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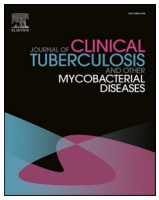

Molecular epidemiology of *Mycobacterium tuberculosis* in Ecuador: Recent advances and future challenges

Bernardo Castro-Rodriguez [a], Greta Franco-Sotomayor [b,c], Solón Alberto Orlando [b,d], Miguel Ángel Garcia-Bereguiain [a,*]

[a] One Health Research Group, Universidad de Las Américas, Quito, Ecuador
[b] Instituto Nacional de Investigación y Salud Pública, Guayaquil, Ecuador
[c] Universidad Católica Santiago de Guayaquil, Guayaquil, Ecuador
[d] Universidad Espíritu Santo, Guayaquil, Ecuador

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ABSTRACT

Tuberculosis (TB) is one of the three leading causes of death from a single infectious agent, *Mycobacterium tuberculosis* (MTB), together with COVID-19 and HIV/AIDS. This disease places a heavy burden on countries with low socio-economic development and aggravates existing inequalities. For the year 2021, estimations for Ecuador were 8500 TB cases, of which 370 were associated to multiple drug resistance (TB-MDR), and 1160 deaths. In the same year, Ecuador notified 5973 total cases, 401 of them were TB-MDR, pointing out an under diagnosis problem. The few molecular epidemiology studies available conclude that L4 is the most prevalent MTB lineage in Ecuador (with LAM as the main L4 sublineage), but L2-Beijing family is also present at low prevalence. Nevertheless, with less than 1 % MTB isolates genetically characterized by either MIRU-VNTR, spolygotyping or WGS to date, molecular epidemiology research must me improved to assist the TB surveillance and control program in Ecuador.

1. Introduction

Tuberculosis (TB) is an infectious disease that affected humans since ancient times and one of the three leading causes of death from a single infectious agent [1–4]. The main causative agent is *Mycobacterium tuberculosis* (MTB), although other species within the *Mycobacterium tuberculosis complex* can also cause TB. MTB is an acid-fast, rod-shaped, aerobic bacteria that do not form spores and spread through the air when sick individuals cough and expel the bacteria. People who live or interact in close contact with infected individuals are at high risk of developing the disease in the lungs (pulmonary TB) or any other organ (extrapulmonary TB) [4,5]. The disease places a heavy burden on low and middle income countries where the global TB estimates by the World Health Organization (WHO) show that a quarter of the world population has been infected with TB [6]. However, some countries have already reduced their TB burden below 10 cases per 100000.

For the region of the Americas, the WHO reported a decline of the TB incidence and from 2000 to 2019, a milestone in the WHO "End TB Strategy". However, this trend was reversed since 2020 due to the negative impact of COVID-19 pandemic in TB surveillance [4,7]. The epidemiology of TB in Latin America varies greatly between countries, with Brazil, Peru and Mexico reporting more than half of TB cases in the region, being considered high burden settings [8]. Moreover, the emergence of drug-resistant and multidrug-resistant (MDR) MTB strains has complicated the treatment of TB early detection drug susceptibility testing before the beginning of a therapy fundamental for an effective control and surveillance strategy [4,9].

Socio-economic and risk factors affecting vulnerable population groups like prisoners, indigenous people, homeless or migrants combined with limited access to health services are the main reasons for the high burden of TB in the Americas [4,7]. As the standard treatment recommended by WHO comprises the use of first-line anti-TB drugs (isoniazid, rifampicin, pyrazinamide, and ethambutol) and second-line anti-TB drugs (fluoroquinolones and injectable drugs) for long periods of time [10,11], loss of adherence to treatment leads to the emergence of multidrug resistance (MDR) and extensively drug resistance (XDR) TB [12]. MDR-TB is defined as failure to respond to at least rifampicin and isoniazid, while XDR-TB refers to resistance to at least one of the second

* Corresponding author.
E-mail address: magbereguiain@gmail.com (M.Á. Garcia-Bereguiain).

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Table 1
Incidence of most important MTB lineages in some countries in Latin America including Ecuador (the values in this table correspond only to studies including MTB samples collected countrywide; (*) we called the atention that values for Beijing has being reported higher for Colombia and Peru for some specific locations as it is detailed in the main text).

Country	LAM	T	Haarlem	S	X	Beijing	Other Lineages	Sample size (year of collection)	Reference
Argentina	33.2	35.9	19.5	3.2	1.5	0.2	1.1	816 (2002–2012)	[40]
Bolivia	26.3	22.2	39.4	2.0	1.0	–	–	134 (2010)	[104]
Brazil	43.6	34.9	18.3	–	0.5	0.5	2.29	218 (2008–2009)	[46]
Chile	40.6	34.1	13.5	–	3.9	0.4	0.44	458 (2011–2012)	[105]
Colombia	40.5	10	19.3	1.1	1.8	3.2*	24,2	741 (1999–2012)	[49]
Ecuador	**45.7**	–	31.8	**13.1**	**4.6**	1.1	3.9	**373 (2013–2016)**	[19]
Honduras	55.0	16.0	16.0	1.0	6.0	0.5	–	206 (1994, 1998, 2002)	[106]
Mexico	11.0	59.6	4.9	–	3.7	2.3	9.2	109 (2003)	[43]
Paraguay	52.3	8.6	18.2	9.5	0.9	0.5	–	220 (2003)	[107]
Peru	23.8	22.3	23.8		4.0	9.3*	–	323 (2004–2006)	[38]
Venezuela	53.0	10.6	5.0	1.9	1.2	0.4	0.2	1298 (1997–2006)	[52]

line drugs in addition to the MDR-TB characteristics [10]. Lack of generalized drug susceptibility testing (DST) for all TB cases in low- and middle-income countries also promotes the spread of drug resistant TB [13]. Nevertheless, DST improved the detection of MDR-TB in the Americas from 48,8% of previously treated cases in 2019 to 63 % in 2022, and from 39.8 % of new MDR-TB cases in 2019 to 58 % in 2022 [7,8]. While DST rates in the Americas improved for countries like Bahamas, Nicaragua, Suriname or Chile, others like Haiti, Colombia and Venezuela fall below the reported regional average [7]. The countries in the South American region with the highest incidence rates (per 100.000 population) of MDR-TB as of 2022 are Peru with 8.3 [14], Bolivia with 2,4 [15], Ecuador with 2.2 [16], Colombia with 2.1 [17], and Venezuela with 1.9 [18].

By molecular epidemiology of TB we referred to the methodologies that focus in genetic characterization of MTB isolates from TB patients. Molecular epidemiology of MTB has evolved from genotyping tools based on DNA amplification by polymerase chain reaction developed from the 90 s and still used (like spoligotyping or MIRU-VNTR typing) to the state of the art next generation sequencing that allows a detailed characterization of MTB genomes. Traditional epidemiology for TB surveillance is based in the patient and information for contact tracing is obtained through surveys, which always have some level of bias. However, molecular epidemiology tools switch the focus to the MTB isolates and obtained information for TB surveillance and control from the genetic characterization of the pathogen. These approach has revolutionized epidemiology not only for TB but for any infectious disease. However, those methods required highly qualified staff, equipment and permanent reagents supplies, specially for whole genome sequencing (WGS). For that reasons, molecular epidemiology tools are still a challenge for low and middle income countries, due to the budget constraints of TB surveillance programs in those settings. In this review, we analyzed the progress done in recent years in Ecuador to improve molecular epidemiology tools from MIRU-VNTR or spoligotyping to WGS and the future challenge to keep improving those methods to reach the goal of end TB.

2. Tuberculosis in Ecuador: a middle-high burden setting with high prevalence of MDR-TB

Ecuador is a 17 million people middle income country that includes multiple ethnics minorities representing more than 10 % of the population. Also, rural population represents almost 40 %. This scenario makes access to health services a challenge for may rural and indigenous communities among other [19–21]. Estimations for Ecuador by the WHO in the year 2022 were 8200 TB cases (45 per 100,000 population), of which 390 were associated to MDR-TB (2.2 per 100 000 population), and 850 deaths between HIV-positive and negative individuals. In the same year, Ecuador notified 7388 total cases, 474 of them were MDR-TB [16]. According to the last Annual TB Bulletin published by the Ecuadorian Health Ministry in 2018, the highest incidence of TB occurs in the Guayas province (55.03 %), El Oro province (7.28 %) and Los Ríos province (6.02 %), where vulnerable population include groups of low economic income and individuals with comorbidities (like HIV-positive or diabetes) [22]. The 2022 Tuberculosis profile for Ecuador reported by WHO [16] reported that TB affects mainly adults in economic productive ages, generating an impact in the national economy and the society. Therefore, Ecuador is considered by the WHO as a middle to high burden country and shows under diagnosis [16] related to limited access to diagnostic tests and insufficient contacts tracing [16,22,23]. Moreover, COVID-19 pandemic produced a decline of 17 % in the reported TB cases for 2020 when comparing to the previous year [7].

Conventional microbiological diagnosis methods based on bacilloscopy and culture are routinely performed in Ecuadorian TB reference laboratories for TB and MDR-TB screening. Nevertheless, the addition of molecular techniques like GeneXpert in combination with conventional methods as part of the TB diagnosis algorithms improved resource optimization to obtain an early diagnosis of MDR-TB [24], as observed with the increase rate of MDR-TB detection in new and previously treated TB patients for 2022 compared to previous years [7,16,25]. The occurrence of MDR strains is a complicated issue that should be addressed by establishing early TB-diagnosis and DST based in molecular methods for a fast and accurate detection of MDR-MTB strains. A successful TB control and surveillance strategy devoted to eradicate TB must be based on early detection of MDR-MTB strains and proper treatment of those patients [9,24].

3. Population structure of *Mycobacterium tuberculosis* in Latin America

TB shows a complex clinical spectrum due to the interaction of diverse factors of the host (immunological state), environment (transmission routes) and the pathogen (genetic characteristics), making the genetic analysis of *Mycobacterium tuberculosis* (MTB) critical for the identification of lineages and their association to virulence factors and resistance profiles, fostering the development of new treatments, vaccines and diagnostic tools [26].

MTB comprises a number of lineages of bacteria that share around 99.9 % of nucleotide identity: L1, East-African-Indian (EAI); L2, East-Asian (includes the Beijing family); L3, Central Asian Strain (CAS); L4, Euro-American (includes Haarlem, X, LAM, S and T); L5 and L6, West African I and II respectively; L7, Ethiopian [27–29]; and L8, East African [30]. These lineages differ in relation to their global distribution and, in some cases, their infective capability, transmission and antibiotic resistance [27,28,31–33]. MTB does not perform horizontal gene transfer nor uses plasmids in comparison to other pathogens, therefore the occurrence of chromosomal mutations are a result of deletion, duplication, insertion and single nucleotide polymorphisms (SNPs), representing the source of the genetic diversity in MTB [26,34]. These

Table 2
Population structure of MTB isolates in different Ecuadorian settings.

Reference	Sample size	Collection years	Lineages (%)													MDR (%)	MDR Lineage
			L2 Beijing	L3 Delhi	L4	L4 LAM	L4 Haarlem	L4 Ghana	L4 Cameroon	L4 S	L4 Uganda I	L4 NEW-1	L4 X	L4 T	L4 Other	Orphan		
Castro-Rodríguez et al. [71] (El Oro proivnce)	56	2012–2016	1.8		98.2	46.4	25	12.5	3.6	8.9	1.8						12	All L4
Castro-Rodríguez et al. [72] (Esmeraldas province)	105	2014–2016			100	49.5	11.4	27.6	4.8	2.9	2.9		0.95				16.3	All L4
Garcés et al. [70]	36	2016	2.8		97.2	58.2	16.7	16.7		5.6							5.6	All L4
Morey-León et al. [69]	88	2019–2021			100	44.3	11.4			11.4			23.9	5.7	3.4		59.1	All L4
Morey-León et al. [54]	21	2020			100	61.9	19			4.8			9.5	4.8			100	All L4
Garzón-Chávez et al. [19]	373	2013–2016	1.1	0.6	98.3	45.6	32.2	0.6		13.2		0.3	4.3		2.4		24.5	76/77 L4 1/77 L3
Zurita et al. [53]	104	2002–2014	1.9		98.1	33.7	3.8	30.8	3.8	5.8						20.2	70.2	56/73 L4 16/73 Orphan 1/73 L2
Jiménez et al. [68]	28	2006–2012	3.6		96.4												7.14	27/28 L4 1/28 L2

events establish a unique pattern of clonal evolution that outlines a common ancestor for the emergence of strains and lineages [26].

Although the diversity of the MTB in Latin America has been studied in several countries like Peru [35–38], Argentina [39,40], Mexico [41–43], Brazil [44–47], Colombia [48–50], Venezuela [51,52] and Ecuador [19,53,54], these studies are scarce compared to other regions. Nevertheless, it has been stablished that the most prevalent lineage is the L4 Euro-American, and within this one, the Latin American-Mediterranean sublineage (LAM) (Table 1) [55,56]; while another widespread lineage in the region is the L2, represented by the Beijing genotype [57–59].

Among the circulating strains in Latin America, the Beijing family have received special attention. Although its less frequent compared to L4 in Latin America, this lineage has a high prevalence worldwide and it is associated with antibiotic resistance by the conjunction of a series of events: higher basal mutation rate, accelerated progression to disease, increased transmission and favorable host-demographics [58,59]. Modern strains of the Beijing family are distributed in Latin America, south of Africa and east of Europe, and are associated with selective advantages to develop antibiotic resistance, hyper virulence and increased transmission [59–61]. According to the study published by Cerezo-Cortés and collaborators in 2019, the prevalence of the Beijing strain in Latin America is 3.9 % and the countries with the highest prevalence are Cuba (17.26 %), Peru (16 %) and Colombia (5 %) [59]; while in Ecuador are three reports that address the prevalence of the Beijing genotype, with values ranging from 0.43 % to 1.6 % [19,62,63].

In summary, knowing the population structure and genetic diversity of MTB strains in the countries of the Americas is critical for a better understanding of MTB transmission dynamics and MDR-MTB strains emergence and spread.

4. Population structure of *Mycobacterium tuberculosis* in Ecuador

4.1. Lineage distribution in Ecuador

Ecuador is a country with a high population diversity and strong socio-economic differences, therefore the study of the molecular epidemiology of the circulating strains facilitates the design and development of public health policies for the prevention and control of TB within different sectors of the population, especially migrants, street inhabitants, indigenous people or prisoners [7,20,64–67]. However, research studies about MTB population structure in Ecuador just started in recent years with a few research groups introducing in the country genotyping methods like MIRU-VNTR and spoligotyping, while the use of genomic sequencing still is a novel approach in the country. Table 2 and Table 3 resume the MTB genotyping research done in Ecuador.

The first study that identified Ecuadorian MTB strains at lineage level was performed in 2017 by Jiménez and collaborators. A sample size of 28 MTB isolates from two hospitals in the region of the Andes (years of collection: 2006–2012) was obtained, and 24-loci MIRU-VNTR was performed to define the population structure of this convenience sample, reporting 1/28 L2 strain (Beijing: 3.6 %) and 27/28 L4 strains. DST identified 2/28 as MDR-MTB strains, both of them belong to L4 [68]. Two years later, Zurita and collaborators performed drug-resistance and genotypic diversity assays in 104 MTB isolates collected between 2002–2014, reporting a prevalence of 33.7 % for LAM isolates, 30.8 % for Ghana, 5.8 % for S, Cameroon sublineage with 3.8 %, Haarlem with 3.8 %, Beijing was represented with 1.9 %, and 20.2 % were reported as orphan types. Drug resistance assays determined a total of 70.2 % MDR-MTB isolates, identifying L4 as the most representative lineage among all antibiotic-resistant isolates (56/73), followed by 16/73 orphan isolates and 1/73 L2-Beijing strain [53].

By 2020, Garzon-Chavez and collaborators published the only country wide and larger study about the population structure of MTB in Ecuador using 24-loci MIRU-VNTR and Regions of Difference (RD) PCR genotyping along with drug resistance information from 373 MTB

Table 3
Molecular techniques used in the analysis of population structure of MTB strains of Ecuador.

Molecular technique	Number of analyzed strains	Years of collection	Reference
24-loci MIRU-VNTR	56	2012–2016	[71]
	105	2014–2016	[72]
	373	2013–2016	[19]
	104	2002–2014	[53]
	28	2006–2012	[68]
15-loci MIRU-VNTR	36	2016	[70]
Spoligotyping	56	2012–2016	[71]
	105	2014–2016	[72]
WGS	88	2019–2021	[69]
	21	2020	[54]
	8	2013–2016	[19]

isolates (years of collection: 2013–2016). They report a prevalent transmission of L4 in Ecuador, of which LAM is the most representative sublineage with 45.6 %, followed by Haarlem with 32.2 %, S with 13.2 %, X with 4.3 %, while other L4 sublineages comprehend 3.9 %, and Beijing prevalence is of 1.1 %. DST identified 24.5 % of MDR strains, of which 1/77 belong to L3-Delhi and the rest were identified as L4. This is the only study performed with a representative sample size to understand the structure of the MTB population at a country-wide level. Interestingly, a very low clustering rate was found between the MTB isolates including in this study, suggesting a potential important role of reactivation of TB latent cases more that active MTB clusters in the transmission dynamics of MTB in Ecuador [19], as it has been suggested for certain regions in Panama [76].

On the other hand, the use of genomic sequencing methods provides a great amount of data for lineage assignation and the identification of mutations associated to resistance against anti-TB drugs. This approach was used by Morey-León et al. in 2023 to analyze the genomic information of 88 samples (years of collection: 2019–2021), most of them collected in the coastal region, while 4/88 were collected in the region of the Andes and 1/88 in the Amazon region. Isolates were identified as LAM (44.3 %), X (23.9 %), S and Haarlem (11.4 % each), T (5.7 %), and Euro-American (3.4 %). Genomic analysis identified a total of 59.1 % of MDR-MTB strains, all of them assigned to lineage L4 [69]. A second study performed by Morey-León et al. in 2022 analyzed genomic sequences from 21 MDR-MTB isolates from the coastal region of Ecuador (year of collection: 2020) for genomic analysis of drug-resistance and reported that all samples belong to lineage 4: LAM (61.9 %), Haarlem (19 %), X (9.5 %), S and T (each 4.8 %). Drug resistance analysis showed six isolates as extensively-drug resistant (XDR), 4 of these were LAM sublineage [54].

In 2023, Garcés and collaborators performed a study in one prison in Guayaquil. 36 MTB isolates collected in 2016 were analyzed by 15-loci MIRU-VNTR method to understand the structure of the MTB population, establishing a high prevalence of L4 strains: 58.2 % LAM, 16.7 % Haarlem, 16.7 % Ghana and 5.6 % S sublineages, and one sample identified as Beijing (2.8 %). A 5.6 % of the analyzed strains were identified as MDR-MTB, all of them belonged to lineage L4 [70].

Latest studies about the population structure of MTB were performed by our research team, focusing on the transmission dynamics at border areas of Ecuador-Peru and Ecuador-Colombia. The first study published in 2024 analyzed MTB isolates from the Ecuadorian province of El Oro located at the border of Ecuador and Peru. A sample size of 56 isolates (years of collection: 2012–2016) were processed to obtain 24-loci MIRU-VNTR and spoligotyping information to compare against data previously published about other provinces of Ecuador and Peru. Results identified 1/56 L2 strain (Beijing: 1.8 %) and 55/56 L4 strains (LAM: 46.4 %, Haarlem: 25 %, Ghana: 12.5 %, S: 8.9 %, Cameroon: 3.6 %, and Uganda I: 1.8 %), while the rest of MTB Ecuadorian isolates presented 4/300 L2 strains (Beijing: 1.3 %) and 296/300 L4 isolates (LAM: 44.6 %, Haarlem: 32.1 %, S: 7.1 %, Cameroon and Ghana: 5.4 % each, Uganda I: 4.7 %, and NEW-1: 0.3 %). DST performed on isolates from El Oro resulted in 12 % MDR-MTB strains, all of them belonged to lineage L4 [71]. The second study published in 2024 analyzed strains obtained from the Ecuadorian province of Esmeraldas at the border of Ecuador and Colombia. 24-loci MIRU-VNTR data and spoligotyping patterns were obtained from a collection of 105 isolates (years of collection: 2014–2016) and compared against previously published information from the other Ecuadorian provinces and Colombia. All 105 isolates from Esmeraldas were determined as L4 strains (LAM: 49.5 %, Ghana: 27.6 %, Haarlem: 11.4 %, Cameroon: 4.8 %, S: 2.9 %, Uganda I: 2.9 %, and X: 0.95 %), while the rest of isolates from Ecuador were organized as follows: 5/385 L2 strains (Beijing: 1.3 %) and 380/385 L4 strains (LAM: 44.9 %, Haarlem: 30.4 %, Ghana: 7 %, S: 6.7 %, Cameroon: 4.7 %, Uganda I: 4.4 %, NEW-1 and X: 0.26 % each). DST on isolates from Esmeraldas identified 16.3 % MDR-MTB strains, all of which were identified as L4 [72].

4.2. *Mycobacterium tuberculosis* strains associated to MDR-TB

Mycobacteria acquire resistance to all drugs used in therapies against them through spontaneous genomic mutations that propagate by replication of resistant bacteria within the host and the subsequent transmission between hosts, as mycobacteria lack the typical mechanisms available in other bacteria, like horizontal gene transfer or mobile resistance elements (plasmids) [10,73]. Strains that belong to lineages L2 and L4 are commonly associated to most historical TB outbreaks [74,75], suggesting that these lineages have genomic characteristics that improve virulence and transmission, increasing the chance to develop drug resistance that affects treatment success [73,75].

The Beijing genotype is a prevalent strain of MTB that belongs to lineage 2 and possess increased transmissibility and virulence [76,77]. As historical immigration of Chinese, Japanese and Korean individuals into Peru in the late 19th century occurred, it is currently considered as the main source of transmission of the Beijing family in the Americas; however, the high prevalence of the genotype in Buenaventura, an important Colombian seaport, it has been suggested as an entry-point of the Beijing strains from Asia [78]. In the region of the Americas, the prevalence of the Beijing genotype shows different prevalence rates from country to country under different settings. Perú and Colombia report the highest prevalence rates in the region, with values in the range of 9 % to 16.4 % [35,36,79–81] and 0.65 % to 15.6 % [48,49,82–85], respectively, while Ecuador reports a 0.43 % prevalence rate [62].

Latin American-Mediterranean (LAM) sublineage belongs to lineage

4 and is the most frequently encountered globally [29,77]. An important genotype found within this sublineage is the RD^{Rio} variant, which is associated to the development of resistance against multiple antibiotics and is distributed in many countries around the world [86–90]. In Latin America, prevalence of RD^{Rio} strains differs from country to country: from 30 % to 51.9 % in different Brazilian settings [86,91,92], 69.8 % LAM-RD^{Rio} strains in Venezuela [51], 9.6 % RD^{Rio} isolates in Colombia [48,49,56], 11.5 % RD^{Rio} strains in Peru [38,56], and Ecuador reported 2.09 % RD^{Rio} strains [108].

Although the prevalence of the RD^{Rio} and Beijing genotypes described to date is low in many countries in the region of the Americas, like Ecuador, the monitoring and surveillance of these strains is highly recommended, as both of these genotypes are strongly associated to increased transmissibility and drug resistance [109]. The highest prevalence of RD^{Rio} isolates occurs in Venezuela, while the highest prevalence of Beijing strains occurs in Peru and Colombia; therefore, the spread of these genotypes to Ecuador and other countries with open-border policies in the region should be addressed, as human migration may induce changes in the dynamics of infectious diseases in the region, especially TB [93]. Venezuelan migration in the past few years after the social and economic collapse in this country, became an important issue to consider as an increase in TB cases has been described in Venezuela due to the failure of the public health system after the dramatic economic crisis that the country is experiencing [94–96].

4.3. Population structure of MTB in border regions

Due to the lack of studies on TB transmission at the border areas of Ecuador with Peru and Colombia, in addition to the high prevalence of the MTB L2-Beijing lineage in Peru and Colombia [59], and the open-borders policy, there is a possibility of active transmission of lineages with dangerous characteristics between Ecuador and its neighboring countries. Two pioneering studies performed by Castro-Rodriguez et al. analyzed the population structure of MTB isolates from the provinces of El Oro (bordering Peru) and Esmeraldas (bordering Colombia) [71,72], and indicate the predominance of L4 strains within the provinces of El Oro and Esmeraldas, particularly the sublineages LAM, Haarlem, Cameroon, and Ghana. LAM and Haarlem sublineages exhibit a broader ecological niche, with greater geographical dispersion and an increased ability to cause disease in different human populations, while the Cameroon and Ghana sublineages show greater adaptability to their host population, limiting their geographical expansion and having reduced transmissibility, reflecting the phenotypic and genetic diversity of L4 [29]. The high genetic variability observed in the analyzed strains from the provinces of El Oro and Esmeraldas does not allow the formation of active transmission clusters with strains from other provinces in Ecuador or with strains from Colombia and Peru used in the analysis. These events may be related to the poor socio-economic conditions of these provinces, extensive rural areas with limited access to health services, and a high presence of vulnerable populations, causing new TB cases in the population to be mainly associated with the reactivation of latent TB infections rather than recent events of active transmission, as it has been described for rural regions in Panama [76].

5. Molecular and genomic typing for *Mycobacterium tuberculosis* in Ecuador: current status and future perspectives

The application of molecular biology techniques to complement the information obtained through classic epidemiology has become an important tool to understand host-pathogen dynamics, identification and determination of TB distribution patterns, and the definition of factors associated to the status of TB in a population [60,94]. Classical molecular epidemiological tools like MIRU-VNTR or spoligotyping has been widely used for more than 20 years to study the population structure and transmission dynamics of MTB in different settings [60,94,95,96,97]. Those techniques allow to identify recent chains of transmission, incidence of TB cases with identical molecular signatures, clustering and other elements associated to emerging and ongoing TB outbreaks [60]. Additionally, the rapid development of new whole genome sequencing (WGS) technologies has allowed the analysis of a massive amount of information to determine the genetic makeup of MTB strains, thus providing insight in all components associated to TB research: diagnosis, treatment, surveillance, drug resistance profiling, emergence, transmission, and evolution of specific outbreak strains [98–103]. Moreover, data derived from WGS of MTB strains has allowed the identification of molecular markers like single nucleotide polimorfisms (SNPs) that are associated to MTB families of concern like Beijing [62] or to MDR-MTB strains of great relevance for MTB dynamic in certain regional settings [110,111]. Based of those SNPs, allele-specific polymerase chain reactions protocols have been successfully implemented for an affordable tracking of MTB strains of concerns in South America [62,101,102].

6. Concluding remarks

Ecuador has made efforts in the last few years to understand the genetic structure of the circulating MTB strains by using classical molecular epidemiology tools and WGS. All published work to date is summarized in Table 3 [19,53,54,62,63,68,69]. Overall, less than 1000 MTB strains have been genotyped by MIRU-VNTR, spoligotyping or WGS, representing less than 1 % of MTB cases in the last 20 years. This limited used of either classic molecular epidemiology techniques or WGS shows a strong technological restriction and limited knowledge of the molecular epidemiology of MTB in Ecuador. Therefore, the use of of affordable and reliable molecular epidemiology tools to monitor the occurrence of dangerous lineages in Ecuador, like MIRU-VNTR and spoligotyping is strongly recommended trough the proper allocation of public investment in infrastructure, laboratory resources and technological update to establish a workflow that can be routinely used within the national TB control program. Additionally, WGS surveillance should be also progressively implemented in Ecuador. As this is still an expensive technique in the context of low and middle income country, institutional collaboration between the public heath authorities and the Academia will help to fund WGS surveillance.

7. Ethics statement

This manuscript is a review paper based on previously published information. No IRB approval is needed.

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CRediT authorship contribution statement

Bernardo Castro-Rodriguez: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Greta Franco-Sotomayor:** Writing – review & editing, Visualization, Investigation, Conceptualization. **Solón Alberto Orlando:** Writing – review & editing, Supervision, Investigation. **Miguel Ángel Garcia-Bereguiain:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Funding acquisition, 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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