



EPIDEMIOLOGY OF TUBERCULOSIS: INVESTIGATION FROM THREE GEOGRAPHICAL AREAS IN GREECE

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Objective

To investigate the epidemiology of tuberculosis in three distinct regions in Greece including molecular typing of isolates.

Background

Tuberculosis (TB) remains a major threat to human health, especially in developing countries. In recent years, Greece has become the destination of a number of immigrants. Many of them originate from countries with high rates of TB and multidrug-resistant TB (MDR-TB), such as Pakistan, Bangladesh, India, Russia, sub-Saharan countries and the countries of Eastern Europe [1]. Under these circumstances, molecular epidemiological studies have been proven useful tools for tracing the transmission patterns of *Mycobacterium tuberculosis* (MTB). MIRU-VNTR (Mycobacterial Interspersed Repetitive Units- Variable Number of Tandem Repeats) typing method has been shown to be a reliable and reproducible typing method with high discriminatory power [2].

Materials & Methods

Demographic, clinical and laboratory data of 211 consecutive tuberculosis patients (111: Athens metropolitan area, 50: Thessaly [Central Greece], 50: Crete [Southern Greece]) were recorded. Patients were diagnosed during 2006-2011.



Region	Greeks	Immigrants
Thessaly	41/50 (82%)	9/50 (18%)
Athens	77/111 (69%)	34/111 (31%)
Crete	28/50 (56%)	22/50 (44%)
TOTAL	146/211 (69%)	65/211 (31%)

Table 1. Distribution of Greek and immigrant patients among the three investigated regions

- Species identification: Genotype MTBDRplus (Hain-Lifescience).
- Identification within the *Mycobacterium tuberculosis complex* (MTBC) species : Genotype MTBC (Hain, Lifescience).
- The susceptibility testing of MTBC isolates was performed by MGIT960 (Becton Dickinson). The antibiotics used were: Isoniazid (INH, 0.1 and 0.4 µg/ml), Rifampicin (RIF, 1 µg/ml), Ethambutol (5 and 7.5 µg/ml), Streptomycin (1 and 4 µg/ml) and Pyrazinamide (100µg/ml) according to the manufacturer's instructions.
- The strains were genotyped using the MIRU-VNTR method by analyzing 24 polymorphic loci [2]. The corresponding lineage of MTB was then determined by the MIRU-VNTRplus database (<http://miru-vntrplus.org>).
- The Hunter-Gaston Index (HGDI) was used to determine the discriminatory power of MIRU-VNTR method [3].

Results

- Among 211 MTBC isolates:
 - 206 were identified as *Mycobacterium tuberculosis*
 - 3 were identified as *M. bovis* BCG
 - 1 isolate was identified as *M. bovis*
 - 1 isolate was identified as *M. africanum* (first isolation of this species in Greece)
- Among 211 patients, 124 were males (58.8%) and 87 females (41.2%).
- The majority (69.2%) of patients were Greeks (Table 1) with higher mean age compared to foreigners (62.8 versus 31 years, p<0.001).
- There were different rates of Greek and foreign patients between regions (Table 1).
- Foreign patients (immigrants) originated from Europe (50.7%), Asia (33.9%) and Africa (15.4%).
- Pulmonary disease was diagnosed among 100/146 of Greek (68.5%) and 50/65 of foreign patients (76.9%).
- Among Greeks, 36% were aged <60 years (Fig.1) indicating continuing transmission in the community.
- The majority of foreign patients (64.3%) were aged between 25 to 44 years.

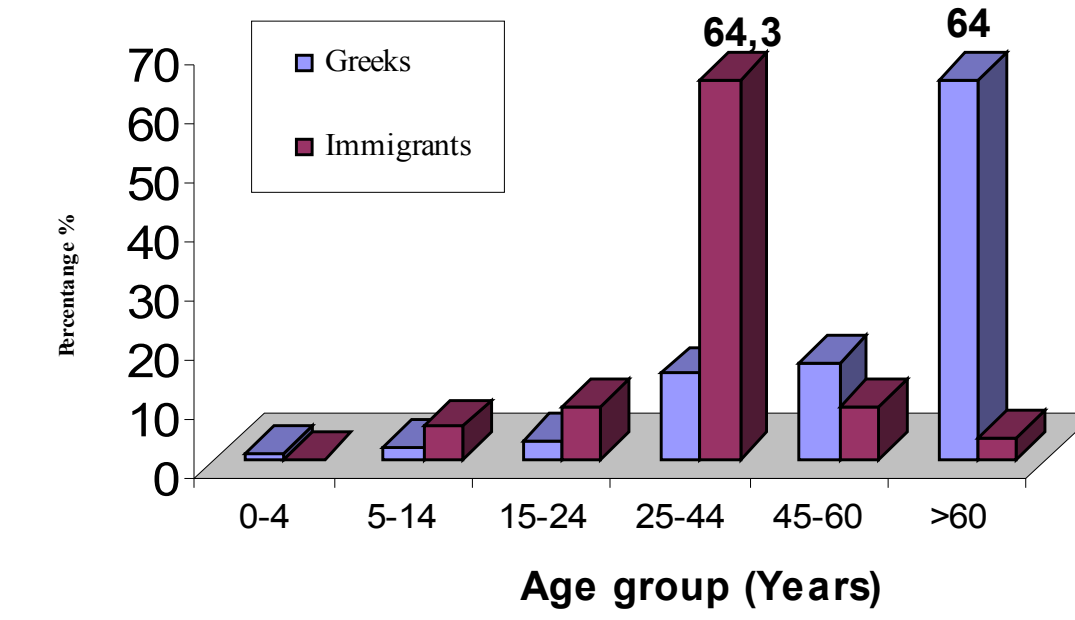


Fig 1. Age distribution of Greek and foreign patients

- Resistance rates were: isoniazid 8.5% (range among regions: 6-10%), rifampin 1.4% (0-2%), ethambutol 1.4% (0-3%), streptomycin 7.6% (4-10%) and pyrazinamide 4.7% (2-8%) (Tables 2 and 3).
- 33 (15.6%) strains were resistant to at least one drug (Table 3).
- Only three strains (1.5%) were multidrug-resistant (MDR).

	Greeks	Immigrants	Total
INH	8/146 (5.5%)	10/65 (15.4%)	18/211 (8.5%)
RIF	0/146 (0%)	3/65 (4.6%)	3/211 (1.4%)
STR	11/146 (7.5%)	5/65 (7.7%)	16/211 (7.6%)
EMB	1/146 (0.7%)	2/65 (3.1%)	3/211 (1.4%)
PZA	8/146 (5.5%)	2/65 (3.1%)	10/211 (4.7%)

Table 2. Resistance rates between Greek and foreign patients

	Athens			Crete			Thessaly		
	Greeks	Immigrants	Total (%)	Greeks	Immigrants	Total (%)	Greeks	Immigrants	Total (%)
INH	5	5	10 (9)	1	4	5 (10)	2	1	3 (6)
RIF	0	2	2 (1.8)	0	1	1 (2)	0	0	0
STR	6	3	9 (8.1)	3	2	5 (10)	2	0	2 (4)
EMB	1	2	3 (2.7)	0	0	0	0	0	0
PZA	3	2	5 (4.5)	4	0	4 (8)	1	0	1 (2)

	Resistance pattern			Total (%)
	Greeks	Immigrants	Total (%)	
INH	2	2	4 (3.6)	4 (8)
STR	3	3	6 (5.4)	6 (11.5)
RIF	0	2	2 (1.8)	2 (4)
PZA	2	1	3 (2.7)	3 (6)
STR/INH/PZA	1	1	2 (1.8)	2 (4)
STR/INH	2	1	3 (2.7)	3 (6)
STR/PZA	1	1	2 (1.8)	2 (4)
INH/RIF/MDR	1	1	2 (1.8)	2 (4)
STR/INH/EMB	1	1	2 (1.8)	2 (4)
MDR STR/EMB/PZA	1	2	3 (2.7)	3 (6)
TOTAL	11	8	19 (17.6)	27 (51.5)

Table 3. Resistance rates and resistance patterns between Greek and foreign patients in the three investigated regions

Locus	Alias	HGDI	Diversity	No. alleles	Locus	Alias	HGDI	Diversity	No. alleles
2163b	QUB-11b	0.80	High	9	3192	MIRU-31	0.41	Moderate	5
4052	QUB-26	0.79	High	12	2401	MTUB30	0.49	Moderate	4
802	MIRU-40	0.76	High	8	580	MIRU-04	0.38	Moderate	8
1955	Mtub21	0.69	High	9	2531	MIRU-23	0.39	Moderate	8
1644	MIRU-16	0.69	High	6	2347	MTUB29	0.3	Poor	4
2165	ETR-A	0.64	High	7	4348	MIRU-39	0.26	Poor	3
424	Mtub04	0.61	High	7	2059	MIRU-20	0.24	Poor	2
960	MIRU-10	0.61	High	10	154	MIRU02	0.18	Poor	3
2996	MIRU-26	0.58	Moderate	9	2461	ETR-B	0.16	Poor	6
3690	Mtub39	0.57	Moderate	10	3171	Mtub34	0.15	Poor	4
577	ETR-C	0.57	Moderate	5	3007	MIRU-27	0.12	Poor	4
4156	QUB-4156	0.54	Moderate	6	2687	MIRU-24	0.05	Poor	2

Table 4. Allelic diversity of all 24 VNTR loci among *M. tuberculosis complex* strains ordered by decreasing diversity.

Results

- Results of MIRU-VNTR genotyping analysis showed that:
 - Number of unique strains: 187
 - Number of clusters: 10, Number of clustered strains: 24 (11.4%), Cluster size range: 2-4
 - Clustering rate: 6.6%, HGDI: 0.998
 - Laboratory contamination was assumed in 2 cases (1.8%).
 - Two suspected incidences of intrafamilial transmission were confirmed (6 patients in total, of which 4 were children).
 - Among the rest of clustered cases (14 cases) no geographical link could be demonstrated.
 - Two cases with mixed infection involving 2-3 strains were diagnosed.

Family	No of strains (%)	Greeks (%)	Immigrants			
			Europe	Asia	Africa	Total (%)
Haarlem	25 (12)	15 (10.3)	9	1		10 (15.4)
S	25 (12)	18 (12.3)	7			7 (10.8)
Delhi/CAS (Central-Asian)	14 (7)			10	4	14 (21.5)
Beijing	11 (5)	5 (3.4)	2	4		6 (9.2)
LAM (Latin-American-Mediterranean)	11 (5)	8 (5.5)	2	1		3 (4.6)
TUR	7 (3.3)	4 (2.7)	2		1	3 (4.6)
H37Rv	4 (1.9)	3 (2)	1			1 (1.5)
EAI (East African Indian)	2 (0.9)	1 (0.7)			1	1 (1.5)
URAL	2 (0.9)	2 (1.4)				
Cameroon	1 (0.5)	1 (0.7)				
New-1	1 (0.5)				1	1 (1.5)
X	1 (0.5)		1			1 (1.5)
<i>M. bovis</i> BCG	3 (1.4)	3 (2)				
<i>M. bovis</i>	1 (0.5)			1		1 (1.5)
<i>M. africanum</i>	1 (0.5)	1 (0.7)				
Unclassified strains	102 (48)	85 (58.2)	9	5	3	17 (26)
Total	211	146				65

Table 5. Distribution of MTB families between Greek and foreign patients.

- MIRU-VNTR classified 104 (52%) of isolates (Athens: 52%, Thessaly: 56%, Crete: 40%) in 12 *M. tuberculosis* families (Table 5)
- The most frequent families were Haarlem (12%), S (12%), Delhi/CAS (7%), Beijing (5%) and LAM (5%).
- Harlem and S families were detected among both Greeks and foreigners.
- All strains that belonged to the Delhi/CAS family were isolated only from foreigners (21.5%) originating from Asia and Africa.
- Eleven strains (5%) belonged to the Beijing family. They were recovered from all three regions (Athens: 8/11, Thessaly: 2/50, Crete: 1/50) demonstrating frequencies of 3.4% in Greeks and 9.2% in foreigners (p=0.08). Among the three MDR isolates, two were Beijing strains.
- The remaining 102 strains did not belong to a known family (unclassified strains). There were differences in rates of unclassified strains between regions (Table 6).
- Unclassified strains were detected more frequently in Crete.
- In Athens there was a considerable difference between rates of unclassified strains between Greek and foreign patients.

Region	Unclassified strains		
	Greeks	Immigrants	Total
Crete	67%	57%	59%
Thessaly	39%	44%	42%
Athens	59%	18%	48%
Total	58%	26%	48%

Table 6. Distribution of Unclassified isolates among Greek and foreigner patients in the three regions

Family	Athens (%)	Thessaly (%)	Crete (%)	Total (%)
Haarlem	13(12)	8(16)	4(8)	25(12)
S-family	12(11)	11(22)	2(4)	25(12)
Delhi/CAS	12(11)		2 (4)	14 (7)
Beijing	8(7)	2(4)	1(2)	11(5)
LAM	4(4)	2(4)	5(10)	11(5)
Total	49 (44)	23 (46)	14 (28)	86 (41)

Table 7. Distribution of the most frequent MTB families in the three investigated regions

Results

Resistance pattern (no of strains)	Greeks	Immigrants
INH (11)	Unclassified (3) LAM (1) S-family (1)	Delhi/CAS (2) LAM (1) H37Rv (1) Unclassified (1) Haarlem (1)
STR (7)	Unclassified (2) TUR (1) URAL (1) S-family (1)	TUR (1) Delhi/CAS (1)
PZA (4)	<i>M. bovis</i> BCG (3) <i>M. bovis</i> (1)	
STR/INH (3)	Unclassified (1) Haarlem (1)	Delhi/CAS (1)
STR/INH/PZA (1)	Haarlem	
STR/INH/EMB (1)	Beijing	
STR/PZA (3)	Unclassified	
MDR STR/EMB/PZA (2)		Beijing (2)
MDR		Unclassified (1)

Table 8. MTBC family distribution of 33 resistant isolates among Greek and foreign patients.

Conclusions

- ✓Apart from Crete, tuberculosis is still more frequently diagnosed among Greeks but not exclusively linked to increased age.
- ✓MDR strains remain uncommon and are associated with the virulent Beijing type.
- ✓A considerable isoniazid resistance rate appears constant between regions.
- ✓This finding in connection to low MDR rates justifies the application of rapid molecular testing targeting isoniazid rather than rifampin resistance genes.
- ✓The application of genotyping for the first time forms a basis for the delineation of molecular epidemiology of MTB in Greece.

References

1. World Health Organization (2010). Global tuberculosis control; Surveillance, Planning, Financing, WHO Report. World Health Organization. Geneva.
2. P. Supply et. al. 2006. J. Clin. Microbiol. 44: 4498-4510.
3. PR Hunter & MA Gaston, 1988 J. Clin. Microbiol. 26: 2465-6.