Population Structure of Multi- and Extensively Drug-Resistant *Mycobacterium tuberculosis* Strains in South Africa

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Genotyping of multidrug-resistant (MDR) *Mycobacterium tuberculosis* strains isolated from tuberculosis (TB) patients in four South African provinces (Western Cape, Eastern Cape, KwaZulu-Natal, and Gauteng) revealed a distinct population structure of the MDR strains in all four regions, despite the evidence of substantial human migration between these settings. In all analyzed provinces, a negative correlation between strain diversity and an increasing level of drug resistance (from MDR-TB to extensively drug-resistant TB [XDR-TB]) was observed. Strains predominating in XDR-TB in the Western and Eastern Cape and KwaZulu-Natal Provinces were strongly associated with harboring an *inhA* promoter mutation, potentially suggesting a role of these mutations in XDR-TB development in South Africa. Approximately 50% of XDR-TB cases detected in the Western Cape were due to strains probably originating from the Eastern Cape. This situation may illustrate how failure of efficient health care delivery in one setting can burden health clinics in other areas.

Drug-resistant tuberculosis (TB) threatens TB control efforts throughout the world (9). In particular, multidrug-resistant TB (MDR-TB), defined by resistance to at least isoniazid (INH) and rifampin (RMP), has profound effects on patient treatment outcomes, since these two most effective anti-TB drugs with the fewest side effects must be replaced by less effective, more expensive, and more toxic drugs (35). South Africa is among the countries worldwide with the highest numbers of MDR-TB cases (an estimated number of 13,000 cases in 2008) (34). In a nationwide survey in 2008, 20.2% of all notified TB cases showed resistance to INH, and nearly half of these (9.6% of all cases) were MDR (37). Six years earlier, in 2002, MDR-TB had been reported in a considerably lower proportion (3.1%) of TB cases, indicating a dramatic increase of MDR-TB in recent years (37, 38). Furthermore, a high rate of extensively drug-resistant TB (XDR-TB; defined as MDR-TB plus additional resistance to a fluoroquinolone and at least 1 second-line injectable drug) of 10.5% was estimated among MDR-TB cases tested for second-line drug resistance (37). MDR-TB is responsible for the majority of the financial burden of TB in South Africa, with approximately 70% of the budget of the national TB control program allocated to the management of MDR-TB (36).

Various reports suggest that the drug-resistant TB epidemic in South Africa is primarily caused by the transmission of MDR strains and the amplification of resistance as a result of the use of inappropriate empirical drug regimens in the context of delayed drug susceptibility testing (DST) (4, 6, 8, 12, 15, 16, 27, 30, 34). As suggested by mathematical models, future levels of MDR- and XDR-TB (M/XDR-TB) will largely be determined by the efficiency of transmission of MDR strains (2, 5). Hence, the elaboration of strategies to contain the M/XDR-TB epidemic requires an understanding of the disease transmission dynamics. Our knowl-

edge in this respect has increased considerably through the application of genotyping techniques such as spoligotyping (17) and IS6110 restriction fragment length polymorphism (RFLP) analysis (29), enabling the detection of TB transmission chains and the study of the evolution of *Mycobacterium tuberculosis* population structures (31). Worldwide, molecular genotyping of *M. tuberculosis* strains has led to the identification of strain families or lineages, some of which are associated with drug-resistant TB, including the Beijing family of strains (11, 16, 19, 30), the LAM genotype (24), and strains belonging to the IS6110 low-copy-number clade (27). However, information on the genetic diversity of *M. tuberculosis* is primarily based on studies of drug-sensitive *M. tuberculosis*, and specific data on the population structure of drug-resistant strains are lacking (10).

This study aimed to decipher the population structure of M/XDR *M. tuberculosis* strains isolated from TB patients in four South African provinces. Our results help provide an understanding of the evolution and spread of M/XDR-TB in a high-incidence setting of drug-resistant TB and provide important baseline data for the assessment of future treatment changes and interventions.

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TABLE 1 Number of isolates analyzed by province and year

Yr	Result by province ^a											
	Western Cape			Eastern Cape			KwaZulu-Natal			Gauteng		
	Isolates	Notified	%	Isolates	Notified	%	Isolates	Notified	%	Isolates	Notified	%
2000	8	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA
2001	67	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA
2002	47	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA
2003	32	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA
2004	40	1,085	3.7	0	NA	NA	0	NA	NA	152	537	34.0 ^b
2005	87	1,192	7.3	0	NA	NA	156	1,024	22.9 ^b	238	676	35.2
2006	106	1,179	9.0	0	NA	NA	77	2,200	10.5 ^b	223	732	30.5
2007	158	1,771	8.9	0	NA	NA	0	NA	NA	152	986	15.4
2008	426	2,220	19.2	220	1,501	29.3 ^b	0	NA	NA	0	NA	NA
2009	566	2,078	27.2	282	1,858	16.6 ^b	0	NA	NA	0	NA	NA
2010	592	NA	NA	0	NA	NA	0	NA	NA	0	NA	NA
Unknown	16	NA	NA	1	NA	NA	0	NA	NA	0	NA	NA
Total	2,145	9,525	14.5 ^c	503	3,359	20.5 ^b	233	3,346	16.5 ^b	765	2,931	26.9 ^b

^a Isolates, number of MDR isolates collected in the indicated year; Notified, number of MDR-TB cases notified by the National Health Laboratory Service (NHLS) in the indicated year; %, estimated proportion of isolates analyzed among MDR-TB cases detected during sampling months; NA, no data available or not applicable.

^b Sampling occurred only during part of the indicated year. To estimate the proportion of notified MDR isolates analyzed, the number of notified cases during the sampling period was calculated *pro rata temporis*.

^c The overall proportion of isolates analyzed considered only years for which NHLS data were available.

MATERIALS AND METHODS

Study population. This study was approved by the ethics committees of the participating universities. Suspect drug-resistant isolates of TB patients attending public health facilities in South Africa are routinely subjected to DST. A random sample of diagnosed MDR isolates from the Western Cape, Eastern Cape, KwaZulu-Natal, and Gauteng Provinces was characterized by spoligotyping. Only one isolate per patient was included in the study. Isolates from patients visiting primary health care clinics in the Western Cape were collected from August 2000 to December 2010. Isolates collected until the end of 2007 covered only two of the four health districts of the Western Cape; however, from 2008, isolates were collected from all four health districts. Isolates from patients attending primary health care clinics in the Eastern Cape, referral hospitals in KwaZulu-Natal, or referral hospitals in Gauteng were collected from July 2008 to November 2009, May 2005 to April 2006, or March 2004 to December 2007, respectively. Altogether, 2,145, 503, 233, and 765 (a total of 3,646) isolates from the Western Cape, Eastern Cape, KwaZulu-Natal, and Gauteng, respectively, were included in the study, corresponding to approximately 15%, 21%, 17%, and 27% of the MDR patients notified for the respective study period and province (Table 1) (22).

Routine culture and drug susceptibility testing. Samples were decontaminated and subjected to bacterial culture using the Bactec MGIT 960 culture system for mycobacteria (28). Isolates from the Western Cape, Eastern Cape, and Gauteng were subjected to routine DST by the National Health Laboratory Services (NHLS) using the indirect proportion method on Middlebrook 7H11 medium (28). In Gauteng, DST was done for INH, RMP, ethambutol (EMB), and streptomycin (SM) only; in the Western Cape and Eastern Cape, DST was extended to ethionamide (ETH), kanamycin (KM), amikacin (AMK), and ofloxacin (OFX). However, in the Western Cape, testing for second-line drugs covered only isolates collected in late 2006 onwards. In KwaZulu-Natal, during the study period, culture and DST were routinely done by the Regional Laboratory Service at Inkosi Albert Luthuli Central Hospital, using the agar proportion method on Middlebrook 7H10 agar plates containing INH, RMP, EMB, SM, KM, and OFX (28).

Genotypic characterization. *M. tuberculosis* isolates from patients were subcultured on Lowenstein-Jensen medium and genotyped using spoligotyping (17). The spoligotype patterns were compared to the data deposited in the fourth international spoligotyping database (SpolDB4)

(3) and grouped according to the different strain families defined in SpolDB4. Subclassification of Beijing genotypes into typical and atypical Beijing strains was performed on a random sample of Beijing genotype strains from the Western Cape, Eastern Cape, and Gauteng using a previously described PCR protocol or IS6110 RFLP analysis (27, 29). Beijing isolates from KwaZulu-Natal were not available for further subclassification. A random sample of 13 typical and 19 atypical XDR Beijing isolates from the Western Cape and 65 atypical XDR Beijing isolates from the Eastern Cape were tested for mutations in the *inhA* promoter using previously described methods (32).

Definitions. MDR and XDR strains were classified according to WHO definitions (37). Pre-XDR-TB isolates were defined as MDR-TB isolates with additional resistance to either a fluoroquinolone (e.g., OFX) or a second-line injectable drug (capreomycin, KM, or AMK) but not both (1). The MDR *sensu stricto* group excluded identified pre-XDR and XDR isolates.

Statistical analysis. Statistical analyses were done using the STATA program (version 10.0; StataCorp LP, College Station, TX). Pearson's chi-square test was performed to test for a significant difference in the proportion of *M. tuberculosis* genotypes between two provinces or drug resistance groups. A *P* value of <0.05 was considered statistically significant.

RESULTS

A sample of 3,646 MDR isolates of *M. tuberculosis* collected between 2000 and 2010 from patients in four South African provinces, namely, the Western Cape, Eastern Cape, KwaZulu-Natal, and Gauteng Provinces, was genotyped by spoligotyping (17). A total of 220 spoligotype patterns were identified, of which 122 could be grouped into 41 strain families according to the international spoligotyping database (SpolDB4) (3). The remaining isolates showed spoligotype patterns that did not match with any of the patterns in SpolDB4 (i.e., 206 isolates representing 98 different spoligotype patterns; 5.7% of all isolates; Table 2). The frequency distribution of isolates from all identified strain families stratified by origin and drug resistance group is presented in Table A1.

Between the four South African provinces, Beijing strains were predominantly localized to the Western and Eastern Cape Prov-

TABLE 2 Frequency distribution of major MDR genotypes in four South African provinces stratified by drug resistance group

		Result by strain family ^a													
		Beijing		LAM4		S		T1		X1		Others		Total	
		No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Province	Drug resistance class	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Western Cape	All MDR	1,174	54.7	52	2.4	51	2.4	158	7.4	181	8.4	529	24.7	2,145	100.0
	MDR <i>sensu stricto</i>	919	50.5	48	2.6	49	2.7	148	8.1	172	9.5	483	26.6	1,819	100.0
	Pre-XDR	161	72.9	4	1.8	2	0.9	6	2.7	9	4.1	39	17.6	221	100.0
	XDR	94	89.5	0	0.0	0	0.0	4	3.8	0	0.0	7	6.7	105	100.0
Eastern Cape	All MDR	348	69.2	52	10.3	10	2.0	13	2.6	0	0.0	80	15.9	503	100.0
	MDR <i>sensu stricto</i>	144	50.5	46	16.1	9	3.2	13	4.6	0	0.0	73	25.6	285	100.0
	Pre-XDR	115	92.7	5	4.0	0	0.0	0	0.0	0	0.0	4	3.2	124	100.0
	XDR	89	94.7	1	1.1	1	1.1	0	0.0	0	0.0	3	3.2	94	100.0
KwaZulu-Natal	All MDR	15	6.4	61	26.2	71	30.5	30	12.9	0	0.0	56	24.0	233	100.0
	MDR <i>sensu stricto</i>	15	7.7	39	19.9	66	33.7	27	13.8	0	0.0	49	25.0	196	100.0
	Pre-XDR	0	0.0	4	33.3	3	25.0	1	8.3	0	0.0	4	33.3	12	100.0
	XDR	0	0.0	18	72.0	2	8.0	2	8.0	0	0.0	3	12.0	25	100.0
Gauteng	All MDR	128	16.7	128	16.7	56	7.3	109	14.2	0	0.0	344	45.0	765	100.0
All provinces	All MDR	1,665	45.7	293	8.0	188	5.2	310	8.5	181	5.0	1,009	27.7	3,646	100.0

^a MDR *sensu stricto*, MDR cases not including identified pre-XDR and XDR-TB cases; No., number of isolates belonging to a given strain family for a given drug resistance group and province; %, proportion of isolates belonging to a given strain family for a given drug resistance group and province. LAM4 is also commonly referred to as F15/LAM4/KZN.

inces, where 55% and 69% of all MDR isolates belonged to this genotype, respectively. Conversely, Beijing strains represented only 6% and 17% of all MDR isolates from KwaZulu-Natal and Gauteng, respectively (Table 2; Fig. 1; $P < 0.001$ for the pairwise comparison of the proportions between all provinces). Differen-

tiation of a subset of the Beijing isolates into the subfamilies of typical and atypical Beijing genotypes highlighted distinct population structures in the different provinces. In the Western Cape, 75% of all analyzed MDR isolates of the Beijing genotype belonged to the group of typical Beijing strains. In contrast, only 8% of the

FIG 1 Frequency distribution of major MDR genotypes in four South African provinces. The proportion of isolates belonging to the Beijing, LAM4, S, T1, X1, or other genotypes is indicated in different shades of gray. The proportion of typical and atypical Beijing strains among the Beijing strains in Western Cape, Eastern Cape, and Gauteng Provinces is shown in Table 2. Beijing strains from KwaZulu-Natal were not available for further characterization. LAM4 is also commonly referred to as F15/LAM4/KZN.

TABLE 3 Proportion of typical and atypical Beijing strains

Province	Drug resistance class	Result by strain type ^a					
		Typical		Atypical		All	
		No.	%	No.	%	No.	%
Western Cape	All MDR	168	75.0	56	25.0	224	100.0
	MDR <i>sensu stricto</i>	129	85.4	22	14.6	151	100.0
	Pre-XDR	25	67.6	12	32.4	37	100.0
	XDR	14	38.9	22	61.1	36	100.0
Eastern Cape	All MDR	16	7.9	186	92.1	202	100.0
	MDR <i>sensu stricto</i>	16	32.0	34	68.0	50	100.0
	Pre-XDR	0	0.0	85	100.0	85	100.0
	XDR	0	0.0	67	100.0	67	100.0
Gauteng	All MDR	67	82.7	14	17.3	81	100.0

^a MDR *sensu stricto*, MDR cases not including identified pre-XDR and XDR-TB cases; No., number of isolates tested belonging to the group of typical or atypical Beijing strains for a given drug resistance group and province; %, proportion of isolates tested belonging to the group of typical or atypical Beijing strains for a given drug resistance group and province.

analyzed Beijing isolates from the Eastern Cape were members of the typical group and 92% belonged to the group of atypical Beijing strains (Table 3; Fig. 1; $P < 0.001$). In Gauteng, 83% of the tested Beijing isolates were members of the typical Beijing genotype (Table 3; Fig. 1). Beijing isolates from KwaZulu-Natal were not available for further characterization.

The concept of distinct population structures within the different provinces is further supported by our observation that the S and LAM4 (also referred to as F15/LAM4/KZN) families together constituted 57% of the isolates in KwaZulu-Natal but only 5%, 12%, and 24% of the isolates in the Western Cape, Eastern Cape, and Gauteng, respectively (Table 2; Fig. 1; $P < 0.001$ for the pairwise comparison of the proportions between all provinces). Interestingly, the X1 family of strains was represented only in isolates from the Western Cape (8% of all isolates) (Table 2; Fig. 1). The strain population structure in Gauteng also differed from that in the other provinces. This was mostly suggested by the more equal frequency distribution of isolates from different strain families. While the two most frequently detected MDR strain families in the Western Cape, Eastern Cape, and KwaZulu-Natal contributed 57% and more of all isolates in these provinces, strains belonging to the two most prevalent genotypes in Gauteng constituted only 33% of the isolates in this province (Table 2; Fig. 1). Of note, the EAI1_SOM and the H3 lineages were significantly more frequently isolated from MDR-TB patients in Gauteng (7% and 12% of the isolates, respectively) than in the other provinces (less than 2%; $P < 0.001$), further illustrating the distinct population structure in Gauteng (see Table A1).

Stratification of the genotyping data for the Western Cape, Eastern Cape, and KwaZulu-Natal by phenotypic drug resistance (no second-line DST results were available for Gauteng) revealed an increasingly marked predomination of one or two strain families from MDR *sensu stricto* to pre-XDR and XDR-TB (Table 2; Fig. 2). In the Western Cape and Eastern Cape Provinces, 90% or more of the XDR isolates belonged to the Beijing family of strains, representing a more than 75% increase compared to MDR *sensu stricto* ($P < 0.001$ for both provinces; Table 2; Fig. 2). Notably, the extrapolated overall proportion of atypical Beijing strains in-

creased from 7% in the MDR *sensu stricto* group to 55% in the XDR group in the Western Cape, while the overall proportion of atypical Beijing strains increased from 34% in the MDR *sensu stricto* group to 95% in the XDR group in the Eastern Cape (Tables 1 and 2; Fig. 2). Similarly, in KwaZulu-Natal, the proportion of isolates belonging to the F15/LAM4/KZN family of strains increased from 20% in MDR *sensu stricto* cases to 72% in XDR-TB cases (Table 2).

Of the 13 typical and 19 atypical XDR Beijing isolates from the Western Cape tested for *inhA* mutations, 11 (85%) and 19 (100%), respectively, showed mutations in the *inhA* promoter. Of the 65 atypical XDR Beijing isolates from the Eastern Cape tested for *inhA* mutation, 62 (95%) showed a mutation in the *inhA* promoter.

DISCUSSION

The present study represents a comprehensive description and comparison of the regional population structures of MDR *M. tuberculosis* strains in a high-burden country. Molecular characterization of MDR isolates from the Western and Eastern Cape, KwaZulu-Natal, and Gauteng Provinces revealed distinct MDR-TB strain population structures in all four provinces and the geographical localization of specific strain families to different regions. In particular, typical and atypical Beijing strains are over-represented in the neighboring coastal Western and Eastern Cape Provinces, and strains of the S and F15/LAM4/KZN families are present at significantly higher frequencies in KwaZulu-Natal (Table 2; Fig. 1).

Distinct population structures between different regions may be explained by the lack of strain exchange between geographically separated populations, resulting in a disconnected evolution and a progressive differentiation of these populations over time (20). Interestingly, the observed differences in the interprovincial MDR strain population structures parallel observations in the population structure of *M. bovis* in other parts of the world (20, 26) but are unexpected, as one might assume that internal migrations in South Africa could homogenize population structures between closely situated regions to a larger extent (18).

However, our data provide evidence to suggest that limited strain exchange between different regions is occurring. This is especially indicated by the presence of most of the strain families in more than one province. Of all 3,646 isolates analyzed within this study, 3,185 isolates (87%, representing 51 spoligotype patterns) exhibited a spoligotype pattern that was present in more than one province. Previous analyses of South Africa's internal migrations showed the highest immigration rates for Gauteng (18); this is also reflected by a more equal frequency distribution of MDR-TB genotypes in this setting (Table 2; Fig. 1). Residents from KwaZulu-Natal mostly immigrated to Gauteng and significantly less often to Western or Eastern Cape Province, perhaps explaining the more frequent isolation of MDR isolates belonging to the S and F15/LAM4/KZN families of strains in Gauteng and the very distinct population structure between KwaZulu-Natal and the Western and Eastern Cape Provinces. Over 50% of the migrations to the Western Cape originated from the Eastern Cape (18), which possibly led to the introduction of the atypical Beijing strain genotype into the Western Cape. It could be speculated that the observed evidence for strain exchange between provinces is mostly based on recent human migrations, while the generally different population structures between provinces could reflect

FIG 2 Frequency distribution of major MDR genotypes stratified by drug resistance group for three South African provinces. The proportion of isolates belonging to the Beijing, LAM4, S, T1, X1, or other genotypes is indicated for MDR *sensu stricto* (not including pre-XDR and XDR isolates), pre-XDR, and XDR cases (different shades of gray). The proportion of typical and atypical Beijing strains among the Beijing strains in Western and Eastern Cape Provinces is extrapolated from Table 2. LAM4 is also commonly referred to as F15/LAM4/KZN. (Map adapted from <http://home.global.co.za/~mercon/map.htm> with permission.)

the situation during earlier periods characterized by less migration (e.g., due to a restrictive migration policy during the apartheid era from 1948 to 1994) (18). Alternatively, the distinct population structures may be a result of multiple factors, including geographically localized outbreaks or epidemics and distinct migration patterns in different settings.

An introduction of the atypical Beijing lineage from the Eastern Cape into the Western Cape and not vice versa is mostly supported by the much higher overall prevalence of this lineage in the Eastern Cape (Fig. 1), a considerably higher diversity of IS6110 RFLP patterns among the atypical Beijing strain population in the Eastern Cape than the Western Cape (data not shown), and a 10 times higher number of human migrations from the Eastern to the Western Cape than vice versa (18). An introduction from other provinces not included in this study, such as the neighboring Northern Cape (Fig. 2), is less likely considering the human migratory behaviors in South Africa (18) and a significantly higher

TB and MDR-TB incidence in the Eastern Cape than the Northern Cape (22), making spillover to the adjacent province more likely. Taken together, the current data suggest that the atypical Beijing lineage, accounting for more than 50% of all XDR-TB cases detected in the Western Cape, was introduced from the Eastern Cape.

XDR-TB cases caused by atypical Beijing strains in the Western Cape are for the most part unlikely to be a result of clonal transmission of XDR strains, as recent data revealed a variety of distinct second-line drug resistance mutations among IS6110 RFLP-clustered isolates (14). Thus, the majority of these XDR cases emerged through the amplification of resistance in primary MDR *sensu stricto* or pre-XDR-TB isolates. The predominance of atypical Beijing strains in XDR-TB in the Western Cape, their significantly reduced contribution to MDR *sensu stricto* TB and pre-XDR-TB (Fig. 2; Table 3), and the lack of evidence for the clonal transmission of XDR strains (14) suggest that a considerable

Result by province and drug resistance group^a^a MDR *sensu stricto*, MDR cases not including identified pre-XDR and XDR-TB cases; No., number of isolates belonging to a given strain family for a given drug resistance group and province; %, proportion of isolates belonging to a given strain family for a given drug resistance group and province. LAM4 is also commonly referred to as F15/LAM4/KZN.

^a MDR *sensu stricto*, MDR cases not including identified pre-XDR and XDR-TB cases; No., number of isolates belonging to a given strain family for a given drug resistance group and province; %, proportion of isolates belonging to a given strain family for a given drug resistance group and province. LAM4 is also commonly referred to as F15/LAM4/KZN.

amount of XDR-TB cases detected in the Western Cape could have developed from patients infected in the Eastern Cape who subsequently migrated or returned to the Western Cape or who were seeking treatment outside their residential province. It seems conceivable that, in particular, patients with complicated disease residing in the economically more depressed Eastern Cape may decide to visit health clinics in the more affluent Western Cape, where they may expect a relatively superior quality of health care services (25). This situation could illustrate how relatively increased disease frequencies or a shortage of access to efficient health care in a given region may burden health systems in adjacent areas. Similar scenarios are found in other regions of the world; e.g., MDR-TB clusters identified in Europe are associated with strains originating in eastern European countries (7). Equal access to functional health care services in closely situated regions is essential to prevent aggravation of circumstances in a given setting and the subsequent overload of health clinics in neighboring regions.

This study shows an intriguing, increasingly marked predominance of one single strain family or two strain families from the MDR *sensu stricto* group to the XDR-TB group in all three provinces analyzed (Table 2; Fig. 2). An association of certain strain families with XDR-TB could be attributed to their relatively more effective transmission as MDR strains or, alternatively, may be explained by an enhanced intrinsic capacity to acquire resistance to second-line anti-TB drugs. We have recently demonstrated a significant association of *inhA* promoter mutations with XDR-TB in the Western and Eastern Cape Provinces of South Africa (21). *inhA* promoter mutations confer low-level resistance to INH (used in first-line therapy for supposedly drug-sensitive TB) and high-level resistance to ETH (commonly used in second-line drug regimens for the treatment of MDR-TB) (39). We hypothesize that MDR strains which have acquired such mutations at a previous instance (e.g., when exposed to INH during first-line therapy) would show an increased probability to gain resistance to other second-line drugs due to the treatment regimen having one less effective drug (21). Our preliminary data from the Western and Eastern Cape Provinces and a previous study in KwaZulu-Natal revealed that *inhA* promoter mutations are strongly linked to strains predominating in XDR-TB in at least three South African provinces (13, 21). Similarly, in a study from Portugal, all of 26 XDR-TB isolates analyzed showed an *inhA* promoter mutation (23), suggesting that this association might be of importance in several parts of the world. These observations could be explained if, within the context of certain standardized second-line drug regimens, the presence of an *inhA* promoter mutation could increase the likelihood for a given strain to acquire additional resistance.

We previously recommended an adapted treatment regimen not including ETH for TB patients infected with strains harboring an *inhA* promoter mutation (21). High-dose INH may be considered for treatment in such cases if no additional high-level resistance mutations (e.g., mutations in *katG* gene codon 315) are present in infecting strains. Importantly, with the use of molecular probe assays such as the GenoType MTBDR_{plus} assay, now regularly used for genotypic DST in many settings, information about the presence of *inhA* promoter mutations in patient isolates is readily available to clinicians (21).

The present study has certain limitations. First, and most importantly, sample collection in the different provinces occurred

during distinct time periods (Table 1), which could have caused artificial dissimilarities between the observed population structures. In Western Cape Province, where isolates were collected over the longest period, an extensive increase in drug-resistant TB cases due to the Beijing family of strains could be observed, with 32% and 63% of the isolates belonging to this family in 2001 and 2009, respectively. This is in accordance with previous reports from this region (16, 27a). Nevertheless, no considerable change with respect to the different strain families comprising the most frequently observed MDR-TB isolates was evident, suggesting that temporal biases were unlikely to artificially create distinct strain population structures between the investigated regions. Second, we may not be able to exclude the possibility that the population bottlenecks occurring during the transitions from MDR *sensu stricto* TB to pre-XDR- and XDR-TB could, in theory, account for the increasingly marked predominance of only one or two strain families from the MDR *sensu stricto* group to the XDR-TB group. More research is needed to investigate the nature of this observation.

In conclusion, the data presented show a distinct population structure and geographical localization of MDR strains of *M. tuberculosis* in four South African provinces. Evidence for provincial migration and, in particular, the introduction of XDR strains from the Eastern to the Western Cape highlights the need for uniformly implemented TB control programs. Only a few strain families contribute to XDR-TB in South Africa, suggesting an enhanced ability of these strains to transmit as primary MDR strains or to acquire resistance to second-line drugs. In either case, inadequate diagnostic and treatment algorithms have led to the programmatic selection of these strains to become XDR strains. South African guidelines for the treatment of MDR-TB have recently been amended, with terizidone replacing EMB for the treatment of MDR-TB and capreomycin, *p*-aminosalicylic acid, and moxifloxacin replacing KM (or AMK), PZA, and OFX, respectively, for the treatment of XDR-TB. It is questionable whether these changes will help curb the emergence of XDR-TB or the predominance of the few transmitting MDR strains accounting for the majority of XDR-TB cases. This study provides important baseline data that may be used to understand and monitor future changes in the population structure of M/XDR strains of *M. tuberculosis* and to assess the impact of such interventions.

APPENDIX

Table A1 presents the frequency distribution of isolates from all identified strain families stratified by origin and drug resistance group.

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REFERENCES

- Banerjee R, et al. 2008. Extensively drug-resistant tuberculosis in California, 1993–2006. *Clin. Infect. Dis.* 47:450–457.
- Borrell S, Gagneux S. 2009. Infectiousness, reproductive fitness and evolution of drug-resistant *Mycobacterium tuberculosis*. *Int. J. Tuberc. Lung Dis.* 13:1456–1466.
- Brudey K, et al. 2006. *Mycobacterium tuberculosis* complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. *BMC Microbiol.* 6:23.

4. Calver AD, et al. 2010. Emergence of increased resistance and extensively drug-resistant tuberculosis despite treatment adherence, South Africa. *Emerg. Infect. Dis.* 16:264–271.
5. Cohen T, Murray M. 2004. Modeling epidemics of multidrug-resistant *M. tuberculosis* of heterogeneous fitness. *Nat. Med.* 10:1117–1121.
6. Cox HS, et al. 2010. Epidemic levels of drug resistant tuberculosis (MDR and XDR-TB) in a high HIV prevalence setting in Khayelitsha, South Africa. *PLoS One* 5:e13901.
7. Devaux I, Kremer K, Heersma H, van Soolingen D. 2009. Clusters of multidrug-resistant *Mycobacterium tuberculosis* cases, Europe. *Emerg. Infect. Dis.* 15:1052–1060.
8. Donald PR, van Helden PD. 2009. The global burden of tuberculosis—combating drug resistance in difficult times. *N. Engl. J. Med.* 360:2393–2395.
9. Dye C, Espinal MA, Watt CJ, Mbiaga C, Williams BG. 2002. Worldwide incidence of multidrug-resistant tuberculosis. *J. Infect. Dis.* 185:1197–1202.
10. Gagneux S, Small PM. 2007. Global phylogeography of *Mycobacterium tuberculosis* and implications for tuberculosis product development. *Lancet Infect. Dis.* 7:328–337.
11. Glynn JR, Whiteley J, Bifani PJ, Kremer K, van Soolingen D. 2002. Worldwide occurrence of Beijing/W strains of *Mycobacterium tuberculosis*: a systematic review. *Emerg. Infect. Dis.* 8:843–849.
12. Hoek KG, Schaaf HS, Gey van Pittius NC, van Helden PD, Warren RM. 2009. Resistance to pyrazinamide and ethambutol compromises MDR/XDR-TB treatment. *S. Afr. Med. J.* 99:785–787.
13. Ioerger TR, et al. 2009. Genome analysis of multi- and extensively-drug-resistant tuberculosis from KwaZulu-Natal, South Africa. *PLoS One* 4:e7778.
14. Ioerger TR, et al. 2010. The non-clonality of drug resistance in Beijing-genotype isolates of *Mycobacterium tuberculosis* from the Western Cape of South Africa. *BMC Genomics* 11:670.
15. Jassal M, Bishai WR. 2009. Extensively drug-resistant tuberculosis. *Lancet Infect. Dis.* 9:19–30.
16. Johnson R, et al. 2010. Drug-resistant tuberculosis epidemic in the Western Cape driven by a virulent Beijing genotype strain. *Int. J. Tuberc. Lung Dis.* 14:119–121.
17. Kamerbeek J, et al. 1997. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. *J. Clin. Microbiol.* 35:907–914.
18. Kok P, O'Donovan M, Bouare O, van Zyl J. 2003. Post-apartheid patterns of internal migration in South Africa. Human Sciences Research Council, Cape Town, South Africa.
19. Mlambo CK, et al. 2008. Genotypic diversity of extensively drug-resistant tuberculosis (XDR-TB) in South Africa. *Int. J. Tuberc. Lung Dis.* 12:99–104.
20. Müller B, et al. 2009. African 1, an epidemiologically important clonal complex of *Mycobacterium bovis* dominant in Mali, Nigeria, Cameroon, and Chad. *J. Bacteriol.* 191:1951–1960.
21. Müller B, et al. 2011. *inhA* promoter mutations: a gateway to extensively drug-resistant tuberculosis in South Africa? *Int. J. Tuberc. Lung Dis.* 15: 344–351.
22. National Health Laboratory Service, National Institute for Communicable Diseases. 2009. National Institute for Communicable Diseases annual report. National Health Laboratory Service, National Institute for Communicable Diseases, Johannesburg, South Africa.
23. Perdigao J, et al. 2010. Genetic analysis of extensively drug-resistant *Mycobacterium tuberculosis* strains in Lisbon, Portugal. *J. Antimicrob. Chemother.* Feb. 65:224–227.
24. Pillay M, Sturm AW. 2007. Evolution of the extensively drug-resistant F15/LAM4/KZN strain of *Mycobacterium tuberculosis* in KwaZulu-Natal, South Africa. *Clin. Infect. Dis.* 45:1409–1414.
25. Sanders D, Chopra M. 2006. Key challenges to achieving health for all in an inequitable society: the case of South Africa. *Am. J. Public Health* 96: 73–78.
26. Smith NH, et al. 2006. Bottlenecks and broomsticks: the molecular evolution of *Mycobacterium bovis*. *Nat. Rev. Microbiol.* 4:670–681.
27. Strauss OJ, et al. 2008. Spread of a low-fitness drug-resistant *Mycobacterium tuberculosis* strain in a setting of high human immunodeficiency virus prevalence. *J. Clin. Microbiol.* 46:1514–1516.
- 27a. van der Spuy GD, et al. 2009. Changing *Mycobacterium tuberculosis* population highlights clade-specific pathogenic characteristics. *Tuberculosis* 89:120–125.
28. Van Deun A, Martin A, Palomino JC. 2010. Diagnosis of drug-resistant tuberculosis: reliability and rapidity of detection. *Int. J. Tuberc. Lung Dis.* 14:131–140.
29. van Embden JD, et al. 1993. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. *J. Clin. Microbiol.* 31:406–409.
30. Van Rie A, et al. 1999. Transmission of a multidrug-resistant *Mycobacterium tuberculosis* strain resembling “strain W” among noninstitutionalized, human immunodeficiency virus-seronegative patients. *J. Infect. Dis.* 180:1608–1615.
31. van Soolingen D. 2001. Molecular epidemiology of tuberculosis and other mycobacterial infections: main methodologies and achievements. *J. Intern. Med.* 249:1–26.
32. Victor TC, et al. 1999. Detection of mutations in drug resistance genes of *Mycobacterium tuberculosis* by a dot-blot hybridization strategy. *Tuberc. Lung Dis.* 79:343–348.
33. Reference deleted.
34. World Health Organization. 2010. Global tuberculosis control. World Health Organization, Geneva, Switzerland. http://whqlibdoc.who.int/publications/2010/9789241564069_eng.pdf. Accessed 10 September 2011.
35. World Health Organization. 2009. Treatment of tuberculosis—guidelines, 4th ed. World Health Organization, Geneva, Switzerland. http://whqlibdoc.who.int/publications/2010/9789241547833_eng.pdf. Accessed 10 September 2011.
36. World Health Organization. 2009. Global tuberculosis control 2009—epidemiology, strategy, financing. World Health Organization, Geneva, Switzerland. http://whqlibdoc.who.int/publications/2009/9789241563802_eng.pdf. Accessed 10 September 2011.
37. World Health Organization. 2010. Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response. World Health Organization, Geneva, Switzerland. http://whqlibdoc.who.int/publications/2010/9789241599191_eng.pdf. Accessed 10 September 2011.
38. The World Health Organization/International Union Against Tuberculosis and Lung Disease (WHO/IUATLD) Global Project on Anti-Tuberculosis Drug Resistance. 2008. Surveillance. Anti-tuberculosis drug resistance in the world. Report no. 4. World Health Organization, Geneva, Switzerland. http://www.who.int/tb/publications/2008/drs_report4_26feb08.pdf. Accessed 10 September 2011.
39. Zhang Y, Yew WW. 2009. Mechanisms of drug resistance in *Mycobacterium tuberculosis*. *Int. J. Tuberc. Lung Dis.* 13:1320–1330.