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High strain diversity among isoniazid-resistant *M tuberculosis* isolates from the Free State and Northern Cape provinces

This study set out to determine the diversity of the isoniazid-resistant *M tuberculosis* isolates causing pulmonary tuberculosis in the Free State and Northern Cape provinces of South Africa. Thirty-seven isoniazid-resistant isolates from a nation-wide survey could be fingerprinted by IS*6110*-based RFLP. Spoligotyping and MIRU-VNTR typing were performed to confirm inconclusive results. The number of IS*6110* copies per isolate varied from 2 to 18, with nine (24.3%) isolates harbouring five insertions or fewer. Nine (24.3%) of 37 cases grouped into four RFLP-clusters. The clustered isolates with fewer than five IS*6110* copies were all shown to be different when subjected to spoligotyping and MIRU-VNTR typing, whereas the clustering of the high-copy isolates was confirmed. The high degree of diversity among H-resistant isolates in the Free State suggests a low recent transmission rate, but this should be investigated by long-term analyses. For the Northern Cape province, representative sampling is needed before proper conclusions can be drawn.

Hoge diversiteit by isoniazide-resistente *M tuberculosis* isolaten van die Vrijstaat en Noordkaap provincies

Het objectief is het bepaling van de diversiteit bij isoniazide-resistente *M tuberculosis* isolaten die pulmonaire tuberculose veroorzaken in de Vrijstaat en Noordkaap provincies in Zuid Afrika. Zevenendertig isoniazide-resistente isolaten van een nationale survey konden worden getypeerd met IS*6110*-RFLP. Spoligotypering en MIRU-VNTR typering werden gebruikt als bijkomende subtypering. Het aantal IS*6110* copiën per isolaat varieerde tussen 2 en 18, en negen (24.3%) isolaten bevatten vijf of minder inserties. Negen (24.3%) van de 37 gevallen groepeerden in vier RFLP-clusters. De isolaten uit de clusters met minder dan vijf IS*6110* inserties werden gedifferentieerd met spoligo-

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typing en MIRU-VNTR typering, terwijl de hoge-copie clusters werden bevestigd. De hoge mate van diversiteit bij de H-resistente isolaten van de Vrijstaat zou een lage recent transmissie kunnen suggereren, maar moet bevestigd worden met lange-termijn analyses. Voor de Noordkaap provincie, is een representative staalname nodig vooraleer conclusies kunnen getrokken worden.

The genome of the causative agent of human tuberculosis (TB), *Mycobacterium tuberculosis*, provides certain pointers towards an understanding of the disease's transmission within populations, and even its dissemination across international borders.[1] Repetitive sequences of variable copy numbers in different loci of the genome are the tools used to track the origins of strains causing outbreaks and active disease in defined areas (Saunders 1999; Van Soolingen 2001). The standardised IS*6110*-based restriction fragment length polymorphism (IS*6110*-RFLP) method (Van Embden *et al* 1993) originally enhanced our understanding of the transmission dynamics of TB bacilli within communities. Since then various DNA-fingerprinting methods have been developed, including spoligotyping (Kamerbeek *et al* 1997) and Mycobacterial Interspersed Repetitive Units (MIRU-VNTR) typing (Supply *et al* 1997). Data on the degree of recent transmission as opposed to the reactivation of TB can be used to re-strategise and allocate resources adequately in affected communities.

Although DNA fingerprinting does not form part of the national TB control programme in South Africa, the genotyping of *M tuberculosis* isolates from the Western Cape province has been utilised to generate data on transmission dynamics in the high-incidence communities around Cape Town (Warren *et al* 1996a). The molecular techniques used in these settings have indicated that recent transmission

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is a significant problem among drug-resistant TB patients (Van Rie *et al* 1999a; Streicher *et al* 2004). To date, there is almost no data on the rate of recent transmission and the genetic heterogeneity of strains circulating in the Free State and Northern Cape. The little that is known comes from the sporadic strain typing and gene studies conducted in communities around Mangaung by the Department of Medical Microbiology at the University of the Free State since 1996 (Van der Spoel-van Dijk *et al* 1996). These studies, performed with non-representative but convenient samples, revealed extremely diverse fingerprint patterns with small clusters.

South Africa, currently rated ninth in global TB rankings, had a TB incidence of 526/100 000 cases in 2002 (Kironde *et al* 2002). Of the 144 910 new cases of pulmonary TB reported in South Africa in 2001, the Free State and Northern Cape contributed 9 978 (352/100 000) and 3 866 (438/100 000) cases, respectively. We have only incomplete data on resistant *M tuberculosis* for South Africa from microbiological studies in a few provinces and a single national study during 2001/2002 (WHO 2004). Data of the latter study reported 6.4% of isoniazid (H)-resistance in new cases and 6.9% in previously treated patients for the Free State. Multidrug-resistant TB (MDRTB), defined as at least resistant to H and rifampicin (R) (Dye *et al* 2002), was detected in 1.8% of new cases and in 1.7% of retreatment patients (WHO, 2004), with 71.9% of cases also being infected with the human immunodeficiency virus. In the Mpumalanga Province, MDRTB rates increased significantly over a four-year period: from 1.5% in 1997 to 2.6% in 2001 in new cases, and from 8.0% in 1977 to 13.7% in 2001 in re-treatment cases (WHO 2004). There is no available data on the extent of drug resistance in the Northern Cape.

In the study presented here, fingerprinting by IS*6110*-RFLP, spoligotyping, and MIRU-VNTR typing were used to determine whether H-resistant *M tuberculosis* isolates from patients with pulmonary tuberculosis in the Free State and Northern Cape provinces were genetically related clones or diverse organisms.

1. Materials and methods

1.1 Study population and bacterial strains

Forty isoniazid-resistant *M tuberculosis* isolates (27 from the Free State and 13 from the Northern Cape province) were cultured and kindly provided by the National Tuberculosis Lead Research Programme, Medical Research Council (MRC), Pretoria. These had been collected during May 2001-April 2002 as part of a nation-wide study, the "National Survey of Tuberculosis Drug Resistance in South Africa". Sputum specimens received for the Free State were collected from all pulmonary tuberculosis suspects at selected Primary Health Care clinics. A total of 628 *M tuberculosis* isolates were grown from 2948 sputum specimens, a sample size according to the WHO guidelines for representative sampling for drug-resistance surveys (WHO 2003). Drug susceptibility to isoniazid (H), rifampicin (R), streptomycin (S), and ethambutol (E) was tested at the MRC using the Proportion method on Löwenstein-Jensen medium (Kleeberg *et al* 1980).

Table 1: Overview of 41 isoniazid-resistant *M tuberculosis* isolates from the Free State province identified at the MRC, Pretoria, and the selection forwarded to the Microbiology Department of the University of the Free State for DNA-fingerprinting

Forwarded to UFS	Resistance profile						
	Mono-resistant to H		Resistant to H and S		MDRTB		
	New case[1]	Previous[1]	New case[1]	Previous[1]	New case[1]	Previous[1]	Total
Yes	11 (73%)	6 (85%)	0	1 (50%)	7 (87%)	2 (67%)	27 (66%)
No	4	1	6	1	1	1	14 (34%)
Total	15	7	6	2	8	3	41
DNA-fingerprint available	9	5	0	1	7	1	23

[1] Treatment status of patients at the time of specimen collection, according to the WHO classification.

H = isoniazid; S = streptomycin; MDRTB = multi-drug-resistant TB; UFS = University of the Free State.

Forty-one of the 628 (6.5%) Free State isolates were resistant to H, with or without resistance to other drugs. Eleven of these tested MDR (Weyer 2002) (Table 1). Twenty-seven could be subcultured and were forwarded to the Department of Medical Microbiology at the University of the Free State. The group of isolates resistant to H and S is clearly underrepresented in this study, whereas for both MDRTB and mono-H-resistant isolates a good proportion of both new and previous cases is represented.

The isolates of the Northern Cape province were not representative due to collection problems, but all thirteen isolates that tested resistant to H were included in the DNA-fingerprinting analyses.

The 40 H-resistant *M tuberculosis* isolates in this study showed variable resistance profiles for the other drugs tested (Table 2). Thirteen were found to be multidrug-resistant tuberculosis (MDRTB).

1.2 DNA fingerprinting

1.2.1 DNA extraction

For the preparation of chromosomal DNA we followed the chloroform/-phenol-extraction method described by Warren *et al* (1996b).

1.2.2 IS*6110*-RFLP

IS*6110*-RFLP was performed according to the standardised IS*6110* technique (Van Embden *et al* 1993) with some minor changes. Briefly, the extracted genomic DNA was restricted with *Pvu* II in a 30 µl reaction mix consisting of 3 µg of genomic DNA and 15 units of *Pvu* II in 3 µl of the prescribed restriction buffer (Amersham biosciences, UK). The restriction mix was incubated overnight (± 16 h) at 37°C. At the end of digestion, the reaction was incubated at 65°C for 10 minutes to terminate any remaining enzyme activity. The restricted products were run on a 200 cm^2 0.8% (w/v) Seakem® ME agarose (BioWhittaker molecular applications, USA) gel using a 1 x Tris-borate-EDTA buffer (TBE). The agarose gel was loaded with 20µl of *Pvu* II restricted genomic DNA, 2 µl loading mix, and 2 µl of molecular weight marker X (Roche, Germany). Fragments of DNA were separated by gel electrophoresis in 1 x TBE buffer at 40 V for 18 hours.

Table 2: Demographic and clinical data on the patients from the 40 H-resistant *M tuberculosis* isolates of the Free State and Northern Cape provinces included in the study

Isolate No	Resistance pattern				Patient category[1]	Province	Town	Clinic
	H	R	E	S				
MRC01	R	S	S	S	New	FS	Thaba Nchu	Gaongalelwe
MRC02	R	S	S	S	New	FS	BFN	Opkoms
MRC03	R	S	S	S	New	FS	BFN	Batho
MRC04	R	S	S	S	New	FS	BFN	Opkoms
MRC05	R	S	S	S	Retreatment	FS	BFN	Opkoms
MRC06	R	S	S	S	Treatment completed	FS	BFN	Opkoms
MRC07	R	R	R	R	New	FS	BFN	Mangaung
MRC08	R	S	S	R	Retreatment	FS	BFN	Opkoms
MRC09	R	S	S	S	Treatment completed	FS	Welkom	Welkom
MRC10	R	S	S	S	Cured	FS	Welkom	Welkom
MRC11	R	S	S	S	New	FS	Wesselsbron	Albert Luthuli
MRC12	R	S	S	S	New	FS	Wesselsbron	Albert Luthuli
MRC13	R	S	S	S	New	FS	Welkom	Welkom
MRC14	R	R	S	S	New	FS	Welkom	Welkom
MRC15	R	R	S	R	New	FS	Wesselsbron	Albert Luthuli
MRC16	R	R	R	S	New	FS	Welkom	Pedisanang
MRC17	R	S	S	S	New	FS	Bethlehem	Bethlehem
MRC18	R	S	S	S	New	FS	QwaQwa	Bolata
MRC19	R	S	S	S	Cured	FS	QwaQwa	Marakong
MRC20	R	R	S	R	New	FS	Welkom	Welkom
MRC21	R	R	R	R	New	FS	Welkom	Welkom
MRC22	R	R	S	S	Treatment completed	FS	Sasolburg	Zamdela
MRC23	R	R	R	S	New	FS	Botshabelo	U/W
MRC24	R	S	S	S	New	FS	BFN	Opkoms
MRC25	R	S	S	S	New	FS	Welkom	Boithusong
MRC26	R	S	S	S	Cured	FS	BFN	Opkoms
MRC27	R	R	S	S	Cured	FS	BFN	Thusong
NC01	R	R	S	S	Cured	NC	NA	Betty Gaetsewe
NC02	R	S	S	S	New	NC	NA	Calvinia
NC03	R	R	S	S	Cured	NC	NA	Kuruman
NC04	R	S	S	S	New	NC	NA	Keimoes Eilande
NC05	R	S	S	S	Treatment interrupted	NC	NA	Montana
NC06	R	S	S	S	New	NC	De Aar	De Aar
NC07	R	S	S	S	New	NC	De Aar	Nonzwakazi
NC08	R	S	S	S	New	NC	Colesberg	Colesberg
NC09	R	R	S	S	New	NC	K/ Ville	Montana
NC10	R	R	R	S	New	NC	Valspan	Valspan
NC11	R	S	S	S	Treatment interrupted	NC	H/ Valley	Montana
NC12	R	R	S	R	New	NC	NA	Progress
NC13	R	S	S	S	Treatment failed	NC	NA	Montana

[1] Treatment status of patients at the time of specimen collection, according to the WHO classification. MDRTB cases are highlighted in yellow.
E = ethambutol; S = streptomycin; FS = Free State province; NC = Northern Cape province.

The DNA in the gel was transferred onto a Hybond-N^+ membrane (Amersham biosciences, UK) using a vacuum blotter (BioRad Laboratories, Hercules, Calif, USA) in accordance with the manufacturer's instructions. Hybridisation was performed with a 245 bp PCR-amplified right arm of the IS*6110* sequence. The probe was labelled with a Gene Images random prime labelling kit in accordance with the manufacturer's instructions. IS*6110* sequences were detected by the Gene Images CDP star detection kit (Amersham biosciences).

The IS*6110* RFLP autoradiographs were scanned using a Hewlett Packard Scanjet II cx/T scanner. Comparison of RFLP profiles was achieved with the aid of the computer software Gelcompar II, version 2.5 (Applied Maths, Kortrijk, Belgium). The DNA fingerprints were analysed using the Dice coefficient unweighted pair-group method with arithmetic averages (UPGMA).

1.2.3 Spoligotyping

Spoligotyping, a technique based on the detection of 43 known spacer sequences in the direct repeat (DR) section of the *M tuberculosis*-complex, was performed using the method described by Kamerbeek *et al* (1997), only changing the PCR mixture to 3.0 mM $MgCl_2$, 50 pmol of each primer and 15 mM Tris, pH 9.0. The mixture was heated for 60 minutes at 37°C for uracil DNA glycosylase incubation; three minutes at 95°C for uracil DNA glycosylase inactivation and DNA denaturation; one minute at 57°C for primer annealing, and one minute at 72°C for primer extension. Twenty-five cycles consisting of one minute at 95°C, one minute at 57°C, and 30 seconds at 72°C were performed. The presence of the respective spacer sequences was detected by using a commercial membrane (Isogen Life Science, Maarsen, The Netherlands) and the ECL detection reagent (Amersham biosciences, UK). Interpretation of the images was done by visual inspection.

1.2.4 MIRU-VNTR typing

MIRU-VNTR typing for 12 loci containing MIRU was carried out according to Mazars *et al* (2001). A 3% NuSieve agarose gel was used instead of the 2% in the method described.

1.2.5 Cluster definition

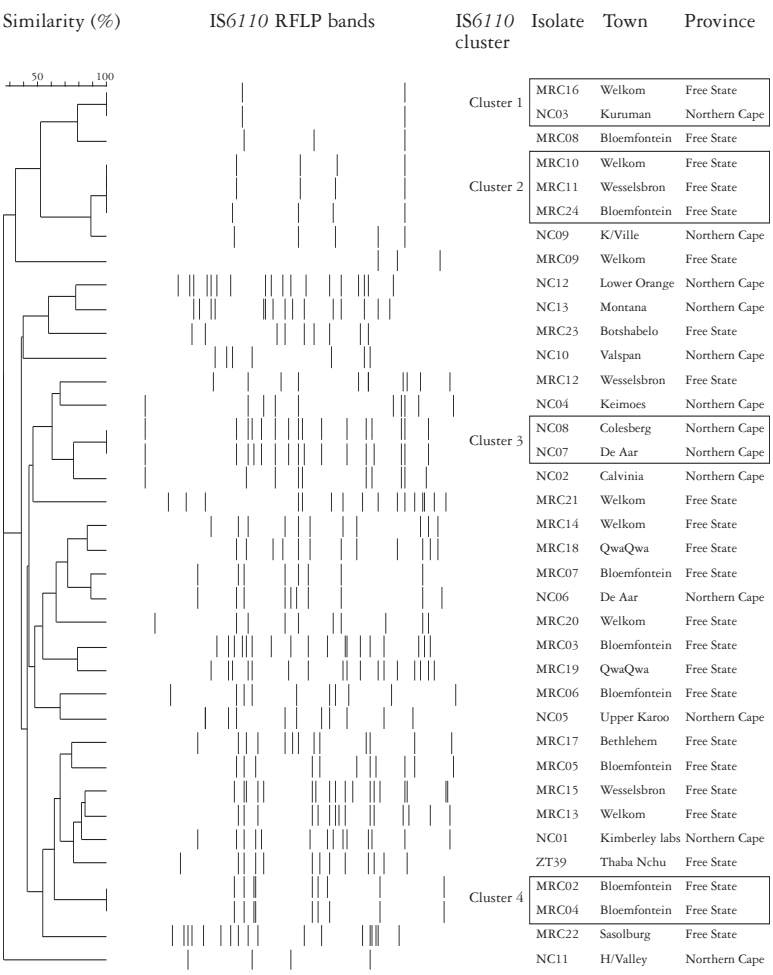
A cluster is defined as *M tuberculosis* isolates showing identical profiles for a specific DNA fingerprinting method. An RFLP-cluster comprises isolates with identical IS*6110*-RFLP profiles (100% match in both the number and the position of the bands).

2. Results

Three of the 27 H-resistant *M tuberculosis* isolates from the Free State that were sent to the laboratory were lost due to contamination (Table 1). A total of 37 H-resistant *M tuberculosis* strains from the Free State and Northern Cape provinces were finally analysed by IS*6110*-RFLP (Figure 1). The number of IS*6110* copies per isolate varied from two to 18, with nine (24.3%) harbouring five insertions or less, and the remaining 28 carrying between 9 and 18 copies. Similarity analyses showed a heterogeneous population of H-resistant *M tuberculosis* isolates in the two provinces. In total, four RFLP-clusters (*ie* groups of isolates with 100% identical profiles) were detected, comprising nine of 37 isolates (Table 3). The remaining 28 (75.6%) each gave a unique pattern. Of the clustered isolates, five had only two or four IS*6110* copies (RFLP-clusters 1 [MRC16 and NC03] and 2 [MRC10, MRC11 and MRC24]), requiring additional typing techniques to confirm clustering. The two remaining clusters had profiles of nine and 16 copies respectively, and each involved two patients. RFLP-cluster 3 (MRC02 and MRC04) was observed in the Free State; RFLP-cluster 4 (NC08 and NC07) in the Northern Cape province. Two of the thirteen MDR strains clustered (RFLP-cluster 1) whereas the remaining 11 showed unique DNA fingerprints.

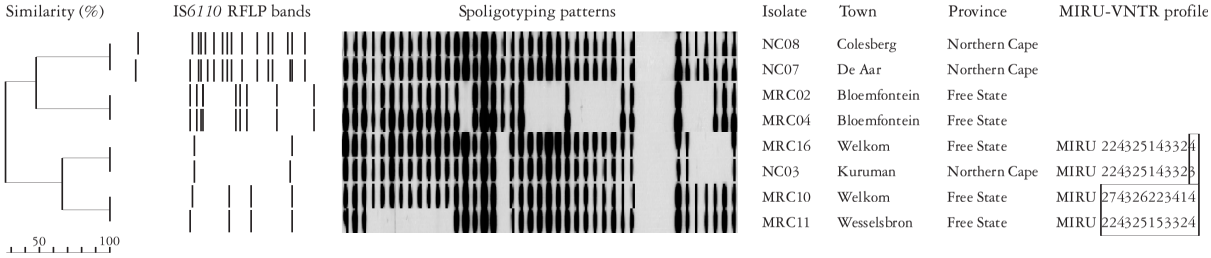
Clustering was confirmed by identical spoligotyping patterns in the case of high-copy RFLP-clusters 3 and 4 (Figure 2), but no direct epidemiological link was found between patients for either of them (Table 3). Clustering of the two MDRTB isolates belonging to RFLP-cluster 1, with only 2 IS*6110* copies (MRC16 and NC03), was confirmed by identical spoligotypes but not supported by MIRU-VNTR typing, with a difference of one allele in locus 40 (Figure 2, Table 3); thus the isolates were considered to be two different strains. The three isolates from RFLP-cluster 2 were differentiated by spoligotyping (only available for isolates MRC10 and MRC11) and/or MIRU-VNTR typing (available for all three), and therefore did not really cluster (Figure 2, Table 3).

Figure 1: Dendrogram of IS*6110*-based patterns for 37 H–resistant *M. tuberculosis* isolates from the Free State and Northern Cape provinces.



The fingerprinting patterns were analysed for similarity using the Dice coefficient and the unweighted pair group method to calculate the dendrogram (Gelcompar software).

Figure 2: Dendrogram of IS*6110*RFLP combined with spoligotyping and MIRU-VNTR typing patterns of 8 *M. tuberculosis* isolates clustered by IS*6110*-RFLP.



Isolate	Town	Province	MIRU-VNTR profile
NC08	Colesberg	Northern Cape	
NC07	De Aar	Northern Cape	
MRC02	Bloemfontein	Free State	
MRC04	Bloemfontein	Free State	
MRC16	Welkom	Free State	MIRU 224325143324
NC03	Kuruman	Northern Cape	MIRU 224325143323
MRC10	Welkom	Free State	MIRU 274326223414
MRC11	Wesselsbron	Free State	MIRU 224325153324

Similarities were determined and the dendrogram calculated using the Dice coefficient unweighted pair-group method with arithmetic averages (UPGMA, Gelcompar software)

Table 3: Patient and isolate characteristics of 9 *M. tuberculosis* isolates with identical IS*6110*-RFLP patterns.

Isolate characteristics							Patient characteristics			
Isolate No.	IS*61110* copies	IS*6110* cluster	Spoligo-typing	MIRU-VNTR profiles	Resistance profile	Conclusion	Gender	Age	Residence	Link
MRC16	2	1	Match	224325143324	HR-resistant	2 different strains	F	49	Welkom FS	Not interviewed
NC03				224325143323	HR-resistant		M	31	Kuruman NC	Not interviewed
MRC10	4	2	No match	274326223414	H-resistant	3 different strains	M	38	Welkom FS	
MRC11			No match	224325153324	H-resistant		M	35	Wesselsbron FS	
MRC24			Not done	224325143322	H-resistant		M	46	Bloemfontein FS	
NC07	16	3	Match	Not tested	H-resistant	Identical strain	M	43	Colesberg NC	Not interviewed
NC08					H-resistant		M	70	De Aar NC	Not interviewed
MRC02	9	4	Match	Not tested	H-resistant	Identical strain	F	17	Bloemfontein FS	No direct link detected
MRC04					H-resistant		M	34	Bloemfontein FS	Same township

FS = Free State province; NC = Northern Cape province; F = female; M = male.
IS*6110* clusters comprise isolates showing 100% similarity after IS*6110*-RFLP analyses.
Differences in spoligotying and MIRU-VNTR profiles are highlighted in yellow.

3. Discussion

The isolates used in this study came from the Free State and Northern Cape provinces. The provinces are among the poorest in South Africa. However, the DOTS strategy has been successfully implemented in both provinces (Dept of Health 2000). Diagnosis of TB is based on direct microscopy and clinical examination. Isolation of the bacilli and determination of the resistance profile of an isolate is only requested in instances of failure to convert to sputum smear negative after two months of treatment or when a patient has been treated for more than a month on a previous occasion (a retreatment case) (Dept of Health 2000). Data obtained from the "National Survey of Tuberculosis Drug Resistance in South Africa" by the MRC, Pretoria, provided a good first indicator of the incidence of drug-resistant TB in the various provinces. In 2001, the prevalence of resistance to any drug among new TB cases was estimated at 8.6% in the Free State province, with MDRTB reaching 1.8% (WHO 2004). However, since resistance phenotypes alone are poor indicators of transmission patterns, the use of molecular typing is essential for epidemiological studies.

IS*6110*-RFLP is considered the gold standard for typing *M tuberculosis*-complex isolates harbouring more than five IS*6110* insertions. In this study, DNA fingerprint profiles with fewer than six IS*6110* insertions were seen in 24.3% of H-resistant isolates. Although more prevalent on the Asian continent (Van Soolingen *et al* 1993), isolates with few copies appear to be globally distributed. They have been reported in many countries, including Botswana, Ethiopia, Britain and the USA (Haas *et al* 1999; Hermans *et al* 1995; Kumar *et al* 2000; Frieden *et al* 1996).

In this study, two RFLP-clusters were present among the low-copy isolates. RFLP-cluster 1 was confirmed by spoligotyping, but differed by one allele in locus 40 after MIRU-VNTR typing. Single allele differences are considered sufficient to differentiate between two *M tuberculosis* isolates (Savine *et al* 2002). The second low-copy cluster (RFLP-cluster 2) showed different spoligotyping profiles for the two isolates tested, and all three isolates were different by MIRU-VNTR typing. Therefore, these isolates definitively did not cluster, which confirms the need for secondary typing in strains with fewer than six IS*6110* insertions.

The majority (75.7%) of the strains had nine to eighteen bands, four of them being found in two clusters whereas the rest had distinct patterns. Clustering was confirmed by spoligotyping in both cases, although epidemiological links either could not be examined (RFLP-cluster 3) or were not clear from informal interviews by clinic staff (RFLP-cluster 4).

Overall, 4 of the 37 H-resistant isolates clustered, and none of the clustered isolates were MDRTB. Studies in the Western Cape have reported that 76.0% of MDR strains were the result of transmission (Van Rie *et al* 2000). With the exception of the HS-resistant *M tuberculosis* isolates, which were clearly under-represented in the DNA-fingerprinting analyses, the Free State sample in our study seems representative for the H-resistant isolates circulating in this province during the study period. However, the study period of collection was too short to draw any definite conclusions on recent transmission, and long-term studies will be needed to determine H-resistant TB and MDRTB transmission in this province. More representative sampling is needed for the Northern Cape province in order to be able to draw any conclusions.

Two phenomena could explain the low clustering rate of H-resistant isolates: reactivation of latent H-resistant infections and inappropriate treatment creating acquired drug resistance after recent transmission. We have no direct evidence to support either, since isolates from previous episodes to document reactivation versus reinfection were not available, and since only H-resistant isolates were included in this study we could not verify if the H-resistant isolates clustered with H-susceptible isolates from the same period.

It is also probable that these results underestimate the importance of recent transmission, since some patients may have contracted the disease outside the two provinces. Such patients would then have formed part of a recent transmission group, extending beyond the catchment area of the study. This premise may well hold true since two-thirds of cases (67.6%) were males, who might have been migrant workers in other provinces at certain times. Since contact could not be established, one can only speculate on loose casual contact or the presence of a common index case beyond the geographical and temporal confines of this study.

Observation of clusters is also associated with patients presenting at clinics with advanced TB. Decrease in the risk of infection for the community may only be obtained by preventing delay on the part of both the patient and the health care workers. There should be an ongoing effort to educate the community on the nature of tuberculosis. In addition, activities aimed at elevating the index of suspicion among health care workers should continue. These efforts, coupled with the amelioration of the socio-economic situation, will reduce delays and retard the spread of the disease. In South Africa tuberculosis awareness is heightened annually by the "International TB Day" celebrated on 24 March. In addition, TB open days are held every year in the Free State as a means of disseminating knowledge to communities. Special emphasis is given to educating the community about the signs and symptoms of the disease. Open days are often preceded by door-to-door information-spreading campaigns by DOT supporters. These activities are reinforced by workshops and in-service training sessions aimed at keeping health care workers well informed.

This study sample proved to be relatively heterogenous with not only a low clustering rate but also a relatively low similarity rate among the isolates. With a similarity index of 65% as a cut-off value, 28 of the isolates could be grouped into nine groups, each comprising only two to eight isolates. In contrast to certain regions of the world, no strains with an IS*6110* pattern characteristic of the Beijing-W type were observed in the samples collected from the Free State and Northern Cape provinces. Studies in the Western Cape and KwaZulu-Natal provinces have identified strains of the Beijing genotype lineage as a significant force (Pillay *et al* 2003). The Beijing genotypes are often associated with multidrug-resistant TB in Asian countries, the USA (W strain), and the Western Cape province (U strain) (Van Rie *et al* 1999). The multi- and polydrug-resistant strain reported by Victor *et al* (<www.promedmail.org>) and designated DRF150 was not detected in either of the areas.

To conclude, the high diversity of H-resistant and MDRTB strains observed in the Free State might suggest a low recent transmission rate, but should be confirmed by long-term analyses. For the Northern Cape province, representative sampling and analyses are needed in order to draw proper conclusions. However, our data emphasises that long-term, continuous monitoring of drug resistance and disease dynamics in the general population is imperative for accurate evaluation of the situation.

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