Journal of Global Antimicrobial Resistance 19 (2019) 301–307

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

First genetic characterisation of multidrug-resistant *Mycobacterium tuberculosis* isolates from Algeria

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ARTICLE INFO

Article history:
Received 8 May 2018
Received in revised form 30 April 2019
Accepted 4 May 2019
Available online 14 May 2019

Keywords:
Mycobacterium tuberculosis
Multidrug-resistant
Algeria
Mutation
Next generation sequencing (NGS)-amplicon

ABSTRACT

Objectives: To characterise the genotypes of multidrug-resistant (MDR) *Mycobacterium tuberculosis* (MTB) isolated in Algeria, where there is a low MDR-MTB incidence rate.
Methods: Ten MDR isolates and one resistant to isoniazid were investigated by PCR-Sanger sequencing for 10 loci involved in resistance. Amplicon-based next generation sequencing (NGS) of 15 loci was additionally performed on isolates harbouring novel mutations.
Results: Sanger and amplicon-NGS provided the same results as with GenoType kits. Mutations known to be associated with resistance were described for most isolates: *rpoB* S531L in seven of 10 rifampicin-R MTB isolates, *katG* S315T in nine of 11 isoniazid-R, and promoter *inhA* c-15t in three of 11, *embB* M306V or M306I in two of two ethambutol-R, *rpsL* K43R in four of eight or *rrs* a514c associated with *gidB* L16R in streptomycin-R, *gyrA* A90V in the ofloxacin-R pre-XDR isolate. New and rare mutations were also described in *rpoB* (deletion 512-513-514), *katG* (S315R, M126I/ R496L), *gidB* (V124G, E92A, V139A, G37V), and *gyrA* (P8A). Mycobacterial interspersed repetitive-unit-variable-number tandem-repeat (MIRU-VNTR) profiles were similar for three isolates (lineage Cameroon), indicating a possible clonal diffusion in epidemiologically unrelated patients.
Conclusions: Resistant MTB isolates in Algeria harbour resistance genotypes similar to other countries, but some rare patterns may result from selection and transmission processes inherent to the country.

1. Introduction

Tuberculosis (TB) remains one of the major infectious diseases, with about 10 million new cases causing 1.6 million deaths worldwide. The emergence of multidrug-resistance (MDR)-TB, and resistance to rifampicin (RIF) and isoniazid (INH) is a major problem in industrialised and developing countries [1] and in the global fight against TB [2]. Algeria is classified by the World Health Organization (WHO) into the group of countries having moderate TB incidence (between 53–88 cases per 100 000 inhabitants in 2017). The number of notified TB cases was 23 224, with a low rate of MDR (1.4 %) in contrast with many other countries. The MDR-*Mycobacterium tuberculosis* (MTB) strains from Algeria have not yet been studied or compared with strains isolated in Africa [3] and the Middle East [4].

In MDR-TB strains, mutations have classically been described within an 81pb region (codons 507-533) of the *rpoB* gene encoding the beta subunit of RNA polymerase. This has been observed in

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https://doi.org/10.1016/j.jgar.2019.05.010

>95% of RIF-resistant strains [5] and serves as a surrogate marker for MDR-TB, since 90% of RIF-resistant isolates also exhibit resistance to INH [6]. Mutations associated with INH resistance are observed in *katG* encoding a catalase-peroxidase enzyme [7] in the promoter region of the mabA (FabG1)-*inhA* operon [8] causing overexpression of *inhA* and, less frequently, in the *inhA* coding sequence [9]. The *katG* S315T mutation in found in 95% of INH-resistant clinical isolates [10] and confers high-level resistance, whereas mutations in the *inhA* promoter result in low-level resistance only [11].

Although considered to be an old anti-TB drug and thus rarely studied, streptomycin (STR) has been an alternative first-line anti-TB drug that is increasingly being used for treating MDR-TB in Algeria. Its resistance is conferred by mutations in *rrs* (in the 530 loop and 912 region) encoding the small ribosomal subunit 16S rRNA, or in *rpsL* (mainly codons 43 and 88) encoding the ribosomal protein S12 [12]. More recently, mutations in *gidB* encoding a conserved 7-methylguanosine methyltransferase specific of the 16S rRNA were shown to confer low-level STR resistance in MTB [13,14].

Other mutations have been reported to be associated with or conferring resistance to other anti-TB drugs. Briefly, resistance to ethambutol (EMB) has been associated with mutations (mostly codon 306) in *embB* encoding arabinosyltransferases involved in the biosynthesis of arabinogalactan and lipoarabinomannan [15,16]. Pyrazinamide (PZA) resistance, which is difficult to phenotypically assess [17], has been associated with mutations in *pncA* encoding pyrazinamidase (PZAse) that activates PZA into the active pyrazinoic acid [18]. Lastly, fluoroquinolone resistance has been found to be conferred by mutations in the quinolone resistance-determining region (QRDR) of the DNA gyrase *gyrA* (most often) and *gyrB* genes [19].

This study aimed to genetically characterise some MDR-TB isolates from Algeria, by epidemiological typing, using mycobacterial interspersed repetitive-unit-variable-number tandem-repeat (MIRU-VNTR) genotyping to distinguish clones and lineages, and searching for mutations associated with resistance. It followed different technical approaches that could be directly performed on crude DNA, such as: LiPA kits, PCR-Sanger sequencing of gene regions known to confer resistance, and amplicon-based next generation sequencing (NGS) [5,20]. The results showed that classical mutations were found, with concordant results with all the techniques, in most strains and lineages. However, rare and new mutations were also detected, showing the specificity of the MDR selection and transmission in this country.

2. Materials and methods

2.1. Mycobacterium tuberculosis *complex clinical strains*

Ten MDR-MTB clinical isolates were studied. They were from 10 patients (aged 21–88 years) diagnosed with pulmonary TB from December 2014 to December 2015 at the National Reference Laboratory for Tuberculosis and Mycobacteria at the Pasteur Institute of Algeria. An additional clinical isolate from another patient was included in the study and was resistant to INH and STR, but susceptible to RIF. The MTB isolates were grown on Löwenstein-Jensen (LJ) media at 37 °C and drug-susceptibility testing was conducted, at Pasteur Institute Algiers, on LJ media by the conventional proportional method [21] with the reference strain *MTB* H37Rv as quality control.

2.2. DNA extraction

DNA was extracted from MTB cultures on LJ by suspending a loop of colonies in 400 μL of TE buffer (10 mM Tris/HCL (pH 8.0), 1 mM EDTA). Samples were vortexed, heated at 95 °C for 120 min, and suspensions were centrifuged at 12 000 rpm for 5 min. DNA extracts were shown to be culture negative. DNA was then stored at −20 °C and brought to the French NRC laboratory where it was used crude for all the molecular experiments.

2.3. Genotype MTBDRplus and genotype MTBDRsl

Isolates were submitted to the detection of mutations conferring INH and RIF resistance by the line probe assay kit GenoType® MTBDRplus VER 2.0 and those conferring resistance to fluoroquinolone and injectable drugs by the kit GenoType® MTBDRsl VER 2.0 (Hain Lifescience - Bruker, Nehren, Germany) according to the manufacturer's protocol.

2.4. PCR amplification and Sanger sequence analysis

Pairs of specific primers were designed to amplify 10 loci (*rpoB, katG, inhA, promoter inhA, rpsL, rrs, gidB, embB, pncA, gyrA*) associated with resistance to anti-TB drugs. Primer sequences are provided in the Supplementary Table. For *rpoB*, *embB*, *rrs and gyrA,* only the regions including mutations known to confer resistance were sequenced. For *rspL, inhA, pncA, gidB*, the entire genes were sequenced to detect resistance-associated mutations throughout the gene. Briefly, a 25 μL PCR reaction mixture was prepared containing 1 μL of each primer at 10 pmol/μL, 12 μL of Go Taq® Green (Promega, WI, USA), 2 μL of sample DNA and 8.5 μL of sterile distilled water. Amplification consisted of an initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing temperatures variations from 50–60 °C for 30 s and extension at 72 °C for 30 s, followed by a final extension at 72 °C for 10 min. PCR product sizes were verified by electrophoresis in 1% agarose gel and further purified by NucleoFast® 96 PCR (Macherey-Nagel, Germany). Sequencing of both strands of the PCR product was performed using the Big DyeTM Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Inc., Foster City, CA, USA) and using a 3313xL sequencing instrument (Applied Biosystems, Inc, Foster City, CA, USA) according to the protocol supplied by the manufacturer.

Sequences were analysed using BioEdit sequence alignment software. The obtained sequences were aligned in multiple sequence alignments with reference sequences of *rpoB, katG, inhA,* promoter *inhA, embB, pncA, rrs, rpsL, gid,* and *gyrA* genes in MTB H37Rv (GenBank accession number NC_000962.3) to identify mutations. All mutations that were found were compared against those included in the drug resistance mutation database [22] (https://tbdreamdb.ki.se/Info/).

2.5. Amplicon-based next generation sequencing

Fifteen genes involved in drug resistance (*rpoB, katG, inhA,* promoter *inhA*, *embB, pncA, rrs, rpsL, gidB, gyrA, gyrB, rplC, tlyA, ethA, ethR*) were sequenced following the amplicon strategy using 454 technologies for two clinical MDR isolates, which showed unknown mutations after Sanger sequencing. The primers that were used are listed in the Supplementary Table. A barcode sequence was added to each primer to distinguish different samples during the analysis.

Each amplicon was amplified and purified twice with Taq DNA polymerase High Fidelity (Invitrogen, Waltham, MA, USA) and Agencourt AMPure XP magnetic beads (Beckman Coulter™, Brea, CA, USA), according to the amplicon library preparation manual of GS Junior (Roche diagnostics, Indianapolis, IN, USA). Purified libraries were pooled in an equimolar mix previously measured with Quant-iT Picogreen dsDNA assay kit (Life technologies, Eugene, OR, USA). The size distribution of amplicons was

determined on an Agilent 2100 Bioanalyzer with D1000 DNA Chips (Agilent Technologies, Santa Clara, USA). Amplicon pools were amplified with GS Junior Lib-A and sequencing using GS Junior titanium sequencing kit in 454-GS Junior instrument. Sequence analysis was performed by the software GS Junior Sequencer v3.0, GS Run Browser v3.0 and GS Amplicon Variant Analyzer v3.0.

2.6. Mycobacterial interspersed repetitive-unit-variable-number tandem-repeat

The isolates from the 11 patients were compared using 24-locus MIRU-VNTR typing, as previously described [22].

3. Results

The results of the genetic characterisation of the 11 MTB isolates are detailed in Table 1 for loci associated with RIF (*rpoB*) and INH (*katG, inhA*, promotor *inhA*) resistance and in Table 2 for other loci associated with STR (*rpsL, rrs, gidB*), ethambutol (*embB*), pyrazinamide (*pncA*), fluoroquinolone (*gyrA*), kanamycin and amikacin (*rrs*) resistance.

3.1. Mutations defining multidrug-resistance as RIF plus INH resistance

Among the 10 MDR-TB isolates, a single nucleotide mutation resulting in Ser531Leu substitution (numbering system in *Escherichia coli* (*E. coli*), the corresponding position is 450 in the numbering system of MTB) was the most prevalent and was observed in seven (70%) isolates. The second most prevalent codon was 526 (numbering system in *E. coli*, the corresponding position is 445 in the numbering system of MTB), with mutations found in two of 10 (20%) isolates, leading to H526D and H526Q substitutions. Surprisingly, a 9pb deletion from 1290 to 1298 nucleotides (nt) corresponding to the codons 512, 513 and 514 (numbering system in *E. coli*, the corresponding positions are 431, 432 and 433 in the numbering system of MTB) (GenBanK accession no. PRJEB24182 or ERP105991) was described in one MDR-TB isolate. The remaining MDR-TB isolate had a mutation leading to D516V (numbering system in *E. coli*, the corresponding position is 435 in the numbering system of MTB). The results obtained by GenoType MTBDRplus were concordant with those of Sanger sequencing (Table 1).

Among the 11 INH-resistant isolates, nine of 11 (88.9%) harboured the classical *katG* S315T mutation, but one isolate showed the rare *katG* S315R. The remaining isolate contained double rare mutations in *katG*, M126I and R496L. Mutations in promoter *inhA* were observed in three of 11 (27.3%) isolates associated with *katG* S315T. In three isolates, the mutation *inhA* A190S was observed (Table 1). The isolate with INH resistance but RIF susceptibility harbours a common *katG* S315T mutation.

3.2. Mutations for other first-line anti-TB drugs

Streptomycin resistance was observed for seven of 10 (70%) MDR-TB and also for the INH-resistant strain. The mutation K43R in *rpsL* was prevalent in four of eight (50%) isolates and co-existed with the mutation V139A in *gidB* (GenBanK accession no. PRJEB24182 or ERP105991) in three isolates (Table 2). Mutations in *rrs* at position 514 (numbering system of MTB, 524 in *E. coli*) was observed in two of the remaining isolates and co-existed with the mutation L16R in *gidB* (Table 2). In addition, a deletion of one nucleotide at position 115 in *gidB* and the mutation V124G in *gidB* (GenBanK accession no. PRJEB24182 or ERP105991) were observed in STR-resistant isolates. Two novel polymorphisms in *gidB* were observed G37V (GenBanK accession no. PRJEB24182 or ERP105991) and E92A (GenBanK accession no. PRJEB24182 or ERP105991) in STR-susceptible isolates.

Contrary to the high frequency of STR resistance, ethambutol resistance was observed in two isolates where *embB* mutations were observed as M306V (n = 1) and M306I (n = 1). Unexpectedly, four MDR-TB determined as ethambutol-susceptible isolates also harboured *embB* mutations at other positions: D328Y (n = 3) and Y319S (n = 1) (Table 2).

Since PZA resistance could not be phenotypically tested, detecting a *pncA* mutation was the only way to know about the frequency of this clinically relevant resistance; *pncA* mutation was found in eight of 10 (80%) of the MDR-TB isolates. Mutations were various: V155A (n = 3), T142M (n = 2), P62R (n = 1), V139L (n = 1), and an insertion of two nucleotides (GG) in the latter isolate (Table 2).

3.3. Mutations for extensively-drug resistant

Of the ten MDR-TB isolates, one was resistant to ofloxacin, with a *gyrA* A90V mutation also detected by GenoType MTBDRsl. This

Table 1
Genotypic resistance patterns of the 11 *Mycobacterium tuberculosis* clinical isolates detailed for rifampicin and isoniazid resistance.

Isolates	RIF			INH					
	pAST	*rpoB* gene		pAST		*katG* gene		p *inhA*	*inhA* gene
		MTBDRplus	Sanger sequencing		MTBDRplus	Sanger sequencing	MTBDRplus	Sanger sequencing	Sanger sequencing
Alg 1	R	MUT2B	**H526D (CAC-GAC)**	*R*	MUT1	**S315T (AGC-ACC)**	WT	WT	WT
Alg 2	R	MUT3	**S531L (TCG-TTG)**	R	MUT1	**S315T (AGC-ACC)**	MUT1	c-15t	WT
Alg 3	R	MUT3	**S531L (TCG-TTG)**	R	MUT1	**S315T (AGC-ACC)**	MUT1	c-15t	WT
Alg 4	R	MUT3	**S531L (TCG-TTG)**	R	MUT1	**S315T (AGC-ACC)**	WT	WT	WT
Alg 5	R	WT7-,WT2-/WT3-	**H526Q (CAC-CAG)**/ Del 512-513-514*	R	MUT1	**S315T (AGC-ACC)**	MUT1	c-15t	WT
Alg 6	S	WT	WT	R	MUT1	**S315T (AGC-ACC)**	WT	WT	WT
Alg 7	R	MUT3	**S531L (TCG-TTG)**	R	MUT1	**S315T (AGC-ACC)**	WT	WT	A190S (GCC-TCC)
Alg 8	R	MUT1	**D516V (GAC-GTC)**	R	MUT-	**S315R (AGC-AGG)**	WT	WT	WT
Alg 9	R	MUT3	**S531L (TCG-TTG)**	R	WT	**M126I (ATG-ATA)**/ **R496L (CGC-CTC)**	WT	WT	WT
Alg 10	R	MUT3	**S531L (TCG-TTG)**	R	MUT1	**S315T (AGC-ACC)**	WT	WT	A190S (GCC-TCC)
Alg 11	R	MUT3	**S531L (TCG-TTG)**	R	MUT1	**S315T (AGC-ACC)**	WT	WT	A190S (GCC-TCC)

Corresponding *Escherichia coli* numbering was used for *rpoB*.
Abbreviations: pAST, phenotypic antibiotic susceptibility testing; R, resistant; S, sensible; RIF, rifampicin; INH, isoniazid.
* Mutations not previously reported are marked with an asterisk.

Table 2
Genotypic resistance patterns of the 11 *Mycobacterium tuberculosis* clinical isolates detailed for aminoglycoside, ethambutol, pyrazinamide, ofloxacin, amikacin and kanamycin resistance.

Isolates	STR				EMB		PZA	OFL			AMK/KAN	
	pAST	Sanger sequencing			Sanger sequencing		Sanger sequencing	pAST	MTBDRsl	Sanger sequencing	MTBDRsl	Sanger sequencing
		rpsL gene	*rrs* gene	*gidB* gene	pAST	*embB* gene	*pncA* gene		*gyrA* gene		*rrs* gene	
Alg 1	R	WT	WT	V124G (GTG-GGC)*	S	WT	**Ins GG 391**	S	WT	E21Q (GAG-CAG) S95T (AGC-ACC)	WT	WT
Alg 2	R	WT	a514c	**L16R (CTT-CGT)**	S	WT	**T142M (ACG-ATG)**	S	WT	E21Q (GAG-CAG) S95T (AGC-ACC)	WT	WT
Alg 3	R	WT	a514c	**L16R (CTT-CGT)**	R	**M306V (ATG-GTG)**	**T142M (ACG-ATG)**	R	MUT1	E21Q (GAG-CAG) A90V (GCG-GTG) S95T (AGC-ACC)	WT	WT
Alg 4	R	**K43R (AAG-AGG)**	WT	WT	S	**Y319S (TAT-TCT)**	**V139L (GTG-CTG)**	S	WT	E21Q (GAG-CAG)	WT	WT
Alg 5	S	WT	WT	E92A (*GAA-GCA*)*	R	**M306I (ATG-ATA)**	**P62R (CCG-CGG)**	S	WT	E21Q (GAG-CAG) S95T (AGC-ACC)	WT	WT
Alg 6	R	WT	WT	Del 1 nt115	S	WT	WT	S	WT	E21Q (GAG-CAG) S95T (AGC-ACC)	WT	WT
Alg 7	R	**K43R (AAG-AGG)**	WT	V139A (GTG-GCG)*	S	**D328Y (GAT-TAT)**	**V155A (GTG-GCG)**	S	WT	E21Q (GAG-CAG)	WT	WT
Alg 8	S	WT	WT	WT	S	WT	WT	S	WT	P8A* (CCT-GCT) E21Q (GAG-CAG) S95T (AGC-ACC)	WT	WT
Alg 9	S	WT	WT	G37V (*GGA-GTA*)*	S	WT	WT	S	WT	P8A* (CCT-GCT) E21Q (GAG-CAG) S95T (AGC-ACC)	WT	WT
Alg 10	R	**K43R (AAG-AGG)**	WT	V139A (GTG-GCG)*	S	**D328Y (GAT-TAT)**	**V155A (GTG-GCG)**	S	WT	E21Q (GAG-CAG)	WT	WT
Alg 11	R	**K43R (AAG-AGG)**	WT	V139A (GTG-GCG)*	S	**D328Y (GAT-TAT)**	**V155A (GTG-GCG)**	S	WT	E21Q (GAG-CAG)	WT	WT

Mutation E21Q and S95T in *gyrA* are known as natural polymorphisms.
Abbreviations: pAST, phenotypic antibiotic susceptibility testing; R, resistant; S, sensible; STR, streptomycin; EMB, ethambutol; PZA, pyrazinamide; OFL, ofloxacin; AMK, amikacin; KAN, kanamycin.
* Mutations/polymorphisms not previously reported are marked with an asterisk.

mutation is known to confer low-level resistance to fluoroquinolones. *gyrA* polymorphisms, not associated with resistance, were also observed: *gyrA* S95T (n = 7), E21Q (n = 11), and P8A (n = 2) (Table 2). Mutations conferring resistance to amikacin, kanamycin or capreomycin in the *rrs* gene were not found, although phenotypic susceptibility could not been tested either.

3.4. Amplicon-based NGS vs. PCR sequencing results

The isolates Alg 3 and Alg 5 were studied by amplicon NGS, since this method could sequence all genes of the 10 loci investigated by PCR-Sanger sequencing (see above) plus five additional loci (*gyrB, ethA, ethR, tlyA, rplC*) in one experiment (Table 3). The maximum coverage was > 50X and the minimum coverage was 40×. Amplicon NGS found the same mutations as in Sanger sequencing.

3.5. MIRU–VNTR genotyping

MIRU-VNTR identified four different strain lineages: Cameroon (four isolates), Haarlem (four isolates), LAM (two isolates), and S (one isolate). Three of the Cameroon lineage isolates (isolates Alg 7, Alg 10 and Alg 11) shared the same MIRU-VNTR code and exhibited identical genotypic resistance patterns, even rare mutations (A190S *inhA*, undescribed V139A *gidB*, D328Y *embB*, V155A *pncA*, E21Q *gyrA*). This suggests the transmission of the same strain between these three patients. However, since they were not living in the same area (Fig. 1) and were not epidemiologically related, this may suggest diffusion of this clone in a broader population.

The isolates Alg 2 and Alg 3 LAM lineage were also very similar since they had almost the same MIRU-VNTR code (only two differences) and resistance genotypes were identical for *rpoB, katG, inhA, rrs, gid* and *pncA*. However, the isolate Alg 3 evolved towards drug resistance since it harboured additional mutations conferring ethambutol resistance (M306 V *embB*) and ofloxacin resistance (A90V *gyrA*) and a status of pre-XDR strain. The four Haarlem lineage strains (Alg 5, Alg 6, Alg 8 and Alg 9) showed different MIRU-VNTR codes and different resistance genotypes, indicating that these strains were not related.

4. Discussion

Studies, performed in different countries, on drug-resistant isolates of MTB have reported gene mutations conferring resistance to anti-TB drugs [23]. No studies were carried out in Algeria before the current study. There was one study on 50 sputum-positive cases of pulmonary TB from Western Algeria submitted to GeneXpert MTB/RIF assay, which confirmed RIF resistance without detailing the mutations [24].

The current study reported the genotypes of 10 MDR and one INH-resistant MTB isolated from patients diagnosed with pulmonary TB in Alger. All isolates showed at least one typical mutation known to confer resistance. In addition, novel mutations associated with resistance and polymorphisms (gene mutations associated with susceptibility) were described, indicating that MTB strains circulating in Algeria are not the result of the world endemic situation. Several molecular methods are used in routine conditions for the detection and genetic characterisation of mutations in MDR-TB. The line probe assays (GenoType MTBDRplus and MTBDRsl), recommended by WHO and which are easy to implement in the field, can detect classical mutations associated with INH resistance – such as *katG* S315T, promoter *inhA* c-15T – but cannot detect mutations at codon 126 and 496 in *katG* for instance, since they are out of the hybridisation region tested. PCR-based DNA sequencing is still a highly reliable method with which to detect and describe mutations responsible for drug resistance

Table 3
Comparison of genotypic results obtained by Sanger PCR sequencing and by amplicon-based next generation sequencing.

Gene	*Mycobacterium tuberculosis* Alg 3			*Mycobacterium tuberculosis* Alg 5		
	Sanger	NGS	Percentages of mutations NGS (%)	Sanger	NGS	Percentages of mutation NGS (%)
rpoB	S531L	S531L	98,73%	H526Q/Delcodon512-513-514	H526Q/ Delcodon512-513-514	100%/100%
KatG	S315T	S315T	100%	S315T	S315T	100%
Promoter inhA	c-15t	c-15t	100%	C-15T	C-15T	100%
inhA	WT	WT		WT	WT	
rpsL	WT	WT		WT	WT	
rrs	a514c	a514c	100%	WT	WT	
gidB	L16R	L16R	100%	E92A	E92A	100%
embB	M306V	No detected		M306I	M306I	100%
pncA	T142M	T142M	100%	P62R	P62R	97.07%
gyrA	E21Q	E21Q	98.46%	E21Q	E21Q	100%
	A90V	A90V	100%/	S95T	S95T	100%
	S95T	S95T	100%			
gyrB	-	WT		-	WT	
ethA	-	WT		-	WT	
ethR	-	WT		-	WT	
tlyA	-	WT		-	WT	
rplC	-	WT		-	WT	

- Not tested; NGS, next generation sequencing.

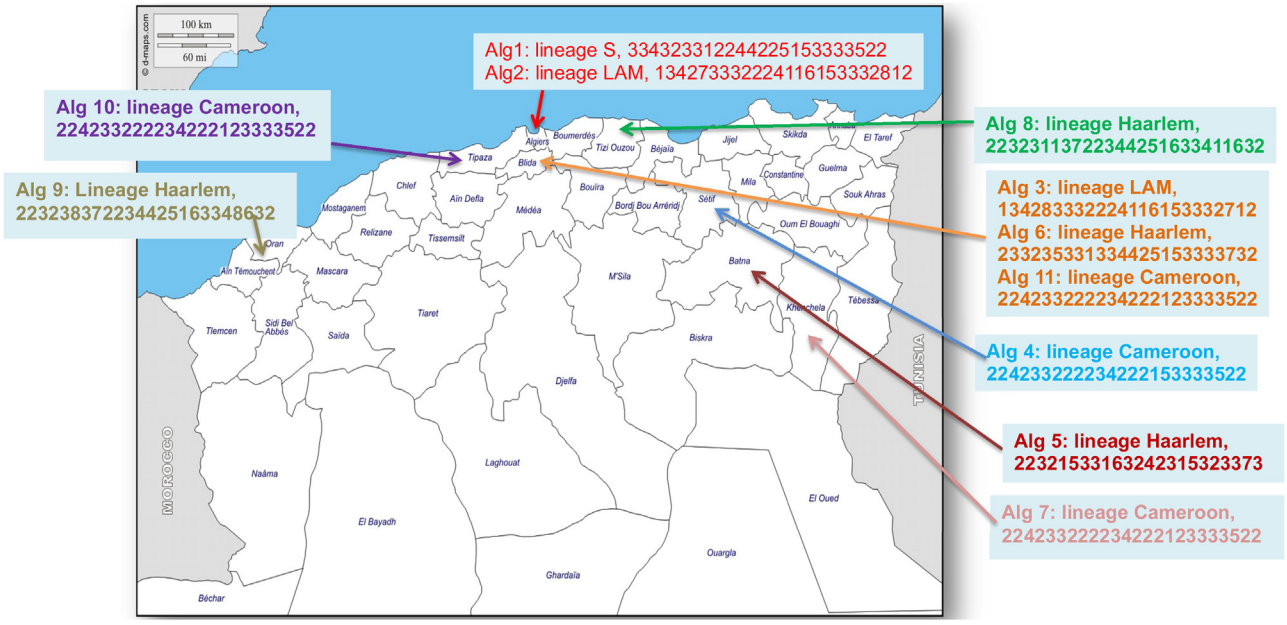

Fig. 1. Results of MIRU-VNTR for the 11 *Mycobacterium tuberculosis* isolates from Algeria.

[25]; however, Sanger equipment is disappearing from research labs. Next generation sequencing used for determining the whole genome sequence (WGS) or amplicon sequences is rapidly becoming popular [26]. Next generation sequencing is still more costly than commercial methods and needs technical skills as well as bioinformatics tools and expertise when performed outside a research laboratory or with low resources. Amplicon-NGS of loci involved in MTB drug resistance can appear to be simpler compared with PCR Sanger sequencing, since multiple loci can be screened in one sequencing reaction, and can appear more feasible than WGS, since it can be performed with crude DNA present in specimens, as performed in the current study [27].

The current results on MDR isolates from Algeria are consistent with previous studies for most of the strains. All (10 of 10) RIF-resistant MTB isolates had mutations within the RRDR, as shown for isolates tested in India (146 of 149, 97.98%) [28] and Brazil (79 of 82, 96.3%) [29], and the codons 531 or 526 were mainly involved [30]. All 11 INH-resistant isolates harboured *katG* mutation at codon 315 and some isolates had mutations in *inhA* promoter. In the clinical MTB strains of patients living in a European country, *katG* mutations were the most prevalent mutation (85%) in strains resistant to INH, specifically S315T in 96% of the cases [27]. In Tunisia, *katG* S315T mutations were present in 85.7% of INH-resistant isolates [31]. Similarly, the current study found that the majority of INH-resistant isolates harboured *katG* S315T mutations (88.9%). Mutations in promoter *inhA* are less frequent [13] with 8–43% of INH- resistant MTB isolates in various countries [11,23] and 27.3% in the current study. In the current study, one INH-resistant isolate did not harbour the *katG*315 mutation, but a double *katG* mutation M126I and R496L. R496L was previously reported in combination with *katG* S315T mutation in one MTB clinical strain resistant to INH [25] but never with *katG* M126I. It is unclear whether R496L is solely capable of conferring INH resistance, but it may concomitantly with M126I [32]. The deletion of nine

nucleotides from 1290 to 1298 (codons 512, 513 and 514) in *rpoB* found in one MDR-TB strain has not previously been reported. Similar deletions, but uncomplete (512–513) or at different positions, were reported in isolates from Korea: deletion of 10 nucleotides from 1287 to 1296 in one isolate XDR-TB and deletion of nine nucleotides from 1282-1290 in one isolate MDR-TB [25].

For STR, 75% of the STR-resistant isolates harboured mutations in *rpsL* (L43R mostly) or *rrs,* described as conferring resistance [12,33]. The current study detected *rrs* mutations at the nucleotide 514 (loop 530) in 25% of STR-resistant isolates, which was similar to previous reports [33,34]. These data are similar to those of other studies strongly confirming that mutations in the rrs 912 region are rare [35]. From the few studies on *gidB* mutation associated with low level STR resistance [13], six variants were identified but none of the four substitutions found in the current isolates (G37V, E92A, V124G, V139A) [13]. However, *gidB* V139A was observed to co-exist with L43R in *rpsL* and *gidB* L16R with *rrs* mutations at position 514 in STR-resistant isolates.

Classical mutations in *embB* [36], as M306I or V, were identified in six of 10 MDR-TB isolates, but other rare mutations were also previously described at codons 328 and 319 [36]. In the current study, the *embB* mutations involving these codons were D328Y and Y319S and were identified in EMB-susceptible MDR-TB, suggesting that they do not confer resistance but may be polymorphisms associated with MDR-TB. Since the phenotypic test for PZA was not performed in Algeria, the report of *pncA* mutations in 80% of the MDR-TB isolates revealed that eight isolates may be PZA-resistant [18], which confirmed a recent report of a high prevalence of PZA resistance in Sub-Saharan Africa countries [37]. The mutations that were identified in *pncA* have previously been described to confer resistance to PZA (V155A and T142M) [18]. Lastly, natural *gyrA* polymorphisms (E21Q and S95T) were found and it was confirmed that they are not associated with resistance to fluoroquinolones [19].

Limitations of this study were: only ten MDR-TB strains were studied, but since the rate of resistance in Algeria is low, it is preferable to study recent isolates than working on a complete dataset of several years. In addition, the difficulty in studying strains isolated in Algeria was also due to limitations in techniques and cost. With collaboration with the NRC in France, molecular tools could be applied that could not be performed in the Algerian laboratory. Therefore, this study will now enable implementation of these genotyping techniques in the TB reference laboratory of Algeria. The difficulty in exchanging MDR-TB cultures explains why there was not a complete phenotypic DST for PZA and second-line drugs. For the same reason, the WGS technique could not be applied because only a small amount of crude DNA extract can be brought from Algeria to France. Since there are no publications about the genotype of MDR-TB strains from Algeria to refer to, the database of the NRC in France had to be searched and 16 MDR-TB isolates were found from patients born in Algeria but who were diagnosed in France; none of them were isolated in 2014 or 2015. These data were not included in the analysis since it could not be determined whether the MTB infection was acquired in Algeria, except for one strain isolated in 2007 that shares the same MIRU pattern with the three clustered isolates of genotype Cameroon. This shows that the transmission was already ongoing in 2007. Moreover, materials and methods were different from the 10 MDR-TB isolates cultivated in Algiers and detailed in this paper.

5. Conclusions

Resistant MTB isolates from Algeria harboured similar resistance genotypes as other countries. However, MDR-TB isolates showed some rare patterns, which were probably the result of selection and transmission processes inherent to the country, such as long-term use of STR. Tuberculosis has been monitored very well in Algeria [24,38] and some of the strains may originate from the 1990s, contrary to what is observed in Asia and other African countries [3,39]. This kind of study can help physiologists and public health authorities to track transmission and recent drug resistance selection.

Funding

This work was supported by Faculty of Nature and Life Sciences, University Hassiba Ben Bouali, Chlef, Algeria and by the CNR-MyRMA, APHP, Hopital Lariboisière (Santé publique France).

Competing interests

None declared.

Ethical approval

Not required.

Acknowledgments

We are grateful to all personnel of CNR-MyRMA- Paris and National Reference Laboratory for Tuberculosis (NRL-TB) of Pasteur Institute - Algiers.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jgar.2019.05.010.

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