JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1997, p. 3015–3020 Vol. 35, No. 12
0095-1137/97/$04.00+0

IS*6110* Fingerprinting of Drug-Resistant *Mycobacterium tuberculosis* Strains Isolated in Germany during 1995

STEFAN NIEMANN, SABINE RÜSCH-GERDES,* AND ELVIRA RICHTER

Forschungszentrum Borstel, National Reference Center for Mycobacteria, D-23845 Borstel, Germany

Received 9 July 1997/Returned for modification 19 August 1997/Accepted 6 September 1997

The epidemiological relatedness of drug-resistant *Mycobacterium tuberculosis* strains isolated in Germany in 1995 was evaluated by the standardized IS*6110* fingerprinting method. Altogether, 196 *M. tuberculosis* isolates from 167 patients were analyzed. A large degree of IS*6110* polymorphism was found, ranging from 1 to 20 copies. Multiple isolates from one patient generally remained stable over a period of up to 1 year. However, one strain showed an additional fragment 7 months after the first isolate was obtained. Isolates from 55 patients (33%) showed identical fingerprint patterns or fingerprint patterns that differed only in one band, and thus they were clustered in 22 fingerprint groups. Specific transmission links could be established between members of four groups, e.g., transmission by family contacts. In one case, transmission of a multidrug-resistant strain to a patient initially infected with a drug-susceptible strain could be shown. Besides these fingerprint groups, 30 of the 167 isolates (approximately 18%) could be grouped in two fingerprint clusters with a similarity of at least 78%. Approximately 60% of the patients of these two clusters were known to be immigrants from the former Soviet Union, and one patient is still living in Belarus. In conclusion, our results indicate that (i) transmission of drug-resistant strains contributes substantially to the emergence of drug-resistant tuberculosis in Germany and (ii) drug-resistant *M. tuberculosis* strains were presumably carried over from the former Soviet Union to Germany by immigrants.

Germany is a country with a low incidence of tuberculosis, namely, 15 cases per 100,000 in 1995 (6). However, tuberculosis remains a major source of morbidity and mortality throughout the world and is increasing worldwide (19, 35). In recent years, the treatment of tuberculosis has become complicated by the rising emergence of drug-resistant strains of *Mycobacterium tuberculosis* (5, 14, 35). The proportion of drug-resistant *M. tuberculosis* strains in Germany remained stable at approximately 5% between 1991 and 1995 (6). However, in our laboratory the proportion of drug-resistant *M. tuberculosis* strains rose from 5.9% in 1993 to 8.4% in 1995.

Recently, some epidemiological studies have been performed to analyze the spread and transmission of drug-resistant *M. tuberculosis* strains (1, 7, 8, 10, 11, 17, 18, 20). These studies indicated that drug-resistant *M. tuberculosis* strains were transmitted as frequently as drug-susceptible strains, and recent transmission of drug-resistant strains was assumed to contribute substantially to the increase of tuberculosis. For Germany, no epidemiological data based on molecular methods concerning the transmission routes of drug-resistant strains were available.

The basis for the epidemiological analysis of tuberculosis is the reliable identification and differentiation of bacterial isolates on the strain level (16, 33). For *M. tuberculosis* the IS*6110* fingerprinting method has been suggested as a standard tool for the characterization of bacterial strains (29). IS*6110*, a transposable sequence belonging to the IS3 family (13), is found in virtually all members of the *M. tuberculosis* complex and is apparently restricted to this group of organisms (4, 27).

To date, IS*6110* fingerprinting has been successfully used to confirm laboratory cross-contaminations (21), to answer the question of endogenous reactivation versus exogenous reinfection (20, 22), and to trace small-scale outbreaks of tuberculosis, as well as outbreaks of drug-resistant tuberculosis, in a large variety of settings, such as hospitals, communities, and shelters for the homeless (2, 3, 9, 11, 32). By taking advantage of computerized processing and analysis of fingerprint data, a rising number of population-based epidemiological studies of tuberculosis using molecular methods have been reported (1, 12, 23–25, 28, 34, 36).

In this work, we performed the first study to analyze the epidemiology of drug-resistant tuberculosis in Germany by molecular methods. All drug-resistant *M. tuberculosis* strains sent to the German National Reference Center for Mycobacteria in 1995, representing at least one-third of all cases in Germany, have been investigated by fingerprinting with IS*6110*. On the basis of these data, we aimed to find possible epidemiological links among the isolates and to identify transmission routes of drug-resistant *M. tuberculosis* strains in Germany.

MATERIALS AND METHODS

Bacterial strains. One hundred ninety-six drug-resistant *M. tuberculosis* strains received by the National Reference Center for Mycobacteria during 1995 were investigated by DNA fingerprinting. Drug resistance means resistance to at least one drug; multidrug resistance means resistance to at least isoniazid and rifampin. The isolates were obtained from 164 patients living in Germany, one patient living in Belarus, and two patients living in Saudi Arabia. *M. tuberculosis* Mt.14323, which served as a control strain in each hybridization experiment, was obtained from the National Institute of Public Health and Environmental Protection (Bilthoven, The Netherlands).

DNA techniques. Extraction of DNA from mycobacterial strains and DNA fingerprinting using IS*6110* as a probe were performed by the standardized protocol described by van Embden et al. (29) and van Soolingen et al. (30). Briefly, for isolation of genomic DNA, *M. tuberculosis* strains were grown on Löwenstein-Jensen slants for 3 to 5 weeks. All bacterial cells from one slant were transferred in 400 μl of TE buffer (0.01 M Tris-HCl, 0.001 M EDTA [pH 8]), and the solution was heated at 80°C for 20 min to kill the bacteria. Fifty microliters of lysozyme (10 mg/ml) were added, and the tube was incubated for 1 h at 37°C. Seventy microliters of sodium dodecyl sulfate (SDS; 10%) and 6 μl of proteinase K (10 mg/ml) were added, and the mixture was incubated for 10 min at 65°C. A 100-μl volume of 5 M NaCl and of a CTAB (*N*-cetyl-*N*,*N*,*N*-trimethyl ammonium bromide)-NaCl solution (4.1 g of NaCl and 10 g of CTAB per 100 ml) was added.

* Corresponding author. Mailing address: Forschungszentrum Borstel, National Reference Center for Mycobacteria, Parkallee 18, D-23845 Borstel, Germany. Phone: (49) 4537-188211. Fax: (49) 4537-188311.

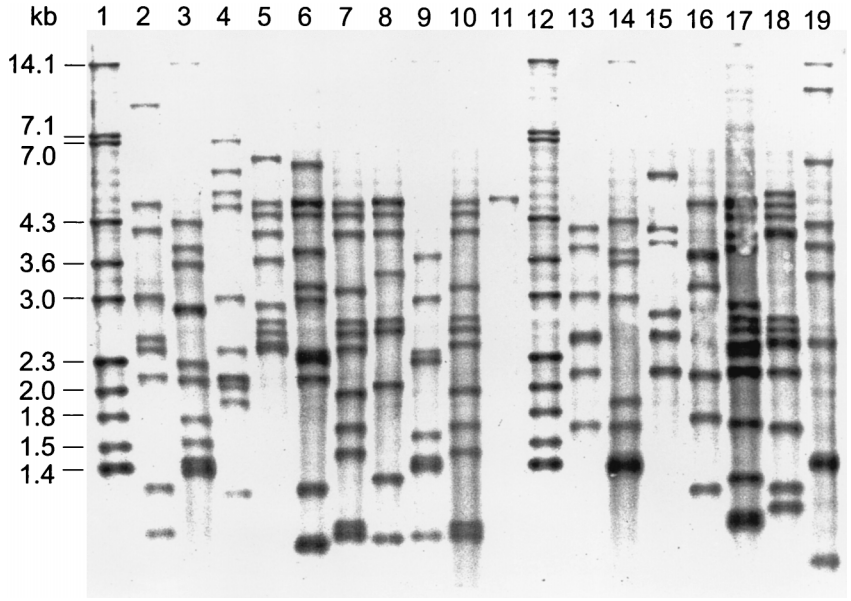

FIG. 1. IS*6110* DNA fingerprint patterns of 17 drug-resistant *M. tuberculosis* strains isolated from Germany and of *M. tuberculosis* reference strain Mt.14323 (lanes 1 and 12). *Pvu*II-digested chromosomal DNA was hybridized with a 245-bp PCR fragment of IS*6110*.

The cups were vortexed and incubated for 10 min at 65°C. An equal volume of chloroform-isoamyl alcohol (24:1) was added, the mixture was centrifuged for 5 min at 12,000 × *g*, and the aqueous supernatant was carefully transferred into a fresh tube. The total DNA was precipitated with isopropanol and redissolved in an appropriate volume of distilled water. For fingerprinting, *Pvu*II-digested total DNA was separated by using horizontal 1% agarose gels in Tris-acetate buffer and was vacuum blotted onto a nylon membrane. Hybridization of the DNA was carried out with a 254-bp internal PCR fragment of IS*6110* (amplified with the primer pair INS1 and INS2) as a probe by using the ECL system (Amersham, Little Chalfont, Buckinghamshire, United Kingdom). *Pvu*II-digested total DNA of reference strain Mt.14323 was used in each Southern blot experiment as an external size standard.

Computer analysis. IS*6110* fingerprint patterns of mycobacterial strains were analyzed with Gelcompar software (Windows 95, version 4.0; Applied Maths, Kortrijk, Belgium) as described previously (12). Autoradiograms were digitized by using a scanner with an optical resolution of 190 dpi (HP ScanJet 4p; Hewlett-Packard, Greely, Colo.). The sizes of IS*6110* restriction fragment length polymorphism (RFLP) fragments were calculated by comparison of their mobilities with those of a set of internal markers of known molecular sizes by superimposing the IS*6110* autoradiogram on the autoradiogram of the internal markers (29). This procedure allows the positions of each IS*6110* fingerprint band to normalize irrespective of the experimental variations in different fingerprinting experiments. The accuracy of the normalization procedure was controlled by comparing the IS*6110* fingerprint patterns of reference strain Mt.14323, which were present as an external marker in the first and last lane on each autoradiogram, with those stored in the database. The fingerprint patterns were analyzed for similarity by using the Dice coefficient, and a dendrogram was calculated with the unweighted-pair group method using average linkage (UPGMA) according to the supplier's instructions. Band positions were determined by using the peak-finder function of the Gelcompar software and were controlled manually by comparison with the original IS*6110* autoradiogram.

RESULTS

IS*6110* fingerprint analysis of drug-resistant *M. tuberculosis* isolates. In this study 196 drug-resistant *M. tuberculosis* isolates from 167 patients were analyzed by the standardized DNA fingerprinting method described by van Embden et al. (29), with IS*6110* as a genetic marker. All isolates were identified as *M. tuberculosis* with gene probes (ACCU Probe; Gen-Probe, San Diego, Calif.) and biochemical tests. The generated IS*6110* fingerprint patterns were highly variable (Fig. 1). All 196 IS*6110* fingerprint patterns were digitized, stored in a fingerprint database, and analyzed for similarity by using the Dice coefficient. Based on these data, the UPGMA method was used to calculate a dendrogram showing the degree of relatedness among strains (Fig. 2). All fingerprint patterns of the reference strain included as a control in each hybridization gel showed a similarity of 100% in the dendrogram and were consequently clustered in one fingerprint group (Fig. 2).

In addition, 53 multiple isolates from 24 patients were analyzed to further test the accuracy of the applied method. In all cases, specimens from one patient could clearly be identified and differentiated from the various other fingerprint patterns. These results demonstrate that the standardized IS*6110* fingerprinting used is well suited for the comparison of large numbers of fingerprint patterns. *M. tuberculosis* strains isolated in succession from 23 patients during 1995 had identical IS*6110* patterns. One isolate from a further patient showed an additional fragment 7 months after the first isolate was obtained (seven identical bands; data not shown). This additional fragment probably derives from a transposition event.

The number of IS*6110* copies per isolate varied from 1 to 20. The majority, 147 of the 167 strains (88%), contained 6 to 16 IS*6110* copies, with a mean of 10 bands (data not shown). No strains lacking IS*6110* were found. One strain showed only a single copy, and three strains each had two IS*6110* copies. Of these strains, two were directly obtained from patients living in Saudi Arabia and two were obtained from patients known to be born in Africa.

One hundred thirty-four distinct IS*6110* fingerprint patterns were observed in the 167 analyzed isolates (multiple isolates from one patient excluded). Banding patterns differing only in one band were considered to be the same. Of these 134 patterns, 112 patterns were observed only once each in this investigation, whereas 22 were shared by two or more isolates (fingerprint groups A through V; Table 1). Strains of fingerprint groups G, P, and Q were isolated on the same day in the same laboratory. Thus, their identical fingerprint patterns may be due to cross-contamination and were excluded from further analyses. For the other strains clustered in fingerprint groups, it is very unlikely that their identical fingerprint patterns were due to cross-contamination, because these strains were isolated on different days. Groups of strains with identical or nearly identical fingerprint patterns varied in size from two to

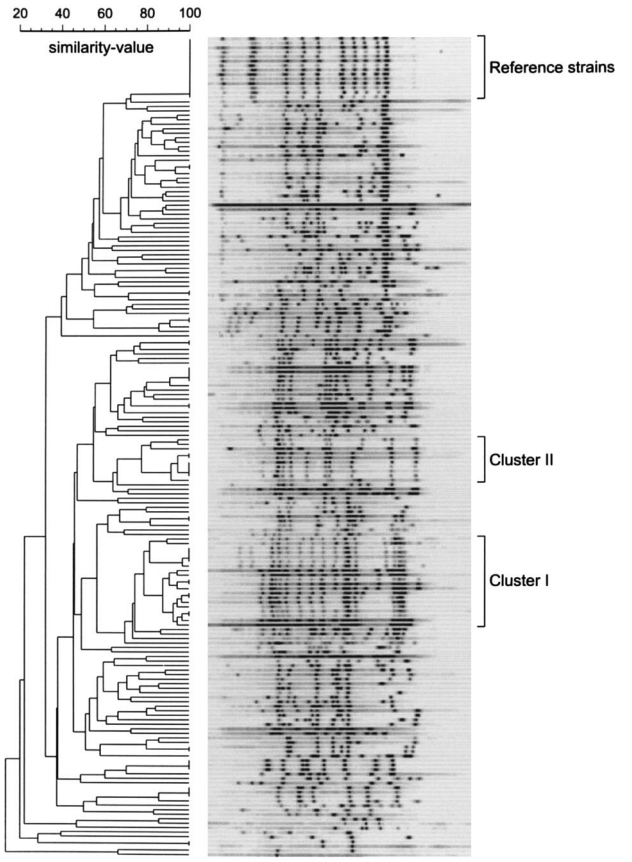

FIG. 2. IS*6110* DNA fingerprint patterns of the 167 drug-resistant *M. tuberculosis* strains and the corresponding dendrogram (multiple isolates from one patient excluded). Banding patterns are ordered by similarity. The position of each IS*6110* band is normalized so that banding patterns of all strains are mutually comparable. Scale depicts similarity of patterns calculated as described in Materials and Methods. Cluster of identical strains on top represents the patterns of the reference strain Mt.14323, which was present in two lanes on each autoradiogram to test the accuracy of the fingerprint database. Two clusters of related strains with fingerprint patterns showing a similarity of more than 78% were designated cluster I and II.

TABLE 1. IS*6110* fingerprint groups among 167 patients with drug-resistant tuberculosis

Group	Patient	Drug resistance pattern[a]	No. of IS*6110* bands
A	1	INH, RMP, PZA, PTH	2
	2	INH, RMP	2
B	3	INH, RMP, PZA	11
	4	INH, RMP, PZA	11
C	5	INH, RMP, EMB	17
	6	INH, RMP, PZA	17
	7	INH, RMP, PZA	18
D	8	INH, EMB, RMP, PZA	15
	9	INH	15
E	10	INH, EMB	15
	11	INH, EMB	15
F	12	INH, EMB, RMP, PTH	16
	13	INH, EMB, RMP, PTH	16
	14	INH, EMB, RMP, PTH	16
	15	INH, RMP	16
	16	INH, RMP, PZA	17
G	17	INH	12
	18	INH	12
H	19	INH, EMB, RMP, PZA, PTH	10
	20	INH, EMB, RMP	10
	21	INH, EMB, RMP, PZA, PTH	10
	22	INH, EMB, RMP, PZA, PTH	10
I	23	INH	9
	24	INH	9
	25	INH, RMP	10
J	26	INH	10
	27	INH, EMB, RMP, PTH	9
K	28	INH, EMB, RMP, PZA	8
	29	INH, EMB, RMP	8
L	30	INH, PTH	6
	31	INH	6
	32	INH, RMP	6
M	33	INH, RMP	11
	34	INH	11
N	35	INH, EMB, RMP, PZA, PTH	11
	36	INH, EMB, RMP, PZA, PTH	11
	37	INH, EMB, RMP, PZA, PTH	11
	38	INH, EMB, RMP, PZA, PTH	11
O	39	INH, EMB, RMP, PZA, PTH	5
	40	INH, RMP, PZA, PTH	5
P	41	PTH	8
	42	PTH	8
	43	PTH	8
Q	44	RMP	12
	45	RMP	12
R	46	INH	18
	47	INH, EMB	17
S	48	INH, RMP	15
	49	INH, RMP	16
T	50	INH, PTH	6
	51	INH, PTH	6
U	52	INH, PTH	8
	53	INH, PZA	9
V	54	RMP, PTH	10
	55	PTH	11

[a] INH, isoniazid; EMB, ethambutol; RMP, rifampin, PZA, pyrazinamide; PTH, protionamide.

five strains, but most groups (68%) consisted of pairs of isolates. The total amount of clustering according to the method of Small et al. (23) was approximately 18% (excluding strains of ≤4 bands and cross-contaminations).

Correlation between IS*6110* RFLP patterns and drug resistance patterns. The drug resistance patterns of strains clustered in one fingerprint group were mostly identical or differed by one or two drugs (Table 1). Only strains of groups D and J differed markedly in their drug resistance patterns, although their IS*6110* fingerprint patterns showed 15 (D) or 9 (J) identical bands. In these cases, tuberculosis infections may have been acquired several years before and the different drug resistance patterns of the *M. tuberculosis* strains may have developed during the patients' histories. The good correlation overall between IS*6110* fingerprint and drug resistance data strongly supports the close relationship between strains of one fingerprint group and indicates transmission of strains from one patient to another or infection of several patients by contacts to one or more index patients.

Epidemiological investigation of fingerprint groups. The epidemiological relationship among patients was investigated for some fingerprint groups. Specific transmission links could be established between patients of groups B, F, H, and L.

The patients of group B were married, and the wife was probably infected by her husband, who had a longer history of tuberculosis.

Patients 12, 14, 15, and 16 of group F were citizens of the former Soviet Union who immigrated to Germany, whereas

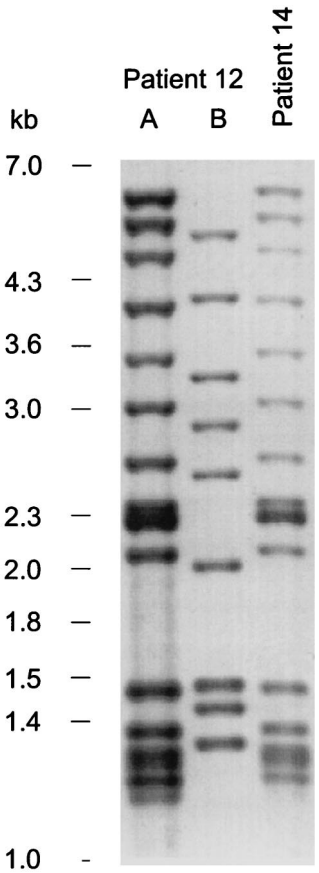

FIG. 3. RFLP patterns of the drug-susceptible (patient 12, lane B) and MDR (patient 12, lane A) strains of patient 12 and the MDR strain of patient 14.

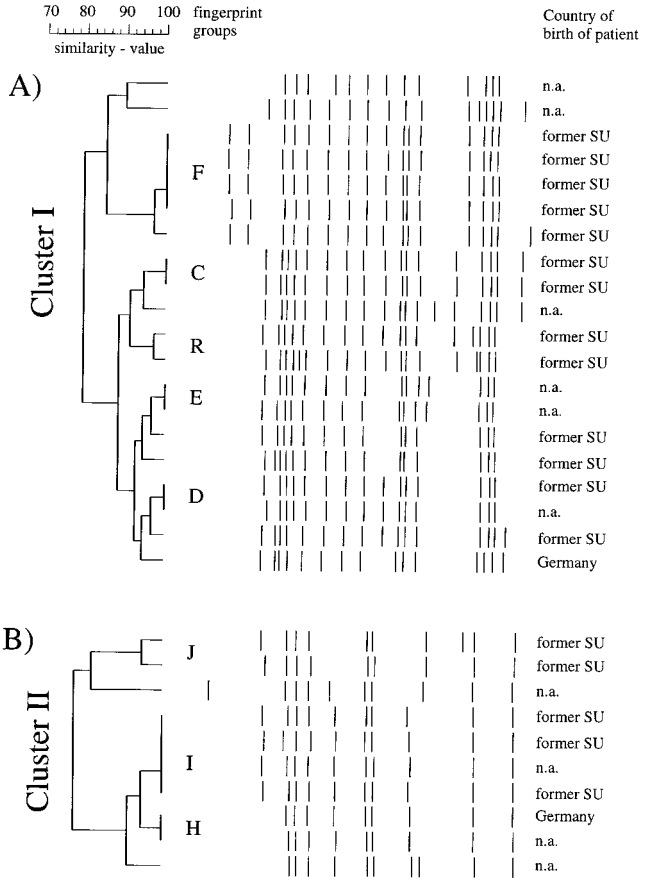

FIG. 4. IS*6110* banding patterns and dendrograms of IS*6110* fingerprint clusters I (A) and II (B). The positions of IS*6110* bands were normalized and displayed as lanes. Scale depicts similarity of patterns calculated as described in Materials and Methods. SU, Soviet Union; n.a., data not available.

patient 13 is still living in Belarus. Patients 12 and 14 stayed in the same hospital at the same time and were accommodated in the same room. Analyses of patients' histories revealed that patient 12 initially had a drug-susceptible strain and was subsequently superinfected during tuberculosis therapy with a multidrug-resistant (MDR) strain. This strain showed the same drug resistance and the same IS*6110* fingerprint pattern as the MDR strain of patient 14, whereas the drug-susceptible strain showed a completely different fingerprint pattern (Fig. 3). Thus, patient 12 was infected with an MDR *M. tuberculosis* strain although he had been under tuberculosis therapy. The *M. tuberculosis* strain obtained from the patient living in Belarus showed a fingerprint pattern identical or nearly identical to those of the strains from patients 14, 15, and 16. Hence, their infections seem to have been acquired in the former Soviet Union.

Members of group H are immigrants from the former Soviet Union too. Patients 21 and 22 were a married couple who either infected each other or were both infected by a third index patient. Patient 19 had an MDR *M. tuberculosis* strain with the same drug resistance and the same IS*6110* fingerprint pattern as the strains of patients 21 and 22, and stayed in the same hospital ward as patient 21. Thus, since patients lived in the same region and belonged to the same social group, patient 19 probably was infected by contacts with patients 21 and 22 or with a fourth common index patient. For patient 20, no obvious transmission link could be established.

Group L consists of two brothers and a third patient who belonged to the same social group (Turkish) living in the same area.

Relatedness between the banding patterns of all drug-resistant *M. tuberculosis* strains. To further analyze the relatedness between the banding patterns of all drug-resistant *M. tuberculosis* strains, a similarity matrix based on the IS*6110* fingerprint data was generated. This matrix shows the degree of relatedness of each IS*6110* banding pattern with any other in the collection.

Two clusters of related strains could be determined. The IS*6110* banding patterns of the clustered strains showed at least a similarity of 79% (cluster I) or 78% (cluster II) (Fig. 4). These clusters contained 20 and 10 isolates, respectively. Thus, 30 of the 167 strains analyzed in this study (approximately 18%) belonged to two clusters of related strains sharing more than two-third of their IS*6110* hybridization bands (Fig. 4). The patients of these two clusters mostly were immigrants from the former Soviet Union (13 patients of cluster I and 5 patients of cluster II). This fact suggests an association between RFLP type and geographical origin of *M. tuberculosis* strains.

DISCUSSION

In the present study we analyzed all available drug-resistant *M. tuberculosis* strains sent to the German National Reference Center for Mycobacteria in 1995 by the standardized IS*6110* fingerprinting method (29). Since approximately 32% of all

culture-positive tuberculosis cases in Germany were differentiated and tested for drug susceptibility in our laboratory, we estimate that the strains investigated in this study represent at least one-third of all German drug-resistant tuberculosis cases. In accordance with the results observed for other low-incidence countries (12, 25, 30), we found a high degree of IS*6110* polymorphism. The copy number of IS*6110* ranged from 1 to 20, and the majority of strains had 6 to 16 bands, similar to what had been reported previously for the Netherlands (30).

The IS*6110* fingerprint patterns of strains isolated in succession from one patient generally remained stable over a period up to 1 year, although the drug resistance patterns varied slightly for some strains obtained from one patient (data not shown). This result is in accordance with the generally accepted opinion that altering of drug resistance patterns of *M. tuberculosis* strains is not connected with a change in their IS*6110* patterns. Only for one patient did an additional IS*6110* fingerprint band occur within 7 months, indicating a transposition event. This high stability of IS*6110* fingerprint patterns is consistent with previously published data (4, 25).

Recent population-based studies had shown that patients with *M. tuberculosis* strains showing identical IS*6110* fingerprint patterns are likely to have become infected recently (9, 23, 25). In our study, 22 fingerprint groups with identical or nearly identical patterns have been found. Transmission links could be established between members of four fingerprint groups. The close relationship of *M. tuberculosis* strains clustered in one fingerprint group was further confirmed by the overall good correlation between IS*6110* fingerprint and drug resistance data. The clustering index according to Small et al. (23) among the strains analyzed was 18%. This indicates that, besides failures of tuberculosis treatment, transmission of drug-resistant tuberculosis contributes considerably to the problem of drug resistance in Germany. The clustering index determined here was low, compared with that observed in other studies (9, 23, 34). This may be due to the short study period and the fact that not all drug-resistant tuberculosis cases in Germany were analyzed. Thus, not all cases of transmission could be detected, and consequently, the observed transmission index is likely to be underestimated.

In one case it could clearly be demonstrated that an immunocompetent patient with drug-susceptible tuberculosis was superinfected with an MDR strain, although he was under treatment. Some cases of superinfection were previously identified by indirect epidemiological methods (15, 26) and by molecular methods (20, 22). Shafer et al. (20) reported a superinfection of a patient who was not immunocompromised and who initially had a rifampin-resistant strain, with a strain resistant to isoniazid and rifampin. In this study we demonstrated superinfection of a patient with a strain resistant to four drugs. Thus, in contrast to common opinion, MDR *M. tuberculosis* strains seem to be as infectious as drug-susceptible strains. In addition, regular tuberculosis therapy is not sufficient to protect a patient against superinfection with a drug-resistant strain.

Altogether, one strain with a single IS*6110* band and three strains with two IS*6110* bands were detected. Strains with a single or a few copies of IS*6110* have been previously reported from Tunisia and Ethiopia (12). In accordance with this, two of the four patients were known to be born in Africa and two isolates were directly obtained from patients living in Saudi Arabia. Thus, distinct fingerprint types seem to be restricted to distinct geographical regions and may be useful for the analysis of a global tuberculosis epidemiology. However, since one or two IS*6110* bands were not sufficient to determine the relatedness of *M. tuberculosis* strains (25, 31), further analyses of these strains by other molecular methods, e.g., fingerprinting with the repetitive polymorphic GC-rich sequence (31) are necessary to confirm the relation between the strains analyzed here and those analyzed by Hermans et al. (12).

A correlation between *M. tuberculosis* DNA type and geographical origin was further confirmed by the two clusters of related strains observed in this investigation. The clustered strains originated mainly from patients who are immigrants from the former Soviet Union and whose infections were probably acquired in their homeland. Therefore, these two RFLP types seem to be typical for *M. tuberculosis* strains originating in the former Soviet Union. At this time, no data concerning the variability of IS*6110* fingerprint patterns of *M. tuberculosis* strains from the former Soviet Union are available. As a consequence, it is not yet clear if the restricted variability of IS*6110* banding patterns found for strains from the former Soviet Union in this study reflects the general situation in the former Soviet Union or is restricted to our study group. However, previous studies indicate that the variability of IS*6110* fingerprint patterns in high-incidence countries is not as high as in low-incidence countries such as the Netherlands (12, 30). Strains from the former Soviet Union may therefore exhibit less IS*6110* DNA polymorphism, and perhaps only a limited number of families of *M. tuberculosis* strains circulate in the former Soviet Union or in some regions of the former Soviet Union. The two clusters of related strains comprise approximately 18% of all isolates analyzed. As a consequence, drug-resistant *M. tuberculosis* strains were presumably carried from the former Soviet Union to Germany by immigrants and may contribute to a great extent to the emergence of drug-resistant tuberculosis in Germany. However, since only a limited number of strains in a short period were analyzed, our data may undervalue or overvalue the percentage of drug-resistant tuberculosis due to immigrants. Thus, further long-term studies are necessary to give a clearer picture of the epidemiology of drug-resistant tuberculosis in Germany.

ACKNOWLEDGMENT

Parts of this work were supported by the Robert-Koch-Institut, Berlin, Germany.

REFERENCES

1. **Alland, D., G. E. Kalkut, A. R. Moss, R. A. McAdam, J. A. Hahn, W. Bosworth, E. Druckner, and B. R. Bloom.** 1994. Transmission of tuberculosis in New York City. An analysis by DNA fingerprinting and conventional epidemiological methods. N. Engl. J. Med. **330:**1710–1716.
2. **Aznar, J., H. Safi, J. Romero, A. Alejo, A. Gracia, and J. C. Palomares.** 1995. Nosocomial transmission of tuberculosis infection in pediatrics wards. Pediatr. Infect. Dis. J. **14:**44–48.
3. **Barnes, P. F., H. El-Hajj, S. Preston-Martin, M. D. Cave, B. E. Jones, M. Otaya, J. Pogoda, and K. D. Eisenach.** 1996. Transmission of tuberculosis among the urban homeless. JAMA **275:**305–307.
4. **Cave, M. D., K. D. Eisenach, P. F. McDermott, J. H. Bates, and J. T. Crawford.** 1991. IS*6110*: conservation of sequence in the *Mycobacterium tuberculosis* complex and its utilization in DNA fingerprinting. Mol. Cell. Probes **5:**73–80.
5. **Demissie, M., M. Gebeyehu, and Y. Berhane.** 1997. Primary resistance to anti-tuberculosis drugs in Addis Ababa, Ethiopia. Int. J. Tuberc. Lung Dis. **1:**64–67.
6. **Deutsches Zentralkommitee zur Bekämpfung der Tuberkulose.** Informationsbericht 22. 1996. pmi-Verlagsgruppe, Frankfurt am Main, Germany.
7. **Edlin, B. R., J. I. Tokars, M. H. Grieco, J. T. Crawford, J. Williams, E. M. Sordillo, K. R. Ong, J. O. Kilburn, S. W. Dooley, K. G. Castro, W. R. Jarvis, and S. D. Holmberg.** 1992. An outbreak of multidrug-resistant tuberculosis among hospitalized patients with the acquired immunodeficiency syndrome. N. Engl. J. Med. **326:**1514–1521.
8. **Frieden, T. R., T. Sterling, A. Pablos-Mendez, J. O. Kilborn, G. M. Cauthen, and S. W. Dooley.** 1993. The emergence of drug-resistant tuberculosis in New York City. N. Engl. J. Med. **328:**521–526.
9. **Genewein, A., A. Telenti, C. Bernasconi, C. Mordasini, S. Weiss, A. M. Maurer, H. L. Rieder, K. Schopfer, and T. Bodmer.** 1993. Molecular ap-

proach to identify route of transmission of tuberculosis in the community. Lancet **342:**841–844.
10. **Gordin, F. M., E. T. Nelson, J. P. Matts, D. L. Cohn, J. Ernst, D. Benator, C. L. Besch, L. R. Crane, J. H. Sampson, P. S. Bragg, and W. El-Sadr.** 1996. The impact of human immunodeficiency virus infection on drug-resistant tuberculosis. Am. J. Respir. Crit. Care Med. **154:**1478–1483.
11. **Goyal, M., L. P. Ormerod, and R. J. Shaw.** 1994. Epidemiology of an outbreak of drug-resistant tuberculosis in the U.K. using restriction fragment length polymorphism. Clin. Sci. **86:**749–751.
12. **Hermans, P. W. M., F. Messadi, H. Guebrexabher, D. van Soolingen, P. E. W. de Haas, H. Heersma, H. de Neeling, A. Ayoub, F. Portaels, D. Frommel, M. Zribi, and J. D. A. van Embden.** 1995. Analysis of the population structure of *Mycobacterium tuberculosis* in Ethiopia, Tunisia, and the Netherlands: usefulness of DNA typing for global tuberculosis epidemiology. J. Infect. Dis. **171:**1504–1513.
13. **McAdam, R. A., P. W. M. Hermans, D. van Soolingen, Z. F. Zainuddin, D. Catty, J. D. A. van Embden, and J. W. Dale.** 1990. Characterization of a *M. tuberculosis* insertion sequence belonging to the IS*3* family. Mol. Microbiol. **4:**1607–1613.
14. **Mendoza, M. T., A. J. Gonzaga, C. Roa, M. A. Velmonte, M. Jorge, J. C. Montoya, T. T. Torres, M. Ong, M. Y. C. Barrez, and C. F. Ang.** 1997. Nature of drug resistance and predictors of multidrug-resistant tuberculosis among patients seen at the Philippine General Hospital, Manila, Philippines. Int. J. Tuberc. Lung Dis. **1:**59–63.
15. **Nardell, E. A., B. McInnis, B. Thomas, and S. Weidhaas.** 1986. Exogenous reinfection with tuberculosis in a shelter for the homeless. N. Engl. J. Med. **315:**1570–1575.
16. **Poulet, S., and S. T. Cole.** 1995. Repeated DNA sequences in mycobacteria. Arch. Microbiol. **163:**79–86.
17. **Rigouts, L., and F. Portaels.** 1994. Restriction fragment length polymorphism analysis of drug-resistant *Mycobacterium tuberculosis* strains isolated in Belgium. Acta Clin. Belg. **49:**5–11.
18. **Rigouts, L., M. Kubin, M. Havelková, and F. Portaels.** 1994. DNA fingerprinting analysis of drug resistant *Mycobacterium tuberculosis* strains isolated in the Czech Republic. Centr. Eur. J. Publ. Hlth. **1:**58–59.
19. **Saunders, N. A.** 1995. State of the art: typing *Mycobacterium tuberculosis*. J. Hosp. Infect. **29:**169–176.
20. **Shafer, R. W., S. P. Singh, C. Larkin, and P. M. Small.** 1995. Exogenous reinfection with multidrug-resistant *Mycobacterium tuberculosis* in an immunocompetent patient. Tubercle Lung Dis. **76:**575–577.
21. **Small, P. M., N. B. McClenny, S. P. Singh, G. K. Schoolnik, L. S. Tompkins, and P. A. Mickelsen.** 1993. Molecular strain typing of *Mycobacterium tuberculosis* to confirm cross-contamination in the mycobacterial laboratory and modification of the procedures to minimize occurrence of false-positive cultures. J. Clin. Microbiol. **31:**1677–1682.
22. **Small, P. M., R. W. Shafer, P. C. Hopewell, S. P. Singh, M. J. Murphy, E. Desmond, M. F. Sierra, and G. K. Schoolnik.** 1993. Exogenous reinfection with multidrug-resistant *M. tuberculosis* in patients with advanced HIV infection. N. Engl. J. Med. **328:**1137–1144.
23. **Small, P. M., P. C. Hopewell, S. P. Samir, A. Paz, J. Parsonnet, D. C. Ruston, G. F. Schecter, C. L. Daley, and G. K. Schoolnik.** 1994. Epidemiology of tuberculosis in San Francisco. N. Engl. J. Med. **330:**1703–1709.
24. **Sola, C., L. Horgen, K. S. Goh, and N. Rastogi.** 1997. Molecular fingerprinting of *Mycobacterium tuberculosis* on a Caribbean island with IS*6110* and DRr probes. J. Clin. Microbiol. **35:**843–846.
25. **Strässle, A., J. Putnik, R. Weber, A. Fehr-Merhof, J. Wüst, and G. E. Pfyffer.** 1997. Molecular epidemiology of *Mycobacterium tuberculosis* strains isolated from patients in a human immunodeficiency virus cohort in Switzerland. J. Clin. Microbiol. **35:**374–378.
26. **Styblo, K.** 1978. Epidemiology of tuberculosis. Bull. Int. Union Tuberc. **53:**141–152.
27. **Thierry, D., A. Brisson-Noël, V. Vincent-Lévy-Frébault, S. Nguyen, J.-L. Guesdon, and B. Gicquel.** 1990. Characterization of a *Mycobacterium tuberculosis* insertion sequence, IS*6110*, and its application in diagnosis. J. Clin. Microbiol. **28:**2668–2673.
28. **Torrea, G., G. Levee, P. Grimont, C. Martin, S. Chanteau, and B. Gicquel.** 1995. Chromosomal DNA fingerprinting analysis using the insertion sequence IS*6110* and the repetitive element DR as strain-specific markers for epidemiological study of tuberculosis in French Polynesia. J. Clin. Microbiol. **33:**1899–1904.
29. **Van Embden, J. D. A., M. D. Cave, J. T. Crawford, J. W. Dale, K. D. Eisenach, B. Gicquel, P. Hermans, C. Martin, R. McAdam, T. M. Shinnick, and P. M. Small.** 1993. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. J. Clin. Microbiol. **31:**406–409.
30. **Van Soolingen, D., P. W. M. Hermans, P. E. W. de Haas, D. R. Soll, and J. D. A. van Embden.** 1991. Occurrence and stability of insertion sequences in *Mycobacterium tuberculosis* complex strains: evaluation of an insertion sequence-dependent DNA polymorphism as a tool in the epidemiology of tuberculosis. J. Clin. Microbiol. **29:**2578–2586.
31. **Van Soolingen, D., P. E. W. de Haas, P. W. M. Hermans, P. M. A. Groenen, and J. D. A. van Embden.** 1993. Comparison of various repetitive DNA elements as genetic markers for strain differentiation and epidemiology of *Mycobacterium tuberculosis*. J. Clin. Microbiol. **31:**1987–1995.
32. **Van Soolingen, D., and P. W. M. Hermans.** 1995. Epidemiology of tuberculosis by DNA fingerprinting. Eur. Respir. J. **8**(Suppl. 20)**:**649–656.
33. **Versalovic, J., V. Kapur, T. Koeuth, G. H. Mazurek, T. S. Whittam, J. M. Musser, and J. R. Lupski.** 1995. DNA fingerprinting of pathogenic bacteria by fluorophore-enhanced repetitive sequence-based polymerase chain reaction. Arch. Pathol. Lab. Med. **119:**23–29.
34. **Warren, R., J. Hauman, N. Beyers, M. Richardson, H. S. Schaaf, P. Donald, and P. van Helden.** 1996. Unexpectedly high strain diversity of *Mycobacterium tuberculosis* in a high-incidence community. SAMJ (S. Afr. Med. J.) **1:**45–49.
35. **World Health Organization.** Report of the tuberculosis epidemic. 1996. World Health Organization, Geneva, Switzerland.
36. **Yang, Z. H., P. E. W. de Haas, C. H. Wachmann, D. van Soolingen, J. D. A. van Embden, and A. B. Andersen.** 1995. Molecular epidemiology of tuberculosis in Denmark in 1992. J. Clin. Microbiol. **33:**2077–2081.