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Genetic diversity and drug resistance of *Mycobacterium tuberculosis* in Ecuador

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SUMMARY

BACKGROUND: The genetic diversity of *Mycobacterium tuberculosis* in Quito, Ecuador is not well known.
OBJECTIVE: To investigate mutations related to drug resistance and bacterial genotypes in *M. tuberculosis* strains in Ecuador.
DESIGN: This was a retrospective study of *M. tuberculosis* isolates from 104 patients. Isolates were phenotypically resistant to rifampicin (RMP) and/or isoniazid (INH). The genotype was determined using 24-locus mycobacterial interspersed repetitive units-variable-number tandem repeats (MIRU-VNTR).
RESULTS: Isolates showed mutations in the *rpo*B and *kat*G genes, and the *inh*A promoter. In *rpo*B, we found 13 genetic alterations at codons 511, 513, 514, 515, 516, 526 and 531. Forty-six (44.2%) RMP-resistant isolates belonged to codon 531. In *kat*G, there were nine genetic alterations at codons 296, 312, 314, 315, 322, 324 and 351. Fifty-three (51%) INH-resistant isolates belonged to codon 315. Five mutations not previously described were identified in *kat*G: Thr324Ser, Thr314Ala, Ala312Pro, Trp351Stop and deleted G at 296 codon. The Latin American Mediterranean (LAM) (33.7%) and Ghana (30.8%) lineages presented most of the main mutations observed.
CONCLUSION: This is the first report from Ecuador; it describes five new mutations in *kat*G and indicates that LAM is the most prevalent lineage.
KEY WORDS: genotypes; *Mycobacterium tuberculosis*; Ecuador

ACCORDING TO THE World Health Organization (WHO), there were more than 10 million new cases of tuberculosis (TB) and 1.7 million of deaths in 2016, and half a million patients with the disease were resistant to treatment.[1] Of the estimated number of incident cases in 2016, 3% occurred in the WHO Region of the Americas. In the same year, Ecuador (population ~16 million) had a TB incidence of 8200, at a rate of 50 per 100 000 population. The mortality rate was 2.4/100 000, and the incidence of multidrug-resistant TB (MDR-TB) was 4.4/100 000.[1,2]

Molecular profiles of *Mycobacterium tuberculosis* may be used to describe the global spread of different *M. tuberculosis* lineages; these allow us to understand the dynamics of transmission of circulating clones locally and internationally.[3] The genotyping method based on mycobacterial interspersed repetitive units-variable number tandem repeat (MIRU-VNTR), which is highly discriminatory and reproducible, is the 'gold standard'.[4] The repetitive units have a length of 40–100 base pairs (bp) and are located in 41 loci scattered throughout the genome of the H37Rv strain of *M. tuberculosis*.[5]

Previously analysed Latin American isolates have shown the presence of the Mediterranean family and the Haarlem family, both from Euro-American lineages, in the region.[6–8] Population genetics in *M. tuberculosis* epidemiology have been well described in several countries of the region; however, there is little information on the genetic diversity of *M. tuberculosis* in Ecuador.[9]

To address this lack of information about the genetic basis of MDR-TB in Ecuador, we identified the main lineages and most common mutations associated with MDR-TB in clinical isolates from Ecuador.

MATERIALS AND METHODS

Selection of clinical isolates

A total of 5779 cultures were performed using the BACTEC™ MGIT™ 960 system (BD, Sparks, MD, USA) between January 2002 and December 2014. All testing was performed in a routine diagnostic Tuberculosis Laboratory in Vozandes Hospital, Quito, Ecuador. Of these, 750 (13.0%) isolates from extra-pulmonary and pulmonary samples were pos-

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Table 1 PCR primers for *rpo*B and *kat*G, and the *inh*A promoter

Gene	Sequences (5′-3′)	Size bp	Annealing temperature	Resistance	Reference
*rpo*B	*rpo*B-F: AGGACGTGGAGGCGATCA *rpo*B-R: GGTTTCGATCGGGCACAT	245	58°C	RMP	10
*kat*G	*kat*G-F: TGGCCGCGGCGGTCGACATT *kat*G-R: GGTCAGTGGCCAGCATCGTC	420	62°C	INH	10
*inh*A promoter	*inh*A-P-F: CCTCGCTGCCCAGAAAGGGA *inh*A-P-R: ATCCCCCGGTTTCCTCCGGT	249	60°C	INH	10

PCR = polymerase chain reaction; bp = base pair; RMP = rifampicin; INH = isoniazid.

itive for *M. tuberculosis*. All isolated strains were maintained at –80°C in a strain bank. We re-cultured 130 consecutive non-repetitive *M. tuberculosis* isolates in Löwenstein-Jensen solid medium (35°C, 5% carbon dioxide) for 8 weeks. These isolates were resistant to isoniazid (INH) or rifampicin (RMP) or both (critical concentrations used: INH 0.1 μg/ml and RMP 1.0 μg/ml) on MGIT 960; second-line drug susceptibility testing (DST) was performed in only some isolates (data not shown). Of the 130 isolates, 26 specimens were excluded from the study (10 were contaminated, 16 failed to grow). Isolates resistant to INH and/or RMP were confirmed to be *M. tuberculosis* using Sanger sequencing of the *rrs* rRNA gene performed at Macrogen (Seoul, South Korea).

Polymerase chain reaction amplification and sequencing

Genomic DNA was extracted using the chemical lysis plus column-based method with the High Pure PCR Template Preparation Kit (Roche, Basel, Switzerland) according to manufacturer specifications. We analysed the nucleotide positions 2073–2318 of the RMP resistance determining region (RRDR) of *rpo*B. In *kat*G, the nucleotide regions studied were at positions 725–1144, and in the *inh*A promoter the region was between –168 and 81. Polymerase chain reaction (PCR) amplifications were achieved using the primers and cycling conditions given in Table 1.[10] The H37Rv strain was used as the amplification control in all PCRs. The amplification products were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) in accordance with manufacturer specifications. Sanger sequencing was performed at Macrogen. The sequences were aligned and analysed using Geneious Pro 5.6.4 (Biomatters, Auckland, New Zealand). The GenBank accession number NC_000962 sequence, described as the H37Rv strain, was used as the control in all aligned analyses. All mutations found in the *rpo*B and the *kat*G genes and the *inh*A promoter were compared based on the TB Drug Resistance Mutation Database (https://tbdreamdb.ki.se/Info/) and the comprehensive resistance gene mutation analysis undertaken by Miotto et al.[11]

Molecular typing method

MIRU-VNTR genotyping was performed using PCR amplification of 24 loci according to the procedure described by Supply et al.[12] Amplicons were evaluated in 3% agarose gel and sized with ImageLab v 4.1 (Bio-Rad Laboratories, Hercules, CA, USA). A numerical value profile was assigned to each strain according to the number of repeats of each VNTR allele. To determine the *M. tuberculosis* strain lineages, 24-locus MIRU-VNTR profiles were analysed in the open access database MIRU-VNTR*plus*.[13] An unweighted pair group method with arithmetic mean (UPGMA) dendrogram was constructed with the MIRU-VNTR*plus* web application using a categorical measure of genetic distance.

Statistical analyses

We calculated the Hunter-Gaston Index (HGDI) of MIRU-VNTR types, as described elsewhere.[14] A cluster was defined as two or more isolates sharing the same MIRU-VNTR genotypic profile. The clustering rate was defined as $(nc–C)/N$, where nc is the total number of clustered cases, C the number of clusters and N the total number of cases in the sample.[14]

Ethics statement

The study protocol was approved by the Ethics Committee of Pontificia Universidad Católica del Ecuador, Quito (Document CEI: 026-2015). Permission to conduct the study at the Hospital Vozandes, Quito, Ecuador (DINVES 2013-0014) was also obtained.

RESULTS

Bacterial strains and drug susceptibility testing

According to the DST records of Hospital Vozandes, of the 104 strains included in this study, 80/104 (73.9%) were RMP-resistant (7/80 strains were RMP-monoresistant), 97/104 (93.3%) were INH-resistant (24/97 strains were INH-monoresistant); 73/104 (70.2%) isolates were categorised as MDR-TB (i.e., resistant to RMP and INH). Hence, between 2002 and 2014, the incidence of MDR-TB in this hospital was 9.7% (73/750 isolates).

Table 2 Mutations found in *M. tuberculosis* isolated from an Ecuadorian population

Gene	Codon	Base mutation	Amino acid change	Strains *n*	Number of resistant strains (number of MDR-TB strains)	Lineages (number of resistant strains)	Susceptible strains *n*
*rpo*B	None	None	None	30	6 (5)	LAM (2), Ghana (1), Haarlem (1), Cameroon (1), Orphan (1)	24
*rpo*B	531	TCG→TTG	Ser→Leu	46	46 (42)	Ghana (18), LAM (16), Haarlem (1), Cameroon (1), S (1), Beijing (1), Orphan (8)	0
*rpo*B	515/516	ATG→ATC GAC→TAC	Met→Ile Asp→Tyr	3	3 (3)	Ghana (2), Orphan (1)	0
*rpo*B	513	CAA→CTA	Gln→Leu	1	1 (1)	Orphan (1)	0
*rpo*B	526	CAC→AGC	His→Ser	2	2 (2)	LAM (1), Orphan (1)	0
*rpo*B	526	CAC→CAA	His→Gln	1	1 (1)	LAM (1)	0
*rpo*B	526	CAC→CGC	His→Arg	3	3 (3)	LAM (1), Orphan (2)	0
*rpo*B	526	CAC→CTC	His→Leu	5	5 (4)	LAM (3), Ghana (1), Orphan (1)	0
*rpo*B	526	CAC→TAC	His→Tyr	4	4 (3)	LAM (2), Ghana (1), Beijing (1)	0
*rpo*B	526	CAC→GAC	His→Asp	3	3 (3)	LAM (1), Ghana (1), Orphan (1)	0
*rpo*B	516	GAC→GTC	Asp→Val	4	4 (4)	Ghana (2), Cameroon (1), Orphan (1)	0
*rpo*B	514	TTC→TTT	Phe→Phe (synonymous)	1	1 (1)	Orphan (1)	0
*rpo*B	511	CTG→CCG	Leu→Pro	1	1 (1)	Ghana (1)	0
Total				104	80 (73)		24
*kat*G	None	None	None	41	34 (26)	LAM (14), Ghana (7), Cameroon (2), Haarlem (2), S (2), Orphan (7)	7
*kat*G	314	ACC→GCC	Thr→Ala*	2	2 (2)	Ghana (2)	0
*kat*G	324	ACC→TCC	Thr→Ser*	1	1 (1)	Ghana (1)	0
*kat*G	322	ACG→GCG	Thr→Ala	1	1 (0)	S (1)	0
*kat*G	315	AGC→ACA	Ser→Thr	2	2 (2)	LAM (2)	0
*kat*G	315	AGC→ACC	Ser→Thr	53	53 (38)	Ghana (21), LAM (17), S (3), Haarlem (1), Beijing (1), Orphan (10)	0
*kat*G	315	AGC→GGC	Ser→Gly	1	1 (1)	LAM (1)	0
*kat*G	296	del G	Frameshift*	1	1 (1)	Orphan (1)	0
*kat*G	312	GCG→CCG	Ala→Pro*	1	1 (1)	Orphan (1)	0
*kat*G	351	TGG→TGA	Trp→stop*	1	1 (1)	Cameroon (1)	0
Total				104	97 (73)		7
*inh*A promoter		None		83	76 (56)	LAM (27), Ghana (25), S (6), Haarlem (2), Cameron (1), Orphan (15)	7
*inh*A promoter		C(-15)T		20	20 (16)	LAM (6), Ghana (6), Cameroon (2), Haarlem (1), Beijing (1), Orphan (4)	0
*inh*A promoter		C(-9)T		1	1 (1)	LAM (1)	0
Total				104	97 (73)		7

* Mutations not previously described.
MDR-TB = multidrug-resistant tuberculosis; LAM = Latin American Mediterranean.

Genetic mutations associated with drug resistance in M. tuberculosis

Mutations in *rpo*B were observed in 74/80 (92.5%) of RMP-resistant isolates. In six (7.5%) strains that were phenotypically RMP-resistant, mutations in *rpoB* could not be identified. Mutations in *katG* were observed in 63/97 (65%) of INH-resistant isolates. Mutations in the *inhA* promoter were observed in 21/97 (21.6%) of INH-resistant isolates; 4/97 (4.1%) INH-resistant isolates had a mutation in the *inhA* promoter and *kat*G; 17/97 (17.5%) INH-resistant isolates did not have any mutations in *kat*G or the *inh*A promoter.

Twelve types of missense mutations and one synonymous mutation were identified in *rpo*B. These mutations involved the codons 511, 513, 514, 515, 516, 526 and 531. The most prevalent mutation was the Ser531Leu substitution, present in 46/74 isolates (62.2%), of which 42 isolates were MDR-TB. The next most prevalent mutation was the His526Leu modification present in 5/74 isolates (6.8%), 4 of which were MDR-TB. Three (4.1%) isolates presented the double mutation, Met515Ile/Asp516Tyr. We identified six different mutations in codon 526. The synonymous mutation was Phe514Phe (TTC→TTT) (Table 2).

We registered nine different mutations in *kat*G, involving codons 296, 312, 314, 315, 322, 324 and 351. The most prevalent change was Ser315Thr, present in 53/63 (84.1%) isolates. The 315 codon also presented two additional mutation types. We identified five mutations in *kat*G not previously

described: Thr314Ala (GenBank Accession number KP732540), Thr324Ser (GenBank Accession number KP732539), Ala312Pro (GenBank Accession number KU669031), a deleted G at codon 296 and the change of Trp to a Stop codon at 351 (Table 2). All isolates with *kat*G mutations that had not been previously described were MDR-TB.

We found two mutations in the *inhA* promoter. The mutation [C(-15)T] was present in 20/21 INH-resistant isolates (95.2%), including 16 isolates that were MDR-TB; the mutation [C(-9)T] was found in 1/21 (4.8%) INH-resistant isolates (Table 2).

MIRU-VNTR genotype profiles of the M. tuberculosis *isolates*

We applied the 24-locus MIRU-VNTR panel approach to all isolates included in this study. All samples tested could provide information in each locus (Figure 1). Six different genotypes were found: LAM (35/104, 33.7%) and Ghana (32/104, 30.8%), followed by S-type (6/104, 5.8%), Cameroon (4/104, 3.8%), Haarlem (4/104, 3.8%) and Beijing (2/104, 1.9%). Twenty-one genotypes (20.2%) could not be identified in the database MIRU-VNTR*plus* web, and these strains were classified as orphan types.

The UPGMA tree based on 24-locus MIRU-VNTR showed 103 different VNTR genotypes. Two isolates were grouped to form one cluster. These two strains (612 and 617) belonged to the Ghana lineage and were MDR-TB; also, both strains had the Ser531Leu mutation in *rpo*B and the C(-15)T mutation in the *inh*A promoter. These were isolated from sputum cultures of two patients from Santo Domingo de los Tsachilas, a city located 133 km west of Quito; the patients were not related. The HDGI was 0.999, and the clustering rate was 0.98% (Figure 1).

Association between the genotype and drug resistance profiles of M. tuberculosis *strains*

Drug-resistant isolates in the main lineages found in our study were LAM, 26/35 MDR-TB (74.3%), followed by Ghana, 26/32 MDR-TB (81.2%) and orphans, 16/21 (76.2%) MDR-TB (16.8%) (Figure 2). INH-monoresistant strains were observed in 8/35 (22.9%) LAM, 5/32 (15.6%) Ghana and 5/6 (83.3%) S-type strains. RMP-monoresistant strains were present in one isolate in all lineages, except for S-type lineages. The two Beijing lineage strains presented one MDR-TB and one RMP-monoresistant strain (Figure 2). The major lineages found in the most prevalent mutation in *rpo*B, the Ser531Leu mutation, were Ghana in 18/32 (56.2%) isolates (all of these isolates were MDR-TB) and LAM in 16/35 (45.7%) isolates (15 MDR-TB). In the most prevalent *kat*G mutation, Ghana was the most common lineage, with 21/32 (65.6%) isolates (18 MDR-TB), followed by LAM with 17/35 (48.6%) isolates (12 MDR-TB) (Table 1). The two Beijing isolates presented different mutation profiles (Table 2 and Figure 1).

Mutations not previously described belonged to the Ghana (Thr314Ala with two isolates and Thr324Ser with one), Cameroon (Trp to a Stop codon at 351) and orphan (Ala312Pro with one isolate and a deleted G at 296 with one isolate) lineages. All these isolates were MDR-TB (Figure 1).

DISCUSSION

RMP is one of the principal first-line drugs used in combination chemotherapy; RMP resistance is a valuable surrogate marker for MDR-TB. Mutations at codons Ser-531 and His-526 in the hypervariable region of 81 bp of *rpo*B are the most frequent, and their relative frequency is very similar in different geographical regions.[13,15] The variations found at codons 531, 515, 516 and 526 comprised 96% of RMP-resistant strains. Our findings are in line with those from studies carried out in other countries, which reported that >96% of RMP-resistant *M. tuberculosis* strains have mutations in a hypervariable region of *rpo*B and that 70% of these mutations occur at codons 531 and 526.[15,16]

In this study, 6/74 (8.1%) RMP-resistant strains had no mutations in *rpo*B. RMP-resistant isolates were associated with mutations in the *rpo*B gene in almost all the cases, with 95% of the strains located in the RRDR region.[17] Telenti et al. did not observe mutations in the RRDR region of *rpo*B in 2/66 (3%) RMP-resistant isolates.[18] Williams et al. found that 7% of RMP-resistant *M. tuberculosis* strains did not have mutations in the RRDR region of *rpo*B.[16] A low-frequency mutation found outside the RRDR is the 1491F mutation. Rukasha et al. reported three RMP-resistant strains with mutations outside the RRDR region of *rpoB*.[19]

It is important to consider the possibility of alternative resistance mechanisms, such as efflux pumps, which are responsible for eliminating a large variety of cytoplasmic compounds from bacteria. The efflux pumps that are involved in resistant *M. tuberculosis* are Rv1258c, Rv1410c, Rv1819c and Rv2136c.[20]

INH is one of the most active compounds used to treat TB worldwide. INH has also been used as a prophylactic drug for individuals with latent tuberculous infection to prevent active TB.[21] In the present study, of 97 INH-resistant isolates, 63 (64.9%) presented a mutation in *kat*G and 21/97 (21.6%) in the *inh*A promoter. The mutation most prevalent in *kat*G was found to be Ser315Thr (84.1%), which is also the most frequently described worldwide,[22] followed by the C(-15)T mutation in the *inhA* promoter in 20/97 (20.2%) isolates. No mutations were found in *kat*G or the *inh*A promoter in 17/97 (17.5%) INH-resistant strains. Other mutations, such

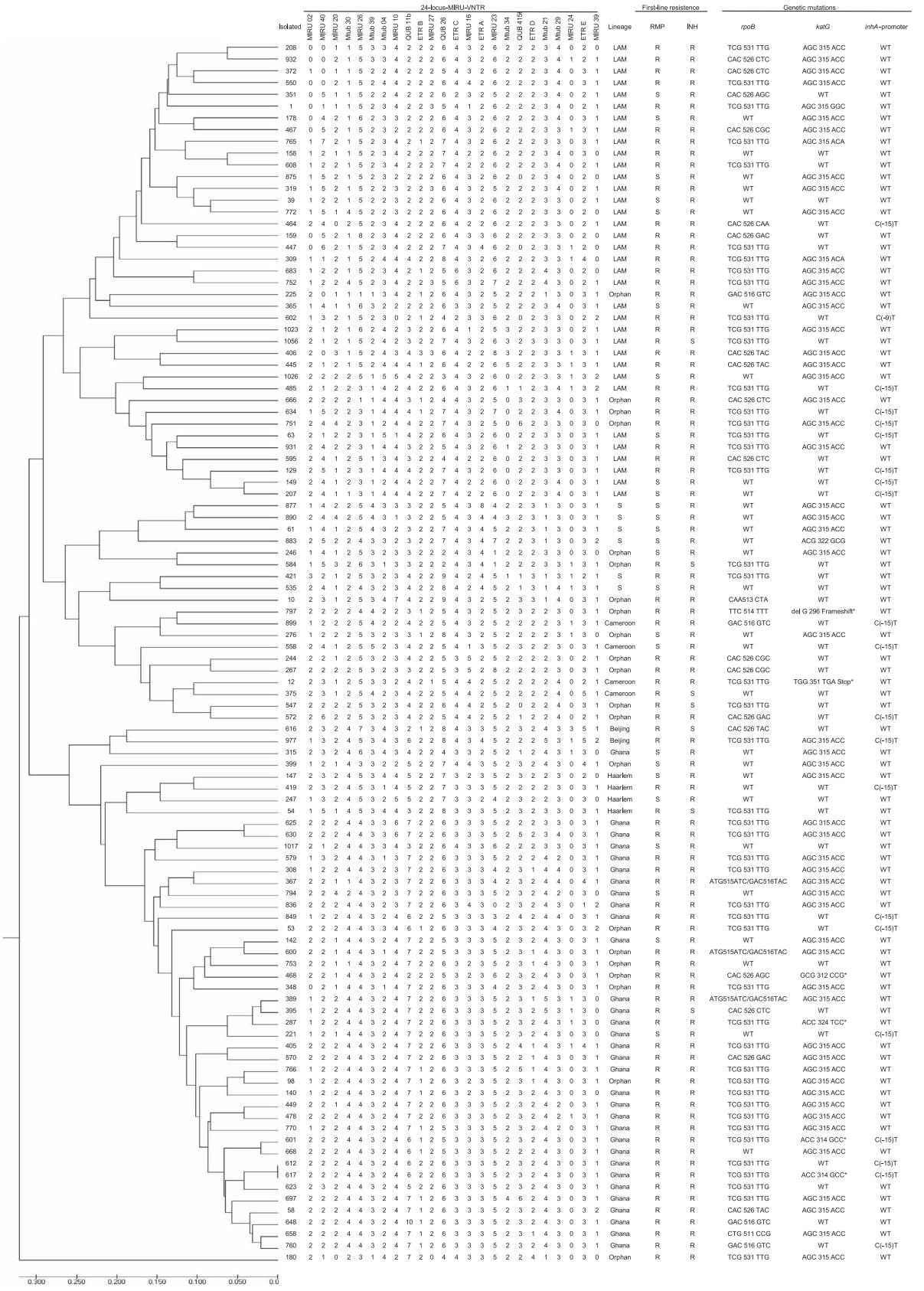

Figure 1 UPGMA dendogram based on 24-locus MIRU-VNTR analysis of Ecuadorian *M. tuberculosis* strains. * New mutation found in *kat*G. MIRU = mycobacterial interspersed repetitive unit; VNTR = variable number of tandem repeats; RMP = rifampicin; INH = isoniazid; R = resistant; WT = wild type; S = susceptible; UPGMA = unweighted pair group method with arithmetic mean.

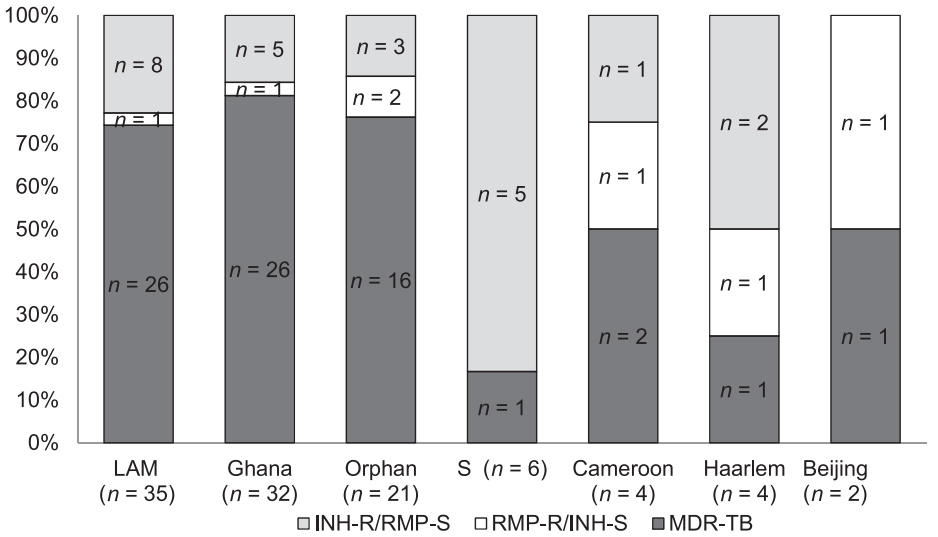

Figure 2 Phenotypic resistance according to *M. tuberculosis* lineage in Ecuador. LAM = Latin American Mediterranean; INH-R = isoniazid-resistant; RMP-S = rifampicin-susceptible; RMP-R = rifampicin-resistant; INH-S = isoniazid-susceptible; MDR-TB = multidrug-resistant tuberculosis.

as *ahp*C, *kas*A, *ndh*, *ini*ABC, *fad*E, *fur*A, Rv1592c and Rv1772, together with the recent association of efflux genes that may have been involved in INH resistance here, were not searched.[23]

We found five mutations in the TB Drug Resistance Mutation Database and in the comprehensive resistance gene mutation analysis by Miotto et al. that had not been previously reported.[11,13] Further analysis of the enzymatic activity of the new *kat*G mutants is needed to understand the true effect of the novel *kat*G mutations in their ability to cause INH resistance.[24]

Although experimental data support the hypothesis that the mutations that cause RMP resistance can reduce the transcriptional capacity of mutants,[25] the reason why RMP-resistant strains are so common is not known. However, we found more INH- than RMP-resistant strains (97 vs. 77). This observation may be explained by the mathematical model suggested by Lipsitch and Levin,[26] in which the existence of different *M. tuberculosis* compartments in different metabolic states (and therefore, with differing susceptibility to drugs) could explain the emergence of mutants resistant to a particular drug and, in later stages, of secondary resistance. In our study, resistance to INH (the most potent anti-tuberculosis drug) emerged and became predominant if there was poor treatment adherence.[26]

In Ecuador, TB incidence decreased from 132/100 000 in 1990 to 50/100 000 in 2016.[1] This was probably due to the introduction of two programmes: the DOTS strategy started in 2001, and a robust and well-coordinated National Tuberculosis Programme started in 2011, covered by government funds, with subsidised medications and a monetary incentive for TB patients. However, despite the efforts made, the rate of loss to follow-up of the anti-tuberculosis treatment is currently 12%, which could explain in part the higher rate of INH than RMP resistance observed, in addition to other factors.[27,28]

Phylogenetic analyses of *M. tuberculosis* strains led to identification of six main lineages, which suggests that the genetic background plays a role in the appearance and spread of MDR-TB.[29–31] Different phylogenetic lineages of *M. tuberculosis* have been associated with different types of drug resistance.[30] The relatively low incidence of MDR-TB strains (9.7%) identified at Vozandes Hospital, compared with the high incidence of MDR-TB 'hotspot' regions such as China, Russia and India, might be related to the regional distribution of *M. tuberculosis* lineages.[1,32] Ford et al. measured the frequency of resistance in isolates belonging to Lineage 2 (which includes the Beijing family) and Lineage 4 (which includes LAM, Ghana, Cameroon, S and Haarlem) and demonstrated that Lineage 2 strains had a higher rate of acquired resistance to RMP and INH than Lineage 4 strains.[33] Lineage 2 is recognised as the main lineage found in large parts of Asia, whereas Lineage 4 is mainly described in the Latin American region.[6–8,32] Further analysis is needed 1) to elucidate the rate of acquired resistance in Lineage 4; and 2) to understand the possible relationship between the novel *kat*G mutations, Thr314Ala and Thr324Ser, and the Ghana lineage. In addition, strains harbouring identical resistance mutations to RMP but belonging to different *M. tuberculosis* lineages might show different levels of fitness-cost.[34] As only susceptible *M. tuberculosis* strains were assessed here, this observation is pending verification.

Research on the distribution of the genotypic *M. tuberculosis* lineage in South America has shown that, although the Euro-American Lineage 4 is the

most widely represented, regional differences in lineage/sublineage distribution are frequently observed in some countries.[6,7,35] The LAM, Haarlem and T families are the most commonly observed members of the Euro-American lineage in South and Central America, apart from the Caribbean, a distribution profile shared with Europe and Central Africa.[36] However, some regional specificities exist, such as the almost exclusive presence of the Beijing family in Peru and Colombia (which is associated with MDR-TB, as well as extensively drug-resistant TB cases)[8,35] and the LAM family designated RDRio (which is associated with MDR-TB in Brazil).[37] In the Andean countries, except Bolivia,[38] the prevalent *M. tuberculosis* genotype is LAM.[7,8]

Finally, MIRU-VNTR, which is considered the gold standard for genotyping, is a reliable method for assessing epidemiological and phylogenetic studies.[39] Techniques using next-generation sequencing, such as whole-genome sequencing (WGS), have recently been gaining importance because they provide the most comprehensive source of information on the genome content of a given clinical isolate.[40] However, MIRU-VNTR remains a valuable method for *M. tuberculosis* genotyping, particularly in low-income countries, as WGS requires adequate bioinformatic support and specialised expertise, and is expensive.[39,41]

The main limitation of our study was the relatively small sample size, so statistical results should be interpreted with caution. Also, the limitations of our clinical data did not allow us to differentiate between samples from new patients and those from failures in the national health care system.

This was the first study on resistance to describe five new mutations and the distribution of lineages in *M. tuberculosis* isolates from Ecuador, and to investigate the relationship between drug resistance and genetic diversity of isolates.

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Conflicts of interest: none declared.

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RÉSUMÉ

CADRE : La diversité génétique de *Mycobacterium tuberculosis* en Quito, Equateur n'est pas bien connu.
OBJECTIF : Examiner les mutations liées à la pharmacorésistance et aux génotypes bactériens des souches de *M. tuberculosis* en Equateur.
SCHÉMA : Ceci a été une étude rétrospective des isolats de *M. tuberculosis* de 104 patients. Les isolats ont été phénotypiquement résistants à la rifampicine (RMP) et/ou à l'isoniazide (INH). Le génotype a été déterminé par la méthode des unités répétitives dispersées sur le génome mycobactérien-nombre variable de répétitions en tandem en nombre variable à 24-loci (MIRU-VNTR).
RÉSULTATS : Ces isolats ont exhibé des mutations sur les gènes *rpo*B et *kat*G et sur le promoteur d'*inh*A. Sur le gène *rpo*B, nous avons trouvé un total de 13 altérations génétiques sur les codons 511, 513, 514, 515, 516, 526 531. Quarante-six (44,2%) souches résistantes à la RMP appartiennent au codon 531. Sur le gène *kat*G, nous avons trouvé un total de neuf altérations génétiques différentes sur les codons 296, 312, 314, 315, 322, 324 et 351. Cinquante-trois (51%) des isolats résistants à l'INH appartiennent au codon 315. Cinq mutations, qui n'avaient jamais été décrites, ont été identifiées sur le gène *kat*G : Thr324Ser, Thr314Ala, Ala312Pro, Trp351Stop et délétion de G sur le codon 296. Les lignées Amérique Latine Méditerranée (LAM) (33,7%) et Ghana (30,8%) ont présenté le plus fréquemment les mutations majeures trouvées dans cette étude.
CONCLUSION : Ce rapport est le premier en Equateur qui décrit cinq nouvelles mutations du gène *kat*G et démontre que la lignée LAM est la plus prévalente.

RESUMEN

MARCO DE REFERENCIA: Diversidad genética de *Mycobacterium tuberculosis* in Quito, Ecuador, no es bien conocido.
OBJETIVO: Investigar los genotipos y las mutaciones relacionadas con farmacoresistencia en cepas de *Mycobacterium tuberculosis* en Ecuador.
DISEÑO: Este fue un estudio retrospectivo de *M. tuberculosis* aislados de 104 pacientes. Los aislamientos fueron fenotípicamente resistentes a rifampicina (RMP) y/o isoniazida (INH). El genotipo se determinó a través de repeticiones en tandem de 24 unidades loci micobacterianas intercaladas repetidas (MIRU-VNTR).
RESULTADOS: Estos aislamientos mostraron mutaciones en el gen *rpo*B y *kat*G, y el promotor *inh*A. En gen *rpo*B encontramos un total de 13 alteraciones genéticas en los codones 511, 513, 514, 515, 516, 526 y 531. Cuarenta y seis (44,2%) de los aislados resistentes a RMP pertenecen al codón 531. En el gen *kat*G encontramos un total de nueve alteraciones genéticas diferentes en los codones 296, 312, 314, 315, 322, 324 y 351. Cincuenta y tres (51%) de los aislados resistentes a INH pertenecen al codón 315. Se identificaron cinco mutaciones no descritas previamente en el gen *kat*G: Thr324Ser, Thr314Ala, Ala312Pro, Trp351Stop y deleción de G en el codón 296. Los linajes Latinoamericano y Mediterráneo (LAM) (33,7%) y Ghana (30,8%) han presentado con mayor frecuencia las principales mutaciones encontradas en este estudio.
CONCLUSIÓN: Este es el primer informe de esta naturaleza en Ecuador que describe cinco nuevas mutaciones en el gen *kat*G y demuestra que LAM es el linaje más prevalente.