were considered matched. Other isolates were classified as unique (unmatched).

Genetic distance

Genetic distance analysis quantifies the relation between any pair of isolates by estimating the putative amount of time (in months) since the two isolates diverged from a hypothetical common ancestor and assigning a numerical value for the distance between each pair (13). The genetic distance measure reflects the assumption that mutations occur independently throughout the *M. tuberculosis* genome and that the events are exponentially distributed over time. Therefore, the time of the next mutation is assumed to be independent of the times of past mutations. Some number of such changes, beginning from a hypothetical shared ancestor, characterizes the relation between each pair of isolates. Hence, any given isolate is associated with its own distribution of genetic distances—the set of distances to every other isolate of interest. The lowest of these distances is termed the

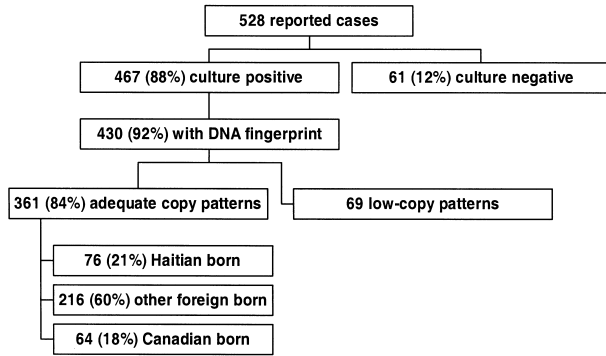


FIGURE 1. Flow chart for tuberculosis cases in Montreal, Quebec, Canada, 1996–1998.

“nearest genetic distance,” and it indicates the degree of homology with the most similar isolate of interest.

Analysis of distinct patterns

To capture the full range of genotypes over the study period and to perform an analysis based on all *distinct* isolates, we added a single isolate from each matched group to the unique isolates—the isolate associated with the earliest date of diagnosis. We then calculated the genetic distance from each distinct isolate to every other isolate, among the isolates from Haitian-born patients and those from other foreign-born patients. We restricted the comparison to non-Haitian foreign-born patients because the diversity in RFLP patterns from so many countries of origin should have provided a maximum standard of diversity against which to compare the patterns of the Haitian-born patients. Canadian-born tuberculosis patients were not included because of their distinctive demographic profile: Typically older, they are even more likely to reactivate remotely acquired infection than are the foreign-born. Furthermore, there is emerging evidence of a major genetic family of *M. tuberculosis* organisms among the Canadian-born in Quebec (16).

We calculated and compared the median nearest genetic distance among all isolates, among isolates from Haitian-born and non-Haitian foreign-born patients, and among the unique isolates from these two subgroups. We compared the nearest genetic distance for distinct isolates from Haitian-born and other foreign-born patients to evaluate the extent of background genetic diversity. A substantially lower nearest genetic distance for the set of distinct isolates from Haitian-born patients would suggest that the infecting organisms were less diverse genetically, and hence more closely related overall.

We performed DNA fingerprint analysis and genetic distance calculations according to the “align and count” method (17) using Molecular Fingerprint Analyzer software (version 2.0; available from the Stanford Center for Tuberculosis Research (<http://molepi.stanford.edu>)). We used SPSS for Windows (version 11.0.1; SPSS, Inc., Chicago, Illinois) for calculation of summary statistics and compari-

sons, including means, medians, interquartile ranges, proportions, and odds ratios and 95 percent confidence intervals, as well as for graphic illustration of the distribution of nearest genetic distances. For statistical comparison of median nearest genetic distances between isolates from Haitian-born patients and those from non-Haitian-born patients, we approximated a 95 percent confidence interval for the differences in median values using a straightforward nonparametric bootstrap (18). For high accuracy, we used 100,000 iterations programmed in S-Plus for Windows (version 6.0; Insightful Corporation, Seattle, Washington).

For purposes of visual illustration, we also displayed and printed IS6110 banding patterns using GelCompar software (Applied Maths, Austin, Texas). The GelCompar software generates accompanying dendrograms (tree diagrams). These identify possible “clusters” of matching isolates, based on the unweighted pair group method using arithmetic averages (19, 20). The graphic output from GelCompar includes a Dice coefficient for each adjacent pair of isolates in each tree, which is an index of similarity for pairs of banding patterns ranging from zero to 100 percent (19). Neither the unweighted pair group method using arithmetic averages nor the Dice coefficient has been validated for the quantification of differences between *M. tuberculosis* RFLP patterns. However, as a further means of comparing pattern diversity among Haitian-born patients with that observed among other foreign-born patients, we also generated a matrix of Dice coefficients to accompany the dendrogram for each group and compared their distributions using summary statistics, as previously described (16).

RESULTS

During 1996–1998, 528 new cases of active tuberculosis were reported to the Montreal Public Health Department. Of these, 61 were culture-negative and therefore not suitable for DNA typing. For the 467 culture-positive cases, we were able to regrow 430 isolates (92 percent) from frozen samples stored at the provincial public health laboratory. Three hundred and sixty-one of the 430 isolates (84 percent) had five or more IS6110 copies and were therefore suitable for further analysis; 69 were low-copy (fewer than five bands) isolates.

Of the 361 isolates, 76 (21 percent) were from Haitian-born patients, 216 (60 percent) were from other foreign-born patients (from 70 different countries), 64 (18 percent) were from Canadian-born patients, and five were from persons whose country of birth was unknown. Figure 1 summarizes these data. Clinical and demographic data for these patient groups are presented in table 1.

Among the Haitian-born patients retained for the analysis, 49 percent (37/76) had isolates with matching IS6110 fingerprint patterns as opposed to 21 percent (46/216) of the non-Haitian foreign-born patients (odds ratio for clustering = 3.5, 95 percent confidence interval (CI): 1.9, 6.3). The matched isolates from Haitian-born patients grouped into eight clusters of 2–11 each; hence, there were 47 distinct patterns (one from each of the eight clusters plus the 39 unmatched). Similarly, there were 191 distinct patterns (one from each of 21

TABLE 1. Demographic and clinical characteristics of Haitian-born tuberculosis patients with adequate copy isolates as compared with other foreign-born and Canadian-born tuberculosis patients, Montreal, Quebec, Canada, 1996–1998

	Patient origin					
	Haitian-born (n = 76)		Other foreign-born (n = 216)		Canadian-born (n = 64)	
	No.	%	No.	%	No.	%
Age (years)						
Range	10–84		1–95		1–90	
Mean	36		44		60	
Median	34		38		66	
Female sex	37	49	87	41	31	48
Pulmonary tuberculosis	50	68	139	66	50	77
Chest radiograph						
Normal*	2	4	2	1.4	2	4
Abnormal, noncavitary	31	62	98	70	33	66
Abnormal, cavitary	17	34	38	27	15	30
Smear-positive*	32	64	81	58	36	72
Medication sensitivity						
Sensitive to all drugs	64	86	181	84	59	92
Resistant to one or more drugs	10	14	27	13	5	8
HIV† serostatus						
Positive	17	22	10	5	10	16
Negative	25	33	37	17	9	14
Unknown	34	45	169	78	45	70
Years since immigration						
Range	0–27		0–55			
Mean	8.0		11			
Median	5.0		5.0			

* Among cases with pulmonary tuberculosis.

† HIV, human immunodeficiency virus.

clusters plus 170 unmatched) in the non-Haitian foreign-born group.

Among all 361 isolates in the study, the median nearest genetic distance was 98 months (range, 10–244 months). Nearest genetic distances were lower (median, 48 months; range, 15–242 months) for the 76 isolates from Haitian-born patients, reflecting the high frequency of DNA matching, while in the non-Haitian foreign-born, the median nearest genetic distance was 110 months (range, 10–244 months).

To study whether the 47 distinct genotypes among the Haitian-born reflected subtle variants of common strains or truly different isolates, we repeated the nearest genetic distance analysis with restriction to only one member of each genotype. Here the median nearest genetic distance was 130 months (interquartile range, 98–201 months) among the 47 distinct isolates from Haitian-born patients; among the non-Haitian foreign-born, the median nearest genetic distance for the 191 distinct isolates was 128 months (interquartile range, 103–170 months). Hence, the difference in median nearest genetic distances was 2 months among Haitians versus non-

Haitians (95 percent CI: 23, –13 (by bootstrapping)). Indeed, the distributions of these nearest genetic distances were similar in the two groups, as illustrated in figure 2.

Visual inspection of the DNA fingerprint patterns (figure 3) also suggests that the isolates from Haitian-born patients were as heterogeneous as those found among other foreign-born patients. Indeed, the associated mean Dice coefficient among isolates from Haitian-born patients was 35 percent (95 percent CI: 34, 36), while for other foreign-born patients it was 34 percent (95 percent CI: 33, 35); median Dice coefficients were 33 percent (interquartile range, 24–44 percent) and 33 percent (interquartile range, 23–44 percent), respectively, indicating nearly identical distributions of Dice coefficients within the two groups.

DISCUSSION

A previous report from Montreal highlighted the frequency of clustering among Haitian-born tuberculosis patients, based on IS6110 typing (12). However, the most appropriate interpretation of this finding was unclear. While

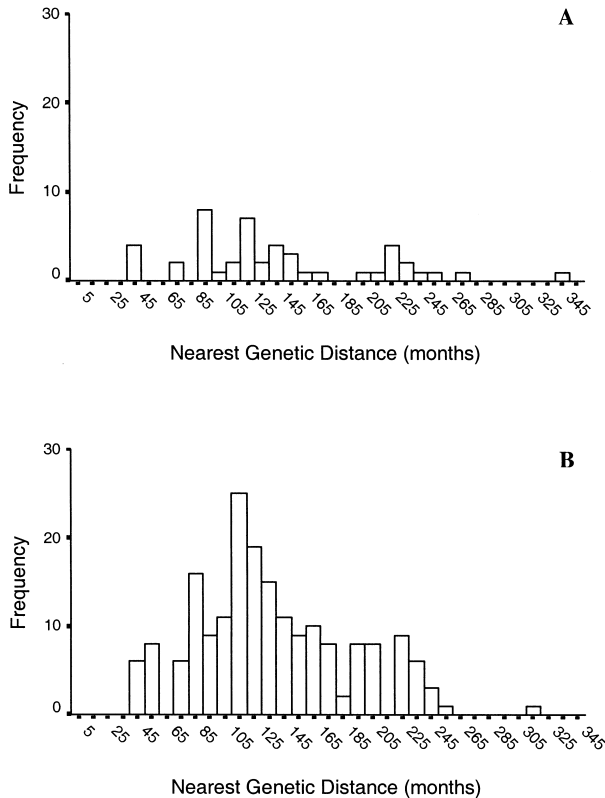


FIGURE 2. Frequency distributions of nearest genetic distance values for *Mycobacterium tuberculosis* isolates obtained from tuberculosis patients, Montreal, Quebec, Canada, 1996–1998. Part A, distribution of the 47 distinct fingerprint patterns obtained from Haitian-born patients; part B, distribution of the 191 distinct fingerprint patterns obtained from non-Haitian foreign-born patients.

there may indeed be ongoing transmission, these results could also reflect reactivation in a population subgroup in which there is limited heterogeneity of infecting *M. tuberculosis* organisms, as has been suggested in other relatively isolated populations (16, 21). We explored the degree of similarity among isolates from Haitian-born and other foreign-born tuberculosis patients in Montreal using both a categorical (identical/near-identical vs. unique) measure of similarity and a continuous (genetic distance) measure of similarity. Our results indicate that frequent clustering among Haitian-born tuberculosis patients in Montreal is unlikely to reflect limited genetic diversity of *M. tuberculosis* organisms.

We identified 47 distinct DNA fingerprint patterns among isolates from 76 Haitian-born patients who were diagnosed during 1996–1998. If the infecting organisms were in fact closely related, as a result of a recent common ancestor, these patterns should have been more similar than those obtained for isolates from a diverse group of foreign-born patients. The nearest genetic distance measure allowed us to further characterize the diversity of these “distinct” isolates. Their diversity was similar to that seen among isolates from patients born in 70 other countries.

These results support the interpretation that Haitian-born tuberculosis patients in Montreal whose *M. tuberculosis* isolates have similar patterns upon RFLP typing are involved in transmission after their arrival in Canada. It is conceivable that some of this transmission occurred in Haiti, but this is not very likely, since this group had not been living in refugee camps before immigration. In addition, half of the Haitian-born patients with matching patterns had resided in Canada for 5 or more years and therefore were not new arrivals.

While human immunodeficiency virus infection is known to promote rapid progression to active tuberculosis following initial infection with *M. tuberculosis* (22), rapid progression to active disease did not appear to be the reason for the greater amount of clustering among Haitian-born immigrants in Montreal (12). Moreover, the similarity or diversity of the “unclustered” patterns examined in this report is not likely to have been affected by the rate of progression to active disease.

The genetic distance measure, which simultaneously accounts for number and position of bands that form the fingerprint pattern, permits a refined assessment of the genetic relation between isolates on a continuum from identical to very different, through varying degrees of similarity. Different patterns are no longer simply classified together: Genetic distance quantifies those differences, making analysis of “unique” patterns possible.

This study is but one example of a more refined use of molecular typing data, which could be extended to other settings. We identified the number of distinct *M. tuberculosis* isolates within two clinical subgroups and assessed their genetic similarity. Use of the genetic distance measure allowed us to study the extent of diversity among isolates that did not match according to categorical criteria and then to explore an alternate hypothesis. Analysis based only on the traditional dichotomous approach comparing “matched” and “unmatched” isolates does not permit such characterization.

Furthermore, we were able to compare the heterogeneity of isolates among Haitian-born patients with that of isolates from patients born around the world. Because unmatched isolates from Haitian-born patients are as distinct as those from other patients, a bacterial “founder effect” is unlikely to account for the higher proportion of matching isolates. Recent transmission remains the most credible interpretation.

The results of this study have important implications for public health. The standard approach to contact identification and evaluation has clearly not been as successful in preventing tuberculosis transmission in the Montreal Haitian community as in other groups. There are several potential reasons for this limited success. In populations with a high baseline prevalence of latent tuberculosis infection, tuberculin testing of close contacts cannot reliably distinguish recent infection from remote infection. Distinctive social networks may be associated with undetected transmission in settings other than the household or workplace. Shame or stigma associated with tuberculosis may also cause patients to withhold contact information. For similar reasons, identified contacts with latent tuberculosis infection may be reluctant to accept tuberculin testing and treatment.

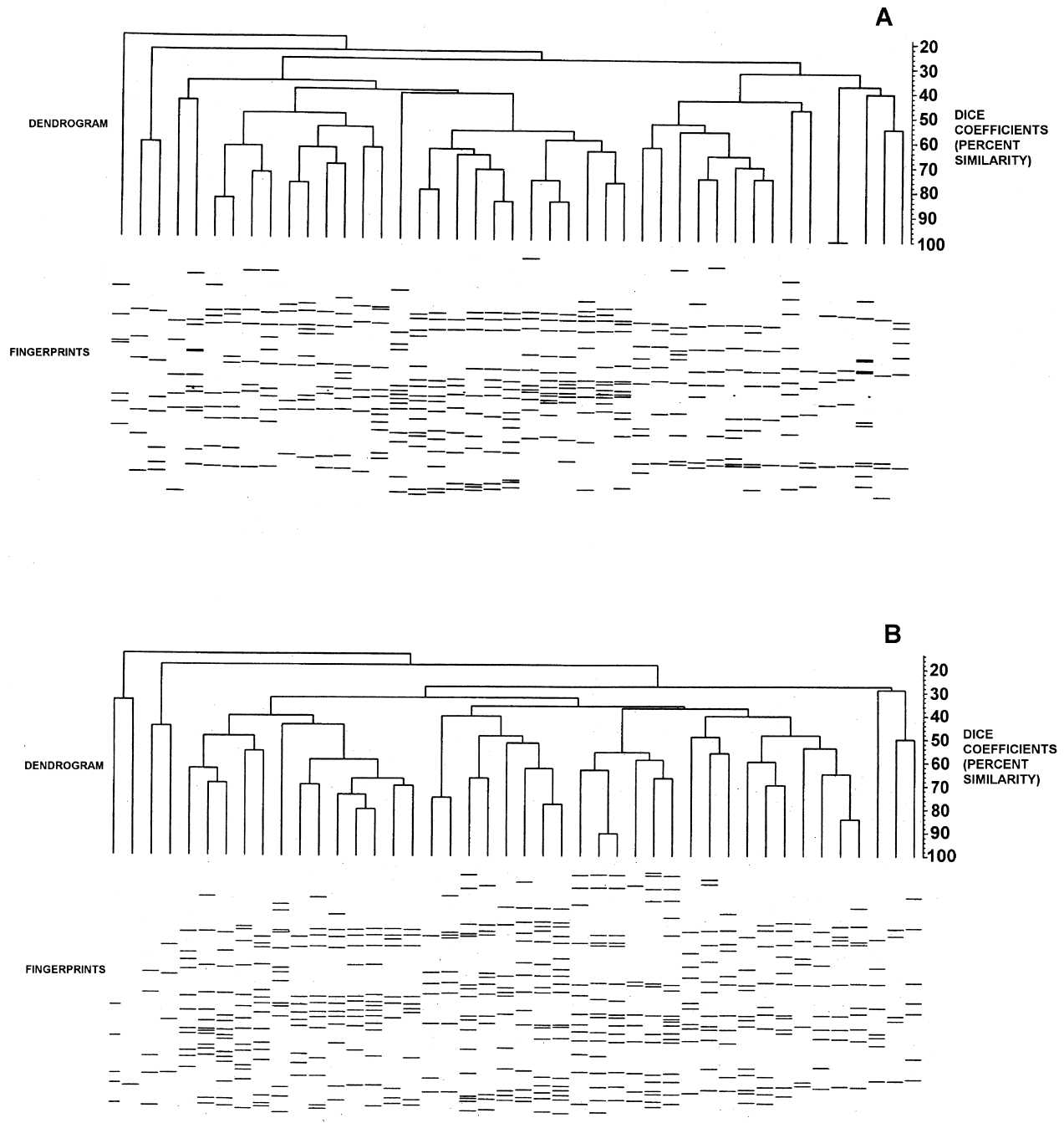


FIGURE 3. IS6110 fingerprint patterns of distinct *Mycobacterium tuberculosis* isolates obtained from (A) Haitian-born tuberculosis patients and (B) a random sample of non-Haitian foreign-born tuberculosis patients, Montreal, Quebec, Canada, 1996–1998. Fingerprints for four isolates from the Haitian-born patients could not be reproduced in this graphic format, so part A displays data for 43 fingerprint patterns. The dendrograms above the fingerprint patterns indicate the similarity of adjacent fingerprints based on Dice coefficients, which range from zero to 100%, as illustrated by the scale to the right of each dendrogram.

The Montreal Public Health Department has already taken steps to better comprehend patterns of tuberculosis in the Haitian community. However, it is not yet clear whether

these steps have improved the identification of infected contacts or the delivery of treatment for latent tuberculosis. Preliminary information from more recent years suggests

that transmission in Montreal's Haitian community continues (Dr. Terry Tannenbaum, Montreal Public Health Department, personal communication, 2003). Therefore, there is a need to further examine current public health approaches. Potential changes might include a broader approach to contact identification, with emphasis on promoting acceptance of and adherence to treatment for latent tuberculosis.

ACKNOWLEDGMENTS

This project was funded by operating grants from the Association Pulmonaire du Québec (to Drs. Kevin Schwartzman and Marcel Behr) and the Sequella Global Tuberculosis Foundation (to Dr. Marcel Behr). Sophie Kulaga is the recipient of a Studentship Award from the Faculty of Medicine, McGill University. Drs. Marcel Behr and Paul Brassard are the recipients of New Investigator Career Awards from the Canadian Institutes of Health Research. Dr. Dao Nguyen is the recipient of a postdoctoral fellowship from the Fonds de la Recherche en Santé du Québec. Dr. Lawrence Joseph is the recipient of a Senior Investigator Career Award from the Canadian Institutes of Health Research. Drs. Dick Menzies and Kevin Schwartzman are the recipients of a Chercheur-Boursier Clinicien Career Award from the Fonds de la Recherche en Santé du Québec.

The authors acknowledge the technical assistance of Michael Purdy and Robert Kozak and the administrative assistance of Esther TomKee.

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