Genotypic and Phenotypic Characterization of Drug-Resistant *Mycobacterium tuberculosis* Isolates from Rural Districts of the Western Cape Province of South Africa

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Received 12 June 2003/Returned for modification 1 September 2003/Accepted 22 October 2003

Genotypic and phenotypic analysis of drug-resistant *Mycobacterium tuberculosis* isolates from the Western Cape Province of South Africa showed that drug resistance is widespread and recently transmitted. Multidrug-resistant (MDR) isolates comprise 40% of this collection, and a large pool of isoniazid monoresistance may be a future source of MDR tuberculosis.

By using genotyping methods, outbreaks of multidrug-resistant tuberculosis (MDR-TB) have been identified in hospitals, among health care workers, in prisons, and in communities (1, 3–5, 8, 11, 17, 21), thus focusing attention on MDR-TB as a major public health issue. The most-extensive MDR-TB outbreak of Beijing/W-like isolates (9) occurred in New York among 267 patients, the majority of whom were coinfecting with human immunodeficiency virus. The Beijing or W-like family of *Mycobacterium tuberculosis* continues to be the focus of extensive investigations as such strains are widely spread throughout the world (2, 10), including South Africa (23) and Russia (15), where it constitutes the major family of MDR-TB isolates. There is a rising concern about the spread of MDR-TB strains from developing to developed countries. However, the bacterial population structure of resistant isolates from both developed and developing countries is not well documented. It is therefore possible that the drug-resistant Beijing/W-like strain or other MDR outbreak-associated strains are widespread but have not been recognized and reported as such. MDR-TB was first recorded in 1985 in the Western Cape Province of South Africa, but no genotype data are available from these early isolates. Although drug surveillance studies have been done in the Western Cape, they have only provided information on the drug-resistant phenotype (24).

This study describes the genotypic and phenotypic characteristics of drug-resistant isolates ($n = 482$) from 328 cases collected from Jan 2001 to Feb 2002 (14 months) from 72 clinics in the Boland-Overberg and Southern Cape-Karoo re-

gions of the Western Cape Province, South Africa. The first available drug-resistant isolate of each case as determined by the indirect proportion method on Lowenstein-Jensen medium (13) was used in this study. The age of patients varied from 15 to 73 years (mean, 37 years), and 59% of patients were male. The isolates were categorized into those that were isoniazid (INH) monoresistant (48%), MDR (40%), and MDR with rifampin monoresistance also included (12%). Each isolate was genotypically characterized by spoligotyping, using the internationally standardized method (12, 16). Sixty-nine different spoligotype patterns were identified and deposited in the international database at The Pasteur Institute of Guadeloupe (7). Of these, 34 were previously listed, while the remaining 35 types were newly added to this database. The genotypic and phenotypic data were analyzed to identify characteristics of the drug-resistant-TB epidemic in the region. The results indicate that MDR-TB forms a significant part (40%) of the drug-resistant-TB epidemic and that drug-resistant TB was spread throughout the region. MDR-TB is present in the whole area but is more prevalent in one town in the Southern Cape region. INH monoresistance is more prevalent in the Boland, Overberg, and Karoo regions but less represented in the Southern Cape region (Fig. 1). Detailed analysis of the genotypic data showed that more than 80% of the isolates can be grouped based on genotypic spoligotypes and phenotypic drug resistance patterns (Fig. 2). The numerous clusters suggest that transmission of drug-resistant strains contributes to the spread of drug-resistant TB. This may be an overestimation of the extent of transmission of drug-resistant TB in the region, as the discriminatory power of spoligotyping is less than that of IS6110 restriction fragment length polymorphism analysis (14). However, the results are similar to those of a IS6110 restriction fragment length polymorphism analysis study which showed that more than 60% of

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FIG. 1. Map of South Africa with the distribution of the three drug resistance groups in 72 clinics of the Western Cape Province.

drug-resistant TB occurred by transmission in urban communities in Cape Town, South Africa (22). We speculate that the high level of transmission may in part be exacerbated by the relatively slow culture-based diagnostic procedures. Application of PCR techniques for rapid diagnosis of drug resistance may help to control the ongoing transmission. Based on the spoligotype patterns, the isolates could be grouped into families (Fig. 2), and the results showed that four strain families were responsible for more than 70% of the drug-resistant-TB epidemic in the region. The Beijing/W-like and the IS6110 low-copy-number clade spoligotype patterns were the most prevalent drug-resistant isolates. It has been suggested that Beijing isolates are more frequently resistant due to their ability to mutate more rapidly than other strains (10). In neighboring communities in Cape Town, 17% of all patients with TB are infected with a Beijing strain (drug resistant or susceptible) (18). Patients infected with a Beijing/W-like drug-resistant isolate in this study may be overrepresented (28%), suggesting that the Beijing strain family acquires drug resistance muta-

tions more frequently than strain families F11 (12%) and F28 (5%). This is still speculative and needs further investigation. The large pool of INH-mono-resistant isolates (48%) is of great concern. INH-mono-resistant isolates are mostly from the Beijing/W-like family (29%). However, many genotypes of the INH mono-resistance group are also present in the MDR group (Fig. 2), suggesting that MDR may have developed predominantly from INH-mono-resistant isolates by selection. Such selection may easily result in additional MDR-TB in the future. This is supported by the observation that 20% of patients with MDR-TB in this region (since 1990) have had previous infections with mono-resistant or MDR strains (C. Kewley, personal communication). Results from the present investigation showed that cases of MDR-TB are overrepresented in the IS6110 low-copy-number clade (54%) in comparison to the results seen with the Beijing/W-like (38%), F11 (38%), and F28 (28%) families. Conversely, the IS6110 low-copy-number clade underrepresents INH-mono-resistant isolates (27%). Although the IS6110 low-copy-number clade isolates are found

FIG. 2. Spoligotypes of drug-resistant isolates from two health districts of the Western Cape Province of South Africa. Explanation of footnote symbol a: Int. type, international type (according to the international database at the Pasteur Institute of Guadeloupe [7]); explanation of footnote symbol b: four strain families: Beijing/W-like (direct variable repeats 1 to 34 deleted; correlates to share type 1 in reference 20); F11 (family 11) (direct variable repeats 9 to 11, 21 to 24, and 33 to 36 deleted; correlates to LAM3 family in reference 6); F28 (family 28) (direct variable repeats 9, 10, and 33 to 36 deleted; correlates to the S family in reference 6); LCC (IS6110 low-copy-number clade) (direct variable repeats 18 and 33 to 36 deleted; correlates to the X family in reference 19). Abbreviations: np1, not previously identified in spoligotypes DB3 and DB4 (7, 20); np1 (LT115), not previously identified in spoligotypes DB3 and DB4 (7, 20), but local type number is 115; INH mono^R, INH-mono-resistant isolates; Multiple drug^R, MDR isolates (rifampin-mono-resistant isolates included).

Int. type ^a	Spoligotype pattern	INH mono ^R	MDR	Multiple Drug ^R	Family ^b
1		46	33	12	Beijing n=91 (28%)
npi		1	0	0	F11 n=40 (12%)
npi		0	1	0	
npi		1	0	0	
npi		1	0	0	
npi		0	1	0	
211		1	0	0	
npi		1	0	0	
npi		0	1	0	
npi		0	0	1	
npi		0	2	0	
130		1	0	1	F28 n=18 (5%)
33		16	10	1	
npi		2	0	0	
71		2	0	0	
npi		2	0	0	
npi		1	1	0	LCC n=85 (26%)
npi		0	1	0	
34		4	3	2	
npi		0	1	0	
npi		1	0	0	
92		9	2	1	
npi		1	0	0	
npi		1	0	0	
348		1	3	0	
npi		1	0	0	
npi		0	0	1	Unknown n=94 (29%)
npi (LT115)		0	21	6	
137		3	3	1	
336		0	5	1	
119		8	9	6	
2		0	0	1	
npi		0	1	0	
npi		0	1	0	
20		1	0	0	
npi		1	0	0	
npi		2	0	0	
21		1	0	0	
26		0	1	0	
npi		1	0	0	
npi		1	0	0	
npi		3	2	0	
npi		1	0	0	
npi		1	0	0	
npi		0	1	0	
npi		0	0	1	
npi		1	0	0	
35		0	1	0	
158		1	0	0	
766		0	1	0	
npi		1	0	0	
npi		0	1	0	
39		15	3	0	
811		1	1	0	
npi		2	4	2	
60		0	1	0	
42		3	2	1	
44		0	3	0	
373		2	0	1	
602		0	0	1	
62		1	0	0	
48		1	0	0	
237		0	2	0	
50		0	4	0	
519		1	0	0	
521		1	1	0	
52		0	1	0	
53		8	3	0	
54		3	0	0	
TOTAL		157	131	40	
(n=328)		(48%)	(40%)	(12%)	

throughout the region, most of the isolates (66%) originate from the Southern Cape. One particular spoligotype (local spoligotype 115) is unique to isolates from the largest town in the region, and this spoligotype was previously not identified in the international spoligotyping database of Guadeloupe, France (7). More than 75% of these isolates are MDR and may represent an outbreak of an emerging MDR-TB strain in this town. This study highlights the drug-resistant-TB epidemic in the Western Cape. It also raises the concern that drug resistance is transmitted and that there is a need for enhanced control strategies, which may include efficient, rapid molecular-based diagnostics.

We thank the IAEA (project SAF 6003 and RAF 6025), the NIH (grant number R21 AI 055800-01), and the Harry Crossley Foundation for financial assistance.

We thank T. Dolman and H. Pretorius for preparation of isolates and A. Jordaan for technical assistance.

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