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Characterizing *Mycobacterium tuberculosis* isolates from Karachi, Pakistan: drug resistance and genotypes

Afsheen Ayaz [a], Zahra Hasan [a], Sana Jafri [a], Raunaq Inayat [a], Rafique Mangi [b], Abid Ali Channa [b], Faisal Riaz Malik [a], Asho Ali [a], Yasraba Rafiq [a], Rumina Hasan [a,*]

[a] *Department of Pathology and Microbiology, Aga Khan University, Stadium Road, PO Box 3500, Karachi 74800, Pakistan*
[b] *Marie Adelaide Leprosy Centre, Karachi, Pakistan*

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SUMMARY

Objectives: To study the prevalence, risk factors, and genotypes of drug-resistant *Mycobacterium tuberculosis* in Karachi.
Methods: Pulmonary tuberculosis (TB) patients were recruited in a cross-sectional study (2006–2009). Drug susceptibility testing was performed for culture-positive cases (n = 1004). Factors associated with drug resistance were evaluated using logistic regression analysis. Strains were typed using spoligotyping and mycobacterial interspersed repetitive units–variable number tandem repeat (MIRU–VNTR). The associations of genotype and drug resistance were explored using the Chi-square test.
Results: Resistance rates – new and previously treated – were as follows: multidrug-resistant (MDR)-TB, 2.4% and 13.9%, respectively; rifampin (RIF) monoresistance, 0.1% and 0.6%, respectively; any isoniazid (INH) resistance, 8.9% and 28.5%, respectively; and INH monoresistance, 3.0% and 6.3%, respectively. Prior TB treatment was a risk factor for MDR-TB (adjusted odds ratio (AOR) 6.8, 95% confidence interval (CI) 3.5–13.1) and INH monoresistance (AOR 2.4, 95% CI 1.1–5.2). Additional risk factors included low socioeconomic status for INH monoresistance (AOR 3.3, 95% CI 1.7–6.5), and belonging to Balouchi (AOR 9.2, 95% CI 2.5–33.4), Sindhi (AOR 4.1, 95% CI 1.2–13.5), or Pakhtun (AOR 3.4, 95% CI 1.0–11.2) ethnicity for MDR-TB. Although Central Asian strain (55.6%) was the most prevalent genotype, MDR-TB was significantly associated with Haarlem (H) genogroup (crude OR 9.2, 95% CI 3.6–23.8).
Conclusions: An MDR-TB rate of 2.4% is reported in new patients. Low RIF monoresistance supports the use of RIF as a marker for MDR-TB in this population. The need to strengthen TB care in the identified at-risk groups is emphasized. Based on INH resistance rates, a review of national treatment/prevention regimens relying on INH is suggested.

1. Introduction

Emerging drug resistance poses a serious threat to the control of tuberculosis (TB), which remains a leading infectious cause of death worldwide.[1,2] The World Health Organization (WHO) estimated 440 000 (95% confidence interval (CI) 390 000–510 000) cases of multidrug-resistant (MDR)-TB globally in 2008 (3.6% of all incident TB cases), causing an estimated 150 000 deaths.[3] Pakistan ranks eighth on the list of 27 high MDR-TB burden countries, with an estimated 15 000 cases occurring in 2008 (2.9% in untreated and 35.4% in treated patients).[4] A hospital-based study from Pakistan reported an MDR-TB prevalence of 1.8% amongst untreated TB patients.[5] In the absence of a national prevalence survey, information from community-based studies is required to help estimate the burden of MDR-TB in the country. Globally, resistance to isoniazid (overall) is estimated at 13.3% (10.3% in new and 27.7% in treated patients).[6] Resistance to isoniazid (INH) has also been reported from neighboring India (11% in new and 37% in treated patients)[7] and Bangladesh (23% in new patients).[8] A three-drug regimen, i.e., INH, rifampin (RIF), and ethambutol (EMB), is recommended during the continuation phase for new TB patients in populations with high levels of INH resistance.[9] In view of limited local information on INH resistance in untreated patients, the national guidelines in Pakistan currently advocate INH and RIF during the continuation phase.[10] However, local INH resistance data are essential for the selection of an appropriate regimen for such patients. This information will also help in deciding the role of INH prophylactic therapy for patients with latent TB in this area.

Over the last 10 years, molecular typing of *Mycobacterium tuberculosis* (MTB) has emerged as an important tool for estimating

* Corresponding author. Tel.: +92 21 34861640/34861641; fax: +92 21 34934294.
E-mail address: rumina.hasan@aku.edu (R. Hasan).

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ongoing TB transmission.[11] Spacer oligonucleotide typing (spoligotyping) of MTB strains from Pakistan has shown a heterogeneous population structure, with a predominance of the Central Asian Strain (CAS) genotype.[12–14] An association between MDR-TB and Beijing strains has also been reported from the country.[12,13] These studies have established the baseline diversity and population structure of MTB isolates from Pakistan. Given a population of over 160 million, an expansive and diverse terrain, and multiple ethnic groups, there is a need to study strains from defined geographic areas within Pakistan.

An earlier interim analysis of this study reported the prevalence of and risk factors associated with MDR-TB.[15] These were reassessed in light of this completed study and a larger sample size. Moreover, the prevalence of INH resistance and risk factors for INH-monoresistant TB were determined. Prevalent MTB genotypes and their associations with drug resistance were also investigated.

2. Methods

This was a cross-sectional study conducted in Karachi from July 2006 to September 2009. Karachi is the economic hub and largest city of Pakistan, with a population of around 17 million. It is divided into 18 administrative units.[16] The study subjects were recruited from 10 field clinics run by the Marie Adelaide Leprosy Centre (MALC) located in 10 of the 18 administrative units of Karachi, as previously described.[15] These clinics are accessible to the population from across the city, including individuals from the low to middle socioeconomic groups. They provide care to a mixed population representing all the major ethnic groups of the city. The clinic patients are either self-referred or are referred by their general practitioners. All patients presenting to the clinics with a clinical suspicion of pulmonary TB during the study period and who consented to participation were recruited into the study and interviewed on their initial visit. A pre-tested structured questionnaire was administered to glean information on socio-demographic and other characteristics. Early morning sputum specimens were collected for smear examination, culture, and drug susceptibility testing.

Drug susceptibility was tested by agar proportion method on enriched Middlebrook 7H10 medium (BBL) using the following drug concentrations: RIF 1 μg/ml and 5 μg/ml, INH 0.2 μg/ml and 1 μg/ml, streptomycin (STR) 2 μg/ml and 10 μg/ml, EMB 5 μg/ml and 10 μg/ml, ofloxacin 1 μg/ml, amikacin 6 μg/ml, kanamycin 6 μg/ml, and capreomycin 10 μg/m. Pyrazinamide (PZA) sensitivity testing was carried out using the BACTEC 7H12 medium pH 6.0 at 100 g/ml (BACTEC™ PZA Test Medium, Becton Dickinson, USA); MTB H37Rv was used as control with each batch of susceptibility testing. Resistance to RIF 1 μg/ml, INH 0.2 μg/ml, STR 2 μg/ml, and EMB 5 μg/ml was used as the cut-off for this study. MDR-TB was defined as a TB isolate resistant to at least INH and RIF.[3] Extensively drug-resistant (XDR)-TB was defined as MDR isolates with additional resistance to a fluoroquinolone (ofloxacin) and to any one of the second-line injectable anti-TB drugs (amikacin, capreomycin, or kanamycin).[3]

2.1. Genotyping methods

2.1.1. Spoligotyping

Culture-positive isolates were typed by spoligotyping using a commercially available kit (Isogen Bioscience BV, Maarssen, the Netherlands).[17] Results were analyzed using BioNumerics software (BioSystematica, UK). Dendrograms were generated using the unweighted pair group method with arithmetic averages (UPGMA) calculation. The spoligotypes were compared with the most prevalent MTB subfamilies as identified by the World Spoligotyping Database SpolDB4.0 of the Pasteur Institute, Guadeloupe.[18]

2.1.2. Mycobacterial interspersed repetitive unit–variable number tandem repeat (MIRU-VNTR) typing

MDR-TB isolates were further genotyped by PCR amplification of the 15 MIRU-VNTR loci, as previously described by Supply et al.[19] PCRs were carried out using 40–60 ng of DNA per reaction in 25 μl volume using 0.4 μM specific primers, 0.5 mM dNTPs mix, 1 mM $MgCl_2$, 1× PCR buffer, 4% dimethyl sulfoxide (DMSO), and 1 U of Super Tth Taq DNA polymerase. PCR was performed as follows: 15 min at 95 °C, 35 cycles, 1 min at 94 °C, 1 min at 59 °C, 1 min 30 s at 72 °C, 10 min at 72 °C. The PCR products were electrophoresed on a 3% agarose gel and sized with a 100-bp ladder (Promega). All the reactions were performed in duplicate using standard positive and negative controls supplied with the MIRU-VNTR validation kit. Sizing of the PCR fragments and assignment of the various VNTR alleles were also done using the standard protocol (Philip Supply INSERM U629, Institut de Biologie/Institut Pasteur de Lille; May 2005; http://www.genoscreen.com). Results of the MIRU-VNTR typing were analyzed using BioNumerics software (BioSystematica, UK).

2.2. Data analysis

Data were double-entered in EpiData and compared for errors. Analysis was done on 1004 culture-positive specimens using SPSS version 19 (IBM SPSS, Chicago, IL, USA). Descriptive statistics were computed for all variables. Means (± standard deviation) were calculated for continuous variables, while frequencies with percentages were calculated for categorical variables.

The prevalence of MDR-TB and INH-resistant TB with 95% CI was calculated. Socio-demographic and other factors associated with MDR-TB and INH- monoresistant TB were evaluated using the Chi-square test of independence. Adjusted odds ratios (AOR) and their 95% CI were estimated using logistic regression analysis.

Patients with MDR and INH-monoresistant MTB were compared to patients with drug-susceptible MTB strains. Individuals with resistance patterns other than MDR and INH-monoresistant TB were excluded from the analysis. Variables with a p-value of <0.25 in the univariate analysis were considered for multivariable analysis. The final model was constructed after checking for confounding and interaction. Model adequacy checks were performed. Variables with a p-value of <0.05 were retained in the final model. INH-monoresistant TB was defined as resistance to only INH.

Some independent variables were re-coded into meaningful categories, while a few new variables were developed. A variable for crowding index was constructed by dividing the number of individuals per household by the number of bedrooms. A participant's household crowding was defined as 'low' if they scored an index of 0–1.9, moderate if 2–3.9, and high if $\geq$4. Another variable termed socioeconomic status was constructed based on the possession of household commodities in accordance with the National Health Survey of Pakistan 1990–1994 criteria. Ethnicity was assessed using mother tongue as the proxy variable.

The association of strain types and drug resistance was explored using the Chi-square test of independence. Crude odds ratios (COR) were calculated for significant associations found.

3. Results

3.1. Study population

A total of 1229 subjects with a clinical suspicion of pulmonary TB were recruited. Specimens from 16 cases grew *Mycobacterium* species other than MTB, while 209 samples were culture-negative. These 225 samples were excluded from the analysis. MTB culture-positive patients (n = 1004) were studied. The mean age of the

Table 1
Characteristics of *Mycobacterium tuberculosis* culture-positive subjects: comparison of patients with multidrug-resistant and isoniazid-monoresistant *M. tuberculosis* with drug-susceptible *M. tuberculosis* patients

Characteristic	Total (N = 1004), n (%)	Fully drug susceptible (n = 770), n (%)	MDR (n = 43), n (%)	p-Value	INH- monoresistant (n = 35), n (%)	p-Value
Age mean (± SD)	32.3 (± 15.5)	33 (± 16)	34 (± 13)		32 (± 16)	
Age group, years						
9–14	32 (3.2)	22 (2.9)	2 (4.7)		1 (2.9)	
15–29	521 (51.9)	409 (53.1)	16 (37.2)	0.167	19 (54.3)	0.907
30–44	209 (20.8)	148 (19.2)	13 (30.2)		8 (22.9)	
≥45	242 (24.1)	191 (24.8)	12 (27.9)		7 (20)	
Gender						
Male	531 (52.9)	415 (53.9)	18 (41.9)	0.124	20 (57.1)	0.706
Female	473 (47.1)	355 (46.1)	25 (58.1)		15 (42.9)	
Marital status						
Single	443 (44.1)	351 (45.6)	10 (23.3)	0.004	15 (42.9)	0.751
Married	561 (55.9)	419 (54.4)	33 (76.7)		20 (57.1)	
Ethnicity						
Urdu-speaking	296 (29.5)	239 (31.0)	9 (20.9)	0.001	5 (14.3)	0.269
Punjabi	233 (23.2)	182 (23.6)	5 (11.6)		7 (20)	
Pakhtun	149 (14.8)	104 (13.5)	8 (18.6)		6 (17.1)	
Sindhi	108 (10.8)	81 (10.5)	8 (18.6)		7 (20)	
Balouchi	47 (4.7)	29 (3.8)	7 (16.3)		1 (2.9)	
Other[a]	171 (17.0)	135 (17.5)	6 (14.0)		9 (25.7)	
Education						
Literate[b]	475 (47.3)	379 (49.2)	13 (30.2)	0.018	11 (31.4)	0.039
Non-literate	529 (52.7)	391 (50.8)	30 (69.8)		24 (68.6)	
Employment status						
Employed	296 (29.5)	223 (29.0)	12 (27.9)	0.882	14 (40)	0.161
Unemployed	708 (70.5)	547 (71.0)	31 (72.1)		21 (60)	
Household income,[c] rupees/month						
≤5000	412 (48.3)	294 (45.0)	24 (68.6)	0.006	23 (69.7)	0.005
>5000	441 (51.7)	360 (55.0)	11 (31.4)		10 (30.3)	
Socioeconomic status[d]						
Upper	14 (1.4)	13 (1.7)	1 (2.4)	0.002	0	0.001
Middle	659 (67.7)	522 (69.9)	18 (42.9)		16 (45.7)	
Lower	300 (30.8)	212 (28.4)	23 (54.8)		19 (54.3)	
Crowding index[e]						
≥4 (high)	440 (45.2)	324 (43.3)	24 (57.1)	0.079	19 (54.3)	0.201
0–3.9 (low/moderate)	534 (54.8)	424 (56.7)	18 (42.9)		16 (45.7)	
Contact exposure present	284 (28.3)	225 (29.2)	8 (18.6)	0.166	9 (25.7)	0.655
BCG scar present	412 (41.0)	316 (41.0)	16 (37.2)	0.637	13 (37.1)	0.642
Previous history of TB present	158 (15.7)	104 (13.5)	22 (51.2)	<0.001	10 (28.6)	0.012

MDR, multidrug-resistant; INH, isoniazid; SD, standard deviation; BCG, bacille Calmette–Guérin; TB, tuberculosis.
[a] Other includes: Hindko, Saraiki, Bangali, Hindu, Persian, etc.
[b] Literate is defined as the ability of the individual to read.
[c] n = 151 (15%) missing values.
[d] n = 31 (3.1%) missing values.
[e] n = 30 (3.0%) missing values.

patients was 32.3 (± 15.5) years (range 9–91 years), and 531 (52.9%) were males. Both literacy and employment rates were higher amongst males (50.7% and 51.6%, respectively) than females (43.6% and 4.7%, respectively). Five major ethnic groups were identified: Urdu-speaking (29.5%), Punjabi (23.2%), Pakhtun (14.8%), Sindhi (10.8%), Balouchi (4.7%), and others (17.0%). A bacille Calmette–Guérin (BCG) scar was present in 412 (41.0%) patients, while 284 (28.3%) gave a history of contact with a TB patient. Overall 158 (15.7%) patients had previously been treated for TB (Table 1).

3.2. Drug resistance

Resistance to one or more of the five first-line anti-TB drugs was noted in 23.3% (n = 234) of patients. The INH resistance rate was 12.0% (95% CI 10.0–14.0%); 8.9% in untreated and 28.5% in treated patients. Resistance to other first-line drugs was as follows: STR 16.6%, PZA 6.5%, EMB 4.7%, and RIF 4.6%. The overall MDR-TB rate was 4.3% (95% CI 3.1–5.5%); 2.5% in untreated and 13.9% in treated patients (Table 2). All MDR-TB isolates (n = 43) were also resistant to PZA, 32 (74.4%) to EMB, and 23 (53.5%) to STR. No XDR-TB isolates were detected, although five isolates among the MDR-TB group were also resistant to ofloxacin.

RIF monoresistance was seen in only 0.2% of cases. INH resistance other than MDR was 7.7% (n = 77); 6.5% in untreated and 14.5% in treated cases. These included 35 (3.5%) INH-monoresistant and 42 (4.2%) polyresistant cases (Table 2).

3.3. Factors associated with drug resistance

3.3.1. Factors associated with MDR-TB

MDR-TB patients (n = 43) were compared to patients with drug-susceptible MTB strains (n = 770). Univariate analysis revealed a higher proportion of females (p = 0.124), married persons (p = 0.004), and individuals aged more than 27 years (p = 0.036) amongst MDR-TB patients. MDR patients were more likely to be non-literate (p = 0.018), have a lower income (p = 0.006), and belong to the lower socioeconomic status group (p = 0.002). A higher proportion of MDR-TB patients reported living in overcrowded houses (p = 0.079) (Table 3). The final multivariable logistic regression model identified prior history of TB treatment (AOR 6.8, 95% CI 3.5–13.1), being married (AOR 2.7, 95% CI 1.3–5.6), and belonging to Balouchi (AOR 9.2, 95% CI 2.5–33.4), Sindhi (AOR 4.1, 95% CI 1.2–13.5), and Pakhtun (AOR 3.4, 95% CI 1.0–11.2) ethnic groups to be associated

Table 2
Pattern of drug resistance in study isolates

	New cases		Previously treated cases		Combined	
	n	%	*n*	%	*n*	%
Number of strains tested	846	100	158	100	1004	100
Strain susceptibility/resistance						
Susceptible to all 5 drugs	666	78.7	104	65.8	770	76.7
Resistant to 1 drug	128	15.1	19	12.0	147	14.6
Resistant to 2 drugs	18	2.1	6	3.8	24	2.4
Resistant to 3 drugs	14	1.7	9	5.7	23	2.3
Resistant to 4 drugs	10	1.2	9	5.7	19	1.9
Resistant to 5 drugs	10	1.2	11	7.0	21	2.1
Any resistance	180	21.3	54	34.2	234	23.3
Isoniazid (INH)	75	8.9	45	28.5	120	12.0
Rifampin (RIF)	23	2.7	23	14.6	46	4.6
Pyrazinamide (PZA)	35	4.1	30	19.0	65	6.5
Ethambutol (EMB)	27	3.2	20	12.7	47	4.7
Streptomycin (STR)	136	16.1	31	19.6	167	16.6
Monoresistant	128	15.1	19	12.0	147	14.6
Isoniazid (INH)	25	3.0	10	6.3	35	3.5
Rifampin (RIF)	1	0.1	1	0.6	2	0.2
Pyrazinamide (PZA)	1	0.1	0	0	1	0.1
Ethambutol (EMB)	3	0.4	1	0.6	4	0.4
Streptomycin (STR)	98	11.6	7	4.4	105	10.5
Multidrug-resistant	21	2.5	22	13.9	43	4.3
INH + RIF + PZA	4	0.5	5	3.2	9	0.9
INH + RIF + PZA + EMB	6	0.7	5	3.2	11	1.1
INH + RIF + PZA + STR	1	0.1	1	0.6	2	0.2
INH + RIF + PZA + EMB + STR	10	1.2	11	7.0	21	2.1

with MDR-TB, after adjusting for other variables in the model (Table 3).

3.3.2. Factors associated with INH-monoresistant TB

Comparison of patients with INH-monoresistant ($n = 35$) and drug-susceptible MTB strains ($n = 770$) using univariate analysis showed that INH-monoresistant TB patients included a higher proportion of individuals who were non-literate ($p = 0.039$), had a lower income ($p = 0.005$), and a lower socioeconomic status ($p = 0.001$). A higher proportion of INH-monoresistant TB patients lived in overcrowded houses ($p = 0.201$) (Table 4). After adjusting for other variables, the final multivariable logistic regression

Table 3
Logistic regression analysis of factors associated with multidrug-resistant tuberculosis

Characteristic	Crude OR[a] (95% CI)	Adjusted OR (95% CI)	*p*-Value[a]
Age[b]			
>27 years	1.966 (1.034–3.740)		
Gender			
Female	1.624 (0.871–3.025)		
Marital status			
Married	2.764 (1.343–5.688)	2.655 (1.253–5.627)	0.011
Ethnicity			0.007
Punjabi	Reference		
Sindhi	3.595 (1.141–11.327)	4.104 (1.244–13.539)	0.020
Pakhtun	2.8 (0.893– 8.782)	3.424 (1.048–11.186)	0.042
Urdu-speaking	1.371 (0.452–4.160)	1.606 (0.514–5.016)	0.415
Balouchi	8.786 (2.613–29.544)	9.184 (2.523–33.432)	0.001
Socioeconomic status[c]			
Upper/middle	Reference		
Lower	3.035 (1.620–5.687)		
Education			
Non-literate	2.237 (1.149–4.354)		
Monthly income			
≤5000 rupees/month	2.672 (1.287–5.544)		
Crowding index			
Low/moderate	Reference		
High	1.745 (0.931–3.270)		
Household exposure			
Present	0.554 (0.253–1.212)		
Previous history of TB			
Present	6.709 (3.564–12.630)	6.797 (3.516–13.139)	<0.001

OR, odds ratio; CI, confidence interval; TB, tuberculosis.
[a] Significance level 0.05.
[b] Age is dichotomized at median after scale examination.
[c] Socioeconomic status categories are based on National Health Survey of Pakistan (1990–1994).

Table 4
Logistic regression analysis of factors associated with INH-monoresistant TB

Characteristics	Crude OR (95% CI)	Adjusted OR (95% CI)	*p*-Value[a]
Socioeconomic status[b]			
Upper/middle	Reference		
Lower	3.435 (1.733–6.808)	3.289 (1.653–6.545)	0.001
Education			
Illiterate	2.115 (1.022–4.378)		
Employment status			
Unemployed	0.612 (0.306–1.224)		
Monthly income			
≤5000 rupees/month	2.816 (1.319–6.011)		
Crowding index			
Low/moderate	Reference		
High	1.554 (0.787–3.069)		
Previous history of TB			
Yes	2.562 (1.196–5.488)	2.408 (1.110–5.224)	0.026

OR, odds ratio; CI, confidence interval; TB, tuberculosis.
[a] Significance level 0.05.
[b] Socioeconomic status categories are based on National Health Survey of Pakistan (1990–1994).

model identified prior history of TB treatment (AOR 2.4, 95% CI 1.1–5.2) and lower socioeconomic status (AOR 3.3, 95% CI 1.7–6.5) to be associated with INH-monoresistant TB (Table 4).

3.4. Genotypic pattern of MTB isolates

Of the 1004 isolates, 987 were spoligotyped. The remaining 17 samples either did not grow or were contaminated. The most prevalent genogroup was CAS (55.6%), followed by the East African Indian (EAI) (9.6%), T clade (4.9%), Haarlem (H) (3.1%), Beijing (2.7%), U clade (2.5%), MANU (0.2%), Lam American Mediterranean (LAM) (0.4%), X (0.2%), orphan clusters (6.9%), and unique isolates (13.5%) (see Supplementary Material Figure S1 and Table S1).

Amongst the MDR isolates ($n = 41$), prevalent genogroups observed were CAS1_Delhi (46.3%), CAS (2.4%), H1 (2.4%), H4 (19.5%), Beijing (4.9%), LAM (4.9%), T3 (2.4%), and U (2.4%), in addition to unique strains (14.6%) (Figure 1). H strains were found to be significantly higher amongst the MDR-TB group (COR 9.2, 95% CI

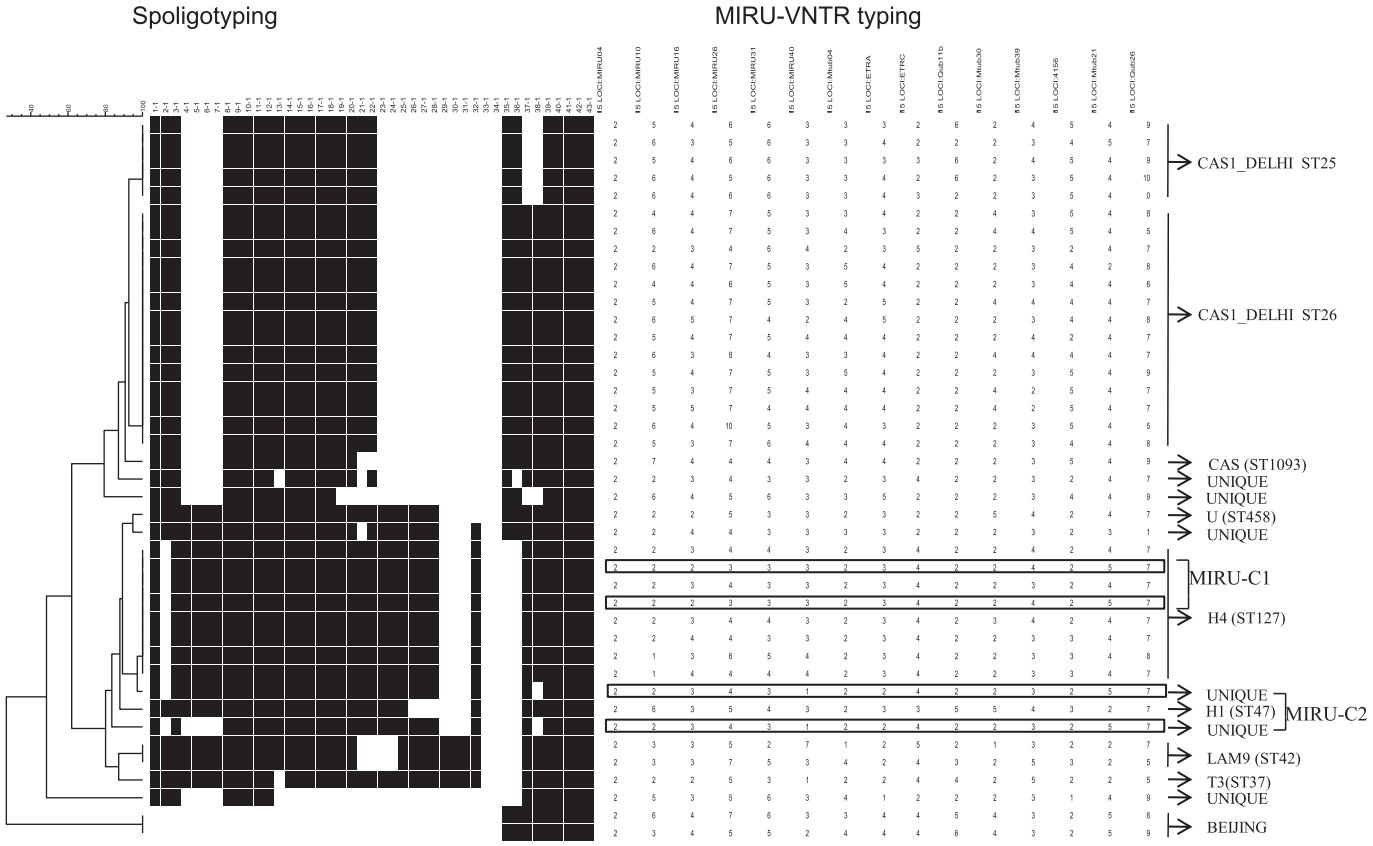

Figure 1. MIRU-VNTR pattern of multidrug-resistant *Mycobacterium tuberculosis* isolates. Forty-one multidrug-resistant *M. tuberculosis* isolates were subjected to 15 loci MIRU-VNTR typing. These comprised Beijing ($n = 2$), CAS ($n = 1$), CAS1_Delhi ($n = 19$), H1 ($n = 1$), H4 ($n = 8$), LAM9 ($n = 2$), T3 ($n = 1$), U458 ($n = 1$), and unique ($n = 6$) spoligotypes. A composite dendrogram of *M. tuberculosis* spoligotypes and MIRU-VNTR types was created using BioNumerics software. Two separate clusters identified amongst 'H' and 'unique' strains are indicated as MDR-C1 and MRD-C2.

3.6–23.8). Fifteen loci MIRU-VNTR typing was performed on MDR-TB strains to investigate further the clustering between isolates. One cluster of two strains was found in H genogroup (H4; MDR-C1), while an additional cluster of two strains was observed between two unique MIRU types (MDR-C2) (Figure 1).

INH-monoresistant isolates included 58.8% CAS and 20.6% EAI genogroup, while STR-monoresistant isolates included 58.7% CAS, 8.7% EAI, and 7.7% T strains. However, in both cases no significant association with any specific genotype was seen.

4. Discussion

This study reports the prevalence, risk factors, and genotypes of drug-resistant *M. tuberculosis* in Karachi, a city with a population of over 17 million. The MDR-TB rate of 2.4% amongst untreated patients is compatible with the earlier interim analysis showing 2.3% MDR in such patients.[15] These results are consistent with a previous study reporting 1.8% MDR amongst new TB patients in Pakistan.[5] The diagnosis of MDR-TB has been greatly facilitated by the recently launched GeneXpert, which relies on RIF resistance as a marker to detect MDR-TB.[20] The fact that 99.9% of RIF-resistant new patients in this study were also MDR, augers well for the performance of GeneXpert. However, the INH resistance rate of 8.9% amongst new patients (12.0% overall) reported in this study is concerning. The findings suggest that using only INH and RIF during the continuation phase for new patients may well lead to an increase in MDR-TB. Such information becomes more relevant given that the detection of INH resistance is difficult in this population where 15% of all new TB cases are extrapulmonary[2] and where there is a considerable disease burden in children.[21] The data further suggest that the role of INH prophylaxis in the area requires review.

The finding of high resistance to STR among the first-line anti-TB drugs is consistent with data from other countries,[22–24] and highlights the importance of susceptibility testing in cases where STR use is planned.

The association of multidrug resistance with Sindhi and Pakhtun ethnic groups reported in the interim analysis[15] is confirmed. Using a larger sample size we further found an association with the Balouchi ethnic group that was not detected previously. However, INH monoresistance (which was not explored in the earlier analysis) did not appear to be related to ethnicity, but only to low socioeconomic status. It is likely that limited health access in these marginalized at-risk populations plays a role in the development of MDR-TB. The data from the current study using a larger sample size did not confirm the association of MDR-TB with female sex, which was reported in the interim analysis. The association between MDR-TB and married individuals is likely to be age-related, the highest frequency of MDR-TB cases being in the 28–42 years age group, thus including a significant proportion of married individuals. This finding is consistent with data from China and Europe also reporting a higher risk of MDR-TB in the 35–44 years age group.[22,25]

Genotyping results support earlier reports of a predominance of CAS genogroup, particularly CAS1_Delhi, which appears to play a significant role in disease transmission in this area, together with other clusters – Beijing, EAI, and Haarlem.[12–14] Previous studies from Pakistan have suggested an association between Beijing strains and MDR.[12,13] In this study, however, we observed a significantly higher proportion of H strains amongst MDR isolates. Our data thus highlight the need to analyze strain populations from different geographic locations within the country in order to better understand the transmission dynamics of MTB. The high frequency of H strains amongst MDR-TB patients has also been reported from Afghanistan and Iran.[26] Cross-border traffic between these neighboring countries is likely the reason for the presence of the H genogroup amongst MDR isolates in the study population.

BCG is part of the universal vaccination in Pakistan. The fact that 59% of patients had no evidence of a vaccination scar is concerning; a focusing of vaccination systems to increase population access is required. The high unemployment figures among TB patients identified in this study highlight the need to integrate economic opportunities into TB control programs.

In summary, while the study is consistent with earlier estimates of MDR-TB, it further suggests that methodologies relying on RIF resistance as a marker to detect MDR-TB are likely to be successful. A greater focus on individuals identified to be at risk of drug-resistant TB (including patients with H genogroup) is required. Finally, based on this initial report of the community-based INH resistance rate, a review of treatment and prophylaxis regimens relying on INH in this population is suggested.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2011.12.015.

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