Prevalence, Risk Factors, and Treatment Outcomes of Isoniazid- and Rifampicin-Mono-Resistant Pulmonary Tuberculosis in Lima, Peru

Leonela Villegas1*, Larissa Otero2*, Timothy R. Sterling1*, Moises A. Huaman1‡, Patrick Van der Stuyft3,4‡, Eduardo Gotuzzo2‡, Carlos Seas2‡

1 Vanderbilt University School of Medicine, Nashville, TN, United States of America, 2 Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru, 3 Unit of General Epidemiology and Disease Control, Department of Public Health, Institute of Tropical Medicine, Antwerp, Belgium, 4 Department of Public Health, Faculty of Medicine, Ghent University, Ghent, Belgium

These authors contributed equally to this work.

Current Address: Division of Infectious Diseases, Vanderbilt University School of Medicine, A2209 Medical Center North, Nashville, TN, United States of America

‡ These authors also contributed equally to this work.

* Leonela.a.villegas@vanderbilt.edu

Abstract

Background

Isoniazid and rifampicin are the two most efficacious first-line agents for tuberculosis (TB) treatment. We assessed the prevalence of isoniazid and rifampicin mono-resistance, associated risk factors, and the association of mono-resistance on treatment outcomes.

Methods

A prospective, observational cohort study enrolled adults with a first episode of smear-positive pulmonary TB from 34 health facilities in a northern district of Lima, Peru, from March 2010 through December 2011. Participants were interviewed and a sputum sample was cultured on Löwenstein-Jensen (LJ) media. Drug susceptibility testing was performed using the proportion method. Medication regimens were documented for each patient. Our primary outcomes were treatment outcome at the end of treatment. The secondary outcome included recurrent episodes among cured patients within two years after completion of the treatment.

Results

Of 1292 patients enrolled, 1039 (80%) were culture-positive. From this subpopulation, isoniazid mono-resistance was present in 85 (8%) patients and rifampicin mono-resistance was present in 24 (2%) patients. In the multivariate logistic regression model, isoniazid mono-resistance was associated with illicit drug use (adjusted odds ratio (aOR) = 2.10; 95% confidence interval (CI): 1.1–4.1), and rifampicin mono-resistance was associated with HIV infection (aOR = 9.43; 95%CI: 1.9–47.8). Isoniazid mono-resistant patients had a higher...
risk of poor treatment outcomes including treatment failure (2/85, 2%, p-value < 0.01) and death (4/85, 5%, p < 0.02). Rifampicin mono-resistant patients had a higher risk of death (2/24, 8%, p < 0.01).

**Conclusion**

A high prevalence of isoniazid and rifampicin mono-resistance was found among TB patients in our low HIV burden setting which were similar to regions with high HIV burden. Patients with isoniazid and rifampicin mono-resistance had an increased risk of poor treatment outcomes.

**Introduction**

Tuberculosis (TB) has been one of the leading infectious agents worldwide throughout the past century, but drug resistance has emerged more recently as a major concern [1–4]. The prevalence of mono-resistance to isoniazid, one of the most potent first-line anti-TB agents, has been reported in ranges from 4–12% for all TB cases with a global average of 8.1% for new TB cases [3–5]. There is less evidence for rifampicin mono-resistance because it is less studied, but prevalences under 1% for new TB cases have been reported within Europe in 2010 and 3.2% in Zambia [6, 7]. Meanwhile, multidrug-resistant TB (MDR-TB; defined as resistance to at least isoniazid and rifampicin) continues to be an extensive problem, with an estimated 3.5% of new cases globally in 2014 [4]. The optimal treatment of drug-resistant TB is unclear due to limited data from randomized, controlled trials, with a focus on aggressive regimens [8–10].

Early reports isoniazid and rifampicin mono-resistance have been presumed to have minimal clinical impact, causing it to be a topic of debate [1, 2, 11, 12]. A meta-analysis demonstrated a strong association between initial isoniazid mono-resistance and treatment failure [9]. There have also been concerns about rifampicin mono-resistant patients acquiring multidrug resistance, specifically in populations where resistance is frequent [13]. Studies have noted previous TB treatment as isoniazid mono-resistant risk factors [14, 15]. Due to few rifampicin mono-resistant patients, risk factors have not been well characterized; but prior studies have identified prior TB treatment and HIV co-infection [1, 16, 17].

Treatment outcomes of isoniazid mono-resistant TB have been well defined in countries with high HIV prevalence, but there is limited data from low HIV prevalent regions [2, 5, 8]. Minimal studies have also been focused on rifampicin mono-resistance in both high and low HIV prevalent countries. Our study addressed the prevalence, risk factors, and treatment outcomes associated with isoniazid and rifampicin mono-resistance among persons diagnosed with their first episode of culture-confirmed pulmonary TB in a high TB incidence and low HIV prevalence district in Lima, Peru.

**Methods**

**Study Setting**

The study was conducted in San Juan de Lurigancho (SJL), a densely-populated district of Lima, Peru (population: 1,069,566 persons) representing 3.5% of the Peruvian population [18]. It is one of the poorest districts in Peru, and contains one of the largest prisons in the country. The SJL district has 33 health care centers and one referral hospital managed by the Ministry of Health, with each site having a TB unit to provide TB treatment under directly observed therapy short-course (DOTS) following National TB Program (NTP) guidelines; the study was
conducted throughout the 34 facilities within the district of SJL. In 2007, SJL reported 7.0% (2,004/29,393) of all TB cases notified to the National TB Program (NTP) and 14.2% (116/818) of the MDR-TB cases [19].

Study Design

We performed a prospective observational cohort study among adults with a smear-positive pulmonary TB diagnosed between March 2010 and December 2011 with no history of previous TB treatment or diagnoses. Patients initiated anti-TB treatment at the NTP site and were followed through the end of their treatment regimen. Treatment outcomes were prospectively obtained from the NTP registers (see outcome definitions below). TB registers were monitored monthly up to two years after the end of treatment of the last enrolled case for TB recurrence. If a recurrent episode was found among an enrolled and cured TB patient, a sputum sample was gathered if available.

We ascertained demographic, epidemiological, and clinical characteristics of all study participants through interviews using a structured questionnaire. Participants were asked about their education, use and type of general public transportation, employment, prison exposure, illicit drug use, MDR-TB contacts, and comorbidities. The CAGE alcoholism-screening test was used to determine if a patient is at low or high risk of alcohol abuse [20]. It is a standardized four-question test that has been extensively validated as a screening technique. Socioeconomic status (SES) was determined by a scale validated by the Peruvian Ministry of Finance to determine poverty, extreme poverty, and non-poverty in urban and rural households (SISFOH) [21]. All patients were offered HIV screening as part of their routine NTP care. We registered the results of those tested.

TB diagnostic laboratory tests

A single sputum specimen for each patient was collected at diagnosis. Sputa were cultured on Löwenstein-Jensen (LJ) media at the Tuberculosis Laboratory of the Instituto de Medicina Tropical Alexander von Humboldt (Universidad Peruana Cayetano Heredia) in Lima, Peru. Drug susceptibility testing (DST) was performed using the 7H10 agar method with the following concentrations: Isoniazid low level (0.2 μg/ml), high level (1 μg/ml), and rifampicin (1.0 μg/ml). Patients were also recorded if they had a recurrent TB episode.

Definitions

Isoniazid mono-resistance was defined as isoniazid resistant and rifampicin susceptible, while rifampicin mono-resistance included patients that were rifampicin resistant and isoniazid susceptible [4]. Ethambutol and Streptomycin resistance were also tested, but not utilized within this study. Isoniazid resistance was classified as low-level or high-level based on presence of growth of M. tuberculosis at 0.2 μg/ml or 1 μg/ml of isoniazid, respectively. Simultaneous resistance to isoniazid and rifampicin is defined as multidrug-resistant TB (MDR-TB) case. For the documented treatment regimens, the time frame in the table in S1 Table represents treatment start and end dates. Adherence was not measured. Treatment outcomes were based on World Health Organization classifications (cured + treatment completion, failure, lost to follow up, death, and transfer). The latter four were considered poor treatment outcomes. An additional category of “treatment change” was created because participants who were continuously smear-positive during their first four months of their regimen or did not improve clinically post treatment initiation, may have had a change in regimen. The patients physician and a decentralized expert committee from the NTP who review all drug resistant cases would have indicated this change based on clinical presentation or DST. With regards to the treatment regimens, standard regimen I was defined as initiating treatment with two months of isoniazid,
rifampicin, ethambutol, and pyrazinamide, followed by four months of isoniazid and rifampicin. The other treatment regimens included medications from each of the five groups of second line drugs following national and international guidelines. Treatment changes were recorded for each patient. Recurrent TB episodes were assessed two years after completion of the treatment among cured patients.

Data Management and Analysis

The data was entered in Microsoft Access (Microsoft Corporation, Redmond, WA, US) and statistical analyses were performed in STATA 13.1 for Mac (Stata Corporation, College Station, Texas). Bivariate analyses were conducted using the chi-squared and the Mann-Whitney rank-sum test for dichotomous and continuous variables, respectively. If the variables had a p-value of <0.2 in the univariate logistic regression, or were considered to be clinically important, they were included in a backward elimination method of logistic regression to generate isoniazid and rifampicin mono-resistance prediction models. Unadjusted and adjusted odds ratios (ORs) with 95% confidence intervals (CI) were also recorded. In addition, to determine the association between isoniazid and rifampicin mono-resistance and treatment outcomes a forward logistic regression analysis was performed, accounting for potential confounders.

Ethical considerations

The cohort study was approved by the Institutional Review Boards at Universidad Peruana Cayetano Heredia, University of Antwerp and at the District Health Direction for East Lima. This subanalysis was also approved by the Vanderbilt University School of Medicine. All participants signed informed consent and were issued a copy of the signed consent form. DST results were given to each patient’s physician in each health facility, for patient management.

Results

Study Population

There were 1,292 persons enrolled in the cohort, of who 1039 (80%) had culture-confirmed TB. Among the 253 that were not confirmed: 115 (45.5%) were culture negative, 49 (19.4%) had a contaminated culture, 31 (12.3%) had an insufficient sample to allow growth, 11 (4.3%) were not processed, and 47 (18.6%) did not submit a sample. The enrolled patients who had culture-confirmed TB were similar to those that were culture negative except that the culture-confirmed TB patients were more likely to report current or former tobacco use and were less likely to have had an undergraduate or post-graduate education level.

Drug Resistant Tuberculosis

There were 85/1039 (8.2%, 95%CI: 6.57–10.07) cases of isoniazid mono-resistant TB. Of these, 45 (53%) had high-level isoniazid resistance and 40 (47%) had low-level isoniazid resistance. Rifampicin mono-resistant TB was found in 24/1039 (2.3%, 95%CI 1.51–3.39) and MDR-TB in 69/1039, (6.6%, 95%CI 5.24–8.28). Table 1 demonstrates the characteristics of isoniazid mono-resistant, rifampicin mono-resistant, MDR-TB, and isoniazid and rifampicin susceptible cases. The MDR-TB group had higher proportion of males and reported a higher proportion of prior rehabilitation center placement.

Characteristics Associated to Isoniazid and Rifampicin Mono-resistance

The bivariate and multivariate analyses for characteristics associated with isoniazid and rifampicin mono-resistance are shown in Tables 2 and 3, respectively. In the multivariable analysis
Table 1. Demographic and Clinical Characteristics of Isoniazid and Rifampicin Susceptible, Isoniazid Mono-resistant, Rifampicin Mono-resistant, and Multidrug-Resistant Pulmonary Tuberculosis Patients.

|                          | Isoniazid & RifampicinSusceptible (n = 861) | RifampicinMono-resistance (n = 24) | IsoniazidMono-resistance (n = 85) | Multi-DrugResistance (n = 69) |
|--------------------------|---------------------------------------------|-----------------------------------|---------------------------------|-----------------------------|
|                          | n   | %    | n   | %    | n   | %    | n   | %    |
| Age                      |     |      |     |      |     |      |     |      |
| <40                      | 674 | 78.3 | 19  | 79.2 | 70  | 82.4 | 56  | 81.2 |
| >40                      | 187 | 21.7 | 5   | 20.8 | 15  | 17.5 | 13  | 18.8 |
| Sex                      |     |      |     |      |     |      |     |      |
| Male                     | 528 | 61.3 | 16  | 66.7 | 49  | 57.6 | 53  | 76.8 |
| Female                   | 333 | 38.7 | 8   | 33.3 | 36  | 42.4 | 16  | 23.2 |
| Use of public transportation|   |      |     |      |     |      |     |      |
| Small/large bus          | 629 | 73.1 | 17  | 70.8 | 61  | 71.8 | 47  | 68.1 |
| Taxi/Shared taxi         | 189 | 22.0 | 7   | 29.2 | 20  | 23.5 | 17  | 24.6 |
| Does not use             | 43  | 5.0  | 0   | 0    | 4   | 4.7  | 5   | 7.2  |
| Tobacco use              |     |      |     |      |     |      |     |      |
| Never                    | 506 | 58.8 | 12  | 50   | 48  | 56.5 | 36  | 52.2 |
| Former/current           | 353 | 41.0 | 12  | 50   | 37  | 43.5 | 33  | 47.8 |
| Alcoholism               |     |      |     |      |     |      |     |      |
| 0–1 (low suspicion)      | 645 | 74.9 | 17  | 70.8 | 71  | 83.5 | 48  | 69.6 |
| 2–4 (high suspicion)     | 184 | 21.4 | 7   | 29.2 | 12  | 14.1 | 19  | 27.5 |
| Illicit drug use         |     |      |     |      |     |      |     |      |
| No                       | 723 | 84.0 | 20  | 83.3 | 67  | 78.8 | 51  | 73.9 |
| Yes                      | 138 | 16.0 | 4   | 16.7 | 18  | 21.2 | 18  | 26.1 |
| Past rehabilitation center admission |   |      |     |      |     |      |     |      |
| No                       | 800 | 92.9 | 23  | 95.8 | 78  | 91.8 | 61  | 88.4 |
| Yes                      | 60  | 7.1  | 0   | 0    | 7   | 8.2  | 8   | 11.6 |
| MDR contact              |     |      |     |      |     |      |     |      |
| No/unknown               | 804 | 93.4 | 24  | 100  | 78  | 91.8 | 58  | 81.2 |
| Yes                      | 57  | 6.6  | 0   | 0    | 7   | 8.2  | 11  | 15.9 |
| HIV status               |     |      |     |      |     |      |     |      |
| Negative                 | 623 | 72.4 | 13  | 54.2 | 58  | 68.2 | 56  | 81.2 |
| Sero-positive            | 11  | 1.3  | 2   | 8.3  | 3   | 3.5  | 4   | 5.8  |
| Unknown                  | 227 | 26.4 | 9   | 37.5 | 24  | 28.2 | 9   | 13   |
| Prior use of isoniaiazid prophylaxis |   |      |     |      |     |      |     |      |
| No                       | 842 | 97.8 | 23  | 95.8 | 83  | 97.6 | 66  | 95.7 |
| Yes                      | 18  | 2.1  | 0   | 0    | 2   | 2.4  | 2   | 2.9  |
| Socioeconomic status     |     |      |     |      |     |      |     |      |
| No poverty               | 595 | 69.1 | 18  | 75   | 51  | 60   | 43  | 62.3 |
| Poverty                  | 210 | 24.4 | 5   | 20.8 | 28  | 32.9 | 21  | 30.4 |
| Prior Imprisonment       |     |      |     |      |     |      |     |      |
| No                       | 820 | 95.2 | 21  | 87.5 | 83  | 97.6 | 65  | 94.2 |
| Yes                      | 40  | 4.8  | 3   | 12.5 | 2   | 2.4  | 4   | 5.8  |

doi:10.1371/journal.pone.0152933.t001
Table 2. Multivariate Analysis of Characteristics Associated with Isoniazid Mono-resistance.

| Variables                        | Isoniazid Monoresistance | Isoniazid and Rifampicin Susceptible | Unadjusted Odds Ratio [95%CI] | Adjusted Odds Ratio [95%CI] |
|----------------------------------|--------------------------|-------------------------------------|-------------------------------|----------------------------|
| Age                              |                          |                                     |                               |                            |
| <40                              | 70                       | 82.3                                | 681 79.1                      |                            |
| >40                              | 15                       | 17.7                                | 180 20.9                      | 0.71 [0.4–1.3]             |
| Sex                              |                          |                                     |                               |                            |
| Male                             | 49                       | 57.6                                | 528 61.3                      | 0.91 [0.6–1.4]             |
| Female                           | 36                       | 42.4                                | 333 38.7                      |                            |
| Diabetes                         |                          |                                     |                               |                            |
| No                               | 82                       | 96.5                                | 825 95.8                      |                            |
| Yes                              | 3                        | 3.5                                 | 36 4.1                        | 0.83 [0.3–2.8]             |
| Use of public transportation     |                          |                                     |                               |                            |
| Small/large bus                  | 61                       | 71.8                                | 629 73.1                      | 3.91 [0.5–29.0]            |
| Yes                              | 18                       | 23.5                                | 189 22.0                      | 4.40 [0.6–34.0]            |
| Tobacco use                      |                          |                                     |                               |                            |
| No                               | 48                       | 56.5                                | 506 58.8                      |                            |
| Former/current                   | 37                       | 43.5                                | 353 41.0                      | 1.17 [0.7–1.9]             |
| Alcoholism                       |                          |                                     |                               |                            |
| 0–1 (low suspicion)              | 71                       | 83.5                                | 645 74.9                      |                            |
| 2–4 (high suspicion)             | 12                       | 14.1                                | 184 21.4                      | 0.60 [0.3–1.2]             |
| Illicit drug use                 |                          |                                     |                               |                            |
| No                               | 67                       | 78.8                                | 723 84.0                      |                            |
| Yes                              | 18                       | 21.2                                | 138 16.0                      | 1.48 [0.8–2.7]             |
| Past rehabilitation center admission |                  |                                     |                               |                            |
| No                               | 78                       | 91.8                                | 800 92.9                      |                            |
| Yes                              | 7                        | 8.2                                 | 60 7.0                        | 1.37 [0.6–3.3]             |
| MDR contact                      |                          |                                     |                               |                            |
| No/unknown                       | 78                       | 91.8                                | 804 93.4                      |                            |
| Yes                              | 7                        | 8.2                                 | 57 6.6                        | 1.38 [0.6–3.2]             |
| HIV status                       |                          |                                     |                               |                            |
| Negative                         | 58                       | 68.2                                | 623 72.4                      |                            |
| Sero-positive                    | 3                        | 3.5                                 | 11 1.3                        | 3.20 [0.9–12.0]            |
| Unknown                          | 24                       | 28.2                                | 227 26.4                      | 1.23 [0.7–2.0]             |
| Prior use of isoniazid prophylaxis |                      |                                     |                               |                            |
| No                               | 83                       | 97.6                                | 843 97.9                      |                            |
| Yes                              | 2                        | 2.4                                 | 18 2.1                        | 1.09 [0.2–4.8]             |
| Socioeconomic status             |                          |                                     |                               |                            |
| No poverty                       | 51                       | 60                                  | 595 69.1                      |                            |
| Poverty                          | 28                       | 32.9                                | 210 24.4                      | 1.53 [0.9–2.5]             |
| Prior imprisonment               |                          |                                     |                               |                            |
| No                               | 83                       | 97.6                                | 821 95.4                      |                            |
| Yes                              | 2                        | 2.4                                 | 40 4.6                        | 0.29 [0.04–2.1]            |

doi:10.1371/journal.pone.0152933.t002
of isoniazid mono-resistant individuals, the best prediction model consisted of HIV status, illicit drug use, prior imprisonment, alcoholism, socioeconomic status, and type of transportation. The report of illicit drug use was the only factor significantly associated (aOR 2.06, 95% CI 1.1–4.1) with isoniazid mono-resistance. In the multivariate analysis of rifampin mono-resistance, the HIV positive status was the only factor found to be weakly associated with mono-resistance (aOR 9.43, 95% CI 1.9–47.8), however, this was based on only two patients.

### Treatment Regimens and Outcomes

The primary endpoint was the outcome at the end of treatment. This analysis demonstrated that isoniazid-mono-resistant patients were more likely to die (4/85, 5%, p = 0.014) and to fail treatment (2/85, 2%, p < 0.01) compared to persons with isoniazid-susceptible TB (Table 4). Rifampicin mono-resistant patients also had an increased risk of death (2/24, 8%, p < 0.01), as shown in Table 4, when compared to rifampicin-susceptible TB cases. The proportion of high

---

**Table 3. Multivariate Analysis of Characteristics Associated with Rifampicin Mono-resistance.**

| Variables                   | Rifampicin Mono-resistance | Isoniazid and Rifampicin Susceptible | Unadjusted Odds Ratio [95%CI] | Adjusted Odds Ratio [95%CI] |
|-----------------------------|----------------------------|--------------------------------------|------------------------------|----------------------------|
| Age                        | n  | %        | n  | %        |                              |                            |
| <40                         | 19 | 82.6     | 681 | 79.1     | 0.75 [0.3–2.2]               | -                          |
| >40                         | 4  | 17.4     | 180 | 20.9     | 1.28 [0.5–3.1]               | -                          |
| Sex                        |    |          |    |          |                              |                            |
| Male                       | 16 | 66.7     | 528 | 61.3     | 1.28 [0.5–3.1]               | -                          |
| Female                     | 8  | 33.3     | 333 | 38.7     |                            |                            |
| Use of public transportation|    |          |    |          |                              |                            |
| Small/large bus            | 17 | 70.8     | 629 | 73.1     | 0.65 [0.3–1.6]               | -                          |
| Taxi/shared taxi           | 7  | 29.2     | 189 | 22.0     |                            |                            |
| Does not use               | 0  | 0.0      | 43  | 5.0      |                            |                            |
| Tobacco                    |    |          |    |          |                              |                            |
| Never                      | 12 | 50.0     | 506 | 58.8     |                            |                            |
| Former/Current             | 12 | 50.0     | 353 | 41.0     | 1.42 [0.6–3.3]               | -                          |
| Alcoholism                 |    |          |    |          |                              |                            |
| 0–1 (low suspicion)        | 17 | 70.8     | 645 | 74.9     |                            |                            |
| 2–4 (high suspicion)       | 7  | 29.2     | 184 | 21.4     | 1.31 [0.5–3.4]               | -                          |
| Illicit drug use           |    |          |    |          |                              |                            |
| No                         | 20 | 83.3     | 723 | 84.0     |                            |                            |
| Yes                        | 4  | 16.7     | 138 | 16.0     | 1.33 [0.4–4.0]               | -                          |
| HIV status                 |    |          |    |          |                              |                            |
| Negative                   | 13 | 54.2     | 623 | 72.4     |                            |                            |
| Sero-positive               | 2  | 8.3      | 11  | 1.3      | 9.43 [1.9–47.8]              | 9.43 [1.9–47.8]            |
| Unknown                    | 9  | 37.5     | 227 | 26.4     | 2.13 [0.9–5.1]               | 2.13 [0.9–5.1]             |
| Socioeconomic status       |    |          |    |          |                              |                            |
| No poverty                 | 18 | 75.0     | 595 | 69.1     |                            |                            |
| Poverty                    | 5  | 20.8     | 210 | 24.4     | 0.78 [0.3–2.1]               | -                          |
| Prior imprisonment         |    |          |    |          |                              |                            |
| No                         | 21 | 87.5     | 821 | 95.4     |                            |                            |
| Yes                        | 3  | 12.5     | 40  | 4.6      | 2.14 [0.5–9.5]               | -                          |

doi:10.1371/journal.pone.0152933.t003
level and low level isoniazid mono-resistant patients who were cured are 76% and 73%, respectively, with minor differences in the poor outcome categories.

Additionally, poor treatment outcomes were more frequent among the isoniazid mono-resistant population than in the isoniazid and rifampicin sensitive group (22/85, 26% vs. 126/861, 15%, p < 0.01) when compared to the successfully treated patients. The rifampicin mono-resistant subgroup had a higher risk of poor treatment outcomes than the isoniazid and rifampicin sensitive group (7/24, 29% vs. 126/861, 15%, p < 0.05).

Standard regimen I was started in 78 isoniazid-mono-resistant patients, of which 36 patients completed the course of six months and 33 patients were switched to a drug resistant regimen detailed in the table in S1 Table. Standard regimen I was initiated in all the rifampicin mono-resistant patients, but three cases were subsequently changed to a drug resistant treatment noted in the table in S2 Table. Sixteen out of the 17 total cured rifampicin mono-resistance patients were cured with standard regimen I. A review of those cases, found that two of them were found to be rifampicin susceptible in a different DST from a routine sputum sample submitted within two days of the study sample. Of the 17 cured rifampicin mono-resistant patients, six were followed up within two years after cure, and seven were followed up to one year after cure. In all cases patients reported to be asymptomatic during that period.

None of the isoniazid mono-resistant participants that were cured (63/85, 74%) had a documented recurrent episode of TB. Among the rifampicin mono-resistant participants who were cured (17/24, 71%), one had a documented reinfection confirmed with spoligotyping and MIR-U-VNTR analysis (1/17, 6%), one had a recurrent episode that was not further classified (1/17, 6%), and fifteen had no documented recurrent episodes (15/24, 88%) during the two year follow-up.

**Discussion**

This study, the largest prospective cohort on isoniazid and rifampin mono-resistant TB to date, found high proportions of isoniazid and rifampicin mono-resistance in a setting of low HIV prevalence. Twenty-six percent of the patients with rifampicin resistance (24/93) were found to not be MDR-TB. Illicit drug use was a risk factor for isoniazid mono-resistance, while HIV infection was associated with rifampicin mono-resistance. Deaths and treatment failures were more frequent among patients with isoniazid mono-resistance and deaths were more frequent among rifampicin mono-resistant patients. Meanwhile, high-level and low-level of isoniazid mono-resistance had similar treatment outcomes. The patients within our study were treated with diverse regimens; however, high cure rates were encountered with rifampicin, ethambutol, pyrazinamide, and the addition of levofloxacin.
Primary drug resistance has been increasing with isoniazid mono-resistance being the largest population particularly in the high burden regions [22]. The global prevalence of isoniazid monoresistance has been estimated at 8.1% for new TB cases with a higher percentage in the coinfected-HIV population [4, 23]. We found a similar prevalence in a low HIV burden setting. Findings from the same study district in Lima suggest ongoing transmission of drug resistant strains [24, 25]. There are limited studies on rifampicin mono-resistance; however, Western Europe reported <0.3%, while proportions as high as 1.3% have been reported in Mexico and Zambia [6, 22]. Our study found a relatively high prevalence: 2.3%, however our definition was less stringent than other studies, because we defined mono-resistance solely based on isoniazid and rifampicin without including the other first line drugs. Rifampicin resistance has frequently been considered a proxy for MDR. Our results call for careful evaluation of implementation of DSTs that only test for rifampicin resistance which may result in giving MDR treatment to isoniazid susceptible patients.

Past studies have demonstrated a strong correlation between history of TB treatment with isoniazid mono-resistance [2, 15, 16, 26]. However, none of our patients received prior TB treatment since we were specifically evaluating primary resistance. Our study contained two patients with isoniazid monoresistance that reported prior isoniazid prophylaxis, but illicit drug use was the only significant factor within the prediction model. Previous studies have shown rifampicin mono-resistance associated with history of TB, prior imprisonment, and alcohol abuse [12, 17]. Our study confirmed a weak association between HIV co-infection and rifampicin mono-resistance [1, 3]. We hypothesize that since Peru has a low burden of HIV, HIV care is centralized, this population may be at higher risk of exposure to drug resistant TB due to their frequent attendance at specialized medical facilities.

Isoniazid mono-resistance treatment outcomes have been a topic of debate due to conflicting studies. Our results were compatible with prior reports demonstrating that isoniazid mono-resistant cases are at a higher risk for poor outcomes, specifically failures and deaths. We found no differences in treatment outcomes between the lower and higher concentrations of isoniazid resistance similar to the findings of Espinal et al and Chien et al [8, 27]. A significant association suggested that the rifampicin mono-resistant population was at higher risk of death, however this was only based on two patients and should be evaluated in a larger sample.

One study in France that included 39 cases reported that only 67% were cured. In regards to long-term outcomes, our study found that none of the isoniazid mono-resistant and one of the rifampicin mono-resistant cases had a recurrence, which could not be tested to determine if it was a relapse or a reinfection; a larger population would be more informative to determine the impact of mono-resistance on TB relapse. There are standardized regimens for isoniazid mono-resistant patients and recommendations for the rifampicin mono-resistant population; however, there is limited evidence supporting their benefit. Our study highlighted how poor outcomes may be more frequent with mono-resistant TB emphasizing the need for appropriate treatment regimens and outreach required to apply them into communities.

As this was an observational study, there were limitations inherent to the study design. First, we had missing information for several of our cases, specifically alcoholism and socioeconomic status, causing our sample size to be decreased. Second, the rifampicin mono-resistant sample size was a small subgroup, generating insufficient statistical power to be able to fully address association with risk factors and treatment outcomes. Third, our long-term outcomes only provided information for cured patients, and in many cases it was only a passive follow up. This may have underestimated relapses, which would over estimate long-term success. The study also used mono-resistance based on isoniazid and rifampicin susceptibilities.

In conclusion, isoniazid and rifampicin mono-resistance were frequent in our setting and were associated with an increased risk of death. The knowledge gaps that still need to be
addressed includes proper treatment regimens and improvement of accessibility for the patients to provide better outcomes [22]. The importance of facilitating second-line regimens in a DOTS program when drug resistant TB strains are detected is crucial based on the high risk of developing MDR-TB with standard short-course chemotherapy and propagating drug-resistant TB [28].

Supporting Information

S1 Table. Treatment Regimens and Outcomes in Isoniazid Mono-resistant Cases (N = 85).

S2 Table. Treatment Regimens and Outcomes in Rifampicin Mono-resistant Cases (N = 24).

Acknowledgments

We thank our field workers for data and sample collection and the staff of the health care facilities for their continuous support in the conduction of the study.

Author Contributions

Conceived and designed the experiments: LV LO MAH TRS PVDS EG CS. Performed the experiments: LV LO PVDS CS. Analyzed the data: LV LO TRS MAH. Contributed reagents/materials/analysis tools: LV LO MAH TRS PVDS EG CS. Wrote the paper: LV LO MAH TRS PVDS EG CS.

References

1. Meyssonnier V, Bui T, Veziris N, Jarlier V, Robert J. Rifampicin mono-resistant tuberculosis in France: a 2005–2010 retrospective cohort analysis. BMC Infect Dis. 2014; 14(1):1–7.
2. Cattamanchi A, Dantes RB, Metcalfe JZ, Jarlsberg LG, Grinsdale J, Kawamura LM, et al. Clinical Characteristics and Treatment Outcomes of Isoniazid Mono-Resistant Tuberculosis. Clin Infect Dis. 2009; 48(2):179–85. doi: 10.1086/595689 PMID: 19086909
3. Hoopes AJ, Kammerer JS, Harrington TA, Ijaz K, Armstrong LR. Isoniazid-Monoresistant Tuberculosis in the United States, 1993 to 2003. Arch Intern Med. 2008; 168(8):1984–92.
4. Organization WH. Anti-tuberculosis drug resistance in the world, Report no. 4 Geneva, Switzerland: World Health Organization Press; 2014.
5. Wang T-Y, Lin S-M, Shie S-S, Chou P-C, Huang C-D, Chung F-T, et al. Clinical Characteristics and Treatment Outcomes of Patients with Low- and High-Concentration Isoniazid-Monoresistant Tuberculosis. PLoS ONE. 2014; 9(1). doi: e86316.
6. Mulenga C, Chonde A, Bwaya I, Kapata N, Kakungu-Simpungwe M, Docx S, et al. Low Occurrence of Tuberculosis Drug Resistance among Pulmonary Tuberculosis Patients from an Urban Setting, with a Long-Running DOTS Program in Zambia. Tuberc Res Treat. 2010; 2010.
7. Sandgren A, Hollo V, Huitric E, Kodmon C. Complete republication: Epidemiology of tuberculosis in the EU/EEA in 2010—Monitoring the progress towards tuberculosis elimination. Eur J Microbiol Immunol. 2012; 2(4):232–6.
8. Espinal M, Kim S, Suarez P, Kam K, Khomenko A, al e. Standard short-course chemotherapy for drug-resistant tuberculosis: treatment outcomes in 6 countries. JAMA. 2000; 283:2537–45. PMID: 10815117
9. Menzies D, Benedetti A, Paydar A, Royce, Pai M, Burman W, et al. Standardized Treatment of Active Tuberculosis in Patients with Previous Treatment and/or with Mono-resistance to Isoniazid: A Systematic Review and Meta-analysis. PLoS Med. 2009; 6(9):1–14.
10. Timperi R, Han L, Sloutskey A, Becerra M, Nardell E, Salazar J, et al. Drug resistance profiles of Mycobacterium tuberculosis isolates: five years' experience and insight into treatment strategies for MDR-TB in Lima, Peru. Int J Tuberc Lung Dis 2005. 9(2):175–80. PMID: 15732737
11. Jacobson K, Theron D, Victor T, Streicher E, Warren R, Murray M. Treatment outcomes of isoniazid-resistant tuberculosis patients, Western Cape Province, South Africa. Clin Infect Dis. Aug 2011; 53 (4):369–72. doi: 10.1093/cid/cir406 PMID: 21810750

12. Mukinda E, Theron D, Van der Spuy G, Jacobson K, Roscher M, Streicher E, et al. Rise in Rifampicin-Monoresistant Tuberculosis in Western Cape, South Africa. Int J Tuberc Lung Dis. 2012; 16:196–202. doi: 10.5888/ijtld.11.0116 PMID: 22236920

13. Arentz M, Sorensen B, Home DJ, Walson JL. Systematic Review of the Performance of Rapid Rifampicin Resistance Testing for Drug-Resistant Tuberculosis. PLoS ONE. 2013; 8(10):e76533. doi: 10.1371/journal.pone.0076533 PMID: 24098523

14. Balcells M, Thomas S, Godfrey-Faussett P, Grant A. Isoniazid Preventative Therapy and Risk for Resistant Tuberculosis Emerg Infect Dis. 2006; 12:744–51.

15. Espinal M, Laserson K, Camacho M, al e. Determinants of drug-resistant tuberculosis: analysis of 11 countries. Int J Tuberc Lung Dis. 2001; 5:887–93. PMID: 11605880

16. LoBue P, Moser K. Isoniazid- and Rifampin-resistant Tuberculosis in San Diego County, California, United States, 1993–2002. Int J Tuberc Lung Dis. 2005; 9:501–6. PMID: 15875920

17. Sandman L SN, Davidow A, Bonk S. Risk Factors for Rifampin-monoresistant Tuberculosis. American J of Resp and Critical Care Med 1999; 159(2): 468–72. 1999; 159(2):468–72.

18. Instituto Nacional de Estadistica e Informatica. Poblacion de Lima Metropolitana 2014. Available from: http://www.inei.gob.pe/media/MenuRecursivo/publicaciones_digitales/Est/Lb1168/libro.pdf.

19. Bonilla AC. Situación de la tuberculosis en el Perú: current status. Acta méd peruana. 2008; 25(3):163–7.

20. Ewing J. Detecting Alcoholism: The CAGE Questionnaire. JAMA. 1984; 252(14):1905–7. PMID: 6471323

21. Sistema de Focalizacion de Hogares. SISFOH 2015. Available from: http://www.sisfoh.gob.pe/nosotros.shtml?x=1452.

22. Villa-Rosas C L-LR, Oceguera-Palao L. Primary drug resistance in a region with high burden of tuberculosis. A critical problem. Salud Publica Mex. 57:177–79. PMID: 26235779

23. Farley J, Ram M, Pan W, Waldman S, Cassell G, Chaissin R, et al. Outcomes of Multi-Drug Resistant Tuberculosis (MDR-TB) among a Cohort of South African Patients with High HIV Prevalence. PLoS Med. 2011; 8(7):1–6.

24. al. Be. Predominant Mycobacterium tuberculosis Families and High Rates of Recent Transmission among New Cases Are Not Associated with Primary Multidrug Resistance in Lima, Peru. 2015; 53 (6):1854–63.

25. Otero L, Krapp F, Tomatis C, Zamudio C, Matthys F, Gotuzzo E, et al. High Prevalence of Primary Multidrug Resistant Tuberculosis in Persons with No Known Risk Factors. PLoS One. 2011; 6(10):e26276. doi: 10.1371/journal.pone.0026276 PMID: 22046266

26. Clark C, Li J, Driver C, Munsiff S. Risk Factors for Drug-resistant Tuberculosis Among non-US Born Persons in New York City. Int J Tuberc Lung Dis. 2005; 9:964–9. PMID: 16158888

27. Chien JY CY, Wu SG, Lee JJ, Wang JY, Yu CJ. Treatment Outcome of Patients with Isoniazid Monoresistant Tuberculosis. Clin Microbiol Infect. 2015; 21(1):59–68. doi: 10.1016/j.cmi.2014.08.008 PMID: 25636929

28. Cox H, Niemann S, Ismailov G, Doshetov D, Orozco J, Blok L, et al. Risk of Acquired Drug Resistance during Short-Course Directly Observed Treatment of Tuberculosis in an Area with High Levels of Drug Resistance. Clin Infect Dis. 2007; 44(11):1421–7. PMID: 17479936