

Molecular Analysis of *Mycobacterium kansasii* Isolates from the United States

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We studied the population genetics of *Mycobacterium kansasii* isolates from the United States by PCR restriction enzyme analysis (PRA) of the 441-bp Telenti fragment of the *hsp-65* gene and pulsed-field gel electrophoresis (PFGE) of genomic DNA with the restriction endonucleases *AseI*, *DraI*, and *XbaI*, and we compared the patterns to those previously reported from France and Japan. By PRA, 78 of 81 clinical isolates (96%) from the United States belonged to subspecies I. With PFGE, 28 *AseI* patterns, 32 *DraI* patterns, and 35 *XbaI* patterns were produced. PFGE showed marked clonality of the U.S. isolates, with differences between genotypes involving only one or two bands. Isolates within Texas showed lower pattern diversity than those from different states. With *DraI*, 31 of 71 isolates (44%) had the same common PFGE pattern, which matched the predominant pattern in France (pattern Ia), determined by Picardeau et al. (M. Picardeau, G. Prod'hom, L. Raskine, M. P. LePenec, and V. Vincent, *J. Clin. Microbiol.* 35:25-32, 1997), and in Japan (type M), determined by Iinuma et al. (Y. Iinuma, S. Ichiyama, Y. Hasegawa, K. Shimokata, S. Kawahara, and T. Matsushima, *J. Clin. Microbiol.* 35:596-599, 1997). With *AseI*, 42% of isolates produced a common pattern indistinguishable from the common pattern seen in French isolates (Ia) and with only one band difference from the common pattern (type M) in Japan. This study demonstrates that subspecies I is the predominant subspecies of *M. kansasii* among clinical isolates in the United States, as it is in Europe and Japan, and that genotype I is highly clonal worldwide, with the same major genotype responsible for human infection. The fact that a single clone of *M. kansasii* is responsible for most cases of human disease suggests that specific virulence factors may be associated with this specific genotype.

Mycobacterium kansasii, a nontuberculous mycobacterium, is an opportunistic pathogen of human diseases. It may produce pulmonary or disseminated infections in human immunodeficiency virus (HIV)-infected patients. It is best known as a cause of fibrocavitary lung disease in non-HIV-infected patients, although it has also produced noncavitary nodular disease in the setting of bronchiectasis (3–5, 7, 9–11, 14). *M. kansasii* is rarely recovered from dust, soil, or natural water supplies and is recovered almost exclusively from municipal tap water, which is considered to be its major environmental reservoir. In Texas *M. kansasii* disease is an urban disease (1), and the organism has been recovered from piped water systems in areas where *M. kansasii* is prevalent (16). As with other nontuberculous mycobacteria, human infections are believed to be acquired from the environment.

Over the past 10 years, molecular typing methods have been applied to the study of *M. kansasii*. Ross et al. published one of the earliest studies in 1992, when they characterized isolates from Australia, Belgium, Japan, South Africa, and Switzerland (15). They found that 20 of 105 isolates were pMK1-9 probe negative and that 19 of the 20 probe-negative isolates had a

partial 16S rRNA gene sequence different from that of *M. kansasii*, suggesting the existence of a molecular subspecies or even possibly a new species. Using molecular-based techniques for *M. kansasii* identification and strain typing, including restriction fragment length polymorphism analysis with the major polymorphic tandem repeat probe and IS1652, pulsed-field gel electrophoresis (PFGE), and PCR restriction analysis (PRA) of the *hsp-65* gene, Picardeau et al. (13) studied 38 clinical *M. kansasii* strains and 24 *M. kansasii* strains from water samples, all isolated in France. They found that the clinical and environmental isolates of *M. kansasii* actually belonged to five different *M. kansasii* subspecies, with genotype II corresponding to the subspecies proposed by Ross et al. (15). Alcaide et al. (2) conducted a study by genotypic analysis of 276 *M. kansasii* isolates from various geographic areas within Europe. They identified the same five PRA subtypes as Picardeau et al. did, and they found that genotype I represented the most common isolates from humans. Iinuma et al. typed 84 *M. kansasii* isolates from patients with bronchopulmonary infections in Japan by PFGE with *VspI* digestion, and 21 distinctive PFGE types were identified (6). PRA genotyping was not performed in that study.

In the United States, *M. kansasii* is one of the most frequently isolated pathogenic nontuberculous mycobacteria, second only to *Mycobacterium avium* complex in many areas (1, 3, 10, 16). No previous studies, however, have provided a molecular analysis of *M. kansasii* isolates from the United States similar to those of isolates from Europe and Japan. In the

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TABLE 1. Locations of 69 patients infected with *M. kansasii*

City and/or state (no. of patients)	No. of cities or states	No. of patients (% of total)	Number of PFGE patterns ^a
Texas			
Dallas (16), Houston (6), Austin (6), Fort Worth (5), Amarillo (1), Arlington (1), Brownsville (1), Corpus Christi (1), Galveston (1), Harlingen (1), Longview (1), Lubbock (1), Mexia (1), Paris (1), San Antonio (1)	15	44 (63.8)	33
Other states			
California (3), Alabama (2), Florida (2), Missouri (2), Pennsylvania (2), Tennessee (2), Arkansas (1), Arizona (1), Colorado (1), Connecticut (1), Kansas (1), Louisiana (1), Maryland (1), Minnesota (1), Mississippi (1), North Carolina (1), North Dakota (1), Oklahoma (1)	18	25 (36.2)	24

^a PFGE patterns with combination of *AseI*, *DraI*, and *XbaI*.

present study we analyzed clinical isolates of *M. kansasii* from Texas and elsewhere in the United States by PRA of the *hsp-65* gene and PFGE and compared the results to those from Europe and Japan.

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MATERIALS AND METHODS

Strains. Eighty-one clinical isolates from the United States, collected between 1982 and 1999 from 69 patients with bronchopulmonary infection, were studied with PFGE and PRA. Isolates either came from patients treated at the University of Texas Health Center at Tyler or had been submitted to the Mycobacterium/Nocardia Laboratory for susceptibility testing. Ten patients (14.5%) were known to be HIV positive, and 48 of 81 isolates had been referred for treatment failure associated with rifampin resistance. Forty-four patients (63.8%) were from 15 Texas cities. The remaining 25 patients (36.2%) were from 18 states other than Texas (Table 1). Only single isolates from each of 62 patients were studied, while the remaining 7 patients provided multiple isolates. All of the isolates were identified as *M. kansasii* on the basis of conventional biochemical tests (8) and PRA. Isolates had been stored at -70°C in tryptic soy broth with 15% glycerol following isolation.

Control strains included 2 reference strains, ATCC 12478 and ATCC 35775, obtained from the American Type Culture Collection (ATCC) (Manassas, Va.); 21 *M. kansasii* isolates from France, including five isolates in genotype I, six in genotype II, three in genotype III, four in genotype IV, and three in genotype V, that were part of the study by Picardeau et al. (13); and 16 *M. kansasii* isolates from Japan, including six isolates with PFGE type B, four with type M, and one each with types C, E, F, G, J, and L, that were included in the study by Iinuma et al. (6).

PRA. The Telenti fragment (441 bp) of the 65-kDa heat shock protein gene (*hsp*) was amplified from cell supernatants by PCR with primers TB11 and TB12 and subjected to restriction enzyme analysis with *BstEII* and *HaeIII* as recommended by Telenti et al. (19) and as performed previously (17). Band patterns were characterized as described by Picardeau et al. (13) and Alcaide et al. (2).

PFGE. Patterns of large restriction fragments of genomic DNA were obtained by PFGE as described previously (21). Organisms in low-melting-point agarose plugs were lysed with lysozyme, sodium dodecyl sulfate, and proteinase K. Genomic DNA contained in the plugs was digested with restriction endonucleases *DraI*, *AseI*, and *XbaI* (each enzyme was used alone) and separated by PFGE with a CHEF Mapper system (Bio-Rad Laboratories, Richmond, Calif.). The gel photos were scanned and analyzed by using Advanced Quantifier 1-D Match (Bio Image, Ann Arbor, Mich.).

To simplify the analysis of *XbaI* patterns, because of their large number of bands, only the bands larger than 97.0 kb were counted. The fragment resolution on electrophoresis gels became optimal by doing so. Generally, differences should also be seen among the larger fragments if differences were found in smaller fragments. Thus, counting only *XbaI* fragments larger than 97.0 kb did not reduce the strain discrimination ability.

In this study, patterns with one or more PFGE band differences were consid-

ered different; otherwise they were considered indistinguishable. Isolates were considered clonal if they exhibited six or fewer band differences as defined by Tenover et al. for outbreak strains (20). They were considered unrelated (non-clonal) if they exhibited seven or more band differences.

Statistics. The chi-square test was used for comparison of PFGE patterns produced by Texas and non-Texas isolates, with a *P* value of <0.05 indicating significance.

RESULTS

PRA. Restriction enzyme analysis of the *hsp-65* gene sequence showed that all 81 clinical U.S. isolates and the two ATCC reference strains matched one of the five recognized subspecies patterns of *M. kansasii* (13). Seventy-eight of 81 clinical isolates (96.3%) had indistinguishable patterns (*BstEII* bands at 240 and 210 bp and *HaeIII* bands at 125, 100, and 80 bp), which matched genotype I described by Picardeau et al. (13) and Alcaide et al. (2). The two reference strains (ATCC 12478 and ATCC 35775) also exhibited the genotype I pattern. Of the remaining three U.S. clinical isolates, two belonged to genotype III and one belonged to genotype II. The identity of the PRA pattern was confirmed by electrophoresis of the U.S. isolates and the reference strains of each genotype from France together on the same gel. Examples of the five genotypes are shown in Fig. 1. Eight *M. kansasii* isolates from Japan, including one each of PFGE types B, C, E, F, G, J, L, and M (6), were also tested. All eight isolates belonged to genotype I.

PFGE. Restriction endonucleases *DraI*, *AseI*, and *XbaI* were used to generate restriction patterns, which were then compared by PFGE. With 69 clinical isolates (one isolate per patient) and the two ATCC reference strains, 28 *AseI* patterns, 32 *DraI* patterns, and 35 *XbaI* patterns were produced (Table 2).

With *DraI* digestion, 31 of 71 isolates (43.7%) had the same pattern (pattern Da), while another 13 isolates belonged to four other clusters. Thus, only 27 isolates (38.0%) had unique patterns. Compared with the most common pattern (pattern Da), 33 isolates with 24 patterns generated one to three band differences, and 4 isolates with 4 patterns generated four to six band differences. Thus, 68 of 71 isolates (95.8%) were clonal. Comparison of our major *DraI* pattern (pattern Da) lane by lane on the same electrophoresis gel with the predominant pattern seen in France (genotype Ia) by Picardeau et al. (13) and the pattern of the major *M. kansasii* group with PFGE *VspI* pattern M found in Japanese patients by Iinuma et al. (6)

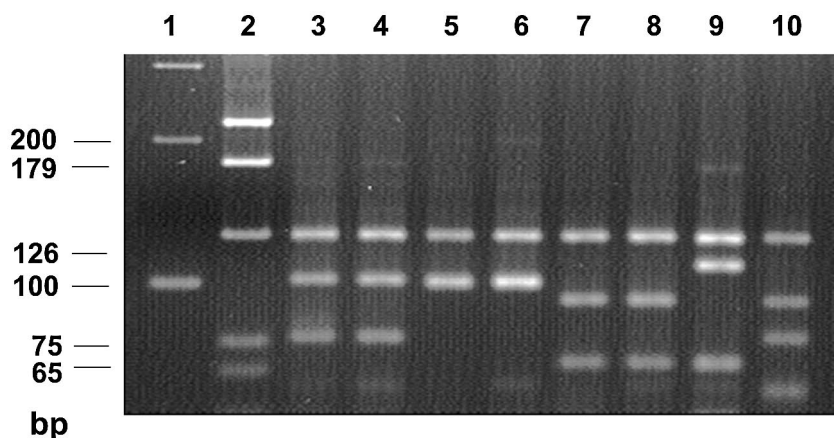


FIG. 1. Examples of the five PRA patterns (genotypes) seen with *Hae*III digestion of the *hsp*-65 gene sequence (441 bp) of *M. kansasii* as described by Picardeau et al. (13). Lanes 1 and 2, 100-bp and pGem markers, respectively. Lanes 3 and 4, genotype 1 (*M. kansasii* 404 and 340, respectively). Lanes 5 and 6, genotype 2 (*M. kansasii* 234 and 335, respectively). Lanes 7 and 8, genotype 3 (*M. kansasii* 241 and 343, respectively). Lane 9, genotype 4 (*M. kansasii* 339). Lane 10, genotype 5 (*M. kansasii* 334).

showed they were indistinguishable. Type strain ATCC 35775 also showed the same major *Dra*I pattern, and another type strain, ATCC 12478, showed only one band difference.

With *Ase*I, 30 of 71 isolates (42.3%) generated a common pattern (pattern Aa), another 19 isolates were in five other clusters, and only 22 isolates (31.0%) had unique patterns. Compared with the most common pattern (pattern Aa), 25 isolates with 14 patterns generated one to three band differences, and eight isolates with five patterns generated four to six band differences. Thus, 63 of 71 isolates (88.7%) were clonal. Lane-by-lane comparison in the same gel showed that our common *Ase*I pattern (Aa) was indistinguishable from the pat-

terns produced by the major group of French isolates (genotype Ia) (13) and had only one band difference with the most frequently observed pattern (type M) in Japan (6) (Fig. 2). Figure 2 also showed that isolates with the same *Dra*I pattern may produce differences with *Ase*I digestion (Fig. 2, lanes 4 to 6).

With *Xba*I, results similar to those with *Dra*I and *Ase*I were obtained (Table 2). Thus, with each of the three restriction enzymes, 32.4 to 43.7% of the isolates fell into a single cluster showing an indistinguishable pattern, with 88.7 to 95.8% of the isolates considered clonal. Most PFGE patterns were highly similar; differences between patterns often involved only one or two bands (Fig. 3 to 5). Some isolates showed indistinguishable patterns with one enzyme but different patterns with another enzyme. These isolates were considered different. Thus, with different combinations of the three enzyme patterns, the strain differentiation was improved. By combining the *Ase*I, *Dra*I, and *Xba*I patterns, 57 different patterns were identified within 71 *M. kansasii* isolates. The use of *Xba*I helped to further subdivide isolates, but the gel patterns were difficult to analyze because of the large number of bands. Counting only the bands larger than 97.0 kb on electrophoresis gels simplified the analysis of *Xba*I patterns.

Even with different combinations of restriction enzyme digestion patterns, there were still 20 isolates in six clusters each having indistinguishable patterns. Forty-four isolates from Texas showed 33 patterns, and 25 isolates from other states had 24 patterns (Table 1).

Of the 44 clinical isolates found in Texas, 26 (59.1%) had the common *Dra*I pattern (pattern Da), while only 4 of 25 isolates (16.0%) found in other states had this pattern ($P < 0.005$). A higher percentage of Texas isolates had the common *Dra*I pattern. However, 18 of 44 Texas isolates (40.9%) and 10 of 25 non-Texas isolates (40.0%) showed the common *Ase*I pattern (pattern Aa) ($P > 0.75$). Our result also showed that 44 isolates from Texas had 33 patterns and 25 isolates from other states had 24 patterns (Table 1) ($P > 0.05$). Isolates within a single

TABLE 2. PFGE patterns and isolate distribution

Restriction endonuclease and pattern(s) ^{a,b}	No. of isolates ^c
<i>Ase</i> I	
Aa	30 (42.3)
Ac	6 (8.5)
Ag	6 (8.5)
Ad	3 (4.2)
Ab, An	2 ^d (2.8)
Other (22 different patterns)	1 ^d (1.4)
<i>Dra</i> I	
Da	31 (43.7)
Dd	5 (7.0)
Dh	4 (5.6)
Dm, Dw	2 ^d (2.8)
Other (27 different patterns)	1 ^d (1.4)
<i>Xba</i> I	
Xa	23 (32.4)
X1	4 (5.6)
Xt	4 (5.6)
Xb, Xf, Xd, Xi, Xk, Xr, Xs, Xdd	2 ^d (2.8)
Other (24 different patterns)	1 ^d (1.4)

^a Restriction patterns were generated for 71 isolates.

^b The total numbers of patterns observed for the restriction endonuclease were as follows: for *Ase*I, 28 patterns; for *Dra*I, 32 patterns; and for *Xba*I, 35 patterns.

^c Values in parentheses are percentages.

^d Number of isolates with each different pattern.

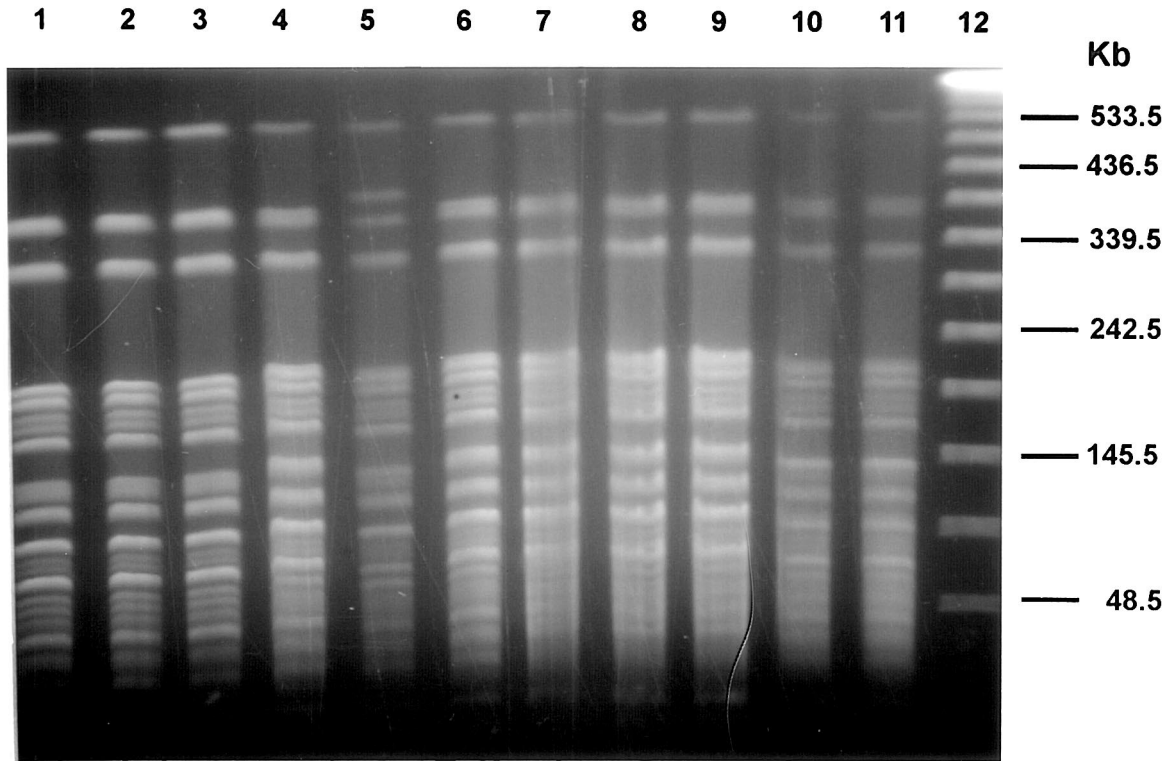


FIG. 2. Comparison of PFGE patterns of major *M. kansasii* groups seen in France, the United States, and Japan, with *AseI* digestion. Lanes: 1 to 3, French isolates (W47, C5, and W45) with major pattern Ia (13); 4 to 6, isolates in the United States (*M. kansasii* 88, 223, and 224) with major pattern Da; 7 to 9, Japanese isolates (65190, 95265, and 95315) of major group with *VspI* pattern M (6); 10, ATCC 35775; 11, ATCC 12478; 12, lambda DNA standard.

state (Texas) were more closely related than those from different states. However, the geographic sources of the isolates with the same common patterns were not actually limited in a single state.

To determine if PFGE patterns over time were stable, 19 isolates from seven patients were chosen. Each patient had two to six isolates, with the time between specimen recovery ranging from 1 to 28.5 months. The results showed that all of the isolates from the same patients over time gave indistinguishable PFGE patterns, with no changes in any visible band.

DISCUSSION

In recent years a number of molecular-based techniques for strain identification and comparison have been developed. These techniques have greatly facilitated investigation of *M. kansasii* identification. Picardeau et al. (13) used five fingerprinting methods, including RFLP analysis with the major polymorphic tandem repeat probe and the *IS1652* probe, PFGE, amplified fragment length polymorphism analysis, and PRA of the *hsp-65* gene. All of these methods showed excellent

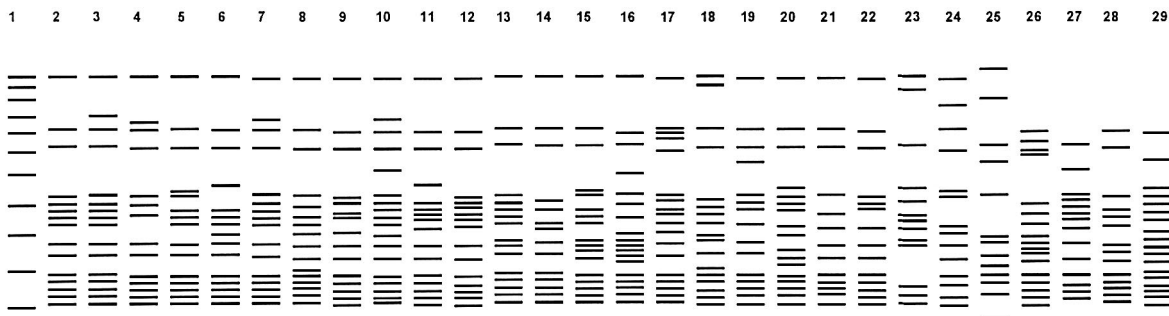


FIG. 3. Schematic representation of PFGE patterns with *AseI* digestion. Lane 1, lambda DNA standards; lane 2, common pattern Aa; all other lanes, clinical isolates with different patterns.

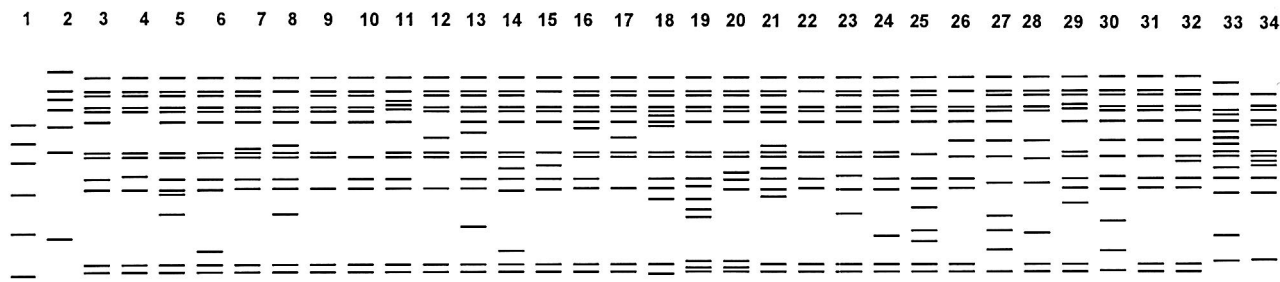


FIG. 4. Schematic representation of PFGE patterns with *DraI* digestion. Lane 1, lambda DNA standards; lane 2, yeast DNA standards; lane 3, common pattern Da; lane 23, ATCC 12478; all other lanes, clinical isolates with different patterns.

typeability and reproducibility. The five methods revealed independent polymorphisms. Nevertheless, the genotypes indicated by each method were in agreement. PRA defined five genotypes, while PFGE allowed recognition of subgroups within the genotypes.

Picardeau et al. (13) studied 63 *M. kansasii* isolates, including 38 clinical strains and 24 water isolates (all recovered in France) and 1 reference strain. The most common of the five genotypes, genotype I, included 25 of 63 strains studied (39.7%). Among clinical isolates only, 16 of 38 (42.1%) belonged to genotype I. Alcaide et al. (2) found the same five genotypes among 276 *M. kansasii* isolates from all over Europe. Genotype I was present among 109 of 163 clinical isolates (66.9%). A recent Swiss national survey (18) and studies of isolates from Bilbao, Spain (5a), found similar results. In the present study, 78 of 81 isolates (96.3%) recovered from the United States had an indistinguishable PRA pattern which matched PRA genotype I of Picardeau et al. (13) and Alcaide et al. (2). The isolates with the eight most common PFGE types from Japan (6) belonged to genotype I as well. Thus, genotype I is the predominant subspecies of *M. kansasii* among clinical

isolates worldwide in all studies to date. Other genotypes are prevalent in Europe and were identified much less commonly in the present study in the United States.

Using PFGE and the restriction enzyme *DraI*, Picardeau et al. (13) found the most prevalent subgroup to be subgroup Ia, which was found in genotype I. This subgroup consisted of 17 clinical isolates and 1 water isolate, and represented 28.6% of the strains studied. Iinuma et al. (6) used restriction enzyme *VspI* and found that 22 of 84 clinical isolates (26.2%) and ATCC 12478 produced their most frequently observed PFGE pattern (type M) in Japan. In our PFGE study, both *DraI* and *AseI* (an isoschizomer of *VspI*) were used. The most prevalent PFGE pattern with *DraI* was pattern Da, and it comprised 31 of 71 strains (43.7%). The most common *AseI* pattern was pattern Aa, which was seen in 30 of 71 (42.3%) of the strains. Lane-by-lane comparison on the same electrophoresis gel showed that the isolates with common PFGE patterns found in the United States (pattern Da), France (pattern Ia), and Japan (type M) gave the same patterns with *DraI*. The comparison also showed that with *AseI* our common pattern (pattern Aa) was indistinguishable from the pattern produced by the major

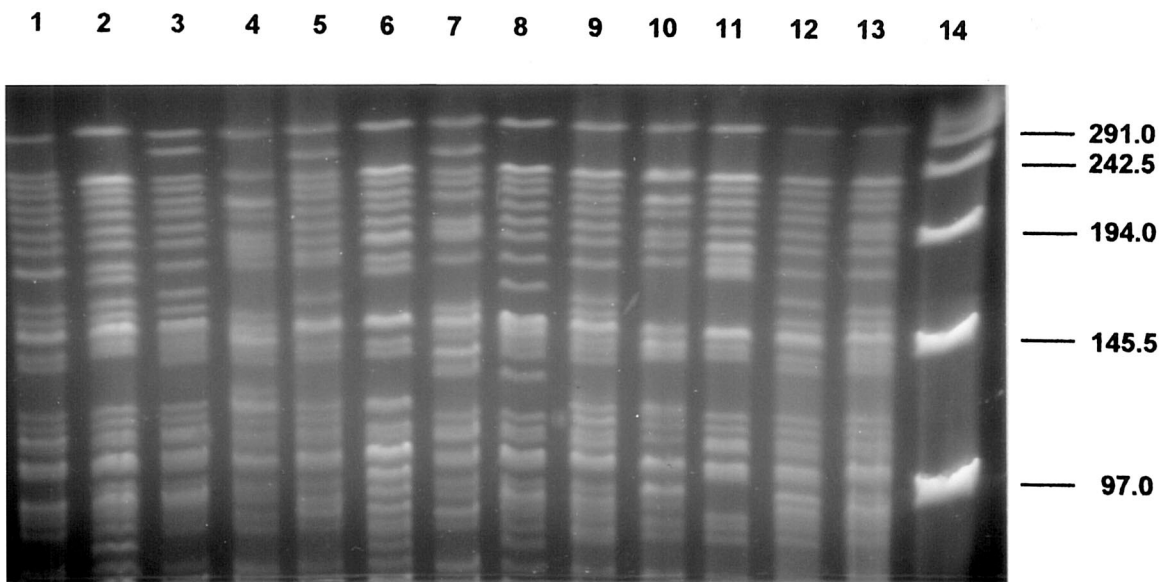


FIG. 5. PFGE patterns of *M. kansasii* isolates with *XbaI* digestion. Lanes 1 through 11, clinical U.S. isolates; lane 12, ATCC 12478; lane 13, ATCC 35775; lane 14, lambda DNA standard.

group of French isolates (subgroup Ia) and had only one band difference with the most frequently observed pattern (type M) in Japan. Thus, a single common PFGE genotype with *DraI* or *VspI*, representing approximately 30% of studied strains, has been observed in all PFGE studies of *M. kansasii* to date.

In our study, the isolates which had the common *DraI* pattern (pattern Da) were not in entire accordance with the isolates which produced the common *AseI* pattern (pattern Aa). As shown in Fig. 2, isolates with the same *DraI* pattern may exhibit minor band differences with *AseI* digestion. Of the 31 isolates with the common *DraI* pattern (pattern Da), only 17 had both the common *DraI* pattern (pattern Da) and the common *AseI* pattern (pattern Aa). The geographic sources of the 17 isolates were mainly in Texas. Only 3 of the 17 isolates were from states other than Texas (one from Arkansas, one from Alabama, and one from North Dakota). When the *XbaI* results were combined with the *DraI* and *AseI* results, 7 of the 17 isolates were still indistinguishable. The seven isolates were from three states (five from Texas, one from Arizona, and one from North Dakota) and were discovered in 1987 to 1997. There was no evidence of an epidemiologic relationship between these seven isolates, considering the sources and the years that the isolates were discovered.

Inuma et al. (6) found that the largest group (type M) within 85 strains tested by PFGE with *VspI* contained 22 clinical isolates. These isolates were collected from four different districts in Japan. In the study by Alcaide et al. (2), 11 of 51 genotype I clinical isolates from Spain (21.5%) showed a PFGE pattern indistinguishable from those of both type strains and one of *M. kansasii* genotype I isolates from Switzerland. Inuma et al. (6) analyzed the 16S rRNA gene sequences of six strains included in their study and verified that four strains, including one with a type M pattern, had sequences identical to that of an *M. kansasii* genotype isolated principally in Europe (presumably subspecies type I). These findings were quite similar to the results in the present study.

Using an *M. kansasii*-specific DNA probe (pMK1-9), Ross et al. (15) tested 105 *M. kansasii* isolates from Australia, Belgium, Japan, South Africa, and Switzerland. Eighty-five probe-positive strains shared most bands in common, with the only very minor variations occurring in the highest-molecular-weight band. This indicated that within the typical *M. kansasii* group, genetic variability is low. Picardeau et al. (13) found that within each of the five fingerprinting genotype PFGE patterns seen in French isolates, there was a relatively low degree of genotypic divergence. In our study, the PFGE patterns showed that approximately 90% of the isolates collected from 19 states were clonal. Differences between PFGE patterns often involved only one or two bands. Even with different combinations of restriction digestion patterns, there were still 20 isolates in six clusters having indistinguishable patterns. All of these findings indicated that *M. kansasii* isolates had a marked clonal structure, especially in the most prevalent genotype found in humans, regardless of the geographic sources of the isolates worldwide.

Tenover et al. (20) proposed PFGE criteria for interpreting the relatedness of isolates epidemiologically involved in outbreaks. By their definition, if two or three fragment differences compared with the outbreak pattern are found, the isolate is probably part of the outbreak. If four to six fragment differences are found, the isolate is possibly part of the outbreak.

The isolate is considered different only if more than six band differences are seen (presumably with any given enzyme). Obviously, however, these definitions could not be used for investigating outbreaks involving *M. kansasii*, which has a very tight clonal structure within each subspecies. Isolates belonging to unusual subspecies or with unique PFGE patterns would be relatively easily identified as different by the definition of Tenover et al. Isolates belonging to subspecies I would be difficult to compare.

A key component of studying organism relatedness is the stability of the organisms in individual patients or outbreaks. Inuma et al. (6) tested additional strains isolated at different times from two patients. The *VspI* PFGE patterns of each of two strains from the two patients remained unchanged. Our results also showed that multiple isolates from the same patients over time gave indistinguishable PFGE patterns. However, additional studies are needed to establish this observation.

This is the first subspecies molecular characterization of *M. kansasii* isolates from the United States. The study demonstrates that genotype I is the predominant subspecies, as it is in Europe. PFGE with *DraI* and *AseI* showed marked clonality of the clinical isolates, with patterns indistinguishable from or close to those from Europe and Japan. Epidemiological studies of strain relatedness of *M. kansasii* in suspected outbreaks will be difficult given the higher pattern similarity of most clinical isolates by PFGE, a finding similar to that for other nontuberculous mycobacteria such as *Mycobacterium celatum* type I and *Mycobacterium xenopi*.

The majority of U.S. isolates chosen for this study were rifampin resistant. Most had detailed histories that have previously been published (22) and that clearly established them as definite pathogens. Such a selection factor could have biased the molecular genotypes observed if one genotype more than another tends to be associated with rifampin resistance. We have no evidence of such a genotype difference, and the same genotype with each restriction enzyme was the predominant one with both rifampin-resistant and -susceptible isolates (data not shown).

The finding of a single clone of *M. kansasii* being responsible for most cases of human disease is surprising for an environmental species. Studies of most environmental species, such as *M. avium* complex, have shown almost as many PFGE patterns as there are isolates (12, 23). A few species, such as *M. xenopi*, appear to have limited genetic diversity like *M. kansasii*, however, suggesting that specific factors may select for one or more specific genotypes. These could be colonization factors within piped water systems or, more likely, could be related to one or more virulence factors present in this clone of organisms. This is supported by the finding that five subspecies and multiple organism clones are present in tap water (2, 13) but that only one subspecies and one clone are responsible for up to 90% of clinical disease. It is also possible that *M. kansasii* is a relatively recently derived species or that the time for genetic change is very slow. The presence of one or more virulence factors in this clone seems the most likely explanation, although little has been learned about these factors.

REFERENCES

1. Ahn, C. H., J. R. Lowell, G. D. Onstad, E. H. Shuford, and G. A. Hurst. 1979. A demographic study of disease due to *Mycobacterium kansasii* or *M. intracellulare-avium* in Texas. *Chest* 75:120-125.

2. Alcaide, F., I. Richter, C. Bernasconi, B. Springer, C. Hagenau, R. Schulze-Röbbecke, E. Toptoli, R. Martin, E. C. Böttger, and A. Telenti. 1997. Heterogeneity and clonality among isolates of *Mycobacterium kansasii*: implications for epidemiological and pathogenicity studies. *J. Clin. Microbiol.* **35**:1959–1964.
3. Block, K. C., L. Zwerling, M. J. Pletcher, J. A. Hahn, J. L. Gerberding, S. M. Ostroff, D. J. Vugia, and A. L. Reingold. 1998. Incidence and clinical implications of isolation of *Mycobacterium kansasii*: results of a 5-year, population-based study. *Ann. Intern. Med.* **129**:698–704.
4. Carpenter, J. L., and J. M. Parks. 1991. *Mycobacterium kansasii* infections in patients positive for human immunodeficiency virus. *Rev. Infect. Dis.* **13**:789–796.
5. Corbett, E. L., M. Hay, G. J. Churchyard, P. Herselman, T. Clayton, B. G. Williams, R. Hayes, D. Mulder, and K. M. De Cock. 1999. *Mycobacterium kansasii* and *M. scrofulaceum* isolates from HIV-negative South African gold miners: incidence, clinical significance and radiology. *Int. J. Tuberc. Lung Dis.* **3**:501–507.
- 5a. Gaafar, A., M. J. Unzaga, R. Cisterna, F. E. Clavo, E. Urra, R. Ayarza, and G. Martín. 2003. Evaluation of a modified single-enzyme amplified-fragment length polymorphism technique for fingerprinting and differentiating of *Mycobacterium kansasii* type I isolates. *J. Clin. Microbiol.* **41**:3846–3850.
6. Iinuma, Y., S. Ichihama, Y. Hasegawa, K. Shimokata, S. Kawahara, and T. Matsushima. 1997. Large-restriction-fragment analysis of *Mycobacterium kansasii* genomic DNA and its application in molecular typing. *J. Clin. Microbiol.* **35**:596–599.
7. Jacobson, K. L., R. Teira, H. I. Libshitz, I. Raad, K. V. I. Rolston, J. Tarrand, and E. Whimbey. 2000. *Mycobacterium kansasii* infection in patients with cancer. *Clin. Infect. Dis.* **30**:965–969.
8. Kent, P. T., and G. P. Kubica. 1985. Public health mycobacteriology: a guide for the level III laboratory. Centers for Disease Control and Prevention, Atlanta, Ga.
9. Klein, J. L., E. L. Corbett, P. M. Slade, R. F. Miller, and R. J. Coker. 1998. *Mycobacterium kansasii* and human immunodeficiency virus co-infection in London. *J. Infect.* **37**:252–259.
10. Lillo, M., S. Orengo, P. Cernoch, and R. L. Harris. 1990. Pulmonary and disseminated infection due to *Mycobacterium kansasii*: a decade of experience. *Rev. Infect. Dis.* **12**:760–767.
11. Lortholary, O., F. Deniel, P. Boudon, M. P. Le Pennec, M. Mathieu, M. Soilleux, C. Le Pendeven, P. Loiseau, V. Vincent, D. Valeyre, et al. 1998. *Mycobacterium kansasii* infection in a Paris suburb: comparison of disease presentation and outcome according to human immunodeficiency virus status. *Int. J. Tuberc. Lung Dis.* **3**:68–73.
12. Mazurek, G. H., S. Hartman, Y. Zhang, B. A. Brown, J. S. R. Hector, D. Murphy, and R. J. Wallace, Jr. 1993. Large DNA restriction fragment polymorphism in the *Mycobacterium avium-M. intracellulare* complex: a potential epidemiologic tool. *J. Clin. Microbiol.* **31**:390–394.
13. Picardeau, M., G. Prod'hom, L. Raskine, M. P. LePennec, and V. Vincent. 1997. Genotypic characterization of five subspecies of *Mycobacterium kansasii*. *J. Clin. Microbiol.* **35**:25–32.
14. Pintado, V., E. Dómeç-Mampaso, P. Martín-Dávila, J. Cobo, E. Navas, C. Querada, J. Fortún, and A. Guerrero. 1999. *Mycobacterium kansasii* infection in patients infected with the human immunodeficiency virus. *Eur. J. Clin. Microbiol. Infect. Dis.* **18**:582–586.
15. Ross, B. C., K. Jackson, M. Yang, A. Sievers, and B. Dwyer. 1992. Identification of a genetically distinct subspecies of *Mycobacterium kansasii*. *J. Clin. Microbiol.* **30**:2930–2933.
16. Steadham, J. E. 1980. High-catalase strains of *Mycobacterium kansasii* isolated from water in Texas. *J. Clin. Microbiol.* **11**:496–498.
17. Steingrube, V. A., J. L. Gibson, B. A. Brown, Y. Zhang, R. W. Wilson, M. Rajagopalan, and R. J. Wallace, Jr. 1995. PCR amplification and restriction endonuclease analysis of a 65-kilodalton heat shock protein gene sequence for taxonomic separation of rapidly growing mycobacteria. *J. Clin. Microbiol.* **33**:149–153.
18. Taillard, C., G. Greub, R. Weber, G. E. Pfyffer, T. Bodmer, S. Zimmerli, R. Frei, S. Bassetti, P. Rohner, J. Piffaretti, E. Bernasconi, J. Bille, A. Telenti, and G. Prod'hom. 2003. Clinical implication of *Mycobacterium kansasii* species heterogeneity: Swiss national survey. *J. Clin. Microbiol.* **41**:1240–1244.
19. Telenti, A., F. Marchesi, M. Balz, F. Bally, E. C. Böttger, and T. Bodmer. 1993. Rapid identification of mycobacteria to species level by polymerase chain reaction and restriction enzyme analysis. *J. Clin. Microbiol.* **31**:175–178.
20. Tenover, F. C., R. D. Arbeit, R. V. Goering, P. A. Mickelsen, B. E. Murray, D. H. Persing, and B. Swaminathan. 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field electrophoresis: criteria for bacterial strain typing. *J. Clin. Microbiol.* **33**:2233–2239.
21. Wallace, R. J., Jr., Y. Zhang, B. A. Brown, V. Fraser, G. H. Mazurek, and S. Maloney. 1993. DNA large restriction fragment pattern of sporadic and epidemic nosocomial strains of *Mycobacterium chelonae* and *Mycobacterium abscessus*. *J. Clin. Microbiol.* **31**:2697–2701.
22. Wallace, R. J., Jr., D. Dunbar, B. A. Brown, G. Onyi, R. Dunlap, C. H. Ahn, and D. T. Murphy. 1994. Rifampin-resistant *Mycobacterium kansasii*. *J. Infect. Dis.* **18**:736–743.
23. Wallace, R. J., Jr., Y. Zhang, B. A. Brown, D. Dawson, D. T. Murphy, R. Wilson, and D. Griffith. 1998. Polyclonal *Mycobacterium avium* complex infections in patients with nodular bronchiectasis. *Am. J. Respir. Crit. Care Med.* **158**:1235–1244.