Tropical Medicine and International Health doi:10.1111/j.1365-3156.2007.01840.x

VOLUME 12 NO 6 PP 700–708 JUNE 2007

Endogenous reactivation and true treatment failure as causes of recurrent tuberculosis in a high incidence setting with a low HIV infection

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Summary

OBJECTIVE To determine the relative frequencies of reinfection *vs*. reactivation or treatment failure in patients from a high tuberculosis incidence setting with a low prevalence of HIV infection.
METHOD We performed DNA fingerprinting on serial isolates from one and multiple TB episodes from 97 retreatment patients; 35 patients had been previously cured, whereas 62 had not.
RESULTS DNA fingerprinting patterns of recurrence *Mycobacterium tuberculosis* isolates of 5 of the 35 previously cured patients did not match with those of the corresponding initial isolates, indicating reinfection. We did not document reinfection during treatment. Isolates from each of the remaining 30 previously cured patients had identical DNA fingerprinting results, indicating reactivation. DNA fingerprinting patterns of isolates from the 62 patients with persistently positive sputum smears were identical, suggesting treatment failure.
CONCLUSION These findings suggest that reinfection is not a common cause of relapse and treatment failure in this rural predominantly HIV-free population despite the high incidence of TB.

keywords tuberculosis, reinfection, reactivation, treatment failure, DNA fingerprinting

Introduction

Tuberculosis (TB) remains a major cause of morbidity and mortality worldwide despite the availability of effective chemotherapy. The situation is worse in developing countries, where multidrug resistance (MDR) and recurrent TB are on the increase (WHO 1997). Recurrent TB is generally higher in high incidence settings (9.6–38.1%) (Van Rie *et al.* 1999b; Johnson *et al.* 2000; Sonnenberg *et al.* 2001; Verver *et al.* 2005) than in low incidence settings (0–6.7%) (Bandera *et al.* 2001; Caminero *et al.* 2001; Garcia de Viedma *et al.* 2002). The recurrence has mainly been considered to be as a result of endogenous reactivation (hereafter referred to as relapse) after seemingly adequate treatment (Katz 1958). Using molecular methods, it is possible to distinguish recurrent disease as a result of relapse of a remotely acquired infection (March *et al.* 1997; Strassle *et al.* 1997), exogenous reinfection (Nardell *et al.* 1986; Chaves *et al.* 1999; Verver *et al.* 2005; Garcia de Viedma *et al.* 2002) or concomitant (mixed) infections (Theisen *et al.* 1995; Shamputa *et al.* 2004, 2006). Prior investigations on recurrent TB mainly focused on previously cured patients or cases with discordant drug resistance patterns between sequential isolates.

In this study, we investigated the relative frequencies of reactivation *vs*. reinfection and of treatment failure *vs*. reinfection in retreatment patients who were previously cured, or not cured, respectively, from the districts covered by Damien Foundation on behalf of the Bangladesh National Tuberculosis Program (NTP). We used spoligotyping, IS*6110*-based restriction fragment length polymorphism (RFLP) and/or typing based on variable number of tandem repeats of mycobacterial interspersed repetitive units (MIRU-VNTR) as DNA fingerprinting techniques.

Materials and methods

Patient population data collection

Our study subjects were drawn from a predominantly low (< 1%) HIV-prevalence rural population of the

Greater Mymensingh, Greater Rajshahi and Greater Faridpur Districts of Bangladesh (Van Deun *et al.* 2004). The estimated incidence of new smear-positive TB cases in the region is 106 cases per 1 00 000 inhabitants per year (Salim *et al.* 2001). A total of 62 785 sputum smear-positive cases (51 783 new cases and 11 002 who had taken anti-TB drugs for at least 1 month) were registered for treatment over the study period (January 1995 to December 2002). During this period of service expansion, the population covered increased from 1.3 to 20 million and the annual total smear-positive case registration rose from 2 336 to 11 502, whereas the proportion of females increased from 21% to 29%. The mean and median age of women remained similar i.e. nearly 40 and 38 years, respectively. The number of TB diagnostic and treatment facilities increased from 4 to 102. Together with a very high population density and a completely free service, this assured an excellent accessibility.

Tuberculosis patients from the study area were treated according to the NTP guidelines in line with previous World Health Organization (WHO) recommendations (WHO 1997). New smear-positive cases received a daily Category I (Cat I) regimen comprising isoniazid (INH), rifampin (RIF), pyrazinamide (PZA) and ethambutol (EMB) for 2 months followed by INH and thioacetazone for 6 months. Retreatment patients with a history of at least 1 month drug intake were treated with INH, RIF, STR, EMB and PZA for 2 months daily followed by INH, RIF, EMB and PZA for 1 month daily and INH, RIF and EMB for 5 months administered three times per week (Category II) (Cat II) (WHO 1997).

Throughout the study period, 825 treatment failures and 1 199 relapses (2 024 patients) had bacteriologically unfavourable treatment outcomes. These were de facto eligible for culture and drug-susceptibility testing (DST) for drug resistance monitoring (Van Deun *et al.* 2004). DST results were available for 1 568 (77.5%) of these patients, whereas for the remaining 456 patients no sample was sent, cultures remained negative or were contaminated. Moreover, two random surveys conducted in 1995 and 2001 resulted in 1 397 new patient isolates tested by DST (Van Deun *et al.* 1999, 2001). Among the 1 568 patients with failure or relapse isolates tested, 198 were eligible for inclusion in the study as they had one or more previously isolated *Mycobacterium tuberculosis* strains, collected at an interval of at least 3 months. Ninety-nine (50%) of these patients were randomly selected, but two patients were later excluded because their isolates were lost in subculture, thereby bringing the total number of patients included to 97 (Figure 1). The clinical data obtained from the patients comprised age, sex, sputum smear and DST results, treatment data and outcome. HIV testing was not performed on TB patients because of its very low prevalence in the study setting.

Isolation of *Mycobacterium tuberculosis* and DST

Sputum samples were collected at diagnosis or at suspicion of failure during treatment or relapse after treatment. Culture and DST was performed at the Prince Leopold Institute of Tropical Medicine (ITM) in Antwerp, Belgium, on Ziehl-Neelsen positive sputa transported in 1% cetylpyridinium chloride. Samples were decontaminated using the adapted Petroff method (Van Deun *et al.* 2001), culture and DST was performed by the standard 1% proportion method on Löwenstein-Jensen (L-J) for INH (0.2 μg/ml), RIF (40 μg/ml), STR (4 μg/ml) and EMB (2 μg/ml) (Canetti *et al.* 1969). All isolates were identified as *M. tuberculosis* by standard phenotypic identification tests and were maintained at −70 °C in Dubos liquid medium supplemented with 10% glycerol.

Minimum inhibitory concentration (MIC) testing

The MICs for INH (0.05, 0.2, 0.8 and 3.2 μg/ml) and RIF (10, 20, 40 and 80 μg/ml) were determined on L-J medium according to previous methods (Shamputa *et al.* 2004; WHO 2004).

Polymerase chain reaction (PCR) and sequencing

Polymerase chain reaction and sequencing for detection of mutations conferring resistance to INH and RIF were performed as described by Telenti *et al.* (1997).

DNA extraction

DNA extraction was performed by boiling bacteria suspended in 400 μl of TE buffer [10 mM Tris-HCl, 1 mM EDTA (pH 8.0)] (for spoligotyping and/or MIRU-VNTR), or as described by Van Embden *et al.* 1993 (for spoligotyping, RFLP or MIRU-VNTR).

DNA fingerprinting

All sequential isolates from each of the 97 patients were typed by spoligotyping (Kamerbeek *et al.* 1997). Isolates from most of these patients were also typed by RFLP (Van Embden *et al.* 1993). Where differences in hybridization patterns among isolates from the same patient were observed in one or both methods, additional typing using MIRU-VNTR was performed (Mazars *et al.* 2001). The

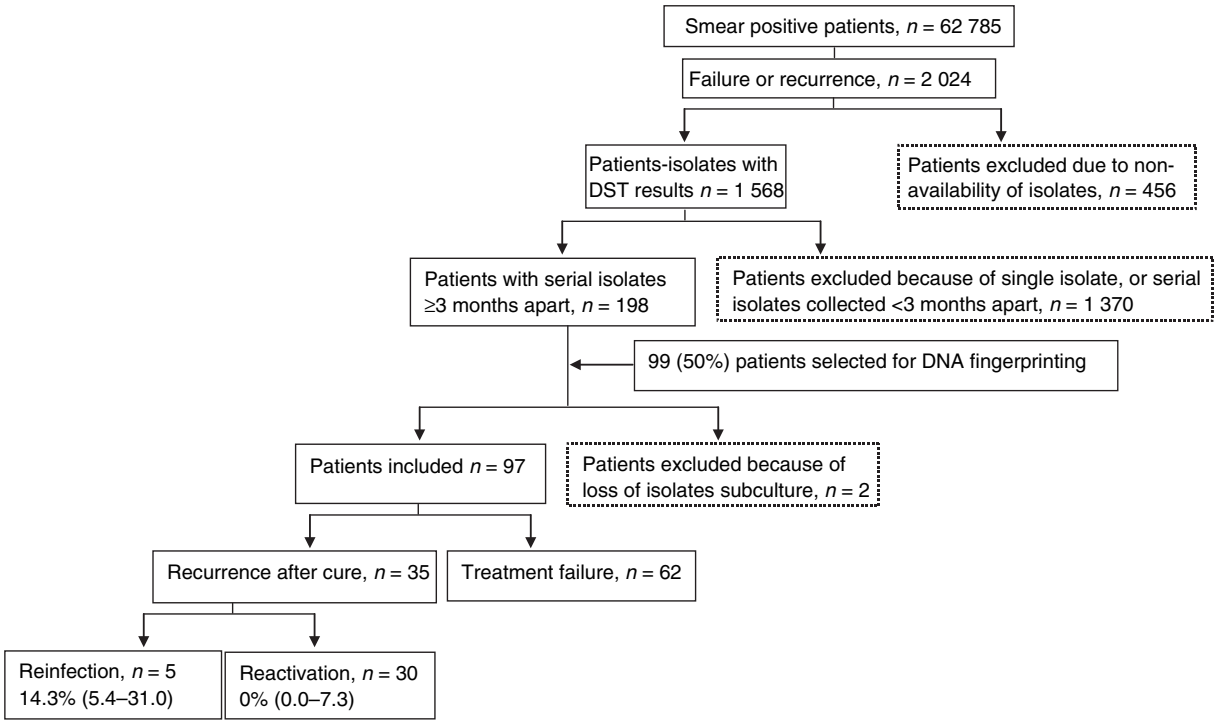


Figure 1 Schematic representation of patients studied.

latter technique was also used as a secondary typing technique where insufficient DNA was available for RFLP.

Isolates were considered identical if they had identical spoligotyping and RFLP profiles or showed only minor differences in RFLP (one or two bands), but identical MIRU-VNTR patterns. Isolates were considered to be different if they had different spoligotyping, MIRU-VNTR and RFLP patterns.

Investigations of laboratory cross contamination

To rule out the possibility that cross contamination may have caused the misassignment of reinfections, we typed by spoligotyping and MIRU-VNTR the strains from all the specimens that were processed in the laboratory on the same day as the specimens in our analysis. Where available, we also typed multiple sequential isolates from the patients with presumed reinfection to confirm our observation.

Computer-assisted analysis

Spoligotyping, RFLP and MIRU-VNTR patterns were compared using the Bionumerics software version 3.0 (Applied Maths, St Martens-Latem, Belgium).

Results

The 97 retreatment patients studied included 35 patients who were cured and 62 patients who were not cured between collection of the initial and last isolate, respectively. Cure was defined as completion of a full course of chemotherapy with negative sputum smears at the end of treatment and at least one preceding follow-up examination.

Previously cured patients

Of the 35 previously cured patients, 29 were men and 6 were women. Their median age was 35 years (range 16–80 years). At collection of initial samples, 20 of these patients were new cases, 4 were treatment failures, 6 were relapses and 5 others were defaulters. The median time interval between the initial and last isolate was 21 months (range 10–83 months). Initial isolates from 7 of the 35 patients were MDR, whereas those from the other 28 patients were non-MDR of which isolates from 18 patients were pan-susceptible. Isolates from the other 10 patients were resistant to at least one drug but non-MDR. A change in drug resistant profiles of isolates from pan-susceptible to MDR was observed in one patient (patient 10) (Table 1).

Table 1 Patient characteristics, treatment history, drug resistance, treatment and fingerprinting results of the five reinfections cases

Patient number	Age/sex	Treatment history§	Isolate number	Drug resistance	Date of sampling (month/year)	Treatment regimen‡‡	Treatment outcome (month/year)	DNA fingerprinting	Cause of recurrence
5	39/M†	Recurrent	99-2151	MDR¶	06/99	Cat II	Cured (02/00)	Different	Reinfection
			00-1553	MDR	10/00	MDR	Failure (10/01)	Identical	
			00-1554						
			01-2676	MDR	05/02	MDR	Failure (11/02)	Identical	
			02-2031						
			03-0548	MDR	11/02	MDR	Died	Identical	
10	45/F‡	Defaulter	96-0666	Susceptible	03/96	Cat II	Cured (11/96)	Different	Reinfection
			97-1002	MDR	07/97	MDR	Cured (04/99)	Identical	
			97-0990						
13	33/M	Failure	95-0413	INH†† resistant	02/95	Cat II	Cured (10/95)	Different	Reinfection
			97-1160	INH resistant	08/97	Cat II	Defaulted (03/98)	Identical	
			01-1241	INH resistant	05/01	Cat II	Defaulted 08/01	Identical	
			02-0581	INH resistant	01/02	Cat II	Cured (09/02)	Identical	
17	18/M	New	96-0816	Susceptible	04/96	Cat I	Cured (01/97)	Different	Reinfection
			99-1744	Susceptible	04/99	Cat II	Cured (12/99)	Different	
30	30/M	New	95-0796	Susceptible	05/95	Cat I	Cured (07/96)	Different	Reinfection
			01-2001	Susceptible	07/01	Cat I	Cured (03/01	Different	

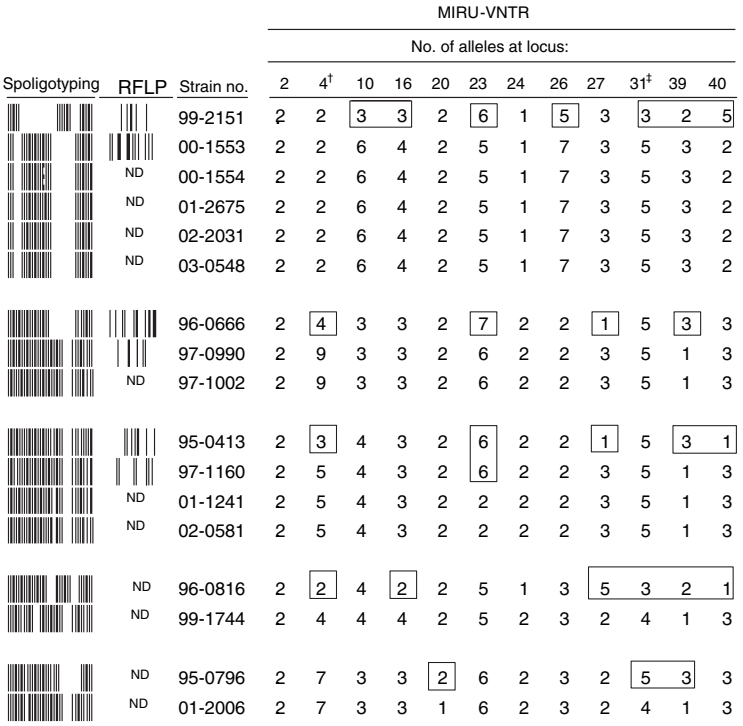
†Male.
‡Female.
§At collection of initial isolate.
¶Multidrug resistance i.e. resistance to at least isoniazid and rifampicin.
††Isoniazid.
‡‡Treatment regimens of the World Health Organisation (2004).

For 30 of the 35 patients, spoligotyping, RFLP and/or MIRU-VNTR patterns of the *M. tuberculosis* strains responsible for the TB recurrence were identical (28 cases) or showed an addition and a deletion of one IS6110 fragment (two cases), but were identical by MIRU-VNTR, indicating endogenous reactivation. The minor changes in RFLP profiles occurred in isolates with a high (>10) IS6110 copy number (data not shown).

For the other 5 (patients 5, 10, 13, 17 and 30) of the 35 patients, DNA fingerprinting patterns of the *M. tuberculosis* strains responsible for the disease were different for the two episodes, indicating exogenous reinfection or initial mixed infection (Table 1, Figure 2). Reinfection in three of these patients (patients 5, 10, 13) occurred within 8 months after cure from the previous episode. In the other two cases reinfection occurred more than 3 years after the previous episode. We did not find any evidence suggesting that cross contamination was responsible for these five reinfection cases.

Patient 5 was a recurrent case from whom the first sample was collected in June 1999. The patient received Cat II treatment and was declared cured in February 2000. The patient returned with smear-positive positive TB in October of the same year and was put on MDR treatment, but remained culture positive 1 year later. MDR treatment was repeated from May 2002, but because of poor compliance the patient was declared a treatment failure in November 2002 and died shortly afterwards. All samples collected between the initial and last isolates (intervening isolates) yielded *M. tuberculosis* strains that were genetically different from the initial isolate, but identical to the last isolate. All 6 isolates were MDR.

Patient 10 was a defaulter recruited into the study in March 1996. The patient received Cat II treatment and was declared cured in November of the same year. However, the patient reported with TB again in June 1997, was put on MDR treatment and declared cured in April 1999. The patient remained culture negative after 2 years of follow-up. Isolates taken 14 months after the initial sample were genetically identical to each other, but different from the initial isolate (Figure 2). The initial isolate was pan-susceptible to all drugs tested, whereas the latter isolates were MDR. RIF resistance in the latter isolates was confirmed by the presence of a His526Leu mutation in the *rpoB* gene and MIC testing (>80 μg/ml). Resistance to INH was confirmed by the presence of a Ser315Asn mutation in the *katG* gene and a C $\rightarrow$ T mutation at position −15 (designated relative to the mRNA initiation start site) in the *inhA* gene and also by MIC testing (> 3.2 μg/ml). The sequence of the *rpoB, katG* and *inhA* genes of the initial isolate for this patient was of the wild type and the MIC for both drugs was below the cut



Spoligotyping	RFLP	Strain no.	MIRU-VNTR No. of alleles at locus: 2	4†	10	16	20	23	24	26	27	31‡	39	40	Strain identity	Sputum collection date	Patient no.
		99-2151	2	2	3	3	2	6	1	5	3	3	2	5	Initial isolate	14 June 1999	5
		00-1553	2	2	6	4	2	5	1	7	3	5	3	2	Intervening isolate	26 October 2000	
	ND	00-1554	2	2	6	4	2	5	1	7	3	5	3	2	Intervening isolate	26 October 2000	
	ND	01-2675	2	2	6	4	2	5	1	7	3	5	3	2	Intervening isolate	22 October 2001	
	ND	02-2031	2	2	6	4	2	5	1	7	3	5	3	2	Intervening isolate	22 May 2002	
	ND	03-0548	2	2	6	4	2	5	1	7	3	5	3	2	Last isolate	24 November 2002	
		96-0666	2	4	3	3	2	7	2	2	1	5	3	3	Initial isolate	3 April 1996	10
		97-0990	2	9	3	3	2	6	2	2	3	5	1	3	Intervening isolate	30 June 1997	
	ND	97-1002	2	9	3	3	2	6	2	2	3	5	1	3	Last isolate	1 July 1997	
		95-0413	2	3	4	3	2	6	2	2	1	5	3	1	Initial isolate	13 February 1995	13
		97-1160	2	5	4	3	2	6	2	2	3	5	1	3	Intervening isolate	4 August 1997	
	ND	01-1241	2	5	4	3	2	2	2	2	3	5	1	3	Intervening isolate	8 May 2001	
	ND	02-0581	2	5	4	3	2	2	2	2	3	5	1	3	Last isolate	21 January 2002	
	ND	96-0816	2	2	4	2	2	5	1	3	5	3	2	1	Initial isolate	17 April 1996	17
	ND	99-1744	2	4	4	4	2	5	2	3	2	4	1	3	Last isolate	6 April 1999	
	ND	95-0796	2	7	3	3	2	6	2	3	2	5	3	3	Initial isolate	18 May 1995	30
	ND	01-2006	2	7	3	3	1	6	2	3	2	4	1	3	Last isolate	25 July 2001	

Figure 2 DNA fingerprinting results of repeat *M. tuberculosis* strains from reinfection patients. The patterns presented were generated by computer analysis using the Bionumerics software version 3.0 (Applied Maths, St Marten-Latem, Belgium). Polymorphic MIRU-VNTR loci are shown in boxes. ND, Not determined; †also called VNTR D (Frothingham & Meeker-O'Connell 1998), ‡also called VNTR E (Frothingham & Meeker-O'Connell 1998).

off for resistance for both INH (0.2 μg/ml) and RIF (40 μg/ml).

Patient 13 was a Cat I treatment failure at collection of the first sample in February 1995. The patient received Cat II treatment and was declared cured afterwards. However, he presented with smear positive TB in August 1997 and again received Cat II treatment, but defaulted 7 months later. In May 2001, Cat II treatment was restarted, but the patient defaulted again after 3 months. Cat II treatment was repeated from January 2002. The patient was declared cured thereafter and no further follow-up samples yielded a positive culture after 2 years. All the isolates from this patient were resistant to INH and had similar spoligotyping patterns. The two latter isolates were identical by MIRU-VNTR typing and had only minor spoligotype differences with the initial and the first intervening isolate. However, by MIRU-VNTR typing, the initial isolate was very different from all the follow-up isolates and only shared the same number of alleles at locus 23 with the first intervening isolate compared with both subsequent isolates. RFLP results, available for the first two isolates showed completely different patterns. In accordance with the interpretation of DNA fingerprinting data in this study, the follow-up isolates were considered to be genetically identical to each other, but different from the initial isolate. This patient was therefore designated a reinfection case.

Patient 17 was a new case from whom the first sample was collected in April 1996. The patient received Cat I treatment and was declared cured 9 months later. The patient was diagnosed with TB again after 3 years and the isolate cultured was genetically different from the first, but were both pan-susceptible. This patient was consequently considered to be a reinfection case (Table 1). This patient received Cat II treatment, was cured and has remained smear-negative.

Patient 30 was also a new TB case diagnosed in May 1995. The patient received Cat I treatment and was declared cured in July 1996. Six years later, the patient presented with TB again. The recurrent isolate was genetically different from the initial isolate, suggesting reinfection (Table 1 and Figure 2). Both isolates were pan-susceptible. The patient was successfully retreated and has remained smear negative.

Patients not cured between isolates

These 62 patients included 42 males and 28 females with a median age of 40 years (range 12–68 years). The median time interval between the initial and last isolate was 8 months (range 3–69 months). The treatment history of these patients included treatment failures (39), relapses (12), defaulters (7) and new cases (4). Initial isolates from 49 patients were MDR, whereas isolates from the other 13 patients were non-MDR, including 6 pan-susceptible and 7 resistant to at least one drug.

A change in DST patterns between the initial and latter isolate(s) among these 62 patients were observed in 14 cases. For 13 of these patients, the change only involved additional resistance to EMB and/or STR (data not shown). Among the polyresistant isolates, amplification of resistance to RIF was observed in one patient by routine DST and confirmed by sequencing of the *rpoB* gene (wild type for the initial isolate and Asp516Tyr mutation for the last isolate) and MIC testing (<10 μg/ml for the initial and > 80 μg/ml for the last isolate). INH resistance was confirmed by MIC testing (>3.2 μg/ml) for both isolates.

Minor changes in RFLP profiles among serial isolates were observed in 6 cases, which all occurred in high (>10) IS6110 copy number isolates. Spoligotyping and MIRU-VNTR patterns of sets of isolates from each of these patients were identical (data not shown).

Repeat isolates from all, but one of the 62 patients were considered identical, based on identical spoligotyping and RFLP profiles or only minor differences in RFLP (one or two bands), but identical MIRU-VNTR patterns, indicating treatment failure. For one of these patients, DNA fingerprints of the last isolate differed from that of the corresponding initial isolate, suggesting exogenous reinfection with a susceptible isolate, which is unlikely to occur during treatment. This observation was further strengthened by data obtained from investigations for laboratory cross-contamination where it became evident that a sputum sample from the last isolate of this patient had been switched with a specimen from a different patient processed on the same day following DNA fingerprinting. Consequently, disease in all these 62 patients was considered to be as a result of treatment failure.

Discussion

Most reports on TB recurrences only studied cases with proved cure of the previous disease episode. Our study included 35 patients with two different TB episodes, and 62 patients with persistently sputum positive smears. Under normal program conditions, samples from patients who receive treatment for at least 5 months are supposed to yield smears negative for *M. tuberculosis*. If the smears remain positive after this period, treatment failure is usually suspected, but reinfection or initial mixed infection should not be excluded. Reinfection is known to occur after successful treatment (Van Rie *et al.* 1999b; Verver *et al.* 2005) and even during treatment (Katz 1958; Small *et al.* 1993). Several investigators have also demonstrated initial mixed infection (Theisen *et al.* 1995; Shamputa *et al.* 2004, 2006). The proportion of reinfection cases among recurrent TB cases in our study sample was demonstrated in 5 (14.3%; 95% confidence interval of 5.3–31.0) of 35 patients with two different TB episodes.

A review of records showed that three (patients 5, 10 and 13) of the five reinfection cases were hospitalized for other infections at a designated hospital in between the DNA fingerprint profile change, suggesting that they may have been reinfected while in hospital. The possibility that the presumed reinfection cases were as a result of initial mixed infection became unlikely after identical spoligotyping patterns were obtained following analysis of 10 isolated colonies prepared from initial isolates of the three patients by making strikes on Dubos agar (data not shown, Shamputa *et al.* 2004), although this can not completely rule out the involvement of mixed infection. In addition, at least two follow-up isolates from most of these patients were genotyped and all found to have identical profiles, which were different from that of the respective initial isolates.

The rate of reinfection in this study was 14.3% (with a 95% C.I. of 5.4–31.0) among previously cured patients and 0.0% (95% C.I. 0.0–7.3) among treatment failures, which is at the lower limit of the range reported from high incidence settings (23–77%) (Das *et al.* 1995; Strassle *et al.* 1997; Van Rie *et al.* 1999b; Kruuner *et al.* 2002; Glynn *et al.* 2004). This could be explained in part by the virtual absence of HIV infection in our study setting, which has been shown to be a risk factor for disease recurrence (Strassle *et al.* 1997; Murray *et al.* 1999; Bandera *et al.* 2001), and exogenous reinfection in particular (Sonnenberg *et al.* 2001). Besides, the lower incidence of TB (106 cases 1 00 000 persons) in this study compared with other settings (1 000 cases per 1 00 000 persons per year) (Van Rie *et al.* 1999b) could have contributed. Our selection particularly for patients with persistently sputum positive smears also favoured those carrying a drug resistant strain (causing the initial episode failure or recurrence) with high risk of reactivation or repeat failure. In addition, different in our setting was the rural, hot and humid setting, with people living mostly outdoors, thereby reducing chances of transmission. It is also possible that the strains examined may represent a higher proportion of

early recurrences, as at some point intake was stopped, which may have resulted in proportionally higher exclusion of very late recurrences. Late recurrences may be more likely to represent reinfection.

The seemingly high rate of treatment failures may be explained by the fact that most of the isolates analysed from these patients were MDR, a factor that compounds treatment outcome (Mukherjee *et al.* 2004). In contrast, the average treatment failure outcome among new cases during the study period was 1.4% for Cat 1 and 2.9% for Cat II treatment, which is quite low for such a setting. One of the treatment failure cases initially suggested reinfection, but was discounted because of evidence suggestive of a switch of specimens in the laboratory with co-processed samples, a phenomenon that has been documented (Fitzpatrick *et al.* 2004; Glynn *et al.* 2004).

A change in DST patterns between the initial and latter isolates was observed in one of the previously cured patients and among 14 treatment failures. The change in the former case was because of reinfection with a MDR strain, whereas that the change among isolates from the 14 treatment failures involved additional resistance to EMB and/or STR. However, as routine drug resistance results for these two drugs are not always reliable (Laszlo *et al.* 2002) and obtainable information from genes involved in these drugs is limited, these changes were not investigated further.

Acquired drug resistance to RIF, confirmed by sequencing of the *rpoB* gene and MIC testing, was observed in 1 of 2 polyresistant non-MDR strains from a treatment failure case, but not from a monoresistant or pan-susceptible case. Although numbers are too low to draw conclusions, these results nonetheless support previous findings that observed amplification of resistance to RIF in 5 (17.8%) of 28 cases (Portaels *et al.* 2006). Taken together, these results show that acquired resistance to RIF may occur in polyresistant cases receiving Cat II treatment. However, second-line drug-resistance acquisition was not studied.

Repeat isolates from three patients among the previously cured patients and six patients among treatment failures had slightly different RFLP, but identical MIRU-VNTR profiles, as previously reported by De Boer *et al.* (2000) and Small *et al.* (1993). The median time between profile change in isolates from previously cured and chronic patients was also similar.

In conclusion, our findings documented persistence of strains in chronic treatment failure cases and suggest that reinfection is not a common cause of recurrence and treatment failure in this rural predominantly HIV-free population despite the high incidence of TB.

Acknowledgements

This work was financed by the Damien Foundation (Brussels, Belgium), and was also supported in part by the Fund for Scientific Research of Flanders (Brussels, Belgium, grant no. G.0471.03N). I. C. S. acknowledges financial support from Ackermans & van Haaren NV.

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Réactivation endogène et véritable échec de traitement comme causes de tuberculose récurrente dans une région à incidence élevée pour la tuberculose et à prévalence basse pour le VIH

OBJECTIF Déterminer les fréquences relatives de la réinfection par rapport à la réactivation ou à l'échec de traitement chez les patients d'une région à incidence élevée pour la tuberculose (TB) et à prévalence faible pour l'infection par le VIH.
MÉTHODES Nous avons déterminé les empreintes génétiques à partir de l'ADN de souches consécutives isolées au cours d'un ou de plusieurs épisodes de TB chez 97 patients en retraitement. 35 patients étaient rapportés comme précédemment guéris alors que 62 ne l'étaient pas.
RÉSULTATS Pour 5 des 35 patients précédemment guéris, les profiles des empreintes de l'ADN des isolats de *Mycobacterium tuberculosis* provenant d'épisodes récurrentes ne correspondaient pas à ceux des isolats initiaux, ce qui indique des cas de réinfection. Nous n'avons pas documenté la réinfection pendant le traitement. Les isolats de chacun des 30 autres patients précédemment guéris avaient des résultats identiques pour l'empreinte de l'ADN, indiquant des cas de réactivation. Les profils des empreintes de l'ADN des isolats des 62 patients avec des frottis de crachats constamment positifs étaient identiques, suggérant l'échec de traitement.
CONCLUSION Ces résultats suggèrent que la réinfection n'est pas une cause commune de rechute et d'échec de traitement dans cette population rurale à prédominance VIH négative malgré une incidence élevée de la TB.

mots clés Tuberculose, réinfection, réactivation, échec de traitement, empreinte génétique de l'ADN

Reactivación endógena y fallo terapéutico como causas de tuberculosis recurrente en un lugar con una alta incidencia de tuberculosis y con una baja prevalencia de VIH

OBJETIVO Determinar las frecuencias relativas de reinfección *vs.* reactivación o fallo terapéutico en pacientes de un lugar con alta incidencia de tuberculosis y baja prevalencia de VIH.
MÉTODOS Hemos realizado un '*fingerprinting*' de ADN en aislados seriados, tanto de uno como de múltiples episodios de TB, en 97 pacientes con retratamiento; 35 pacientes habían sido previamente curados, mientras que los restantes 62 no.
RESULTADOS Los patrones de '*fingerprinting*' de ADN de aislados recurrentes de *Mycobacterium tuberculosis* de 5 de los 35 pacientes previamente curados no correspondían a los aislados iniciales, indicando una reinfección. No se documentó la reinfección durante el tratamiento. Aislados de cada uno de los 30 pacientes restantes curados tenían unos resultados de '*fingerprinting*' idénticos, indicando una reactivación. Los patrones de '*fingerprinting*' de ADN de aislados de 62 pacientes con esputo positivo persistente eran idénticos, sugiriendo un fallo terapéutico.
CONCLUSIÓN Estos resultados sugieren que la reinfección no es una causa común de recaída y fallo terapéutico en esta población rural y predominantemente VIH negativa, a pesar de la alta incidencia de TB.

palabras clave Tuberculosis, reinfección, reactivación, fallo terapéutico, '*fingerprinting*' de ADN