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-coinfected 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 coinfected 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 coinfected 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 *rpoB* S450L. In addition, in our study, L4 strains were associated with low fitness *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 coinfected 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 *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 coinfected patients have been reported (40). Such outbreaks might be explained by i) a higher risk of *Mtb* infection and reinfection due to diminished host immunity, ii) on-going transmission of drug-resistant *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 coinfected individuals and DR-TB patients are more likely to co-exist, and iv) *Mtb* strains carrying high-fitness drug resistance mutations.

In summary, using a global sample of drug-resistant *Mtb* clinical strains from HIV coinfected and HIV negative TB patients, we showed that low-fitness *rpoB* variants were overrepresented in HIV coinfected 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 *Mtb*.

Data availability. The *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

We thank all the sites that participated in this study and the patients whose data were used in this study. We thank Dr. Jan Hattendorf for providing statistical help and Dr. Sebastian M. Gygli for critically reading the manuscript. Calculations were performed at the sciCORE (http://scicore.unibas.ch/) scientific computing core facility at University of Basel.

Funding

This research was supported by the Swiss National Science Foundation (grant numbers 153442, 310030_188888, 174281 and IZRJZ3_164171). The International Epidemiology Databases to Evaluate AIDS (IeDEA) is supported by the U.S. National Institutes of Health's National Institute of Allergy and Infectious Diseases, the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development, the National Cancer Institute, the National Institute of Mental Health, the National Institute on Drug Abuse, the National Heart, Lung, and Blood Institute, the National Institute on Alcohol Abuse and Alcoholism, the National Institute of Diabetes and Digestive and Kidney Diseases, the Fogarty International Center, and the National Library of Medicine: Asia-Pacific, U01AI069907; CCASAnet, U01AI069923; Central Africa, U01AI096299; East Africa, U01AI069911; NA-ACCORD, U01AI069918; Southern Africa, U01AI069924; West Africa, U01AI069919. Informatics resources are supported by the Harmonist project, R24AI124872. This work is solely the responsibility of the authors and does not necessarily represent the official views of any of the institutions mentioned above. RJW is supported by Francis Crick Institute which receives funding from Wellcome (FC0010218), CRUK (FC0010218) and UKR1 (FC0010218). He is also supported by Wellcome (104803,203135).

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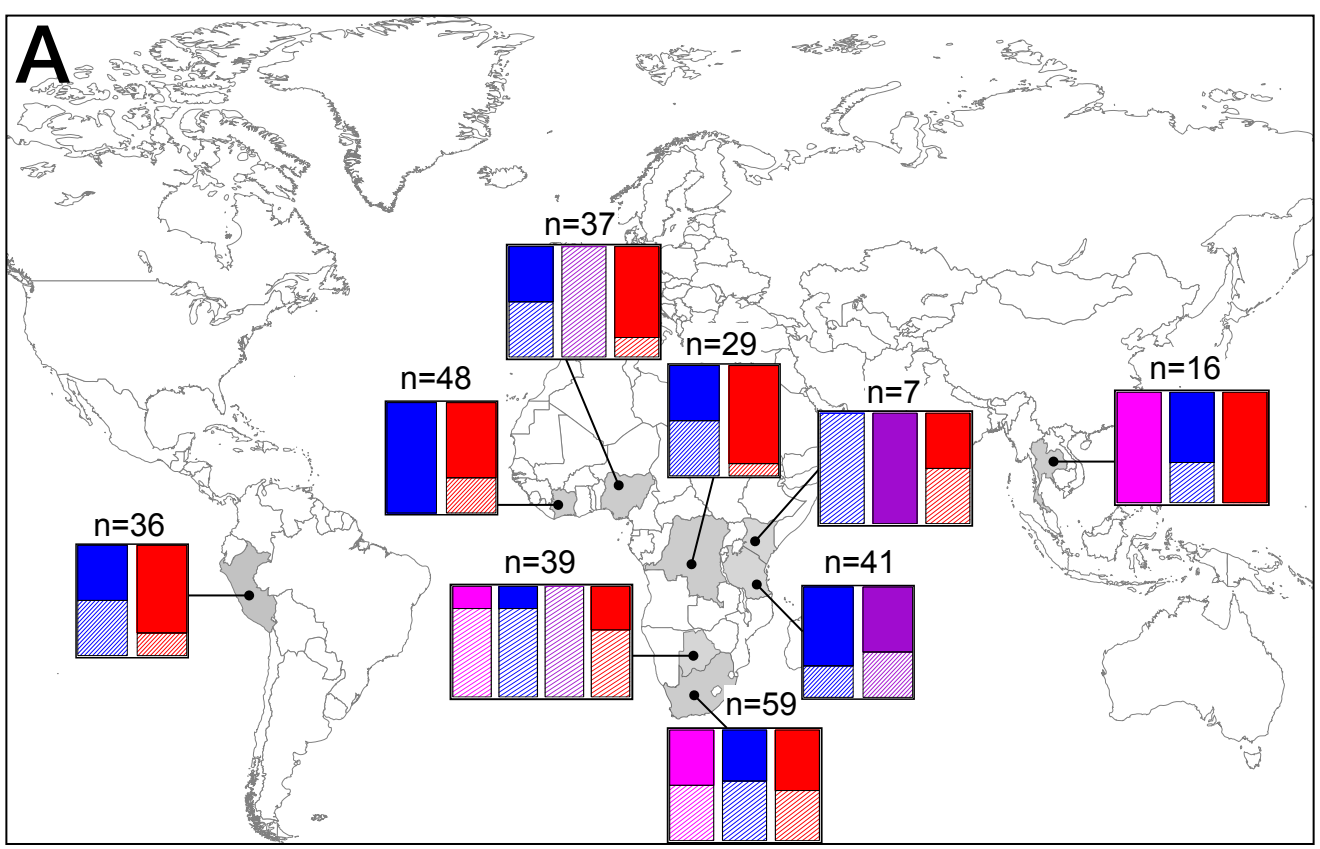
List of Tables and figures

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

Figure 1. A. Frequency of *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 *Mtb* lineage 1, blue corresponds to *Mtb* lineage 2, purple corresponds to *Mtb* lineage 3 and red corresponds to *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 *M. canettii*. *Mtb* isolated from HIV coinfected patients are indicated by black dots. The peripheral ring depicts the country of isolation of the strains sequenced.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n=16
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n=59

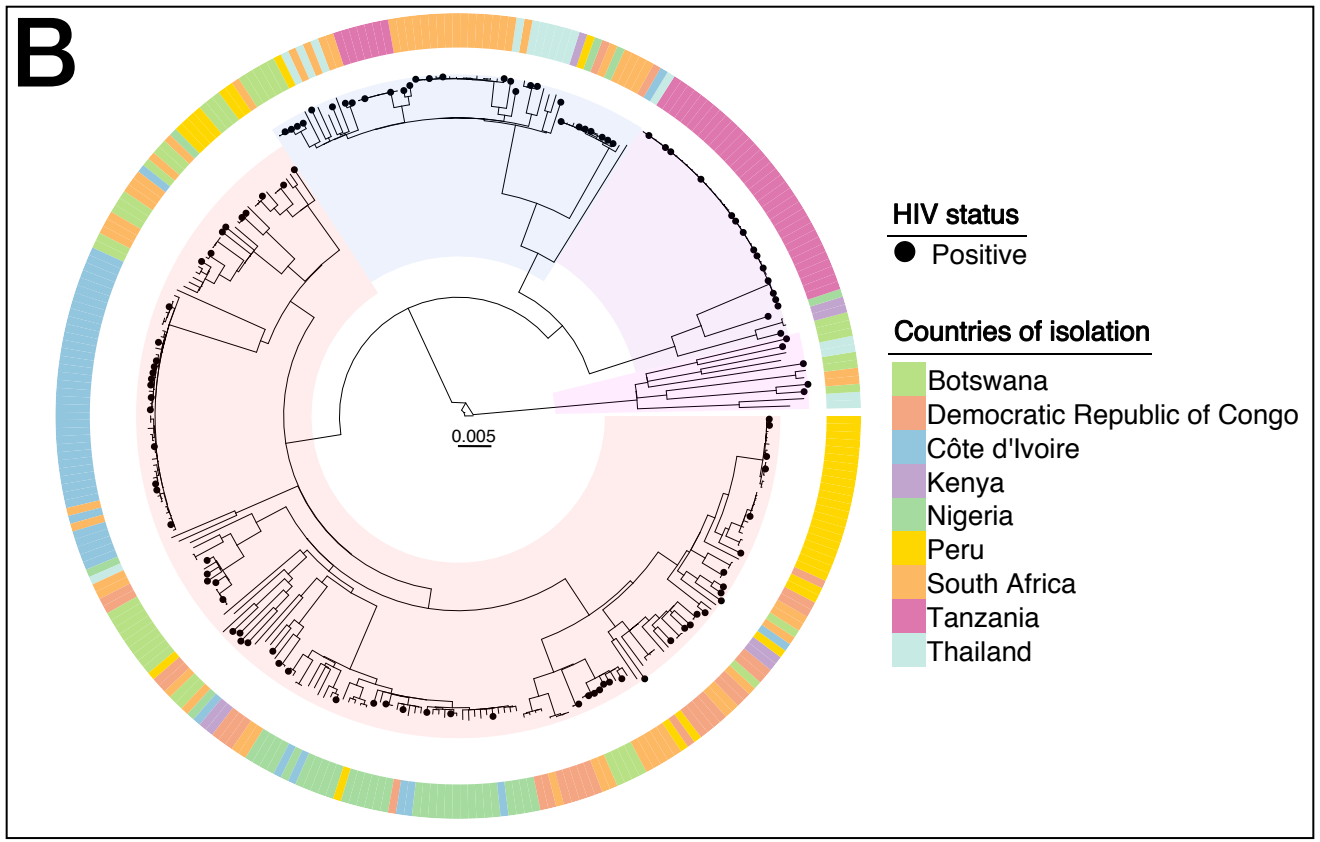
B
0.005
HIV status
Positive
Countries of isolation
Botswana
Democratic Republic of Congo
Côte d'Ivoire
Kenya
Nigeria
Peru
South Africa
Tanzania
Thailand

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

Dependent: fitness of *rpoB* variants		Low-fitness	High-fitness	univariable		multivariable	
				OR (95% CI)	*P*	OR (95% CI)	*P*
HIV status	HIV-	71 (51.4)	67 (48.6)	reference		reference	
	HIV+	47 (72.3)	18 (27.7)	2.46 (1.30-4.66)	0.006	4.58 (1.69-12.44)	0.003
Presence of a compensatory mutation in *rpoA/C*	No	117 (71.3)	47 (28.7)	reference		reference	
	Yes	1 (2.6)	38 (97.4)	0.01 (0.00-0.08)	< 0.0001	0.01 (0.00-0.06)	< 0.0001
Mtb lineage	Lineage 2	16 (44.4)	20 (55.6)	reference		reference	
	Lineage 4	99 (61.5)	62 (38.5)	2.00 (0.96-4.14)	0.06	3.10 (0.94-10.21)	0.06
	Other (L1 or L3)	3 (50.0)	3 (50.0)	1.25 (0.22-7.05)	0.80	0.97 (0.11-8.31)	0.98
Clustering of the genome	No	109 (59.6)	74 (40.4)	reference		reference	
	Yes	9 (45.0)	11 (55.0)	0.56 (0.22-1.41)	0.21	1.05 (0.28-3.90)	0.94
Country of isolation	South Africa	29 (55.8)	23 (44.2)	reference		reference	
	Democratic Republic of Congo	11 (37.9)	18 (62.1)	0.48 (0.19-1.23)	0.13	0.39 (0.12-1.34)	0.14
	Côte d'Ivoire	35 (79.5)	9 (20.5)	3.08 (1.24-7.70)	0.02	2.04 (0.58-7.23)	0.27
	Kenya	4 (66.7)	2 (33.3)	1.59 (0.27-9.44)	0.61	0.94 (0.10-8.42)	0.96
	Nigeria	20 (58.8)	14 (41.2)	1.13 (0.47-2.72)	0.78	1.00 (0.29-3.40)	0.99
	Peru	16 (53.3)	14 (46.7)	0.91 (0.37-2.23)	0.83	1.49 (0.33-6.70)	0.60
	Thailand	3 (37.5)	5 (62.5)	0.48 (0.10-2.20)	0.34	0.42 (0.07-2.65)	0.36
Age	Mean (SD)	32.5 (10.4)	34.3 (12.3)	0.99 (0.96-1.01)	0.25	0.97 (0.94-1.01)	0.10
Sex	Female	47 (59.5)	32 (40.5)	reference			
	Male	71 (57.3)	53 (42.7)	0.91 (0.51-1.62)	0.75	0.77 (0.34-1.71)	0.52
History of TB disease	No	35 (52.2)	32 (47.8)	reference			
	Yes	83 (61.0)	53 (39.0)	1.43 (0.79-2.58)	0.23	0.96 (0.34-2.73)	0.94

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