Identification of a W Variant Outbreak of *Mycobacterium tuberculosis* via Population-Based Molecular Epidemiology

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Context Typing of *Mycobacterium tuberculosis* could provide a more sensitive means of identifying outbreaks than use of conventional surveillance techniques alone. Variants of the New York City W strain of *M tuberculosis* were identified in New Jersey.

Objective To describe the spread of the W family of *M tuberculosis* strains in New Jersey identified by molecular typing and surveillance data.

Design Population-based cross-sectional study.

Setting and Subjects All incident culture-positive tuberculosis cases reported in New Jersey from January 1996 to September 1998, for which the W family was defined by insertion sequence (IS) IS*6110* DNA fingerprinting, polymorphic GC-rich repetitive sequence (PGRS) typing, spacer oligotyping (spoligotyping), and variable number tandem repeat (VNTR) analysis.

Main Outcome Measure Identification and characterization of W family clones supplemented by surveillance data.

Results Isolates from 1207 cases were analyzed, of which 68 isolates (6%) belonged to the W family based on IS*6110* and spoligotype hybridization patterns. The IS*6110* hybridization patterns or fingerprints revealed that 43 patients (designated group A) shared a unique banding motif not present in other W family isolates. Strains collected from the remaining 25 patients (designated group B), while related to W, displayed a variety of IS*6110* patterns and did not share this motif. The PGRS and VNTR typing confirmed the division of the W family into groups A and B and again showed group A strains to be closely related and group B strains to be more diverse. The demographic characteristics of individuals from groups A and B were specific and defined. Group A patients were more likely than group B patients to be US born (91% vs 24%, $P<.001$), black (76% vs 16%, $P<.001$), human immunodeficiency virus positive (40% vs 0%, $P = .007$), and residents of urban northeast New Jersey counties ($P<.001$). Patients with group B strains were primarily non-US born, of Asian descent, and more dispersed throughout New Jersey. No outbreak had been detected using conventional surveillance alone.

Conclusions The implementation of multiple molecular techniques in conjunction with surveillance data enabled us to identify a previously undetected outbreak in a defined geographical setting. The outbreak isolates comprise members of a distinct branch of the W family phylogenetic lineage. The use of molecular strain typing provides a proactive approach that may be used to initiate, and not just augment, traditional surveillance outbreak investigations.

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DESPITE THE INTRODUCTION OF the first antituberculin drugs almost 50 years ago, morbidity and mortality associated with *Mycobacterium tuberculosis* remains a major public health threat. Recently, the study of tuberculosis (TB) epidemiology and transmission, traditionally accomplished by patient contact tracing, has been augmented by the use of molecular strain typing. A striking example was the identification of the W strain, a multidrug-resistant (MDR) clone that caused disease in more than 350 patients in New York City and accounted for more than 25% of all MDR cases in the United States in the early 1990s.[1-4] This MDR and successful clone, associated with high mortality rates in both New York prisons and hospitals, has since become the "index" strain in the Public Health Research Institute (PHRI) TB Center (New York, NY) and has been the focus of a number of molecular epidemiological studies.[1-6]

It is generally accepted that *M tuberculosis* isolates with identical inser-

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tion sequence (IS) IS*6110* fingerprint patterns, such as the 350 W isolates responsible for the New York City outbreak, are clonal and indicative of recent transmission while isolates with unique patterns represent cases of reactivation and are unrelated.[7,8] However, the use of multiple typing techniques has provided insight into the relatedness of strains with similar, but not identical, IS*6110* patterns.

The use of multiple molecular tools in combination has demonstrated the phylogenetic relatedness of strains from diverse temporal and geographic areas that have genetic markers similiar to the MDR W strain. These strains, which are grouped in the W family lineage, have a common genotype with the previously described Beijing clones, which are the predominant strains in China and found throughout Asia.[9-11] These strains are now viewed as being members of the same phylogenetic lineage and recent ancestors to the MDR W strain. Together, the W and Beijing families share distinctive chromosomal markers, as they all belong to genotypic group 1, have the identical spacer oligotype (spoligotype) pattern S00034, and have in common unique IS*6110* chromosomal insertions.[4,12,13]

In the past, molecular epidemiological studies of TB have primarily focused on the analysis of disease spread in small areas in which molecular data had been used to confirm epidemiological linkage or test hypotheses.[1,7,8,14-16] The PHRI TB Center, in collaboration with the New Jersey Department of Health and Senior Service (NJDHSS), has been genotyping all viable *M tuberculosis* cultures from reported TB cases in the state of New Jersey. The molecular analysis is routinely combined with patient surveillance data. A major goal of this collaboration has been to develop public health strategies and TB control protocols that integrate *M tuberculosis* molecular information and case surveillance data on a state population.

We conducted an investigation of the spread of the *M tuberculosis* W family in the New Jersey TB population during the years 1996-1998, a time when no outbreaks of TB had been identified by conventional contact tracing methods.

METHODS

Study Population

The study population included all culture-positive TB cases reported to the NJDHSS between January 1996 and September 1998. All available isolates from culture-positive cases were genotyped by IS*6110* fingerprinting as part of the National Tuberculosis Genotyping and Surveillance Network, Centers for Disease Control and Prevention (CDC). During the study period, 1575 culture-positive TB cases were reported and isolates from 1207 cases (77%) were genotyped. Isolates from 368 cases (23%) were nonviable or not available. Out of 1207 total isolates, 68 belonged to the W family based on their IS*6110* pattern similarities to the index W strain. These 68 isolates were further analyzed.

IS*6110* DNA Fingerprinting

Mycobacterium tuberculosis isolates were cultured on Lowenstein-Jensen slants and grown at 37°C for 3 to 5 weeks. Right and left IS*6110* DNA fingerprint analysis was performed as previously described.[17] The hybridization patterns were compared on a Sun Sparc 5 workstation (Sun Microsystems, Palo Alto, Calif) with BioImage Whole Band Analyzer software version 3.4 (BioImage, Ann Arbor, Mich). Classification of the DNA fingerprint patterns was previously described.[18] Isolates with identical banding patterns were assigned the same arbitrary letter code (eg, W, C, BE) to indicate that at least 2 TB cases were caused by the same strain. The IS*6110* patterns that resembled, but were not identical to, 1 of the strain types were denoted by the addition of a number to the cluster letter (eg, W4, W79). Since 1992, PHRI has characterized nearly 10 500 *M tuberculosis* isolates of which 80% were cultured from New York City and New Jersey patients. The other isolates were from 7 additional states in the United States, the former Soviet Union, Singapore, South Africa, Romania, Egypt, Israel, Venezuela, Honduras, Mexico, India, Chile, and Kenya.

Other Molecular Analysis

The W family isolates were further characterized using a number of previously described secondary typing methods. Sequence determination of codons 463 and 95 in the genes encoding catalase peroxidase (*katG*) and the A subunit of DNA gyrase (*gyrA*), respectively, cataloged the isolates into 1 of 3 principal genotypic groups.[12,19] The unique direct repeat region of the *M tuberculosis* chromosome was compared for each isolate using the spoligotype membrane format.[20,21] Specific IS*6110* insertion site mapping probes were used to determine the presence of insertions in the origin of replication and in the NTF chromosomal region.[12,13] The DNA was also compared on the basis of Southern blot hybridization using a consensus polymorphic GC-rich repetitive sequence (PGRS) probe.[22,23] Polymerase chain reaction was used to determine the exact number of tandem DNA repeats at each of 5 chromosomal loci containing variable numbers of tandem repeats (variable number tandem repeat [VNTR] loci ETR-A through ETR-E), as previously described.[24]

Epidemiological Analysis

The demographic and clinical data were obtained from the TB surveillance system in New Jersey. This includes data from the NJDHSS contact investigation reports, which were used to evaluate epidemiological links between the patients. Routine contact investigations were conducted on all proven or suspected pulmonary TB cases. Investigations included an index patient interview and identification of close contacts. Patient contacts were interviewed, and tuberculin tests and chest radiography were performed if necessary. Cases identified through the contact investigation were considered to have epidemiologic links to the index case. Tuberculosis patients from Essex, Hudson, and Passaic counties were defined in this study as residents of urban northeast New Jersey counties.

Analysis was carried out using SAS, version 6.12 (SAS Institute, Cary, NC).

Fisher exact and χ^2 tests were used to compare the proportions of categorical variables between groups. Crude odds ratios were calculated with SAS; a value of 0.5 was added when tables consisted of 0 values.

Geographic Information System mapping was carried out for all cases included in this study. Mapping coordinates were abstracted from the New Jersey Topologically Integrated Geographic Encoding and Referencing file (US Bureau of Census and US Geological Survey) and linked to 1990 Census of Population and Housing. Maps were generated using ARC/INFO (v 7.12; Environmental Systems Research Institute Inc, Redlands, Calif).

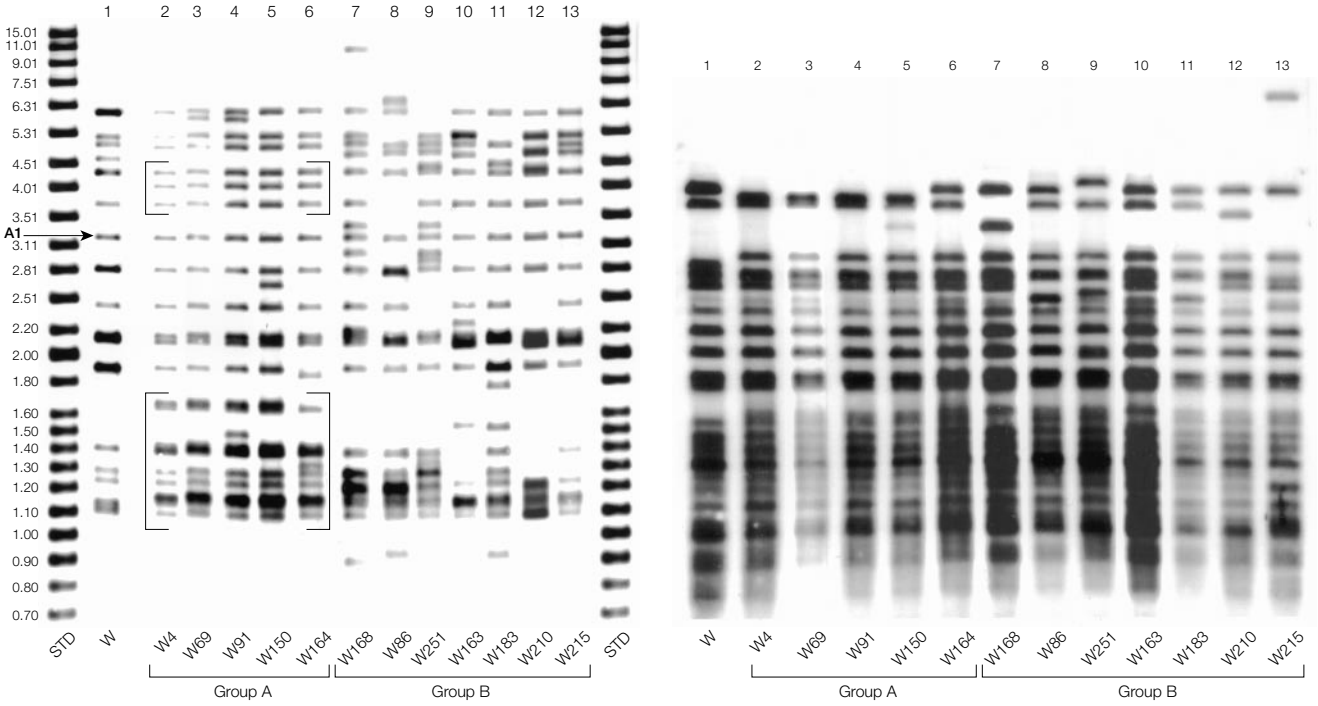
RESULTS

New Jersey Population

Of 1207 New Jersey TB cases typed by IS*6110* DNA fingerprinting, isolates from 433 cases (36%) had IS*6110* hybridization patterns that were unlike any others in the New Jersey collection or the PHRI TB Center archive (unique isolates). Among the remaining 774 cases, 237 (31%) were assigned to 11 major strain types (defined as ≥10 cases each during the 45-month study period). In addition, 179 isolates fell into 40 strain types with 3 to 9 TB cases each and 90 cases segregated into 45 strain types with 2 cases each.

Compared with the entire population of New Jersey TB patients, those with unique isolates and those with isolates related to other cases were similar in sex (men, 248/433 [57%] vs 459/774 [59%]; women, 185/433 [43%] vs 315/774 [41%]; $P = .50$) and proportion of Hispanic (95/433 [22%] vs 155/744 [20%]; $P = .21$) and white persons (90/433 [21%] vs 155/744 [20%]; $P = .77$). Patients with related isolates were younger than those with unique patterns (mean age, 47 vs 45 years; $P<.02$). Patients with unique isolates were more likely to be Asian (146/433 [34%] vs 122/774 [16%]; $P<.001$), whereas those with related isolates were more likely to be non-Hispanic black (102/433 [23%] vs 342/744 [44%]; $P<.001$). Among patients with known human immunodeficiency virus (HIV) status, there was a higher percentage of HIV-seropositivity in the related-isolate group (43/433 [10%] vs 175/744 [23%]; $P<.001$). Unique isolates were more likely to come from non–US-born persons (287/433 [62%] vs 288/744 [37%]) and related isolates were more likely to be seen in US-born patients (146/433 [38%] vs 486/774 [63%]; $P<.001$). Related isolates were also more likely to come from residents of urban northeast New Jersey counties (175/433 [40%] vs 426/774 [55%]; $P<.001$).

Figure 1. Southern Blot Hybridization of *Mycobacterium tuberculosis* Isolates



Left panel is a Southern blot of *Pvu*II-restricted *M tuberculosis* chromosomal DNA hybridized with the *Bam*HI-*Sal*/1 fragment of IS*6110*. Lane 1 is W multidrug resistant (MDR); lanes 2-6, group A; lanes 7-13, group B. The 2 characteristic W4 motifs are in brackets. W164 is the product of restriction site length polymorphism of W4 resulting in a shift of an IS*6110* band. The A1 *dnaA-dnaN* IS*6110* insertion found in the W family is denoted by an arrow. The IS*6110* image was composed of different exposures of the same experiment. Right panel is a Southern blot hybridization of *Alu*I-restricted *M tuberculosis* chromosomal DNA probed with a polymorphic GC-rich repetitive sequence (PGRS). Lane1 is W-MDR; lanes 2-6, group A; and 7-13, group B. Group A isolates share a common PGRS pattern.

W Family Genotype and Spoligotyping

Solely on the basis of IS*6110* DNA fingerprints that resembled the 18-band W strain pattern signature, a total of 68 isolates (6%) with 29 different patterns were assigned to the W family. Genotypic grouping, multiplex polymerase chain reaction, and IS*6110* insertion site mapping, all molecular methods previously used to distinguish the W family,[4] confirmed the identity of the W family isolates in this study.

A sample of 234 isolates from New Jersey, including all strains with limited copies of IS*6110*, were spoligotyped, and the patterns were analyzed against the Wadsworth database, which contains an additional 847 samples from the Northeast TB population. All 68 isolates grouped to the W family had spoligotype S00034; this pattern was not found among other New Jersey isolates analyzed.

In summary, all 68 W family isolates were genotypic group 1, had the A1 IS*6110* insertion in the origin of replication, the single IS*6110* copy in the NTF, and spoligotype S00034.

Table 1. Grouping W Family Strains by Multiple Molecular Techniques*

IS*6110* DNA Fingerprint Pattern	Patients, No.	US Born, No.	Country or Region of Birth	No. of IS*6110* Insertions	PGRS Genotype	VNTR Genotype†
			Group A‡			
W4	25	22	United States§	21	P0002	32435
W69	10	9	United States‖	22	P0002	32435
W79	3	3	United States	20	P0002	32435
W91	1	1	United States	23	P0002	32435
W150	1	1	United States	22	P0002	32435
W152	2	2	United States	20	P0002	32435
W164	1	1	United States	21	P0003	32435
			Group B‡			
W	1	1	United States	18	P0001	42435
W37	1	0	South Korea	18	P0018	42435
W65	1	0	China	20	P0017	32435
W68	1	0	Vietnam	19	P0011	42435
W70	1	0	Taiwan	19	P0011	42435
W86	2	1	Taiwan	20	P0006	42437
W87	1	0	South Korea	16	P0015	42435
W88	1	0	China	18	P0014	42435
W90	1	0	Vietnam	18	P0011	42436
W126	1	0	Vietnam	24	P0009	42435
W163	3	3	United States	19	P0005	42435
W168	1	1	United States	25	P0004	42435
W183	1	0	China	21	P0008	42435
W184	1	0	China	21	P0009	42435
W190	1	0	South Korea	16	P0009	42435
W205	1	0	Vietnam	18	P0009	42435
W210	1	0	China	17	P0007	42435
W211	1	0	South Korea	20	P0012	42435
W212	1	0	Brazil	22	P0013	42535
W215	1	0	Mongolia	21	P0016	42435
W250	1	0	Hong Kong	22	P0010	42435
W251	1	0	Philippines	22	P0009	42435

*PGRS indicates polymorphic GC-rich repetitive sequence; VNTR, variable number tandem repeat.
†Each digit of the VNTR allele profile represents the number of tandem repeats at a particular chromosomal locus.[25]
‡All spacer oligotyping for both groups was S00034.
§Non–US-born patients: 1 from Colombia, 2 from Portugal.
‖Non–US-born patient from Peru.

IS*6110* Fingerprint Pattern Analysis

Among the 29 IS*6110* hybridization patterns similar to the index W strain (FIGURE 1), 2 subtypes, W4 and W69, represented 25 and 10 individual cases, respectively. Their closely related fingerprint patterns had a common signature-banding motif (the W4 motif) and an IS*6110* copy number ranging between 20 and 22 insertions. This motif was also identified in 5 additional types (W79, W91, W150, W152, and W164) isolated from 1 to 3 cases each. These 7 types, cultured from a total of 43 patients, defined the relatively homogeneous group A samples in our study. The group A strains are viewed as a distinct branch of the W family.

Twenty-three additional patterns in 25 patients lacked the W4 motif and therefore were assigned to group B. These isolates had 16 to 25 IS*6110* insertions (TABLE 1 and Figure 1). The MDR W strain prototype (ie, New York City outbreak strain) fingerprint pattern and the Beijing family strains isolated throughout Asia were also assigned to group B.

PGRS and VNTR Subtyping

As shown in Table 1, the PGRS and VNTR genotypes among the 29 IS*6110* subtypes divided the samples into groups A and B in agreement with the fingerprint pattern distinctions. Only 2 PGRS hybridization patterns (42/43 isolates were type P0002) were found among the group A strains; all group A strains had the 32435 VNTR pattern. The 23 group B IS*6110* subtypes had 3 different VNTR subtypes and 14 PGRS patterns. Among group B strains, only W65, isolated from a single individual, had the VNTR pattern typical of group A.

Epidemiological Analysis of the W Family

Of the 68 cases associated with W family strains, 49 (72%) occurred in men, 23 (34%) in non–US-born individuals, and 37 (54%) among non-Hispanic blacks. Among the 33 per-

sons for whom HIV status was known, 17 (52%) were co-infected with HIV.

TABLE 2 shows a comparison of characteristics of group A and B patients. Group A patients were more likely to be non-Hispanic black (odds ratio [OR], 17.33; 95% confidence interval [CI], 4.8-62.4) and US born (OR, 30.88; 95% CI, 9.2-103.4). Among cases for whom data were available, group A patients were 21.7 times more likely to be HIV-seropositive. Four group A patients were born outside the United States. (Table 1), all of whom had known risk factors for TB acquisition (defined as at least 1 of the following: HIV seropositivity, history of incarceration, intravenous drug use) that suggested recent transmission.

FIGURE 2 illustrates the geographic occurrence of W family cases in New Jersey. Group A and group B patients were from 8 and 10 New Jersey counties, respectively. Eighty-one percent of group A patients were from 3 neighboring northeast New Jersey counties (see "Methods" section), of which 94% were present in 5 neighboring cities. In comparison, only 16% of group B patients resided in these 3 counties. Forty-two percent (18 cases) of all group A cases were prevalent in Paterson, NJ. Furthermore, 25% of all cases reported in this city (n = 71) were either W4 or a closely associated variant (8, W4 cases; 8, W69; 1, W150; and 1, W152). Most counties with group A cases were located in northeastern New Jersey. Conversely, group B strains were geographically more dispersed throughout New Jersey.

COMMENT

In this study, a combination of molecular techniques segregated a large population of related *M tuberculosis* strains into 2 epidemiologically significant groups. Among a genetically well-characterized family of strains drawn from a population-based sample from New Jersey, we showed that 1 set of closely related strain variants, group A, appeared on molecular grounds to be a separate phylogenetic branch of the W family. Epidemiological characteristics of group A isolates such as geographical aggregation, near absence of non–US-born patients, and high prevalence of specific demographic factors indicate a locally produced cluster. In contrast to group A, group B variants comprised a heterogeneous set of distantly related isolates from the W family. This group exhibited epidemiological correlates of an endemic and globally prevalent disease as defined by geographical dispersion, high proportion of non–US-born patients, and a lack of demographic uniformity.

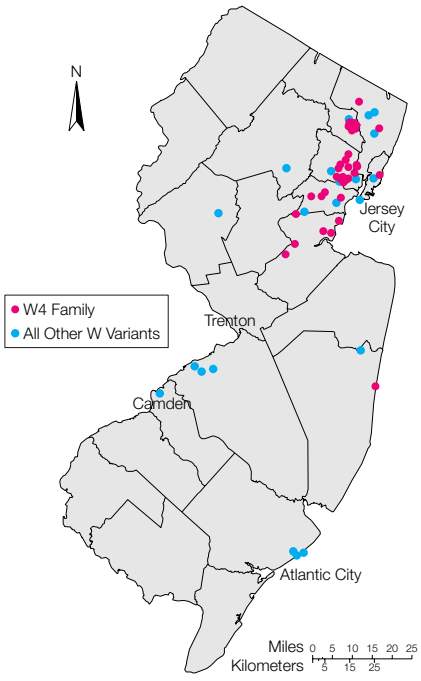
This study emphasizes the usefulness of grouping strains with similar, but not identical, IS*6110* fingerprint patterns to identify variants that may represent the extension of an outbreak. In New Jersey, neither routine contact investigations nor relating strains by strict IS*6110* fingerprint interpretation would have recognized the extent of the large group A cluster. The identification of group A was made possible by a combination of W4-signature pattern analy-

Table 2. Univariate Comparison of Characteristics of Groups A and B*

	Group A (n = 43)	Group B (n = 25)	New Jersey (n = 1575)	*P* Value† (Group A vs B)
Age, y				
Mean	40	47	46	
<50	33 (77)	15 (60)	987 (63)	.17
≥50	10 (23)	10 (40)	588 (37)	
Sex				
Men	31 (72)	18 (72)	937 (60)	>.99
Women	12 (28)	7 (28)	638 (40)	
Race/ethnicity‡				
Non-Hispanic white	5 (12)	3 (12)	332 (21)	.15
Non-Hispanic black	33 (76)	4 (16)	559 (35)	<.001
Asian	0 (0)	18 (72)	375 (24)	<.001
Hispanic	5 (12)	0 (0)	309 (20)	.15
HIV serology§				
Positive	17 (40)	0 (0)	271 (17)	.007
Negative	10 (23)	6 (24)	450 (29)	
Unknown	16 (37)	19 (76)	854 (54)	
Birthplace				
US born	39 (91)	6 (24)	809 (51)	<.001
Foreign born	4 (9)	19 (76)	766 (49)	
Residence in urban northeast New Jersey counties‖	35 (81)	4 (16)	734 (47)	<.001

*All data are presented as number (percentage) except mean age.
†Based on 2-tailed Fisher exact test.
‡*P* values are for the comparison of each race/ethnic group with all other races/ethnic groups.
§Unknown human immunodeficiency virus (HIV) status not included in *P* value.
‖See "Methods" section. Counties include Passiac, Essex, and Hudson.

Figure 2. Location of W Variant Tuberculosis Cases in New Jersey



Data source is Environmental Protection Region II and Public Health Research Institute Tuberculosis Center (New York, NY).

sis in conjunction with additional molecular techniques.

The results presented in this study are of methodological significance. They show that molecular methods for characterizing *M tuberculosis* are valid not only in elucidating transmission patterns when the epidemiological situation is known, as in recognized outbreaks[14-16,26] or epidemic situations in small areas,[7,8] but are relevant on a large-scale population level when outbreaks are not suspected. Indeed, group A cases associated by genotype analysis display clustering predominantly in black men in cities in urban northeast New Jersey counties that is suggestive of an ongoing or recent outbreak in that area. This assertion is further supported by the particularly strong local clustering of group A cases (representing 25% of all reported cases) in 1 city. Significantly, this clustering is in contrast to the background of diverse strain types associated with other cases from this location (data not shown). Without molecular studies this cluster would not have been suspected against the background of a generally high TB case rate in that part of the state.

Our findings affirm and extend those of a recent study in which IS*6110* fingerprinting was used in combination with geographic analysis to assess *M tuberculosis* transmission in Baltimore, Md.[25] In the Baltimore study, strains clustered by fingerprint analysis were primarily found in localized areas of low socioeconomic status and in a patient population with high rates of HIV infection, alcohol and drug abuse, and homelessness. In contrast, the unclustered isolates were found in middle-class neighborhoods. Extensive contact investigation identified only 24% of epidemiological links within the cluster population. Based on these results, the investigators concluded that location-based strain identification might render routine contact investigation more effective. Similar conclusions were drawn from a recent Texas study showing the spread of TB in frequented social settings.[27] Our findings, which cover fewer TB cases but refer to an even larger and more diverse geographic area and invoke a wider array of molecular results for strain characterization, support the conclusions of the Baltimore and Texas studies.

These results also indicate that other factors, in addition to an MDR phenotype, contributed to the appearance of the W strain outbreak. Indeed, the outbreak strain that wreaked so much havoc in New York prisons[5] and hospitals[1,3] and across the United States[6] in the early 1990s appears to have been a branch of a lineage that continues to develop, evolving into variants that could be clearly differentiated. In this study, the phylogenetic relatedness observed in group A isolates represents the local spread and evolution of a particular strain variant. Individually, these variants carry outbreak potential that can be augmented in certain situations, as was the case with the W strain and drug resistance.

Consistent with the finding that these strains are geographically clustered, only 3 TB cases from group A were identified in the New York population from 1993-1999. In addition, IS*6110* fingerprint patterns that define the group A cases have not been reported from the other states that participate in the CDC National Tuberculosis Genotyping and Surveillance Network.

Our study has a number of limitations. First, patient data linking cases that appeared on molecular grounds to be related were not available to us. This limits the inference that geographically or epidemiologically clustered cases represent an outbreak or local spread. We purposely blinded ourselves to the results of contact investigation to avoid biasing the analysis of the correlation of surveillance data with molecular results. Second, we were only able to fingerprint 77% of all culture-positive cases. Most of the cases with no available isolates were reported from private clinics in southern New Jersey. The demographic features of the patients may differ from those reported in our study. However, this sampling bias is unlikely to alter the main conclusions in this study, particularly the demographic contrasts between groups A and B within the W family.

The integration of molecular and surveillance data has allowed public health workers to focus their epidemiological investigation on patients infected with related strains suspected to be the product of recent transmission vs unique isolates that are most probably cases of reactivation. Consequently, several molecularly guided cluster investigations have been initiated, including a reinvestigation of group A cases. Since our study was completed, 5 additional group A cases (4, W4; and 1, W69) from New Jersey have been identified. All molecular and surveillance data are in agreement with the work presented here. All 5 patients are US-born from urban northern New Jersey, and 3 of them are HIV positive.

In our study, molecular analysis identified the spread of *M tuberculosis* variants not previously recognized by classic epidemiology and provided a better understanding of both endemic and outbreak strain transmission. We believe that relating strains with the use of molecular typing may facilitate a proactive approach to TB investigation that, guided by knowledge of strain information, will allow a more rapid as well as rational application of traditional contact investigation and better use of limited public health resources.

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