High clonality of *M. tuberculosis* strains among MDR-TB patients in Gauteng, South Africa

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ABSTRACT

BACKGROUND: The problem of multidrug and extensively drug resistant tuberculosis (M/XDR-TB) was first highlighted in the 1990's with an outbreak of MDR-TB in New York City, followed a decade later by an XDR-TB outbreak (KZN strain) in Tugela Ferry, South Africa. Both were due to highly clonal highly resistant strains of *M. tuberculosis* (Mtb), spread mainly in HIV infected populations. However, the relative contributions to the M/XDR-TB epidemic in South Africa of clonal spread and de novo acquired resistance are unclear. To better understand the dynamics of MDR-TB transmission and resistance amplification in Mtb, we carried out a cluster analysis of Mtb strains from patients initiating treatment for MDR-TB at a referral hospital in Gauteng, South Africa.

METHODOLOGY: Mtb isolates from 89 patients were genotyped by *IS*6110-RFLP and direct DNA sequencing of 9 resistance determining genes: *rpoB*, *katG*, *mabA-inhA*, *gyrA*, *gyrB*, *pncA*, *tlyA*, *rpsL*, and *rrs*. Clusters were defined by 2 or more isolates with an identical *IS*6110-RFLP. Diagnosis of MDR was confirmed by drug susceptibility testing as part of routine clinical management.

RESULTS: Among the 74 patients, 36% were HIV infected and 29% had been treated for at least one prior TB episode. The Mtb isolates represent 17 strain families and 47 *IS*6110 genotypes, including 11 (15%) pre-XDR and 1 (1.4%) XDR strains. Clustering is high overall (40/74, 54%). Pre-XDR and XDR isolates are more highly clustered (10/12, 83%) than MDR *sensu stricto* (30/62, 48%). Isolates from HIV-positive patients show more clustering (17/26, 65%) than those from HIV-negative patients (23/47, 49%). The majority of clustering is accounted for by 4 families (26/40, 65%) which comprise 47% of the total Mtb population analyzed. The largest strain family (14/74 isolates, 19%) has the KZN genotype and is highly clustered (10/14, 71%). One pre-XDR isolate is nearly identical to the KZN strain; the other 13 show diverse mutation profiles, as seen in other South African variants, likely due to independent evolutionary trajectories from a less resistant precursor. Our data shows evidence of multiple importations of KZN strains to Gauteng, with further amplification of drug resistance and ongoing transmission in the region. The other 3 highly clustered strains show similar clonal characteristics with evidence of resistance amplification.

CONCLUSIONS: MDR-TB is spreading in Gauteng, as indicated by high levels of clustering. Moreover, a few strains with relatively low diversity predominate in the MDR Mtb population. Rather than showing a fitness cost, pre-XDR and XDR are more highly clustered than MDR strains. There is a high level of MDR-TB transmission among HIV-positive patients. The KZN strain is circulating in Gauteng and shows evidence of ongoing resistance amplification.

BACKGROUND

- HIV and the emergence of multidrug and extensively drug resistant (MDR & XDR) TB are threatening global TB control.
- TB is a major cause of death in HIV; South Africa has among the highest rates of HIV-TB co-infection and MDR-TB.
- M/XDR-TB treatment is complicated and costly, with poor outcomes and elevated mortality rates.
- Recent reports indicate that primary transmission is a significant source of MDR and XDR-TB, evidenced by a relatively low diversity of Mtb strains among M/XDR-TB cases.
- While this may indicate an intrinsic ability of some Mtb strains to acquire drug resistance, the identity of clonally spread M/XDR-TB strains seems to be regionally specific.
- We sought to investigate this question by genotypic characterization and cluster analysis of MDR-TB isolates obtained from patients in Gauteng province, South Africa.

METHODS

STUDY POPULATION: Patients initiating treatment for pulmonary MDR-TB at a large referral hospital in Johannesburg, South Africa were enrolled in a prospective observational study currently ongoing. The patient ages ranged from 19-62 years; 27 (36%) were HIV+, 48 (64%) were HIV- and 1 was unknown; 31 (45%) were female and 18 (29%) had been previously treated for one or more past episodes of TB.

- Diagnosis of MDR was based on drug susceptibility testing confirming phenotypic resistance to isoniazid (INH) and rifampicin (RIF).
- Mtb isolates were cultured from sputum specimens obtained from 76 patients at the start of treatment.
- Strain isolates were characterized by IS*6110* RFLP genotyping and direct DNA sequencing after PCR of genes conferring resistance to RIF (*rpoB*), INH (*katG*, *mabA-inhA*), fluoroquinolone (*gyrA*, *gyrB*), pyrazinamide (*pncA*), streptomycin (*rpsL*, *rrs*) and aminoglycosides (*rrs*).
- Clusters were defined as 2 strains having an identical IS*6110* RFLP or no more than 1 band difference (and >6 bands) with an identical pncA mutation.

RESULTS

- The 76 MDR Mtb isolates were widely diverse, representing 18 strain families and 49 IS*6110* genotypes, with clustering seen in 47 (52%) of the isolates.
- Clustering was seen in 40 out of the 76 (52%) isolates, with fewer strain families (14/76, 39%) and fewer specific IS6110 RFLP-defined (18/76, 34%) strains represented among clustered strains.

TABLE 1: Characteristics Associated with Strain Clustering

		CLUSTERED (n=40)		UNCLUSTERED (n=36)		Chi-square X^2	df	*P*
AGE	Median (IQR)	35	(25-44)	38	(26-44)			
SEX	Female	19	(49%)	15	(42%)	0.14	1	0.7083
	Male	20	(51%)	21	(58%)			
HIV SEROSTATUS	Positive	15	(38%)	12	(33%)	0.05	1	0.8231
	Negative	24	(62%)	24	(67%)			
PRIOR HISTORY OF TB	Yes	0	(0%)	26	(54%)	18.07	1	<0.0001
	No	24	(100%)	22	(46%)			
DRUG RESISTANCE CATEGORY	Pre-XDR or XDR	10	(25%)	2	(6%)	4.02	1	0.045
	MDR	30	(75%)	34	(94%)			
inhA MUTATION	Yes	26	(65%)	4	(11%)	20.83	1	<0.0001
	No	14	(35%)	32	(89%)			
Mtb STRAIN FAMILY	HP, BW, MH	5	(14%)	22	(55%)	12.24	1	0.0005
	Others	31	(86%)	18	(45%)			

- 22 (55%) of all clustered isolates were accounted for by 3 strain families (HP/LAM4/KZN, BW and MH-atypical Beijing).
- Strain clustering was more strongly associated with prior history of TB ($P<0.0001$) and the presence of mutations in the *mabA-inhA* locus ($P<0.0001$), which confer resistance to ethionamide and INH.
- Pre-XDR and XDR strains were significantly more clustered than MDR *sensu stricto* ($P=0.045$) and represented a large proportion of the MDR-TB cases (12/76, 16%).
- Although W-Beijing strains are highly represented (10/76, 13%), these strains were negatively associated with clustering (X^2 3.53, $P=0.0603$).

HP/KZN/LAM4 STRAIN FAMILY: ISOLATE GENOTYPES

RFLP	*rpoB*	*katG*	*inhA*	*gyrA*	*gyrB*	*rpsL*	*pncA*	*rrs*	Bands
*Ref**	*D516G_L533P_I1106T*	*S315T*	*-8T>A*	*A90V*		*wt*	*ins C pos456*	*A1401G*	*14*
HP81	D516G_L533P	S315T	-8T>A	A90V	wt	wt	ins C pos*456*	wt	15
HP134	S531L	S315T	-8T>A	wt	wt	wt	del T pos*388*	wt	13
HP132	S531L	S315T	-8T>A	wt	wt	wt	del T pos*388*	wt	14
HP112	S531L	S315T	-8T>A	wt	wt	wt	del T pos*388*	wt	14
HP135	I572F_S574T	S315T	-8T>A	wt	wt	wt	G132A	wt	14
HP81	I572F_S574T	S315T	-8T>A	S91P	wt	wt	G132A	wt	15
HP81	I572F_S574T	S315T	-8T>A	S91P	wt	wt	G132A	wt	15
HP72	D516Y	S315T	-8T>A	wt	H571Q	wt	G132A	wt	14
HP72	S531L	S315T	-8T>A	wt	wt	wt	V139G	wt	14
HP72	S531L	S315T	-8T>A	wt	wt	wt	V139G	wt	14
HP72	S531L	S315T	-8T>A	wt	wt	wt	V139G	wt	14
HP81	L533P	S315T	-8T>A	nd	nd	wt	nd	nd	15
HP124	S531L	S315T	wt	wt	wt	wt	nd	A908G	15
HP100	S531L	S315T	wt	wt	wt	wt	nd	wt	15
HP139	S531L	S315T	wt	wt	wt	wt	nd	wt	15
HP150	S531L	S315T	wt	nd	nd	wt	nd	nd	15
HP82	S531L	S315T	wt	nd	nd	K43R	nd	nd	15
HP72	S531L	S315T	nd	nd	nd	wt	nd	nd	14

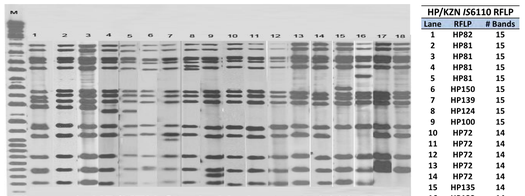


Figure 1. HP/KZN Strain Variants, *IS*6110 RFLP

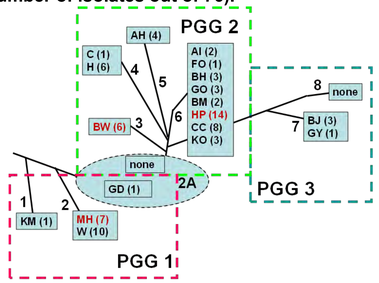
HP/KZN *IS*6110 RFLP

Lane	RFLP	# Bands
1	HP82	15
2	HP81	15
3	HP81	15
4	HP81	15
5	HP81	15
6	HP150	15
7	HP139	15
8	HP124	15
9	HP100	15
10	HP72	14
11	HP72	14
12	HP72	14
13	HP72	14
14	HP72	14
15	HP135	14
16	HP132	14
17	HP112	14
18	HP134	13

MH – ATYPICAL BEIJING STRAIN FAMILY: ISOLATE GENOTYPES

RFLP	*rpoB*	*katG*	*inhA*	*gyrA*	*gyrB*	*rpsL*	*pncA*	*rrs*	Bands
*Ref***	*D516V*	*S315T*	*-17G>T*				*insG pos172*	*A513C_A1401G*	
MH	D516V	S315T	-17G>T	wt	wt	wt	insG pos*172*	A513C_A1401G	13
MH6	D516V	S315T	-17G>T	D94G	wt	wt	insG pos*172*	A513C_A1401G	14
MH	S531L	S315T	wt	D94G	wt	wt	C14R	A513C	13
MH	S531L	S315T	wt	D94G	wt	wt	C14R	A513C	13
MH	S531L	S315T	wt	D94G	wt	wt	C14R	A513C	13
MH	S531L	S315T	wt	D94G	wt	wt	C14R	A513C	13
MH	S531L	S315T	wt	wt	wt	wt	wt	wt	13
MH	S531L	S315T	wt	nd	nd	wt	nd	nd	13
MH	S531L	S315T	T-35G	nd	nd	wt	nd	nd	13

Figure 2. Phylogenetic relatedness of Mtb strains (number of isolates out of 76).



CONCLUSIONS

- Clustering was relatively high among MDR-TB strains, suggesting that transmission is an important contributor to the epidemic in the region.
- There is a strong tendency towards reduced strain diversity among clustered, as compared to unclustered, isolates indicated by fewer strain families and specific genotypes represented in the two groups. A large proportion of clustering (22%) was accounted for by 3 strains alone: HP/LAM4/KZN, MH and BW.
- The high clustering among HP/KZN MDR Mtb and highly clonal nature of these strains, with diversity of drug resistance genotypes, suggests a scenario of multiple importations of these strains to Gauteng, with further amplification of drug resistance and ongoing transmission in the region.
- A large proportion of the MDR-TB cases were additionally pre-XDR or XDR-TB (16%), suggesting the importance of second-line drug susceptibility testing at the start of treatment to ensure that all patients receive appropriately targeted treatment regimens.
- We were not able to establish the relative contributions of transmission and treatment failure to MDR-TB among HIV-positive patients with this study. Further studies are needed to address this important question.

**Gandhi et al.* Nosocomial transmission of extensively drug-resistant tuberculosis in a rural hospital in South Africa. JID; 2013;207(1):9-17.
***Klopper et al* . Emergence and Spread of Extensively and Totally Drug Resistant Tuberculosis,South Africa. EID; 2013;19(3):449-55.
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