Reinfection and mixed infection cause changing *Mycobacterium tuberculosis* drug-resistance patterns.

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Online Data Supplement

MATERIALS AND METHODS.

Cloning of IS6110 insertion sites.

M. tuberculosis DNA from the respective strains were digested with RsaI and the resulting chromosomal fragments were ligated into EcoRV linearized and dephosphorylated pBluescript. The ligation reaction was then subjected to PCR amplification using the forward primer GCTCAACGCCAGAGACCAGC (complementary to the IS*6110* element) in combination with the T3 primer CAATTAACCCTCACTAAAGG (complementary to the pBluescript vector). Amplification products were then subjected to a second round of amplification with a nested forward primer GGACTCACCGGGGCGGTTC together with the T3 primer. These products were fractionated in 1% agarose and visualised after staining with ethidium bromide. The respective amplification products were extracted from the agarose and ligated into pGEM-Teasy (Promega) according to the manufacturers instructions. Positive clones were subjected to DNA sequencing to determine the points of IS*6110* insertion. Primers complementary to the IS*6110* insertion junctions were designed from the sequence data. Each IS*6110* insertion junction primer was used together with the universal forward primer in a PCR amplification assay to determine the specificity of amplification by amplification of *M. tuberculosis* DNA from a panel of isolates representative of the 30 different strain families identified in the epidemiological field site (E1). IS*6110* insertion junction primers which only produced an amplification product on amplification of the strain from which they were cloned were used in the PCR assay to determine the strain population in serial sputum cultures from patients with MDR-tuberculosis.

FIGURE LEGENDS.

Figure E1. Phenotypic and genotypic characterization of sputum cultures from patients 3, 4, 5, 6, 7 and 8.

Serial *M. tuberculosis* cultures were obtained from patients diagnosed with MDR tuberculosis. Phenotypic culture-based drug susceptibility testing was performed by the direct proportion method. Treatment regimen implemented at each visit is indicated, while adherence was measured for the period between each visit. Mutations conferring resistance were detected by DNA sequencing or PCR dot-blot (E2). All sputum cultures were genotyped by IS*6110* DNA fingerprinting (E3) and the strain(s) present was randomly assigned an alphabetical designation according to their strain family classification. Presence of multiple strains in each culture was determined using strain-specific PCR amplification (1). Abbreviations: S drug sensitive; R drug resistant; nd not determined; Inh (isoniazid) ; Rif (rifampin); Pza (pyrazinamide); Emb (ethambutol); Eth (ethionamide); Kana (kanamycin); Inat (isoniazid & thiacetazone); Oflox (ofloxacin); Sm (streptomycin); Teri (terizidone); thia (thiacetazone); D default (stopped therapy for a period of > 2 months); U unknown; + mutation present; - mutation absent; * internal positive PCR control (Rv3875).

REFERENCES.

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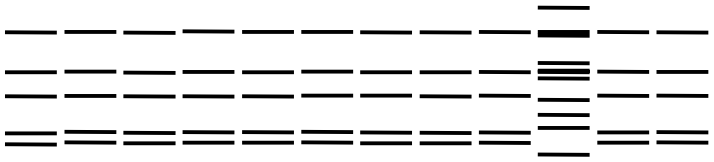
E3. van Embden, J. D., M. D. Cave, J. T. Crawford, J. W. Dale, K. D. Eisenach, B. Gicquel, P. Hermans, C. Martin, R. McAdam, and T. M. Shinnick. 1993. Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: recommendations for a standardized methodology. *J.Clin.Microbiol.* 31:406-409.

Figure E1a

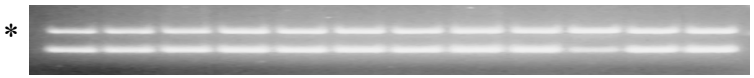
		Patient 3 (HIV neg)													Patient 4 (HIV neg)							
		A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**	**M**	**A**	**B**	**C**	**D**	**E**	**F**	**G**	**H**
Date	**Dav**	U	25	20	16	29	05	09	31	29	04	15	05	14	U	14	09	20	21	28	05	08
	Month	11	05	07	08	09	10	11	01	02	04	10	02	08	11	04	06	06	07	07	01	06
	Year	94	95	95	95	95	95	95	96	96	96	96	98	98	93	94	94	94	94	94	95	95
Susceptibility - isoniazid		nd	nd	R	R	R	R	R	S	R	R	S	R	R	R	R	S	R	R	S	R	R
- rifampin		nd	nd	R	R	R	R	R	S	R	R	S	R	R	R	R	S	R	R	S	R	R
- streptomycin		nd	nd	R	R	R	R	R	nd	R	R	nd	R	R	nd	nd	nd	R	nd	nd	S	R
- ethambutol		nd	nd	S	S	S	S	S	nd	S	S	nd	S	S	nd	nd	nd	S	nd	nd	S	R
Treatment - Rifafour (Inh, Rif, Pza, Emb)		U	X	X	X	X	X															
- Rifater (Inh, Rif, Pza)															X							
- Emb, Eth, Inat, Kana								X	X	X	X	X	X	X								
- Emb, Eth, Inat, Kana, Teri																X						
- Eth, Inat, Kana, Teri																	X	X	X	X		
- Emb, Eth Inat,																					X	
- Emb, Eth, Inat, Teri, Pza																						X
Adherence (%)			87	79	90	90	95	99	71	79	53	49	D	U		83	80	82	100	100	68	57
Point mutations - *KatG*315		nd	nd	+	+	+	+	+	+	+	+	-	+	+	nd	+	-	+	+	+	+	+
- *rpoB*531		nd	nd	-	nd	nd	nd	nd	nd	nd	nd	-	-	-	nd	+	-	+	+	+	+	+
- *rpoB*516		nd	nd	+	+	+	+	+	+	+	+	-	+	+	nd	nd	-	nd	nd	nd	nd	nd
- *rpsL*43		nd	nd	+	+	+	+	+	+	+	+	-	+	+	nd	-	-	-	-	-	-	-
- *rrs*513		nd	nd	-	-	-	-	-	-	-	-	-	-	-	nd	-	nd	nd	+	+	+	+
- *emb*306		nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	-	-	-	nd	+	-	+	+	+	+	+
Strain designation			**b**	**b**	**b**	**b**	**b**	**b**	**b**	**b**	**b**	**e**	**b**	**b**		**d**	**f**	**d**	**d**	**d**	**d**	**d**

IS*6110* DNA fingerprinting

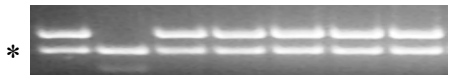


Strain-specific PCR

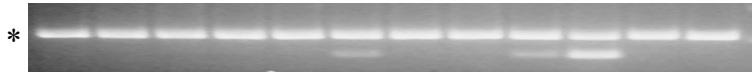
PCR (strain b)



PCR (strain d)



PCR (strain e)



PCR (strain f)

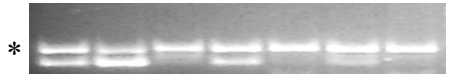


Figure E1b

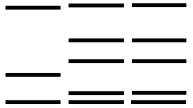
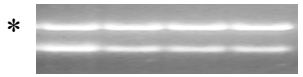
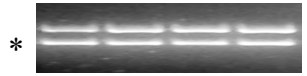
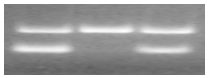
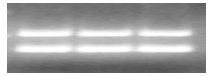
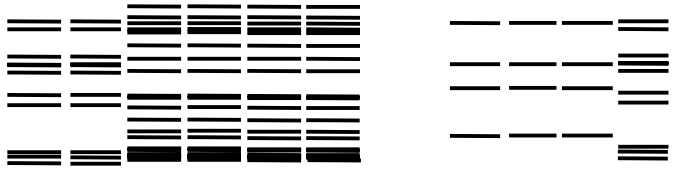
		Patient 5 (HIV nd)				Patient 6 (HIV nd)		
		A	**B**	**C**	**D**	**A**	**B**	**C**
Date	**Day**	03	26	05	05	17	23	10
	Month	06	11	01	01	06	09	10
	Year	93	93	95	95	93	93	93
Susceptibility - isoniazid		S	nd	R	R	R	R	R
- rifampin		S	nd	R	R	R	R	R
- streptomycin		S	nd	R	R	nd	R	R
- ethambutol		S	nd	R	R	nd	nd	nd
Treatment - Rifater (Inh, Rif, Pza)		X				X	X	X
- Emb, Eth, Kana, Inat, Oflox, Sm, Teri, Pza			X	X	X			
Adherence (%)			U	78	78		97	U
Point mutations - *KatG*315		-	+	+	+	-	+	+
- *rpoB*531		-	+	+	+	-	+	+
- *rpsL*43		-	-	-	-	-	+	+
- *rrs*513		-	+	+	+	-	-	-
- *emb*306		-	+	+	+	-	-	-
Strain designation		**g**	**d**	**d**	**d**	**h**	**b**	**b**
IS*6110* DNA fingerprinting								
Strain-specific PCR		PCR (strain g)  PCR (strain d) 				PCR (strain h)  PCR (strain b) 		

Figure E1c

		Patient 7 (HIV pos)						Patient 8 (HIV nd)			
		A	**B**	**C**	**D**	**E**	**F**	**A**	**B**	**C**	**D**
Date	**Day**	01	02	07	15	15	21	19	24	24	05
	Month	07	07	12	12	12	12	02	02	02	03
	Year	98	98	98	98	98	98	98	98	98	99
Susceptibility	- isoniazid	nd	nd	S	R	R	R	nd	R	R	R
	- rifampin	nd	nd	S	R	R	R	nd	R	R	R
	- streptomycin	nd	nd	S	R	nd	R	nd	S	S	R
	- ethambutol	nd	nd	S	R	nd	R	nd	S	S	S
Treatment	- Emb, Pyrifin								X	X	
	- Rifinah		X	X	X	X	X				
	- Emb, Eth, Sm, Inat, Pza										X
Adherence (%)				95	85	85	100			38	D
Point mutations	- *KatG*315	-	-	+	+	+	+	+	+	+	-
	- *rpoB*531	-	-	+	+	+	+	+	+	+	-
	- *rpsL*43	-	-	-	-	-	-	-	-	-	-
	- *rrs*513	-	-	+	+	+	+	-	-	-	-
	- *emb*306	-	-	+	+	+	+	-	-	-	-
Strain designation		**e**	**e**	**d**	**d**	**d**	**d**	**c**	**c**	**c**	**e**

IS*6110* DNA fingerprinting



Strain-specific PCR