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Genetic diversity of *Mycobacterium tuberculosis* strains isolated in Algeria: Results of spoligotyping



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ABSTRACT

Objective/background: Molecular typing tools, including spoligotyping, are currently widely used in the monitoring and study of the dynamics of tuberculosis epidemics.

Methods: A study of the molecular profile of a sample of 129 *Myobacterium tuberculosis* strains isolated during 2011 was carried out in the National Reference Laboratory for Tuberculosis and Mycobacteria at the Pasteur Institute of Algeria. This sample was selected at random from a set of 350 strains isolated from tuberculosis patients from central and eastern areas of the country.

Results: Genotypic analysis helped to clarify the frequencies of the different genotypes in the current study population: H family, 29%; LAM family, 26%; T family, 25%; S family, 5%, and other genomic families, including orphan strains, 15%.

Conclusion: The study of strains isolated between January and December 2011 has allowed insight into the frequency of different genomic families and the importance of existing clusters in the population of central and eastern Algeria.

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Introduction

Tuberculosis (TB) is an infectious and contagious disease that remains endemic in Algeria despite a long and well-administered national TB control program (NTBP). Algeria is classified by the World Health Organization into the group of countries having moderate TB prevalence, with an annual incidence of between 20 and 99 cases per 100,000 people [1,2]. In 2011, the incidence of microscopy-positive pulmonary TB cases was 19.4 out of 100,000 inhabitants, and the annual incidence of total TB was 57.7 out of 100,000 that same year. Molecular typing tools, including spoligotyping, are currently

widely used in the monitoring and study of the dynamics of tuberculosis epidemics. Using these tools, a retrospective study of the strains isolated during the year 2011 was conducted, involving a sample of 129 strains of *Mycobacterium tuberculosis* isolated from TB patients diagnosed in the central and eastern regions of the country.

Materials and methods

The study was performed on 129 strains of *M. tuberculosis* identified from January 1st to December 31st, 2011. The study material was drawn from 14 departments of central and

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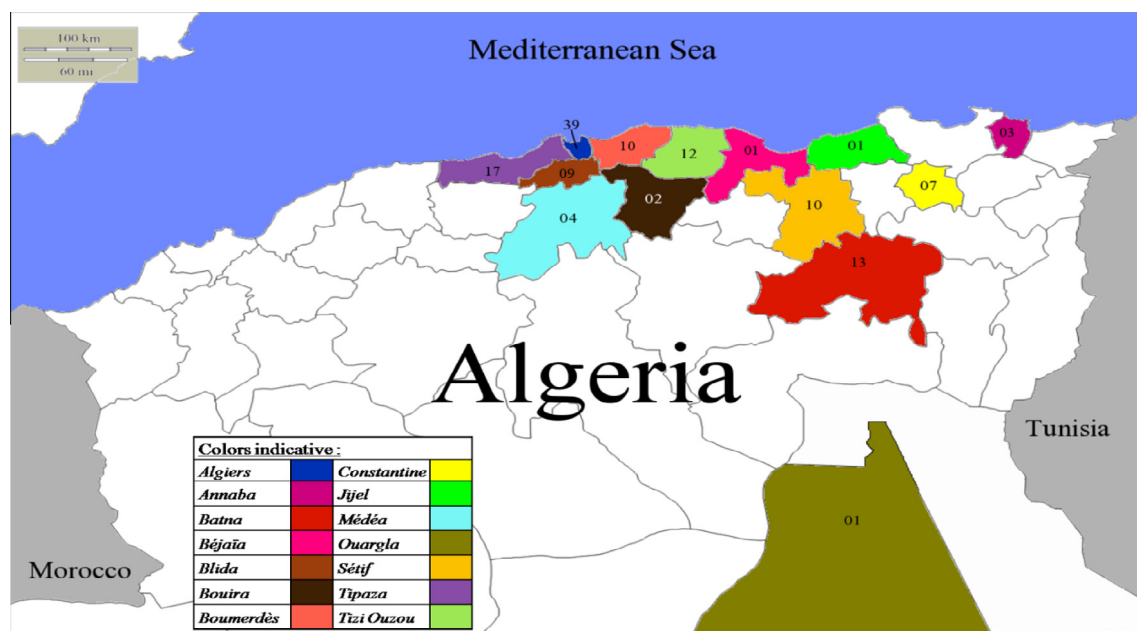


Fig. 1 – Distribution of individuals in the study population between the 14 departments from central and eastern Algeria.

eastern Algeria (Fig. 1). *M. tuberculosis* complex strains were confirmed by biochemical tests (nitratase, catalase) and by sensitivity to chemical substances, such as 2-thiophene carboxylic acid (TCH), Sodium 4-aminosalicylate (PAS), 4 nitrobenzoic acid (PNB). Each strain corresponds to a patient. The sensitivity tests for antituberculosis (isoniazid, streptomycin, rifampicin, ethambutol, kanamycin, and ofloxacin) were performed using the proportion method on Löwenstein-Jensen medium.

The strains included in the study were typed by spoligo-typing [3,4]. The profiles obtained were analyzed by using the SPOLDB4 database [5]. A dendrogram was produced with the MIRU-VNTR software available on the website (<http://www.MIRU-VNTRplus.org>, August 2008).

Results

Study population

The study was performed on 129 strains randomly selected from a sample of 350 strains isolated from TB patients between January and December 2011. Strains isolated from pulmonary localization represent 88% of cases, while 12% of cases represent extrapulmonary tuberculosis strains. The sex ratio was 1.43 and the average age of patients was 39 years. The proportion of previously treated patients was 41% (53 patients) of the sample. The demographic, clinical, and bacteriological characteristics of patients are reported in Table 1.

Antituberculosis drug resistance

The results of the sensitivity tests were available for 126 isolates (97.7%), of which 113 cases (89.7%) were sensitive to all of the first-line antituberculosis drugs. The majority of these

strains (76.6%) were isolated from new TB patients and 53 strains were isolated from previously treated patients. Analysis of drug resistance profiles in patients previously treated or new patients shows that among 13 drug-resistant strains, nine were isolated from patients previously treated. Among these, six are multidrug resistant. Of the four strains isolated from new patients, one showed resistance to four major antibiotics tested. The results appear in Table 1.

Table 1 – Demographic, clinical, and bacteriological characteristics of patients.

Specifications	General data		
TB patients	129		
Male	76 (58%)		
Female	53 (42%)		
Sex ratio (M/F)	1.43		
Average age (y)	39		
Patients with unknown age	9		
Location of the TB			
Pulmonary	114 (88%)		
Extrapulmonary	15 (12%)		
History of the disease			
New cases	76 (59%)		
Previously treated cases	53 (41%)		
Results of susceptibility tests			
Total strains tested	129	76NC	53PC
Sensitive strains	113	72	44
Resistant strains	13	4	9
H	1	0	1
S	2	1	1
HS	3	2	1
MDR (at least H+R)	7	1	6
Contaminated	3		

E = ethambutol; F = female; H = isoniazid; M = male; MDR = multidrug resistant; NC = new cases; PC = previously treated cases; R = rifampicin; S = streptomycin; TB = tuberculosis.

Table 2 – Strain distribution according to families, subfamilies, genomic, and dominant spoligotypes.

Genomic family	Number of strains	Frequency (%) of genomic families	Number of subfamilies	Number and % of strains clustered by genomic family	Number of clusters by genomic family	Number of spoligotypes	The dominant clusters	Number of strains per dominant cluster	Frequency (%) of dominant clusters
H	38	29.5	3	32/24, 80%	7	13	SIT No. 50	16	19.3
LAM	33	25.6	5	20/15, 50%	3	16	SIT No. 42	16	19.3
T	32	24.8	3	27/20, 93%	3	8	SIT No. 53	21	25.3
S	7	5.4	1	4/03, 10%	1	4	-	-	-
U	3	2.3	1	-	0	3	-	-	-
X	1	0.8	1	-	0	1	-	-	-
USA	1	0.8	1	-	0	1	-	-	-
LAM-CAM	1	0.8	-	-	0	1	-	-	-
H-LAM	1	0.8	-	-	0	1	-	-	-
Orphan	12	9.3	-	-	0	12	-	-	-

H = Haarlem; LAM = Latin-American-Mediterranean; S = Sicily-Sardinia.

Spoligotyping results

Referring to the SPOLDB4 database [5], common spoligotypes found within more than one strain were referred to as “shared types” and designated by a shared international type number (SIT). The analysis of 129 strains revealed a very high genetic diversity of 48 different spoligotypes. Among these, 117 strains (90.7%) are known profiles and 12 (9.3%) are not yet listed (orphan profiles). The profile analysis revealed seven large genomic families (clades). The predominance of Haarlem clade (H) with 38 strains, Latin-American-Mediterranean (LAM) with 33 strains, the T clade with 32 strains, and Sardinia-Sicily (S) with seven strains. Rare clades with lower effectiveness corresponded to the U clade with three strains, the X clade with one strain, the USA clade with one strain, LAM10-CAM with one strain, and H3-LAM9 with one strain. Genomic families are more diverse, with 15 subclades in total. LAM is the most diverse family, with five subclades, while the H and T families have three subclades each. The LAM and H families are the most diverse, with 16 and 13 different spoligotypes, respectively. The total frequency of strains clustered in relation to the total strains studied is 64.3% (83 strains), represented primarily by H, LAM, T, and S clades.

The total number of observed clusters is 14, with the H family having seven different clusters for 32 strains (24.8% frequency), the LAM family having three different clusters for 20 strains (15.5% frequency), and T family having 27 strains (20.9% frequency).

The dominant clusters were represented primarily by the SIT n° 53/T1 with 25.3% (21 strains), SIT n° 50/H3 with 19.3% (16 strains), and SIT n° 42/LAM9 with 19.3% (16 strains). The percentage of dominant clusters was determined relative to the total number of clustered strains (Table 2).

Other lineages such as U, X, USA, LAM-CAM, and CAM-H, were represented by 2.3% for U and 0.7% for each of the other lineages, respectively. Additional details for the different profiles can be found in Fig. 2.

Comparison of the 12 strain profiles (9.3%) not listed in the SPOLDB4 database [5] and the SITVITWEB database (Pasteur Institute of Guadeloupe, 2012) (Fig. 2), has allowed us to relate these profiles to certain clades. We were able to link the strains n° 006, 035, 050, 064, 090, and 126 to the H family, the strain n° 027 to the S family, strains n° 029 and 096 to the U family, and strains n° 048, 062, and 066 to the LAM family, knowing that the orphan profile n° 066 was also found and published in Portugal [6].

Discussion

Besides their advantages in determination of genetic diversity of circulating strains, molecular typing tools can detect potential epidemiological links between TB patients. Comparisons were established between the results of our study and those obtained in four Mediterranean countries, specifically Portugal [6], Tunisia [7], Italy [8], and France [9] (Table 3).

This comparison aims to determine the largest genomic families and dominant spoligotypes in the region. The

Table 3 – Comparison of dominant clusters from the present study with results from Four Mediterranean countries.

Studies	Study period	Dominant clusters/clades	
Algerian study—129 strains	2011	42/LAM9	12.4%
		50/H3	12.4%
		53/T1	16.3%
		34/S	3.1%
Tunisia—378 strains [7]	2001–2005	42/LAM9	16.0%
		50/H3	39.0%
		53/T1	10.5%
		34/S	1.3%
Italy (Tuscany)—248 strains [8]	2004	42/LAM9	5.2%
		50/H3	5.0%
		53/T1	11.7%
		34/S	1.2%
France (Limousin)—259 strains [9]	1998–2006	42/LAM9	–
		50/H3	8.0%
		53/T1	13.0%
		34/S	–
Portugal—655 strains [6]	1999–2005	42/LAM9	10.5%
		50/H3	2.5%
		53/T1	8.0%
		34/S	2.9%

H = Haarlem; LAM = Latin-American-Mediterranean; S = Sicily-Sardinia.

analysis shows very similar results, indicating the predominance of LAM, T, and H clades and the similar frequencies of spoligotypes SIT n° 42, 50, and 53. This distribution is likely the result of the mixed populations located around the Mediterranean basin.

The results obtained in this study were also compared to the new SITVITWEB database (Pasteur Institute of Guadeloupe, 2012) [10]. A first comparison was made between all dominant spoligotypes from this study and the Algerian

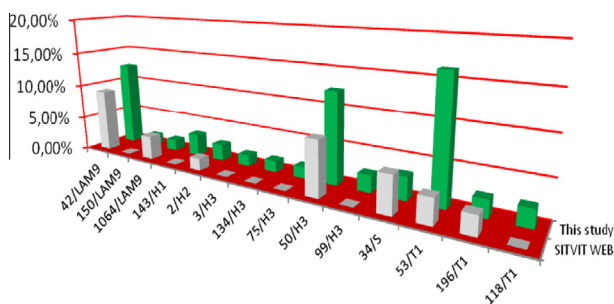


Fig. 3 – Comparison of dominant shared types from this study with Algerian data in the SITVITWEB database (Pasteur Institute of Guadeloupe, 2012) [10].

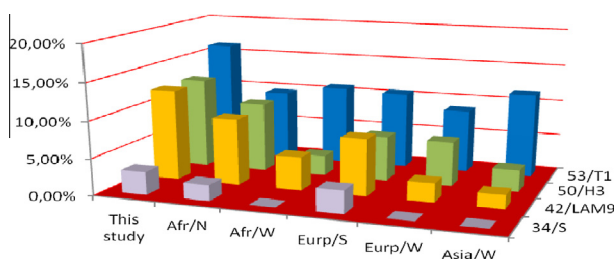


Fig. 4 – Comparison of dominant shared types in this study with data from bordering geographic areas.

data. This comparison indicated similarities particularly between shared types n° 42, 50, 53, and 196 (Fig. 3).

Another comparison between results from the five regions bordering Southern and Western Europe, North and West Africa, Western Asia, and the analysis of data from the current study indicates that our results are similar to those observed for North Africa, with approximately the same order of frequency of the dominant clusters n° 42, 50, and 53.

However, clade S, which appears in two geographically adjacent regions (North Africa and Southern Europe), indicates possible relationships between these two regions. The discovery of clade S in our country is likely the result of frequent travel and its past history of colonization (Fig. 4).

Conclusion

Genotypic analysis on a sample of 129 typed strains isolated from TB patients in 2011 helped to reveal the genetic diversity of strains circulating in our study population (14 departments from the central and eastern regions of Algeria). We identified three genomic dominant families, H, LAM, and T represented by 28.7%, 25.6%, and 25.6%, respectively. The frequency of certain dominant clusters (cluster SIT n° 42/LAM (12.4%), cluster SIT n° 50/H3 (12.4%), and cluster SIT n° 53/T1 (16.9%)) has been evaluated and indicates tuberculosis transmission. The spoligotypes obtained can be used to enrich the genotyping database of our country, which is currently insufficient.

A more exhaustive study covering the western and southern regions of the country researching a larger number of strains over a longer time period will be launched to provide more representative results of the epidemiological situation.

Conflict of interest

None declared.

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