MUTATIONS CONFERRING RESISTANCE TO FIRST-LINE ANTI-TUBERCULOSIS DRUGS IN PERU: A SYSTEMATIC REVIEW OF THE LITERATURE

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ABSTRACT

Objective. To systematize available information regarding mutations that confer resistance to first-line anti-tuberculosis drugs. ***Materials and Methods.*** A systematic review of the scientific literature was conducted to identify articles that reported mutations conferring resistance to first-line anti-tuberculosis drugs. This search emphasized resistance to isoniazid and rifampicin drugs in *M. tuberculosis* strains of Peruvian patients. The search was performed on PubMed and LILACS (Latin American and Caribbean Health Sciences Literature). ***Results.*** Fourteen (14) articles were included, of which three reported mutations associated with resistance to isoniazid, six to rifampicin, eight to pyrazinamide and one to ethambutol. All mutations to isoniazid or rifampicin were identified directly or indirectly by the molecular diagnostic test GenoType MTBDRplus® v2.0. The greatest variability of mutations was determined in resistance to pyrazinamide. ***Conclusions.*** There is a great variability of mutations associated with resistance to anti-tuberculosis drugs that have been reported in Peru, and they are systematized in this report. These mutations must be taken into account for the development of diagnostic devices or selection of diagnostic tests to be applied in our country.

Keywords: Mycobacterium tuberculosis; Drug Resistance; Genotype; Mutation (source: MeSH NLM).

INTRODUCTION

Drug-resistant tuberculosis (TB) is a major global public health problem. In 2018, the World Health Organization (WHO) estimated that ten million people developed the disease. Of these cases, 500,000 had rifampicin-resistant tuberculosis, of which 78% was multidrug-resistant tuberculosis (MDR-TB); that is, they had simultaneous resistance to isoniazid and rifampicin [1]. In addition, it was determined that 7.3% of the diagnosed MDR-TB cases were extensively drug-resistant tuberculosis (XDR-TB) cases; that is, they were additionally resistant to at least one fluoroquinolone (levofloxacin, ciprofloxacin, or moxifloxacin) and a second-line aminoglycoside (capreomycin, amikacin, kanamycin). Peru is no stranger to MDR-TB and XDR-TB epidemics, being among the 30 countries with the highest MDR-TB burden in the world [1]. and reporting around 3000 prevalent cases of MDR-TB annually [2].

Resistance to anti-tuberculosis drugs is determined by mutations in the *M. tuberculosis* genome, which occur spontaneously or induced by selective drug pressure [3]. Patients who take inadequate therapeutic schemes or who are not very adherent can generate, in the presence of subtherapeutic concentrations of the drugs, proliferation of the strains that present these genetic resistance mutations [3]. These strains can then be transmitted by air to other people, who develop drug-resistant tuberculosis without having previously received any therapeutic regimen.

Mutations present in the coding gene of the "RNA polymerase subunit β" *(rpoB gene)* are now known to confer resistance to rifampicin. Likewise, mutations present in the "catalase-peroxidase" gene *(katG gene)* and in the promoter region of the "enoyl-ACP reductase" gene *(inhA gene)* are associated with resistance to isoniazid [4]. On the other hand, molecular diagnostic devices used in our health system, such as the GenoType MTBDRplus® v2.0 (Hain Lifescience) and Xpert® MTB/RIF (Cepheid, USA) detect resistance to the abovementioned drugs. There are also mutations in other genes that have been identified as responsible for resistance to other anti-tuberculosis drugs

such as pyrazinamide *(pncA)*, aminoglycosides *(rrs)*, fluoroquinolones *(gyrA)*, among others.

The presence and/or absence of mutations can vary significantly from one country to another, which could mean that the diagnostic devices that identify the most frequent mutations in one country does not have the same diagnostic performance in another [3]. Therefore, knowledge of mutations existing in strains of *M. tuberculosis* which circulate at the national level can help develop new diagnostic devices that improve resistance detection at a local level [5].

The objective of this study is to review the available information regarding mutations that confer resistance to first-line anti-tuberculosis drugs, with emphasis on isoniazid and rifampicin, in publications that describe strains of *M. tuberculosis* from Peruvian patients.

MATERIALS AND METHODS

A systematic review of the scientific literature related to the molecular characterization of mutations existing in *M. tuberculosis* strains with resistance to first-line anti-tuberculosis drugs in Peru was performed, according to PRISMA guidelines [6]. The study focused on the analysis of resistance to isoniazid and rifampicin, as well as strains of *M. tuberculosis* isolated from peruvian patients.

INCLUSION AND EXCLUSION CRITERIA

The inclusion criteria for the publications considered in the study were first, language (articles in english, spanish, french, and portuguese); second, study design (case reports or series, observational or experimental studies); third, that they are articles with explicit information on the nucleotide sequence of the mutation conferring resistance to first-line drugs, mainly isoniazid and rifampicin (independently of any additional tests they may have done, including non-commercial and commercial tests); fourth, that they include strains from Peruvian patients; and finally, that they have been published in the last ten years, with the aim of obtaining a better reflection of the diversity of mutations conferring resistance today.

Articles that only included phenotypic tests for the diagnosis of resistance, strains of patients from countries other than Peru, and articles aimed at studying the genetic lineage of mycobacteria, but not their resistance to anti-tuberculosis drugs, were excluded.

KEY MESSAGES

Research Motivation. Resistance to anti-tuberculosis drugs is increasing in the country. It is important to systematize the existing information on the genetic characteristics of the resistant microorganisms that have been reported in our environment.

Main Findings. Fourteen (14) studies were found, which, together, reported 94 changes in the genetic structure of the microorganism that generated resistance to the most important drugs used in the treatment of tuberculosis.

Implications. These changes in the genetics of the tuberculosis bacillus must be taken into account when designing the best devices to diagnose resistant tuberculosis or choosing commercial methods that can adequately identify them.

BIBLIOGRAPHIC SEARCH STRATEGY

Bibliographic databases searched to identify studies to be included were: PubMed, the Library of Congress, and LILACS (Latin American and Caribbean Literature in Health Sciences).

For the PubMed search, the following strategy was used: "(polymorphism OR genotype OR variant OR mutation OR allele) AND Peru AND tuberculosis", words that were selected based on previous systematic reviews about mutations [7,8]. The LILACS search used the following strategy: «mutaciones» y «tuberculosis» y «Peru» ("mutations" and "tuberculosis" and "Peru").

In the case of multiple publications on the same study, the study with the largest sample or first to be published was included.

STUDY SELECTION AND DATA COLLECTION

AV and LS independently selected the studies to be included. The list of included and excluded studies was then reviewed. Where there was conflict, it was discussed, and consensus was reached. References and articles related to the initially identified publications were also reviewed.

Once the studies to be included were defined, information about the characteristics of the publication (author, journal, year), the study (type of design, characteristics of patient), and the strains (evaluated drugs, evaluated genes and existing mutations) was extracted into tables previously designed for data collection. Finally, "summary tables" were elaborated with the collected information.

The quality of the publications was not formally evaluated since they are often specific reports of mutations and the medical literature for the reporting of this type of publications is heterogenous (initiatives to homogenize the reports of publications about mutations in *M. tuberculosis* are just emerging). For that reason, it was decided not to carry out a risk of bias assessment in the studies.

The main aspect to report was the nucleotide sequences of specific mutations associated with resistance to first-line anti-tuberculosis drugs, particularly isoniazid and rifampicin. Finally, once the information on the mutations found was extracted, they were graded as minimal, moderate and high confidence for association with resistance according to the WHO's technical guide on "The use of next-generation sequencing technologies for the detection of mutations associated with drug resistance in *Mycobacterium tuberculosis* complex" [9]. The results are reported according to the anti-tuberculosis drug analyzed.

It is worth mentioning that the protocol of this review was not registered and, since it was a purely descriptive topic, the respective PICO question was not structured.

RESULTS

Seventy (70) articles were retrieved from PubMed and two from LILACS, of which one was duplicated. In addition, other seven articles were obtained from the references in the selected articles. Sixty-four (64) articles were excluded, and, finally, 14 studies met our inclusion/exclusion criteria and were admitted for review. Figure 1 shows the selection flowchart for the included studies. The studies of Moradigaravan [10] and Barletta [11] were excluded for studying second-line drugs (para-amino salicylic acid, and quinolones and aminoglycosides, respectively).

The main characteristics of these studies are shown in Table 1. Of the 14 articles, 13 were published in English. Nine articles focused on the evaluation of mutations that conferred resistance to a single anti-tuberculosis drug and seven evaluated more than one drug at a time. Characteristics such as population type and specimens are also described in Table 1.

First-line anti-tuberculosis drugs (isoniazid and rifampicin) are discussed in Tables 2 and 3, respectively.

In addition, information concerning pyrazinamide is included in the supplementary material. Studies evaluating several drugs simultaneously were included in all the respective tables.

Of the three studies that reported resistance to isoniazid, two evaluated the *inhA* and *katG* genes, and one evaluated the *katG* and *kasA* genes.

Of the seven mutations reported in these studies, two had a high likelihood of association with resistance and one had a moderate likelihood of association with resistance [26]. The most frequent mutation was reported in codon 315 of the *katG* gene. The location of these mutations in the *inhA* and *katG* genes, as well as the gene region evaluated by the commercial test for resistance diagnosis (GenoType MTBDRplus® v2.0), is shown in Figure 2.

Of the six studies that reported mutations associated with resistance to rifampicin, all showed mutations in the rpoB gene, and it could be seen that Linger's study [15] identified the most mutations. The studies of Maha, Gygli, and Galarza [12, 13, 20] mostly presented mutations with a
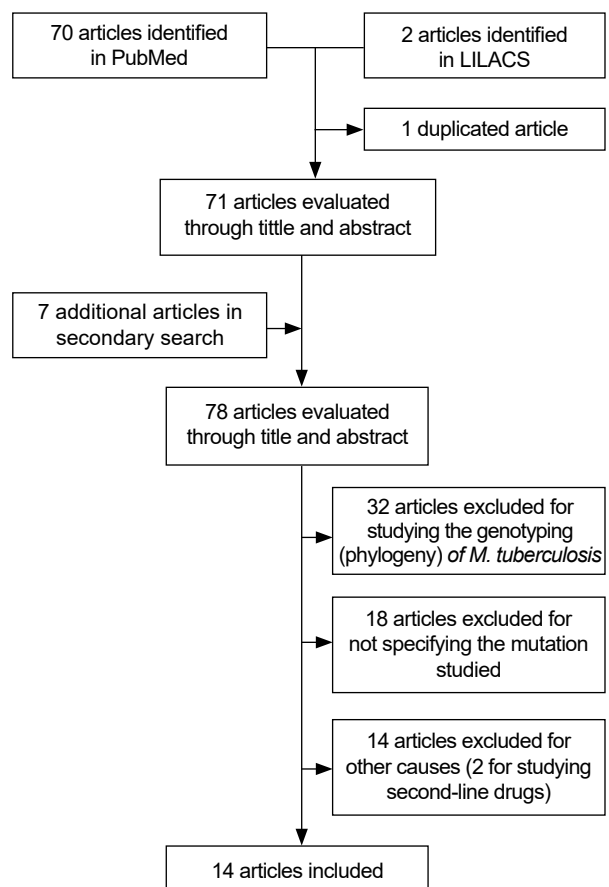

Figure 1. Flowchart of the publications included in the systematic review about mutations conferring resistance to anti-tuberculosis drugs in Peru.

Table 1. Main characteristics of the studies on mutations conferring resistance to first-line anti-tuberculosis drugs in Peru

Study	Author, year of publication, institution	Objective	Population	Type and number of specimens included
1	Gygli, 2019, UPCH [12]	To compare the diagnosis of anti-tuberculosis resistance based on the whole genome with the diagnosis based on cultures of *M. tuberculosis*	Strains from patients with drug-resistant TB between 2004-2015	189 strains
2	Maha, 2019, SES [13]	To describe the observed *rpoB* variants, evaluate the degree of cross-resistance between rifampicin and rifabutin, and develop mutant *rpoB* in a laboratory strain to study the in vitro effect of D435V on the minimum inhibitory concentrations of rifampicin and rifabutin	Sputum from patients with MDR-TB	1003 sputum samples
3	Jaramillo, 2018, INS [14]*	To identify resistance to anti-tuberculosis by mismatch detection mutation in gene fragments associated with resistance.	Strains from patients with MDR-TB and XDR-TB.	16 strains
4	Linger, 2018, SES [15]	To develop a closed-amplicon, microarray-based consumable for the detection of MDR-TB, to characterize the device's analytical and shelf-life behavior and evaluate the test	Sputum from suspected and confirmed tuberculosis-positive patients	25 sputum samples
5	Rueda, 2018, UPCH [16]*	To determine mutations associated with resistance to pyrazinamide	Strains resistant and sensitive to pyrazinamide	23 23 strains (11 resistant and 12 sensitive)
6	Tessema, 2017, UPCH [17]	To describe the resistance profile of cryopreserved strains from the FIND TB strain bank	Strains from patients with MDR-TB, XDR-TB and mono-resistant strains.	118 strains (102 MDR-TB, 10 XDR-TB and 6 Mono-resistant)
7	Sheen, 2017, UPCH [18]*	To determine which mutations are associated with resistance to pyrazinamide	Strains from patients with TB resistant and sensitive to pyrazinamide	68 strains (26 resistant and 42 sensitive)
8	Dudley, 2016, UPCH [19]*	To study the utility of *pncA* mutation typing, along with phenotypic resistance testing and spoligotyping, to detect nosocomial outbreaks	Adults diagnosed with TB in Lima, Peru, 1999-2005.	794 strains
9	Galarza, 2016, INS [20]	To determine resistance to isoniazid and rifampicin using the HRM method.	Strains from Patients with MDR-TB and Sensitive.	167 strains (89 sensitive and 78 TB-MDR)
10	Galarza, 2014, INS [21]	To compare the genome of Peruvian MDR strains with that of the KZN 1435 strain	Multi-drug resistant TB strain, African strain KZN 1435*.	2 strains
11	Sheen, 2013, UPCH [22]*	To analyze the individual and adjusted contribution of pyrazinamidase activity, *pncA* expression, and pyrazinoic acid efflux rate on pyrazinamide resistance	Strains from patients with TB that are sensitive and resistant to *M. tuberculosis*.	30 strains
12	Zimic, 2010, UPCH [23]*	To determine and compare the distribution, at the secondary structure level, of amino acid substitutions of *M. tuberculosis* pyrazinamidase from pyrazinamide-resistant strains found in Peru with those reported globally	Strains from patients with pyrazinamide-resistant TB previously studied.	108 strains
13	Sheen, 2009, UPCH [24]*	To examine the correlation between the kinetic parameters of recombinant mutated pyrazinamidase cloned from pyrazinamide-resistant *M. tuberculosis* clinical strains and the microbiological pyrazinamide resistance level	Strains from patients with pyrazinamide-resistant TB previously studied.	13 strains (12 pyrazinamide-resistant strains and 1 control H37Rv)
14	Sheen, 2009, UPCH [25]*	To evaluate a rapid molecular test for pyrazinamide resistance	Sputum from patients with TB and TB negative microscopy.	181 sputum samples (147 with TB and 34 sputum samples for negative control)

* Studies addressing other first-line anti-tuberculosis drugs.
UPCH: Universidad Peruana Cayetano Heredia; SES: Partners in Health; INS: National Institute of Health; TB: tuberculosis; MDR-TB: multidrug-resistant tuberculosis; XDR-TB: extremely resistant tuberculosis.
No strains that were not from peruvian patients were considered

Table 2. Studies describing mutations conferring resistance to isoniazid in peruvian patients.

Author, year	Method	Reference standard	Evaluated Genes	Mutations found	Mutation detected by GenoType MTBDRplus® v2.0	Confidence for association with resistance [9]
Linger, 2018 [15]	IS 6110 - Specific Quantitative PCR Microarrays	MGIT 960	katG	S315T	Direct	High
				S315N	Indirect	High
			inhA*	c-15t	Direct	Moderate
				t-8a	Direct	Not reported
				t-8c	Direct	Not reported
				g-17t	Indirect	Not reported
Galarza, 2016 [20]	High-resolution melting assays (PCR-HRM); DNA sequencing	MODS, 7H10	inhA*	c-15t	Direct	Moderate
			katG	S315T	Direct	High
Galarza, 2014 [21]	MIRU-VNTR; DNA sequencing	7H10	katG	S315T	Direct	High
			kasA	G269S	Not detected	Not reported

* The change at the nucleotide level is noted. PCR: polymerase chain reaction; HRM: high-resolution melting assays; MODS: microscopic observations drug susceptibility; MGIT: mycobacterial growth indicator tube.

high likelihood of association with resistance. The two most frequent mutations, D516V and H526Y, had a high association with resistance (Table 3, Figure 3).

With regards to mutations in the pncA gene associated with resistance to pyrazinamide, eight studies that reported a high number of mutations were identified. In addition, it could be seen that the mutations showed a high association with resistance to this drug. The most frequently found mutation was H51R, which is highly associated with resistance. Mutations conferring resistance to pyrazinamide already classified by WHO and those pending classification are reported in the supplemental material.

With respect to the other first-line anti-tuberculosis drugs, one study [12] evaluating the rpsL gene for resistance to streptomycin was observed. This study used Whole Genome Sequencing and 7H10 and MGIT BACTEC 960 as phenotypic method, finding the K43R mutation, which is highly associated with resistance. Likewise, Galarza also used Whole Genome Sequencing to evaluate resistance to ethambutol through the embB gene, finding the Y319S mutation, which does not confer resistance [21].

DISCUSSION

This systematic review identified 14 studies reporting mutations in the genome of *M. tuberculosis* in genes associated with resistance to first-line anti-tuberculosis drugs. In total, seven mutations in relation to resistance to isoniazid were reported (two of them with high and one with moderate confidence for association with
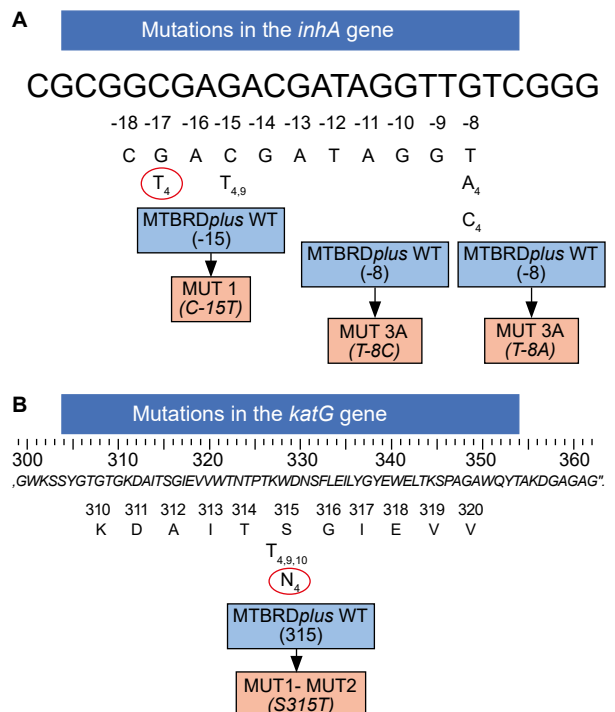

Figura 2. A. Nucleotide-level mutations reported for the inhA gene promoter region. **B.** AA (amino acids)-level mutations reported for the katG gene

The small numbers show the studies where these mutations were reported according to Table 1. The blue and red boxes show the mutation reported by the GenoType MTBDRplus® v2.0 methodology, however, the red circle shows a mutation that is not directly identified by such methodology.

Table 3. Studies describing mutations that confer resistance to rifampicin in Peruvian patients.

Author, year	Method	Reference standard	Genes assessed	Mutations found **	Mutation detected by		Confidence of association to resistance [9]
					GenoType MTBDRplus® v2.0	Xpert® MTB/RIF	
Gygli, 2019 [12]	Whole genome sequencing	7H10 MGIT 960	*rpoB*	L533P (452)	Indirect	Indirect	Moderate
				H526L (445)	Indirect	Indirect	High
				H526Y (445)	Direct	Indirect	High
Maha, 2019 [13]	Whole genome sequencing	7H10	*rpoB*	D516V (435)	Direct	Indirect	High
				S531L (450)	Direct	Indirect	High
				H526Y (445)	Direct	Indirect	High
Linger, 2018 [15]	IS *6110*- PCR Specific quantitative PCR; Microarrays	MGIT 960	*rpoB*	del507(426)	Indirect	Indirect	Not reported
				Q510H (429)	Indirect	Indirect	Not reported
				L511P (430)	Indirect	Indirect	Minimal
				L511R (430)	Indirect	Indirect	Not reported
				S512T (431)	Indirect	Indirect	High
				S512R (431)	Indirect	Indirect	Not reported
				Q513L (432)	Indirect	Indirect	High
				Q513K (432)	Indirect	Indirect	High
				Q513P (432)	Indirect	Indirect	High
				M515I (434)	Indirect	Indirect	High
				D516E (435)	Indirect	Indirect	Not reported
				D516Y (435)	Indirect	Indirect	Moderate
				D516G (435)	Indirect	Indirect	High
				D516V (435)	Direct	Indirect	High
				S522L (441)	Indirect	Indirect	Moderate
				L524S (443)	Indirect	Indirect	Not reported
				H526D (445)	Direct	Indirect	High
				H526R (445)	Indirect	Indirect	High
				H526L (445)	Indirect	Indirect	High
				H526Q (445)	Indirect	Indirect	Not reported
				H526C (445)	Indirect	Indirect	High
				H526N (445)	Indirect	Indirect	Minimal
				H526P (445)	Indirect	Indirect	Moderate
				H526Y (445)	Direct	Indirect	High
				S531W (450)	Indirect	Indirect	High
				S531L (450)	Direct	Indirect	High
				S531Q (450)	Indirect	Indirect	High
				S531C (450)	Indirect	Indirect	Not reported
				L533P (452)	Indirect	Indirect	Moderate
Tessema, 2017 [17]	Whole genome sequencing		*rpoB*	S531L (450)	Direct	Indirect	High
				D516V (435)	Direct	Indirect	High
Galarza, 2016 [20]	HRM DNA sequencing	7H10	*rpoB*	S531L (450)	Direct	Indirect	High
				D516V (435)	Direct	Indirect	High
				H526D (445)	Direct	Indirect	High
				Q513L (432)	Indirect	Indirect	High
				Q513P (432)	Indirect	Indirect	High
				M515I (434)	Indirect	Indirect	High
				D516G (435)	Indirect	Indirect	High
				D516Y (435)	Indirect	Indirect	Moderate
				N518H (437)	Indirect	Indirect	Not reported
				H526R (445)	Indirect	Indirect	High
				H526Y (445)	Direct	Indirect	High
				S531F (450)	Indirect	Indirect	High
Galarza, 2014 [21]	DNA sequencing	7H10	*rpoB*	D516V (435)	Direct	Indirect	High

PCR: polymerase chain reaction; HRM: high-resolution melting assays; MGIT: mycobacterial growth indicator tube.
** The number of the codon used in Miotto's classification is reported in parenthesis [26].
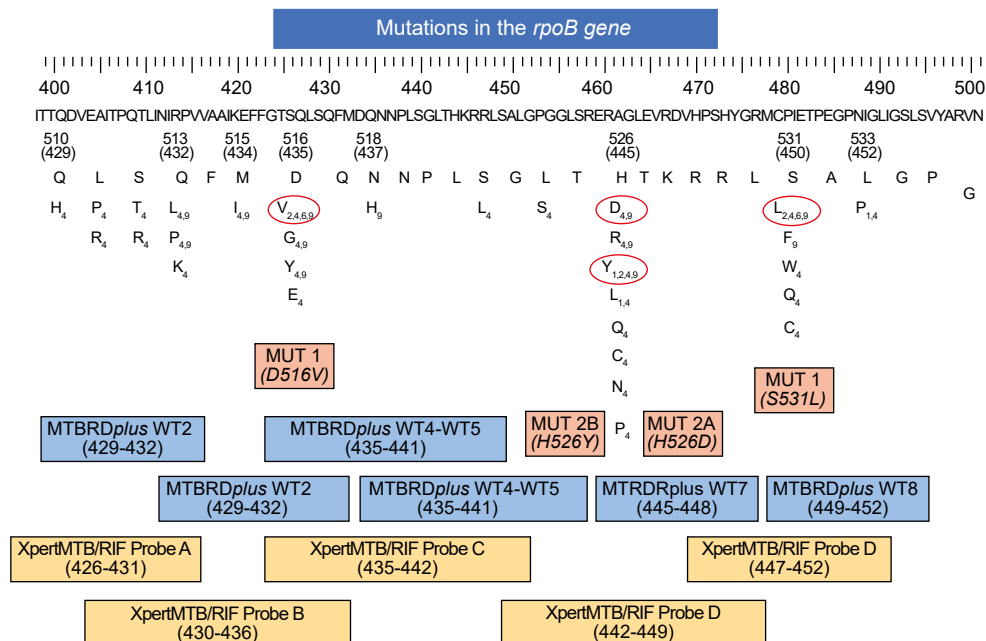
Figure 3. Nucleotide level mutations reported for the *rpoB* gene

The small numbers show the studies where these mutations were reported according to Table 1. The blue and red boxes show the mutation reported by the GenoType MTBDRplus® v2.0 methodology and the yellow boxes, the ones reported by the Xpert® MTB/RIF methodology. The red circles show mutations that are reported by the GenoType MTBDRplus® v2.0 test directly.

resistance). For rifampicin, of the 31 mutations found, 21 have already been confirmed as associated with resistance (15 with high, four with moderate, and two with minimal confidence for association with resistance). Additionally, only one mutation associated with resistance to ethambutol, one to streptomycin, and 48 to pyrazinamide —a drug on which a significant number of research studies have been conducted— were found. In addition to the objectives of the study, we were able to identify three mutations associated with aminoglycoside resistance and five with quinolone resistance.

This denotes an important effort from the tuberculosis research groups present in our country, many of which have been very active during the last decades, producing studies to deepen the knowledge of the epidemiology, clinical and microbiology of tuberculosis in Peru. In recent years, the branch that has gained the most relevance is molecular biology, which works to build the foundations not only for new diagnostic methodologies by detecting mycobacterial genetic material, but also for the development of new drugs [26].

It is important to point out that, as part of the control of drug-resistance in tuberculosis, the priority must be the identification of resistance to the two pillars of treatment:

isoniazid and rifampicin, which is why these drugs were the focus of the study. For detection of rifampicin resistance, commercially available and recommended tests are: GenoType MTBDRplus® v2.0 line probe assay and Xpert® MTB/RIF. Both tests detect mutations in the "Rifampicin Resistance-Determining Region" (RRDR) using DNA probes that bind specifically to that region. A comparative study carried out in 2014 between GenoType MTBDRplus® v2.0 and Xpert® MTB/RIF determined the superiority of the former for the detection of rifampicin mono-resistant *M. tuberculosis* in terms of sensitivity [27]. Furthermore, isoniazid resistance can only be detected using the GenoType MTBDRplus® v2.0 test taking into consideration two genes associated with resistance: the *katG* gene and the promoter region of *inhA* gene. The Xpert® MTB/RIF test does not detect this resistance.

At the *katG* gene level, codon 315 was the only position that evidenced the appearance of two mutations highly associated with isoniazid resistance: S315T and S315N [9,15]. When evaluating the mutations detected by GenoType MTBDRplus® v2.0 (Figure 2) we can see that only the S315T mutation is directly identified, whereas the S315N mutation [15] is indirectly identified. Similarly, when comparing the information collected on mutations found

in the inhA gene by the GenoType MTBDRplus® v2.0 test, the mutation present in the g-17t nucleotide position is indirectly identified. S315N and g-17t mutations in the *katG* and *inhA* genes, respectively, are not detected directly by the GenoType MTBDRplus® v2.0 test, but indirectly by the absence of some wild type band. On the other hand, the G269S mutation of the *kasA* gene is not analyzed by this molecular test.

With respect to the mutations reported in the *rpoB* gene, in general, all mutations can be detected by molecular tests GenoType MTBDRplus® v2.0 and Xpert® MTB/RIF. In addition to the mutations usually associated with resistance, mutations L511R, D516E, N518H, Q510H, S512R, L524S, H526Q and S531C were identified [15, 20], which are not reported in the WHO technical guide [9]. These mutations D516E, D516G, D516Y, del507, H526C, H526L, H526N, H526P, H526Q, H526R, L511P, L511R, L524S, L533P, M515I, N516H, Q510H, Q513K, Q513L, Q513P, S512T, S512R, S522L, S531C, S531F, S531Q, S531W are not defined in the GenoType MTBDRplus® v2.0 mutation bands; however, they are detected indirectly by this methodology, due to the absence of wild type bands covering the respective codons.

Although some mutations are not directly determined by the GenoType MTBDRplus® v2.0 test for both isoniazid and rifampicin, they will be reported as inferred resistance due to the absence of wild type bands. Something similar occurs with the Xpert® MTB/RIF test, as it works with wild probes and does not identify specific mutations [28]. The Xpert® MTB/RIF hybridization probes A, B, C, D, and E indirectly identify all rifampicin mutations reported in this systematic review [29,30].

Another important finding is the large number of mutations conferring resistance to pyrazinamide (Annex 3). Microbiological detection of resistance to this drug has always been a challenge [26], and, to date, the most widely used method is Wayne's [31]. The fact that there are a large number of mutations associated with pyrazinamide resistance makes it difficult to develop molecular methods of resistance detection.

In 2018, the use of whole genome sequencing for the detection of resistance-associated mutations in *M. tuberculosis* was recommended by WHO for implementation in national reference laboratories [9]. Because of this, the use of whole genome sequencing of *M. tuberculosis*—under usual or routine conditions—will provide more complete information about the significance of mutations for the identification of mycobacterial species, detection

of anti-tuberculosis drug resistance, and the dynamics of microorganisms' transmission [32, 33].

As part of the strengths of our study, it should be noted that there are only few systematic reviews on molecular biology issues, and specifically on resistance to anti-tuberculosis drugs. Previous studies have been published, such as the one by Vásquez-Loarte [34]; but they are more focused on the association of polymorphisms to evaluate the phylogenetic origin of *M. tuberculosis.* However, our study specifically addresses the issue of mutations existing in the strains which circulate in our country. This may be more useful from a practical point of view, since this information gathers data on the mutations that are present in Peruvian strains to date. On the other hand, it can serve as a basis for the development of tests to detect resistance to anti-tuberculosis drugs in our country, considering high-prevalence mutations at the global level, as well as those that are not yet included in commercial diagnostic devices.

We must also point out some aspects of the study that limit its scope. First, because not all publications recorded mutation frequencies, it was not possible to determine the mutations that are most prevalent in our territory. We can only mention that they are present. On the other hand, despite our effort to verify, according to WHO recommendations, whether mutations conferred minimal, moderate or high confidence for association with resistance, or were not associated with resistance, there were numerous mutations that could not be classified. Therefore, the clinical implications of the latter are uncertain for now, until more studies are conducted and more evidence on their impact is generated.

In conclusion, there is a wide variety of mutations associated with resistance to first-line anti-tuberculosis drugs in peruvian patients. For the drugs isoniazid and rifampicin, the mainstays of anti-tuberculosis treatment, the number of resistance-associated mutations is lower compared to pyrazinamide. A positive aspect is that the vast majority of mutations for both isoniazid and rifampicin are directly or indirectly identified (by the absence of wild type probe hybridization) by the GenoType MTBDRplus® v2.0 and Xpert® MTB/RIF tests. These considerations must be taken into account for the development of diagnostic devices to be used locally in our country.

the data, the writing of the article, and the approval of the final version. All authors claim responsibility for the study.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

Supplementary Material: Available in the electronic version of the RPMESP.

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