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Molecular investigation of recurrent tuberculosis in patients from Rwanda

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SUMMARY

SETTING: Pulmonary tuberculosis (TB) patients enrolled in four provinces of Rwanda.

OBJECTIVE: To determine the cause of recurrent TB.

DESIGN: Serial *Mycobacterium tuberculosis* isolates obtained from patients with recurrent TB from January 2002 to September 2005 were genotyped by spoligotyping and mycobacterial interspersed repetitive unit-variable number of tandem repeat (MIRU-VNTR) typing. Drug resistance was determined by phenotypic susceptibility testing and sequencing of *rpoB, katG, inhA* and *embB* genes.

RESULTS: Among 710 culture-positive TB patients enrolled in the study, initial drug susceptibility testing results were available for 638. Sixty-nine of these had multidrug-resistant (MDR) TB and 569 were non-MDR-TB. Among the MDR-TB patients, 22 had follow-up isolates after cure (n = 12) or chronic infection (n = 10). The DNA patterns of sequential isolates from 4 of the 12 previously cured MDR-TB patients were different, indicating re-infection. DNA patterns of isolates from the remaining 8 previously cured and 10 chronic MDR-TB patients were identical, suggesting reactivation and treatment failure, respectively. Among the non-MDR-TB patients, disease recurrence was observed in one case; this was determined to be due to reactivation after initial mixed infection.

CONCLUSION: These results document a high treatment failure/reactivation rate for MDR-TB and suggest that re-infection within 2 years may not be a common cause of recurrent TB in this setting.

KEY WORDS: DNA fingerprinting; *M. tuberculosis*; reactivation; recurrence; re-infection

RECURRENCE OF DISEASE is common among tuberculosis (TB) patients. It is generally more frequent in high-incidence settings (9.6–38.1%)[1–3] than in low-incidence settings (0%–6.7%).[4–6] Factors that affect the recurrence rate include the prevalence of active TB in the community (risk of infection) and the presence of conditions that favour progression to active disease after exposure to new strains (most commonly, advanced human immunodeficiency virus [HIV] disease). Although recurrent TB is primarily thought to result from the reactivation of a *Mycobacterium tuberculosis* strain responsible for an initial disease episode,[7–9] re-infection[3,6,10–12] and even initial mixed infections[13,14] have also been shown or suggested to be frequent causes. Knowledge of the common cause of recurrent TB in a particular setting is useful for the effective management of TB programmes. To date, no molecular epidemiological data on TB have been available for Rwanda.

The present study assessed the proportion of recurrent TB attributable to reactivation, re-infection or treatment failure among patients in four provinces of Rwanda, using spoligotyping and typing based on mycobacterial interspersed repetitive unit-variable number tandem repeats (MIRU-VNTR), in combination with clinical data.

STUDY POPULATION AND METHODS

From January 2002 to September 2005, all smear-positive pulmonary TB patients were recruited from four provinces (Kigali, Butare, Ruhengeri and Rwamagana) in Rwanda. Patients were identified by screening individuals with cough of at least 2 weeks. Three sputum samples were collected from each individual suspected of having TB at diagnosis, after 2 months, 5 months and at the end of treatment for individuals who remained smear-positive. All new and previously treated patients were included.

Patients were treated according to National Tuberculosis Programme (NTP) guidelines in line with previous World Health Organization (WHO) recommendations.[15] New smear-positive cases re-

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ceived the Category I regimen, which comprises daily doses of isoniazid (INH, H), rifampicin (RMP, R), pyrazinamide (Z, PZA) and ethambutol (E, EMB) for 2 months followed by INH and RMP three times weekly for 4 months ($2HRZE/4H_3R_3$). Retreatment patients with a history of at least one month of drug intake were treated with daily INH, RMP, streptomycin (S, SM), EMB and PZA for 2 months followed by daily INH, RMP, EMB and PZA for 1 month and INH, RMP and EMB for 5 months administered three times per week ($2HRSEZ/1EZ/5H_3R_3E_3$, Category II).[15,16] Consenting TB patients were tested for HIV after counselling.

Demographic data for each patient, including age, sex and previous history of TB, were collected by standard questionnaire. Clinical data were obtained by searching patient treatment charts. Patients were followed for 24 months after completing treatment. Sputum collection in subsequent disease episodes was performed as in the initial episodes for all smear-positive patients.

The study was approved by the Ethics Review Board of the Centre Hospitalier Universitaire de Kigali, Rwanda.

Culture and drug susceptibility testing

Sputum samples collected from each patient during the initial and subsequent TB episodes were mixed with 1% cetylpyridinium chloride (CPC) and shipped to the National Reference Laboratory of Kigali for analysis. Each sample was cultured on Löwenstein-Jensen (LJ) medium and Coletsos after decontamination, using the Petroff procedure.[17] The cultures were incubated at 37°C and read weekly for growth for a maximal duration of 10 weeks. Primary cultures that resembled *M. tuberculosis* were sent to the St Pierre Laboratory, Brussels, Belgium, for species identification and drug susceptibility testing (DST). Identification was done using classical methods[18] and DST was performed for RMP (2 μg/ml), INH (0.2 μg/ml), SM (4 μg/ml) and EMB (15 μg/ml) using the BACTEC 460 radiometric method, as described by Siddiqi et al.[19,20] Phenotypic susceptibility testing for PZA was not performed because the results of this test can be difficult to reproduce and may not correlate well with in vivo drug susceptibility.[21] Duplicate DST of 20% of the *M. tuberculosis* isolates was also performed at the Mycobacteriology Unit at the Institute of Tropical Medicine in Antwerp, Belgium. The results of this external quality control system gave the same results.

DNA extraction

The genomic DNA used for polymerase chain reaction (PCR) analysis was obtained by resuspending mycobacterial colonies into 200 μl 1× buffer Tris-EDTA (ethylenediaminetetraäcetic acid disodium) (10 Mm Tris-HCl, 1 Mm EDTA [pH 8.0]) followed by boiling for 10 min.

DNA fingerprinting techniques

Spoligotyping was performed using a commercial kit (Isogen Bioscience BV, Maarssen, The Netherlands), as previously described.[22] DNA amplification for MIRU-VNTR was carried out using the HotStartTaq DNA Polymerase Kit (Qiagen, Hilden, Germany), according to previously described methods.[23] The PCR fragments were analysed by agarose gel electrophoresis using 3% NuSieve agarose (Cambrex Bio Science Rockland Inc., Rockland, ME, USA).

PCR amplification and sequencing

Direct DNA sequencing of resistance-determining regions of first-line anti-tuberculosis drugs (*rpoB, katG, mabA-inhA* [including upstream regions] *embB, rpsL, rrs*) was performed using the method described elsewhere[24–26] for a selection of 20 isolates spanning various phenotypic resistance profiles.

Interpretation of molecular typing data

Reactivation was considered the cause of recurrence when the strains isolated in the sequential episodes were identical, as determined by identical or very similar spoligotypes and identical MIRU-VNTR patterns. Re-infection was considered if the strains isolated from sequential episodes for each patient showed different DNA fingerprints by both methods. Mixed infection was suspected when more than one allele was detected at more than one MIRU-VNTR locus in one sample.[27]

Steps taken to prevent cross-contamination

To minimise laboratory cross-contamination, preparation of the PCR reaction mixes (prepared in a laminar flow cabinet), addition of the DNA, DNA amplification and electrophoresis were conducted in physically separated rooms. *M. tuberculosis* H37Rv DNA was included as a positive control in each amplification run, while water (negative control) was included to detect reagent contamination.

Computer-assisted analysis

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

Data management and analysis

On a weekly basis during the inclusion period, data were double entered by two different people to ensure accuracy using SPSS version 11.5 (SPSS, Chicago, IL, USA). These data were later linked to DST results. Both data sets were compared using Epi Info version 6.04d (Centers for Disease Control and Prevention, Atlanta, GA, USA) and cleaned by verifying the paper-based questionnaires, sample transportation forms and DST results. Analysis was done according to WHO/International Union Against Tuberculosis and

Lung Disease recommendations.[20] Fisher's exact test was used to identify significant differences between patient groups.

RESULTS

Study population

A total of 710 patients resident in the four epidemiological field sites were found to be culture-positive for *M. tuberculosis* from January 2002 to September 2005. Initial DST results were available for 638 of these 710 patients (Figure 1). Cultures from the remaining 72 patients were contaminated, lost in subculture or failed to produce valid DST results. Initial phenotypic DST classified 569 patients as non-MDR-TB and 69 as having MDR-TB (defined as resistance to at least INH and RMP). A single *M. tuberculosis* isolate was available for 47 of the 69 MDR-TB patients, whereas multiple isolates were available from the other 22 patients, of whom 12 had TB again after achieving cure of the initial episode and 10 patients who remained sputum smear-positive throughout the evaluation period (chronic patients). Among the 47 patients with a single disease episode, 21 patients were cured, 19 died and 7 could not be traced for follow-up. Among the 569 non-MDR-TB patients, 557 patients were cured, of whom one had TB after cure, while the remaining 12 patients were lost to follow-up (Figure 1).

DNA fingerprinting

Spoligotyping was performed on all isolates from the 22 recurrent MDR-TB patients with multiple isolates (12 previously cured and 10 chronic TB patients) and from one non-MDR-TB case with recurrent disease. Sequential isolates from 4 of the 12 previously cured MDR-TB patients showed different spoligotypes, suggesting re-infection as the cause of their recurrence (Figures 1 and 2). In contrast, the spoligotyping patterns of isolates from the other eight previously cured MDR-TB patients were indistinguishable, indicating reactivation. As expected, identical fingerprints were obtained among isolates from the 10 chronic MDR TB cases, corroborating the conclusion of clinical failure among these patients (data not shown).

To reconfirm these results, all isolates from the 22 MDR-TB patients and the previously cured non-MDR-TB patient were genotyped using MIRU-VNTR, a technique with a higher discriminatory power. In agreement with spoligotyping, major MIRU-VNTR differences (at >3 loci) in patterns of the sequential isolates from 4 of the 12 previously cured MDR-TB patients were obtained among these cases. In addition, this latter technique also detected double repeats at two loci (31 and 40) from an initial isolate of a non-MDR-TB (patient 9) with identical spoligotypes, indicating initial mixed infection.[13,27] One of the double repeats detected in the initial isolate was also detected in the follow-up isolate from the same patient at the respective loci. This suggests that the subsequent isolate was also present during the initial disease episode, but was now solely responsible for the subsequent disease episode (Figure 2). The drug susceptibility of the two isolates differed: the first was resistant to RMP and EMB and the second to all first-line drugs tested.

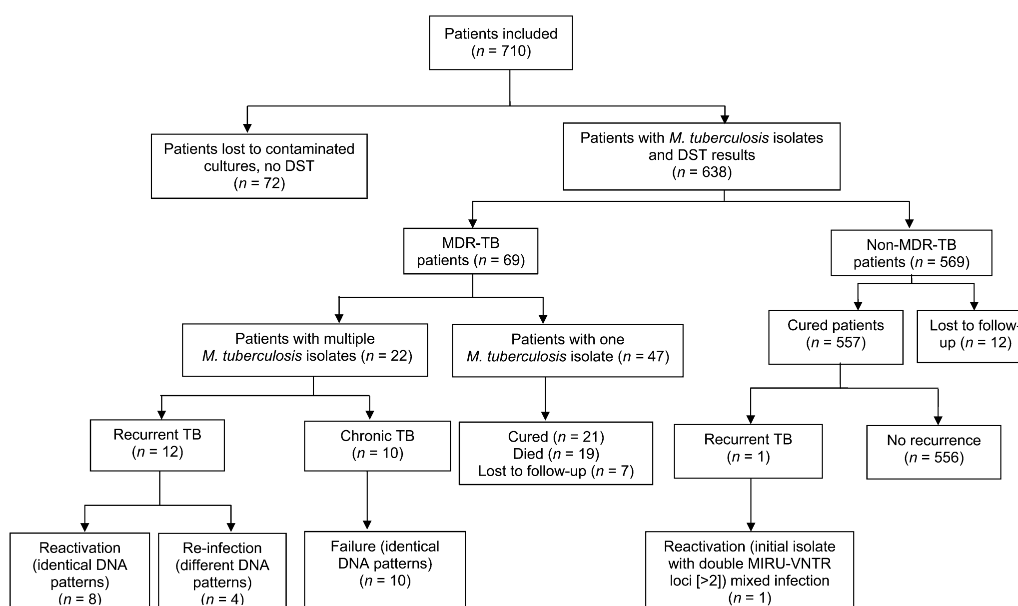

Figure 1 Diagram showing the grouping of patients according to disease episode, drug susceptibility results and DNA fingerprinting. DST = drug susceptibility testing; MDR-TB = multidrug-resistant tuberculosis; MIRU-VNTR = mycobacterial interspersed repetitive units-variable number tandem repeat.

Patient no.	Strain no.	Spoligotyping	MIRU-VNTR Alleles at locus: 2	4	10	16	20	23	24	26	27	31	39	40
1	1064	7 7 6 7 7 7 6 0 6 0 6 0 7 3 1	2	2	4	1	2	6	1	5	2	3	2	1
	1342	7 7 6 7 7 7 6 0 6 0 6 0 7 3 1	2	2	4	1	2	6	1	5	2	3	2	1
	1390	7 7 6 7 7 7 6 0 6 0 6 0 7 3 1	2	2	4	1	2	6	1	5	2	3	2	1
2	1057a	7 3 4 3 7 7 7 7 7 7 7 7 7 7 1	2	2	3	3	2	5	1	5	3	4	2	1
	1057b	7 3 4 3 7 7 7 7 7 7 7 7 7 7 1	2	2	3	3	2	5	1	5	3	4	2	1
3	1118	7 3 4 3 7 7 7 7 7 7 7 7 7 7 1	2	2	3	3	2	5	1	5	3	4	2	1
	1346a	7 3 4 3 7 7 7 7 7 7 7 7 7 7 1	2	2	3	3	2	5	1	5	3	4	2	1
	1346b	7 3 4 3 7 7 7 7 7 7 7 7 7 7 1	2	2	3	3	2	5	1	5	3	4	2	1
4	1247	7 7 6 7 7 7 6 0 6 0 6 0 7 3 1	2	2	4	1	2	6	1	5	2	3	2	1
	1267	7 7 6 7 7 7 6 0 6 0 6 0 7 3 1	2	2	4	1	2	6	1	5	2	3	2	1
	1269	7 7 7 7 7 7 7 7 7 7 6 0 7 3 1	2	2	3	3	2	5	1	5	3	4	2	3
	1382	7 7 6 7 7 7 6 0 6 0 6 0 7 3 1	2	2	4	1	2	6	1	5	2	3	2	1
5	1032	7 7 7 7 7 7 7 7 7 7 6 0 7 3 1	2	2	3	3	2	5	1	5	3	4	2	3
	1166	7 7 6 7 7 7 6 0 6 0 6 0 7 3 1	2	2	4	1	2	6	1	5	2	3	2	1
	1343	7 7 7 7 7 7 7 7 7 7 6 0 7 3 1	2	2	3	3	2	5	1	5	3	4	2	3
	1383	7 7 7 7 7 7 7 7 7 7 6 0 7 3 1	2	2	3	3	2	5	1	5	3	4	2	3
6	1246	7 7 7 7 7 7 7 7 7 7 6 0 7 3 1	2	2	3	3	2	5	1	5	3	4	2	3
	1266	7 7 7 7 7 7 7 7 7 7 6 0 7 3 1	2	2	3	3	2	5	1	5	3	4	2	3
7	1268	7 7 6 7 7 7 6 0 6 0 6 0 7 3 1	2	2	4	1	2	6	1	5	2	3	2	1
	1385	7 7 6 7 7 7 6 0 6 0 6 0 7 3 1	2	2	4	1	2	6	1	5	2	3	2	1
	1598	7 7 7 7 7 7 7 7 7 7 6 0 7 3 1	2	2	3	3	2	5	1	5	3	4	2	3
8	1271	7 7 7 7 7 7 7 7 7 7 6 0 7 3 1	2	2	3	3	2	5	1	5	3	4	2	3
	1389	7 7 7 7 7 7 7 7 7 7 6 0 7 3 1	2	2	3	3	2	5	1	5	3	4	2	3
9	1274	7 7 7 7 7 7 7 7 7 7 6 0 7 3 1	2	2	3	3	2	5	1	5	3	4+5	2	3+4
	1501	7 7 7 7 7 7 7 7 7 7 6 0 7 3 1	2	2	3	3	2	5	1	5	3	4	2	3
10	1315	7 7 6 7 7 7 6 0 6 0 6 0 7 3 1	2	2	4	1	2	6	1	5	2	3	2	1
	1484	7 7 6 7 7 7 6 0 6 0 6 0 7 3 1	2	2	4	1	2	6	1	5	2	3	2	1
11	1244	7 7 6 7 7 7 6 0 6 0 6 0 7 3 1	2	2	4	1	2	6	1	5	2	3	2	1
	1402	7 7 6 7 7 7 6 0 6 0 6 0 7 3 1	2	2	4	1	2	6	1	5	2	3	2	1
	1428	7 7 6 7 7 7 6 0 6 0 6 0 7 3 1	2	2	4	1	2	6	1	5	2	3	2	1
12	1104	7 7 6 7 7 7 6 0 6 0 6 0 7 3 1	2	2	4	1	2	6	1	5	2	3	2	1
	1131	7 7 7 7 7 7 7 7 7 7 6 0 7 3 1	2	2	3	3	2	5	1	5	3	4	2	3
	1279	7 7 7 7 7 7 7 7 7 7 6 0 7 3 1	2	2	3	3	2	5	1	5	3	4	2	3
13	1013	7 7 6 7 7 7 6 0 6 0 6 0 7 3 1	2	2	4	1	2	6	1	5	2	3	2	1
	1096	7 7 6 7 7 7 6 0 6 0 6 0 7 3 1	2	2	4	1	2	6	1	5	2	3	2	1

Figure 2 DNA fingerprints of *M. tuberculosis* isolates from some patients representing reactivation (patients 1, 2, 3, 6, 8, 10, 11 and 13), re-infection (patients 7 and 12), reinfection+reactivation (patients 4 and 5) and reactivation after initial mixed infection (patient 9). The presented patterns were obtained by computer-assisted analysis (Bionumerics software version 3.0, Applied Maths, Sint-Martens-Latem, Belgium). MIRU-VNTR = mycobacterial interspersed repetitive units-variable number tandem repeat.

Clinical and demographic characteristics of MDR-TB patients

Among the MDR-TB cases, no significant differences were found between patients with one or more *M. tuberculosis* isolates with regard to sex, age, type of TB, HIV status and treatment outcome (Table 1). We cannot, however, rule out that this might be due to a lack of statistical power to detect differences, given the small number of patients. Re-infection was documented among both HIV-seronegative and HIV-seropositive patients, but the differences in HIV status were not significant between the re-infection and reactivation groups (relative risk [RR] = 1.08, 95% confidence interval [CI] 0.56–2.09).

Drug resistance investigation among the MDR-TB patients

Genotypic drug resistance profiles were determined for 13 selected MDR-TB cases representing the following patterns: H+R, H+R+S, H+R+E and H+R+E+S resistance. Resistance to RMP was confirmed by the presence of known mutations in the *rpoB* gene for all isolates. In five isolates (patients 2, 3, 6, 12 and 13), additional previously unknown mutations (T563A, T480I, T481A and T481S) were detected in the *rpoB* gene (Table 2).

All isolates had the same single-nucleotide substitution in the *katG* gene (S315T), two of which also harboured a C15T mutation in the promoter region

Table 1 Characteristics of 69 MDR-TB patients with and without disease recurrence

Patient characteristics	Patients with a single isolate (n = 47) %	Patients with multiple isolates (n = 22) %	P value
Male	47.8	32.0	0.3103
Female	52.2	68.0	
Mean age, years	32.3	32.4	0.9708
Disease classification			
Pulmonary	93.2	96.0	1.0000
Extra-pulmonary	6.8	4.0	
Treatment history			
New case	53.2	54.2	0.8031
Retreatment	46.8	45.8	
HIV status			
Positive	38.3	54.5	0.8185
Negative	36.2	45.5	
Unknown	25.5	0.0	
Outcome			
Culture positive	65.9	60.0	0.3231
Culture negative	2.3	12.0	
Death	20.5	24.0	
Unknown	11.4	4.0	

MDR-TB = multidrug-resistant tuberculosis; HIV = human immunodeficiency virus.

of *mabA-inhA*. Genotypic resistance to SM was not observed, but in one strain of the 25 phenotypically resistant isolates an unknown mutation was observed in the *sprL* gene (S78L). Phenotypic resistance to EMB was observed in 29 isolates, while sequence analysis of the *embB* gene, the genetic target of EMB, revealed resistance mutations in 28 of the isolates (Table 2).

DISCUSSION

This report presents an analysis of *M. tuberculosis* isolates from 619 pulmonary TB patients with complete follow-up over a period of 2 years. Based on clinical data and microscopic evidence, the overall recurrence rate was 2.1% (13/619), and 1.6% (10/619) of patients had chronic TB. DST results showed recurrence rates of 0.18% (1/557) among non-MDR-TB patients and 19.4% (12/62) among MDR-TB patients. All chronic cases proved to be MDR-TB.

The DNA fingerprinting results suggest that reactivation (8/12, 66.7%) is the main cause of recurrent TB after clinical cure among MDR-TB patients receiving a standard retreatment regimen in our study population. Data on recurrent TB available for comparison are scarce, are generally from old series or from selected groups of the population, and results depend on setting-specific variables such as anti-tuberculosis treatment regimens used, prevalence of HIV/AIDS (acquired immune-deficiency syndrome) and MDR-TB. In general, recent figures for recurrence range from 1% to 11%,[28,29] and for one of the classic studies the recurrence proportion (6%) was nearly twice as high as that reported here.[29]

A re-infection rate of 33.3% (4/12) among recurrent MDR-TB patients was found in our study. Although the rate of re-infection among MDR-TB patients appears to be relatively high, it should be interpreted with caution because the reported rate represents only four cases. A previous study by Van Rie et al. in South Africa found a large proportion of re-infection (75%) in a selected population of HIV-negative patients after curative treatment; these patients resided in an area with very high TB incidence (1000 cases per 100 000 population per year).[2] On the contrary, our study area has a moderate incidence of TB (mean incidence for the study period: 77 cases/100 000)[15] and the majority of our recurrent TB cases had MDR-TB. Furthermore, our study included both HIV-seropositive and HIV-seronegative cases. Re-infection was recorded in both HIV-negative and -positive patients, but differences in HIV status were not significant (RR 1.08, 95%CI 0.56–2.09) for either group. The above observation may partially be explained by the fact that most HIV-positive patients were treated with potent antiretroviral therapy during the period of the study, and the possible improvement in immunity could have further reduced the risk of recurrent disease, if re-infected.

Studies from other areas of the world have shown that re-infection is increasingly becoming a cause of recurrent disease as TB incidence rates increase.[30] In low- to moderate-incidence countries (i.e., TB case rates <50/100 000/year), re-infection has ranged from 10% to 33% (in Spain).[5,6,9] In high-burden countries (i.e., >200 cases/100 000/year), re-infection rates have ranged from 23% to 60%.[2,6,31]

The single case of recurrent TB among non-MDR-TB patients (patient 9) proved to be a reactivation of one of the strains initially present in the mixed infection, as evidenced by the presence of double alleles at two MIRU-VNTR loci in the first isolate and the presence of only one of these alleles in the second isolate. The subsequent drug resistance observed in the follow-up isolate of this patient could be a case of reactivation in combination with acquired drug resistance.

Our study has some limitations. First, it is a study of patients enrolled from multiple sites but not a national survey. It is possible that the selection bias associated with enrolment affected the risk of reactivation versus re-infection and the high rate of resistance. Second, the period of follow-up after treatment completion for the patients was 2 years, and the proportion of cases caused by re-infection may be higher among cases of recurrent TB occurring more than 2 years after treatment completion.

Within the above stated limitations, this study shows that treatment failure is common in patients with MDR-TB in Rwanda and that re-infection might not be a common cause of recurrent TB in this setting.

Table 2 Epidemiological and clinical characteristics of 13 patients with recurrent TB after cure

Patient	Age years/sex	HIV status	Culture date	Interval between episodes days*	Isolate no.	Drug resistance status SM, INH, RMP, EMB	Genotypic drug resistance *rpsL rrs, katG inhA, rpoB, embB*	Re-infection or reactivation after fingerprint result
1	37/F	Positive	13 January 2002	1112	MR 1064	SRRR	*wt-wt*; S 315T-*wt*; S 531L; G 406A	Reactivation
			28 July 2003[†]		MR 1342	RRRR	S78 L-*wt*; S 315T-*wt*; S 531L; G 406A	
			30 January 2005		MR 1390	RRRR	*wt-wt*; S 315T-*wt*; S 531L; G 406A	
2	22/F	Negative	21 October 2002[†]	534	MR 1057	SRRR	*wt-wt*; S 315T-*wt*; S 531L+T563; M 306A	Reactivation
			10 April 2004		M1057 IP	RRRR	*wt-wt*; S 315T-*wt*; S 531L; M 306A	
3	23/F	Negative	31 January 2001[†]	1027	MR 1118	RRRR	*wt-wt*; S 315T-*wt*; S 531L+480I; M 306V	Reactivation
			20 June 2003		MR 1346a	RRRR	*wt-wt*; S 315T-*wt*; S 531L+480I; M 306V	
			28 November 2003		MR 1346b	RRRR	*wt-wt*; S 315T-*wt*; S 531L+480I; M 306V	
4	38/F	Positive	11 December 2002[†]	727	MR 1247	RRRR	*wt-wt;* S 315T-*wt*; S 531L; M 306V	Reactivation
			29 June 2003		MR 1267	RRRR	*wt-wt*; S 315T-*wt*; S 531L; M 306V	Re-infection
			22 February 2004[†]		MR 1269	RRRR	*wt-wt;* S 315T-*wt*; S 531L; S 297A	
			9 December 2004		MR 1382	RRRR	wt-wt; S 315T-*wt*; S 531L; M 306V	
5	24/F	Negative	27 January 2002[†]	820	MR 1032	SRRR	*wt-wt;* S 315T-*wt*; S 531L; M 306V	Re-infection + Reactivation
			22 July 2002[†]		MR 1166	SRRR	*wt-wt;* S 315T-*wt*; S 531L; S 297A	
			27 April 2003[†]		MR 1343	SRRR	*wt-wt*; S 315T-*wt*; S 531L; M 306V	
			19 July 2004[†]		MR 1383	RRRR	*wt-wt*; S 315T-*wt*; S 531L; M 306V	
6	Ad/M	Positive	3 June 2002[†]	923	MR 1246	RRRR	*wt -wt;* S 315T-*wt*; S 531L+T480I; M 306V	Reactivation
			19 December 2004		MR 1266	RRRR	*wt-wt; S* 315T+*pos-15*; S 531L+T480I*;* M 306V	
7	37/F	Positive	2 November 2002[†]	939	MR 1268	RRRR	*wt-wt;* S 315T-*wt*; S 531L; S 297 A	Re-infection
			20 October2003		MR 1385	RRRR	*wt-wt;* S 315T-*wt*; S 531L; S 297A	
			20 June 2005		MR 1598	RRRR	*wt-wt*; S 315T-*wt*; S 531L; M 306 V	
8	30/F	Positive	23 March 2002[†]	850	MR 1271	SRRS	*wt-wt;* S 315T-*wt*; S 531L; *wt*	Reactivation
			28 July 2004[†]		MR 1389	RRRS	*wt-wt*; S 315T-*wt*; S 531L; *wt*	
9[‡]	23/M	Unknown	3 July 2003[†]	605	MR 1274	SSRR	*wt- wt*; *wt-wt*; S 531L; M 306V	Mixed infection (with MDR strain)
			11 March 2005		MR 1501	RRRR	*wt-wt*; S 315T-*wt*; S 531L; S 297A	
10	34/M	Positive	13 December 2002[†]	635	MR 1315	RRRR	*wt-wt*; S 315T-*wt*; S 531L; S 297A	Reactivation
			2 September 2004		MR 1484	RRRR	*wt-wt*; S 315T-*wt*; S 531L; S 297A	
11	30/F	Positive	11 March 2002[†]	1092	MR 1244	RRRS	*wt-wt;* S 315T-*wt*; A 516V; *wt*	Reactivation
			30 May 2003[†]		MR 1402	RRRS	wt-wt; S 315T-*wt*; A 516V; *wt*	
			9 March 2005		MR 1428	RRRS	*wt-wt*; S 315T-*wt*; A 516V; *wt*	
12	45/F	Negative	1 September 2001[†]	1173	MR 1104	SRRR	*wt-wt;* S 315T-*wt*; S 531L; S 297 A	Re-infection
			2 October 2002[†]		MR 1131	SRRR	*wt-wt*; S 315T-*wt*; S 531L+T481A; M 306V	
			19 November 2004		MR 1279	SRRR	*wt-wt*; S 315T-*wt*; S 531L; M 306V	
13	30/F	Positive	24 September 2002[†]	790	MR 1013	RRRR	*wt-wt*; S 315T-*wt*; S 531L+T 563A; M 306V	Reactivation
			20 December 2004		MR 1096	RRRR	*wt-wt*; S 315T-*wt*; S 531L; M 306V	

Spoligotypes and MIRU-VNTR patterns for these patients are shown in Figure 2.
* Period between cure of the previous and the subsequent disease episode.
[†] Culture dates for patients declared cured based on negative smear microscopy results.
[‡] For patient 9, the subsequent episode was diagnosed as MDR-TB. This patient was not tested for HIV infection.
TB = tuberculosis; HIV = human immunodeficiency virus; SM = streptomycin; INH = isoniazid; RMP = rifampicin; EMB = ethambutol; F = female; M = male; wt = wild type; S = susceptible; R = resistant; Ad = adult; MDR = multidrug-resistant; MIRU-VNTR = mycobacterial interspersed repetitive unit-variable number of tandem repeat typing.

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RÉSUMÉ

CONTEXTE : Tous les patients atteints de tuberculose (TB) pulmonaire enrôlés dans quatre provinces du Rwanda.

OBJECTIFS : Déterminer la cause de récurrence de la TB.

MÉTHODOLOGIE : De janvier 2002 à septembre 2005, les isolats successifs des patients atteints d'une TB récurrente ont été génotypés par spoligotypage et typage MIRU-VNTR (mycobacterial interspersed repetitive unit-variable number tandem repeat). La résistance aux antituberculeux a été déterminée de manière phénotypique par les antibiogrammes et génotypique par séquençage des gènes *rpoB, katG, inhA* et *embB.*

RÉSULTATS : Les résultats de l'antibiogramme sont connus chez 638 des 710 patients à culture positive enrôlés dans l'étude (69 patients TB-MDR et 569 non TB-MDR). Parmi les patients TB-MDR, 22 des isolats de suivi ont été obtenus dont 12 patients chez des cas déclarés guéris et 10 dans des cas chroniques. Les profils d'ADN par spoligotypage et MIRU des isolats de suivi de 4 des 12 patients précédemment guéris ont été différents, indiquant une réinfection. Les profils d'ADN des isolats de suivi de 8 patients précédemment guéris et des 10 patients TB-MDR chroniques ont été identiques, suggérant respectivement la réactivation et des échecs du traitement. Parmi les patients non TB-MDR, on a observé un cas de TB récurrente, lequel serait dû à une réactivation après infection initiale mixte.

CONCLUSION : Ces résultats montrent un taux élevé de cas de réactivation et d'échec du traitement chez les patients TB-MDR et suggère qu'après 2 ans de suivi de cette cohorte, la réactivation n'est pas une cause courante de TB récurrente.

RESUMEN

MARCO DE REFERENCIA : Los pacientes con tuberculosis (TB) pulmonar registrados en cuatro provincias de Rwanda.

OBJETIVO : Determinar la causa de la TB recurrente.

MÉTODO : Se realizó el genotipado de los aislados seriados de TB provenientes de pacientes con TB recurrente que se presentaron entre enero de 2002 y septiembre de 2005. Se llevó a cabo la tipificación con oligonucleótidos espaciadores del elemento de inserción secuencia repetitiva directa (spoligotyping) y con unidades repetitivas intercaladas de micobacterias y elementos repetitivos en tándem de número variable (MIRU-VNTR). La resistencia a los medicamentos se determinó mediante pruebas fenotípicas y secuenciación de los genes *rpoB, katG, inhA* y *embB.*

RESULTADOS : De los 710 pacientes incluidos con baciloscopia positiva, 638 contaban con resultados iniciales de sensibilidad. Se encontraron 69 pacientes con tuberculosis multidrogorresistente (TB-MDR) y 569 sin TB-MDR. De los pacientes con TB-MDR, en 22 se recuperaron aislados clínicos durante el seguimiento así : 12 después de haber alcanzado la curación y 10 que presentaron infección crónica (persistencia de cultivos positivos). En cuatro de los 12 pacientes inicialmente curados, la tipología del ADN de sus aislados consecutivos fue diferente, lo cual indicó reinfección ; en los 8 restantes la tipología observada fue idéntica, signo de reactivación y en los 10 pacientes con TB-MDR crónica la tipología del ADN fue también idéntica, lo cual sugirió fracaso terapéutico. Se observó recurrencia en uno de los pacientes sin TB-MDR, cuya causa fue reactivación posterior a una infección inicial mixta.

CONCLUSIÓN : Estos resultados confirman una alta tasa de fracaso terapéutico y de reactivación en los pacientes con TB-MDR e indica que la reinfección tal vez no representa una causa frecuente de TB recurrente durante los 2 primeros años, en este contexto.