UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL YACHAY

Escuela de Ciencias Biológicas e Ingeniería

ANALYSIS OF DRUG SUSCEPTIBILITY PATTERNS IN TUBERCULOSIS DIAGNOSED CASES FROM ECUADOR DURING 2012-2017

Trabajo de integración curricular presentado como requisito para la obtención del título de Biólogo

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Urcuquí, abril 2020

Urcuquí, 27 de abril de 2020

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(Vicerrectorado Académico/Cancillería)
ESCUELA DE CIENCIAS BIOLÓGICAS E INGENIERÍA
CARRERA DE BIOLOGÍA
ACTA DE DEFENSA No. UITEY-BIO-2020-00009-AD

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Dedication

To Ana and Aurora, two women in my life who I owe everything I am and all I have accomplished in my life.

To my brother Diego, for supporting me whenever I needed his help and advice.

To Cristina, Paco, and Mercy. All of them have contributed to a lot of aspects of my personal and academic life.

Alexander Paolo Vallejo Janeta

Acknowledgments

To all my professors in Yachay, all of them have taught everything that I now right now, and have made me passionate about science and research.

To my tutor, for all the support and effort for the success of this project.

To my dear friends in Yachay, thanks for all the experiences and for authentic and unconditional friendship. Especially to Ronny and Romel, who always have been there to hear me and accompany along the career.

And last but not least, special thanks to Nataly, for being the support, motivation, and inspiration in the last semester of the University, and during the process of this work.

Alexander Paolo Vallejo Janeta

ABSTRACT

Mycobacterium tuberculosis is the principal causative agent of pulmonary tuberculosis. It constitutes a serious public health problem worldwide, especially in developing countries (e.g., Ecuador) where control strategies are scarce or inefficient. Some strains of this pathogen have high virulence and have acquired multiple drug resistance to standard first- and second-line drugs. In Ecuador, the National Institute for Research in Public Health (INSPI) collects national data from tuberculosis cases and performs drug susceptibility tests to assess the different resistant profiles. In the present work, national surveillance data from INSPI was used to evaluate the prevalence and evolution of the drug susceptibility patterns and multidrug-resistant tuberculosis (MDR-TB) in the cases diagnosed from 2012 to 2017. Data were analyzed using different statistical methods, and a variety of risk factors were considered to observe their influence in the development of any drug resistance and MDR-TB. A decrease from 2012 to 2014, followed by an increasing trend during the last years (2015-2017), were observed in the emergence of any resistance, as well as higher MDR-TB prevalence than the estimates by WHO. In Ecuador, the most important factor associated with the emergence of resistant TB is the previous treatment. These analyses are crucial to allow the implementation of efficient treatments and control strategies adapted to Ecuador. This study is one of the most recent studies performed in the region to take into account the epidemiology of different resistance patterns in tuberculosis.

KEYWORDS

Mycobacterium tuberculosis, multidrug-resistant tuberculosis, public health, drug resistance, epidemiology, developing countries, Ecuador.

RESUMEN

Mycobacterium tuberculosis es el principal patógeno causante de la tuberculosis pulmonar. Este constituye un serio problema de salud pública a nivel mundial, especialmente en los países en Desarrollo (p. ej., Ecuador) donde las estrategias de control son escasas o ineficientes. Algunas cepas de este patógeno presentan alta virulencia y han adquirido resistencia a múltiples drogas estándar de primera y segunda línea. En Ecuador, el Instituto Nacional de Investigación en Salud Pública (INSPI) recolecta información sobre los casos de Tuberculosis a nivel nacional, y ejecuta pruebas de susceptibilidad a fármacos para evaluar los perfiles de resistencia. En este trabajo, datos de vigilancia nacional obtenidos del INSPI se usaron para valorar la prevalencia y la evolución de los patrones de susceptibilidad a drogas y de la Tuberculosis Multidrogoresistente (MDR-TB) en los casos diagnosticados de 2012 a 2017. Los datos fueron analizados usando diferentes métodos estadísticos, y una variedad de factores de riesgo se consideraron para observar su influencia en el desarrollo de cualquier drogorresistencia y de MDR-TB. Se observó un decrecimiento desde 2012 a 2014, seguido por una tendencia creciente durante los años posteriores (2015-2017), en la emergencia de cualquier resistencia, así como una prevalencia de MDR-TB superior a las estimaciones de la OMS. En Ecuador, el principal factor asociado a la emergencia de Tuberculosis resistente es el tratamiento previo. Este tipo de análisis son cruciales para la implementación de tratamientos eficientes y estrategias de control adaptadas al contexto ecuatoriano. El presente estudio es uno de los más recientes ejecutados en la región en los que se toma en cuenta la epidemiología de los patrones de drogorresistencia en Tuberculosis.

PALABRAS CLAVE

Mycobacterium tuberculosis, tuberculosis multidrogorresistente, salud pública, drogorresistencia, epidemiología, países en desarrollo, Ecuador.

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Introduction

Mycobacterium tuberculosis (TB) is the causative pathogen of Tuberculosis, a pulmonary disease that constitutes a serious public health issue, especially in developing countries, causing millions of deaths around the world (Pai et al., 2016; World Health Organization, 2018). It is the principal cause of death due to infectious agents, surpassing HIV/AIDS (World Health Organization, 2018). However, the distribution of TB is heterogeneous, with the developing countries having higher incidence rates (between 150-500 new cases per 100,000 population) when compared to the same parameters in developed countries (< 10 new cases per 100,000 population) (Dheda et al., 2017; Pai et al., 2016; World Health Organization, 2018). Another heterogeneity evidence is the fact that men present a higher incidence of active TB than women, with around 64% of the cases occurring in the adult male population during 2017 (World Health Organization, 2018). Furthermore, the risk of developing the disease varies along the lifetime of individuals being high in exposed infants, lower in children until ten years old, and remaining high from 25 years of age (Pai et al., 2016).

The causative agent of TB might inhabit the system of the host without clinical manifestations. In such a case, the patient has a latent TB infection (LTBI) (Pai et al., 2016; World Health Organization, 2018). The essential characteristics of this state are that the pathogen is unable to spread, but the different tests used for diagnostic may be positive (Pai et al., 2016). Around 23% of the world population was estimated to have LTBI in 2014; this percentage accounts for a prevalence estimate of 1.7 billion of patients with potential progression to active TB (Houben & Dodd, 2016). This progression can occur suddenly, which makes the LTBI population crucial for preventive treatment (World Health Organization, 2018). This prevention becomes vital when the lifetime risk of developing TB from LTBI varies between 5% and 10% of the infected individuals (Vynnycky, 2000; World Health Organization, 2018).

Regarding the progression to active TB, the risk factors become crucially important, including the role of tumor necrosis factor (TNF), inborn errors in immunity, diabetes, and HIV infection (Dheda et al., 2017; Pai et al., 2016). This last factor accounts for around 300,000 deaths (World Health Organization, 2018). Although HIV has been considered a driver of multidrug-resistance (MDR) in TB (jointly with diabetes), there is no clear relationship when socioeconomic factors are considered (Dheda et al., 2017; Eldholm et al., n.d.).

According to the World Health Organization (WHO), the mortality rate for TB is unacceptably high, displaying in their reports the same estimated number of deaths in 2012 than in 2017 with 1.3 million deaths (Organization, 2013; World Health Organization, 2018). General statistics concerning the burden of TB depends on the reliability of data provided by health institutions, which drive to the underrepresentation of cases in different countries. The principal reasons for this underrepresentation include failure in diagnostic and notification of cases by laboratories and health-staff, as well as the lack of access to health providers (World Health Organization, 2013, 2018). Even though actions have been taken to reduce this global burden, the quantities lie far from the target of the End TB Strategy. WHO defined these targets at the reduction of 90% in TB deaths and 80% in TB incidence for 2035 (milestones for 2020 set at 35% reduction in deaths and 20% in incidence) (World Health Organization, 2014, 2018).

In the Americas, the reports illustrate a breach in the TB statistics. A deficit in the number of notified and estimated cases is still present. Around 82% of estimated cases are reported, translating into a diagnosis breach of about 53,000 cases (Panamerican Health Organization, 2018). The lack of use of rapid diagnostic equipment explains this breach in the statistics. Only 13% of the cases were diagnosed using that equipment, despite its number increasing considerably in the region, although heterogeneously (Panamerican Health Organization, 2018). Moreover, this deficit is also present for the cases of HIV-associated TB (68%) (Organización Panamericana de la Salud, 2018; Panamerican Health Organization, 2018).

In Ecuador, the scenario is not favorable. The available information is not complete, and it is estimated a prevalence of 32 per 100,000 population (5,157 reported cases in 2014), considering it an upper-middle-income country (Maita-Zapata, 2018). The mentioned statistics contrast with the data from Panamerican Health Organization (PAHO), which estimates around 7,200 cases for 2018, along with other reports suggesting a higher incidence from field studies (Giacomazzi et al., 2010; Panamerican Health Organization, 2018; Romero-Sandoval et al., 2007). Regarding diagnostic equipment, Ecuador has approximately one rapid diagnostic equipment per 100 cases (Panamerican Health Organization, 2018). In addition, the treatment success rate also remained low with respect to previous years, being 55% for retreatments and 75% for new patients (Panamerican Health Organization, 2018; Sripad et al., 2014).

Problem Statement

Multidrug-resistant tuberculosis (MDR-TB) is defined as the pathogen being resistant to at least two out of the four first-line drugs used in the treatment of TB, which are isoniazid (H) and rifampicin (R). The other first-line antibiotics used for TB treatment include pyrazinamide (Z) and ethambutol (E). Furthermore, streptomycin (S) is also considered as an additional first-line drug.

In the Americas region, around 11,000 MDR-TB cases were estimated for 2017, with Peru, Brasil, Mexico, and Ecuador leading the burden statistics. Only 37% of those cases were notified, yielding a deficit of 6,900 untreated cases (Panamerican Health Organization, 2018). Ecuador accounts for 1/8 of the multidrug-resistant (MDR) cases in South America (Migliori et al., 2010). MDR-TB supposes an emerging challenge in matters of public health in Ecuador. With 650 estimated cases, our country occupies the 4th position among the highest in the Americas, and it has been considered a MDR hotspot (Migliori et al., 2010; Panamerican Health Organization, 2018).

Information regarding this topic in Ecuador is centered on the genetic analysis of the different resistances (Franco-Sotomayor & León-Benitez, 2017; Nicola-Salas et al., 2018). Epidemiologic analyses of drug resistance patterns are limited, in terms of periods and geographical regions, even though this country has one of the highest burdens of MDR-TB in South America (Franco-Sotomayor & León-Benitez, 2017). Studies in such small populations offer an idea to estimate and compare field data with the estimates by WHO and PAHO. For instance, a study performed in two hospitals in Highlands and Amazon regions of Ecuador displayed a high prevalence of MDR-TB in two groups of patients; 8.7% in new patients and 16.7% in previously treated patients (Mertz et al., 2000). Another analysis conducted on 45 samples showed a MDR incidence of 21% (Giacomazzi et al., 2010).

The limited data not only refers to MDR-TB and extensively drug-resistance tuberculosis (XDR-TB), but it also takes into account the overall cases and types of TB occurring in different regions of Ecuador. The problem with the overall data of Ecuador is the discordance between the official government reports and the data reported by independent laboratories and agencies as it has been evidenced by evaluations in the Ecuadorian borders. The study determined a TB incidence of 125 per 100,000 inhabitants in the southeastern border and 140 in the Andean southern border, while the official reports stated an incidence of 38.23 per 100,000 inhabitants (Ortiz-Rico

et al., 2015). This underrepresentation problem is manifested even in small studies. One that was performed in an indigenous community of Cotopaxi province showed a prevalence of 6.7%, which was higher than the data reported from the Ecuadorian government institutions (Romero-Sandoval et al., 2007).

The problems with official reports regarding MDR- and XDR-TB rely not only on the reported data. The Public Health Ministry informed about the lack of equipment to perform a rapid test for the detection of XDR, although its implementation has already been recommended (Ministerio de Salud Pública del Ecuador, 2018). Because of this, the traditional test takes around two to three months to complete. Hence, resistance tests are not carried out for every case registered in the Public Health System (Franco-Sotomayor & León-Benitez, 2017; Ministerio de Salud Pública del Ecuador, 2018).

More information about drug-resistant TB in Ecuador is imperative. This information becomes essential to confirm the efficiency of the strategies of control. Ecuador executes its National Tuberculosis Program through the Directly Observed Treatment, Short-course (DOTS) scheme, and also implementing a monetary bonus of $240 monthly for those drug-resistant TB patients who adhere to the treatment scheme (Ministerio de Salud Pública del Ecuador, 2018; Romero-Sandoval et al., 2009; Sripad et al., 2014). Nevertheless, this strategy is not entirely effective due to a variety of challenges in the treatment adherence, including payment delays, lack of education, length of treatment, poverty, and social stigma (Sripad et al., 2014). The relevance of epidemiologic information on MDR is crucial, as it might well serve as a tool to understand the evolution of resistance profiles, and to assess the adherence to treatment in the population.

General and Specific Objectives

General Objective

Analyze the pattern of drug susceptibility for Tuberculosis along five years in Ecuador.

Specific Objectives

- Identify the evolution of MDR profiles for Tuberculosis reported cases during the 2012-2017 period.
- Define differences in the resistance burden according to the treatment adherence in different populations.

Methodology

Data collection and case selection

A database containing 23,680 cases of tuberculosis was provided by the *Instituto Nacional de Salud Pública e Investigación* - INSPI (National Institute of Research in Public Health). Eight entries were deleted from this database due to unknown sample origin, so a total of 23,672 cases were left for the study. All cases in the database were anonymized at the beginning of the study, thus observing the counseling of the Helsinki Declaration (WMA) regarding human experimentation. The notified cases corresponded to the period from January 2012 to December 2017.

Original clinical records contained numerous variables, including Report date, Sample, Age, Sex, Provenance, Doctor/Area, Bacilloscopy results, Culture results, Drug-susceptibility test (DST) results for First-Line Drugs, DST results for Second-Line Drugs, Atypical case, Diabetes case, Previous Treatment, HIV coinfection, and MDR result. Because some of these parameters contained either incomplete or useless information for the objectives of this thesis, they were finally be fixed or removed from the final database. Regarding the "Report date", the cases were grouped considering only the year of their report. The parameter "Sample" was simplified to "Pulmonary and Extra-pulmonary", to thus reflect the site of infection in this study. These data became the variable "Site of Disease" in the final database. "Culture" and "DST" data were summarized as either positive or negative, indicating the presence or absence of TB infection and the performance of DST for any first-line drug, respectively. Because the information provided for the MDR result was inaccurate due to missing MDR reports in the original database, further correction of this parameter was carried out by exhaustive revision of the reported individual DST results. DST results for Second-Line Drugs were not included given that this information was unreliable and incomplete because the lack of equipment to perform this analysis for second-line drugs in the country.

Additionally, entries only containing Bacilloscopy results were excluded from the final study population. To be included in the final study population, cases had to meet three conditions: be reported in the database by INSPI between 2012 and 2017, be culture positive, and have been tested for all first-line drugs. A total of 12,238 cases (51.7%) were culture positive (Figure 1). The final study population included 8,932 cases, which account for a total of 73% of the culture-

positive cases. The final study population was reviewed in detail for the different patterns of drug resistance.

Some observations about the whole database took the fact that INSPI is the unique institution in Ecuador performing drug susceptibility tests. Furthermore, some bias was intrinsically present due to the performance of DST largely in risk groups, including children, elderly, diabetes, and HIV patients, as well as in people with active TB infection having physical contact with previously TB-treated patients.

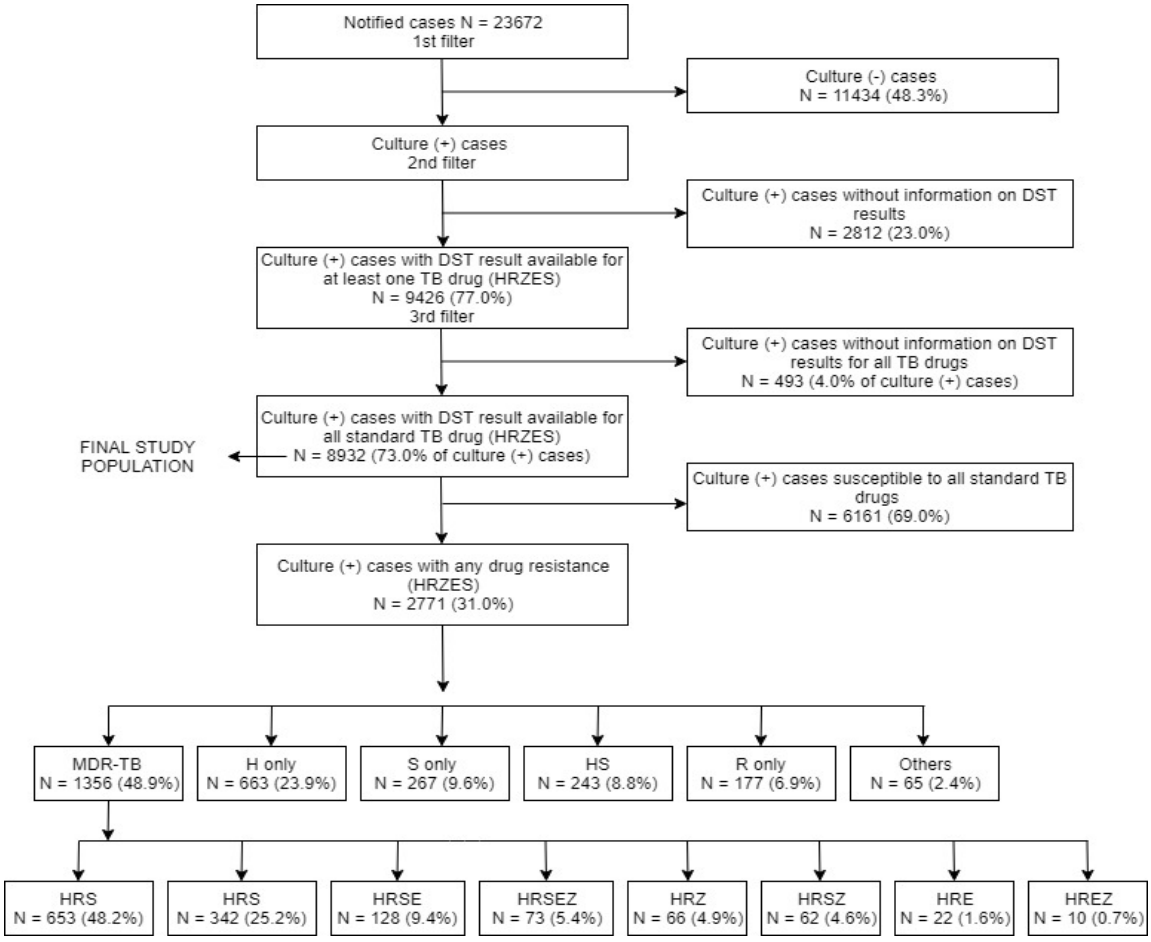

Figure 1: Workflow describing the case selection process, predominant resistance patterns, and MDR-TB patterns in Ecuador, 2012-2017.

Variables considered for the analysis

Due to their informative accuracy, only the following parameters were considered from the database for the statistical analysis of the drug resistance: Site of disease, Age, Sex, Previous TB treatment, HIV, presence of MDR-TB, and the individual DST results for all the following five drugs: Isoniazid (H), Rifampicin (R), Streptomycin (S), Ethambutol (E), and Pyrazinamide (Z),

jointly referred as HRSEZ. As to the site of disease, the information was simplified in the following terms: Pulmonary vs. Extrapulmonary and data deducted from the nature of sample for the DST withdrawn from the patient. Up to four different age groups were defined for the analysis: Children (0 to 15 years), two Adult groups (15 to 39 years and 40 to 59 years), and the Elderly group (> 60 years). In the database, the parameter referring to "previous treatment" was annotated as VT for patients naive to treatment and AT for treatment-experienced patients. The individual DST results were classified in the following categories: "any drug resistance" as the resistance to at least one of the HRSEZ, "mono-resistance" as the resistance to only one of the HRSEZ, "MDR-TB" as the resistance to at least H and R simultaneously (resistance to all HRSEZ may also be included in this category), and "others" as any other resistance pattern found in the database.

Statistics

Data descriptive analysis was performed considering each of the variables independently. For each of the variables, the descriptive results were presented as numbers of cases and percentages. Drug monoresistance patterns, MDR-TB, and their trends throughout the period 2012-2017 were analyzed using the Chi-Squared Test for linear trends and presented as the percentages for each year. Percentages between the different values of the variables were compared using the Chi-Squared Test for statistically relevant differences in their proportions (Glasauer et al., 2019). A further multivariate analysis was carried out with pooled data using the Multivariate Logistic Regression to compute the level of association between sex, age groups, site of disease, previous treatment, and HIV co-infection as the independent variables with the principal resistance patterns: any resistance, MDR-TB, HS resistance, and monoresistances against H, R, and S as the dependent variables. For the descriptive univariate analyses, the alpha value was set to 0.05. For the logistic regression, confidence intervals were determined at 95%. All the statistical analyses were conducted using RStudio Version 1.1.419.

Results

Firstly, three filters were applied to the data, based on the requirements mentioned in the previous section (Fig. 1). From the total number of reported cases, 12,238 (51.7%) were TB culture-positive cases, while 9,426 (77.0% of culture-positive cases) had available the DST results for at least one of the standard first-line drugs. The number of cases with a complete DST profile

available for the first-line drugs was 8,932 (73% of culture-positive cases). That was of cases included in the Final Study Population (FSP) of this research project.

Regarding the FSP, a total of 2,771 cases (31.0% of FSP) presented resistance to any of the HRZES (either alone or combined), while the majority of cases (6,161, 69.0%) were found to be pan-susceptible to first-line TB antibiotics (Fig. 1). As expected, the percentage of cases corresponding to males (6,382/8,932, 71.5% of total FSP cases) was higher than to females (2550/8932, 28.5%). Most of the culture-positive TB cases were found in the adult cohort, particularly in the 15 to 39-year group, with a total of 4,304 out of 8,932 cases (48.2%). Only 2.0% of TB cases (175) were reported in children (0-14 years old) from the FSP. Concerning the previous treatment-experience, information was unavailable in 163 cases. The majority of positive cases never received TB treatment (5,691/8,932, 64.9%). Most of the cases had no HIV co-infection (7,678/8,932, 86.0%). The characteristics of the FSP are summarized in Table 1.

In overall, 23 out of the 31 resistance patterns were found in Ecuador. Among them, they highlighted two in terms of percentage (Fig. 1). The most represented patterns were MDR-TB, (1,356/2,771 cases, 48.9%) and H-mono-resistance (663/2,771 cases, 23.9%). S-mono-resistance and HS-combined resistance jointly represented an 18.4% of the cases. The HR combined resistance pattern was the most prevalent of the MDR-TB (653 per 1,356, 48.2%, Fig. 1). The MDR-TB, HS-combined resistance, and H, R, and S monoresistances were then selected for statistical analyses. The eight resistance patterns with no cases reported in Ecuador were HEZ, RSE, RSEZ, RE, REZ, SEZ, SZ, and EZ.

	Total number of cases		*Pan-susceptible*		*Monoresistance*		*Multidrug resistance*		*Other resistances*	
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Total	8932		6161		1119		1356		296	
Sex										
Female	2550	28.5%	1789	29.0%	263	23.5%	429	31.6%	69	23.3%
Male	6382	71.5%	4372	71.0%	856	76.5%	927	68.4%	227	76.7%
Age ranges										
0-14	175	2.0%	134	2.2%	18	1.6%	20	1.5%	3	1.0%
15-39	4304	48.2%	3003	48.7%	600	53.6%	569	42.0%	132	44.6%
40-59	2854	31.9%	1924	31.2%	340	30.4%	483	35.6%	107	36.2%
60+	1599	17.9%	1100	17.9%	161	14.4%	284	20.9%	54	18.2%
Previous treatment (n=8769)										
Yes (AT)	5691	64.9%	4263	70.6%	674	61.5%	601	44.9%	153	52.2%
No (VT)	3078	35.1%	1778	29.4%	422	38.5%	738	55.1%	140	47.8%
Unknown	163		120		23		17		3	
Site of disease										
Pulmonary	8352	93.5%	5706	92.6%	1059	94.6%	1303	96.1%	284	95.9%
Extrapulmonary	580	6.5%	455	7.4%	60	5.4%	53	3.9%	12	4.1%
HIV										
No	7678	86.0%	5271	85.6%	955	85.3%	1185	87.4%	267	90.2%
Yes	1254	14.0%	890	14.4%	164	14.7%	171	12.6%	29	9.8%

Table 1: Characteristics of the final study population from 2012-2017 according to the variables under study. AT: Previously treated. VT: Non-previously treated.

Drug resistance during the period 2012-2017

From a total of 2,771 resistant cases in this period, 682 out of 1,845 cases (37.0%) were of 2012. This number constituted the highest prevalence of drug resistance for that period. Conversely in 2014, it was found the lowest percentage of resistant cases (461 out of 1,754, 26.3%). However, in terms of absolute frequency, the lowest amount of resistant cases was found in 2016, with 262 cases per 838 (31.3%). The distribution of the cases is shown in the Table 2.

According to the statistical analysis, significant differences ($p < 0.05$) in the incidence of any resistance were observed between the periods of 2012-2013 (χ-squared=16.8) and 2013-2014 (χ-squared=6.7). Drug resistance reached the lowest percentage during 2014 and then showed a trend of rising in the following years ($p < 0.05$, χ-squared=6.4, Figure 2). The increase was nevertheless smooth since 2014, with no significant differences in terms of percentage between the 2014-2015, 2015-2016, and 2016-2017 years.

	Monoresistances							
	H	R	S	E	Z	MDR-TB	Others	Total
2012	140	39	77	0	1	341	84	**682**
2013	118	36	50	1	0	240	55	**500**
2014	110	23	30	0	3	254	41	**461**
2015	112	27	40	1	1	165	32	**378**
2016	62	21	18	1	0	129	31	**262**
2017	121	31	52	2	2	227	53	**488**
Total	**663**	**177**	**267**	**5**	**7**	**1356**	**296**	***2771***

Table 2: Distribution of the resistance patterns (Monoresistances and MDR-TB) in Ecuador during the period 2012-2017. Others: Resistance patterns different than monoresistances and MDR-TB.

Further analysis was run to assess the trends along the period, relative to the most characteristic resistance patterns, including all monoresistances and MDR-TB (Figure 3). Although no statistically significant trend was observed for any of the monoresistance patterns, some significant differences emerged in the patterns along the time. In H-monoresistance, a significant increase from 110/1,754 cases (6.3%) to 112/1,321 cases (8.5%) was revealed across 2014 and 2015 (χ-squared=5.2) (Fig. 3). S-monoresistance showed significant differences between 2013 and 2014 (50/1,648 cases, 3.0% and 30/1,754, 1.7% respectively, χ-squared=5.9), and between 2014 and 2015 (30/1,754 cases, 1.7% and 40/321 cases, 3.0% respectively, χ-squared=5.3). R, E, and Z monoresistances had no significant differences among the period of study.

The only pattern showing a statistically significant increasing trend was MDR-TB. It had an overall increasing trend from 2015 to 2017 (χ-squared=7.2), despite of having no significant differences between any years, with the exception of 2012-2013 (χ-squared=9.4) (Fig. 3), where it was detected a change from 341 per 1,845 cases (18.5%) to 240 per 1,648 cases (14.6%).

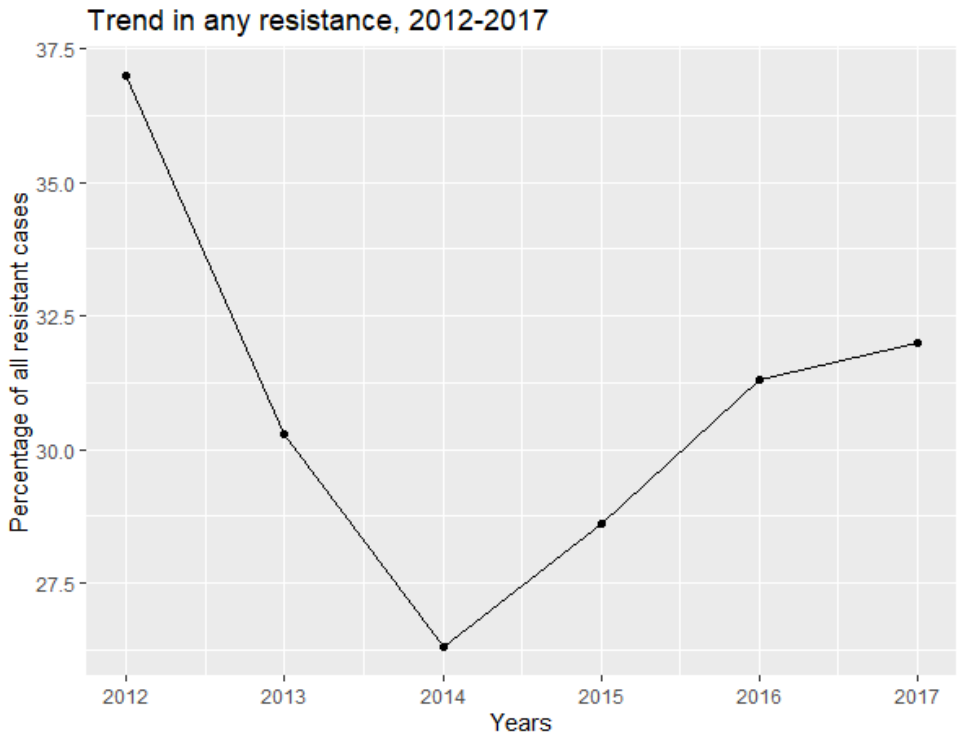

Figure 2: Development of the resistance prevalence in Ecuador during 2012-2017.

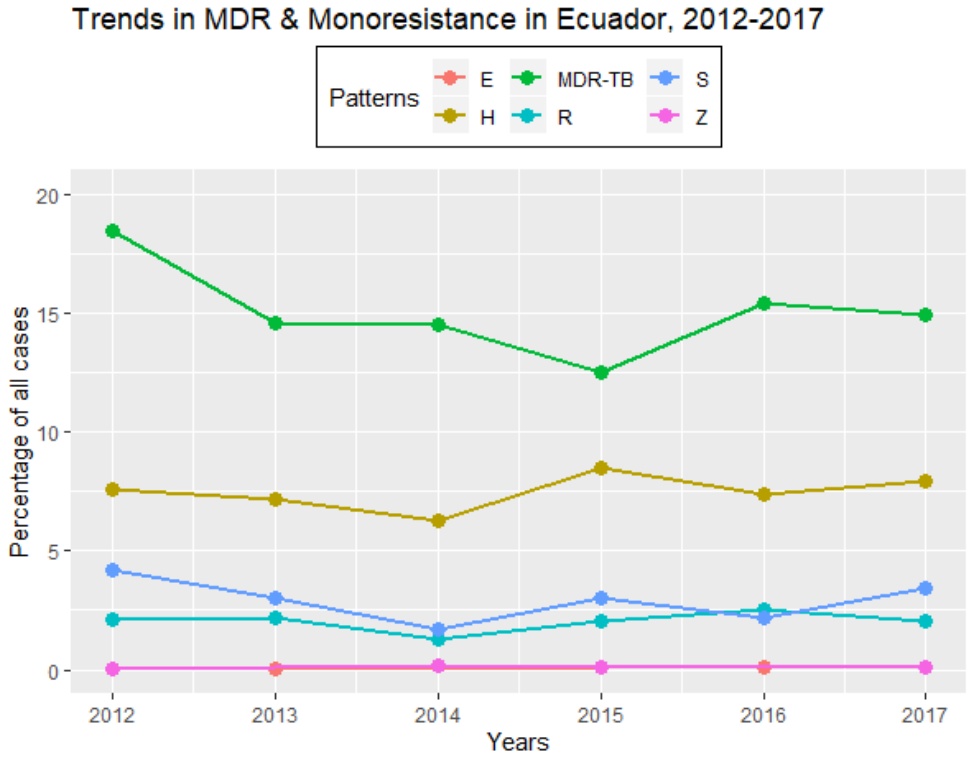

Figure 3: Trends in monoresistance patterns and MDR-TB in Ecuador during 2012-2017.

Resistance according to sex

Males accounted for the vast majority of TB cases in the FSP. In the study period, 6,382 out of 8,932 cases (71.5%) were male, and 2,550 out of 8,932 cases (28.5%) were female. Regarding any resistance, the distribution maintains with 2,010 per 6,382 resistant cases (31.5%) in male and 761 per 2,550 resistant cases (29.8%) in the female. However, sex-dependent differences in the ratios was not significant (p-value > 0.05, χ-squared=2.3).

About the three most important monoresistance patterns, all of them presented significant differences between males and females. From the 761 resistant cases in females, 132 (17.4%) belonged to the H-monoresistance, and 56 (7.4%) were S-monoresistant. Male showed significantly higher rates of H and S monoresistances, with 531 per 2,010 (26.4%, χ-squared=24.5) and 211 per 2,010 (10.5%, χ-squared=5.9) respectively. Females had a significantly higher proportion of R monoresistance than males (χ-squared=10.8). Specifically, females accounted for 68/761 R-monoresistant cases (8.9%) and males accounted for 109/2,010 R-monoresistant cases (5.4%).

For the HS-combined resistance pattern, again, the male had a significantly higher proportion (p < 0.05, χ-squared=3.4) with 189/2,010 cases (9.4%), while the female had 54/761 cases (7.1%). MDR-TB accounted for 56.4% of resistant cases in females (429/761), in contrast with the 46.1% in male (927/2,010), presenting a significant difference (χ-squared=22.8). The distribution and proportions for this section are summarized in Table 3.

			Monoresistances				
	Total cases	Any resistance	H	R	S	HS	MDR-TB
Female	2550 (28.5%)	761 (29.8%)	132 (17.4%)*	68 (8.9%)*	56 (7.4%)*	54 (7.1%)*	429 (56.4%)*
Male	6382 (71.5%)	2010 (31.5%)	531 (26.4%)*	109 (5.4%)*	211 (10.5%)*	189 (9.4%)*	927 (46.1%)*
Total	**8932**	**2771 (31.0%)**	**663 (23.9%)**	**177 (6.4%)**	**267 (9.6%)**	**243 (8.8%)**	**1356 (48.9%)**

Table 3: Proportions and distribution (based on sex) of the five predominant resistance patterns in Ecuador between 2012 and 2017. *p < 0.05. Resistance percentages are calculated in function of the number of resistant cases ("Any resistance").

Distribution of the drug resistance across ages

The rates for any class of drug resistance in the four age groups were compared to evaluate the association between age and resistance, and which group had the greatest drug resistance rate. Notably, children accounted for a low quantity of all TB cases (175 out of 8,932, 2.0%), and a very low quantity of all resistant cases (41 out of 2,771, 1.5%). In children, the number of MDR-TB

cases was only 20 (11.4%) from a total of 175 TB cases. The age group ranging from 15 to 39 years old presented the majority of all TB cases (4,304 out of 8,932, 48.1%), and the majority of any drug resistant cases (1,301 out of 2,771, 46.9%). The group from 40 to 59 years old had 2,854 TB cases out of 8,932 (32.0%) and 930 resistant cases out of 2,771 (32.6%), and therefore the highest rates. Elderly presented 1,599 TB cases out of 8,932 (17.9%) and 499 resistant cases out of 2,771 (18.0%).

The rates used for the comparison of any drug resistance between the four groups were related to the total number of TB cases in each group as follows: 0-15 years: 41/175, 23.4%, 15-39 years: 1301/4,304, 30.2%, 40-59 years: 930/2,854, 32.6%, and > 60 years: 499/1,599, 31.2%. A pooled analysis of the four proportions mentioned before revealed an association between the age and the incidence of any TB resistance ($p < 0.05$, χ-squared=9.3). Moreover, the chi-squared pairwise post hoc analysis showed significant differences ($p < 0.05$) between children and every other age group (for 15-39: χ-squared=3.4, for 40-59: χ-squared=5.9, for > 60: χ-squared=4.2) and between the 15-39 and 40-59 age groups (χ-squared=4.3).

A detailed count of each possible resistance pattern allowed the comparison of the most characteristic patterns for each age group (Table 4). Children (0-14 years old) were not considered for the comparison of the proportions because of the low representation of resistant cases. Concerning the MDR-TB, significantly lower proportions were found among 15-39 and other groups, when compared with the 40-59 (χ-squared=18.5) and > 60 years old groups (χ-squared=19.1). Also, significant differences were found in the case of H-monoresistance, except that proportions were higher for the 15-39 years old group compared with 40-59 (χ-squared=19.5) and > 60 years old groups (χ-squared=32.9), and the percentage was also significantly greater for 40-59 group compared to the elderly (χ-squared=4.8). In R-monoresistance, the only significant difference was found between the 15-39 years old group and the elderly (χ-squared=2.8), with 70 cases per 1,301 (5.4%) compared to 38 per 499 (7.6%) respectively. The 15-39 years old group also showed a proportion significantly higher (χ-squared=4.2) than the 40-59 years old group for S-monoresistance. Meanwhile, no significant differences are present for HS-combined resistance.

			Monoresistances				
	Total cases	Any resistance	H	R	S	HS	MDR-TB
0-14	175	41 (23.4%)	6 (14.6%)	4 (9.8%)	4 (9.8%)	-	20 (48.8%)
15-39	4304	1301 (30.2%)	382 (29.4%)	70 (5.4%)	143 (11.0%)	115 (8.8%)	569 (43.7%)
40-59	2854	930 (32.6%)	195 (21.0%)	65 (7.0%)	77 (8.3%)	84 (9.0%)	483 (51.9%)
60+	1599	499 (31.2%)	80 (16.0%)	38 (7.6%)	43 (8.6%)	44 (8.8%)	284 (56.9%)
Total	**8932**	**2771 (31.0%)**	**663 (23.9%)**	**177 (6.4%)**	**267 (9.6%)**	**243 (8.8%)**	**1356 (48.9%)**

Table 4: Proportions and distribution, based on age, of the five predominant resistance patterns in Ecuador between 2012-2017. Resistance percentages are calculated in function of the number of resistant cases ("Any resistance").

Resistance according to the previous treatment

In this variable, 163 cases had no information about whether they received previous treatment, so there were 8,769 TB cases considered. Most of the cases (5691/8,769, 64.9%) received no previous treatment for TB (VT), in contrast to 3,078 cases (35.1%) that in effect had previous treatment against TB (AT). A total of 1,428 out of 5,691 (25.1%) cases being naive to treatment did not have any drug resistance, while a higher proportion of previously treated patients had resistance (1,300 per 3,078, 42.2%). This difference between the two groups was significant ($p < 0.05$, χ-squared=273.12), which denoted a relationship between a previous experience with TB treatment and the development of any resistance to TB drugs.

Those cases that were previously treated had significantly a higher proportion (χ-squared=276.9) of MDR-TB, with 738 TB cases out of 3,078 cases that did receive treatment (24.0%) compared to the 601 TB cases out of 5,691 treatment-naive cases (10.6%). No significant differences ($p > 0.05$) are present for HS-combined resistance (AT: 114/1,300, 8.8%. VT: 128/1,428, 9.0%, χ-squared=0.01) and R-monoresistance (AT: 85/1,300, 6.5%. VT: 86/1,428, 6.0%, χ-squared=0.23). New TB cases had a proportion of H-monoresistance (410/1,428, 28.7%, χ-squared=36.9) and S monoresistance (168/1,428, 11.8%, χ-squared=16.8), higher than previous cases (H-only: 243/1,300, 18.7%. S-only: 92/1,300, 7.1%).

			Monoresistances				
	Total cases	Any resistance	H	R	S	HS	MDR-TB
No (VT)	5691	1428 (25.1%)*	410 (28.7%)*	86 (6.0%)	168 (11.8%)*	128 (9.0%))	601 (10.6%)*
Yes (AT)	3078	1300 (42.2%)*	243 (18.7%)*	85 (6.5%)	92 (7.1%)*	114 (8.8%)	738 (24.0%)*
Total	**8769**	**2728 (31.1%)**	**653 (23.9%)**	**171 (6.3%)**	**260 (9.5%)**	**242 (8.9%)**	**1339 (49.1%)**

Table 5*:* Proportions and distribution (according to previous treatment) of the five predominant resistance patterns in Ecuador between 2012 and 2017. *p < 0.05. Except for MDR-TB, resistance percentages are calculated in

function of the number of resistant cases ("Any resistance"). For MDR-TB, percentages are relative to total TB cases (Total cases).

Resistance according to previous treatment and age

Cases were counted in groups sorted by age, and within each group, it was considered the AT and VT groups according to previous treatment. The case counts and proportions for the main resistance patterns are presented in Table 6. For the children (0-14), the proportions between AT and VT were not statistically different. Nevertheless, a noticeable outcome from this count was the presence of more resistant cases in the newly diagnosed (29 per 127, 22.8%) rather than in the previously treated patients (12 per 41, 29.3%).

As to the 15-39 years old group, significant differences ($p < 0.05$) in the rates were observed for any resistance (χ-squared=97.4), H and S-monoresistances (χ-squared=9.9, χ-squared=6.6, respectively), and MDR-TB (χ-squared=87.9). As a whole, previous cases presented a higher rate of any resistance (546/1,344, 40.6%) than new cases (735/2,874, 25.6%). Whereas in H and S mono-resistances, new cases exhibited a larger proportion of resistance (H-only: 241/735, 32.8%. S-only: 95/735, 12.9%) than previous cases (H-only: 134/546, 24.5%. S-only: 45/546, 8.2%). For MDR-TB, AT had greater proportion (276/1,344, 20.5%) compared to the new cases (286/2,874, 10.0%).

In the group of 40 to 59 years old, significant differences in proportions were observed for the same patterns as the 0-15 years old group. One more time, the previous cases (459/1,087, 42.2%) presented a percentage of any resistance larger than new cases (450/1,713, 26.3%, χ-squared=76.5). In contrast, new cases (120 out of 450, 26.7%) presented more H-mono-resistance than previous cases (72 out of 459, 15.7%, χ-squared=15.8). The same comparison applied to S-mono-resistance (χ-squared=6.4). The VT group (47/450, 10.4%) had a percentage of S-mono-resistance higher than AT (26/459, 5.7%). For MDR-TB, AT had a higher proportion compared to VT (271/1,087, 24.9% vs. 203/1,713, 11.9%, χ-squared=79.9).

Among the elderly, significant differences in proportions were found for any resistance (χ-squared=105.6), H-monoresistance (χ-squared=7.4), and MDR-TB (χ-squared=108.4). When any resistance and MDR-TB were considered, previous cases (Any resistance: 283/606, 46.7%. MDR-TB: 186/606, 30.7%) presented larger percentages of each pattern than new cases (Any resistance:

214/977, 21.9%. MDR-TB: 97/977, 9.9%), while in H-monoresistance, new cases (46/214, 21.5%) presented more resistance than previous cases (34/283, 12.0%).

Age group: [0-14]							
			Monoresistance				*Others*
Previous treatment	**Total number of cases**	**Any resistance**	**H**	**R**	**S**	**MDR-TB**	**HS**
AT	41	12 (29.3%)	3 (25.2%)	2 (16.7%)	1 (8.3%)	5 (12.2%)	-
VT	127	29 (22.8%)	3 (10.3%)	2 (6.9%)	3 (10.4%)	15 (11.8%)	-
Unknown	7						
Total	**168**	**41 (24.4%)**	**6 (14.6%)**	**4 (9.8%)**	**4 (9.8%)**	**20 (48.8%)**	**-**

Age group: [15-39]							
			Monoresistance				*Others*
Previous treatment	**Total number of cases**	**Any resistance**	**H**	**R**	**S**	**MDR-TB**	**HS**
AT	1344	546 (40.6%)*	134 (24.5%)*	36 (8.6%)	45 (8.2%)*	276 (20.5%)*	44 (8.1%)
VT	2874	735 (25.6%)*	241 (32.8%)*	32 (4.4%)	95 (12.9%)*	286 (10.0%)*	70 (9.5%)
Unknown	86	20	7	2	3	7	1
Total	**4218**	**1281 (30.4%)**	**375 (29.3%)**	**68 (5.3%)**	**140 (10.9%)**	**562 (43.9%)**	**114 (8.9%)**

Age group: [40-59]							
			Monoresistance				*Others*
Previous treatment	**Total number of cases**	**Any resistance**	**H**	**R**	**S**	**MDR-TB**	**HS**
AT	1087	459 (42.2%)*	72 (15.7%)*	29 (6.3%)	26 (5.7%)*	271 (24.9%)*	49 (10.7%)
VT	1713	450 (26.3%)*	120 (26.7%)*	33 (7.3%)	47 (10.4%)*	203 (11.9%)*	35 (7.8%)
Unknown	54	21	3	3	4	9	-
Total	**2300**	**909 (32.5%)**	**192 (21.1%)**	**62 (6.9%)**	**73 (8.0%)**	**474 (52.2%)**	**84 (9.2%)**

Age group: [60+]							
			Monoresistance				*Others*
Previous treatment	**Total number of cases**	**Any resistance**	**H**	**R**	**S**	**MDR-TB**	**HS**
AT	606	283 (46.7%)*	34 (12.0%)*	18 (6.4%)	20 (7.1%)	186 (30.7%)*	21 (7.4%)
VT	977	214 (21.9%)*	46 (21.5%)*	19 (8.9%)	23 (10.8%)	97 (9.9%)*	23 (10.8%)
Unknown	16	2	-	1	-	-	-
Total	**1583**	**497 (31.4%)**	**80 (16.1%)**	**37 (7.5%)**	**43 (8.7%)**	**283 (56.9%)**	**44 (8.9%)**

Table 6: Rates and distribution, according to previous treatment and age groups jointly, of the five predominant resistance patterns in Ecuador between 2012 and 2017. *p < 0.05. Except for MDR-TB, resistance percentages are calculated in function of the number of resistant cases ("Any resistance"). For MDR-TB, percentages are relative to total number of TB cases.

Resistance according to the site of disease

As expected, the majority of cases fell into the Pulmonary TB (8,352/8,932, 93.5%) rather than to Extrapulmonary TB (580/8,932, 6.5%) categories. Data showed a significant association (χ-squared=25.5) between the presence of any resistance and the site of the infection, although this association was likely to be due to the characteristics of the disease. Pulmonary TB cases had a

higher proportion of any resistance (2,646/8,352, 31.7%) compared to Extrapulmonary TB (125/580, 21.6%, Table 7). MDR-TB also showed significant difference (χ-squared=17.1) between the pulmonary (1303/8352, 15.6%) and extrapulmonary group (53/580, 9.1%) No significant differences were found amid the proportions for other of the most important resistance patterns.

			Monoresistances				
	Total cases	Any resistance	H	R	S	HS	MDR-TB
Pulmonary	8352	2646 (31.7%)*	637 (24.1%)	166 (6.3%)	249 (9.4%)	236 (8.9%))	1303 (15.6%)
Extrapulmonary	580	125 (21.6%)*	26 (20.8%)	11 (8.8%)	18 (14.4%)	7 (5.6%)	53 (9.1%)
Total	**8932**	**2771 (31.0%)**	**663 (23.9%)**	**177 (6.4%)**	**267 (9.6%)**	**243 (8.8%)**	**1356 (48.9%)**

Table 7: Proportions and distribution, according to the site of disease, of the five predominant resistance patterns in Ecuador between 2012 and 2017. *p < 0.05. Except for MDR-TB, resistance percentages are calculated in function of the number of resistant cases ("Any resistance"). For MDR-TB, percentages are relative to total number of TB cases ("Total cases").

Resistance according to HIV co-infection

Most of the cases in the FSP were negative for HIV (7,687 out of 8,932, 86.0%), in contrast to just 1,254 out of 8,932 (14.0%), which were HIV-positive. The proportions of resistant cases for each group had no significant differences (χ-squared=108.4) for HIV-Negative (2,407/7,678, 31.4%) compared to HIV-positive 364/1,254 (29.0%). For any resistance pattern, no significant differences were found between the percentages of these above groups. Case counts and proportions for this variable are summarized in Table 8.

			Monoresistances				
	Total cases	Any resistance	H	R	S	HS	MDR-TB
HIV Negative	7678	2407 (31.4%)	571 (23.7%)	150 (6.2%)	223 (9.3%)	218 (9.1%)	1185 (15.4%)
HIV Positive	1254	364 (29.0%)	92 (25.3%)	27 (7.4%)	44 (12.1%)	25 (6.9%)	171 (13.6%)
Total	**8932**	**2771 (31.0%)**	**663 (23.9%)**	**177 (6.4%)**	**267 (9.6%)**	**243 (8.8%)**	**1356 (48.9%)**

Table 8: Proportions and distribution (according to HIV co-infection) of the five predominant resistance patterns in Ecuador between 2012 and 2017. Except for MDR-TB, resistance percentages are calculated in function of the number of resistant cases ("Any resistance"). For MDR-TB, percentages are relative to total number of TB cases ("Total cases").

Multivariate Logistic Regression (MLR) analysis

A regression analysis was carried out to elucidate the actual effect of each variable in the presence of any resistance and MDR-TB (Table 9). Cases with unavailable information for any of the variables taking as risk factors were removed from the database for this analysis. The Odds Ratio (OR) represented the probability of developing any resistance or MDR-TB for each of the levels of the risk factors. These variables included age, sex, prior TB treatment, HIV infection, and

site of the disease. According to the MLR, only young adults had significantly lower probabilities of developing MDR-TB (OR: 0.741; 95% CI: 0.631-0.871) in comparison with the elderly. For the other age segments, the probabilities were also low but not significant, especially for children. Taking males as the reference group, females had a significantly higher probability of developing MDR-TB (OR: 1.235; 95% CI: 1.084-1.406). Yet not significant, the probability of developing any drug resistance was lower in females.

Previously treated patients have twice the odds of acquiring any drug resistance, with an OR of 2.127 (95% CI: 1.935-2.339). This probability for previously treated cases has a slight increase of MDR-TB (OR: 2.611; 95% CI: 2.313-2.948) compared to treatment-naive patients. According to this analysis, another significant risk factor turned out to be the site of the disease. Extrapulmonary patients had half of the odds for developing any resistance (OR: 0.700; 95% CI: 0.563-0.870) and MDR-TB (OR: 0.652; 95% CI: 0.474-0.898) than “pulmonary-site” patients. Finally, HIV-negative patients had no statistically significant results in this analysis compared to HIV-positive patients, although they suggest a lower probability for the presence of both MDR-TB and any resistance.

Risk Factor	*Any resistance*			*MDR-TB*		
	OR	95% CI		OR	95% CI	
		Lower	Upper		Lower	Upper
Age						
[0-14]	**0.822**	0.565	1.195	**0.746**	0.456	1.221
[15-59]	**1.001**	0.881	1.138	**0.741**	0.631	0.871
[40-59]	**1.046**	0.913	1.198	**0.924**	0.748	1.091
[60+]	**ref**					
Sex						
Male	**ref**					
Female	**0.953**	0.860	1.057	**1.235**	1.084	1.406
Previous treatment						
No	**ref**					
Yes	**2.127**	1.935	2.339	**2.611**	2.313	2.948
HIV						
Yes	**ref**					
No	**0.969**	0.841	1.116	**0.929**	0.769	1.123
Site of the disease						
Pulmonary	**ref**					
Extrapulmonary	**0.700**	0.563	0.870	**0.652**	0.474	0.898

Table 9: MLR results for the risk factors associated with any drug resistance and MDR-TB in Ecuador during 2012-2017. OR=1, no association between risk factor and emergence of resistance. OR>1, positive association between risk factor and emergence resistance (more probability of resistance). OR<1, negative association between risk factor and emergence of resistance (less probability of resistance).

Discussion

This study is one of the first epidemiological studies on drug-resistant tuberculosis performed in Ecuador and Latin America with compiled national surveillance data. The overall resistance found by this study reached 31.0% of the final study population. In Ecuador, most of the previous studies on the subject of drug-resistant tuberculosis rely on the genetic characterization of resistant strains or the identification of genes associated with the resistance (Franco-Sotomayor & León-Benitez, 2017; Nicola-Salas et al., 2018). Studies about the epidemiology of MDR-TB or any resistant tuberculosis are scarce, and the available information considers small geographical populations and short periods (1 to 3 years maximum) rather than a longer time interval or the whole Ecuadorian territory. Furthermore, other non-published studies consider only the information provided by small territorial healthcare units (Centros de Salud), where the data management could be even less reliable than from INSPI.

The most common resistance pattern found was MDR-TB, which represented 48.9% of the resistant cases. It was followed by H-monoresistance, which accounts for 23.9% of all resistant cases. This percentage was higher that other similar studies performed abroad. In Germany, a study by Glasauer et al. (2019) found a proportion of 12.7% of resistant cases among the population, and 16.7% of MDR-TB cases amid resistant cases. Throughout the period under study, a decrease in the overall drug resistance was observed from 2012 (37.0%) to 2014 (26.3%). This fact could be due to the implementation, in this period, of an economic bonus for the adherent drug-resistant patients by the Ecuadorian National Tuberculosis program (Sripad et al., 2014).

However, the proportion of resistant TB increased in 2015 and kept growing until 2017, producing a rising trend during that period. A possible explanation of this fact could again be the possible ending of the money bonus, lack of bonus payment, or lack of public investment for the National Tuberculosis Program, along with the lack of local surveillance by the public healthcare personal of the TB patients. One limitation of this study was that the information about which patients received the mentioned bonus was missing.

The main resistance pattern leading the trend in the overall resistance was MDR-TB, despite having a great difference between the two first years. This trend was likely to be due to the constant growth rate of resistance in the last (2015 and 2016) years (no significant differences since 2013). The overall proportion of MDR-TB between those years reached 15.18% of all TB cases (1,356 out of 8,932 cases). This proportion is higher than the estimated by PAHO, which stated 9% of rifampicin resistant TB (RR-TB)/MDR-TB (650 cases) of a total of TB cases in Ecuador during 2018 (Panamerican Health Organization, 2018).

Furthermore, the proportion during 2017 (14.9%) was also higher than the estimated. The number of MDR-TB cases reported for this year was 227, which was in contrast with the estimated 650 cases, suggesting a lack of diagnosis of MDR-TB or a lack of efficient reports from the health authorities in Ecuador. Nonetheless, the number of MDR-TB cases stayed close to the MDR/RR-TB incidence estimated by WHO. This gave a total of 220 cases in Ecuador and 11,000 cases for the Americas Region during 2018 (World Health Organization, 2019, 2020). From a total of 4084 RR/MDR-TB cases reported in the Americas Region in 2017 (Panamerican Health Organization, 2018), Ecuador contributes with 227 cases of MDR-TB (5.6%) and 31 cases of RR-TB (0.8%).

Among mono-resistances, the most important pattern between these years was H-monoresistance. It showed no trend because their proportions had kept almost similar frequencies during the period (7.59% in 2012 vs. 7.93 in 2017). Significant findings of this study were the low resistances against two first-line drugs, ethambutol and pyrazinamide. Such low proportions can be explained by the unreliability of phenotypic DST for these two drugs (Andres et al., 2019), and because the pathogen is less prone to generate those resistances, especially against E (The CRyPTIC Consortium and the 100,000 Genomes Project, 2018).

As expected from previous studies, TB cases were more prominent in men rather than in women, though no association with the development of any drug resistance was demonstrated by comparison of proportions and regression analysis. Diverse studies support the finding that sex is not a risk factor for MDR-TB or any other resistance (Demile et al., 2018; Desissa et al., 2018; Dessalegn et al., 2016; Glasauer et al., 2019; Gobena et al., 2018; Gomes et al., 2014). However, MDR-TB was more presented in females, suggesting a relationship (Liu et al., 2013; O'Donnell et al., 2011). This notion was supported by the regression analysis of this study, where females had 1.244 times more probability of acquiring MDR-TB than males.

The univariate analysis suggested an association between the age of the patients and the development of any resistance, with the adult (40-59 years old) group having the highest proportion of resistant cases. In agreement with other studies, the majority of resistant patients were < 60 years old (Faustini et al., 2006; Law et al., 2008). Unfortunately, this association was not supported by the regression analysis, where the only group having a significant association was the young adults (15-39 years old) group. They showed a lower probability of developing resistance than the others, and also lower MDR-TB proportion than older groups. A similar pattern was valid for the children (0-14 years old) since they had no significant association, but a lower probability of developing resistance. Several studies make associations between younger age and MDR-TB (Chen et al., 2013; Faustini et al., 2006; Liu et al., 2013; Shao et al., 2011; Workicho et al., 2017), affirming that the acquisition of TB strains by older age groups before the emergence of drug resistance would explain it (Dheda et al., 2017), a finding that our multivariate study could not support.

Moreover, the comparison of proportions showed a lower proportion of MDR-TB in children and young adults than in adults and the elderly. Drug resistance in children was meager

in Ecuador, accounting for only 41 cases (41 out of 2,771, 1.5% of all resistant cases) during the whole 2012-2018 period. Half of resistant cases were MDR-TB.

Although several abroad studies have encountered an association of HIV co-infection with drug resistance (Campos et al., 2003; Joseph et al., 2006; Mesfin et al., 2014; Suchindran et al., 2009), the current study disagrees that finding. According to other foreign studies, including the present one, HIV constitutes no risk factor for TB drug resistance (Faustini et al., 2006; Kibret et al., 2017; Lv et al., 2017; Workicho et al., 2017).

According to the univariate and multivariate analysis, pulmonary cases have an association with the development of any resistance. Nonetheless, this result can be explained due to the nature of the disease rather than by an actual association of the site of disease with the acquisition of resistance. Additionally, an overrepresentation of pulmonary cases exists in the database, with more than 90% of cases being pulmonary, which could also impact on the results for the "Site of disease" variable.

The most important risk factor for the development of any drug resistance, including MDR-TB, was the previous experience with treatment. More than 40% of the patients who previously received treatment (1,428 out of 3,078 previously treated cases) had any drug resistance. For MDR-TB, this proportion reached 24.0% of all previously treated TB cases. This MDR-TB rate was lower than the estimated proportion in previously treated cases (28%) in Ecuador in 2018 (World Health Organization, 2020), but higher than the 20% found in Peru in 2017 (World Health Organization, 2019). In contrast, the proportion of new cases of MDR-TB (10.6%) was higher than the estimated by WHO (2.1%, World Health Organization, 2020), and also higher than 6.3% reported in the most recent study in Peru (World Health Organization, 2019). For the non-previously treated cohort, the proportion of any resistance reached 25.1%, higher than in other countries (Lemus et al., 2013; World Health Organization, 2019).

Both univariate and multivariate analysis allowed to confirm that the previous administration of treatment constituted a consistent, significant risk factor for the acquisition of any resistance and MDR-TB — the probability of developing any resistance doubles when a patient had previously received any treatment. The probability of developing MDR-TB was also double for previously treated patients. Other studies estimated this probability to be up to 6 times higher (Glasauer et al., 2019). A large body of evidence have also recognized the prior treatment

as one of the main risk factors and predictors of drug resistance (Chen et al., 2013; Dheda et al., 2017; Faye et al., 2018; Glasauer et al., 2019; Shamaei et al., 2009; Zhao et al., 2015). Consequently, the resistance in Ecuador (particularly in adults) could be explained by the non-fulfillment of the treatment regime or the administration of inadequate treatment dosage. This inadequate treatment for some resistant patients could be sustained by the bias in the performance of DST. The DST test is not conducted for every single case of TB in Ecuador. Some at risk (priority) groups like children and adults with co-morbidities, are the only individuals eligible for DST. This biased the DST performance and it was likely to boost the spread of the resistant strains.

The joint analysis between age and previous-treatment groups gave an idea of the actual rates of any resistance and MDR-TB that could spread out in Ecuador. Given the bias in the database, the non-previously treated children were considered as the reference for the actual rates of resistance. In this case, the MDR-TB in non-previously treated children was 11.8% of all children cases, while the "any resistance" rate was 22.8%. This percentage was lower than the estimated one in a prior study in Colombia (Llerena et al., 2010). The proportion of MDR-TB for previously-treated children (12.2%) was similar to non-previously treated children, which pointed to other causes for the acquisition of MDR-TB in this group, such as the susceptibility to new resistant TB strains, rather than the no compliance with the treatment. Nevertheless, the number of cases in children was still very low like to be considered significant. The percentage of MDR-TB in non-previously treated adults was similar to children. In non-previously treated young adults, the MDR-TB decreases down to 10.0% of all TB cases in this age category. A similar proportion was observed in non-previously treated elderly. The proportions for previously treated patients were higher for any age group (except for children), which suggests the no adherence to the treatment as the most likely explanation for the development of MDR-TB and any other resistance in these age groups.

Once again, the fact that DST were not performed in every individual had an impact in the differences found in the adults and elderly. In children, all cases received DST, and subsequently the appropriate treatment. Hence in this group, the differences in the resistance between non-treated and treated children could be completely explained by the non-fulfillment of the treatment. However, the differences observed in adults were affected by an inadequate treatment as a consequence of the lack of DST for every case (i.e. some adults were administered drugs for which

they had previous resistance). If we assume a similar abandonment of the treatment in children and adults, this difference between previously and non-previously treated adults would be lower than the described in our study, and the infection by resistant strains would constitute an important explanation to the rates of resistant cases in Ecuador.

The present study shows a more recent picture of the TB burden in Ecuador, where the last national surveillance data was from 2012 (World Health Organization, 2019). These data covered only the MDR-TB burden in previously treated TB cases. Although this study may be useful to know the current status of TB resistance in Ecuador, some limitations were also observed, including the lack of reliable information regarding the current status of TB in Ecuador or neighbor countries, the poor quality of data management, and the inconsistency between government information and the one provided by independent studies. This study depended on the quality of the data that different health institutions reported to INSPI as well as on the accuracy of the first-line DST data generated from INSPI. Hence, a similar study considering not only a wider period, but also socioeconomic variables would improve the knowledge of the findings described in this work.

Conclusions

- Epidemiology studies of drug resistance/susceptibility patterns in Tuberculosis are useful to assess the current burden of TB in Ecuador since the last reported data was from 2012.
- The overall resistance proportion found in the Final Study Population was 31.0%, with almost half of the resistant cases being MDR-TB.
- A decrease in TB resistance was observed from 2012 to 2014, matching the implementation of the DOTS strategy and economical payment for the fulfillment of TB treatment.
- An overall increasing trend was observed in TB resistance from 2014 to 2017.
- No clear trend was observed for any of the most characteristic patterns, except for the MDR-TB, which showed an increasing trend.
- Regarding the variables under scrutiny, only the previous TB treatment constituted a risk factor for the development of any resistance and MDR-TB, with previously treated patients being two times more susceptible to develop resistance patterns.
- In Ecuador, the burden of any resistance and MDR-TB for previously treated patients was 42.2% and 24.0%, respectively. For non-previously treated patients was 25.1% and 10.6%.
- For non-previously treated children, the burden of MDR-TB was 11.8%, similar to the 12.2% for the previously treated children. Resistance in children was likely to be explained by the infection with new resistant TB strains circulating in Ecuador.
- For the other age groups, proportions of MDR-TB and any resistance were higher for previously treated patients, suggesting a non-adherence to the treatment or the administration of inadequate doses like the explanation for the development of MDR-TB, as well as overall resistance.

Recommendations

- More reliable drug susceptibility tests are mandatory to fulfill the 80% of the recommendations suggested by WHO.
- Implementation of more functional rapid test equipment, based in automated real-time PCR (i.e. GeneXpert®) (WHO Global TB Programme, 2014), to evaluate the resistance in the majority of patients.
- A better strategy to surveil the treatment should be applied by the National Tuberculosis Program to ensure the attainment of the treatment by the majority of TB patients.
- Customized treatments should be implemented according to the needs of each patient. Such treatments should be based on the resistance profile for each patient.
- Further studies regarding the epidemiology of resistant TB are also mandatory to keep an updated control and surveillance of the TB burden in Ecuador.

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