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Research article

Rapid resistance detection is reliable for prompt adaptation of isoniazid resistant tuberculosis management

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ABSTRACT

Objectives: Appropriate tuberculosis (TB) management requires anti-TB drugs resistance detection. We assessed the performance of rapid resistance detection assays and their impact on treatment adaptation, focusing on isoniazid resistant (Hr) TB.
Methods: From 2016 to 2022, all TB cases enrolled in 3 hospitals were reviewed for phenotypic drug susceptibility testing (p-DST) and genotypic DST (g-DST) performed by rapid molecular testing, and next generation sequencing (NGS). Clinical characteristics, treatment and outcome were collected for Hr-TB patients. The concordance between g-DST and p-DST results, and delay between treatment initiation and results of g-DST and p-DST were respectively recorded to assess the contribution of DST results on Hr-TB management.
Results: Among 654 TB cases enrolled, 29 were Hr-TB. Concordance between g-DST by rapid molecular methods and p-DST was 76.9 %, whilst concordance between NGS-based g-DST and p-DST was 98.7 %. Rapid resistance detection significantly fastened Hr-TB treatment adaptation (median delay between g-DST results and treatment modification was 6 days). It consisted in fluoroquinolone implementation for 17/23 patients; outcome was favourable except for 2 patients who died before DST reporting.
Conclusion: Rapid resistance detection fastened treatment adaptation. Also, NGS-based g-DST showed almost perfect concordance with p-DST, thus providing rapid and safe culture-free DST alternative.

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1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (Mtb), is the leading cause of death from a single infectious agent worldwide [1]. Effective TB control is threatened by the global emergence and spread of anti-TB drug resistant Mtb, which hampers antibiotics efficacy and therefore requires treatment adaptation to avoid treatment failure or relapse. Mtb drug resistance may present either as resistance to rifampicin (R) or isoniazid (H) alone, or as multi-drug resistant (MDR) TB (meaning that Mtb is resistant to both major first-line anti-TB drugs R and H) [1].

The resistance to H, alone or combined with other drugs, is now the most common type of resistance to anti-TB drugs; the frequency of H-resistant R-susceptible TB (Hr-TB) is greater than the one of R-resistant TB, and estimated at 7.4 % of new and 11.4 % of previously treated TB cases [2]. Evidence indicates additional risk of poor treatment outcomes, such as treatment failure, relapse, and progression to MDR-TB, when patients with Hr-TB are treated with the standard anti-TB regimen [3].

To enable a prompt treatment adaptation, the World Health Organization (WHO) recommends an early detection of drug resistant TB through the use of a rapid diagnostic test [4]. Accurate rapid detection of H resistance is challenging, as H resistance is linked to multiple genetic variations in different loci, resulting in technical difficulties, which explains the heterogeneous performance of rapid H resistance detection tests [5]. Depending on the series and the genetic particularities of Mtb isolates tested, 30 %–70 % of H resistant strains remain undetected [6].

Though time-consuming and requiring level 3 biological safety laboratories (BSL3), the standard reference for H susceptibility detection still relays on phenotypic culture-based drug susceptibility detection (p-DST). Testing using different critical concentrations offers the possibility to distinguish between low- and high-level of H resistance and may guide the decision for treatment regimen adaptations. *In vitro* data suggest that high-dose H may be effective for the treatment of patients having low-level of Hr-TB [7], while high-level of Hr-TB requires the replacement of H by a fluoroquinolone (FQ), mainly levofloxacin (LFX) [3]. As recommended by the WHO, it is essential that the resistance to R is excluded and, when possible, the resistance to FQ should also be excluded, prior to the implementation of LFX containing regimen, to help avert the acquisition of additional drug resistance [4].

Interestingly, recent progress has been done in the field of resistance detection based on next generation sequencing (NGS) to perform targeted or whole genome sequencing (WGS). The Deeplex Myc-TB® (GenoScreen, Lille, France) NGS-based tool provides a rapid identification of a large set of mutations in different resistance associated loci, up to 13 antibiotics, including all first-line anti-TB drugs and some second-line molecules such as FQ, linezolid, bedaquilin and injectable drugs, with performances equalizing those of p-DST [8]. Likewise, WGS allows exhaustive drug resistance detection by using a mutation database validated by the WHO [9]. These NGS based diagnosis tests not only enable accurate detection of Hr-TB, but may also provide information on pyrazinamide (Z), ethambutol (E) and FQ susceptibility, enabling a secure usage of LFX as recommended by the WHO guidelines [10].

France is a high-income country with low prevalence of TB cases (less than 10/100,000 inhabitants); in a previous retrospective study from 2016 to 2017, only 26 % of patients diagnosed with TB had rapid molecular detection of H resistance, which correlated with early adaptation of Hr-TB treatment [11]. Thus, the first objective of the present study was to explore the impact of rapid resistance detection tests, including NGS-based approach on the management of the patients and on the TB disease outcome, with a focus on Hr-TB cases. The second objectives were to assess the performances of H resistance diagnosis tests and the compliance of Hr-TB treatment regimens with international guidelines.

2. Methods

2.1. Data collection

This is a retrospective study conducted in one tertiary-care (Hospices Civils de Lyon, France, hosting 5362 hospitalisation beds) and two secondary-care hospitals (Bourg-en-Bresse and Valence, France, hosting respectively 860 and 800 hospitalisation beds). Were included, between November 2016 and July 2022, all patients diagnosed with TB and having a positive Mtb culture (726 TB patients diagnosed, among whom 654 having Mtb positive culture, Fig. S1A), at the central laboratory equipped with BSL3 and NGS facilities (Institut des Agents Infectieux, Lyon, France). For all Mtb clinical isolates, WGS analysis was performed in routine practice as part of the laboratory diagnosis. For patients with Hr-TB, demographic data (age, sex, continent of birth), clinical presentation (symptoms, comorbidities, pulmonary, extra-pulmonary TB), anti-TB treatment, outcome and microbiological data (sputum smear results, time to positivity, p-DST and genotypic DST (g-DST), lineage) were collected. All the data were implemented in a database, in accordance with the decision 20–216 of the ethics committee of the Lyon University Hospital and the French legislation in place at the time of the study (Reference methodology MR-004).

2.2. Mtb culture process

Mtb clinical isolates culture and p-DST were performed as previously described [12]. Of note, Hr was high when resistant at critical concentration of 0.1 and 0.4 mg/L, and low when resistant at critical concentration of 0.1 mg/L and susceptible at critical concentration of 0.4 mg/L [12]. G-DST by rapid molecular methods were performed on cultured Mtb on a routine basis as previously described, using the line probe assay GenoType MTBDRplus v2.0 test (Hain Lifescience, Nehren, Germany) from November 2016 to August 2020 [12] and the MDR/MTB ELITe MGB® Kit (ELITechGroup SpA, Torino, Italy) on the ELITe InGenius® platform (ELITechGroup SpA) [13] from September 2020 to July 2022. When clinically suspected, the R resistance was also performed using Xpert MTB/RIF Ultra (Cepheid, Sunnyvale, CA, USA) on the primary sample as recommended [14]. Finally, all R and H resistant isolates

were retrospectively analysed using Deeplex Myc-TB® (GenoScreen, Lille, France) according to the manufacturer's instructions [15].

2.3. Whole-genome sequencing and bioinformatic analysis

Genomic DNA extraction, library preparation, short-read WGS using Illumina technology (San Diego, CA, USA) and bioinformatic analyses were performed as previously described [16]. The reference genome coverage breadth was at least 92 % with a mean depth of coverage of at least 30×. Variant calling with MuTect2 in microbial mode was performed. Variant calls were processed using the open access SNP-IT tool (https://github.com/samlipworth/snpit) to identify Mtb complex lineage based on WGS SNP calling [16]. Mykrobe [17] command-line version is included in the pipeline to detect antibiotic resistance. Moreover, the WHO catalogue of mutations in Mtb complex and their association with drug resistance was also used as a database to annotate variants and to detect antibiotic resistance [9].

2.4. Statistical analysis

Data were expressed as count (percentage, %) for dichotomous variables and as median (interquartile range [IQR]) for continuous values. The number of missing values was excluded from the denominator. For dichotomous variables, $\chi 2$ test was used and for continuous values, the paired *t*-test was used to compare groups. Statistical analyses were performed using GraphPad Prism® for Windows version 5.02 (GraphPad Software, La Jolla, CA, USA). A p-value <0.05 was considered significant.

3. Results

3.1. Microbiological characterisation of Mtb isolates

Among the 654 patients with TB diagnosed at the Institut des Agents Infectieux of Lyon University Hospital from November 2016 to July 2022, 29 (4.4 %) had Hr-TB, 6 (0.9 %) had R resistant TB and 9 (1.4 %) had MDR-TB, according to p-DST (Figs. S1A and B). Hr-TB was found in all lineages of Mtb *stricto sensu* (Fig. S1D). The WGS revealed that the prevalence of FQ resistance was very low, only 3 cases were detected (0.46 %); 1 strain was susceptible to first-line anti-TB drugs, 1 was an Hr-TB strain, and 1 was an MDR-TB strain (Fig. S1C).

A total of 39 patients presented at least an H resistance, the most common mutations were the S315T on the *katG* gene, which is associated with high-level resistance, and the C-15T on the *inhA* promoter, which is associated with low-level resistance. Herein, rapid molecular methods failed to accurately identify 5 mutations associated with H resistance, concerning 9/39 (23 %) TB patients with H resistance. Three mutations on the *katG* gene, that are associated with high-level resistance, were not detected, or gave an uninterpretable result. Moreover, 2 mutations on the *inhA* gene associated with the C-15T mutation on the *inhA* promoter were considered as low-level resistance mutations instead of high-level resistance (Table 1). Regarding NGS-based methods, the Deeplex Myc-TB method just failed to identify a single nucleotide deletion at the end of the coding region of the *katG* gene, at amino acid 975. WGS correctly

Table 1
R and H resistance detection by phenotypic and genotypic methods.

Resistance mutations	No isolates	Phenotypic DST	Rapid molecular methods	NGS based methods	
				Deeplex	WGS
Mutations associated with R resistance					
rpoB C761139A H445 N	1	Resistant	Resistant	Resistant	Resistant
rpoB C761139T H445Y	2	Resistant	Resistant	Resistant	Resistant
rpoB C761155T S450L	10	Resistant	Resistant	Resistant	Resistant
rpoB CA761139TG H445C	1	Resistant	Resistant	Resistant	Resistant
rpoB CG761155TC S450F	1	Resistant	Resistant	Resistant	Resistant
rpoB T761161C L452P	1	Susceptible	**Resistant**	**Resistant**	**Resistant**
Mutations associated with H resistance					
katG DEL amino acids 1–492	1	Resistant	**Undetermined**	Resistant	Resistant
katG T2155849G Q88P	1	Resistant	**Susceptible**	Resistant	Resistant
katG DEL CG2155137C, amino acid 975	1	Resistant	**Susceptible**	**Susceptible**	Resistant
katG C2155168G S315T	19	Resistant	Resistant	Resistant	Resistant
inhA C1673425T −15 + katG C2155168G S315T	1	Resistant	Resistant	Resistant	Resistant
fabG1 G1674048A L203L + katG C2155168G S315T	2	Resistant	Resistant	Resistant	Resistant
inhA C1673425T −15 + inhA T1674481G S94A	4	Resistant	**Resistant**	Resistant	Resistant
inhA C1673425T −15 + inhA T1674481G I194T	2	Resistant	**Resistant**	Resistant	Resistant
inhA C1673425T −15	7	Resistant	Resistant	Resistant	Resistant
inhA C1673423T −17	1	Resistant	Resistant	Resistant	Resistant

Genotypic DST by rapid molecular methods were performed on cultured Mtb, using the line probe assay GenoType MTBDRplus v2.0 test from November 2016 to August 2020 and the MDR/MTB ELITe MGB® Kit on the ELITe InGenius® platform from September 2020 to July 2022. For R resistant isolates, genotypic DST was also performed using Xpert MTB/RIF Ultra. Bold: discordant results between p-DST and g-DST. Underlined: H high-level resistance.

identified all H-resistant isolates; overall the sensitivity of NGS-based methods for H resistance detection was 98.7 %.

Regarding R resistance, among the 6 mutations detected, the most common was the S450L mutation on the *rpoB* gene. All mutations associated with R resistance were detected by g-DST, both by rapid molecular methods and by NGS-based methods. The use of these methods even allowed to identify a low-level R resistance mutation, the L452P mutation on the *rpoB* gene, which is not always detected by p-DST (Table 1).

3.2. Epidemiological and clinical characteristics of Hr-TB patients

Among the 29 patients with Hr-TB, the clinical data and information on treatment and outcome were available for 23 patients (Table S1); these data were missing for 6 patients who had been transferred to distant care centres upon TB management, and were therefore excluded from the analysis.

The median [IQR] age at diagnosis was 37 [21–53.5] years old, there were 16 (69.6 %) male patients, and 19 (82.6 %) were not born in France, coming from various countries within Africa, Asia, and Europe (Table 2). Five patients (21.7 %) had comorbidities at TB diagnosis; 3 patients (13.0 %) presented diabetes mellitus, and 2 of them (8.7 %) also presented underlying chronic pulmonary conditions. Two patients (8.7 %) had chronic viral hepatitis B and C, respectively, and a single patient (4.3 %) was recently diagnosed with Human Immunodeficiency Virus (HIV) infection.

The patients presented asthenia (n = 13; 56.5 %), weight loss (n = 13; 56.5 %), fever (n = 12; 52.1 %), and night sweats (n = 5; 21.7 %). Pulmonary TB occurred in 18 patients (78.3 %) while 5 patients (21.7 %) had extrapulmonary TB. Among the patients with pulmonary TB, 9 patients (50 %) showed an isolated pulmonary involvement, 4 patients (22.2 %) had an associated lymph nodes involvement, 2 patients (11.1 %) had an associated vertebral involvement, 2 patients (11.1 %) had a central nervous system involvement, and 1 patient (5.5 %) had a pleural involvement. Among the 5 patients with extrapulmonary TB, 2 patients (40 %) exhibited osteoarticular TB, 2 patients (40 %) had lymph nodes TB and 1 patient (20 %) had pleural TB.

Among the 23 patients with Hr-TB, 10 (43.5 %) had smear positive samples, and the median [IQR] time to positivity of the culture was 11 days [6–16]. According to p-DST, 8 (34.7 %) patients had low-level H resistance and 15 (65.3 %) had high-level H resistance. The median [IQR] delay between sampling and g-DST performed on cultured Mtb was 15.5 days [10–20] and was significantly lower than the median [IQR] delay between sampling and p-DST of 36 days [30–50] ($p < 0.0001$).

Table 2
Main characteristics of the patients diagnosed with Hr-TB.

Patients Characteristics	
Median age at diagnosis [IQR]	37 [21–53.5]
Male (%)	16 (69.6 %)
French born/Not French born	4/19
Comorbidities	
Diabetes mellitus	3 (13 %)
Underlying pulmonary condition	2 (8.7 %)
Active chronic viral hepatitis (HBV or HCV)	2 (8.7 %)
HIV infection	1 (4.3 %)
Clinical presentation	
General symptoms	
Fever	12 (52.1 %)
Night sweats	5 (21.7 %)
Asthenia	13 (56.5 %)
Loss of weight	13 (56.5 %)
Pulmonary TB	18 (78.2 %)
Pulmonary lesions exclusively	9 (50 %)
Pulmonary lesions + lymph nodes involvement	4 (22.2 %)
+ bone and joint involvement	2 (11.1 %)
+ central nervous system involvement	2 (11.1 %)
+ pleuritis	1 (5.5 %)
Extrapulmonary TB	5 (21.7 %)
Bone and joint	2 (40 %)
Lymph nodes	2 (40 %)
Pleural	1 (20 %)
Microbiological characteristics	
Smear positive samples	10 (43.5 %)
Time to positivity (days)	11 [6–16]
H resistance level	
Low-level resistance	8 (34.7 %)
High-level resistance	15 (65.3 %)
Delay between sampling and g-DST by rapid molecular methods (days)	15.5 [10–20]
Delay between sampling and p-DST (days)	36 [30–50]

HBV: Hepatitis B Virus; HCV: Hepatitis C virus; HIV: Human Immunodeficiency Virus; p-DST: phenotypic drug susceptibility testing; g-DST: genotypic drug susceptibility testing.

3.3. Treatment and outcome of Hr-TB patients

Among the 23 patients with Hr-TB, 16/23 (69.6 %) received the standard regimen RHZE upon treatment initiation (Table 3, detailed in Table S1). An early detection of Hr-TB on highly positive sputum in 2 (8.7 %) patients resulted in the initiation of a FQ containing, H-free regimen from the start. Toxicity concerns were responsible for the start of non-conventional regimen for other patients. Following the molecular detection of H resistance in all patients, H doses were increased in 3 (13 %) patients and H was switched to FQ in 10 (43.4 %) patients. Additional phenotypic detection of H resistance resulted in 2 more switch from H to FQ. In total, 17 (73.9 %) Hr-TB patients received a FQ-containing regimen (13 received LFX, 3 received moxifloxacin (MOX) and 1 received both sequentially, Table S1). Toxicity or adverse events related to treatment modifications occurred in 5 (21.7 %) patients, either due to hepatic cytolysis (n = 2), epileptic seizure (n = 1), tendinopathy (n = 1), or neutropenia (n = 1). During the continuation phase, 13 (56.5 %) patients underwent treatment simplification combining two drugs, including R associated with either a FQ (n = 8; 35 %), Z (n = 2; 8.7 %), H (n = 2; 8.7 %) or E (n = 1; 4.3 %). The median (min-max) duration of these simplified regimens (from treatment modification to the end of anti-TB therapy) was 6 months (2–20). Overall, the median [IQR] treatment duration was 9 [6–12] months including 6 (26.1 %) patients treated for 6 months, 8 (34.7 %) patients treated for 9 months, and 7 (30.4 %) treated >9 months (range 10–22). In 8 patients, a longer therapy was explained by the extrapulmonary localisation and/or by risk factors of treatment failure (extensive cavitary disease and/or slow conversion of smear/culture). However, the 7 patients who were treated for >9 months had no associated risk factors for relapse or treatment failure.

Among the 23 patients, the outcome was favourable in 21 (91.3 %) patients, while 2 (8.7 %) patients died from TB unrelated cause before treatment completion, after 2 days and 21 days respectively (Table S1).

3.4. Impact of microbiological results on Hr-TB treatment

The anti-TB treatment was mostly initiated before the obtention of both p-DST and g-DST results; the median [IQR] delay between treatment initiation and p-DST was 32 days [28–45 days] and the median [IQR] delay between treatment initiation and g-DST was 10.5 days [4–12 days]. In 2 patients the g-DST was reported before treatment initiation, thus allowing an appropriate Hr-TB management from the start (Fig. 1A). Among the 23 patients with Hr-TB, 19 (82.6 %) were accurately detected using g-DST by rapid molecular methods. A total of 4 high-level H resistant cases were missed; 2 cases were presumed to have low-level H resistant, while 2 were presumed H susceptible (82.6 % concordance between g-DST and p-DST).

The g-DST results generally led to the modification of anti-TB treatment, by replacing H by FQ or increasing the doses of H; the median [IQR] delay between g-DST results and treatment modification was 6 days [1–13 days]. In 2 patients, the treatment was only modified after p-DST results (Fig. 1B); in 1 patient, the g-DST by rapid molecular method did not detect any H resistance associated mutation (*katG* deletion of amino acid 975, Table 1). In the other patient, for whom only the presumed low-level H resistance C-15T mutation on the *inhA* promoter was detected, the pursuit of the standard regiment combined with pharmacological monitoring was decided. In the other 2 patients, where H resistance mutations were not accurately detected by rapid molecular methods, 1 died before g-DST results (*katG* Q88P mutation), and 1, for whom g-DST only detected a mutation associated with low-level H resistance, LFX was implemented and then continued when p-DST revealed high-level H resistance (supported by NGS-based DST showing a double

Table 3
Main characteristics of the Hr-TB patients' treatment regimens.

Treatments characteristics	
Initial treatment	
Standard regimen (RHZE)	16 (69.6 %)
FQ containing regimens	5 (21.7 %)
Other regimens	2 (8.7 %)
Treatment modification	
Switch H to FQ after H resistance detection	15 (65.2 %)
H doses increased after H resistance detection	3 (13.0 %)
Toxicity/Adverse events	5 (21.7 %)
Standard drug susceptible TB regimen continuation phase	1 (4.3 %)
No secondary modification	2 (8.7 %)
Overall FQ containing regimens	17 (73.9 %)
Regimens simplification (continuation phase with 2 molecules)	13 (56.5 %)
- R + FQ	8 (34.7 %)
- R + Z	2 (8.7 %)
- R + H	2 (8.7 %)
- R + E	1 (4.3 %)
Duration of treatment	
Median in months [IQR]	9 [6–12]
6 months	6 (26.1 %)
9 months	8 (34.7 %)
>9 months	7 (30.4 %)
Death before completion of treatment	2 (8.7 %)

R: Rifampicin; H: Isoniazid; Z: Pyrazinamide; E: Ethambutol; FQ: fluoroquinolone.

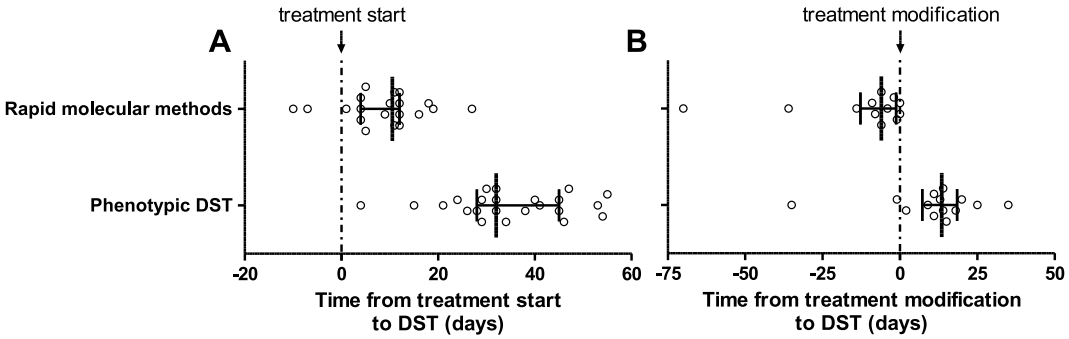

Fig. 1. Delay between anti-TB treatment and drug susceptibility testing results.

mutation of *inh*A locus: *inh*A C1673425T −15 + *inh*A T1674481G I194T).

4. Discussion

The present study showed that rapid resistance detection tests allowed to accelerate the treatment adaptation in Hr-TB patients. H resistance remains the most common type of resistance to anti-TB drugs. Global data on H resistance without concurrent R resistance were 7.4 % in new TB cases and 11.4 % in previously treated TB cases [2], whilst this series included 29 TB patients with Hr-TB representing 4.4 % of TB managed cases. Until recently, Hr-TB was thought to have similar treatment outcomes compared to drug-susceptible TB. However, many studies support that Hr-TB is associated with higher rates of treatment failure and relapse than drug-susceptible TB [18]. The increasing risk of MDR-TB in case of relapse, urged the elaboration of guidelines for appropriate management of Hr-TB [3]. To fasten the treatment adaptation, rapid testing for H resistance, in addition to R resistance, is largely encouraged by the WHO [4]. The present results thus confirm previous reports showing that g-DST compared to p-DST significantly accelerates Hr-TB diagnosis and appropriate anti-TB implementation, and should therefore be performed on routine basis by all TB diagnosis laboratories.

Though g-DST by rapid molecular methods may speed results by several weeks, the lack of sensitivity of H resistance remains a challenge [12]. This could be explained by the fact that clinical diagnosis companies mainly focus on R resistance detection as (i) MDR-TB always include resistance to R; (ii) the genetic support of R resistance is restricted to a short nucleotide sequence of *rpo*B locus; (iii) as a corollary there is an excellent feasibility of molecular tests [4,19]. Herein, the performances of rapid molecular R resistance detection were excellent. Conversely, regarding H resistance, the majority of rapid molecular methods only target codon 315 of *katG* locus (associated with high-level H resistance), leaving undetected other polymorphisms, frameshifts, and deletions on *katG,* though probably associated with phenotypic resistance [5]. This is linked to the genetic basis of H resistance; the heterogeneous performance of currently developed PCR-based assays for H-resistance detection can be explained by multi-locus spread genetic modifications [6, 13]. This issue seems to have been overpassed by the NGS-based assay Deeplex Myc-TB®, which herein displayed performances very similar to p-DST. Nevertheless, this assay needs every-day access to NGS services to be able to provide results in a time frame similar to rapid molecular tests.

Previously very useful to detect the different levels of H-resistance, p-DST was essential to determine Hr-TB treatment regimens; using high-dose of H in patients having low-level H-resistance, or replacing H by LFX in patients having high-level H-resistance. In order to prevent inappropriate Hr-TB management in case of misdiagnosis of H high-level resistance, possibly linked to the *in vitro* resistance expression variability, the WHO pragmatically recommends the replacement of H by LFX within the anti-TB regimen, regardless of the H resistance level [3]. In the present series, 17/23 patients received FQ containing regimen in accordance with international guidelines. Since LFX containing regimen are increasingly implemented, it is more important to perform an accurate detection of H resistance than evaluating the level of H resistances. Although FQ resistance is still very low, 3/654 herein, to ensure safe implementation of FQ treatment, there is a need for a rapid detection of FQ resistance, in addition to a rapid and accurate resistance detection of the first-line anti-TB drugs. From this respect, to address the need of combined detection on multiple resistance markers, exhaustive NGS-based methods such as Deeplex® Myc-TB or WGS reveal to be more useful than prior developed real-time PCR-based methods. Herein, the excellent performances of NGS-based methods were confirmed for anti-TB drugs resistance detection, consistent with the target product profile requested for Mtb DST [20], and thus providing a culture-free DST solution for TB patients.

In this study, the median duration of anti-TB therapy was higher than the one recommended by the WHO 2018 guidelines: a duration of 6 months for LFX containing regimen [21]. However, previous guidelines (2014) recommended a treatment duration of 6–9 months [22], thus, the prolongation to 9 months of treatment in absence of associated risk factors observed in our patients might be explained by the heterogeneous comprehension of successive guidelines. Unexpectedly, a regimen reduction to bitherapy was observed in 11 patients not managed with the RHZE standard regimen, including 8 patients with R + FQ regimen. This therapeutic option may be explained by the misinterpretation of the WHO guidelines, tending to mimic the switch to bitherapy performed upon the standard regimen, as well as by the intolerance or fear of intolerance in 6 months multi-antibiotic treatment. However, the favourable outcome observed for all the patients concerned by this simplification, may suggest that FQ containing regimens could be simplified

upon the continuation phase.

Our study has limitations, firstly the enrolling centres were close geographically and linked to a single laboratory diagnosis, thus, it did not entirely reflect the French TB diagnosis and management landscape. Secondarily, during the 5 years of the enrolling period, changes of the diagnosis methods were performed as well as changes of TB management guidelines, probably explaining the heterogeneity of treatments. However, our results were in line with a previous French multi-centre assessment covering the first part of our enrolling period [11], revealing, in our case, a better implementation of g-DST and also a better compliance with the international Hr-TB management guidelines, consistent with the 2018 WHO new recommendations.

The present study highlighted the importance of g-DST to safely engage Hr-TB patients on adapted treatment. Also, NGS-based g-DST showed almost perfect concordance with p-DST, thus providing rapid and safe culture-free DST alternative. Moreover, this study revealed an important heterogeneity regarding patient management, probably due to the clinical complexity of patients, but also to misinterpretation of recent guidelines. We identified a need of simplified regimens to prevent toxicity and improve treatment adherence, in the context of TB management secured by accurate NGS-based resistance detection tools.

Ethics statement

All patient-related data were collected and processed in accordance with the reference methodology MR-004. French government deliberation No. 2018-155 of May 3, 2018 approved the reference methodology MR-004 for the processing of personal data implemented in the context of research not involving the human person, studies and evaluations in the field of health. Patient data recording form for MR-004 processing was registered under the number 20–216 by the data protection officer of Lyon University Hospitals.

Relevant approval regarding access to patient-identifiable information are granted by the French data protection agency (*Commission Nationale de l'Informatique et des Libertés*, CNIL).

Data availability statement

Whole-genome sequences of all isolates were submitted to European Nucleotide Archive (ENA) under the accession number PRJEB42621.

CRediT authorship contribution statement

Olivier Bahuaud: Writing – original draft, Visualization, Validation, Resources, Investigation, Formal analysis, Data curation. **Charlotte Genestet:** Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Elisabeth Hodille:** Visualization, Resources, Methodology, Investigation, Formal analysis, Data curation. **Maxime Vallée:** Visualization, Software, Resources, Investigation, Formal analysis, Data curation. **Quentin Testard:** Visualization, Software, Resources, Methodology, Formal analysis, Data curation. **Caroline Tataï:** Visualization, Validation, Resources, Investigation, Formal analysis, Data curation. **Julien Saison:** Validation, Resources, Investigation, Formal analysis, Data curation. **Jean-Philippe Rasigade:** Visualization, Validation, Resources, Investigation, Formal analysis. **Gérard Lina:** Visualization, Validation, Resources, Investigation, Formal analysis, Data curation. **Florence Ader:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Oana Dumitrescu:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29932.

Genotypic DST by rapid molecular methods were performed on cultured Mtb, using the line probe assay GenoType MTBDRplus v2.0 test from November 2016 to August 2020 and the MDR/MTB ELITe MGB® Kit on the ELITe InGenius® platform from September 2020 to July 2022. Anti-TB treatment switch: replacement of H by FQ or increase in the dose of H.

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