HIV coinfection is associated with low fitness rpoB variants in rifampicin-resistant *Mycobacterium tuberculosis*
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Abstract
We analysed 312 drug-resistant genomes of *Mycobacterium tuberculosis* (*Mtb*) collected from HIV coinfected and HIV negative TB patients from nine countries with a high tuberculosis burden. We found that rifampicin-resistant *Mtb* strains isolated from HIV coinfected patients carried disproportionally more resistance-conferring mutations in *rpoB* that are associated with a low fitness in the absence of the drug, suggesting these low fitness *rpoB* variants can thrive in the context of reduced host immunity.
Tuberculosis (TB), caused by members of the Mycobacterium tuberculosis (Mtb) Complex, is a leading cause of death worldwide, killing more people than any other infectious disease. Among the many factors driving the global TB epidemics, two factors stand out as particularly important: antibiotic resistance and HIV coinfection (1). Although the impact of both of these factors individually is well recognized, the interaction between them is less clear and likely depends on the particular epidemiologic setting (2). HIV coinfection and drug-resistant TB often coexist in severe epidemics, which could indicate spread of drug-resistant Mtb strains from immune-compromised patients (3–5). The propensity of drug-resistant Mtb strains to spread is influenced by the fitness cost associated with drug resistance determinants (6). Specifically, bacterial strains that have acquired drug resistance-conferring mutations may be less transmissible than their susceptible counterparts, although this fitness cost can be ameliorated by compensatory mutations (7–10). Moreover, the effect of different resistance-conferring mutations on fitness can be heterogeneous (11). In the clinical setting, there is a selection for high-fitness and/or compensated drug-resistant Mtb strains in TB patients (12). However, in immune-compromised hosts, such as HIV coinfected patients, even strains with low-fitness resistance mutations might propagate efficiently (13–15), which could partially explain why drug-resistant TB has been associated with HIV co-infection (16, 17). However, to date, no evidence directly supports the notion that the immunological environment created by HIV co-infection modifies the fitness of drug-resistant Mtb (5, 18, 19).

In this study, we tested the hypothesis that resistance-conferring mutations with low fitness in Mtb are overrepresented among HIV-coinfected TB patients. We focused our analysis on isoniazid and rifampicin, the two most important first-line anti-TB drugs, for which resistance-conferring mutations have been shown to differ in their fitness effects when measured in the laboratory (11). In addition, the frequency of the resistance alleles found in a clinical setting correlates well with the in vitro fitness of strains (20, 21). To explore the association between HIV coinfection and the fitness effect of different drug resistance-conferring mutations in Mtb, we compiled a collection of drug-resistant strains using the global International epidemiology Databases to Evaluate AIDS (IeDEA, http://www.iedea.org) consortium (22, 23) as a platform. For this study, 312 strains were collected.
from HIV-coinfected and HIV uninfected TB patients originating from nine countries on three continents: Peru, Thailand, South Africa, Kenya, Côte d’Ivoire, Botswana, Democratic Republic of the Congo, Nigeria and Tanzania (supplemental methods, Figure 1 and supplemental Table S1). The association between the fitness of isoniazid resistance-conferring mutations and HIV coinfection was tested in a univariate analysis (Figure S1). Isoniazid resistance-conferring mutations were divided into three groups, as previously described (24): katG S315T mutation, katG mutations other than S315T, and inhA promoter mutations only. The S315T substitution in katG causes high-level isoniazid resistance, while retaining some catalase/peroxidase functions (25). Conversely, the inhA promoter mutation does not affect KatG activity. Other substitutions/deletions in katG have been associated with a lower fitness in the laboratory and are observed only rarely among clinical isolates (24, 26, 27).

In the case of rifampicin, the association between the fitness of rpoB variants and HIV coinfection was tested in both a univariate and multivariate analysis (Table 1). Resistance-conferring variants in rpoB were classified into two groups based on their fitness effects documented previously (11, 28, 29). The mutation rpoB S450L was considered ‘high-fitness’, since this mutation was previously shown to confer a low fitness cost in the laboratory (11) and is generally the most common in clinical strains (30). Any other resistance-conferring mutation affecting rpoB was considered ‘low-fitness’ (11). The multivariable logistic regression model with outcome “low fitness rpoB variants” was adjusted for host-related factors (history of TB, country of isolation, sex and age) (31) and bacterial factors (Mtb lineage, presence of a rpoA/C compensatory mutation, clustering of the genome inferred by genetic relatedness). Seventy-six patients from Tanzania and Botswana were excluded from the model due to missing or unknown clinical data.

Out of 312 patients, 113 (36.2%) were HIV-coinfected, 120 (38.5%) were women, 115 (37%) were newly diagnosed TB cases (therefore treatment naïve), 276 (88.5%) harboured isoniazid resistance-conferring mutations, with or without additional resistance, and 282 (90.4%) harboured rifampicin resistance-conferring mutations, with or without additional resistance. In total, 78.8% (n= 246) of the strains were classified as being at least MDR, defined as resistance to isoniazid and rifampicin with or without additional resistance to 2nd line drugs. Amongst the 113 HIV coinfected individuals, 34 (30%)
were on antiretroviral therapy (ART), 26 (23%) were not, and 53 (47%) had unknown ART start date. Four of the seven known *Mtb* lineages were represented in the following proportions: 11 L1 (3.5%), 57 L2 (18.3%), 38 L3 (12.2%), 206 L4 (66.0%). After dividing a total of 276 isoniazid-resistant strains into the three groups of isoniazid resistance-conferring mutations defined above, we found similar proportions in HIV-infected and HIV-uninfected patients (chi-square test, p=0.54, Figure S1), and as expected, the *katG* S315T mutation was the most frequent mutation in both categories (overall, found in 80% of isoniazid-resistant strains). In the case of rifampicin resistance, a univariate and multivariate analysis of 203 strains with complete clinical records, indicated that HIV coinfection TB patients carried a higher proportion of low-fitness *rpoB* resistance variants in comparison to HIV negative patients (72.3% vs. 51.4%). The univariate analysis showed higher odds of having a low-fitness *rpoB* variant in HIV coinfection patients (Odds Ratio 2.46 [95% Confidence Interval 1.30-4.66], p=0.006, Table 1). Our multivariable regression analysis confirmed these results and showed an association between low-fitness *rpoB* variants and HIV-coinfection, whilst controlling for other factors (Odds Ratio 4.58 [95% Confidence Interval 1.69, 12.44], p=0.003, Table 1). This association can be explained at least in two ways. Firstly, HIV-coinfected patients are thought to have fewer lung cavities on average and lower sputum bacillary load (32, 33). The resulting smaller *Mtb* population size would lead to fewer replication events, possibly reducing the number of mutations available for selection to act upon. In other words, low-fitness variants and high-fitness variants would co-occur less often in an HIV-coinfected patient, such that competition between them would be less likely. This scenario would be relevant for de novo acquisition of low-fitness drug-resistant variants within an HIV-coinfected patient. Secondly, following the transmission of a drug-resistant strain with low fitness to a host with reduced immunity, weaker immune pressure acting on this strain might lead to better bacterial survival. The association between low-fitness *rpoB* variants and HIV coinfection remained significant even after adjusting for the different epidemiologic settings (i.e. countries) and the strain genetic background (i.e. *Mtb* lineages). We also observed that strains carrying the *rpoB* S450L resistance-conferring mutation were more likely to also carry a compensatory mutation in *rpoA/C* (97.4% vs. 2.6%, Table 1). Even though this phenomenon seems counter-intuitive, it has been described multiple times (7, 9, 34–36) and might thus point to different mechanisms of compensation.
in strains carrying resistance mutations other than \textit{rpoB} S450L. In addition, in our study, L4 strains were associated with low fitness \textit{rpoB} variants, compared to L2 (Odds Ratio 3.10 [95% Confidence Interval 0.94, 10.21], p=0.06, Table 1), indicating that the strain genetic background could play a role in shaping the cost of resistance, as was previously shown for other bacterial species (37) and for other drugs (38). In the regression analysis, we had several categorical variables with only few observations. Therefore, statistical power especially for country of isolation was low and the results should be interpreted with care.

HIV coinfectected TB patients are generally thought of having a reduced potential for TB transmission (32, 39) because these patients have reduced formation of lung cavities, more extrapulmonary disease, and a shorter period of infectiousness due to earlier diagnosis or higher mortality, especially in the absence of anti-retroviral treatment and if antibiotic resistance is already present (4). Based on the over-representation of low-fitness \textit{rpoB} mutations in the context of HIV coinfection, one would expect a further reduction of the transmission potential of drug-resistant TB in this context. Yet, outbreaks of drug-resistant TB in HIV coinfectected patients have been reported (40). Such outbreaks might be explained by i) a higher risk of \textit{Mtb} infection and reinfection due to diminished host immunity, ii) on-going transmission of drug-resistant \textit{Mtb} from a larger pool of immune-competent TB patients to immune-compromised patients, iii) transmission occurring in conducive environments such as health care settings where both HIV coinfectected individuals and DR-TB patients are more likely to co-exist, and iv) \textit{Mtb} strains carrying high-fitness drug resistance mutations.

In summary, using a global sample of drug-resistant \textit{Mtb} clinical strains from HIV coinfectected and HIV negative TB patients, we showed that low-fitness \textit{rpoB} variants were overrepresented in HIV coinfectected patients, and that this association was independent from other potential confounding factors. Taken together, our results provide new insights into how HIV coinfection can impact the fitness of drug-resistant \textit{Mtb}.

\textbf{Data availability.} The \textit{Mtb} whole-genome sequences from the patients are available on NCBI under several project IDs. The accession number for each genome is indicated in the supplemental Table 1.
Acknowledgments

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Table 1. Results of the univariate and multivariate analysis showing host and bacterial factors 
associated with low fitness \textit{rpoB} variants in 203 TB patients.

Figure 1. A. Frequency of \textit{Mtb} lineages by HIV status for countries sampled. Countries coloured 
in grey were sampled. The barplots indicate the proportion of each lineage represented in this study. 
Magenta corresponds to \textit{Mtb} lineage 1, blue corresponds to \textit{Mtb} lineage 2, purple corresponds to \textit{Mtb} 
lineage 3 and red corresponds to \textit{Mtb} lineage 4. Solid colour corresponds to HIV negative and hatches 
correspond to HIV coinfected TB patients. The number of genomes sampled in each country is 
indicated on top of the barplots. B. Phylogenetic tree of the dataset used in the study. Maximum 
likelihood phylogeny of 312 whole-genome sequences based on 18,531 variable positions. The scale 
bar indicates the number of substitutions per polymorphic site. The phylogeny was rooted on \textit{M. 
canettii}. \textit{Mtb} isolated from HIV coinfected patients are indicated by black dots. The peripheral ring 
depicts the country of isolation of the strains sequenced.
HIV status
Positive
Countries of Isolation
Botswana
Democratic Republic of Congo
Côte d'Ivoire
Kenya
Nigeria
Peru
South Africa
Tanzania
Thailand

A

B

0.005
Table 1. Results of the univariate and multivariate analysis showing host and bacterial factors associated with low fitness rpoB variants in 203 TB patients.

<table>
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<tr>
<th>Dependent variable</th>
<th>Low fitness</th>
<th>High fitness</th>
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<th>Multivariable</th>
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<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
<td>OR (95% CI)</td>
<td>P</td>
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<tr>
<td>HIV status</td>
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<td></td>
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<td>71 (51.4)</td>
<td>67 (48.6)</td>
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<td>HIV+</td>
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<td>4.58 (1.69-12.44)</td>
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<td>38 (97.4)</td>
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<td>Lineage 4</td>
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<td>3 (50.0)</td>
<td>1.25 (0.22-7.05)</td>
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<td></td>
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<td></td>
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<td>0.97 (0.11-8.31)</td>
</tr>
<tr>
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<td>1.05 (0.28-3.90)</td>
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<td>Country of isolation</td>
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<td>29 (55.8)</td>
<td>23 (44.2)</td>
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<td>Democratic</td>
<td>11 (37.9)</td>
<td>18 (62.1)</td>
<td>0.48 (0.19-1.23)</td>
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<td>Republic of Congo</td>
<td>35 (79.5)</td>
<td>9 (20.5)</td>
<td>3.08 (1.24-7.70)</td>
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<td>53 (42.7)</td>
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<td>83 (61.0)</td>
<td>53 (39.0)</td>
<td>1.43 (0.79-2.58)</td>
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</table>

Number of observations in model = 203; CI = confidence interval; The odds ratio and p-value are obtained from the regression model.