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Evidence from Molecular Fingerprinting of Limited Spread of Drug-Resistant Tuberculosis in Texas

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To determine the contribution of recent transmission to spread of drug-resistant tuberculosis in Texas, we performed IS*6110*-based and pTBN12-based restriction fragment length polymorphism (RFLP) analyses on *Mycobacterium tuberculosis* isolates. Isolates collected from 201 patients in Texas between 1992 and 1994 were studied. The distribution of cases was strikingly focal. All cases were reported from 35 of the 254 counties in Texas, and 74% (148 of 201) were reported from only 9 counties. One hundred sixty-one (80%) of the patients had *M. tuberculosis* isolates with unique RFLP patterns, and 41 (20%) patients were in 20 clusters, each comprising 2 to 3 patients. The largest number of cases of drug-resistant tuberculosis were reported in counties bordering Mexico, but the percentage of clustered cases was highest in northeast Texas and in counties that included the cities of Dallas, Fort Worth, and Houston. Compared to nonclustered patients, clustered patients were more likely to be African American and to have been born in the United States. Clustered patients were significantly more likely to be from the same geographic area, and clustered patients from the same geographic area were more likely to have isolates with identical drug susceptibility patterns, suggesting that they were linked by recent transmission. In 11 of 20 clusters, clustered patients were from geographically separate regions, and most isolates did not have identical drug susceptibility patterns, suggesting that tuberculosis was contracted from a common source in the remote past. Based on the low percentage of clustered cases and the small cluster size, we conclude that there is no evidence for the extensive transmission of drug-resistant tuberculosis in Texas.

Texas consistently ranks third in the United States in the number of tuberculosis cases reported to the Centers for Disease Control and Prevention, behind New York and California (6). In addition, Texas ranks third in the number of reported cases of multidrug-resistant tuberculosis (resistant to isoniazid and rifampin) (6). Because southern Texas borders Mexico, where drug resistance is more common than in the United States (7), the transmission of drug-resistant tuberculosis in Texas is a major public health concern.

Researchers' understanding of the dynamics of the transmission of tuberculosis has been greatly enhanced by restriction fragment length polymorphism (RFLP) analysis of *Mycobacterium tuberculosis* isolates, allowing the identification of specific genotypes. Recent studies based on RFLP analysis have demonstrated that 19 to 54% of tuberculosis cases in urban areas of the United States result from recent disease transmission (1, 2, 4, 18). Most isolates in these studies were fully drug susceptible, and no data are available on the contribution of recent transmission to the spread of drug-resistant tuberculosis in the United States, except during outbreaks of the disease (9, 10). To investigate this issue, we performed RFLP analysis on drug-resistant isolates from 201 tuberculosis patients in Texas.

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MATERIALS AND METHODS

***M. tuberculosis* strains.** From January 1992 through December 1994, 5,987 patients with culture-confirmed tuberculosis were diagnosed in Texas (19–21). We studied 334 *M. tuberculosis* isolates from 201 patients diagnosed in Texas from May 1992 through August 1994. Two or more isolates were evaluated for 44 patients, and one isolate was evaluated for 157 patients. These represented all isolates with resistance to isoniazid, rifampin, ethambutol, or streptomycin available from the mycobacteriology laboratories of the Texas Department of Health or the University of Texas Health Center at Tyler. During this period, 304 cases of drug-resistant tuberculosis were reported in Texas. Isolates not included in the study were those that were no longer viable ($n = 19$), those that could not be located ($n = 13$), and those that were processed at other laboratories ($n = 71$). Of the 103 isolates that were excluded from the study, 81 were from patients in Harris, Dallas, or Tarrant counties, where specimens were often processed by local hospitals or health departments.

All isolates were identified as *M. tuberculosis* by using a commercial DNA probe (ACCUPROBE; Gen-Probe, San Diego, Calif. [16]) or high-performance liquid chromatography to determine the mycolic acid profile (5). Isolates were screened for susceptibility to isoniazid (1.0 μg/ml), rifampin (1.0 μg/ml), and ethambutol (5.0 μg/ml) by the Bactec radiometric method (Becton Dickinson, Mountain View, Calif.). For isolates that were resistant to any of these three agents, susceptibilities to isoniazid (1.0 μg/ml), rifampin (1.0 μg/ml), ethambutol (5.0 μg/ml), and streptomycin (2.0 μg/ml) were tested by the proportion method on 7H10 agar. Drug resistance was defined as the presence of at least 1% growth on the drug-containing agar compared to growth on the control agar (12).

RFLP analysis. *M. tuberculosis* isolates were subcultured in 5 ml of Dubos medium supplemented with albumin (Difco, Detroit, Mich.) and incubated at 37°C for 2 to 3 weeks prior to DNA extraction. All isolates were subjected to IS*6110*-based RFLP analysis, as previously described (22, 24). Briefly, chromosomal DNA was prepared by chloroform-isoamyl alcohol DNA extraction, and 1 μg of DNA from each isolate was restricted with *Pvu*II and then hybridized to the IS*6110* probe. The molecular size standard was *Pvu*II-restricted chromosomal DNA of *M. tuberculosis* H37Rv and two additional DNA fragments which hybridize to IS*6110* (24). IS*6110*-based RFLP results were considered inconclusive if RFLP patterns with fewer than six fragments were identical or if RFLP patterns with six or more fragments were identical except that one isolate showed an additional fragment or a fragment that differed in size. In these cases,

TABLE 1. Characteristics of clustered and nonclustered patients with drug-resistant tuberculosis

Characteristic	Total (n = 201) (%)	Clustered (n = 41) (%)	Not clustered (n = 160) (%)	P value[a]
Male sex	131 (65)	32 (78)	99 (62)	0.07
Ethnicity				0.06
White	29 (14)	7 (17)	22 (14)	
African American	28 (14)	11 (27)	17 (11)	
Hispanic	116 (58)	19 (46)	97 (61)	
Asian	27 (13)	4 (10)	23 (14)	
Age in years				0.45
<15	3 (1)	1 (2)	2 (1)	
15–24	21 (10)	2 (5)	19 (12)	
25–44	107 (53)	24 (59)	83 (52)	
>44	70 (35)	14 (34)	56 (35)	
Country of birth				0.06
United States	75 (37)	22 (63)	53 (39)	
Mexico	62 (31)	9 (26)	53 (39)	
Vietnam	17 (8)	3 (9)	14 (10)	
Other[b]	18 (9)	1 (3)	17 (12)	
Unknown	29 (14)	6 (15)	23 (14)	
HIV infection[c]	25 (12)	7 (17)	18 (11)	0.35
Drug resistance[d]				0.68
INH only	49 (24)	12 (29)	37 (23)	
INH, SM only	28 (14)	6 (15)	22 (14)	
RIF only	23 (11)	6 (15)	17 (11)	
INH, RIF	82 (41)	15 (37)	67 (42)	
Other patterns	19 (10)	2 (5)	17 (11)	
County				0.001
Dallas or Tarrant	39 (19)	12 (29)	27 (17)	
Harris	33 (16)	8 (20)	25 (16)	
U.S.-Mexican border[e]	76 (38)	14 (34)	62 (39)	
Northeast[f]	9 (4)	5 (12)	4 (3)	
Other	44 (22)	2 (5)	42 (26)	

[a] Two-sided Fisher's exact test comparing clustered and non clustered patients.
[b] Other, Asian countries not including Vietnam, Central and South America, and Africa.
[c] This represents a minimum estimate, as not all patients were tested for HIV.
[d] INH, isoniazid; SM, streptomycin; RIF, rifampin.
[e] Cameron, El Paso, Hidalgo, Maverick, Starr, and Webb counties.
[f] Anderson, Rusk, Shelby, Smith, and Upshur counties.

pTBN12-based RFLP analysis was performed, as described previously (8). Briefly, chromosomal DNA was restricted with *Alu*I and then hybridized to the pTBN12 probe. The molecular size standard was a 1-kb DNA ladder.

To analyze IS*6110*-based RFLP patterns, hybridized blots were exposed to a phosphor screen, which was scanned with ImageQuant software (Molecular Dynamics, Sunnyvale, Calif.). The patterns were analyzed with Whole Band Analyzer software (version 3.3; BioImage, Ann Arbor, Mich.), allowing a fragment size deviation of 2.5% when patterns were matched pTBN12-based RFLP patterns were evaluated by visual comparison in adjacent lanes.

We considered *M. tuberculosis* isolates from different patients to be the same strain if the IS*6110*-based RFLP patterns (i) revealed six or more fragments of identical sizes, (ii) revealed six or more fragments of identical sizes except that one isolate showed one additional fragment or one fragment of a different size and the pTBN12-based RFLP patterns were identical, or (iii) revealed five or fewer fragments of identical sizes and the pTBN12-based RFLP patterns were identical. Two or more patients infected with the same *M. tuberculosis* strain constituted a cluster.

Demographic data. Demographic data on the 201 study patients were obtained from the tuberculosis reporting forms sent to the Texas Department of Health and from laboratory records.

Statistical analysis. To compare the distributions of categorical variables among clustered and nonclustered patients, the two-sided Fisher exact test was used. To determine if patients in a cluster were more likely to have the same ethnicity than patients randomly selected in groups the sizes of the clusters, we assumed a binomial distribution for the number of clusters in which all patients were matched for ethnicity, based on the distribution of ethnicity among nonclustered patients. We then computed the probability of obtaining at least the observed number of clusters in which all patients were matched for ethnicity. The same method was used to determine if all patients in a cluster were more likely than randomly selected patients to be from the same group of counties.

RESULTS

Demographics of drug-resistant tuberculosis patients. Demographic features of the patients in the study population are shown in Table 1. Hispanic and Asian patients comprised 71% of those with drug-resistant tuberculosis, but only 46% of reported tuberculosis patients in Texas from 1992 to 1994 (19–21). At least 48% of patients with drug-resistant tuberculosis were foreign-born, compared to 23% of tuberculosis patients in Texas (19–21). Twelve percent of patients with drug-resistant tuberculosis were known to be infected with human immunodeficiency virus (HIV), similar to the 13% HIV coinfection rate among tuberculosis patients in Texas (19–21). Counties were divided into five groups, based on geographic location as follows: (i) adjacent Dallas and Tarrant counties in north-central Texas, which include the cities of Dallas and Fort Worth, respectively; (ii) Harris county on the Gulf coast, which includes the city of Houston; (iii) counties along the U.S.-Mexico border; (iv) counties in northeast Texas; and (v) all other counties. Thirty-eight percent of patients with drug-resistant tuberculosis were reported from six counties along the U.S.-Mexico border compared to only 14% of tuberculosis patients in Texas (19–21).

The distribution of cases of drug-resistant tuberculosis was strikingly focal, with all 201 cases reported from only 35 of the 254 counties in Texas. In contrast, during 1992 to 1994, tuberculosis cases were reported from 176 counties (19–21). One case of drug-resistant tuberculosis was reported from each of 17 counties, and two to nine cases were reported from each of 11 counties (total, 36 cases). Seventy-four percent (148 of 201) of the cases of drug-resistant tuberculosis were reported from only seven counties, which contributed 62% of the reported tuberculosis cases in 1992 to 1994 (19–21).

Comparison of clustered and nonclustered patients. Results of RFLP analysis with IS*6110* and pTBN12 are shown in Table 2. IS*6110*-based RFLP analysis alone revealed 25 clusters comprising 68 patients. pTBN12-based RFLP analysis was performed for all isolates with one to five IS*6110* fragments and those isolates with more than five IS*6110* fragments that were similar but not identical, as outlined in Materials and Methods. Secondary typing with pTBN12 differentiated many isolates with identical IS*6110*-based RFLP patterns of one to five fragments, reducing the number of clustered patients from 41 to 11 and the number of clusters from eight to five.

TABLE 2. Number of clusters, based on RFLP analysis with IS*6110* and pTBN12

IS*6110* copy no.	IS*6110* only		IS*6110* and pTBN12	
	No. of patients in clusters	No. of clusters	No. of patients in clusters	No. of clusters
1–5	41[a]	8	11	5
>5, similar	6	3	6	3
>5, identical[b]	24	12	24	12
Total	71	23	41	20

[a] Thirty-six isolates had identical RFLP patterns; five had similar RFLP patterns.
[b] pTBN12-based RFLP analysis was not performed for isolates with more than five identical IS*6110* fragments.

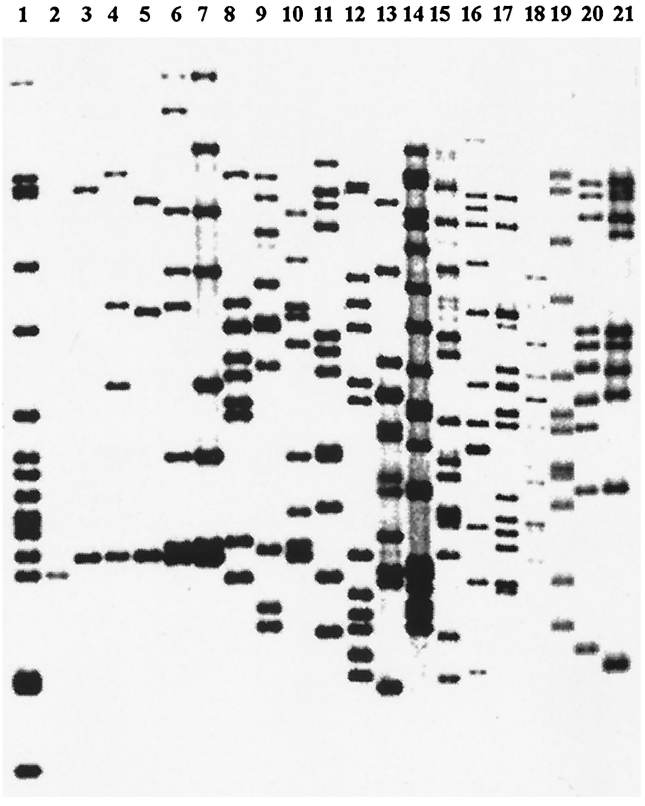

FIG. 1. IS*6110*-based RFLP patterns of 20 drug-resistant isolates of *M. tuberculosis* from clustered patients. One isolate from each cluster of patients is shown. Lane 1, DNA of *M. tuberculosis* H37Rv to which was added two additional IS*6110*-containing markers of known molecular weights; lanes 2 to 21, DNA of isolates from patients.

The IS*6110*-based RFLP patterns of one isolate from each cluster are shown in Fig. 1. Forty-one (20%) patients were clustered, and 160 (80%) were not. Clustered patients were more likely than nonclustered patients to be African American and less likely to be Hispanic (Table 1). In addition, clustered patients were more likely to have been born in the United States than nonclustered patients. In clustered and nonclustered patients, the percentages of patients coinfected with HIV and the patterns of drug resistance were similar. Sixty-one percent of clustered patients were reported from northeast counties and from Dallas, Tarrant, and Harris counties compared to only 36% of nonclustered patients. Only 2% of clustered patients were reported from other counties, representing many geographically separate locations, compared to 26% of nonclustered patients.

Characteristics of clustered patients. Forty-one patients with drug-resistant isolates were in 20 clusters. Nineteen clusters consisted of two patients, and one cluster consisted of three patients. Table 3 shows the demographic characteristics of the clustered patients and the drug resistance patterns of their *M. tuberculosis* isolates. All patients in 9 of the 20 clusters were reported from counties in the same geographic area ($P = 0.008$, binomial test), suggesting that clustering represented recent transmission of tuberculosis.

All patients in 12 of 20 clusters were of the same ethnicity (Hispanic in 7, African American in 4, and Asian in 1; $P = 0.025$, binomial test). Of the 11 clusters in which patients were not from the same geographic region, both patients in 6 clusters were of the same ethnicity (Hispanic in 4, African American in 2; $P = 0.09$, binomial test). Because people generally associate more with members of their own ethnic groups, these findings suggest that some transmission resulted from travel between geographically separate parts of the state or that clustering reflected the reactivation of remote infection with *M. tuberculosis* strains that were prevalent in specific ethnic groups.

Drug susceptibility patterns of isolates from six clusters (1 through 6) were identical. Drug susceptibility patterns of isolates from seven clusters (clusters 7 through 13) differed for one drug, and those of isolates from seven clusters (clusters 14 through 20) differed for two or three drugs. Of the 29 patients in 14 clusters in which the drug susceptibility patterns differed, drug susceptibility testing was performed for two or more isolates from 15 patients. Susceptibility results were the same for all isolates from each of these 15 patients, indicating that susceptibility testing results were reproducible. Of the nine clusters in which all patients were from the same geographic area, four (44%) had identical drug susceptibility patterns. In contrast, of the 11 clusters in which patients were from different geographic regions, only 2 (18%) had identical drug susceptibility patterns.

DISCUSSION

This report demonstrates that the distribution of drug-resistant tuberculosis in Texas is more focal than the distribution of tuberculosis in general and that there is no evidence for extensive transmission of drug-resistant tuberculosis. Assuming that all clustered patients developed tuberculosis from recent infection, a maximum of 20% of drug-resistant cases with available isolates were due to recent infection. Making the more conservative assumption that one patient in each cluster had reactivation tuberculosis and that the others were recently infected (2, 4, 18), only 11% (22 of 201) of these cases were due to recent infection. Most clustered patients were born in the United States and were from three urban areas (Dallas, Fort Worth, and Houston) and northeast Texas.

The significance of clustering in population-based studies is controversial. In most urban areas, epidemiologic data strongly suggest that clustering indicates recent transmission of tuberculosis (1, 2, 18). In contrast, in geographically stable, rural populations, clustering may result from the reactivation of infection acquired from the same source in the distant past, as has been observed in Arkansas (3). Both phenomena probably accounted for some clustering among drug-resistant tuberculosis patients in Texas. Clustered patients were significantly more likely to be from the same geographic area, and clustered patients from the same geographic area were more likely to have isolates with identical drug susceptibility patterns, suggesting that they were linked by recent transmission. However, in 11 of the 20 clusters, clustered patients were from geographically separate parts of the state, and most isolates did not have identical drug susceptibility patterns. Although there are many potential reasons for this finding, the most likely explanation is that tuberculosis was transmitted from a common source in the distant past, providing time for infected persons to travel to different locations and for *M. tuberculosis* organisms to develop different patterns of drug resistance.

In New York City, Los Angeles, and San Francisco, 38 to 59% of tuberculosis cases were clustered, and there were many large clusters comprising up to 43 patients (1, 2, 18). In contrast, in the present study, only 20% of cases were clustered and all clusters included only two or three patients. This striking difference indicates that extensive transmission of drug-resistant tuberculosis did not occur in Texas. Isolates from 81

TABLE 3. Characteristics of clustered patients with drug-resistant tuberculosis

Patient[a]	Ethnicity	Country of birth	County[b]	Sensitivity to indicated drug[c]				IS*6110* copy no.
				INH	RIF	SM	EMB	
1A	African American	U.S.	Harris	R	S	S	S	12
1B	Hispanic	Mexico	Border	R	S	S	S	12
2A	African American	U.S.	Harris	R	S	S	S	20
2B	African American	U.S.	Harris	R	S	S	S	20
3A	Asian	Phillipines	Tarrant	R	S	S	S	12
3B	White	U.S.	Tarrant	R	S	S	S	12
4A	Hispanic	Unknown	Border	R	R	R	R	11
4B	Hispanic	Mexico	Border	R	R	R	R	10
5A	Hispanic	Mexico	Dallas	S	R	S	S	2
5B	White	U.S.	Dallas	S	R	S	S	2
6A	Hispanic	Mexico	Dallas	R	R	R	R	4
6B	Hispanic	Unknown	Border	R	R	R	R	4
7A	Hispanic	U.S.	Northeast	R	S	S	S	11
7B	Hispanic	Mexico	Border	R	R	S	S	11
8A	African American	U.S.	Northeast	R	S	S	S	8
8B	African American	U.S.	Northeast	R	S	R	S	8
9A	Hispanic	Mexico	Border	R	R	S	S	11
9B	White	U.S.	Dallas	S	R	S	S	10
10A	Hispanic	Mexico	Border	R	R	R	S	9
10B	White	U.S.	Dallas	R	S	R	S	9
11A	African American	U.S.	Other	R	R	S	S	10
11B	African American	U.S.	Northeast	R	S	S	S	10
12A	Hispanic	U.S.	Border	R	S	R	S	13
12B	Hispanic	Unknown	Border	R	R	R	S	13
13A	Hispanic	Unknown	Border	R	S	R	S	10
13B	Hispanic	U.S.	Harris	R	R	R	S	10
14A	White	U.S.	Harris	R	S	S	S	12
14B	African American	U.S.	Harris	S	R	S	S	12
15A	Hispanic	U.S.	Border	R	R	R	S	13
15B	White	U.S.	Harris	S	R	R	R	13
16A	Hispanic	Mexico	Other	R	S	R	S	8
16B	Hispanic	Unknown	Border	R	R	R	R	8
17A	African American	U.S.	Northeast	S	R	S	S	6
17B	White	U.S.	Tarrant	R	S	S	S	6
18A	African American	U.S.	Dallas	R	S	S	S	3
18B	African American	U.S.	Harris	S	R	S	S	3
19A	Hispanic	Mexico	Border	R	R	R	R	4
19B	Hispanic	Unknown	Border	R	R	S	S	4
20A	Asian	Vietnam	Dallas	R	S	R	S	1
20B	Asian	Vietnam	Dallas	R	R	R	R	1
20C	Asian	Vietnam	Tarrant	S	S	R	S	1

[a] Clusters are numbered 1 through 20; patients are identified as A, B, and C. Drug resistance patterns are identical in clusters 1 through 6 and differ by one drug in clusters 7 through 13 and by two or more drugs in clusters 14 through 20.

[b] Border counties are those along the U.S.-Mexico border (El Paso, Hidalgo, Starr, and Webb counties); northeast counties are Anderson, Rusk, and Smith counties; other counties are Midland and Walker counties.

[c] INH, isoniazid; RIF, rifampin; SM, streptomycin; EMB, ethambutol; R, resistant; S, sensitive.

patients from Dallas, Tarrant, and Harris counties were not available for our study. Because 28% of the cases in these counties were clustered (Table 1), it is possible that the percentage of clustered drug-resistant cases may be slightly higher than we observed. Most cases of drug-resistant tuberculosis in Texas were diagnosed along the U.S.-Mexico border, in northeast Texas, and in other parts of the state where widespread transmission of tuberculosis is unlikely to occur because most persons live in stable family settings in small cities and rural areas. A minority of drug-resistant tuberculosis cases were diagnosed in the cities of Dallas, Fort Worth, and Houston, where significant numbers of persons are homeless or live in unstable social settings. These settings favor the extensive transmission of tuberculosis (2, 13), and large clusters of drug-susceptible tuberculosis cases have been observed in Tarrant county (5a). The lack of extensive transmission of drug-resistant tuberculosis in these locations during the study period may simply be fortuitous. Alternatively, because most drug-resistant isolates are resistant to isoniazid, a more speculative possibility is that the transmission potential of isoniazid-resistant organisms is less than that of drug-susceptible organisms (11). Isoniazid-resistant organisms have an increased frequency of katG gene mutations (17), which reduce catalase-peroxidase activity and may inhibit the ability of *M. tuberculosis* to resist the antimycobacterial activity of macrophages. In addition, studies in the 1950s showed that isoniazid-resistant *M. tuberculosis* was less virulent for guinea pigs than were drug-susceptible organisms (15), and more recent work revealed that katG mutations attenuated the virulence of *Mycobacterium bovis* and H37Rv for guinea pigs and mice, respectively (14, 23). Further studies are needed to evaluate the epidemiologic and microbial factors that influence the transmission dynamics of drug-susceptible and drug-resistant tuberculosis.

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